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INTERPRETATION OF GENEXPERT RESULTS AND THEIR ASSOCIATION WITH SPUTUM SMEAR STATUS IN SUSPECTED PULMONARY TUBERCULOSIS PATIENTS

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Abstract

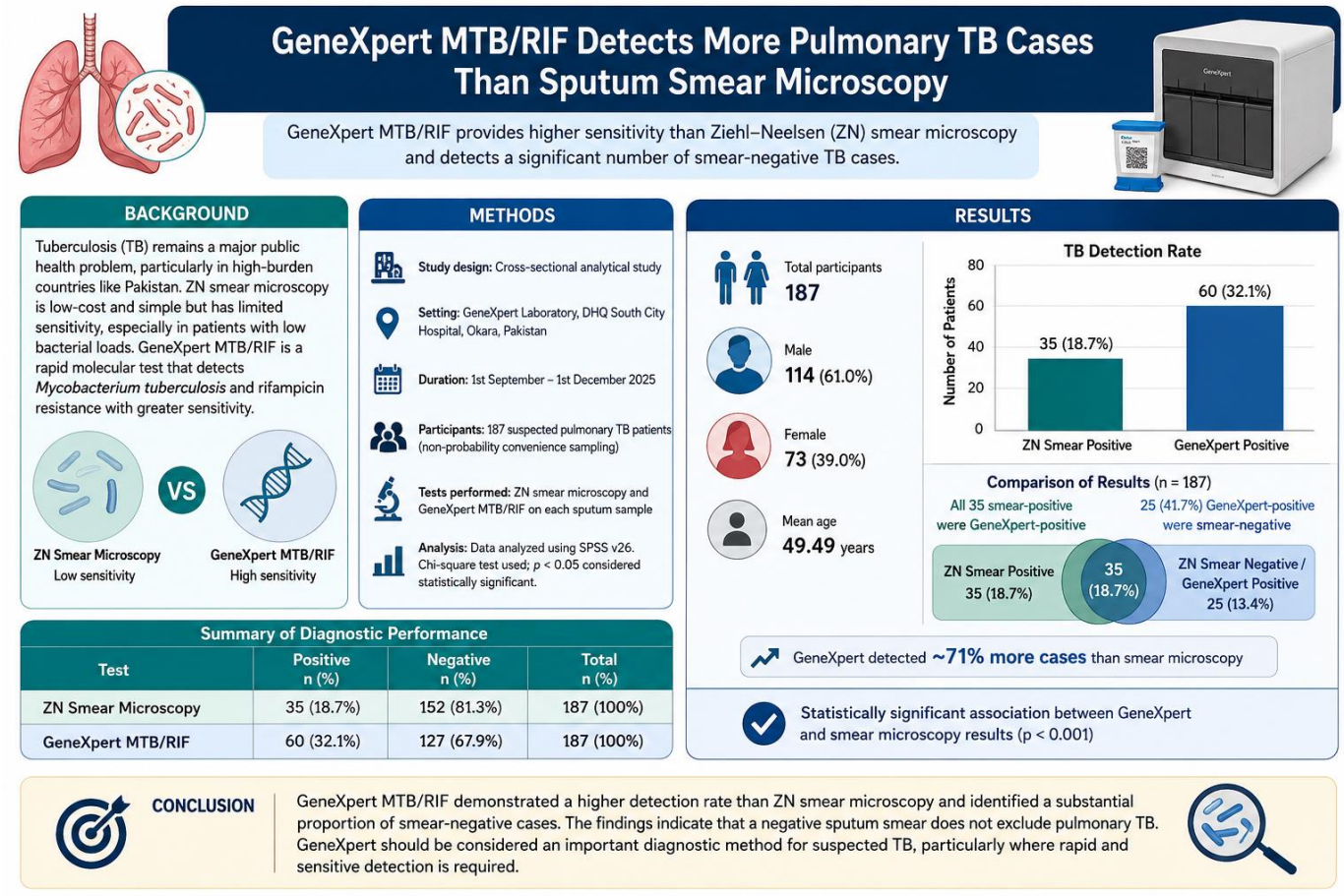
Background: *Tuberculosis (TB) remains a major public health problem, particularly in high-burden countries such as Pakistan. Ziehl-Neelsen (ZN) sputum smear microscopy is widely used because of its low cost and simplicity, but its sensitivity is limited, especially in patients with low bacterial loads. GeneXpert MTB/RIF is a rapid molecular method that can detect Mycobacterium tuberculosis and rifampicin resistance with greater sensitivity. This study was conducted to determine the relationship between GeneXpert MTB/RIF results and sputum smear microscopy among suspected pulmonary TB patients.*

