

Diagnostic Performance of Xpert MTB /RIF in Comparison with LED Fluorescence Microscopy and Culture in Suspected Cases of Pulmonary Tuberculosis

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Abstract

GeneXpert MTB/RIF assay (GeneXpert) is relatively new technology in India and it is important to generate data on its efficacy for diagnosis of Tuberculosis. Thus; present study was undertaken to compare the diagnostic performance of GeneXpert for diagnosis of pulmonary tuberculosis (PTB) in comparison with Auramine O staining based Light Emitting Diode- Fluorescence Microscopy (LED-FM) considering culture as gold standard. A total of 662 (451 BAL, 211 Sputum) respiratory samples obtained from patients suspected of PTB were tested for smear microscopy and GeneXpert assay and were compared with culture results. Rifampicin resistance was compared with Genotype MTBDR *plus* assay. Sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) of the assays were calculated using SPSS version 22. On comparing with culture the sensitivity, specificity, PPV and NPV of GeneXpert MTB/RIF was found to be 97.25%, 98.54%, 96.20% and 98.95% respectively whereas sensitivity, specificity, PPV and NPV of smear microscopy was found to be 73.08%, 100%, 100% and 90.74% respectively. Rifampicin resistance was detected in 11 (1.66%) samples by GeneXpert. Five (0.75%) samples among them were found to be MDR whereas one sample showed false positive result for rifampicin resistant when compared by Genotype MTBDR *plus* assay. GeneXpert MTB/RIF assay offers much higher sensitivity as compared to Auramine O staining based LED-FM and seems to be a promising tool for TB diagnosis. Further longitudinal studies are required to evaluate the potential of this assay at primary health care settings and diagnosis of Extrapulmonary TB.

Keywords: Culture, Diagnosis, GeneXpert, Microscopy, *Mycobacterium tuberculosis*.

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(Received: 28 July 2019; accepted: 02 September 2019)

Citation: Vishnu Prasad Shenoy, Ajay Kumar and Kiran Chawla, Diagnostic Performance of Xpert MTB /RIF in comparison with LED Fluorescence Microscopy and Culture in Suspected Cases of Pulmonary Tuberculosis, *J Pure Appl Microbiol.*, 2019; **13**(3): 1461-1465. <https://doi.org/10.22207/JPAM.13.3.16>

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INTRODUCTION

Tuberculosis (TB) remains a major health care issue in India. India accounted for highest burden for TB (2.74 million) and multi drug resistant TB in 2017, which accounted for 4.1 lakhs lives¹. Though TB is curable with appropriate treatment such huge number of deaths signifies the need of early accurate diagnosis, prompt treatment and proper monitoring of such cases. Over years lack of inadequate tools for rapid diagnosis of TB remained major obstacle in TB control programmes especially in resource poor countries like India. For diagnosis of TB, Mycobacterial culture is the gold standard but it takes 6-8 weeks to obtain final result and requires sophisticated laboratory facility and technically skilled person to perform the test^{2,3}. Thus, majority of TB control programmes were based on Ziehl-Neelsen (ZN) staining based smear microscopy which has suboptimal sensitivity⁴. In 2011, WHO endorsed auramine based light-emitting diode fluorescent microscopy (LED-FM) for national tuberculosis control programmes in developing countries⁵. Auramine-LED-FM has shown increased sensitivity as compared to conventional Ziehl-Neelsen microscopy under programmatic conditions in India⁶. Recently WHO endorsed GeneXpert (Xpert[®] MTB/RIF assay); a fully automated cartridge based nucleic acid amplification (CB-NAAT) assay which simultaneously detects *Mycobacterium tuberculosis* and most common genetic mutations associated with Rifampicin resistance with a turnaround time of less than 2 hours⁷. Preliminary studies assessing the performance of GeneXpert have reported very high sensitivity and specificity in Pulmonary TB (PTB) cases⁸⁻¹⁰. GeneXpert is relatively new technology in India and it is important to generate data on utility of this assay for diagnosis of TB. Thus, present study was undertaken to evaluate the diagnostic efficacy of GeneXpert (Cepheid, US) assay for diagnosis of PTB in comparison with Auramine O staining based LED-FM.

