RJNMSR
Review Journal of Neurological & Medical Sciences Review

Volume 3, Issue 7, 2025

EVALUATION OF GENEXPERT VERSUS CONVENTIONAL DIAGNOSTIC METHODS FOR DETECTION OF PULMONARY AND EXTRA-PULMONARY TUBERCULOSIS

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DOI: https://doi.org/10.5281/zenodo.17836439

Received	Accepted	Published
09 October 2025	15 November 2025	29 November 2025

ABSTRACT

Introduction: *Tuberculosis (TB) remains a major global health challenge, necessitating rapid and accurate diagnostic methods. While conventional techniques like acid-fast bacilli (AFB) smear and culture are widely used, their limitations in sensitivity and turnaround time highlight the need for more efficient tools. The GeneXpert MTB/RIF assay offers a molecular-based solution for the simultaneous detection of Mycobacterium tuberculosis and rifampicin resistance.*

Objectives: *This study aimed to evaluate the diagnostic accuracy of GeneXpert in comparison to AFB smear and fluorescent staining, focusing on their sensitivity, specificity, and overall diagnostic performance.*

Material and Methods: *This prospective cross-sectional study was conducted at Mahaban Medical and Research Hospital, Topi, District Swabi. A total of 227 samples (115 males and 112 females) were analyzed, categorized by age and sample type (pulmonary and extra-pulmonary). All samples underwent diagnostic testing with GeneXpert, AFB smear, and fluorescent staining.*

Results: *Of the 227 samples, 36 (15.85%) were positive for M. tuberculosis, comprising 19 (52.77%) pulmonary and 17 (47.3%) extra-pulmonary specimens. The most affected age group was 1-20 years (36.11%). The GeneXpert assay demonstrated a sensitivity of 97.2%, specificity of 100%, positive predictive value (PPV) of 100%, and negative predictive value (NPV) of 99.5%. In comparison, AFB smear showed sensitivity, specificity, PPV, and NPV of 72.2%, 99.5%, 96.3%, and 95.02%, respectively, while fluorescent staining showed corresponding values of 83.33%, 98.96%, 93.75%, and 97.44%, respectively. The incidence of rifampicin resistance was 2.8%, detected in one sputum sample by GeneXpert.*

Conclusion: *GeneXpert demonstrates superior sensitivity and specificity compared with conventional smear microscopy. Its ability to rapidly and accurately detect both M. tuberculosis and rifampicin resistance makes it an invaluable tool for the timely diagnosis and effective management of tuberculosis. Incorporating GeneXpert into routine diagnostic protocols can significantly enhance TB control efforts*

Keywords: *GeneXpert, rifampicin, Mycobacterium tuberculosis*

INTRODUCTION

Tuberculosis (TB), caused by the bacterium Mycobacterium tuberculosis, is a disease of profound historical significance, with evidence of its existence traced back to ancient

human remains (1). Despite being a preventable and curable disease, TB continues to be a major global health threat and a leading cause of death from a single infectious agent. Pathogenesis begins with the inhalation of droplet nuclei into the alveoli, where macrophages engulf the bacilli (2). The complex interaction between the host immune response and the bacterial evasion mechanisms, facilitated by the unique lipid-rich cell envelope of M. tuberculosis, can lead to either latent infection or active disease (3) (4).

The cornerstone of TB control lies in early and accurate diagnosis, which enables prompt initiation of treatment and interrupts the chain of transmission (5). For decades, the diagnosis of TB has relied on conventional methods. Sputum smear microscopy, using either Ziehl-Neelsen (ZN) or fluorescent staining, is a rapid and low-cost technique but suffers from limited sensitivity (requiring 5,000-10,000 bacilli/mL) and an inability to distinguish between M. tuberculosis and other acid-fast bacilli (6). While culture remains the gold standard with a sensitivity of over 99%, its utility is hampered by a prolonged turnaround time of several weeks, leading to delays in diagnosis and treatment (7).