Methods: *A cross-sectional analytical study was conducted at the GeneXpert Laboratory of DHQ South City Hospital, Okara, Pakistan, from 1st September to 1st December 2025. A total of 187 sputum samples from patients suspected of pulmonary TB were selected through non-probability convenience sampling. Each sample was tested using both ZN smear microscopy and GeneXpert MTB/RIF. Demographic and laboratory data were recorded and analyzed using SPSS version 26. The Chi-square test was used to assess the association between GeneXpert and smear microscopy results, with $p < 0.05$ considered statistically significant.*

Results: *Among the 187 participants, 114 (61.0%) were male and 73 (39.0%) were female, with a mean age of 49.49 years. ZN smear microscopy detected TB in 35 (18.7%) patients, whereas GeneXpert detected M. tuberculosis in 60 (32.1%) patients. All 35 smear-positive samples were also GeneXpert-positive,*

while 25 (41.7%) of the GeneXpert-positive cases were smear-negative. GeneXpert therefore detected approximately 71% more cases than smear microscopy. A statistically significant association was observed between GeneXpert and smear microscopy results ($p < 0.001$).

Conclusion: *GeneXpert MTB/RIF demonstrated a higher detection rate than ZN smear microscopy and identified a substantial proportion of smear-negative cases. The findings indicate that a negative sputum smear does not exclude pulmonary TB. GeneXpert should therefore be considered an important diagnostic method for suspected TB, particularly where rapid and sensitive detection is required.*



Graphical Abstract

INTRODUCTION

Tuberculosis (TB) is a major infectious disease caused mainly by *Mycobacterium tuberculosis*. Although it can affect different organs, pulmonary TB is the most common and transmissible form. Transmission occurs mainly through airborne droplets released during coughing, sneezing, or talking. TB continues to cause considerable morbidity and mortality, particularly in low- and middle-income countries where poverty, overcrowding, malnutrition, limited healthcare access, delayed diagnosis, and drug-resistant strains contribute to ongoing transmission. In 2022, approximately 10.6 million people developed TB and about 1.3 million died from the disease worldwide (Bedzina

et al., 2025). Pakistan remains a high-TB-burden country, with delayed diagnosis contributing to continued transmission and the development of drug resistance. Important risk factors for progression to active TB include HIV infection, diabetes, chronic kidney disease, immunosuppression, malnutrition, smoking, low socioeconomic status, and overcrowded living conditions (Getachew et al., 2026). Therefore, early and accurate diagnosis is essential for appropriate treatment and disease control. Sputum smear microscopy using Ziehl-Neelsen (ZN) staining remains widely used because it is inexpensive, simple, and suitable for resource-limited laboratories. The acid-fast property of *M. tuberculosis*, mainly related to mycolic acids in its cell wall, allows bacilli to retain carbol fuchsin after acid-alcohol treatment. However, microscopy has limited sensitivity and generally requires a high bacterial concentration of approximately 5,000–10,000 bacilli/mL. Consequently, patients with early, paucibacillary, or immunocompromised disease may have active TB despite a negative smear result. Moreover, smear microscopy cannot identify rifampicin resistance (Umair et al., 2020; Hatiya et al., 2025). Culture methods such as Lowenstein-Jensen and MGIT provide high sensitivity and specificity and are considered reference methods, but their long turnaround time, cost, technical requirements, and biosafety demands limit their routine use. Molecular testing has therefore become increasingly important. The GeneXpert MTB/RIF assay is an automated real-time PCR-based test that detects *M. tuberculosis* DNA and mutations associated with rifampicin resistance within approximately two hours. Its closed-cartridge system reduces contamination and operator-dependent errors and provides faster diagnostic information than conventional culture (Kumbi et al., 2025). Previous studies have consistently demonstrated better TB detection by GeneXpert compared with smear microscopy. Arora and Dhanashree (2020) reported GeneXpert sensitivity of 88.6%, compared with approximately 65–68% for smear microscopy, while Khan et al. (2020) reported sensitivities of 93.6% and 71.3%, respectively, in Pakistan. A major advantage of GeneXpert is its ability to detect TB among smear-negative patients with low bacterial loads. Such cases may otherwise remain undiagnosed and untreated, contributing to disease progression and transmission (Boehme et al., 2010; Zhengyu et al., 2025). Studies from Pakistan have similarly shown that GeneXpert improves pulmonary TB detection and identifies a substantial proportion of smear-negative cases (Sasikumar et al., 2020). In addition, GeneXpert provides information related to bacterial burden and detects rifampicin resistance, which can assist in identifying patients at risk of multidrug-resistant TB. Nevertheless, its implementation may be limited by cartridge costs, equipment maintenance, calibration, electricity requirements, and laboratory infrastructure (Meyer et al., 2017). The diagnostic performance of both methods can also be influenced by specimen quality, disease stage, bacterial load, nutritional and immune status, comorbidities, staining quality, and variation in microscopic interpretation (Elbrolosy et al., 2021; Mechal et al., 2019). Although culture remains important for comprehensive drug susceptibility testing, current evidence supports GeneXpert as an important initial diagnostic test, particularly in high-burden settings.

