



Comparison of Sputum Gene Xpert and Sputum Smear by Fluorescent Microscope for Diagnosis of Pulmonary Tuberculosis

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Abstract: Background: Pulmonary tuberculosis remains a major public health concern in developing countries, particularly in high-burden regions such as Pakistan. **Objective:** To compare the diagnostic accuracy of sputum GeneXpert and sputum smear by fluorescent microscopy for the diagnosis of pulmonary tuberculosis using sputum culture as the gold standard. **Methodology:** This cross-sectional study was conducted in the Department of Pulmonology, Allama Iqbal Medical College / Jinnah Hospital, Lahore from June 2025 to October 2025. A total of 208 clinically suspected pulmonary tuberculosis patients aged 18–75 years were included using non-probability consecutive sampling. Baseline demographic and clinical characteristics were recorded. **Results:** Among 208 participants, sputum culture confirmed pulmonary tuberculosis in 118 (56.7%) patients. Fluorescent microscopy detected 94 (45.2%) positive cases, while GeneXpert detected 110 (52.9%) positive cases. The sensitivity and specificity of fluorescent microscopy were 71.2% and 88.9%, respectively, with an overall diagnostic accuracy of 78.8%. GeneXpert demonstrated higher sensitivity (89.8%) and specificity (95.6%) with an overall diagnostic accuracy of 92.3%. **Conclusion:** GeneXpert showed superior diagnostic performance compared with sputum fluorescent microscopy for the detection of pulmonary tuberculosis.

Keywords: Mycobacterium, Global scale, Patients, GeneXpert, Microscopy, Sensitivity.

INTRODUCTION

Mycobacterium tuberculosis complex is the name of a group of nine species, with the most widespread being Mycobacterium tuberculosis (MTB), the cause of tuberculosis (TB) among humans [1]. On the international level, tuberculosis (TB) prevalence fell by 144.12 to 97.56 cases per 100,000 individuals between 1990 and 2019. The average rate of this decrease was 1.28 percent per annum, though there has been an increasing trend in the prevalence of extensively drug-resistant and multidrug-resistant TB [2]. Mycobacterial culture [3] is the gold standard for TB diagnosis and can detect as few as 10 bacilli/ml. Acid-fast bacilli (AFB) smear microscopy is also a cheaper and widely used diagnostic test, with a detection threshold of

5,000 bacilli/ml [4]. Another diagnostic modality is fluorescent microscopy, which can successfully identify Mycobacterium tuberculosis in sputum samples in resource-limited settings where high-throughput tests such as GeneXpert are not available [5]. Mycobacterium tuberculosis /rifampicin (MTB/RIF) assay (a nucleic acid amplification test) has a significantly lower detection limit of 136 bacilli/ ml of media and it can detect the resistance to rifampicin [6]. The diagnosis of pulmonary TB using gold standard diagnostic test is sputum culture, which possesses the lowest threshold (10bacilli/ml) to detect MTB [7]. In relation to the comparative analysis of the diagnostic accuracy of the GeneXpert sputum and the sputum fluorescent microscopy, past research has depicted mixed findings as detailed below. One of the studies reported that prevalence

of pulmonary TB (PTB) was 72% with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of sputum GeneXpert to diagnose PTB of 71.43%, 90.28%, 74.07%, 89.04% and 85.00% which was lower as compared to sputum fluorescent microscopy which had the values of 86.11% 78.5 In comparison to this, a study had given conflicting results with sensitivities and negative predictive values (NPV) of sputum GeneXpert of diagnosing PTB of 97.25% and 98.95, respectively that was far much higher than that of sputum fluorescent microscopy that had the following values of 73.08 and 90.74, respectively [9]. On the same note, in a different study, it was established that geneXpert is much more sensitive (73 percent) than fluorescent microscopy (40 percent) to identify MTB [10]. This discrepancy in the outcomes of past researches renders ambiguous the fact that whether the use of fluorescent microscopy can effectively substitute geneXpert under resource limited conditions in which the patients have either limited access to geneXpert or cannot afford it.