This diagnostic gap is particularly critical in high-burden, resource-limited settings, where timely results are essential for effective public health intervention. The limitations of microscopy often result in a significant number of false-negative cases, especially in patients with paucibacillary disease, such as children or individuals with extra-pulmonary TB or HIV co-infection. Furthermore, the global rise of multidrug-resistant tuberculosis (MDR-TB) necessitates diagnostic methods that can not only detect the bacterium but also provide information on drug susceptibility to guide appropriate therapy from the outset (8).

The evolution of molecular diagnostics has revolutionized TB management. The GeneXpert MTB/RIF assay, an automated, cartridge-based nucleic acid amplification test, can simultaneously detect M. tuberculosis complex and rifampicin resistance directly from clinical samples within two hours (9). This addresses critical gaps left by conventional methods. Rifampicin resistance is a key marker for MDR-TB, and its early detection is vital for guiding appropriate therapy.

Given the critical need for efficient diagnostic tools in high-burden settings, this study was conducted to evaluate the diagnostic performance of the GeneXpert MTB/RIF assay in comparison to conventional AFB smear and fluorescent staining techniques.

Materials and Methods

This prospective cross-sectional study was conducted at Mahaban Medical & Research Hospital, Topi, District Swabi, following approval from the institutional review board. Participants were enrolled using a convenience sampling technique. The sample size of 227 was determined using the World Health Organization (WHO) sample size calculator, with parameters set at an assumed population proportion of 18%, a 95% confidence level, and a 5% margin of error. The study included all clinical samples (pulmonary and extra-pulmonary) received in the laboratory for concurrent smear microscopy and GeneXpert MTB/RIF testing. Samples submitted for only one of the two tests were excluded. The final cohort comprised 227 samples, including sputum (n=170), various body fluids such as pleural fluid (n=32), early morning gastric aspirate (n=11), stool (n=5), cerebrospinal fluid (CSF, n=4), pus (n=3), and urine (n=2).

All samples underwent parallel diagnostic testing. For smear microscopy, samples were processed to prepare smears for both ZN staining and fluorescent staining with auramine-O. Stained slides were examined microscopically, and the presence of acid-fast bacilli (AFB) was graded according to the International Union Against Tuberculosis and Lung Disease (IUATLD) scale. A sample was considered smear-positive if any smear result was graded as scanty (1-9 AFB per 100 fields) or higher (+1, +2, +3). For molecular testing, the GeneXpert MTB/RIF assay (Cepheid, USA) was performed as per the manufacturer's protocol. The diagnostic performance of each method—GeneXpert, ZN smear, and fluorescent smear was evaluated by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)

Data Analysis

Diagnostic performance metrics, including sensitivity, specificity, positive predictive value

(PPV), and negative predictive value (NPV), were calculated for each test (GeneXpert, ZN smear, and fluorescent smear).

Results

A total of 227 participants meeting the study's inclusion and exclusion criteria were enrolled. The cohort comprised 115 (50.66%) males and 112 (49.34%) females, resulting in a near-equal gender distribution. Participants were stratified into four age groups. The largest proportion was in the 21-40 years category (77 participants, 33.92%), followed by the 1-20 years group (55 participants, 24.23%), the 41-60 years group (51 participants, 22.47%), and those above 60 years (44 participants, 19.36%). The demographic breakdown is summarized in Table 1.

Table 1: Age-based distribution of the participants

Age Group (Years)	Number of participants	Percentage
01-20	55	24.23%
21-40	77	33.92%
41-60	51	22.47%
>60	44	19.36%

The overall positivity rate for M. tuberculosis across the three diagnostic methods varied significantly. The AFB smear using Zn staining detected the fewest positive cases, identifying 26 samples (11.45% positivity). Fluorescent staining demonstrated improved detection, with 30 positive samples (13.2% positivity). The GeneXpert MTB/RIF assay exhibited the highest diagnostic yield, confirming 35 positive samples (15.41% positivity).

