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Accuracy of Xpert MTB/RIF Assay in Diagnosis of Tuberculosis a Comparison to Culture: Retrospective Analysis from South Kashmir, India

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***Abstract*---**This study was done to assess the performance of CB-NAAT (Cepheid GeneXpert®) system for the diagnosis of MTB, from south Kashmir, India. Pulmonary & extra pulmonary specimens received at the laboratory, from new clinically suspected tuberculosis patients were used. The samples were divided into three portions, one part each for AFB direct smear, CB-NAAT and culture, respectively. The concentrates were cultured on LJ media.CB-NAAT was done using Cepheid GeneXpert® system. Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) were calculated, by considering the culture results as the gold standard.831 patients were included, 682 pulmonary and 149 extra pulmonary samples. The true positive occurrence for smear microscopy was 95.83%.The true positive rate of detection of MTB by CB-NAAT was 75.74%.The sensitivity, specificity, PPV and NPV of smear microscopy were 38.33, 99.54, 95.83 and 85.38% respectively. The sensitivity, specificity, PPV and NPV of CB-NAAT was seen to be 84.45, 94.93, 75.73, and 97.02 % respectively. This study could therefore, favor the use of CB-NAAT, using the Cepheid GeneXpert® system, as a rapid method for the detection of MTB alone. Further research is required for development of a better diagnostic method that can simultaneously distinguish MTB and NTM.

***Keywords*---**accuracy Xpert, CB-NAAT, diagnosis tuberculosis, GeneXpert® system, MTB, MTB/RIF assay.

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Introduction

The world today is known for its advancements in technology in all social sectors, most importantly in the health sector and specifically in the field of Mycobacteriology. Clinically suspected TB cases are normally tested for the presence of *Mycobacterium tuberculosis* in appropriate samples by laboratory diagnostic methods. Conventionally, Acid fast bacilli (AFB) smear microscopy and the culture methods are employed for the diagnosis. Microscopy of smears and liquid culture are the commonly used conventional methods. Culture method is the most sensitive and specific method for the detection of *Mycobacterium tuberculosis*. Despite this, cultures are highly prone to contamination and the process can still take several days and does require expensive equipment, strict biosafety practices and well trained technical staff (McNerney et al., 2017).

Among the currently available Nucleic Acid Amplification Tests (NAAT), the Xpert MTB/RIF Assay (CB-NAAT), the LINE Probe Assay (LPA) and the Loop-Mediated Isothermal Amplification (LAMP) are endorsed by WHO for *in vitro* diagnosis of TB. The GeneXpert® system powered by the Cepheid Innovations, for the CB-NAAT (Cartridge based), is an automated, semi-quantitative, hemi-nested, real-time PCR used for the simultaneous detection of the MTB complex and its rifampicin (RIF) resistance pattern associated with the mutation in the *rpoB* gene, in clinical samples with a 2 h turnaround time. (Sunnyvale, 2012). The present study was carried out to assess the performance of CB-NAAT (Cepheid GeneXpert®) system for the diagnosis of MTB in both pulmonary and extrapulmonary specimens, within the demographic area of south Kashmir, India.

Materials and Method

This study was conducted at the Department of Microbiology, Government Medical College, Anantnag Kashmir India, over a period of 1 year from March 2017 to February 2018. The Department of Microbiology consists of Designated Microscopy Center as per the Revised National Tuberculosis Control Program (RNTCP) under the DOTS (Directly Observed Treatment, Short course). Pulmonary & extra pulmonary specimens received at the Microbiology laboratory, in sterile containers, from the clinically suspected tuberculosis patients were used in the study. Repeat samples, Saliva, blood, and stool samples were excluded from the study.

The samples received at the laboratory were divided into three portions, one part each was used for AFB direct smear preparation, CB-NAAT and culture, respectively. Direct and concentrated smears were prepared, stained using the acid-fast staining method and screened as per the guidelines (*Flourescence Microscopy Manual*, n.d.) A smear was reported positive if either the direct or the concentrated smear showed the presence of AFB. The NALC-NaOH method (*Mgit_manual_nov2006.Pdf*, n.d.) Was used for the sample digestion and decontamination. The concentrates were cultured on to LJ media Cultures were incubated for up to 8 weeks to confirm the negativity of *Mycobacteria* in the sample. CB-NAAT was done using the Cepheid GeneXpert® system, according to the manufacturer's instructions (Sunnyvale, 2012). At the end of the test, the result was reported as MTB detected or MTB not detected along with Rifampin

(RIF) resistance status. The collected data was entered onto Statistical Package for Social Sciences (IBM SPSS Statistics for Windows). Results were expressed as proportions using tables. Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) were calculated, by considering the culture results as the gold standard. For comparison across the CB-NAAT and culture groups, chi-square test was used.

