

Original Research Article

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GeneXpert MTB/RIF Assay: A Revolutionizing Method for Rapid Molecular Detection of Mycobacterium Tuberculosis in Comparison to Other Conventional Methods

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ABSTRACT

Tuberculosis (TB) remains the leading cause of morbidity and mortality worldwide that is caused by Mycobacterium tuberculosis (MTB). Conventional cultures are highly sensitive but time-consuming, whereas Ziehl-Neelsen (ZN) stain is a rapid method with high specificity but has lower sensitivity. The GeneXpert MTB/RIF assay represents a great breakthrough in rapid diagnosis of TB which is not only important in treatment but also has a vital role to prevent its transmission in the community. The aim of this study is to throw a light on the importance of GeneXpert assay as a rapid and reliable test for the diagnosis of TB compared to conventional MGIT960 culture and ZN microscopy. A total of 380 sputum samples and 55 extrapulmonary samples (37 pleural fluids, 11 CSF, 3 peritoneal fluids, 2 pus and 2 synovial fluids) were taken from 435 patients clinically suspected to have TB. GeneXpert, MGIT960 liquid culture and ZN stain were done for all samples. Out of 435 cases, 41 cases (9.4%) were positive for TB by bacteriological, clinical, radiological and/or pathological evidence. From 41 positive TB cases 40, 35, and 30 cases were positive by GeneXpert assay, MGIT960 and ZN stain respectively. The overall sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of GeneXpert were 97.6%, 100 %, 99.7 % and 100% respectively. On the other hand, the sensitivity, specificity, NPV and PPV of MGIT960 culture were 85.4%, 99.4%, 98.5% and 94.6%, while for ZN stain were 73.2%, 99.4%, 99.5% and 93.8% respectively. GeneXpert is a simple, rapid, highly sensitive (97.6 %) and specific (100%) method that greatly speeds up the detection of MTB compared to conventional MGIT960 culture and ZN stain.

Keywords

Tuberculosis,
GeneXpert
MTB/RIF,
MGIT960 culture,
Ziehl-Neelsen stain.

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Introduction

Mycobacterium tuberculosis (MTB) is the causative agent of Tuberculosis (TB) which remains the leading cause of morbidity and mortality worldwide. It is airborne disease that primary infects the lungs (pulmonary TB) (Hosne *et al.*, 2016).

According to WHO report in 2015, there were 10.4 million new TB cases worldwide and 1.8 million TB deaths. Patients with HIV account for 1.2 million of new TB cases and 0.4 million of TB deaths. Most of these new cases were found in India, Indonesia, China,

Nigeria, Pakistan and South Africa (WHO, 2016).

The GeneXpert MTB/RIF assay (Cepheid, Inc., Sunnyvale, CA, USA) represents a great breakthrough in the rapid diagnosis of TB which is essential for both early disease treatment and infection control. It detects both *Mycobacterium tuberculosis* complex (MTBC) and rifampicin (RIF) resistance by PCR amplification of the 81-bp fragment of the MTB *rpoB* gene in 2 hours (Arzu *et al.*, 2011). This gene is known to be specific for TB diagnosis and its mutation is associated with resistance to RIF. The assay not only provides the advantage of rapid diagnosis within 2 hours but also detects even low numbers of MTB in various specimens (Maynard-Smith *et al.*, 2014).

Conventional cultures are highly sensitive but time-consuming, whereas ZN stain is a rapid method with high specificity but has lower sensitivity (Nakwon *et al.*, 2013).

Aim of the work

The aim of this study is to throw a light on the importance of GeneXpert assay as a rapid and reliable test for the diagnosis of TB compared to conventional MGIT960 culture and ZN microscopy.

Materials and Methods

This study was conducted in Microbiology Laboratory, King Khalid Hospital, Hail, KSA between January 2016 and February 2017. A total of 380 sputum samples and 55 extrapulmonary samples (37 pleural fluids, 11 CSF, 3 peritoneal fluids, 2 pus and 2 synovial fluids) were taken from 435 patients clinically suspected to have TB.

Nonsterile clinical specimens were processed by the conventional N-acetyl-L-cysteine-

NaOH method for digestion, decontamination and concentration (Shafiq *et al.*, 2016).

Processed samples were divided into three parts; one part was immediately tested using GeneXpert, second part used for ZN smear microscopy and the third part for MGIT960 liquid culture.

AFB smears

All the smears were stained by the ZN method and examined microscopically (Lombardi *et al.*, 2017).

