ORIGINAL ARTICLE

Diagnostic accuracy of GeneXpert vs. Fluorescence microscopy on pulmonary tuberculosis: High-burden country

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ABSTRACT

Background: Pulmonary tuberculosis (PTB) is a significant public health challenge in developing countries, and it is one of the leading causes of infection-related morbidity and mortality. Early and accurate diagnosis is crucial for controlling its transmission and improving patient outcomes.

Material and Methods: This cross-sectional study was carried out at the microbiology laboratory of Indus Hospital and Health Network, Karachi, from February 1, 2023, to July 31, 2023. A consecutive (non-probability) sampling technique was employed. We performed Xpert MTB/RIF, fluorescence microscopy, and mycobacterial cultures on all pulmonary samples. The sensitivity, specificity, predictive values, and diagnostic accuracy of Xpert MTB/RIF and fluorescence microscopy were calculated and compared with culture as the gold standard.

Results: A total of 386 sputum samples were included in the study. More samples tested positive with Xpert MTB/RIF (n = 77, 20%) than fluorescence microscopy (n = 52, 13%). Xpert MTB/RIF demonstrated higher sensitivity (95%) than fluorescence microscopy (64%), while both methods showed comparable specificity (100%) and positive predictive values (100%). The negative predictive value of Xpert MTB/RIF (99%) exceeded that of fluorescence microscopy (91%). Likewise, the diagnostic accuracy of Xpert MTB/RIF (99%) was higher than fluorescence microscopy (97%).

Conclusion: The Xpert MTB/RIF demonstrated better diagnostic efficacy, highlighting its importance for prompt and accurate diagnosis of PTB. However, the accessibility and utility of fluorescence microscopy in low-resource settings cannot be overlooked.

Keywords: Microscopy, Fluorescence; *Mycobacterium tuberculosis* / microbiology, Nucleic acid amplification techniques, Predictive value of tests, Tuberculosis, Pulmonary, Sensitivity and specificity

BACKGROUND

Tuberculosis (TB), often considered a disease of the past, remains a significant challenge in the modern world. In 2023, the World Health Organization (WHO) reported 10.8 million cases of TB worldwide, a rise from previous years, with a global incidence of 134 cases per 100,000 population. With an estimated 686,000 incident TB cases in 2023, Pakistan stood fifth among 30 high-burden countries, adding 6.3% to the worldwide burden of TB disease.[1] Pulmonary tuberculosis (PTB) is a highly contagious infection, as one smear-positive, untreated case can infect 10-15 persons per year in high-burden countries.[2]

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Early and accurate diagnosis is crucial for curtailing its transmission, improving patient management, and alleviating the financial burden on the healthcare system.[3] The National Guidelines for Tuberculosis Control in Pakistan recommend Acid-Fast Bacilli (AFB) smear testing for non-immunocompromised adults who are not at risk of acquiring drug-resistant TB and for whom Xpert MTB/RIF is inaccessible.[4] Smear microscopy for TB uses two methods: the Ziehl-Neelsen (ZN) and auramine-rhodamine (fluorescent) stains. The auramine-rhodamine stain shows 8% to 10% greater sensitivity than the ZN stain, making it a more reliable diagnostic tool.[5] Fluorescence microscopy is less sensitive compared to other advanced diagnostic tests because of its higher limit of detection, which is 5000-10000 bacilli per milliliter.[6] Therefore, paucibacillary TB, people living with HIV, and children with TB might not be positive when tested for AFB smear only.[7] Observer variability, inability to differentiate between dead and viable bacilli, and inability to perform susceptibility testing are a few additional limitations of smear microscopy.[5]

The Xpert MTB/ RIF was a cutting-edge diagnostic test

for TB and was endorsed by the WHO in 2010.[8] The various benefits offered by Xpert MTB/RIF include enhanced diagnostic accuracy due to its lower limit of detection (131 bacilli per milliliter of sample), simultaneous detection of rifampicin resistance, and a rapid turnaround time of 2 hours.[9] However, it is a nucleic acid amplification test; we cannot assess bacilli's viability, and it cannot identify non-tuberculous mycobacteria (NTM).[10] The gold standard test for TB diagnosis is mycobacterial culture, with an extremely low limit of detection (10 bacilli per milliliter of sample), enabling performance of phenotypic drug susceptibility testing and differentiation of *Mycobacterium tuberculosis* (MTB) from NTM. The requirement for a Biosafety Level 3 (BSL-3) laboratory, longer turnaround times, and MTB's slow growth pose therapeutic challenges.[11]