A detailed analysis of discordant results between the methods is presented in Table 2. AFB smear microscopy yielded one false-positive result (positive by AFB smear but negative by fluorescent stain) and ten false-negative results (negative by AFB smear but positive by GeneXpert). Fluorescent staining resulted in two false-positive and six false-negative cases. Notably, the GeneXpert assay demonstrated a single false-negative result; one sample was positive by AFB smear, fluorescent stain, and subsequent confirmatory culture but was not detected by the molecular assay. No false-positive results were recorded for GeneXpert.

Table 2: Concordance and Discordance Analysis of Diagnostic Methods

Diagnostic method	True positive n(%)	True Negative n(%)	False positive n(%)	False negative n(%)
AFB	26(11.5)	190(83.7)	1(0.4)	10(4.4)
Fluorescent	30(13.2)	189(83.3)	2(0.9)	6(2.6)
GeneXpert	35(15.4)	191(84.1)	0	1 (0.4)

19 (52.77%) of the total positive samples were pulmonary, while the remaining 17 (47.3%) were extra-pulmonary in origin.

A detailed analysis of the demographic and clinical profile of the tuberculosis-positive cohort (n=36) is presented in Table 3. The prevalence of confirmed TB was higher among males, who constituted 22 of the 36 positive cases (61.11%), compared to 14 female cases (38.89%). Analysis by age group revealed that the highest proportion of positive cases was found in the youngest cohort (1-20 years), accounting for 13 cases (36.11%). This was followed by the 21-40 years group with 9 cases (25.00%), while the 41-60 years and >60 years groups each contributed 7 cases (19.44%).

Regarding sample type, pulmonary specimens (predominantly sputum) constituted a slight majority of the positive cases, with 19 samples (52.77%). Extra-pulmonary samples accounted for the remaining 17 positive cases (47.22%).

age	1-20 y	21-40y	41-60y	>60y	total
sputum	3 M	5 M	2 M	2 M	19

	2 F	2 F	3 F	0 F	
stool	1 M	0 M	1 M	0 M	2
	0 F	0 F	0 F	0 F	
EMGA	3 M	0 M	1 M	0 M	6
	1 F	0 F	0 F	1 F	
Fluids	3 M	0 M	0 M	1 M	7
	0 F	1 F	0 F	2 F	
Urine	0	0	0	1F	1
PUS	0	1 M	0	0	1
Total	13(36.11%)	9(25%)	7(19.44%)	7(19.44%)	36
	M=male 22(61.11%)		F= Female 14(38.88%)		

Table 3: Distribution of Positive Tuberculosis Cases by Specimen Type, Age Group, and Gender

Among the 170 sputum samples, the GeneXpert assay identified 19 positive cases (11.2%). This included two samples that were negative by AFB smear microscopy and one sample that was negative by fluorescent staining, all of which were subsequently confirmed as true positives by GeneXpert.

Of the 32 fluid samples, 6 were positive (18.8%) as detected by GeneXpert. Two of these positive samples were not detected by AFB smear microscopy, and one was not detected by fluorescent staining. A single fluid sample presented a discordant result, testing positive by both AFB smear and fluorescent stain but negative by GeneXpert; this sample was later confirmed as a true positive by culture.

Analysis of the 11 early morning gastric aspirate samples revealed 4 positive cases by smear microscopy. GeneXpert detected an additional two positive cases that were smear-negative, for a total of 6 positive EMGA samples. Among the other specimen types, one urine and one pus sample were positive and exclusively detected by GeneXpert, as they were negative by both microscopy methods. Two of the five stool samples were positive for M. tuberculosis complex by GeneXpert, while all four cerebrospinal fluid (CSF) samples tested negative across all diagnostic methods (table 4).

