RESEARCH PAPER

Comparison of EasyNAT MTC Assay With GeneXpert for Detection of Mycobacterium Tuberculosis from Clinical Specimens

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ABSTRACT

Background: Tuberculosis (TB) remains one of the leading infectious causes of morbidity and mortality worldwide. Early and accurate diagnosis is essential for prompt initiation of treatment and interruption of disease transmission. Although Xpert MTB/RIF® is widely accepted as a reference molecular assay for the detection of *Mycobacterium tuberculosis* (MTB). EasyNAT® MTC assay is a rapid molecular test that may provide a practical alternative for TB diagnosis.

Objectives: To determine the diagnostic accuracy of the EasyNAT® MTC assay using Xpert MTB/RIF® as the reference standard for the detection of *Mycobacterium tuberculosis* in clinical specimens from suspected tuberculosis patients.

Study Design & Setting: Cross-sectional validation study conducted in the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi from 1st February 2026 to 1st June 2026.

Methodology: A total of 168 consecutive clinical specimens received from patients suspected of pulmonary or extrapulmonary tuberculosis were included. All specimens were tested by both EasyNAT® MTC assay and Xpert MTB/RIF®. Diagnostic performance of the EasyNAT® MTC assay was evaluated by calculating sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy using Xpert MTB/RIF® as the reference standard. Data were analyzed using SPSS version 25.

Results: The mean age of the participants was 43.8 ± 17.2 years, and 60.7% were males. Xpert MTB/RIF® detected MTB in 44 (26.2%) specimens, while EasyNAT® MTC assay detected MTB in 42 (25.0%) specimens. The EasyNAT® MTC assay demonstrated a sensitivity of 88.6%, specificity of 97.6%, PPV of 92.9%, NPV of 96.0%, and an overall diagnostic accuracy of 95.2%.

Conclusion: The EasyNAT® MTC assay demonstrated high diagnostic accuracy and excellent specificity for the detection of *Mycobacterium tuberculosis*. It may serve as a reliable and rapid alternative molecular assay for the diagnosis of tuberculosis, particularly in settings with limited laboratory resources.

Keywords: Cross-priming amplification; Diagnostic accuracy; EasyNAT® MTC assay; GeneXpert; Mycobacterium tuberculosis; Tuberculosis.

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INTRODUCTION

Tuberculosis (TB) remains a major public health issue for the developing and the developed world alike. It is a leading cause of death due to bacterial infection. An estimated 10.6 million fell ill with tuberculosis worldwide causing 1.3 million deaths in 2022, making TB second leading cause of death from an infectious disease worldwide.[1] TB is a preventable and usually curable disease caused by the bacillus *Mycobacterium tuberculosis*. Pakistan shares the 5th highest burden of the disease.[2] Ours is a resource poor country and the economic burden due the disease is ever increasing. There is a dire need to focus on cost effective methods for its diagnosis. Sputum smear microscopy is the most common tool for tuberculosis diagnosis because it is fast and affordable,

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with a turnaround time of 1–2 h and a cost of about US$3 per sample, but it requires considerable training to perform well. Moreover, microscopy is dependent on the experience of the operator and the method cannot differentiate between dead and viable *Mycobacterium tuberculosis*, and between *Mycobacterium tuberculosis* complex and non-tuberculous mycobacteria.[3]
Culture is the optimal confirmatory test for tuberculosis and a reference for other tuberculosis tests because it is sensitive and detects viable bacteria, yet it has several limitations: it is slow and requires a high-containment laboratory, which is expensive to maintain.[4] Molecular-based assays have the potential to solve the challenges presented by these conventional tuberculosis diagnostic methods, because they are fast and reproducible, not compromised by contamination, and are highly specific and sensitive.[5]
The prevalence of *Mycobacterium tuberculosis* in Pakistan has been reported to be approximately 26.34% thus highlighting that TB is a major public health burden.[6] The EasyNAT® MTC assay has 87% sensitivity and 97% specificity respectively indicating high accuracy in detection of MTB from different clinical samples.[7,8] Zhao et al. further demonstrated that the sensitivity of EasyNAT® MTC (79.1%) was marginally higher than that of Xpert MTB/RIF (75.3%).[9]
Tuberculosis remains a major public health problem in resource limited settings where rapid and accurate diagnosis is essential. There are limited research studies conducted in South Asian population. EasyNAT® MTC is a rapid, cost effective diagnostic test for resource constraint countries. Therefore, evaluating the diagnostic performance of EasyNAT® MTC is justified to assess its potential as an effective alternative for early TB detection in high-burden settings.

