



Utility of GeneXpert MTB/RIF assay for the diagnosis of pulmonary and extra-pulmonary tuberculosis, A report from Egypt

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Abstract

Early diagnosis of tuberculosis continues to be a challenge for clinicians. The World Health Organization (WHO) guidelines recommend the application of GeneXpert MTB/RIF in extra-pulmonary tuberculosis (EPTB) diagnosis. This study aimed to test and compare the accuracy of the GeneXpert MTB/RIF assay to diagnose pulmonary tuberculosis (PTB) and EPTB, compared to bacterial culture and to composite reference standard (CRS). The GeneXpert assay diagnosed tuberculosis (TB) in 19.5 % of patients. With reference to bacterial culture, the sensitivity of this assay for detection of the pulmonary and extra-pulmonary specimens was perfect. For pulmonary specimens, on using CRS; the detected sensitivity and specificity of the GeneXpert assay were 78.3 % and 99.1 %, respectively. However, for extra-pulmonary specimens, the sensitivity and specificity of the GeneXpert assay were 37.1 % and 99 %, respectively. In the current study, the GeneXpert assay showed almost perfect agreement with the bacterial culture for TB diagnosis. The diagnostic accuracy of the GeneXpert assay was high in ruling in, but not in ruling out of EPTB.

Keywords: GeneXpert MTB/RIF, Extra-pulmonary TB; Pulmonary TB, Egypt

1. Introduction

Globally, tuberculosis (TB) persists as a current and leading health concern with an estimated 10 million new cases in 2017, and only 67 % of the cases (6.7 million cases) have been diagnosed. Furthermore, an estimated 6.6 million rifampicin-resistant (RIF-R) cases occurred, but only 2.0 million (30 %) were

identified ([WHO. 2018](#)). The WHO has classified Egypt as a middle/low-level country according to TB prevalence. The estimated TB annual prevalence is 11/100 000 cases with smear-positive (SP) active pulmonary tuberculosis (PTB), and 24/100 000 cases

with all types of TB, as reported by [Moussa *et al.*, \(2016\)](#).

In clinical practice, early diagnosis of TB continues to be a challenge for clinicians, especially with extra-pulmonary TB (EPTB), childhood TB, and TB patients co-infected with HIV ([WHO, 2016](#)). Virtually, EPTB may affect every part of the body away from the lungs ([Sharma and Mohan, 2004](#)). According to a previous study conducted by [Tortoli *et al.*, \(2012\)](#), EPTB has varied clinical manifestations, and atypical presentation, and therefore requires a high index of clinical suspicion as reported recently by [Tag Eldin *et al.*, \(2019\)](#). Besides, the EPTB is difficult to diagnose due to the smaller number of bacteria in the specimens (paucibacillary nature); difficulty in obtaining specimens from deep-seated organs, and inability to get an extra specimen, as revealed by [Bankar *et al.*, \(2018\)](#).

In low-income countries, conventional methods such as Ziehl-Neelsen (ZN) smear microscopy is a cheap and rapid method for the detection of acid-fast bacilli; however, it has poor sensitivity and poor PPV (positive predictive value) ([Chen *et al.*, 2012](#)). Though culture is the gold standard for diagnosis of TB, it often takes weeks to have the results, which causes significant delay. Furthermore, the deficiency of diagnostic infrastructure, experienced staff and specialized laboratories interfere with proper patients care and outcomes, and exacerbate the dilemma of EPTB diagnosis. Thus, recent studies conducted by [Bankar *et al.*, \(2018\)](#), [Rasheed *et al.*, \(2019\)](#) highlighted that rapid and early detection of *Mycobacterium tuberculosis* (MTB), and the multidrug resistance/rifampicin-resistant (MDR/RIF-R) strains is an obligation. The [WHO, \(2013\)](#) has endorsed GeneXpert MTB/RIF (Xpert) assay (Cepheid, CA, USA) for the PTB diagnosis, as it is highly sensitive and specific for CP (culture positive) TB. Additionally, several studies conducted by [Tortoli *et al.*, \(2012\)](#); [Pang *et al.*, \(2017\)](#) have recommended that GeneXpert assay has a hopeful efficacy in detecting EPTB, which assists in following the WHO guidelines.