Table 4: Comparative Diagnostic Yield by Specimen Type

Sample type	Total samples	AFB positive samples(n=26)	AFB (-) later detected (+) on fluorescent stain n=4	Smear (-) later detected (+) by GeneXpert n=5	Samples detected (+) on GeneXpert n=35
Sputum	170	16	2	1	19
Fluids	32	4	2	1	6
EMGA	11	4	0	2	6
PUS	3	0	0	1	1
Stool	5	2	0	0	2
Urine	2	0	0	1	1
CSF	4	0	0	0	0
Total positive	Total of 35, but one later confirmed by culture				36

Among the 36 confirmed cases of tuberculosis, rifampicin resistance was detected in a single sputum sample by the GeneXpert MTB/RIF assay, corresponding to a resistance prevalence of 2.8% (1/36) among the study's positive cohort.

The diagnostic accuracy metrics for the GeneXpert assay were calculated based on its performance against a composite reference standard, which incorporated results from smear microscopy and culture for discordant cases. The assay demonstrated superior performance characteristics. Out of the 36 true positive cases,

GeneXpert correctly identified 35, yielding a sensitivity of 97.2%. There were no false-positive results, resulting in a specificity of 100%. Consequently, the positive predictive value (PPV) was 100%, and the negative predictive value (NPV) was 99.5% table 5.

Table 5: Rifampicin resistance frequency of M. tuberculosis

Table V: Rifampicin Resistant		
	Frequency	%
Sensitive	35	97.2
Resistant	1	2.8
Total	36	100

The diagnostic performance of conventional microscopy techniques was also evaluated. The AFB smear identified 26 true positive cases. It yielded 10 false-negative and 1 false-positive result. Based on these figures, the calculated sensitivity was 72.2%, specificity was 99.5%, positive predictive value (PPV) was 96.3%, and negative predictive value (NPV) was 95.02%.

Fluorescent staining demonstrated a higher detection rate, with 30 true positive cases. This method resulted in 6 false-negative and 2 false-positive results. The performance metrics were calculated as follows: sensitivity of 83.33%, specificity of 98.96%, PPV of 93.75%, and NPV of 97.44%.

The consolidated diagnostic accuracy parameters for all three methods are presented in Table VI for direct comparison.

Table 6: Consolidated Diagnostic Accuracy of GeneXpert, AFB Smear, and Fluorescent Staining

	GeneXpert	**AFB**	**Fluorescent**
Sensitivity	97.2%	72.2%	83.33%
Specificity	100%	99.5%	98.96%
PPV	100%	96.3%	93.75%
NPV	99.5%	95.02%	97.44%

Discussion

This study evaluated the diagnostic performance of GeneXpert MTB/RIF against conventional microscopy techniques for detecting M. tuberculosis in a diverse sample population. The findings corroborate and contrast with existing literature, highlighting the assay's utility and contextual performance. Consistent with several studies, a higher prevalence of TB was observed among males (61.11%) in our cohort, aligning with findings reported by Kabir et al. (10), Mohit Bhardwaj et al study (11) and Muia Pk et al (12). The age group most affected was 1-20 years (36.11% of positives), which contrasts with a study by Oliva Sanchez et al (8), but underscores the variable demographic susceptibility noted by Mahboob et al. (13), suggesting that TB can affect any age group or gender. The distribution of positive samples was nearly equal between pulmonary (52.77%) and extra-pulmonary (47.3%) origins, reflecting the comprehensive diagnostic scope of this study.

The overall positivity rate of 15.41% with GeneXpert falls within the wide spectrum reported globally. It is lower than rates reported by Ejeh et al. (16.1%) (14) Tang et al. (34.0%) (15) and Elbrolosy et al. (24.7%) (16) but higher than those reported by Araya et al. (12.6%) (17) but higher than those reported by Araya et al. (12.6%) (18) but higher than those reported by Araya et al. (12.6%) (19). This variation likely reflects differences in local disease prevalence, patient selection criteria, and sample types.