MGIT960 [Becton Dickinson, Sparks, MD, USA]

MGIT960 is a non-radiometric automated isolation system for recovery of MTB. The MGIT tube contains 7 ml of 7H9 medium and supplemented with 0.8 ml of Oleic Acid-Albumin-Dextrose-Catalase [OADC] along with PANTA [Polymyxin B-Amphotericin B-Nalidixic acid -Trimethoprim - Azlocillin] (Siddiqi *et al.*, 2006). To this tube, 0.5 ml of decontaminated sample was added. If there was no growth of MTB after 6 weeks, the culture was considered negative. When the tubes were flagged positive, ZN staining and culture on 5% sheep blood agar were performed from the tube directly to rule out any contamination as per the manufacturer's instructions (Hosne *et al.*, 2016).

GeneXpert assay method

GeneXpert MTB/RIF assay can detect MTBC and associated RIF resistance directly from clinical samples using ultrasensitive nested real time PCR (Vadwai *et al.*, 2011). The assay integrates sample processing and PCR in a single-use plastic cartridge containing all reagents required for bacterial lysis, nucleic acid extraction, amplification and amplicon detection (Evans, 2011).

GeneXpert testing was performed according to the manufacturer's instructions. Decontaminated samples were added to sample reagent with 1:3 dilutions, manually mixed two times and kept for 15 min at room temperature. Two ml of the processed material was transferred to the test cartridge and loaded into the test platform of the machine (Tang *et al.*, 2017).

Mycobacterium tuberculosis complex is detected by five overlapping probes that collectively are complementary to the entire 81 bp *rpoB* core region of RIF-susceptible MTB (wild type) and are labeled with a differently colored fluorophore (Lawn and Nicol, 2011). Positive results were reported when at least two of the five probes give positive signals with a cycle threshold (CT) of ≤ 38 cycles. Also, a semi-quantitative estimate of positive samples depending on CT range was given (high, <16 ; medium, 16–22; low, 22–28; very low, >28). Each test includes a Sample Processing Control (SPC) and Probe Check Control (PCC). The SPC monitor adequate processing of the samples and the presence of inhibitors in the PCR reaction. The PCC verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability (Blakemore *et al.*, 2011).

Results and Discussion

Out of 435 cases, 41 cases (9.4 %) were positive for TB by bacteriological, clinical, radiological and/or pathological evidence. The upper hand for MTB detection was demonstrated by GeneXpert assay (40/41; 39 sputum and on pus samples), followed by MGIT960 (35/41) and ZN stain (30/41). Only one sample was reported negative by the GeneXpert assay while its MGIT960 culture result was positive, in addition to the presence of clinical and radiological evidence of TB. Also, there were 6 and 11 negative samples by MGIT culture and ZN stain respectively,

but they were positive by the GeneXpert. All these data are shown in table 1 and figure 1.

In table 2, all high and medium positive results by GeneXpert MTB/RIF assay were positive by both MGIT960 culture and ZN stain, whereas all very low positive cases by GeneXpert MTB/RIF assay were negative by MGIT960 culture and ZN stain. Also, all low positive cases by GeneXpert MTB/RIF assay were positive by MGIT960 culture and negative by ZN stain.

Regarding table 3, among the 435 specimens, 30 samples were positive by all three methods, 4 by GeneXpert and MGIT, 6 by GeneXpert only and one by MGIT only.

Of 395 negative cases by GeneXpert assay, 3 and 2 cases were positive by MGIT and ZN stain respectively. The two positive cases by MGIT culture and ZN stain were identified as *Mycobacterium Other Than Tuberculosis* (MOTT). The third positive one by MGIT and negative by ZN stain was MTB by clinical, radiological and pathological evidence.

In order to start appropriate treatment, rapid diagnosis of TB is very important. Turnaround time of GeneXpert assay to detect MTBC is only 2 hours compared to 15 ± 5 days and 2 hours for MGIT culture and ZN stain respectively (Table 4).

By using a combination of bacterial, clinical, radiological and/or histopathological evidence of TB as the reference standard, the sensitivity, specificity, negative and positive predictive values for GeneXpert assay were 97.6, 100%, 99.7% and 100% respectively. Also, the sensitivity, specificity, negative and positive predictive values of MGIT culture were 85.4%, 99.4%, 98.5% and 94.6% and for ZN stain were 73.2%, 99.4%, 99.5% and 93.8% respectively (Table 5). The rapid detection of MTB is essential for both early disease management and infection control.

