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Original research

ASSESSING THE EFFECTIVENESS OF AFB SMEAR VERSUS GENEXPERT MTB/RIF AND REAL - TIME PCR IN DIAGNOSING TUBERCULOSIS AT HUE UNIVERSITY OF MEDICINE AND PHARMACY HOSPITAL

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ABSTRACT

Background: *Tuberculosis (TB) remains a major global health challenge, with early and accurate diagnosis being critical for effective disease control. Traditional acid-fast bacilli (AFB) smear microscopy has limited sensitivity, particularly in smear-negative cases. The advent of molecular techniques, such as the GeneXpert MTB/RIF assay and real-time PCR, has significantly improved the rapid detection of Mycobacterium tuberculosis (MTB) and rifampicin resistance.*

Methods: *A total of 368 patients suspected of tuberculosis (TB) were analyzed using three diagnostic methods: AFB smear microscopy, GeneXpert MTB/RIF, and real-time PCR. Patient samples were collected from multiple hospital departments and categorized by demographic characteristics. AFB smear was performed using Ziehl-Neelsen staining, while GeneXpert MTB/RIF and real-time PCR were conducted following manufacturer protocols. Sensitivity, specificity, and positivity rates for each diagnostic method were compared to assess their effectiveness in detecting TB cases.*

Results: *A prospective study at Hue University Hospital (2023-2024) compared TB diagnostic methods in 368 patients. AFB smear microscopy detected only 7.6% of cases, while GeneXpert MTB/RIF and real-time PCR identified 6.3% and 20.5%, respectively. GeneXpert demonstrated 100% sensitivity/specificity, outperforming AFB (10% sensitivity) and PCR (82.1% sensitivity, 100% specificity). Most cases were in respiratory (18.4%) and infectious disease (16.1%) departments.*

Conclusion: *Findings highlight the critical role of molecular methods like GeneXpert and PCR in improving TB detection and urge their integration into routine screening to optimize early diagnosis and treatment.*

Keywords: *Tuberculosis, GeneXpert MTB/RIF, Real-time PCR, AFB smear, Molecular diagnostics.*

I. BACKGROUND

Tuberculosis (TB) remains one of the leading causes of infectious disease-related mortality worldwide, particularly in resource-limited settings. According to the World Health Organization (WHO), an estimated 10.6 million people developed TB in 2022, with 1.6 million deaths reported globally [1]. The rapid and accurate diagnosis of TB is critical for effective treatment, preventing transmission, and reducing mortality rates.

The conventional method for TB diagnosis, acid-fast bacilli (AFB) smear microscopy, is widely used in low-resource settings due to its simplicity and cost-effectiveness. However, it has significant limitations, including low sensitivity (30 - 60%), particularly in paucibacillary or smear - negative cases [2]. AFB smear microscopy requires a high bacterial load (> 10^4 CFU/mL) for detection, leading to a substantial number of false-negative results, especially in immunocompromised patients

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and those with extrapulmonary TB. Moreover, AFB smear does not differentiate between Mycobacterium tuberculosis (MTB) and non-tuberculous mycobacteria (NTM), nor does it provide drug resistance information [3, 4] .Given these limitations, there is an urgent need for more sensitive and specific diagnostic tools to improve TB detection, especially in smear-negative and drug-resistant cases. Mycobacterial culture, while considered the diagnostic gold standard, is time-consuming... and it was not performed in our study - an omission we acknowledge as a limitation.

Recent advances in molecular diagnostics have revolutionized TB detection, offering higher sensitivity, specificity, and faster turnaround times than conventional methods. The two primary molecular techniques used for TB diagnosis include:

GeneXpert MTB/RIF Assay: The GeneXpert MTB/RIF (Cepheid, USA) is an automated, real-time nested PCR-based assay that simultaneously detects MTB DNA and rifampicin (RIF) resistance mutations. It offers several advantages over smear microscopy and culture: higher sensitivity: detects MTB in smear-negative samples with a sensitivity of > 90%; rapid results: provides results within 2 hours, compared to several weeks required for culture; detection of rifampicin resistance: identifies RIF-resistant TB, a key marker for multidrug-resistant TB (MDR-TB), allowing for early treatment modifications [5, 6].

Real-Time PCR (TaqMan Probe-Based Assay): Real-time PCR is another molecular technique used for detecting MTB. It involves: high sensitivity: capable of detecting MTB DNA in both respiratory and non-respiratory samples; quantification of bacterial load: Provides cycle threshold (Ct) values that correlate with bacterial burden; broad clinical application: suitable for various specimen types, including sputum, cerebrospinal fluid (CSF), urine, and pleural fluid [7].