Pakistan is a TB-endemic country. Despite the broad utility of fluorescence microscopy and Xpert MTB/RIF for diagnosing PTB, comparative data on their diagnostic performance with culture results in routine clinical settings are limited. While Xpert MTB/RIF is known to have superior sensitivity compared to fluorescence microscopy[12], no recent regional studies have validated this in PTB. This lack of evidence hinders the informed selection of diagnostic methods for rapid and accurate disease detection and, consequently, its effective control.

To address this gap, the present study evaluated the diagnostic accuracy of Xpert MTB/RIF and fluorescence microscopy, comparing them with culture in PTB patients in routine clinical settings in Pakistan.

MATERIAL AND METHODS

This cross-sectional study was performed at the microbiology section of the clinical laboratory at Indus Hospital and Health Network, Karachi, from February 1, 2023, to July 31, 2023.

The Sample size was calculated by using Dr. Lin Naing's sensitivity and specificity calculator, with a prevalence of 8%, a margin of error of 5%, and a confidence level of 95%. The sensitivity of Xpert MTB/RIF was 87.1%, and the specificity was 98.7%, respectively. The sensitivity and specificity of the AFB smear were 83.9% and 95%, respectively. The total calculated sample size was 80.[11] Ethical approval was obtained before initiation of the study from the Institutional Review Board of the Indus Hospital & Health Network (Ref# IHHN_IRB_2022_12_009) on January 27, 2023.

We performed Xpert MTB/RIF, fluorescence microscopy, and culture on all sputum samples from patients aged 2–75 years presenting with cough and fever for >2 weeks. Samples not meeting these criteria, as well as duplicates and follow-up specimens, were excluded to ensure uniformity. Each sputum sample was divided into three aliquots for the respective tests. Xpert MTB/RIF was performed following the manufacturer's instructions. Fluorescence microscopy was done using auramine–rhodamine staining and interpreted using WHO grading. All procedures were carried out in an accredited laboratory under standard operating procedures. Fluorescence slides were read independently by two technologists, and discrepancies were resolved by a third reader. Routine internal quality control and blinded re-reading of 10% of slides minimized observational bias. For culture, we inoculated specimens into BD BACTEC™ Mycobacteria Growth Indicator Tube (MGIT) and Lowenstein-Jensen (LJ) medium, and incubated them at 37°C for 42 and 56 days, respectively. We confirmed MTB using an immunochromatography test kit, Mycobacterium protein tuberculosis 64 (MPT 64) by BD™. We extracted patient demographic data from the hospital's electronic database and documented it on a standardized proforma. To maintain confidentiality, we assigned codes to the datasets. Variables recorded include age, gender, and the results of Xpert MTB/RIF, fluorescence microscopy, and culture. We used Xpert MTB/RIF and fluorescence microscopy as index tests, and culture as the gold standard. Data were entered into Microsoft Excel and then transferred to Statistical Package for the Social Sciences (SPSS) version 26.0. Normality was checked for the continuous variable, i.e., age, using the Shapiro–Wilk test, which showed significant deviation from normality ($p < 0.001$). The median and interquartile range were calculated for age, while frequencies and percentages were calculated for gender, culture, Xpert MTB/RIF, and smear results. We categorized age into three groups (0-15 years, 16-29 years, and ≥30 years). We analyzed the diagnostic accuracy of Xpert MTB/RIF and fluorescence microscopy for sensitivity, specificity, and positive and negative predictive values using SPSS v26 and MedCalc Statistical Software version 23.3.7 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org). The

data are presented as numbers and percentages in the main tables. To assess the association between categorical variables (age groups, gender, and culture results) and Xpert MTB/RIF and smear results, a chi-square test was performed. A p-value less than 0.05 was considered statistically significant. These analyses are presented in Supplementary Table S1.

RESULTS

We performed Xpert MTB/RIF, fluorescence microscopy, and culture on 386 sputum samples. Our study population had an average age of 36.8 ± 18.7 years. The age distribution of study participants showed that 56 (15%) were 0–15 years old, 87 (22%) were 16–29 years old, and 243 (63%) were ≥30 years old. Regarding gender, 241 participants (62%) were male, while 145 (38%) were female (Table-I).