Objective

To find the diagnostic accuracy of sputum GeneXpert and sputum Acid- Fast Bacilli (AFB) smear by fluorescent microscope for diagnosis of pulmonary tuberculosis taking culture as gold standard.

METHODOLOGY

This was a cross-sectional study conducted at the Department of Pulmonology, Allama Iqbal Medical College / Jinnah Hospital, Lahore from June 2025 to October 2025. Non-probability consecutive sampling technique was used to recruit eligible participants. A total of 208 patients were included in the study. The sample size was calculated using a 95% confidence level, 72% prevalence of pulmonary tuberculosis, expected sensitivity and specificity of GeneXpert as 71.4% and 90.28% respectively, with a 7.5% margin of error.

Inclusion Criteria:

- Patients aged 18–75 years.
- Male and female patients.
- Clinically suspected cases of pulmonary tuberculosis (as per operational definition).

Exclusion Criteria:

- Patients with previous history of tuberculosis (confirmed by review of medical records).
- Patients with extra-pulmonary tuberculosis.
- Patients presenting with dry cough.
- Patients who did not provide consent to participate in the study.

Data Collection

After approval of the study from CPSP and obtaining informed consent from the participants, a total of 208 patients fulfilling the inclusion criteria and presenting to the indoor and outdoor departments of Pulmonology at Allama Iqbal Medical College / Jinnah Hospital, Lahore were enrolled. Such baseline characteristics as age (years), gender, weight (kg), term of symptoms (weeks), education status (illiterate or school education and above), place of residence (rural or urban), monthly income (<80,000 PKR/month or 80,000 PKR/month), and whether smoked or not were taken. Three samples of sputum collected in an empty stomach were requested of each patient in the mornings. In the operation definitions, these samples were referred to the hospital laboratory to undergo sputum fluorescent microscopy, the sputum GeneXpert testing and the sputum culture in order to confirm pulmonary tuberculosis. Pulmonary tuberculosis patients were treated based on the normal treatment plan. Identification was also with a high degree of confidentiality and anonymity of the participants, all information was documented on a predesigned proforma.

Data Analysis

Data were analyzed using SPSS version 20. Quantitative variables such as age, weight, and duration of symptoms were expressed as mean $\pm$ standard deviation (SD). Qualitative variables such as gender, educational status, area of residence, monthly income, and test results of sputum fluorescent microscopy, GeneXpert, and sputum culture were presented as frequencies and percentages. Diagnostic accuracy was assessed by constructing 2 $\times$ 2 contingency tables to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy for both sputum fluorescent microscopy and GeneXpert, taking sputum culture as the gold standard.

Comparison of Sputum Fluorescent Microscopy with Sputum Culture

	Sputum culture positive	Sputum culture negative
Sputum fluorescent microscopy positive	TP	FP
Sputum fluorescent microscopy negative	FN	TN

Comparison of Sputum GeneXpert with Sputum Culture

	Sputum culture positive	Sputum culture negative
Sputum GeneXpert positive	TP	FP
Sputum GeneXpert negative	FN	TN

RESULTS

Data were collected from 208 patients, mean age of the participants was 41.2 ± 13.6 years. Males constituted the majority of the study population with 132 (63.5%) participants, while females accounted for 76 (36.5%). The mean body weight of the participants was 59.4 ± 9.8 kg, and the

average duration of symptoms was 6.8 ± 2.9 weeks. Regarding educational status, 84 (40.4%) participants were illiterate, whereas 124 (59.6%) had school education or above. In terms of residence, 98 (47.1%) participants belonged to rural areas while 110 (52.9%) were from urban areas.

