

## Diagnostic Evaluation Of Gene Xpert Mycobacterium Tuberculosis Assay For The Detection Of Rifampicin Resistant Mycobacterium Tuberculosis Among Pulmonary Patients Of South Zone Of Odisha



### Microbiology

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### ABSTRACT

*Tuberculosis remains a major global health problem. It ranks as the second leading cause of death from an infectious disease worldwide. Our aim was to evaluate the performance of GeneXpert system for the detection of Mycobacterium tuberculosis with its Rifampicin resistance within shortest possible time. Our assay can simultaneously confirm the presence of M. Tuberculosis and detect the mutations associated with resistance to anti-TB drugs, there by diagnosing M/XDR-TB with good sensitivity and specificity.*

### Introduction:

Tuberculosis remains a major global health problem. It ranks as the second leading cause of death from an infectious disease worldwide. The latest estimates included that there were 8.6 million new Tuberculosis cases in 2013 and 1.5 million Tuberculosis deaths.<sup>1</sup> Globally in 2012, 3.6% of new Tuberculosis cases and 20.2% of previously treated cases were estimated to have MDR-TB; furthermore, there were approximately 170,000 deaths from MDR-TB; however, extensively drug-resistant Tuberculosis (XDR-TB) has been reported by 105 countries in 2014.<sup>2</sup> There is growing concern that the increase in the global prevalence of multidrug- and extensively drug-resistant tuberculosis (M/XDR-TB), as well as the emergence of what is being called "totally drug-resistant TB",<sup>3-5</sup> may compromise the recent successes seen by global TB control efforts.<sup>6</sup> Conventional diagnostic method for Mycobacterium tuberculosis are slow and/or lack sensitivity. A number of new diagnostic approaches have brought incremental improvements to detection and drug susceptibility testing; however, the technical complexity of these assays and their dependence on dedicated laboratory infrastructure have limited their adoption, especially in low-resource, high-burden settings.<sup>7,8,9,10</sup> The recently introduced Xpert MTB/RIF (manufactured and marketed by Cepheid, Sunnyvale, CA) assay simultaneously detects the presence of M. tuberculosis and its susceptibility to the important first-line drug rifampin (RIF).<sup>11</sup> A sample processing system and an automated heminested real-time PCR assay are integrated into a single disposable cartridge. The assay can be performed directly from a clinical sputum sample or from a decontaminated sputum pellet and can generally be completed in less than 2 hrs.<sup>11</sup>

The Xpert MTB/RIF assay detects M. tuberculosis and RIF resistance by PCR amplification of the rifampin resistance-determining region (RRDR) of the M. tuberculosis rpoB gene and subsequent probing of this region for mutations that are associated with RIF resistance. Approximately 95% of RIF resistant tuberculosis cases contain mutations in this 81-bp region.<sup>12</sup> Our previous work has established that the Xpert MTB/RIF assay has a limit of detection (LOD), defined as the minimum number of bacilli that can be detected with 95% confidence) of 131 CFU per ml of clinical sputum.<sup>11</sup> The assay was also able to identify RIF resistance in samples containing 23 common clinically occurring rpoB mutations. None of the 20 nontuberculosis mycobacteria (NTM) species tested, including the NTM species commonly described as causing human disease were falsely identified as M. Tuberculosis,<sup>11</sup> suggesting high specificity. Sever-

al small studies using clinical samples demonstrated 98% to 100% sensitivity overall, 72% sensitivity in smear-negative patients, and a specificity of 100%.<sup>11</sup> Our aim was to evaluate the performance of GeneXpert system for the detection of Mycobacterium tuberculosis with its Rifampicin resistance within shortest possible time.

### Material and methods:

The present study was conducted in the departments of Department of Microbiology, Hi-Tech medical college and hospital, Bhubaneswar during the period from November 2010 to April 2011. Suspected cases of MDR-TB patients who were attended in the OPD and IPD of HMCH were selected as study population. Patients were excluded who were undergoing treatment or having extra-pulmonary tuberculosis or were new pulmonary tuberculosis cases. Fresh sputum were collected from suspected multidrug resistant pulmonary tuberculosis (MDR-TB) patients with all aseptic precaution and sputum samples were digested and decontaminated by N-acetyl-L-Cysteine-Sodium Hydroxide (NALC-NaOH) method.<sup>13</sup> The sediment of processed sputum was used for microscopic examination by Ziehl-Neelsen (Z-N) staining and by auramine staining, culture and drug susceptibility test (DST) on MGIT 960 media.<sup>13</sup> Liquid culture and Drug Susceptibility Test (DST) were performed in BACTEC MGIT 986 Media.<sup>14</sup> MGIT growth supplement/PANTA was aseptically added to the appropriately labeled MGIT tube and then well mixed concentrated specimen was added to each MGIT tube. Inoculated tubes were placed on a rack and were carried to BACTEC MIGT 986 System for loading on the same day.<sup>15</sup> The instrument was monitored for the entered susceptibility test set. The susceptibility Set Carrier was scanned and the report was printed. The instrument printout indicated susceptibility results for each drug. GeneXpert MTB/RIF Assay (GXMTB/RIF-10) was performed. The primers in the Xpert MTB/RIF Assay amplify a portion of the rpoB gene containing the 81 base pair "core" region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with Rifampicin resistance. Each Xpert MTB/RIF cartridge was labeled with the sample ID. The sample was then transferred into the sample chamber of the labeled Xpert MTB/RIF cartridge and lid was closed firmly. The barcode on the Xpert MTB/RIF cartridge was scanned. The instrument module door opened with the blinking green light and the cartridge was loaded. The results were interpreted by the GeneXpert DX System from measured fluorescent signals and embedded calculation algorithms and displayed in the "View Results" window. Lower Ct values represent a higher starting con-