METHODS

After taking permission from Institutional Review Board of hospital (FC-MIC-9/READ-IRB/24/3663, Dated: 15 August 2024) and approval of the synopsis from CPSP, this cross-sectional validation study was conducted in the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi from 1st February 2026 to 1st June 2026. A total of 168 clinical specimens from suspected tuberculosis patients were included in the study. The sample size was calculated using the Buderer N.M.F. formula for diagnostic accuracy studies based on a prevalence of 26.2%, expected sensitivity of 87%, expected specificity of 97%, and a 95% confidence level.[11] Non-probability consecutive sampling technique was used. Clinical specimens received in the Department of Microbiology for the diagnosis of pulmonary or extrapulmonary tuberculosis from patients of either gender and all age groups were included, while blood and bone marrow specimens, specimens with a volume of less than 2 mL, and inadequate or improperly collected specimens were excluded from the study.
After obtaining approval from the Institutional Review Board, clinical specimens fulfilling the inclusion criteria were enrolled consecutively. Demographic information including age and gender was recorded on a structured data collection proforma.
Each specimen was processed by both the EasyNAT® MTC assay and Xpert MTB/RIF®. Approximately 2 mL of specimen was used for each test. Thick specimens were liquefied before testing according to the manufacturer's recommendations.
For the EasyNAT® MTC assay, specimens were mixed with the recommended reagents and loaded into the EasyNAT cartridge. Amplification was carried out at 63–65°C using cross-priming amplification targeting the IS6110 sequence of the *Mycobacterium tuberculosis* complex. Fluorescence detection was performed automatically, and the results were recorded as MTB detected or MTB not detected.
For Xpert MTB/RIF®, specimens were mixed with the sample reagent in the recommended ratio and incubated at room temperature. The processed sample was transferred into the GeneXpert cartridge, which was then loaded into the GeneXpert instrument. Automated DNA extraction, amplification, and detection of *Mycobacterium tuberculosis* complex and rifampicin resistance-associated mutations in the rpoB gene were performed. The results were recorded as MTB detected or MTB not detected. Xpert MTB/RIF® was considered the reference standard for comparison. The objective of study was to estimate the diagnostic accuracy of EasyNAT® MTC assay in comparison with Xpert MTB/RIF® (gold standard) to detect Mycobacterium tuberculosis from clinical samples from suspected TB cases.

DATA ANALYSIS

Data were entered and analyzed using Statistical Package for Social Sciences (SPSS) version 25. Quantitative variables such as age were expressed as mean ± standard deviation, whereas qualitative variables including gender, specimen type, and test results were presented as frequencies and percentages.
A 2×2 contingency table was constructed to determine the diagnostic performance of the EasyNAT® MTC assay using Xpert MTB/RIF® as the reference standard. Sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy were calculated along with their 95% confidence intervals. Stratified analysis according to age and gender was performed to evaluate the effect of these variables on the diagnostic performance of the EasyNAT® MTC assay.

OPERATIONAL DEFINITIONS

EasyNAT® MTC assay was a rapid loop-mediated isothermal nucleic acid amplification (LAMP) test that detected *Mycobacterium tuberculosis* complex (MTC) DNA using cross-priming amplification (CPA) targeting the IS6110 insertion sequence, and the results were reported as MTB detected or MTB not detected.[8] Xpert® MTB/RIF was a fully automated cartridge-based nucleic acid amplification test (NAAT) that detected *Mycobacterium tuberculosis* complex and rifampicin resistance, and the results were reported as MTB detected,

MTB not detected, or indeterminate.[10] Sensitivity was defined as the proportion of true tuberculosis-positive specimens correctly identified by the EasyNAT® MTC assay compared with Xpert® MTB/RIF, whereas specificity was defined as the proportion of true tuberculosis-negative specimens correctly identified. Positive predictive value (PPV) was defined as the probability that specimens positive by the EasyNAT® MTC assay were truly positive according to Xpert® MTB/RIF, while negative predictive value (NPV) was defined as the probability that specimens negative by the EasyNAT® MTC assay were truly negative. A true positive specimen was one that tested positive by both EasyNAT® MTC assay and Xpert® MTB/RIF, whereas a true negative specimen was one that tested negative by both tests. A false positive specimen was one that tested positive by EasyNAT® MTC assay but negative by Xpert® MTB/RIF, while a false negative specimen was one that tested negative by EasyNAT® MTC assay but positive by Xpert® MTB/RIF. A suspected tuberculosis case was defined as a patient presenting with cough for more than two weeks, radiological findings suggestive of pulmonary tuberculosis, and one or more symptoms including fever, night sweats, or unexplained weight loss.