Table 1: Baseline Characteristics of Study Participants (n = 208)

Variable	Category	n (%) / Mean $\pm$ SD
Age (years)	—	41.2 ± 13.6
Gender	Male	132 (63.5%)
	Female	76 (36.5%)
Weight (kg)	—	59.4 ± 9.8
Duration of Symptoms (weeks)	—	6.8 ± 2.9
Education Status	Illiterate	84 (40.4%)
	School education or above	124 (59.6%)
Area of Residence	Rural	98 (47.1%)
	Urban	110 (52.9%)
Monthly Income	< 80,000 PKR	118 (56.7%)
	$\geq$ 80,000 PKR	90 (43.3%)
Smoking History	Yes	82 (39.4%)
	No	126 (60.6%)

Sputum culture, considered the gold standard for diagnosis, confirmed pulmonary tuberculosis in 118 (56.7%) patients, while 90 (43.3%) were culture negative. Sputum fluorescent microscopy

identified 94 (45.2%) patients as positive and 114 (54.8%) as negative. GeneXpert MTB/RIF detected 110 (52.9%) positive cases and 98 (47.1%) negative cases.

Table 2: Distribution of Pulmonary Tuberculosis Diagnosis by Different Tests

Diagnostic Test	Positive n (%)	Negative n (%)
Sputum Culture (Gold Standard)	118 (56.7%)	90 (43.3%)
Sputum Fluorescent Microscopy	94 (45.2%)	114 (54.8%)
GeneXpert MTB/RIF	110 (52.9%)	98 (47.1%)

When the diagnostic performance of the two tests was compared using sputum culture as the reference standard, fluorescent microscopy detected 84 true positive cases and 80 true negative cases, with 10 false positives and 34 false

negatives. This resulted in a sensitivity of 71.2%, specificity of 88.9%, positive predictive value of 89.4%, negative predictive value of 70.2%, and an overall diagnostic accuracy of 78.8%.

Table 3: Diagnostic Accuracy Comparison of Fluorescent Microscopy and GeneXpert Using Sputum Culture as Gold Standard (n = 208)

Parameter	Fluorescent Microscopy	GeneXpert
True Positive (TP)	84	106
False Positive (FP)	10	4
False Negative (FN)	34	12
True Negative (TN)	80	86
Total Tested	208	208
Sensitivity (%)	71.2	89.8
Specificity (%)	88.9	95.6
Positive Predictive Value (PPV) (%)	89.4	96.4
Negative Predictive Value (NPV) (%)	70.2	87.8
Diagnostic Accuracy (%)	78.8	92.3

Stratification analysis based on demographic variables showed that among patients aged ≤ 40 years, 64 (54.2%) were culture positive compared to 54 (45.8%) among those aged > 40 years ($p = 0.62$). Regarding gender, 76 (64.4%) males and 42 (35.6%) females were culture positive ($p = 0.74$).

Smoking history showed a statistically significant association with tuberculosis positivity, where 56 (47.5%) smokers were culture positive compared with 62 (52.5%) non-smokers ($p = 0.01$).

Table 4: Stratification of Pulmonary Tuberculosis by Selected Demographic Variables (Based on Culture Results)

Variable	Category	Culture Positive n (%)	Culture Negative n (%)	p-value
Age	≤ 40 years	64 (54.2%)	52 (57.8%)	0.62
	> 40 years	54 (45.8%)	38 (42.2%)	
Gender	Male	76 (64.4%)	56 (62.2%)	0.74
	Female	42 (35.6%)	34 (37.8%)	
Smoking History	Yes	56 (47.5%)	26 (28.9%)	0.01
	No	62 (52.5%)	64 (71.1%)	
Area of Residence	Rural	62 (52.5%)	36 (40.0%)	0.08
	Urban	56 (47.5%)	54 (60.0%)	