According to [Helb *et al.*, \(2010\)](#), the GeneXpert assay is an automated closed-cartridge system; easy to use, bio-safe, requires minimal training, and its results are acquired within two hours. [Boehme *et al.*, \(2010\)](#) added that the test detects MTB and rifampicin resistance simultaneously, which can thus be used as a representative marker for MDR-TB.

The objectives of this study were to test the accuracy of the GeneXpert assay to diagnose PTB and EPTB, compared to culture on Lowenstein-Jensen (LJ) medium and to a composite reference standard (CRS). This is in addition to detecting the prevalence of RIF resistance among the reported cases.

2. Material and methods

2.1. Study design and settings

This prospective study was carried out at Fayoum University Hospital, in collaboration with Fayoum Chest Hospital, Fayoum, Egypt. Patients enrolled in the current study were from both sexes with suspected PTB or EPTB, during the period from December, 2016 to December, 2019. In this study, we compared the PTB and EPTB detection capabilities of the GeneXpert assay, to bacterial culture on Lowenstein-Jensen (LJ) medium, and to composite reference standards (CRS). About 778 patients with highly suspected TB based on the clinical data; the relative laboratory tests results and the radiological findings; however, they did not start anti-tuberculosis treatment (ATT) yet at the time of registration, were included in this study. Excluded from this study were patients who were reported to have tuberculosis and started ATT; those who were unable to get proper samples for examination, patients refused or were unable to give written valid consent, in addition to patients with an underlying clinical diagnosis other than TB. Patients whose cultures grew as non-tuberculosis mycobacteria (NTM), those who were lost and/or died during follow-up, were banned from the current study.

2.2. Clinical specimen's collection and processing

A total of 571(73.4 %) sputum specimens and 207(26.6%) extra-pulmonary specimens were included in this study. Sputum volume of at least 2-3 ml was considered as optimum and was processed for analysis. The smallest volumes of extra-pulmonary specimens required were as follows: 3 ml for any kind of body fluid including pus, 2.5 ml for cerebrospinal fluid (CSF); and 1 cm by 1 cm for biopsy specimens. After centrifugation of the sterile body fluids, the pellets were used. Non-sterile clinical specimens were processed by the conventional N-acetyl L-cysteine-NaOH (NALC-NaOH) method, for making smears, cultures and GeneXpert tests, according to [Kawai *et al.*, \(2006\)](#); [Zeka *et al.*, \(2011\)](#). The invasively collected specimens were processed directly.

2.3. Acid-fast bacilli smears and culture on Lowenstein-Jensen (LJ) medium

The processed specimens were used for microbiological examination. Sputum specimens were obtained from each patient, and were subjected to smear microscopy by ZN staining and culture on LJ media, following the protocol of [Singh *et al.*, \(2016\)](#). Cultures were dealt with as a reference standard for measuring the accuracy of the GeneXpert assay as a diagnostic test.

2.4. GeneXpert MTB/RIF assay

The GeneXpert assay (Cepheid Inc, USA) was performed according to the manufacturer's instructions. In brief, expectorated sputum specimen (about 0.5 ml) and GeneXpert reagent were added in 1:2 ratio; vortexed twice and then incubated for 15 min. at room temperature until completely homogenized. About 2 ml of this mixture was pipetted into GeneXpert test cartridge, and then the cartridge was loaded into the GeneXpert machine. After 90 min., the GeneXpert system interpreted the results according to the measured fluorescent signals. According to [Khadka *et al.*, \(2019\)](#), results were reported as negative if MTB was not detected, and considered as positive if MTB was detected; with or without rifampicin resistance. All samples that were

culture positive (CP) and GeneXpert assay negative, and samples that were CN (culture-negative) and GeneXpert assay positive were retested twice, and the last result was considered for the study. Bias in reading GeneXpert results were minimized as it were interpreted by an independent observer who didn't know the results of CRS.