The GeneXpert MTB/RIF assay is a cartridge-based, automated and rapid molecular assay that performs rapid TB diagnosis within 2 hours. In this study, our aim is to throw a light on the importance of GeneXpert MTB/RIF assay as a rapid and reliable test for the diagnosis of TB compared to conventional methods.

Out of 435 cases included in our study, 41 cases (9.4 %) were positive for TB by bacteriological, clinical, radiological and/or pathological evidence. Higher percentage of TB around 25% was demonstrated by Nakwon *et al.*, (2013), Arzu *et al.*, (2011) and Agrawal *et al.*, (2016). In India, Raj *et al.*, (2012) and Sajed *et al.*, (2014), found that positive cases represent 51.7% (out of 547

suspected patient) and 37% (out of 100 suspected patients) respectively.

This high percentage of TB in these studies compared to our study could be explained by the fact that South Korea and Turkey were considered as countries with intermediate burden of TB (Kim *et al.*, 2012), while the world's largest burden of TB was found in India (Agrawal *et al.*, 2016).

The highest detection of MTB was demonstrated by GeneXpert assay (40/41; 39 sputum and on pus samples), followed by MGIT960 (35/41) and ZN stain (30/41). Similar results were demonstrated by Agrawal *et al.*, 2016, Bajrami *et al.*, 2016) and Tang *et al.*, 2017.

Table.1 Number of positive cases by different methods in relation to all studied cases and positive TB cases

Methods	Total number of studied cases (n=435)		Total number of positive cases (n=41)	
	Positive	negative	Positive	Negative
GeneXpert assay	40 (9.2%)	395	40	1
MGIT 960culture	37(8.5%)	398	35	6
ZN stain	32(7.4 %)	403	30	11

Table.2 Relation between semi-quantitative GeneXpert assay results and conventional methods (MGIT960 culture and ZN stain).

Semi-quantitative GeneXpert assay (n=40)	Positive MGIT culture	Positive ZN stain
High positive(n=21)	21	21
Medium positive(n=9)	9	9
Low positive(n=4)	4	0
Very low positive(n=6)	0	0
Total	34	30

Table.3 Comparison of GeneXpert assay with MGIT culture and ZN stain

	MGIT culture		ZN stain	
	positive	negative	positive	Negative
GeneXpert assay				
Positive(n=40)	34	6	30	10
Negative(n=395)	3	392	2	393
Total	37	398	32	403

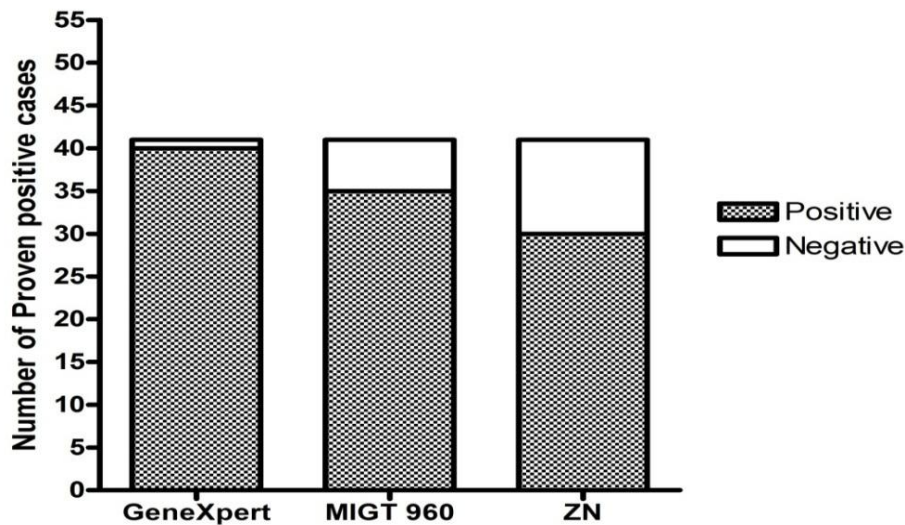
Table.4 Turnaround time (TAT) of TB detection by different methods

Methods	TAT of positive results	TAT of negative results
GeneXpert assay	2 hours	2 hours
MGIT culture	15±5	6 weeks
ZN stain	2 hours	2 hours

Table.5 Sensitivity, specificity and predictive values of different methods in the detection of positive TB cases (41 cases)