A key finding is the superior diagnostic accuracy of GeneXpert. The sensitivity (97.2%) and specificity (100%) in this study are higher than those reported in several other studies. For

instance, Nguyen Thi Quynh Nhu et al. reported a sensitivity of 59.3% and specificity of 99.5% (20), while Tingyu Tang et al. reported 84% and 87.8%, respectively (15). Our sensitivity is notably higher than the 70% reported by Zeka et al. (21). and 88% reported by Alqawwas et al. (22). Although the specificity aligns with these high-specificity reports. The 100% specificity indicates an exceptional ability to rule out non-TB cases, minimizing false positives.

The performance of conventional methods was also assessed. The AFB smear positivity of 11.9% was higher than the 5% reported by Aydemir et al. (18) and 9.61% by Balaji et al. (23) but lower than the 12.88% reported by Mechal et al. (24). Similarly, the fluorescent stain positivity of 13.21% exceeded the 10.57% reported by Balaji et al (23) and 6.9% by Shameem et al. (25) but was lower than the 18.1% reported by Arora et al. (26). This confirms the established trend that fluorescent microscopy generally offers higher sensitivity than ZN staining. The rifampicin resistance rate of 2.8% (1/36) in our study is lower than the 3.1% reported by Habous et al. (27) and the 7.11% reported by Ramachandra et al. (28).

This low prevalence is encouraging but underscores the necessity of continued surveillance.

Our results affirm that GeneXpert significantly outperforms smear microscopy. It detected 35 positive cases compared to 26 by AFB smear and 30 by fluorescent stain, correctly identifying 10 and 6 cases missed by each method, respectively. This superior sensitivity for both pulmonary and extra-pulmonary samples, as demonstrated in a 2024 study (13), is critical for early diagnosis and treatment initiation. While smear microscopy remains a rapid, simple, and cost-effective frontline test, its lower sensitivity, as proven here, can lead to misdiagnosis and delayed care, particularly in paucibacillary or extra-pulmonary disease.

Potential limitations include the single false-negative GeneXpert result from a pus sample, which may be attributed to a very low microbial load or the presence of PCR inhibitors. Nonetheless, the World Health Organization's endorsement of GeneXpert for pulmonary (2010) and extra-pulmonary TB (2013) is strongly supported by our data, which show unremarkably high performance and rapid turnaround time.

Conclusion

This study demonstrates the superiority of the GeneXpert assay over conventional diagnostic methods, highlighting its simplicity, rapidity, and high diagnostic accuracy, characterized by exceptional sensitivity and specificity. A key advantage of GeneXpert is its capacity not only to detect M. tuberculosis but also to concurrently identify rifampicin resistance, thereby facilitating the diagnosis of multidrug-resistant tuberculosis cases. In contrast, acid-fast bacillus smear microscopy, including fluorescent staining, is comparatively time-consuming, exhibits lower sensitivity and specificity, and requires substantial technical expertise and trained personnel. Conversely, the GeneXpert system is notably user-friendly, requiring minimal operational training. This ease of use, combined with its diagnostic performance, renders it a highly suitable option for resource-limited settings.

These findings strongly support the need to scale up the implementation of GeneXpert technology for tuberculosis diagnosis, particularly in high-burden countries. Widespread adoption has the potential to significantly improve the speed and accuracy of TB diagnosis, enable timely initiation of appropriate treatment, and ultimately enhance patient outcomes and strengthen tuberculosis control programs.

Limitations

The study had an unequal number of samples. Not all samples were run on the gold standard culture method, and only compared GeneXpert with AFB and Fluorescent stain, but did not compare it with other diagnostic methods.

Recommendations

Incorporation of GeneXpert as a primary diagnostic tool and Expansion of it in the rural and underserved areas to ensure timely diagnosis and treatment.

Conflict of Interest

The authors declared no conflict of interest.

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