

GeneXpert *MTB*/RIF Assay – A Major Milestone for Diagnosing *Mycobacterium tuberculosis* and Rifampicin-Resistant Cases in Pulmonary and Extrapulmonary Specimens

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Abstract

Background and Objective: Tuberculosis (TB) is an endemic disease in Iraq. Many methods are available to diagnose pulmonary and extrapulmonary TB (EPTB). The most traditional test is the sputum smear for acid-fast bacilli (AFB). However, it is well known for its low sensitivity and specificity. On the other hand, culturing AFB although considered the gold standard for detecting *Mycobacterium tuberculosis* (MTB), yet it takes long time to confirm or exclude the presence of TB. The WHO has recommended the use of a gene-based molecular technique called GeneXpert (GX) MTB/rifampicin (RIF) for rapid and accurate detection of MTB in pulmonary and extrapulmonary (EPTB) sites. GX is a quick, fully automated system that can be easily used with minimal training. The objective of this study was to evaluate the accuracy of the GX test for diagnosing MTB in pulmonary and extrapulmonary sites in Kurdistan/Iraq that is considered as an endemic area for TB, as well as testing the ability of this technique to identify the resistant strains of these bacilli to first-line anti-TB treatment. **Methodology:** A total of 925 (504 males and 421 females) patients attended the TB center in Erbil/Iraq from August 2015 to August 2017. These patients were clinically diagnosed or suspected to have TB. Two sputum samples were collected from each patient and subjected to AFB smear staining. The other portions of the sputum were examined by GX assay, and a number of cases were grown on the Lowenstein–Jensen media. For extrapulmonary fluid samples, the same tests were done. **Results:** Seven hundred and forty-three were pulmonary samples, and the remaining 182 cases were extrapulmonary specimens (cerebrospinal fluid, peritoneal aspirate, pleural fluid, urine, and blood). Of these, 575 had their AFB smears done which was positive for in 184 (32%) and negative in 391 (68%) cases. On the other hand, real-time polymerase chain reaction using GX technology was positive in 228 (39.65%) while negative in 347 (60.34%) cases. The sensitivity and specificity of the GeneX versus AFB smear considering culture as a gold standard were 94.9% and 80.1%, respectively. In addition, GX technique revealed that about 20 (14.3%) of positive MTB cases were resistant to RIF therapy. **Conclusion:** The current study displayed the real significance of using GX test in diagnosing MTB in pulmonary and extrapulmonary specimens to save time and to avoid unnecessary anti-TB treatment.

Keywords: Acid-fast bacilli, false negative, false positive, GeneXpert tuberculosis, pulmonary tuberculosis

INTRODUCTION

Tuberculosis (TB) is still an endemic disease in many countries in the world, particularly in low socioeconomic areas such as India, Indonesia, China, South Africa, and others where the rate is >150/100,000 population.^[1] A recent study showed that in Iraq 8500 TB cases were notified in 2015, with an incidence of 16/100,000 population.^[2] The rapid diagnosis of suspected cases is required to initiate a proper treatment as quick as possible and to prevent spread infection to nearby contacts. Although Ziehl–Neelsen (ZN) staining of sputum

smears or aspirated fluid for acid-fast bacilli (AFB) is still considered the standard method in most of our laboratories to diagnose TB as it is a rapid and inexpensive test, its sensitivity and specificity are low.^[3] On the other hand, culture-based

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technique is more sensitive but takes a long time and needs special facilities for *Mycobacterium tuberculosis* (MTB) to grow safely with weekly checking of the growth. In addition, the diagnosis of MTB in young children can be very difficult due to lack of obvious symptoms beside the difficulty in collecting the sputum samples. Moreover, false positive (FP) results may lead to unnecessary anti-TB treatment. In contrary, false-negative (FN) AFB test, particularly cases complicated with human immunodeficiency virus may lead to missing many cases with active TB and delay their treatment in addition to increase disease burden.^[4,5] To improve management and outcome of suspected patients, the WHO had recommended the use of a gene-based molecular technique called GeneXpert (GX) MTB/rifampicin (RIF) for rapid and accurate detection of MTB in pulmonary and extrapulmonary (extrapulmonary TB [EPTB]) sites. GX is a quick fully automated system that can be easily used with minimal training. Results for detecting MTB can be achieved within 2 h beside it can detect mutant strains of MTB that is resistant to first-line anti-TB treatment such as RIF. On the other hand, GX test yields positive results even with dead and fragmented bacilli, therefore, could not be used to diagnose active cases of TB cases or to follow-up treated cases.^[6,7]