5 of 386 samples were excluded from the analysis due to contamination. Table-II presents a contingency table of Xpert MTB/RIF and fluorescence microscopy with culture, along with the calculated sensitivities, specificities, positive predictive values, and negative predictive values. In culture-positive specimens, Xpert MTB/RIF accurately identified 77 cases but missed 4. These 4 cases were considered false negatives and later identified as NTM on culture. In culture-negative specimens, 300 were correctly reported as negative by Xpert MT/RIF, with no false positives. Of the culture-positive samples, fluorescence microscopy correctly detected 52 cases but missed 29 cases. Among culture-negative samples, 300 were accurately identified as negative, while none were falsely detected as positive. Xpert MTB/RIF showed sensitivities, specificities, positive predictive values, and negative predictive values of 95%, 100%, 100%, and 99%, respectively. In comparison, smear microscopy demonstrated sensitivities, specificities, positive predictive values, and negative predictive values of 64%, 100%, 100%, and 91%, respectively.

Table-I: Demographic characteristics of study participants (n=386).

Age (years)	n (%)
0 – 15	56 (15)
16 – 29	87 (22)
≥ 30	243 (63)
Gender	**n (%)**
Male	241 (62)
Female	145 (38)

Table-II: Contingency table showing the diagnostic performance of Xpert MTB/RIF and smear with culture.

	Xpert MTB/RIF Positive	Xpert MTB/RIF Negative	Smear positive	Smear negative
Culture Positive	77	04	52	29
Culture Negative	00	300	00	300
Sensitivity	95%		64%	
Specificity	100%		100%	
Positive Predictive Value	100%		100%	
Negative Predictive Value	99%		91%	

DISCUSSION

In our study, Xpert MTB/RIF demonstrated excellent performance with a sensitivity of 95%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 99%. These findings are consistent with previous studies. Kiran *et al*. reported a higher sensitivity of Xpert MTB/RIF (90.2%) than fluorescence microscopy (78.5%).[13] Similarly, Khan *et al*. demonstrated that Xpert MTB/RIF has higher sensitivity (73%) and specificity (100%) than fluorescence microscopy (40% and 100%, respectively).[14] Moreover, a Cochrane review reported Xpert MTB/RIF sensitivity ranging from 67% to 98% and a specificity of 99%.[15] Further supporting our findings, Zaporojan *et al*. found that Xpert MTB/RIF detected TB in about 16% of cases, compared to 9% by microscopy. Notably, the Xpert MTB/RIF showed higher sensitivity and specificity.[16] Likewise, Chaudhary *et al*. demonstrated that the Xpert MTB/RIF assay has a sensitivity of 100% and specificity of 99.5%. In comparison, AFB microscopy showed a sensitivity of 45.3% and specificity of 99.5% in 473 pulmonary

samples.[17] Rimal *et al*. evaluated Xpert MTB/RIF in 162 smear-negative sputum samples of presumptive TB patients at the Nepal Tuberculosis Center. Xpert MTB/RIF detected 19.1% out of 21.6% culture-positive samples. The Xpert MTB/RIF showed a sensitivity of 74.3%, specificity of 96.6%, positive predictive value of 86.7%, and negative predictive value of 92%. Xpert MTB/RIF.[11]

Although Xpert MTB/RIF shows higher sensitivity, especially in smear-negative cases, the importance of smear microscopy cannot be denied in resource-limited settings, where it remains a primary diagnostic tool. In our study, we observed a sensitivity of 64% with high specificity (100%), high positive predictive value (100%), but slightly lower negative predictive value (91%) for fluorescence microscopy. The sensitivity of fluorescence microscopy can be improved by testing multiple samples, as demonstrated by Ahmad *et al*. He evaluated three consecutive sputum samples to determine the sensitivity of fluorescence microscopy and found it increased to 93.2%.[18]