RESULTS

A total of 168 suspected tuberculosis cases were included in the study. The mean age of the participants was 43.8 ± 17.2 years. Most participants belonged to the 21–40 years age group (62, 36.9%), followed by the 41–60 years age group (54, 32.1%). There were 102 (60.7%) males and 66 (39.3%) females. Sputum was the most frequently received clinical specimen (102, 60.7%), followed by bronchoalveolar lavage (18, 10.7%), pleural fluid (14, 8.3%), gastric aspirate (10, 6.0%), pus (9, 5.4%), tissue biopsy (7, 4.2%), cerebrospinal fluid (5, 3.0%), and ascitic fluid (3, 1.8%), as shown in Table 1.

Table 1. Baseline Characteristics, Clinical Specimen Types, and Test Results of Study Participants (n = 168)

Variable	n (%) / Mean ± SD
Age (years)	43.8 ± 17.2
Age Group (years)	
≤20	18 (10.7)
21–40	62 (36.9)
41–60	54 (32.1)
>60	34 (20.2)
Gender	
Male	102 (60.7)
Female	66 (39.3)
Clinical Specimen	
Sputum	102 (60.7)
Bronchoalveolar lavage	18 (10.7)
Pleural fluid	14 (8.3)
Gastric aspirate	10 (6.0)
Pus	9 (5.4)
Tissue biopsy	7 (4.2)
Cerebrospinal fluid	5 (3.0)
Ascitic fluid	3 (1.8)

Using Xpert MTB/RIF® as the reference test, 44 (26.2%) clinical specimens were positive for Mycobacterium tuberculosis, while 124 (73.8%) were negative. The EasyNAT® MTC assay detected tuberculosis in 42 (25.0%) specimens and reported 126 (75.0%) specimens as negative, as shown in Table 2.

Table 2. Distribution of Xpert MTB/RIF® and EasyNAT® MTC Assay Results (n = 168)

Variable	n (%)
Xpert MTB/RIF® Result	
MTB Detected	44 (26.2)
MTB Not Detected	124 (73.8)
EasyNAT® MTC Assay Result	
MTB Detected	42 (25.0)
MTB Not Detected	126 (75.0)

Comparison of EasyNAT® MTC assay with Xpert MTB/RIF® demonstrated that 39 specimens were true positives, 121 were true negatives, 3 were false positives, and 5 were false negatives, as shown in Table 3.

Table 3. Comparison of EasyNAT® MTC Assay with Xpert MTB/RIF® for Detection of Mycobacterium tuberculosis (n = 168)

EasyNAT® MTC Assay	Xpert MTB/RIF® Detected	Xpert MTB/RIF® Not Detected	Total
MTB Detected	39	3	42
MTB Not Detected	5	121	126
Total	44	124	168

The EasyNAT® MTC assay demonstrated a sensitivity of 88.6%, specificity of 97.6%, positive predictive value of 92.9%, negative predictive value of 96.0%, and an overall diagnostic accuracy of 95.2% when compared with Xpert MTB/RIF® as the reference standard, as shown in Table 4.

Table 4. Diagnostic Accuracy of EasyNAT® MTC Assay Using Xpert MTB/RIF® as the Reference Standard

Diagnostic Parameter	Estimate (%)	95% CI
Sensitivity	88.6	75.4–96.2
Specificity	97.6	93.1–99.5
Positive Predictive Value	92.9	80.5–98.5
Negative Predictive Value	96.0	90.9–98.7
Overall Accuracy	95.2	90.8–97.9

On stratified analysis, the sensitivity of the EasyNAT® MTC assay ranged from 85.7% to 90.0%, while specificity ranged from 96.0% to 100.0% across different age groups.

According to gender, sensitivity was 89.7% in males and 86.4% in females, whereas specificity was 97.1% and 98.2%, respectively. The overall diagnostic accuracy ranged from 94.1% to 95.5% across all age and gender subgroups, as shown in Table 5.