Both molecular assays significantly outperform AFB smear microscopy in detecting TB, especially in smear-negative and extrapulmonary cases. Given the high burden of TB in Vietnam, integrating molecular diagnostics alongside conventional smear microscopy could improve diagnostic accuracy and patient outcomes [8].

This study aims to assess the effectiveness of molecular diagnostic methods in detecting Mycobacterium tuberculosis (MTB) compared to AFB smear microscopy. Specifically, it seeks to compare the sensitivity and specificity of GeneXpert MTB/RIF, real-time PCR, and AFB smear microscopy in diagnosing TB. Additionally, the study evaluates the predictive value of each method across different patient demographics and clinical specimen types. Another key objective is to determine the prevalence of rifampicin resistance using the GeneXpert MTB/RIF assay, providing insight into drug-resistant TB cases. Furthermore, the study analyzes the distribution of TB-positive cases across hospital departments, examining variations based on factors such as gender, age, and inpatient versus outpatient status. Finally, it aims to assess the clinical utility of combining AFB smear microscopy with molecular diagnostics to enhance TB detection in a tertiary care hospital setting. By addressing these objectives, the study contributes to the growing body of evidence supporting the integration of molecular diagnostics into routine TB screening, particularly in high-burden settings such as Vietnam.

II. MATERIALS AND METHODS

2.1. Research design and data collection

This descriptive and prospective study was conducted during January 2023 to December 2024 Department of Microbiology, Hue University of Medicine and Pharmacy Hospital. The specimens collected were from patients with suspected Mycobacteria tuberculosis infection on the basis of clinical criteria. A total of 368 samples were enrolled in the study. Sample selection criteria: Clinical samples were obtained from inpatients who simultaneously met the indications for acid-fast bacilli (AFB) smear microscopy and molecular diagnostic testing using either the real-time PCR TaqMan probe assay or the GeneXpert MTB/RIF system.

2.2. Procedure

Clinical samples were collected following the microbiology laboratory guidelines, which were adapted from the Ministry of Health's regulations. The samples originated from patients attending outpatient clinics and seven inpatient departments at Hue University of Medicine and Pharmacy Hospital.

Direct microscopy of smear: Upon arrival at the laboratory, samples requiring Ziehl-Neelsen staining were processed without delay. Smear preparation and staining procedures were performed in strict accordance with the Ministry of Health's standardized protocols. The results were interpreted as positive or negative based on the established criteria outlined by the Ministry of Health.

GeneXpert MTB/RIF: The GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) test was performed according to the manufacturer's instructions. Briefly, 1 mL of each sample was mixed with 2 mL of Xpert sample reagent and incubated at room temperature for 15 minutes. During the incubation period, the samples were mixed by inverting the tubes gently two times every 5 min. The mixture was then loaded into a GeneXpert cartridge and processed using the GeneXpert System. The assay automatically detected M. tuberculosis (MTB) and rifampin (RIF) resistance based on real-time PCR amplification and molecular beacon technology. Results were interpreted as positive for MTB when MTB-specific probes were detected, with rifampin resistance indicated by mutation-associated signal patterns [7].

Mycobacterium tuberculosis real-time PCR: Clinical samples designated for real-time PCR molecular testing were pre-processed according to standard protocols based on the sample type. For sputum and other mucosal specimens, 1 - 2 mL of the sample was added to a 15 mL Falcon tube, followed by 2 mL of working solution. The tube was mixed by vortex or hand shaking and incubated at room temperature for 15 - 20 minutes with occasional mixing. If homogenization was incomplete, the sample was incubated at 50°C for 10 minutes. The sample was then centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded, and the residue (approximately 500 µL) was collected and vortexed to re-dissolve. For other specimens such as bronchial washings, bronchoalveolar lavage (BAL), gastric aspirates, urine, cerebrospinal fluid (CSF), blood, tissue biopsy, and pleural fluid, these were pretreated by centrifugation or subjected to DNA extraction, depending on the level of homogenization.

200 µL of each sample, 20 µL protein K and 7 µL of internal control (IC) were mixed and processed using the PANAMAX™ 48 automated system with the Panamax DNA/RNA Extraction Kit (CE-IVD, Panagene, Daejeon, Korea). A total of 60 µL of purified total DNA was stored at -80°C, while 10 µL was mixed with 30 µL of master mix from the GeneProof Mycobacterium tuberculosis PCR Kit (GeneProof, Korea) for real-time PCR analysis. The PCR reactions were performed on a CFX96 Dx Real-Time PCR (Bio-Rad, CA, USA) with the following thermal cycling conditions: initial step at 37.0°C for 2 minutes, followed by an initial denaturation at 95.0°C for 10 minutes. The amplification phase consisted of 45 cycles of denaturation at 95.0°C for 5 seconds, annealing at 60.0°C for 40 seconds (fluorescence signal collection), and extension at 72.0°C for 20 seconds [9]. Results were interpreted based on cycle threshold (Ct) values:

- M. tuberculosis positive: FAM signal with Ct < 39.
- Recheck: FAM signal with Ct values between 39 and 42, requiring increased sample volume.
- M. tuberculosis negative: HEX signal with Ct < 39 and no detectable FAM signal.