MATERIALS AND METHODS

Samples from patients referred to TB center in Erbil, Iraq, suspected clinically and radiologically to have TB during the period from August 2015 to August 2017. The first sputum sample was taken on arrival. A second sample was self-collected overnight sputum to get the highest concentration of *Mycobacterium* to increase the sensitivity of the test. Sputum samples were submitted immediately to ZN staining and examined directly by the microscope following the WHO technical guides.^[8] The next step was to process the rest of the sputum samples in a 15 ml Falcon tube after diluting them with reagents provided in the GX kit (Cepheid, Sunnyvale, CA, USA) in a ratio of 2:1.^[3,9]

Fluid samples from nonrespiratory sites were centrifuged at 400 ×g for 15 min, and then, the deposit was dealt with exactly like sputum samples. The tubes were incubated at room temperature for at least 15 min during that they were shaken manually twice, then 2 ml of this suspension was aspirated using a Pasteur pipette and loaded into the GX cartridge for polymerase chain reaction (PCR) test.^[10] Within 2 h, the results were analyzed automatically depending on special software to detect MTB and RIF-resistant cases.^[11] A red line on the screen indicates positive MTB, whereas green lines mean negative for MTB. Positive cases of MTB can be sensitive to RIF; hence, a green line will appear next to the first one or the sample can be resistant to RIF so the adjacent line will appear red [Figure 1].

Traditional cultures of the specimen using Lowenstein–Jensen (L-J) media were done according to the manufacturer's instructions for MTB-positive cases, particularly those appeared resistant to RIF on the GX screen to check for drug

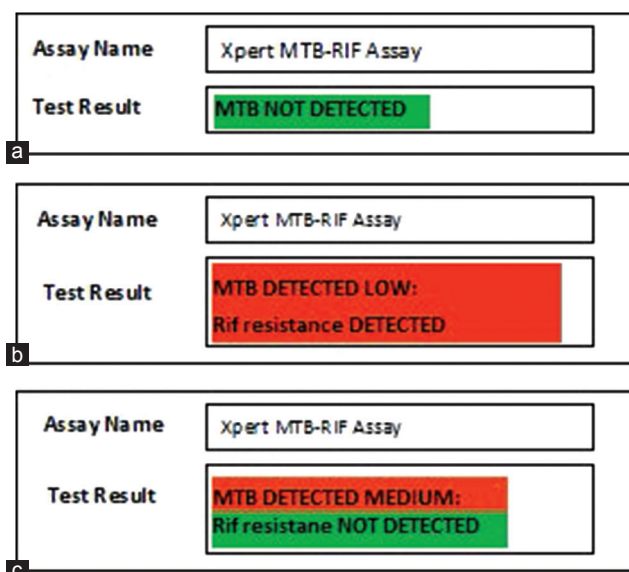


Figure 1: Computer screen of GeneXpert software showing no MTB detected in (a), low MTB detected with rifampicin resistance in (b), *Mycobacterium tuberculosis* detected with no rifampicin resistance in (c)

susceptibility (DS) for RIF, isoniazid, and streptomycin. L-J culture also done for AFB-negative cases but positive for GX or positive for AFB but PCR negative to check for the accuracy of the real-time PCR GX technique.^[12]

Statistical analysis

Statistical analysis of the current data was performed using the SPSS Inc, Chicago, IL, USA. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for GX test vice versa AFB: smear using culture as the gold standard as follows:

Sensitivity = true positive (TP)/TP + FN
 Specificity = true negative (TN)/TN + FP
 PPV = TP/total positive
 NPV = TN/total negative
 Accuracy test = (TP/FN)/(FP/TN).^[13]

Photo for GX screen readings [Figure 1], L-J culture for MTB [Figure 2] and positive MTB smear [Figure 3].