MATERIALS AND METHODS

Study setting

Present research was carried out in Department of Microbiology at a tertiary care centre in Karnataka, India from August 2016 to August 2017 after obtaining approval from the

Institutional Research and Ethics Committee. A total of 662 (451 BAL, 211 Sputum) non repetitive samples obtained from patients suspected of pulmonary TB were examined by Auramine O staining based LED-FM and GeneXpert. All samples were divided into two equal aliquots; one aliquot was used for GeneXpert whereas other aliquot was used for Auramine O staining and Culture.

Smear Microscopy

For all samples AFB smears were prepared and rest of samples were stored at 4°C to use later for validation of GeneXpert and smear microscopy results. The smears were stained with 0.3% Auramine phenol O for 10 minutes and then were decolourised with 1% acid alcohol solution for 2 minutes and finally counter stained with 0.1% potassium permanganate for 1 minute. All stained smears were examined under LED-FM following the guidelines of Revised National Tuberculosis Control Program (RNTCP)¹¹.

GeneXpert Assay

GeneXpert assay was performed following the instructions given in product insert provided by manufacturer along with the kit. Briefly untreated sample was mixed with sample reagent at a ratio of 1:2 and the contents were mixed manually by gentle agitation and sample reagent mixture was kept at room temperature for 15 minutes and 2ml of mixture was transferred to the GeneXpert cartridge. Then cartridge was loaded into the GeneXpert device and finally the results were read after completion of the test. All samples that showed invalid results were repeated.

Validation of results

All samples were further processed using the NALC-NaOH (N-acetyl-L cysteine- sodium hydroxide) method and were inoculated in Mycobacterial Growth Indicator Tubes (MGIT) or Lowenstein Jensen (LJ) medium and were monitored for positive growth till 6- 8 weeks¹². All samples that were positive for culture were subjected to ZN staining and culture on blood agar plates to confirm growth of acid fast bacilli and to rule out the contamination respectively. Confirmation of MTB from the cultures was done using MPT64 antigen test kit (SD Biotec, Korea) using manufacturer's protocol. Samples showing rifampicin resistant by GeneXpert assay were validated by performing Genotype MTBDR *plus* assay following manufacturer's guidelines.

Table 1. Comparison between smear microscopy, GeneXpert and MGIT 960 culture

	Smear Positive	Smear Negative	Culture Positive	Culture Negative
GeneXpert Positive	128	56	177	07
GeneXpert Negative	05	473	05	473
Sensitivity (95% CI)	96.24% (91.44%-98.77%)		97.25% (93.71%-99.10%)	
Specificity (95% CI)	89.41% (86.47%-91.90%)		98.54% (97.02%-99.41%)	
Positive Predictive Value(95% CI)	69.57% (64.03%-74.59%)		96.20% (92.38%-98.14%)	
Negative Predictive Value(95% CI)	98.95% (97.56%-99.55%)		98.95%(97.55%-99.56%)	

Statistical Analysis

Patient demographic data and all test were analysed using SPSS version 22 (SPSS Inc., USA). A *p* value < 0.05 was considered statistically significant for all statistical analysis.

RESULTS

A total of 662 respiratory samples comprising of sputum (211) and BAL (451) from patient suspected of tuberculosis were tested for smear microscopy and GeneXpert. The mean age±SD of the patients was found to be 52.7±13.56 years (Range: 15-85 years), 451 (68.12%) among them were male where as 211 (31.87%) were females. On comparing the results of smear microscopy and GeneXpert 128 (19.33%) samples were found positive by both methods whereas 473 (71.45%) samples were negative by both methods. Fifty six (8.45%) samples were positive by GeneXpert alone, whereas 5 (0.75%) samples were positive by smear microscopy only (Table1). When GeneXpert and smear microscopy results were compared with culture, 49 (7.40%) smear negative but GeneXpert positive samples were found positive for culture; further 5(0.75%) smear positive but GeneXpert negative samples were also found positive for culture and were identified as Non Tuberculous Mycobacteria. Seven (1.05%) samples were found positive alone by GeneXpert (Table 1). On comparing with culture the sensitivity, specificity, positive predictive value and negative predictive value of GeneXpert assay was found to be 97.25% (93.71%-99.10%), 98.54% (97.02%-99.41%), 96.20% (92.38%-98.14%) and 98.95% (97.55%-99.56%) respectively whereas sensitivity, specificity, positive predictive value