Overall, the literature indicates that GeneXpert is more sensitive than ZN smear microscopy and is particularly valuable for detecting smear-negative pulmonary TB. However, smear microscopy remains important in resource-limited settings because of its affordability and simplicity. Evaluating the relationship between GeneXpert and sputum smear results can therefore help laboratories understand the extent to which conventional microscopy may miss TB cases. Since local evidence from Pakistan remains limited, particularly at district-level hospitals, this study was designed to assess the relationship between GeneXpert MTB/RIF results and sputum smear microscopy among suspected pulmonary TB patients at DHQ South City Hospital, Okara.

1.1 Objectives

1. To determine the prevalence of GeneXpert MTB/RIF-positive cases.
2. To determine the prevalence of ZN smear-positive cases.
3. To assess the relationship between GeneXpert and smear microscopy results.
4. To determine the proportion of smear-negative but GeneXpert-positive cases.
5. To compare TB detection by GeneXpert MTB/RIF and ZN smear microscopy.

MATERIALS AND METHODS

A cross-sectional analytical study was conducted to determine the relationship between GeneXpert MTB/RIF and ZN sputum smear microscopy results among patients suspected of pulmonary TB. The study was carried out in the GeneXpert Laboratory of DHQ South City Hospital, Okara, Pakistan, from 1st September 2026 to 1st December2026. The study population consisted of patients suspected of pulmonary TB who presented to the hospital laboratory. A total of 187 sputum samples were included through non-probability convenience sampling. Patients of any age or sex suspected of pulmonary TB, whose sputum was tested by both ZN microscopy and GeneXpert MTB/RIF, were included. Extrapulmonary TB cases, contaminated or insufficient specimens, duplicate samples, and samples with incomplete laboratory or demographic information were excluded.

Ethical approval was obtained from the Ethical Review Committee of DHQ South City Hospital, Okara, and informed consent was obtained from participants. Patient identities were kept confidential through coded data. Patients were instructed to provide deep respiratory sputum rather than saliva or nasal secretions. Samples were collected in clean, dry, leak-proof, labeled sterile containers, preferably using morning sputum, and approximately 3–5 mL was considered adequate. Samples were transported promptly to the laboratory in leak-proof packaging. Specimens with leakage, incorrect labeling, insufficient volume, saliva, contamination, or discrepancies between the specimen and request form were rejected. Samples requiring delayed processing were stored at 2–8°C.

For GeneXpert MTB/RIF testing, approximately 1 mL of sputum was mixed with sample reagent at a 2:1 reagent-to-sputum ratio, followed by vigorous shaking and incubation at room temperature. After liquefaction, the processed specimen was transferred into a GeneXpert cartridge and loaded into the instrument. DNA extraction, amplification, and detection were performed automatically, with results available in approximately two hours. The assay detected *M. tuberculosis* complex DNA and mutations in the *rpoB* gene associated with rifampicin resistance (CDC, 2024). Results were recorded as MTB detected or not detected.