In concordance with our results, Fatima *et al*. reported a sensitivity of 55%, specificity of 99.5%, PPV of 100%, and NPV of 79%.[19] Similarly, in a five-year retrospective study of 6,019 suspected tuberculosis cases at Cape Coast Teaching Hospital, smear microscopy detected TB in 6.6% of first sputum samples and 6.07% of second samples. The Xpert MTB/RIF assay identified an additional 2.93% of cases in first smear-negative samples and 5.44% in second smear-negative samples, uncovering cases that microscopy missed. Although smear microscopy showed low false-negative rates, the Xpert MTB/RIF assay's enhanced sensitivity explains why newer molecular methods should be preferred in the routine diagnostic algorithm for TB.[6]

In our study, 329 cases (86.3%) were smear-negative; 29 (8.8%) were culture-positive, and the Xpert MTB/RIF correctly identified them as positive, indicating that they were false negatives by fluorescence microscopy. The 52 smear-positive cases (13.64%) were identified correctly by Xpert MTB/RIF and culture. The higher diagnostic accuracy of Xpert MTB/RIF (99%) compared with fluorescence microscopy (97%) reinforces its role as a rapid point-of-care diagnostic modality. In a high-burden setting, its ability to deliver fast, accurate results can reduce misdiagnosis, avoid unnecessary treatment of false-positive cases, and ease the burden on the healthcare system.

Our study has some limitations. It was conducted at a single center, and therefore, the results cannot be generalized to a larger population. Although Indus Hospital & Network is one of the largest hospitals in Karachi, Pakistan, serving approximately 2.5 million people, a multi-center study would provide more compelling evidence. Additionally, our study primarily focused on the diagnostic accuracy of the different modalities. It did not incorporate clinical variables, such as comorbidities and treatment history, which may affect interpretation. Furthermore, rifampicin is a key first-line antimicrobial agent; the study did not report rifampicin resistance. We intend to conduct a more comprehensive study involving a broader, more diverse population to enhance the validity of the findings. Incorporating clinical variables and reporting drug resistance patterns will further strengthen the clinical relevance.

CONCLUSION

We observed high efficiency for Xpert MTB/RIF, highlighting its key role in PTB diagnosis. Although fluorescence microscopy was less sensitive in our study population, it remains an indispensable tool in low-resource settings. When fluorescence microscopy is used as a single diagnostic tool, strategies to overcome its limitations are essential to optimize diagnostic accuracy.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Ramlla Hatif: Conceived the idea, designed the study, collected data, final approval, accountable for all aspects of publication.

Nazia Khursheed: Conceived the idea, designed the study, final approval, accountable for all aspects of publication.