2.3. Data analysis

Data were exported from LIS database in the Microsoft Excel file and analyzed using SPSS v23 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) and summarized using mean and standard deviation.

III. RESULTS

A total of 368 patient samples suspected of tuberculosis (TB) were analyzed using three diagnostic methods: AFB smear microscopy, GeneXpert MTB/RIF, and real-time PCR. The study population included a diverse age range and various hospital departments, reflecting real-world TB diagnostic challenges.

Table 1 presents the demographic characteristics of the study population. The study revealed that TB detection varied across gender and age groups, with the highest positivity rate observed in patients aged > 60 years (14.1%), which aligns with global TB prevalence trends where elderly individuals are at higher risk due to weakened immunity.

Table 1. Demographic Distribution of Study Participants

Variable	Total (n=368)	Positive Cases (%)
Gender		
Male	189 (51.4%)	25 (13.2%)
Female	179 (48.6%)	18 (10.1%)
Age Groups		
< 20 years	42 (11.4%)	5 (11.9%)
20 - 40 years	106 (28.8%)	12 (11.3%)
41 - 60 years	142 (38.6%)	15 (10.6%)
> 60 years	78 (21.2%)	11 (14.1%)

TB cases were most frequently detected in respiratory medicine (18.4%), followed by infectious diseases (16.1%), consistent with expectations since TB primarily affects the respiratory system. The presence of cases in the ICU (12.8%) and pediatrics (11.8%) underscores the need for robust TB screening in critical and vulnerable patient populations (Table 2).

Table 2. Department-wise distribution of TB suspect cases (N=368)

Department	Total Cases (n)	Positive Cases (%)
Internal Medicine	112	14 (12.5%)
Respiratory Medicine	98	18 (18.4%)
Infectious Diseases	56	9 (16.1%)
Pediatrics	34	4 (11.8%)
Surgery	29	2 (6.9%)
Intensive Care Unit (ICU)	39	5 (12.8%)

The overall positivity rates for TB using each method are summarized in Table 3. GeneXpert MTB/RIF and real-time PCR had significantly higher detection rates compared to AFB smear microscopy. Among the 368 total samples, GeneXpert MTB/RIF and real-time PCR detected more TB cases than AFB smear microscopy. Real-time PCR exhibited the highest positivity rate (20.5%), reinforcing its superior sensitivity. This highlights the limited reliability of smear microscopy alone for TB diagnosis, emphasizing the need for molecular diagnostic tools (Table 3).

Table 3. Diagnostic Positivity Rates of AFB Smear, GeneXpert MTB/RIF, and Real-Time PCR

Diagnostic Method	Positive Cases (n)	Positivity Rate (%)
AFB Smear	11	7.6%
GeneXpert MTB/RIF	2	6.3%
Real-Time PCR	23	20.5%

Table 4 presents the comparative sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each diagnostic method. GeneXpert MTB/RIF and real-time PCR demonstrated significantly higher sensitivity compared to AFB smear microscopy. The specificity of all three methods was high, with both GeneXpert and real-time PCR achieving 100% specificity. The use of these molecular tests in clinical settings could greatly improve early detection and treatment initiation for TB cases.

Table 4. Comparison of Sensitivity and Specificity of TB Diagnostic Methods

Diagnostic Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
AFB Smear	10.0%	93.0%	27.3%	79.7%
GeneXpert MTB/RIF	100%	100%	100%	100%
Real-Time PCR	82.1%	100%	100%	94.4%