Results

The samples received from suspected TB patients were from different localities within and around the district of Anantnag. 1497 samples were received out of these 831 samples were from new suspected TB cases which were included in this study ,while as repeat samples were excluded from this study. Pulmonary samples were 682 and extra pulmonary samples were 149 .Smears were positive in 72 patient samples, which consisted of 56 pulmonary and 16 extra-pulmonary cases. The true positive occurrence with the use of smear microscopy was 95.83%. A positive culture growth was observed in 148 pulmonary and 32 extra pulmonary samples. Table 1

Table 1
AFB smear comparison with culture

	Culture Positive	Culture Negative
Smear Positive	69	3
Smear Negative	111	648

The GeneXpert system detected the presence of MTB in 136 samples. The true positive rate of detection of MTB by CB-NAAT was found to be 75.74%. Five cases of RIF resistance were also detected. 30 samples positive by CB-NAAT had grown Genus *Mycobacterium* species in culture. Additionally, CB-NAAT detected MTB in three samples which were smear positive and culture negative. CB-NAAT also detected MTB in 61 smear positive and culture positive samples, 42 smear negative and culture positive samples, and 33 smear negative and culture negative samples. The sensitivity, specificity, PPV and NPV of smear microscopy were 38.33, 99.54, 95.83 and 85.38% respectively and that of the CB-NAAT assay is tabulated in Table 2.

Table 2
CB-NAAT assay is tabulated

SAMPLES (n = 831)	SENSITIVITY (%)	SPECIFICITY (%)	PPV (%)	NPV (%)
PULMONARY (n = 682)	82.11	94.76	73.58	96.75
EXTRA-PULMONARY (n = 149)	92.59	95.73	83.33	98.25
OVER ALL	84.45	94.93	75.73	97.02

Discussion

In spite of rapid advances in diagnosis and treatment, TB still continues to be a menace in many developing countries, including India. TB is among the 10

cardinal causes of mortality across the globe. The fight against TB has definitely given notable results. In the last 17 years, about 53 million lives were redeemed from the clutches of TB, mainly through timely diagnosis and effective treatment. The major hindrance in the combat against TB is the lack of early diagnosis and appropriate and timely treatment. Programs like RNTCP and DOTS are efficiently confronting this issue with high priority, thus decreasing complications towards a great extent.

Our study included the assessment of performance of GeneXpert® system in detecting MTB infection among the patients in and around the district of Anantnag. Such an evaluation is the first of its kind in this area and thus the comparison is mainly with other studies across India (Nikam et al., 2014) or abroad (Ioannidis et al., 2011; Kwak et al., 2013; Sharma et al., 2015; Vadwai et al., 2011; Sharma et al., 2014). There are a few studies which have compared the performance of CB-NAAT for both pulmonary and extrapulmonary samples (Ioannidis et al., 2011). Most of the studies focus either on pulmonary samples Nikam et al. (2014); Kwak et al. (2013); Sharma et al. (2015), or on extrapulmonary samples alone (Vadwai et al., 2011; Sharma et al., 2014).

The sensitivity and PPV of the CB-NAAT in our study was lower when compared to previous studies (91.4 and 86.5% respectively) (Ioannidis et al., 2011). The lower sensitivity can be attributed to the false negatives (BAL) obtained with the CB-NAAT. BAL is known to have a lower sensitivity for the detection of MTB by CB-NAAT (Sharma et al., 2015). Another reason could be the very low load of the organism in the sample, lower than the detection limit of CB-NAAT (131 CFU/mL of sample) (Sunnyvale, 2012). The lower PPV can be attributed to the 33 positive cases obtained by CB-NAAT which failed to grow in culture. The inability to grow in culture and ability to be detected by CB-NAAT may have been due to the paucibacillary nature of extra pulmonary specimens. It may also be due to the treated cases where even the dead bacilli were detected by the CB-NAAT or the cases where the use of NALC-NaOH treatment for decontamination of the samples proved excessively harsh resulting in no growth. The specificity and NPV were almost at par with the previous studies (93 and 95.6% respectively) (Ioannidis et al., 2011).

In the case of pulmonary samples, the total sensitivity was similar to other studies (79.5%) and was lesser than that of certain other studies (95.7%) (Kwak et al., 2013; Sharma et al., 2015). While the NPV and the sensitivity for smear positive and culture positive samples were comparable to former studies (94–98.1% and 88.9–99.2% respectively), the specificity, PPV and sensitivity for smear negative and culture positive samples were lower than previous studies (99.6–100%, 99–100% and 73.1–77.7% respectively) (Kwak et al., 2013; Sharma et al., 2015). In the case of extra pulmonary samples, the total sensitivity, sensitivity for smear negative and culture positive samples, smear positive and culture positive samples and NPV were higher than that of former studies (71–83, 66, 95 and 90% respectively) (Vadwai et al., 2011; Sharma et al., 2014). The specificity and PPV were similar to previous studies (95 and 83% respectively) (Vadwai et al., 2011; Sharma et al., 2014).

In the present study, CB-NAAT was found to be better than that of smear microscopy (with a difference of above 45% in terms of sensitivity), as observed by the earlier studies (Zeka et al., 2011). Adding to its advantage, this test is rapid, require minimal training of personnel and lower biosafety level (compared to culture) (Suleiman & Lessem, 2017). CB-NAAT needs to be made more useful by including a distinguishable detection of NTM. Apart from this, the CB-NAAT is also comparatively disadvantageous in terms of its cost, shelf-life of cartridges, requirement of continued power supply and the need for the periodic servicing and calibration of the equipment (Raizada et al., 2015). This study could have been strengthened if all the Mycobacterial isolates in our study were identified to species level.

Conclusion

This study could therefore, successfully favor the use of CB-NAAT (Cepheid GeneXpert® system) as a rapid method for the detection of MTB alone. Further research is required for development of a better diagnostic method that can simultaneously distinguish MTB and NTM, thus rendering to detect and treat the increasing incidence of infections caused by NTM.

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