DISCUSSION

The pulmonary tuberculosis has remained one of the most significant healthcare issues in developing countries especially in Pakistan where the late diagnosis of the disease coupled with the lack of access to modern diagnostic laboratories has led to the further spread of the disease. Pulmonary tuberculosis can be diagnosed early and accurately in order to initiate treatment and properly manage the disease. The current research involved the comparison of diagnostic abilities of sputum fluorescent microscopy and GeneXpert in detecting pulmonary tuberculosis sputum culture as the gold standard [11]. In the present research, sputum culture proved pulmonary tuberculosis in 118 (56.7) of the clinically suspected cases. This observation indicates that tuberculosis has a high load in symptomatic patients attending tertiary care

hospitals. Past studies, particularly in high burden countries on the occurrence of tuberculosis have also reported similar prevalence rates as reported here, with culture proven pulmonary tuberculosis among suspected cases ranging between 50 to 65 percent. These results suggest that a significant percentage of patients with respiratory symptoms in the endemic areas end up diagnosed with active tuberculosis [12-14]. The diagnostic performance which was realised in the given study showed that GeneXpert was more sensitive (89.8%) than sputum fluorescent microscopy (71.2%). This implies that a higher percentage of true cases of tuberculosis were revealed by GeneXpert. It has also been reported in the previous studies that average sensitivity of GeneXpert is between 85% to 92 percent, and the sensitivity of smear microscopy is between about 60 percent to 75 percent. The increased sensitivity

of GeneXpert may be explained by the fact that this method is based on a molecular mechanism of detection that can detect the *Mycobacterium tuberculosis* DNA when the number of bacteria is quite small [15]. GeneXpert was also more specific (95.6) than fluorescent microscopy (88.9). When specificity is higher, fewer false-positive results are produced by GeneXpert. The same findings have been reported in previous studies, where GeneXpert specificity was greater than 90, which supports its reliability as a diagnostic technique. Fluorescent microscopy is more sensitive than the traditional Ziehl-Neelsen staining and also enables visualisation of bacilli, but it may occasionally result in false-positive results due to staining artifacts or non-tuberculosis organisms [16]. The overall diagnostic correctness of GeneXpert in this case was 92.3 which was quite more than that of fluorescent microscopy (78.8%). These results show that GeneXpert has a better diagnostic power in the identification of pulmonary tuberculosis. Besides being more precise, GeneXpert has the benefit of giving the results in a few hours and at the same time determining rifampicin resistance, which is critical in the early detection of multidrug-resistant tuberculosis and how to treat them [17,18].

Although GeneXpert performs better in diagnostic testing, sputum microscopy remains common in most medical institutions due to its low cost, simplicity, and availability in peripheral labs [19]. Fluorescent screening remains a useful initial screening method in resource-limited environments. Nonetheless, these study findings favor the growing use of GeneXpert as a component of standard diagnostic algorithms to enhance early detection of cases and minimize the delays in diagnosis [20]. There were a number of limitations of this study that must be taken into

consideration during interpretation of the findings. The trial was carried out in one tertiary care hospital, and thus, this could narrow the extrapolation of the results to other medical facilities, especially primary care or in the rural areas. Only 208 patients were used as a sample and a bigger sample population would have given stronger estimates in regard to diagnostic accuracy. Moreover, patients capable of using sputum samples were only included whereas those with dry cough or those who could not produce sputum by means of expectoration were not included, which can also create a selection bias. Another limitation of the study was that it only tested two diagnostic techniques, sputum fluorescent microscopy and GeneXpert, and failed to include other diagnostic instruments like radiological findings or other molecular tests which would give a more detailed diagnosis examination. Moreover, no long-term follow up of patients was taken in study to evaluate the outcome of treatment or influence of early detection on transmissions of the diseases.

CONCLUSION

It is concluded that GeneXpert demonstrates higher sensitivity, specificity, and overall diagnostic accuracy compared with sputum fluorescent microscopy for the diagnosis of pulmonary tuberculosis when sputum culture is used as the gold standard. GeneXpert was more effective in detecting true tuberculosis cases and showed fewer false results, making it a reliable and rapid diagnostic tool. Although fluorescent microscopy remains useful as an initial screening method due to its low cost and wider availability, the use of GeneXpert can significantly improve early and accurate diagnosis of pulmonary tuberculosis.

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