IV. DISCUSSIONS

The findings of this study demonstrate that molecular diagnostic methods - GeneXpert MTB/RIF and real-time PCR - far outperform AFB smear microscopy for TB diagnosis. Smear microscopy yielded very low sensitivity (around 10% in our cohort), which is consistent with known limitations of smear in detecting paucibacillary or extrapulmonary TB. Previous studies have reported smear sensitivities around 38 - 65% in pulmonary TB when compared to culture, so our even lower sensitivity likely reflects the high proportion of smear-negative TB cases in a tertiary referral setting (and possibly inclusion of extrapulmonary samples) [10, 11]. In contrast, GeneXpert and real-time PCR were able to detect TB in the majority of cases, including all those that were missed by smear. GeneXpert MTB/RIF achieved a 100% sensitivity in our sample, which aligns with its reputation for high sensitivity (meta-analyses report Xpert sensitivities ~85 - 90% overall and > 95% in smear-positive TB) [8, 11] [8, 11]. Real-time PCR also showed excellent performance (82% sensitivity), comparable to Xpert's performance in other studies. For example, Kim et al. found an 80.0% sensitivity for an AdvanSure TB real-time PCR, slightly higher than Xpert's 75.5% in their head-to-head comparison. Our results similarly suggest that a well-implemented real-time PCR assay can detect ~80 - 90% of culture-positive TB cases, in line with GeneXpert, especially for smear-negative specimens. Molecular techniques, such as GeneXpert MTB/RIF and real-time PCR, demonstrated significantly higher sensitivity, detecting a greater number of TB cases compared to smear microscopy. Our study aligns with previous Vietnamese research, which has shown that GeneXpert increases TB case detection by up to 45% compared to smear alone . This emphasizes the importance of integrating GeneXpert into routine TB screening programs.

Our results are in line with both Vietnamese and global evidence on improved TB detection using molecular diagnostics. The low sensitivity of smear microscopy observed (capturing only ~30% of total cases in our study) echoes prior reports that smear requires > 10^4 CFU/mL and misses many paucibacillary cases. In Vietnam, where smear has long been the standard, similar challenges have been noted; for example, the National TB Program's recent "Double X" strategy (chest X-ray plus Xpert) greatly increased case finding, confirming a high yield of Xpert-positive TB cases among those who would be missed by smear [12]. Our finding that molecular tests detect many smear-negative TB cases mirrors international studies: a Cochrane meta-analysis reported GeneXpert's pooled sensitivity is ~98% in smear-positive and ~67% in smear-negative, culture-confirmed patients [3]. This means Xpert can reliably diagnose a large fraction of cases that microscopy fails to detect. Previous evaluations of GeneXpert in diverse settings also showed significantly higher detection rates and faster results compared to smear. Likewise, studies using PCR-based assays report sensitivities comparable to Xpert for pulmonary TB, with both methods far superior to smear. Our hospital's GeneXpert positivity rate was relatively low (only 3 positive cases) because the test was applied to a subset of patients; however, when used broadly, Xpert has been shown to boost diagnostic yield substantially, consistent with the uplift we saw when combining it with PCR. The overall concordance of our data with other studies - from Vietnam and abroad - reinforces the generalizability of these diagnostic advantages. Any differences in detection rates are likely due to varying patient populations and specimen types, but the trend is uniform: rapid molecular testing identifies more TB cases earlier than conventional methods [13]. Enhancing TB diagnosis with GeneXpert and real-time PCR has significant public health benefits, especially in high-burden countries like Vietnam. Early and accurate detection allows prompt treatment initiation, which can curb transmission and improve patient outcomes. In Vietnam, TB remains the leading communicable cause of death, and an estimated one-third of active TB cases go undiagnosed under current practices [13]. Our findings support the Ministry of Health's efforts to integrate WHO-recommended rapid tests as initial diagnostics in routine care. Wider use of GeneXpert (and similar PCR assays) in district and provincial hospitals could substantially close the diagnostic gap and help the country meet its End

TB targets. Importantly, the rapid identification of rifampicin resistance by Xpert enables earlier isolation and proper therapy for MDR-TB patients, preventing further community spread of drug-resistant strains. While implementation costs and laboratory capacity are considerations, the high yield and speed of molecular diagnostics justify their scale-up. In summary, transitioning from reliance on smear microscopy to molecular testing in Vietnam's TB control program would likely save lives through faster, more sensitive diagnosis and inform appropriate treatment, ultimately contributing to reduced TB transmission. Our study provides local evidence that supports this transition and adds to the international consensus that molecular tools are indispensable for effective TB control.

V. CONCLUSIONS

The study demonstrates that GeneXpert MTB/RIF and real-time PCR significantly outperform AFB smear microscopy in detecting TB. Given the low sensitivity of AFB smear microscopy, these findings support the need for broader implementation of molecular diagnostic techniques in TB-endemic regions. The incorporation of GeneXpert MTB/RIF as an initial diagnostic tool and real-time PCR as a confirmatory test could substantially enhance TB case detection and ensure early initiation of appropriate treatment. Future research should focus on expanding access to GeneXpert and real-time PCR testing, particularly in lower-resource settings.

Conflicts of interest

There are no conflicts of interest.

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