For ZN smear microscopy, a thin smear was prepared from the purulent portion of the sputum, air-dried, and heat-fixed. The smear was stained with 0.3% carbol fuchsin, gently heated, decolorized with 3% acid alcohol, and counterstained with 0.3% methylene blue. After drying, the slide was examined under the 100× oil-immersion objective. Acid-fast bacilli appeared as red or pink rods against a blue background. Smear results were interpreted according to WHO grading criteria after examination of at least 100 microscopic fields. Results were recorded as negative, scanty, 1+, 2+, or 3+ according to the number of AFB observed.

Demographic and laboratory information was recorded using laboratory registers and a data collection form. Data were entered and analyzed using SPSS version 26. Frequencies and percentages were calculated for categorical variables, while the Chi-square test was used to assess the association between GeneXpert and smear microscopy results. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 187 suspected pulmonary TB patients were included, with a mean age of 49.49 years; 114 (61.0%) were male and 73 (39.0%) were female. ZN smear microscopy detected TB in 35

(18.7%) patients, while 152 (81.3%) were smear-negative. GeneXpert MTB/RIF detected *M. tuberculosis* in 60 (32.1%) patients and was negative in 127 (67.9%).

Table : Age Group Distribution Table of Study Participants (N=187)

Age Group (Years)	Frequency	Percentage (%)
10-25	20	10.7%
26-40	45	24.1%
41-55	44	23.5%
Above 55	78	41.7%
Total	187	100.0

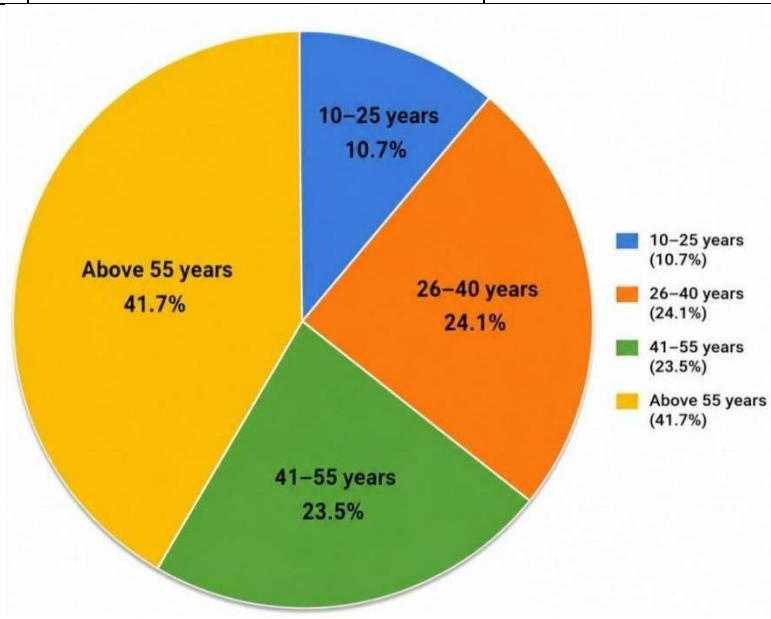
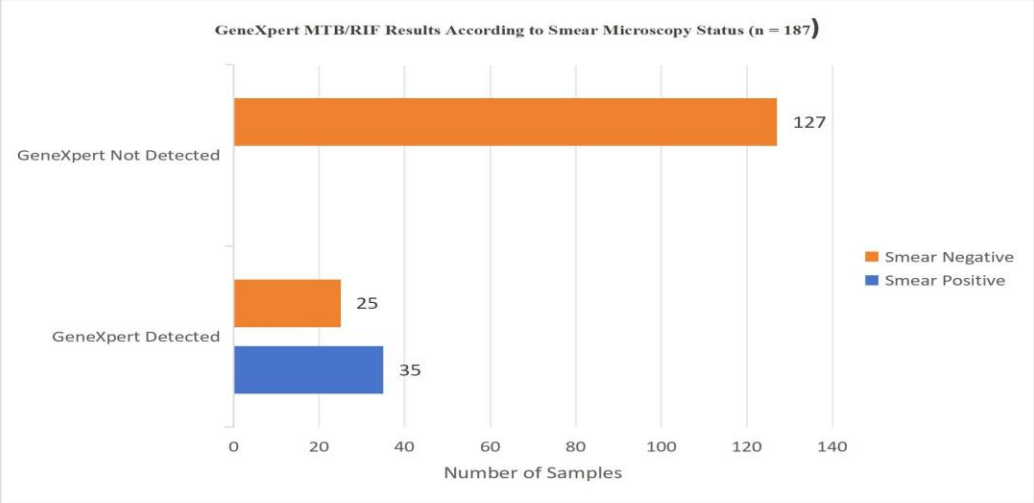