REFERENCES

1. World Health Organization. Global tuberculosis report 2023. Geneva: World Health Organization; 2023.

2. MSD Manuals. Tuberculosis (TB) [Internet]. 2022. Available from: https://www.msdmanuals.com/professional/infectious-diseases/*mycobacteria*/tuberculosis-tb
3. Ponmani P, Sinha R, Das SK. Tuberculosis in India: comprehensive SWOC analysis. Indian J Tuberc. 2025; 72(3): 275-81. DOI: https://doi.org/10.1016/j.ijtb.2024.10.001
4. National TB Control Program, Ministry of National Health Services, Regulations and Coordination. National guidelines for the management of tuberculosis in Pakistan. Islamabad; 2019. Available from: https://ntp.gov.pk/ntp-old/uploads/National_Guidelines_for_TB_Revised_2019.pdf
5. Sharma M, Broor S, Maheshwari M, Sudan DPS. Comparison of conventional diagnostic methods with molecular method for the diagnosis of pulmonary tuberculosis. Indian J Tuberc. 2023; 70(2): 182-9. DOI: https://doi.org/10.1016/j.ijtb.2022.04.006
6. Asare KK, Azumah DE, Adu-Gyamfi CO, Opoku YK, Adela EM, Afful P, *et al*. Comparison of microscopic and Xpert MTB diagnoses of presumptive *Mycobacterium tuberculosis* infection: Retrospective analysis of routine diagnosis at Cape Coast Teaching Hospital. BMC Infect Dis. 2024; 24(1): 660. DOI: https://doi.org/10.1186/s12879-024-09566-9
7. Macaux L, Rasolofo V, Eyangoh S, N'Guessan KR, Tejiokem MC, Rakotosamimanana N, *et al*. Accuracy of Xpert and alternative sampling methods to diagnose childhood pulmonary tuberculosis: A prospective cohort study. Clin Microbiol Infect. 2025; 31(3): 417-24. DOI: https://doi.org/10.1016/j.cmi.2024.11.002
8. Orlando S, Triulzi I, Ciccacci F, Palla I, Palombi L, Marazzi MC, *et al*. Delayed diagnosis and treatment of tuberculosis in HIV+ patients in Mozambique: A cost-effectiveness analysis of screening protocols based on four symptom screening, smear microscopy, urine LAM test and Xpert MTB/RIF. PLoS One. 2018; 13(7): e0200523. DOI: https://doi.org/10.1371/journal.pone.0200523
9. Boldi MO, Denis-Lessard J, Neziri R, Brouillet R, von Garnier C, Chavez V, *et al*. Performance of microbiological tests for tuberculosis diagnostics according to the type of respiratory specimen: A 10-year retrospective study. Front Cell Infect Microbiol. 2023; 13: 1131241. DOI: https://doi.org/10.3389/fcimb.2023.1131241
10. Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J, *et al*. The new Xpert MTB/RIF ultra: Improving detection of *Mycobacterium tuberculosis* and resistance to rifampin in an assay suitable for point-of-care testing. mBio. 2017; 8(4): e00812-17. DOI: https://doi.org/10.3389/fcimb.2023.1131241
11. Rimal R, Shrestha D, Pyakurel S, Poudel R, Shrestha P, Rai KR, *et al*. Diagnostic performance of GeneXpert MTB/RIF in detecting MTB in smear-negative presumptive TB patients. BMC Infect Dis. 2022; 22(1): 321. DOI: https://doi.org/10.1186/s12879-022-07287-5
12. Sabella-Jiménez V, Sabella-Jiménez VL, Restrepo-Espinosa V, Eljadue-Flórez J, Gallardo-Castro CV, Silvera AA, *et al*. 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(1): 491. DOI: https://doi.org/10.1186/s12879-025-10856-z
13. Kiran M, Naqvi SMA, Iqbal HB, Butt ARZ, Shafi N. The diagnostic performance of fluorescent microscopy and GeneXpert MTB/RIF assay in pulmonary tuberculosis at a tertiary care hospital of Lahore. Pak J Med Health Sci. 2022; 16(5): 449. DOI: https://doi.org/10.53350/pjmhs22165449
14. Khan AS, Ali S, Khan MT, Ahmed S, Khattak Y, Abduljabbar, *et al*. Comparison of GeneXpert MTB/RIF assay and LED-FM microscopy for the diagnosis of extra-pulmonary tuberculosis in Khyber Pakhtunkhwa, Pakistan. Braz J Microbiol. 2018; 49(4): 909-13. DOI: https://doi.org/10.1016/j.bjm.2018.02.011
15. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev. 2014; (1): CD009593. DOI: https://doi.org/10.1002/14651858.cd009593.pub3
16. Zaporojan N, Hodișan R, Zaha AA, Zaporojan C, Zaha DC. Performance of conventional laboratory tests and Xpert MTB/RIF in the diagnosis of tuberculosis. Monaldi Arch Chest Dis. 2025. DOI: https://doi.org/10.4081/monaldi.2025.3394
17. Chaudhary R, Bhatta S, Singh A, Pradhan M, Srivastava B, Singh YI, *et al*. Diagnostic performance of GeneXpert MTB/RIF assay compared to conventional culture for pulmonary and extrapulmonary tuberculosis in Nepal. Narra J. 2021; 1(2): e33. DOI: https://doi.org/10.52225/narraj.v1i2.33
18. Alamgir MA, Atif Muhammad F, Akhtar S, Qureshi MA, Rahat W. Diagnostic accuracy of three consecutive sputum smears for AFB among TB patients visiting chest OPD. Proceeding S.Z.P.G.M.I. 2010; 24(2): 91-95. Available from: https://proceedings-szmc.org.pk/public/old-doc/2010/Diagnostic-Accuracy-of-Three-Consecutive-Sputum-Smear-for-AFB-Among-TB-Patients-Visiting-Chest-OPD.pdf
19. Zahra F, Ikram A, Zaman G, Satti L, Lalani F, Khan M. Diagnosis of pulmonary tuberculosis in resource-limited settings of Rawalpindi. Open Microbiol J. 2018; 12: 1-7. DOI: http://dx.doi.org/10.2174/1874285801812010376