Original Article

CBNAAT: a Novel Diagnostic Tool For Rapid And Specific Detection Of Mycobacterium Tuberculosis In Pulmonary Samples

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Abstract : Mycobacterium tuberculosis remains to be one of the most significant causes of death from an infectious agent. Rapid diagnosis of tuberculosis and detection of Rifampicin (RIF) resistance are essential for effective disease management. CBNAAT (Cartridge Based Nucliec Acid Amplification Test) also known as Gene Xpert MTB/RIF assay is a novel integrated diagnostic device for the diagnosis of tuberculosis and rapid detection of RIF resistance in clinical specimens. We determined the performance of the MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in smear-positive and smear-negative pulmonary specimens obtained from possible tuberculosis patients. Aim of the study - To assess diagnostic usefulness of Gene Xpert MTB/RIF assay technique in management of tuberculosis. Material and Methods : This is an Observational study conducted in the department of pulmonary medicine Maharajah's Institute of Medical Sciences, Vizianagaram between June 2012 and December 2013. Two hundred and five Sputum samples were obtained from Tuberculosis suspects. All samples were tested on Gene Xpert for MTB/RIF detection after AFB microscopy. Results : 108(52.68%) sputum samples were AFB smear positive and 96 (47.32%) were negative. In MTB/RIF assay 144 (70.24%) were MTB positive and 61 (29.76%) were negative. Chi-Square test was applied; P value is <0.001. All results are highly significant. The MTB/RIF assay also detected 4 RIF-resistant specimen and 140 RIF-susceptible specimens, and the results were confirmed by drug susceptibility testing. Conclusions: The MTB/RIF test is a simple method, and routine staff with minimal training can use the system. It helps to avoid injudicious use of anti-tuberculosis drug.

Key Words : AFB, Gene Xpert, MTB/RIF, TB and ZN staining.

Introduction

The global burden of TB remains enormous. More than 9 million new M tuberculosis (MTB) cases and 1.7 million deaths occur annually worldwide. Most of them occur in resource-limited settings.[1] Smear microscopy for acid-fast bacilli (AFB) is rapid and inexpensive.

Smear microscopy is the cornerstone for the diagnosis of TB in resource-limited settings but it has only modest (35-80%) sensitivity and a poor positive predictive value (PPV)[2]. Culture is the "gold standard" for final determination, and also permits drug susceptibility testing. However, it remains largely inaccessible in resource-limited settings as a result of infrastructure and financial limitations. Even where accessible, culture results are typically not available for 2-6 weeks. Diagnosis through either smear or culture requires multiple steps that significantly impede program effectiveness. The need is for accurate, feasible, rapid, affordable, and if possible, near-point-of-case TB diagnostic tests for use in resource-limited settings.

Drug resistance is a major issue in the treatment of tuberculosis. Though rare for Rifampicin (RIF) , drug resistance is common in other 1st line drugs Isoniazid (INH), Ethambutol (EMB), and Pyrazinamide (PZA).[2] Multi drug resistance is a reflection of either mismanagement of tuberculous patients wrong diagnosis, delay in diagnosis, wrong or interrupted treatment and mistreatment of both first and second line drugs. Injudicious use of drugs is to be avoided in the better interest of patients[3]. Thus, for rapid identification, which is essential for earlier initiation of treatment and improved outcomes, more effective public health interventions and newer methods of detection are required[4]. Multiple approaches to improve diagnosis of TB are in development. One test, Gene Xpert® MTB/RIF, which was recently endorsed by the World Health Organization (WHO), has the potential to lead a revolution in the diagnosis of active TB disease and multidrug-resistant (MDR) TB.

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Gene Xpert test is a semi-quantitative nested real-time PCR in-vitro diagnostic test with two uses:

(1) The detection of Mycobacterium tuberculosis complex DNA in sputum samples or concentrated sediments prepared from induced or expectorated sputum that are either acid-fast bacilli (AFB) smear positive or negative.

(2) The detection of Rifampicin resistance associated mutations of the rpoB gene in samples from patients of Rifampicin resistance[5, 6].

Among the most important diagnostic techniques the Gene Xpert is one which detects gene mutation (rpoB) associated with RIF resistance. Hence it is of enormous importance in the diagnosis of both drug susceptible and drug resistant cases. Gene Xpert test can be performed on sputum or bronchial washing samples. Results become available in less than 2 hours. The rapid detection of Mycobacterium tuberculosis and its resistance to Rifampicin (RIF's) allows the physician to make critical decisions in the management of patient regarding therapy during the same visit.

The aim of this study is to determine the diagnostic usefulness of the MTB/RIF assay for the diagnosis of tuberculosis and rapid detection of rifampicin resistance in smear-positive and smear-negative pulmonary clinical specimens.

Materials and Methods : This is an observational study conducted in the department of Pulmonary medicine, Maharajah's Institute of Medical Sciences during June 2012-December 2013. CBNAAT, an automated instrument (figure 1) which works on the principle i.e., sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and reverse transcriptase PCR.
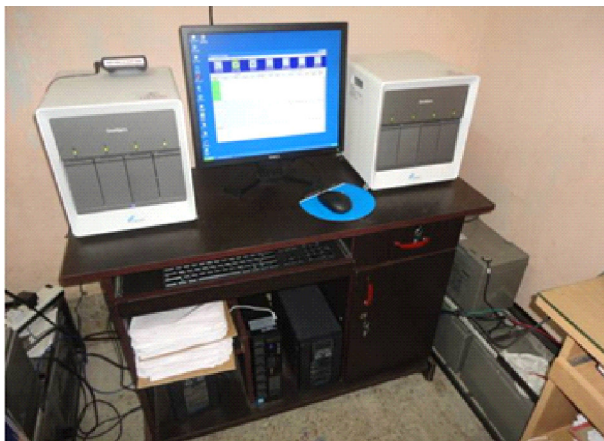

Figure 1 : CBNAAT instrument

205 Patients who presented with symptoms and signs suggestive of pulmonary tuberculosis, chest X ray showing features of pulmonary tuberculosis were included in the study. Sputum samples from these patients were sent for AFB staining as well as Xpert MTB/RIF test. Early morning, deep coughed sputum specimens in sterile containers were included in the study. Specimens were stored at 2-8°C in freezer till further processing. However, the specimen can be safely stored at 35°C for three days. After collection, Ziehl-Neelsen (ZN) staining was done on all samples in department of Microbiology and each sample was run on Gene Xpert.