and negative predictive value of smear microscopy was found to be 73.08% (66.01%-79.37%), 100% (99.23%-100%), 100% and 90.74% (88.52%-92.56%) respectively. Rifampicin resistance was detected in 11 (1.66%) samples by GeneXpert, Five (0.75%) samples among them were found to be MDR whereas one sample showed false positive result for rifampicin resistant when compared by Genotype MTBDR *plus* assay.

DISCUSSION

Early detection of TB and MDR status is one of the most important factors for appropriate management of TB. In majority of resource poor countries the diagnosis of TB is based on conventional methods which are suboptimal, laborious and time consuming. Unavailability of adequate tool for rapid diagnoses of TB remains a major hindrance in achieving the global target of tuberculosis elimination. GeneXpert is a rapid automated assay for detection of TB and Rifampicin resistance directly from clinical samples. Authors in the past have reported utility of GeneXpert assay in rapid diagnosis of TB¹³⁻¹⁴. Present study was performed with an aim to evaluate the diagnostic performance of this assay for diagnosis of pulmonary tuberculosis in comparison with Auramine O staining based LED-FM. The overall sensitivity, specificity, PPV and NPV of GeneXpert in present study was found to be 97.25%, 98.54%, 96.20% and 98.95% respectively, whereas the overall sensitivity, specificity, PPV and NPV for Auramine O staining based LED-FM was found to be 73.08%,100%, 100%, and 90.74% respectively which is comparable with previously published studies^{15,8}. A previous study from India showed

10.8% increment in case detection by GeneXpert when compared to fluorescent microscopy. In the present study also 49 (7.40%) samples which were smear negative were detected positive by GeneXpert and culture¹⁶. Five smear positive samples were found negative by GeneXpert, all these samples were later confirmed as NTM (Non tuberculous mycobacteria) by culture and Hains GenoType Mycobacterium CM assay which suggests that GeneXpert is highly specific for MTB complex. Bacterial count below detection limit of GeneXpert may also attribute to negative results¹⁷. GeneXpert showed false positive result for 7 samples, all these patients had past history of TB treatment and DNA of dead bacteria may have resulted in false positive GeneXpert result. Since rifampicin mono-resistance is rare and 90% cases of rifampicin resistance were reported to have isoniazid resistance as well, thus resistance to rifampicin is considered as a marker for MDR TB¹⁸. In present study, 5 samples out of 11 rifampicin resistant samples were found to be MDR. Authors in past has reported discrepancies in rifampicin resistance detection by GeneXpert; likewise one sample detected rifampicin resistant by GeneXpert in the present study was found to be a sensitive strain when validated on Genotype MTBDR *plus*¹⁹. To conclude GeneXpert offers much higher sensitivity as compared to Auramine O staining based LED-FM although the specificity of both is almost similar. GeneXpert assay seems to be a promising tool for TB diagnosis and has a potential to improve the case detection rates especially in smear negative cases and MDR-TB. Further longitudinal studies are required to evaluate the potential of this assay at primary health care settings and diagnosis of Extrapulmonary TB.

ACKNOWLEDGEMENTS

We would like to thank Technical Staff Miss Suraksha and Mrs Rajini for their various roles and contributions to this project.

CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors have made substantial

contribution to the work and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All data generated during the study are included in the manuscript.

ETHICS STATEMENT

The study was undertaken after obtaining approval from the Institutional Research and Ethics Committee.

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