COMPARISON OF FASTSURE TB DNA AND MGIT 960 FOR THE DETECTION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX IN CLINICAL SPECIMENS

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ABSTRACT

Objective: To compare the efficacy of Fastsure TB DNA with fully automated MGIT 960 method for detection of *Mycobacterium tuberculosis* complex (MTB) in clinical specimens.

Study Design: Comparative cross sectional study.

Methodology: After decontamination procedure, the clinical specimens were subjected to DNA extraction and amplification. Extracted DNA was separated in a separate tube provided with fastsure TB DNA kit and was then inserted into the cartridge provided and results were observed within 30 minutes. For Processing in MGIT 960, OADC and PANTA were added to the clinical specimens after decontamination and then the tubes were processed in MGIT 960.

Results: A total of 80 specimens were tested by both MGIT 960 and fastsure TB DNA. On MGIT 960 system, 57 specimens showed growth of MTB while 23 were negative. On Fastsure TB DNA, 47 Specimens were tested as positive and 33 specimens showed negative result. Sensitivity and specificity of Fastsure TB DNA method was calculated to be 82.45 % and 100 % respectively, while positive and negative predictive values were 100 % and 69.69 % respectively.

Conclusion: Fast sure TB DNA is a rapid and accurate method for the detection of *Mycobacterium tuberculosis* complex (MTB) from clinical specimens.

Keywords: *Mycobacterium tuberculosis* complex (MTB), Fastsure TB DNA.

INTRODUCTION

Tuberculosis(TB) is one of the most deadly infectious diseases of the human beings. Although it is more common in the underdeveloped countries but it still remains a serious health concern for the developed countries of the world due to Acquired Immune Defeciency Virus epidemic. Presently one third of the world's population is infected with *Mycobacterium tuberculosis* (MTB)[1]. In Pakistan incidence of Tuberculosis is 181 per 100000 population per year[2]. Adding fuel to the fire is the emergence of MDR(Multi drug resistant)TB, which has reached an alarming level in our population[3]. This demands not only appropriate treatment with second line ATT drugs but also techniques for the early, accurate and reliable diagnosis[4]. In developing countries lack of diagnostic facilities is the main hurdle in the diagnosis of TB, and many patients in these countries are treated on mere presumptive clinical diagnosis. In the centres where these facilities are available direct microscopy and culture on Lowenstein Jensen (LJ) medium are still considered as gold standard for the diagnosis of TB, but these methods have low sensitivity (direct microscopy) and take longer time (3-4 weeks) for the culture of MTB. In the past few years a lot of research has been carried out to explore new diagnostic modalities for the early and accurate detection of MTB. BACTEC MGIT 960 system is fully automated, broth based culture technique which is now being used as a rapid diagnostic system for tuberculosis in many developed countries[5]. This system though fully automated and easy to use has mean detection time of about 11.2 days in smear positive cases and 14.2 days in smear negative specimens[6].

Fastsure TB DNA is a rapid test recently launched by MP Biomedicals. It is an accurate, rapid and cross contamination proof nucleic acid detection kit which is intended for the qualitative detection of *Mycobacterium tuberculosis*(TB) DNA in different human clinical specimens. FASTSURE TB DNA Rapid Test includes sample preparation, nucleic acid isothermal amplification and hybridization, and detection using a patent cross-contamination proof device. All these steps are completed within 3hrs. This study was carried out with

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objective to compare the efficacy of Fastsure TB DNA with MGIT 960 for the detection of MTB in clinical specimens. This is the first study of its kind to be conducted in Pakistan.

METHODOLOGY

This comparative cross sectional study was conducted at the Microbiology Department, Armed Forces Institute of Pathology Rawalpindi, during July and August 2011. A total of 80 specimens were selected. Out of these 80 specimens 28 were smear positive (Ziehl-Neelsen staining) whereas 52 were smear negative. To reduce the chances of contamination from the resident and transient flora, decontamination procedure of the specimens was performed by Digestion and Decontamination technique. These specimens thereafter were subjected to DNA extraction and amplification. One ml of decontaminated specimen was transferred to an appendorf tube and was than centrifuged at 10,000 rpm for 10 mins. The supernatent was discarded and pellet was given two washes with 1 ml of normal saline by centrifugation at 10,000 rpm for 10 min. The supernatant was discarded and pellet was kept for next step. Thereafter 40 µl of DNA extract solution was added to the tube containing this pellet. This mixture was incubated at 95ºC to 100ºC for 10 min and then cooled at room temp for 5 min. The mixture was centriguged at 10,000 rpm for 5 min and the supernatant was used for amplification. For amplification, 15 µl. Resuspension buffer was added to the tube containg the reaction mixture and incubated at room temp for 2-3 min. To the reaction mixture tube 20 µl of mineral oil was added and centrifuged at 4,000 rpm for 3-5 sec. The mixture was than incubated for 60 min at 63^0C. Extracted DNA was separated in a separate tube provided with fastsure TB DNA kit and was then inserted into the cartridge provided. The results were observed after 30 mins. The formation of two lines indicated positive result while single line indicated negative result.

