



TO STUDY THE DIAGNOSTIC ACCURACY OF GENEXPERT MTB/RIF ASSAY FOR THE DIAGNOSIS OF PULMONARY TUBERCULOSIS IN A TERTIARY CARE HOSPITAL IN NAVI MUMBAI.

Microbiology

Dr Samir Pachpute* PhD. Scholar (Microbiology) MD, DPB, MBBS Associate Professor, Microbiology Department, MGM Medical College, Kamothe, Navi Mumbai. *Corresponding Author

Dr Preeti Pachpute PhD. Scholar, MD MBBS

Dr A.D. Urhekar MD, Head of Microbiology Department, MGM Medical College, Kamothe, Navi Mumbai

ABSTRACT

Introduction: Tuberculosis remains one of the major communicable diseases. There are number of diagnostic tests available for the diagnosis of tuberculosis but conventional microscopy has low sensitivity and culture although gold standard, takes longer time for positivity.

Aim: To evaluate the sensitivity, specificity, PPV & NPV of Nucleic acid amplification assay (GeneXpert) using respiratory samples in patients with suspected pulmonary tuberculosis and compare with AFB smear microscopy and AFB culture.

Materials and Methods: 89 patients from April 2015 to July 2017 for ZN stain, culture and GeneXpert (Xpert® MTB/Rif assay) were studied. The sensitivity, specificity, PPV and NPV of GeneXpert and ZN microscopy were calculated using Liquid culture of Mycobacterium tuberculosis as gold standard.

Results: A total of 89 patient samples were evaluated in final analysis. Of these, 16 samples were positive by all three methods used in our study. The overall sensitivity, specificity, PPV and NPV of GeneXpert were 86.8%, 93.1%, 78.5% and 96%. The overall sensitivity and specificity of AFB smear microscopy were 22.2%, and 78.5% respectively.

Conclusion: GeneXpert can be a useful tool for early diagnosis of patients with high clinical suspicion of pulmonary tuberculosis. The other major advantage of Gene Xpert is that it simultaneously detects Rifampicin resistance and is especially beneficial in patient with MDR and HIV associated tuberculosis and should be studied further.

KEYWORDS

Tuberculosis, GeneXpert MTB/RIF Assay, AFB smear.

Introduction:

As per the Global Tuberculosis report 2014 of World Health Organization (WHO), Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) is one of the major health problem. [1]. The disease usually affects the lungs (pulmonary TB) and spread by air transmission from people with pulmonary TB [2]. In 2013, out of the estimated global annual incidence of 9 million TB cases, India alone shares the incidence of 2.1 million (24%) cases/year (one fourth of global incidence)[3].

Sputum Microscopy, Mycobacterial Tb culture are standard methods used in laboratory diagnosis of TB across the world, but Smear is not highly sensitive and Culture is time-consuming processes, taking up to eight weeks for diagnosis. Doctors in the developing world often rely only on chest X-rays with few patients being sent for sputum smears; confirmation by culture is not done routinely. Early diagnosis is imperative for early patient management and successful patient outcomes. False-negative results and misdiagnosis of TB suspects are common in developing nations, as most TB control programmes use Ziehl-Neelsen (ZN) smear microscopy, which has less sensitivity and multiple visits are required that leads to higher default. Mycobacterial culture, although considered as the gold standard but is slow and usually takes 2-6 weeks time to yield a final result and requires proper infrastructure, Laboratory requirements and technical expertise [1,4,5].

There are number of Nucleic Acid Amplification (NAA) methods that have been developed for rapid detection and identification of *Mycobacterium tuberculosis* (MTB) in clinical specimens of pulmonary and extra-pulmonary tuberculosis cases [6,7]. These techniques not only provide the advantage of rapidity of diagnosis but also detect even low MTB genomic copies in various specimens.

More recently, the WHO endorsed the GeneXpert (Xpert® MTB/Rif assay) for the diagnosis of TB [6]. The GeneXpert utilizes a DNA-PCR technique for simultaneous detection of *Mycobacterium tuberculosis* and Rifampicin resistance related mutations. It is the first fully automated bench top cartridge based nucleic acid amplification (CB-NAAT) assay for TB detection that includes all necessary steps of DNA PCR. It gives results within 2 hours. Diagnostic accuracy of GeneXpert for pulmonary TB has been reported high [8,9]. Patients with high risk of tuberculosis like presumptive HIV-associated TB

patients and pediatric presumptive including extra pulmonary cases in whom AFB smear examination is usually negative, are the most likely to be benefited from GeneXpert [3,9]. The aim of this study was to evaluate Cepheid GeneXpert MTB/RIF assay for direct detection of *M. tuberculosis* and RIF resistance and compare it with conventional Sputum smear and culture methods.