RESULTS

A total of 925 (504 males and 421 females) patients attended the TB center in Erbil, Iraq, from August 2015 to August 2017. These patients were clinically diagnosed or suspected to have TB. Out of the total, 743 samples were pulmonary and the remaining 182 cases were extrapulmonary specimens (cerebrospinal fluid, peritoneal aspirate, pleural fluid, and urine). Of 575 smear staining for AFB, MTB was recovered from 184 (32%) of cases, whereas the remaining 391 (68%) cases were negative for MTB. On the other hand, using the real-time PCR (GX technology), MTB was positive in 228 (39.7%) cases while negative in 347 (60.34%) cases. Of the 184 positive smears, 150 (26%) had positive results by GX test and 34 (5.9%) showed negative results by this test. On the other



Figure 2: *Mycobacterium tuberculosis* grown in (Lowenstein–Jensen) media appearing as dry, rough, raised wrinkled creamy white colonies

side, 313 (54.5%) negative smears had their matched negative PCR, whereas 78 (13.6%) turned to be positive by PCR. GX multidrug-resistant (MDR)/RIF screen detected 20 (14.2%) RIF-resistant cases out of the total positive MTB cases. Culture of sputum or fluids using L-J media was performed for 182 (31.6%) cases restrictively for the following candidates; patients having two controversial results by diagnostic methods for TB (e.g., PCR + ve cases but AFB – ve or vice versa); also to check for drug sensitivity and susceptibility in AFB + ve samples showing resistance to RIF and first-line anti-TB therapy by GX methods, and the results are depicted in Table 1. In addition, 10 of 313 AFB-negative/PCR-negative cases were cultured to confirm the true negativity of GX test. Furthermore, eight smear-positive cases were negative by GX, later proved by culture to be negative for MTB. However, these cultures were positive for non-MTB that could not be detected by the GX machine. Another 26 (18.57%) smear-positive cases were shown to be negative by both PCR and culture methods (FP smear).

Sensitivity, specificity, PPV, NPV, and accuracy of GX versus culture, GX versus AFB, and AFB versus culture are shown in Tables 2-4, respectively. Comparison of current results with other studies is shown in Table 5.

DISCUSSION

Laboratory diagnostic methods for TB are continuously evolving to find a rapid, accurate, and inexpensive tool to diagnose TB and RIF resistant cases to initiate anti-TB treatment once diagnosed and hence limit the spread of this infection and minimize its complications. Although the earliest method was the ZN stain of smears from pulmonary and extrapulmonary specimens which is rapid method, cheap with good PPV, its sensitivity is low (around 87%).^[3] In addition, smears for AFB cannot differentiate between *Mycobacterium* species, also the number of bacilli in the sample has to be (>3000–5000 AFB/mL) to give positive smears.^[7] On the other hand, culturing specimens although considered

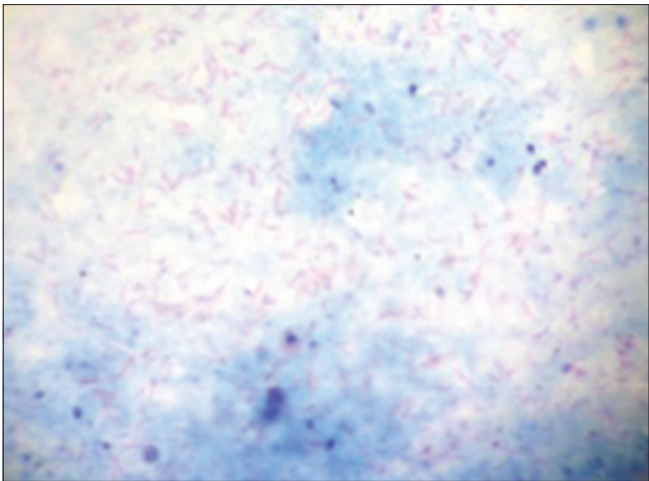


Figure 3: Smear acid-fast bacilli from sputum specimen stained with Ziehl–Neelsen stain showing positive mycobacteria