Methods	Sensitivity	Specificity	NPV	PPV
GeneXpert assay	97.6 % (40/41)	100% (394/394)	99.7% (394/395)	100% (40/40)
MGIT 960 culture	85.4 % (35/41)	99.4 % (394/396)	98.5% (394/400)	94.6% (35/37)
ZN stain	73.2 % (30/41)	99.4% (394/396)	99.5% (394/405)	93.8% (30/32)

Fig.1 Number of positive cases by different methods in relation to all positive TB cases (n=41)



No false positive results were reported by GeneXpert, but there were 2 cases by MGIT culture and ZN stain. These two false positive cases (positive by both MGIT and ZN and negative by GeneXpert) were identified as MOTT. This is because GeneXpert detects only certain part of DNA which is specific to MTBC, but MGIT culture and ZN stain cannot differentiate between MTBC and MOTT. On the contrary, none of MGIT culture and ZN positive samples for MTB was negative by GeneXpert except only one sample that was positive by MGIT and negative by GeneXpert, whereas, there were 6 and 11 false negative samples (positive by GeneXpert) by MGIT culture and ZN stain respectively.

This discordance between GeneXpert and MGIT culture was also reported by Agrawal *et al.*, 2016, Bajrami *et al.*, 2016 and tang *et al.*, 2017 who found that 9, 6 and 19 samples were GeneXpert positive and culture negative respectively. Also, the same previous authors found that 2, 2 and 13 samples were negative by GeneXpert and positive by culture respectively. This false negative result by GeneXpert could be due to the presence of few nucleic acid material or PCR inhibitors in the specimens, meanwhile, false negative results (positive by GeneXpert and negative by culture) could be due to the very low detection range of GeneXpert or death of some bacilli during decontamination (PCR test amplifies DNA of live or dead bacilli, but MGIT culture only detect living MTB).

In our study, by using a combination of bacterial, clinical, radiological and/or histopathological evidence of TB as the reference standard, overall sensitivity, specificity, NPV and PPV of GeneXpert were 97.6%, 100%, 99.7% and 100% respectively. Also, the sensitivity, specificity, negative and positive predictive values of MGIT culture were 85.4%, 99.4%, 98.5% and 94.6% and for ZN stain were 73.2%, 99.4%,

99.5% and 93.8% respectively (Table 5). This higher sensitivity and specificity of GeneXpert compared to MGIT culture and ZN stain is due to the ability of GeneXpert to detect very low level of MTB (Table 2).

In conjunction with our study, Pandey *et al.*, 2017, found that the overall sensitivity, specificity, PPV and NPV of GeneXpert MTB/RIF assay were to be 98.6%, 100%, 100% and 93.8% respectively. Furthermore, Singh *et al.*, 2016, demonstrated a sensitivity of 100% and specificity of 100% for pulmonary samples by using GeneXpert assay. In addition, Agrawal *et al.*, 2016, reported that the sensitivity and specificity of GeneXpert in sputum were 100% and 90% that is compatible with the study of Sharma *et al.*, 2015 who found a sensitivity of 96.9% and a specificity of 99.8%.

On the other side, Moussa *et al.*, 2016, found that the sensitivity and specificity of the GeneXpert assay were 93% and 98.3% respectively. This relatively low sensitivity compared to our results could be due to a low number of patients (218) and different study design. Similarly, according to the study done by Arzu *et al.*, (2011), 110 tuberculosis patients (62 pulmonary TB and 48 extra pulmonary TB) were diagnosed. The sensitivity, specificity, NPV and PPV of the GeneXpert were 70%, 100%, 90.6%, and 100% respectively. This difference in sensitivity between this study and our study could be explained by the presence of large numbers of paucibacillary extra pulmonary TB cases (48 out of 110) with a very low numbers of organisms which were under the limits of detection of the GeneXpert. The detection limit of GeneXpert is about 4.5 genomes per reaction or 131 cfu/ml (Helb *et al.*, 2010).

The time factor is very important in the diagnosis of TB to start appropriate treatment. In the present study, turnaround time of

GeneXpert assay is only 2 hours compared to 15±5 days for MGIT960 culture. Although the turnaround time of ZN smear was as rapid as GeneXpert, the sensitivity of the GeneXpert was much higher.

In a similar study by Sajed *et al.*, (2014) and Tang *et al.*, (2017), the mean detection time of GeneXpert assay was only 2 hours compared to 3.5±0.8 hours for Zn smear, 12±5 days for positive MGIT Culture and 42 days for a negative result of culture.

GeneXpert is a simple, rapid, highly sensitive (97.6%) and specific (100%) method that greatly speeds up the detection of MTB compared to conventional MGIT 960 culture and ZN stain.

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