Materials and Methods: Inclusion criteria: Adult Patients with clinical suspicion of pulmonary tuberculosis including symptoms of cough with or without expectoration for >2 weeks, weight loss, malaise, haemoptysis and loss of appetite. Ethical and Research Committee approval was obtained for conducting the study. **Exclusion Criteria:** Patients who refused to participate in the study. Pulmonary specimens of 89 patients with suspected pulmonary tuberculosis, received retrospectively for the request of ZN microscopy, liquid AFB culture and GeneXpert from MGM respiratory Medicine department & Karjat Subdistrict Hospital to MGM TB Microbiology Laboratory, were reviewed from a period of April 2015 to July 2017. Pulmonary specimens included 89 sputum samples. Patient related information was collected from the Test Requisition Forms (TRF), received with the sample.

Laboratory Methods: Each sputum sample received in the lab from the centers as per the collection and transportation policy of the laboratory, were divided into three parts; one part was immediately tested using GeneXpert, second part used for ZN smear microscopy and third part for MGIT BACTEC liquid culture and performed on same day. GeneXpert testing was performed according to the manufacturer's instructions [10]. Sample reagent was added to untreated sputum at a ratio of 2:1, manually agitated and kept for 10 min at room temperature, then shaken again and kept for 5 min; 2 ml of the inactivated material was transferred to the test cartridge and inserted into the test platform. Only electronic results were used for comparison. Direct Smear microscopy was performed to investigate presence of acid fast bacilli with the second part of the specimen using conventional ZN staining method. Slides showing red coloured acid fast bacilli were taken as positive and negative slides were those without any acid fast bacilli [11]. Third part was processed using the N-acetyl-L cysteine- sodium hydroxide method (NALC-NaOH) as per the manufacturer's instructions, cultured on MGIT media and incubated in MGIT BACTEC liquid culture system [11]. Sodium hydroxide (NaOH) is a decontaminating agent and also acts as

emulsifier and NALC acts as a mucolytic agent and also reduces the concentration of NaOH required [11]. When the tubes were flagged positive by the system, ZN staining and culture on 5% sheep blood agar were performed from the tube directly to see any contamination as per the manufacturer's instructions. All tubes were checked for positivity till 42 days. MOTT and *Mycobacterium tuberculosis* testing from positive culture tubes were done by rapid immunochromatography test kit using MPT 64 antigen according to the manufacturer's instructions.

Analysis: The data was tabulated in Microsoft excel spreadsheet in a master chart and studied for correlation. Sensitivity, specificity, PPV and NPV was calculated. The sensitivity, specificity, PPV and NPV for the diagnosis of Pulmonary tuberculosis was calculated for AFB smear microscopy and the GeneXpert, using culture of *Mycobacterium tuberculosis* from sputum as gold standard. By taking culture method as reference, samples that were positive and negative in culture were considered true positive and true negative. Culture negative and GeneXpert positive samples were taken as false positive samples. GeneXpert negative and culture positive samples were considered false negative.

Results: A total of 89 respiratory specimens (Sputum samples) were tested. Of the 89 specimens, 37 samples were positive and 52 specimens were negative by MGIT Culture Gold standard method used. Among 89 samples, 35 samples (39 %) were GeneXpert TB positive. Among the 89 samples, 35 samples were culture and GeneXpert positive, 2 samples were GeneXpert positive and 1 sample were only culture positive.

Table 1: Comparison of results from GeneXpert with culture as gold standard.

GeneXpert TB Results	MGIT Culture results	
	Positive	Negative
Positive	35	2
Negative	1	51

Statistic	Formula	Value	95% CI
Sensitivity	$\frac{a}{a+b}$	97.22%	85.47% to 99.93%
Specificity	$\frac{d}{c+d}$	96.23 %	87.02% to 99.54%
Disease prevalence	$\frac{a+b}{a+b+c+d}$	40.45%	30.17% to 51.38%
Positive Predictive Value	$\frac{a}{a+c}$	94.59%	81.78% to 98.56%
Negative Predictive Value	$\frac{d}{b+d}$	98.08 %	88.06% to 99.72%
Accuracy	$\frac{a+d}{a+b+c+d}$	96.63%	90.46% to 99.30%

In all, 37 (41%) specimens were culture positive for AFB. 35 (39.3%) isolates were found to belong to MTB, while the remaining 2 (2.2%) strains from samples were identified as *Mycobacterium* other than tuberculosis (MOTT) species. Further speciation of these isolates was not done. Out of 89 samples, only 16 samples were found AFB smear positive. All these AFB smear positive samples were culture and GeneXpert positive.