Table 1: Outcomes by different tuberculosis diagnostic methods			
	Total (%)	Culture positive	Culture negative
PCR positive/AFB positive	150 (26)	60/60	0
PCR negative/AFB positive	34 (5.9)	8	26
PCR negative/AFB negative	313 (54.5)	0	10/10
PCR positive/AFB negative	78 (13.6)	72	6
Total	575 (100)	140	42

PCR: Polymerase chain reaction, AFB: Acid-fast bacilli

fundamental for the accurate diagnosis of TB, the results need 4–6 weeks to be available during which there will be great chance for the infection to spread and the patient’s condition will deteriorate due to delay in the initiation of treatment. The WHO endorsed new technology for the detection of TB bacilli in even very low concentrated specimens by applying nucleic acid amplification method through Cepheid Xpert® MTB/RIF desk machine. Cepheid Xpert® MTB/RIF can provide a great opportunity for rapid laboratory diagnosis of patient with pulmonary and EPTB weeks earlier than cultures and at the same time detect resistant cases to RIF.^[14]

In the current study, 100% of smear and GX-positive cases were positive by culture also, which agree with what was found by Shagufta *et al.* and Shama *et al.*^[9,15] Seventy-two (12.5%) cases with negative smears were in fact positive by PCR and culture (FN smear), and this could possibly be attributed to technical problems such as smearing of saliva instead of sputum, dry and/or scanty sputum sample, improper viewing of the slide (<100 fields), or sometimes the smear is not properly stained or inefficient technologist.^[12] Therefore, to avoid missing TB cases by smear, TB programs should work to improve the quality of direct AFB microscopy test in TB diagnostic centers to avoid delayed treatment of positive TB cases. Regarding the eight (1.4%) AFB-positive cases but negative by PCR, later revealed to be positive by culture;

Table 2: Sensitivity, specificity, and diagnostic accuracy of GeneXpert compared to culture

GX	Culture		Total	Sensitivity	Specificity	PPV	NPV	Accuracy
	Positive	Negative						
Positive	132	6	138	94.2	85.7	95.7	81.8	92.3
Negative	8	36	44					
Total	140	42	182					

PPV: Positive predictive value, NPV: Negative predictive value, GX: GeneXpert

Table 3: Sensitivity, specificity, and diagnostic accuracy of GeneXpert versus acid-fast bacilli smear with culture as the gold standard

GX	AFB		Total	Sensitivity	Specificity	PPV	NPV	Accuracy
	Positive	Negative						
Positive	150	78	228	94.9	80.1	65.8	97.5	84.3
Negative	8	313	321					
Total	158	391	549					

PPV: Positive predictive value, NPV: Negative predictive value, AFB: Acid-fast bacilli, GX: GeneXpert

Table 4: Sensitivity, specificity, and diagnostic accuracy of acid-fast bacilli compared to polymerase chain reaction with culture as the gold standard

AFB	Culture		Total	Sensitivity	Specificity (%)	PPV	NPV	Accuracy
	Positive	Negative						
Positive	68	26	94	48.6	38.1	72.3	18.2	46.2
Negative	72	16	88					
Total	140	42	182					

PPV: Positive predictive value, NPV: Negative predictive value, AFB: Acid-fast bacilli

Table 5: Comparison with other studies

Study ID (first author, year)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Lawn, 2011 ^[11]	72	99	94	94
Reechaipichitkul, 2016 ^[16]	83.9	92.1	81.3	93.3
Agrawal, 2016 ^[17]	86.8	93.1	78.5	96.7
Meawed & Shaker, 2016 ^[18]	98	75	99.15	75
Pandey <i>et al.</i> , 2016 ^[19]	98.6%	100%	100%	93.8%
Iram <i>et al.</i> , 2015 ^[20]	100%	100%	-	-
Ioannidis, 2011 ^[21]	91.4	93	86.5	95.5
Moussa, 2016 ^[22]	92.96	98.3	96.35	96.72
Bunsow, 2014 ^[23]	93	98.3	95.7	99.1
Current study	94.9	80.1	65.8	97.5