Standard Assay Procedure of Gene Xpert: The assay utilizes single-use plastic cartridges with multiple chambers that are preloaded with liquid buffers and lyophilized reagent beads necessary for sample processing, DNA extraction and heminested rt-PCR.[7,8]Clinical sputum samples (or decontaminated sputum pellets) are treated with sodium hydroxide and isopropanol-containing sample reagent (SR). The SR is added to the sample (currently recommended at a 3:1 ratio for sputum pellets and a 2:1 ratio for unprocessed sputum samples) and incubated at room temperature for 15 min. The treated sample is then manually transferred to the cartridge which is loaded into the Gene Xpert instrument.

Subsequent processing is fully automated. The cartridge incorporates a syringe drive, a rotary drive and a filter upon which M. tuberculosis bacilli are deposited after being liberated from the clinical material. The test platform employs a sonic horn that inserts into the cartridge base to cause ultrasonic lysis of the bacilli and release of the genetic material. The assay then amplifies a 192 bp segment of the rpoB gene using a hemi-nested rt-PCR reaction[8,9]. Mycobacterium tuberculosis is detected by the five overlapping molecular probes (probes A–E) that collectively are complementary to the entire 81 bp rpoB core region[8, 9].

M. tuberculosis is identified when at least two of the five probes give positive signals with a cycle threshold (CT) of d"38 cycles and that differ by no more than a prespecified number of cycles. The basis for detection of rifampicin resistance is the difference between the first (early CT) and the last (late CT) M. tuberculosis-specific beacon (ÄCT). The system was originally configured such that resistance was reported when ÄCT was >3.5 cycles and sensitive if d"3.5 cycles.

Results

ZN staining was done for 205 samples of the patients who were having history suggestive of pulmonary tuberculosis. Out of these 108(52.68%) sputum samples were AFB smear positive and 96(47.32%) were negative. (Table 1) Then all the samples were tested on Gene Xpert ® MTB/RIF assay. Out of the 205 sputum samples of the patients having history suggestive of pulmonary tuberculosis, 144 (70.24%) were MTB positive and 61 (29.76%) were negative. The MTB/RIF test detected the agent in 108 out of 109 sputum smear positive cases, and 36 out of 96 sputum smear negative cases.

Table 1.

Gene Xpert	Sputum for AFB+Ve	Sputum for AFB-Ve	Total samples
MTB +ve	108	36	144
MTB - ve	1	60	61
Total samples	109	96	205

The results of Gene Xpert and ZN staining are compared in our study. It is evident from the table that Gene Xpert MTB/RIF is more useful than ZN staining. As compared to ZN staining it can detect MTB even in 1ml of sputum.

The second important advantage of Gene Xpert is that it also detects Rifampicin (RIF) resistance and helps us to diagnose multi-drug resistance tuberculosis (MDR TB). In table2. 4 patients were rifampicin resistant out of 205 (1.9%) suspected cases, which was confirmed with drug susceptibility.

Table 2.

RIF Resistance	MTB+Ve	MTB-Ve	Total samples
NOT DETECTED	105	96	201
DETECTED	4	0	4
Total samples	109	96	

Statistical Analysis : All results were analyzed statistically by applying chi-square test. P value was <0.001 and all the results are highly significant

Discussion

In this study, the performance of the MTB/RIF assay with pulmonary specimens obtained during the clinical routine was compared with AFB staining. In our study, the MTB/RIF test detected the agent in 144 out of 205 pulmonary specimens (70.24 % detection rate) whereas sputum for AFB was able to detect only 108 out of 205 pulmonary specimens (52.68% detection rate). Out of 109 sputum smear positive cases, tuberculosis was detected in 108 cases, by MTB/RIF test and out of 96 sputum smear negative cases 36 cases were detected.

A previous study found that the MTB/RIF assay had a calculated limit of detection of 131colony-forming units(CFU)/ml of sputum and was able to detect as few as 10 CFU/ml of sputum in 35% of samples compared with approximately 10,000 CFU/ml with conventional smear microscopy[10].

The MTB/RIF test is easy to perform and is less dependent on the user's skills. Routine staff with minimal training can use the test. Technicians can be trained in 1-2 days; Only 2 steps (addition of buffer and sputum sample) are manual and the rest of the steps are automated,The results are available within 90 minutes. Each table top-sized module can process 4 samples daily (larger modules can run 200 tests in an 8-hour day), and because it is a closed system, biosafety and contamination concerns are minimized. It has a short turn-around time and simultaneously detects M. tuberculosis and RIF resistance in less than 2 hours. Although the MTB/RIF test could be a useful tool for rapid identification of RIF-resistant M. tuberculosis, especially in smear-positive clinical samples, the test results must always be confirmed by culture and DST.

Conclusion

We concluded that as compared to sputum AFB microscopy, Gene Xpert is more sensitive and specific not only for acid fast bacilli (AFB) detection but also for rifampicin (RIF) resistance. Routine staff with minimal training can use this system. It also helps to avoid injudicious use of anti-tuberculosis drugs.

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