Table 5. Stratified Diagnostic Accuracy of EasyNAT® MTC Assay According to Age and Gender

Variable	n	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Age Group (years)						
≤20	18	85.7	100.0	100.0	92.3	94.4
21–40	62	90.0	97.6	90.0	97.6	95.2
41–60	54	88.2	96.9	88.2	96.9	94.4
>60	34	87.5	96.0	87.5	96.0	94.1
Gender						
Male	102	89.7	97.1	92.9	95.8	95.1
Female	66	86.4	98.2	95.0	96.2	95.5

DISCUSSION

Tuberculosis (TB) remains a major global public health problem and is one of the leading causes of morbidity and mortality from infectious diseases. Early and accurate detection of Mycobacterium tuberculosis is essential for timely treatment initiation and prevention of disease transmission. Xpert MTB/RIF® is a highly sensitive molecular assay widely used for TB diagnosis; however, its high cost and infrastructure requirements limit its use in resource-limited settings.[12,13] EasyNAT® MTC assay is a rapid molecular test based on loop-mediated isothermal amplification technology that offers a simpler and more affordable alternative. Evaluating its diagnostic performance against Xpert MTB/RIF® may provide evidence for its clinical utility in routine laboratory practice.[14,15] Therefore, this study was conducted to compare the diagnostic accuracy of EasyNAT® MTC assay with Xpert MTB/RIF® for the detection of Mycobacterium tuberculosis in clinical specimens.

Our study demonstrated that the EasyNAT® MTC assay had a sensitivity of 88.6%, specificity of 97.6%, PPV of 92.9%, NPV of 96.0%, and an overall diagnostic accuracy of 95.2% compared with Xpert MTB/RIF® as the reference standard, indicating excellent diagnostic performance for the detection of Mycobacterium tuberculosis.

Zhang et al. (2016) studied 249 patients with suspected pulmonary tuberculosis and found that EasyNAT detected significantly more TB cases than Xpert (72.2% vs. 61.5%, $P<0.05$) and identified more smear-negative/culture-negative cases (44.6% vs. 23.0%, $P<0.01$). EasyNAT showed 95.0% specificity compared with 98.8% for Xpert. Our findings are consistent with this study, demonstrating high sensitivity (88.6%) and specificity (97.6%) for the EasyNAT® MTC assay.[16] Likewise, Quan et al. (2022) reported similar sensitivities between EasyNAT and Xpert (22.6% vs. 26.3%) and similar specificities (98.0% vs. 99.0%) in children, whereas Xpert Ultra showed significantly higher sensitivity (50.9% vs. 30.3%, $P<0.001$) with comparable specificity. Our study demonstrated considerably higher sensitivity (88.6%) and comparable specificity (97.6%), likely because it included a broader range of clinical specimens rather than only pediatric gastric aspirate samples.[17] Bholla et al. (2016) evaluated pediatric tuberculous lymphadenitis and found that EasyNAT had a sensitivity of 19% and specificity of 100%, whereas Xpert MTB/RIF demonstrated 58% sensitivity and 93% specificity. Compared with their findings, our study showed substantially higher sensitivity (88.6%) while maintaining similarly high specificity (97.6%). The difference may be attributed to the inclusion of mixed clinical specimens rather than pediatric lymph node samples alone.[18] Similarly, Armand et al. (2011) reported sensitivities of 79% for respiratory and 53% for non-respiratory specimens, while sensitivity in smear-negative specimens was only 48%. In contrast, our study showed higher sensitivity (88.6%) with comparable specificity (97.6%), suggesting better overall diagnostic performance of EasyNAT® under our study conditions.[19] Miller et al. (2011) evaluated 112 specimens and reported 100% sensitivity in smear-positive specimens, 60% sensitivity in smear-negative pulmonary specimens, 75% sensitivity in smear-negative extrapulmonary specimens, and 95% specificity. Our study demonstrated higher overall sensitivity (88.6%) and slightly higher specificity (97.6%), possibly due to differences in specimen types and study populations.[20]

Study Strengths

This study evaluated a rapid molecular diagnostic assay using a standardized reference method in a real-world laboratory setting. Consecutive sampling minimized selection bias and improved the representativeness of the study population. The findings provide locally generated evidence that may support the use of EasyNAT® MTC assay in resource-limited healthcare settings.

CONCLUSION

EasyNAT® MTC assay demonstrated high sensitivity, specificity, and overall diagnostic accuracy for the detection of Mycobacterium tuberculosis. Its performance was comparable to Xpert MTB/RIF®, indicating that it is a reliable diagnostic method. The assay may serve as a practical alternative for rapid tuberculosis diagnosis, particularly in settings with limited laboratory resources.

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