Figure: Age group distribution (n=187)

All 35 smear-positive samples were also GeneXpert-positive, whereas 25 (41.7% of GeneXpert-positive cases) were smear-negative but GeneXpert-positive. GeneXpert therefore detected approximately 71% more cases than smear microscopy. A statistically significant association was observed between GeneXpert and smear microscopy results ($p < 0.001$).



Results

Parameter	Result
Total patients	187
Mean age	49.49 years
Male	114 (61.0%)
Female	73 (39.0%)
Smear positive	35 (18.7%)
GeneXpert positive	60 (32.1%)
GeneXpert positive negative but smear	25 (41.7% of GeneXpert positives)
P-value	< 0.001

GeneXpert detected 71% more cases than smear microscopy alone. One out of every four GeneXpert-positive patients (25 out of 60) was smear-negative and would have been missed by conventional microscopy.

DISCUSSION

This study compared GeneXpert MTB/RIF with ZN smear microscopy among 187 suspected pulmonary TB patients at DHQ South City Hospital, Okara. The findings demonstrated that GeneXpert detected substantially more TB cases than conventional smear microscopy, particularly among smear-negative patients. The mean age of participants was 49.49 years, with a greater proportion belonging to middle-aged and older groups, which is generally consistent with previous reports showing a considerable TB burden among older adults (Akalu et al., 2022). Males constituted 61.0% of participants compared with 39.0% females, which is consistent with the higher TB burden generally reported among men (WHO, 2024).

Only 35 (18.7%) patients were smear-positive, whereas 152 (81.3%) were smear-negative. This relatively low smear positivity may be explained by the limited sensitivity of microscopy, particularly when bacterial load is low. Conventional ZN microscopy generally requires approximately 5,000–10,000 bacilli/mL for reliable detection, making it less effective in early or paucibacillary disease (Steingart et al., 2006). GeneXpert detected 60 (32.1%) positive cases, representing approximately a 71% higher detection rate than smear microscopy. This finding supports previous studies from Pakistan

and other countries reporting a greater diagnostic yield with GeneXpert (Khan et al., 2020; Arora & Dhanashree, 2020; Boehme et al., 2010).

The most important finding was that all 35 smear-positive patients were also GeneXpert-positive, while 25 of the 60 GeneXpert-positive patients (41.7%) were smear-negative. These cases demonstrate the diagnostic limitation of relying solely on smear microscopy. Similar findings have been reported in Pakistan, where a substantial proportion of GeneXpert-positive patients were smear-negative (Khan et al., 2020). The statistically significant association between the two methods ($p < 0.001$) further demonstrates their relationship while also showing that GeneXpert can identify cases that may be missed by microscopy. The findings therefore support the use of GeneXpert as an important diagnostic method for suspected pulmonary TB, while smear microscopy remains valuable because of its low cost and accessibility. Combining both approaches can improve case detection, particularly in high-burden and resource-limited settings(Haider et al., 2025).

The study had some limitations. It was conducted at a single hospital with a relatively small sample size of 187 patients. Culture confirmation was not available, and clinical factors such as HIV status, diabetes, smoking, and nutritional status were not evaluated. Therefore, the findings may not represent the entire Pakistani population. However, the study's strength was that it was conducted in a routine district-level hospital and all participants underwent both GeneXpert and smear testing using standardized laboratory procedures, making the findings relevant to similar healthcare settings.

CONCLUSION

This study demonstrated that GeneXpert MTB/RIF detected substantially more pulmonary TB cases than ZN smear microscopy, including 25 (41.7%) GeneXpert-positive cases that were smear-negative. The findings show that a negative smear result does not exclude pulmonary TB and highlight the value of GeneXpert for improved case detection. Further multicenter studies incorporating culture and relevant clinical factors are recommended to strengthen the evidence from Pakistan.

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