PPV: Positive predictive value, NPV: Negative predictive value

this could be attributed to the sensitivity of the GX machine designed for MTB only and cannot detect *Mycobacterium bovis* or *Mycobacterium leprae* or any other species. On the other hand, the reasons behind positive AFB smears but negative by both PCR and culture (FP smear) could possibly be due to non-TB mycobacteria,^[15] or due to TB bacilli transferred accidentally through the objective lenses to the negative smear, or using unclean stick to spread the smear on the slide, or sometimes miss labeling of a slide and others. While if PCR was positive in smear-negative/culture-negative cases, the most important cause is that the GX can analyze the DNA even for dead and fragmented bacteria which is considered as

a big disadvantage of this test, therefore, GX technique cannot be used for follow-up of patients after the treatment.^[6] For this reason, patients with a high clinical suspicion of pulmonary tuberculosis, but smear-negative should have at least one culture to confirm the diagnosis of TB to avoid unnecessary anti-TB treatment.

Sensitivity and specificity of GeneXpert *Mycobacterium tuberculosis*/rifampicin assay

The current study showed a sensitivity of 94.9% and specificity 80.1% for GX test versus AFB with culture as the gold standard. Our findings are higher than those reported by Lawn

et al., Reechaipichitkul *et al.*, and Agrawal *et al.*, who reported the sensitivity for GX using culture as the gold standard to be 72%, 83.9%, and 86.8%, respectively, and a specificity of 99%, 92.1%, and 93.1%, respectively.^[11,16,17] Whereas, our results agree with that reported by Meawed and Amany in 2016 who reported a sensitivity and specificity of GX of 98.15% and 75% and accuracy of 96.55% in MTB diagnosis^[18] which is in line with other studies^[11,16-18-23] [Table 5].

On the other hand, AFB smear sensitivity and specificity were 48.6% and 38.1% with a PPV and NPV of 72.3% and 46.2%, respectively, the result is higher than that found by Agrawa *et al.* (sensitivity 22.2% and specificity 78.5%) but much lower than the results reported by Moussa *et al.* (sensitivity 86.4% and specificity 99.4%) but comparable with that of Zeka *et al.* (sensitivity 43.5% and specificity 99.5%).^[22,24,25] Differences in sensitivity and specificity could be attributed to geographical variations of the sampling locations, differences in the way of sample collection, and GX cartridge conservation.

To sum up, the sensitivity and specificity of GX MTB/RIF technique in clinical specimens were reported in different studies to range from 72% to 98% and 75% to 99%, respectively, as shown in Table 5, particularly in smear-positive cases.^[16-18,21-26]

Detection of rifampin resistance

Cepheid Xpert MTB/RIF assay detected 14.3% of MTB positive cases resistant to RIF therapy, which is higher to what was found by Okonkwo *et al.* who reported a prevalence for RIF resistance rate of 6.9% and by Atashi *et al.* 3.1% in Iran but lower than that found by Guenaoui *et al.* by GX MTB/RIF.^[26-28] This discrepancy in results could be attributed mostly to the existence of mixed bacterial strains in the sample sputum, particularly in retreated patients or due to gene mutations in MTB, especially *rpo B* gene.^[11] Thus, it is recommended to start the second-line treatment for MDR-TB cases displayed on GX screen, while waiting for results of DS culture.

CONCLUSION

Although direct smear examination for AFB is the most popular test worldwide because it is rapid and costless, yet still missing sensitivity and specificity. GX is a promising diagnostic tool and should be confidently used for early detection of MTB, particularly for smear-negative cases with suspected clinical and radiological findings to start anti-TB treatment and interrupt disease transmission.

As well, GX/MTB can simultaneously detect RIF-resistant TB cases, particularly in immunocompromised patients. Nevertheless, patients with culture negative but GX positive should be interrelated with their clinical status to start their proper therapy.

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Conflicts of interest

There are no conflicts of interest.

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