RESULTS

A total of 80 specimens were selected for the study. The Patients were between the ages of 2 to 80 years with the mean age of 39.5±21.46 years. Fifty five percent (n= 44) specimens were from male patients and 45% (n=36) specimens were from female patients. The patients were between the ages of 2 to 80 years with the mean age of 39.05±21.46 years. Fifty seven specimens showed positive results on MGIT 960, while 23 were negative. Out of 80 clinical specimens tested by Fastsure TB DNA, 58.75 % (n= 47) showed positive results whereas 41.25 % (n=33) showed negative results (Table:1). Sensitivity measures are given in table 2.

DISCUSSION

Progress towards global TB control has remained elusive despite intensified standard measures of TB control[7]. National TB control programmes in most TB endemic countries continue to rely mainly on old, traditional and inaccurate methods such as direct smear microscopy, solid agar culture, chest radiography and tuberculin skin testing[8]. Currently, there is no rapid, accurate and reliable test that allows early detection of active TB at the peripheral health care settings. Thus many patients with active TB, especially in TB endemic areas are either treated on clinical

Table-1: Description of tests (n=80)

FASTSURE TB DNA

Fastsure TB DNA	**MGIT 960**	
	Positive	Negative
Positive	47(TP)	0(FP)
Negative	10(FN)	23(TN)

Table-2: Sensitivity measures of fast sure Tb DNA

Sensitivity Measures	
Sentivity	82.45%
Specificity	100%
Positive Predicative Value	100%
Negative Predicative Value	69.69%
Accuracy	87.5%

grounds and without microbiological proof of TB-infection or remain undiagnosed and are continuing to spread the disease in the community[9]. The need for a more rapid, accurate, point-of-care TB diagnostic test that is applicable in TB endemic areas is crucial for achieving global TB control. Model studies have shown that new diagnostic modalities for TB and (MDR-TB) may have an important impact at the population level in disease endemic countries[10]. Over the past decade the TB diagnostic modalities have expanded, with several technologies showing promise[8]. Simplified PCR-based techniques are some of the methods which have been shown to detect *Mycobacterium tuberculosis* and resistance to rifampicin with good sensitivity and specificity directly from positive cultures or clinical specimens[11]. Recently, several nucleic acid amplification technology (NAAT) tests have also been developed that rapidly detect M. tuberculosis DNA in patient samples. Fastsure TB DNA is one of the nucleic acid detection methods. It is a rapid test recently launched by MP Biomedicals. It is an accurate, rapid and cross contamination proof nucleic acid detection kit which is intended for the qualitative detection of MTB DNA in different human clinical specimens. The whole test starting from receipt of clinical specimen till the final result is achieveable in 3-4 hours. The test also does not require special instruments and can easily be used in smaller laboratories and any technician can be easily trained to perform this test. The study has revealed a sensitivity of about 82% and excellent specificity and positive predictive value of 100 % each. The test is very specific for MTB and will not detect Mycobacterium other than tuberculosis (MOT).

We conducted this study to evaluate the performance of Fastsure TB DNA kit. There was no published study found in the literature about the use of this kit, however many studies have been done previously based on nucleic acid detection techniques. The results of our study indicate that this kit is much superior to direct microscopy as about 60 % of all the smear negative specimens were tested positive by this kit. Culture continues to be the Gold Standard in the diagnosis of *Mycobacterium tuberculosis* but this technique can be a very useful adjuvant to microscopy, especially when the critical decision of starting antituberculosis treatment has to be taken.

One of the limitation of our study was that NAAT assays were performed on frozen aliquots, while smear microscopy and MGIT culture are performed on fresh samples. This may have impaired MTB detection, and reduced the sensitivity of the NAATs in comparison to culture. Studies at more centers are required to further evaluate the effectiveness of this kit, so that it can be recommended for use in our set up.

CONCLUSION

FASTSURE DNA Rapid Test is an accurate, simple, rapid and cost effective nucleic acid detection kit, which can be used for the early detection of Mycobacterium tuberculosis complex (MTB) in different clinical specimens without requirement of any specialized equipment.

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