Table 2: Comparison of results from AFB smear and culture on gold standard.

Smear ZN AFB Results	MGIT Culture results	
	Positive	Negative
Positive	16	0
Negative	22	51

Statistic	Formula	Value	95% CI
Sensitivity	$\frac{a}{a+b}$	42.11%	26.31% to 59.18%
Specificity	$\frac{d}{c+d}$	100.00 %	93.02% to 100.00%
Disease prevalence	$\frac{a+b}{a+b+c+d}$	42.70%	32.26% to 53.63%
Positive Predictive Value	$\frac{a}{a+c}$	100.00%	
Negative Predictive Value	$\frac{d}{b+d}$	69.86 %	63.87% to 75.25%
Accuracy	$\frac{a+d}{a+b+c+d}$	75.28%	65.00% to 83.81%

Discussion

In our study, we have evaluated the diagnostic accuracy of Gene Xpert to detect MTB in respiratory samples and compared it with AFB culture which was taken as gold standard. Mycobacterial cultures for detection of *Mycobacterium tuberculosis* can be done either using solid (Lowenstein Jensen media) or liquid broth system (MGIT 320). Results by MGIT liquid culture medium come earlier as compared to LJ medium [13,14]. In our study, results from MGIT culture were included. GeneXpert is a simple bench top point of care diagnostic assay that can be performed with minimal training. The results are available within 2 hours, much earlier than the culture which usually takes days to come positive [9,15]. Few studies have demonstrated the utility of GeneXpert in diagnosis of pulmonary tuberculosis [16,17]. In our study, overall sensitivity, specificity, PPV and NPV of GeneXpert were 97%, 96%, 95% and 98% respectively that is comparable with other studies [18,19]. Although specificity in our study is 96%, it is because 2 culture samples were positive for MOTT and GeneXpert only detects MTB. In one sample, although MTB growth is in culture but it is possible that the bacterial load may have been too low for the GeneXpert to detect the DNA from MTB- complex. It shows that a patient with a negative GeneXpert can still have TB with MTB or MOTT [18,20,22]. The NPV value of GeneXpert is high in our study in comparison to the study done by Kanwal et al., as LJ media was used in their study whereas in our and other studies, liquid culture method was used [21]. 2 samples which were culture negative and GeneXpert positive, the result of GeneXpert was very low or low positive. As cases were evaluated retrospectively, history of treatment with ATT cannot be ruled out with low bacterial load. PCR test amplifies any DNA, of live or dead bacilli. Therefore while diagnosing a person with active tuberculosis clinicians need to be very cautious using it as a sole method. Clear history of treatment with ATT is required to avoid false positive results [16,25]. In comparison with culture used as gold standard, sensitivity, specificity, PPV and NPV for Smear microscopy for pulmonary sample were recorded as 42.1%, 100%, 100% and 69.86 % respectively, which is in line with other studies [18,20,21]. Our study further strengthens the use of GeneXpert in smear positive pulmonary samples as endorsed by WHO [9]. In patients with incongruous results of smear microscopy and GeneXpert pulmonary samples but high clinical evidence of pulmonary tuberculosis like HIV positive or critically ill, clinicians may exercise their clinical decision to start anti tubercular treatment after sending sample for culture [6]. However, GeneXpert does not eliminate the need of conventional microscopy, culture and anti-tubercular drug sensitivity that are required to monitor the progression of treatment and to detect resistance to drugs other than Rifampicin [6].

Conclusion

The diagnostic accuracy and Sensitivity of GeneXpert is much higher than AFB smear microscopy in respiratory samples. Although culture is considered as a gold standard method but as it takes days to come positive and simultaneous detection of Rifampicin resistance is not possible with it. Also, GeneXpert can be a useful diagnostic method in patients of suspected pulmonary tuberculosis either AFB smear negative or positive due to its rapidity and simultaneous detection of Rifampicin resistance especially beneficial in patient with MDR and HIV associated tuberculosis. Positive GeneXpert, but culture negative results need to be read cautiously and should be well correlated with clinical and treatment history of the patient.

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