centration of DNA template; higher Ct values represent a lower concentration of DNA template. Before collecting specimens, informed written consent was taken from patients. The study protocol was approved by the ethical committee of Hi-Tech medical college and hospital, Bhubaneswar.

### Results and Discussion:

The concentration of *M. tuberculosis* bacteria in clinical sputum samples can vary from over 107 to less than 20 CFU/ml.<sup>16</sup> Sputum samples were collected from 125 clinically suspected multidrug resistant pulmonary tuberculosis (MDR-TB) patients. Liquid culture had yielded the highest growth of *Mycobacterium tuberculosis* which was 84 (67.2%) cases. GeneXpert MTB assay showed 65.6% positive and 34.4% negative (Table-1).

**Table-1: Comparison of results of liquid culture and GeneXpert MTB assay:**

GeneXpert	Culture (n = 125)		Total
	Positive	Negative	
Positive	82	0	82
Negative	2	41	43
Total	84	41	125

**Table-2: Performance of GeneXpert MTB/RIF Assay for detection of M. Tuberculosis:**

Variables	Values
Sensitivity	97.12%
Specificity	100%
Negative Predictive Value (NPV)	95.96%
Positive Predictive Value (PPV)	100%
Accuracy	99.7%

**Table 3. Detection of rifampicin resistant *M. tuberculosis* by GeneXpert MTB/RIF assay and Drug Susceptibility Test (DST) on liquid media (MGIT 960) culture:**

Method	RIF sensitive	RIF resistant
GeneXpert MTB/RIF	65	17
Drug Susceptibility Test	65	19

All samples positive by GeneXpert were also positive by liquid culture and additionally one case was positive by liquid culture (Table-1). Table-2 shows the performance of GeneXpert MTB/RIF assay when compared to liquid culture on MGIT 986 media for detection of *M. tuberculosis*. Table-3 compares the rifampicin resistance detection by GeneXpert MTB/RIF and liquid culture on MGIT 986 media. GeneXpert MTB/RIF detected 17 (20.7%) cases of *Mycobacterium tuberculosis* which were rifampicin resistant among 82 *M. tuberculosis* positive samples. On the other hand liquid (MGIT 986) culture detected 19 (22.6%) out of 84 *M. tuberculosis* positive samples. An alarming increase in the global incidence of drug-resistance *Mycobacterium tuberculosis* infection has created a critical need for methods that can rapidly detect *Mycobacterium tuberculosis* and identify drug-resistant cases. Failure to quickly and effectively recognize and treat patients with drug-resistant tuberculosis, particularly multidrug resistant (MDR) and extensively drug-resistant (XDR) tuberculosis, leads to increased mortality, nosocomial outbreaks, economic burden and resistance to antitubercular drugs. In this study, 19

(22.6%) out of 84 MGIT positive samples and 17 (20.7%) out of 82 GeneXpert MTB positive samples were shown to be Rifampicin (RIF) resistant. So there was one phenotypic (DST on MGIT 986) and genotypic (GeneXpert assay) discordance—liquid culture detected one more sample as MTB positive which was Rifampicin resistant on culture-based drug susceptibility testing (DST). Ocheretina et al.<sup>17</sup> reported the phenotypic and genotypic characterization of 153 consecutive clinical *Mycobacterium tuberculosis* strains diagnosed as RIF-resistant by molecular tests in Port-au-Prince, Haiti. 133 (86.9%) isolates were resistant to both RIF and Isoniazid and 4 (2.6%) isolates were RIF mono-resistant in MGIT SIRE liquid culture-based DST. However the remaining 16 isolates (10.5%) were RIF-sensitive by the assay. According to CGXMTB/RIF-10 manual, 2009, GeneXpert MTB detected Rifampicin resistance from 16.86% and liquid culture detected from 17.44% sputum samples. The Xpert itself contains amplicons within the sealed cartridge, reducing or eliminating the need for the precautions typically associated with other nucleic acid amplification tests.

### Conclusion:

These findings suggest that the GeneXpert MTB/RIF is a rapid and highly dependable technique for identification of *M. tuberculosis* and rifampicin resistance from clinical sputum sample. The results are obtained within 2 hours with GeneXpert MTB/RIF assay. GeneXpert MTB/RIF assay should be used routinely for detection of *M. tuberculosis* and Rifampicin resistant *M. tuberculosis* from sputum sample of clinically suspected MDR patients where facilities are available. Our assay can simultaneously confirm the presence of *M. Tuberculosis* and detect the mutations associated with resistance to anti-TB drugs, thereby diagnosing M/XDR-TB with good sensitivity and specificity.

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