2.5. Composite reference standard for comparison

Culture on LJ medium is considered as an accepted reference standard, which is widely recognized as the best available and accurate method for isolation and detection of MTB. However, in paucibacillary diseases such as EPTB, growth of mycobacteria on this culture medium may be limited, as reported by [Vadwai *et al.*, \(2011\)](#). The molecular tests including polymerase chain reaction (PCR) can detect DNA from dead bacterial cells with a limit of detection ranging from 5- 100 bacilli/ ml; thus it may be used to identify CN samples. As a result of the seriously suboptimal reference standard of culture on LJ medium for EPTB, thus we also compared the GeneXpert results to a composite reference standard (CRS) assay; to test its true diagnostic potential for EPTB, in reference to [Denkinger *et al.*, \(2014\)](#). The CRS may have poor specificity, hence, both CRS and culture on LJ medium were considered as reference standards to attain the best sensitivity and specificity. For PTB, diagnosis was used if any two of the following tests were positive: smear/ culture/ response to treatment/ radiological findings. Similarly, for EPTB, diagnosis was carried out if any two of the following assays were positive: smear/ culture/ histopathology/ cytology/ biochemical analysis/ response to treatment/ adenosine deaminase (ADA) levels; for sterile body fluids, pleural fluid, ascetic fluid, cerebrospinal fluid (CSF)/ and radiological findings ([Vadwai *et al.*, 2011](#)). The patients were followed for 3 months after diagnosis and initiation of treatment. Improvement in signs and symptoms was considered as an adequate response to treatment. Meanwhile, if the case was improved on non-anti-tuberculosis treatment (non-ATT), it was considered negative.

2.6. Patients categories

According to the CRS and ATT follow-up, the patient's cases were categorized into four diagnostic groups by two experts, who were blinded to results of the GeneXpert test, in reference to [Moussa *et al.*, \(2016\)](#). These four groups include: confirmed TB cases (CP, with SP or SN), probable TB cases (CN but has clinical symptoms, cytology/ histology and/or radiological findings, indicative of TB), possible TB cases (culture and other tests are negative with a clinical assumption of TB, and response to empirical ATT after 3 months follow-up), and no TB (all tests were negative for TB, and the case has improved without having ATT).

2.7. Statistical analysis

Statistical analysis was carried out with the SPSS for Windows (version 16.0). Numerical variables were summarized with mean \pm SD (standard deviation). The significant differences among groups were assessed by the Student t-test, whereas analysis of categorical variables was examined by the chi-square test. Fisher Exact test was used for two by two tables if expected values were less than 5. A value of $p \leq 0.05$ was considered significant. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the GeneXpert assay for TB diagnosis were considered, and 95 % CI (confidence interval) was also calculated. The Kappa co-efficient was calculated to indicate level of agreement between readings of any two tests. It was described as follows: 0.2= none, 0.21-0.39= minimal, 0.40-0.59=weak, 0.60-0.79= moderate, 0.80-0.89=strong, 0.90-11=almost perfect ([Ou *et al.*, \(2015\)](#)).

3. Results

After exclusion of contaminated cultures (n= 12), insufficient specimens (n= 9), NTM positive specimens (n= 10), patients who were missing to follow-up (n= 22), and those who died (n= 6); about 778 samples (571 pulmonary specimens and 207 extra-pulmonary specimens) from 778 patients were analyzed for the study. The socio-demographic data of

the patients are described in Table (1). Patients aged between 4-88 years old, mean \pm SD is 44.3 ± 14.9 years. According to their residence, 447 (57.4 %) were living in rural areas, 321 (41.3 %) in urban areas, and 10 (1.3 %) were prisoners. Patients from rural areas significantly predominate over those from urban areas ($p = 0.004$). The extra-pulmonary specimens from 207 patients were variably distributed, as demonstrated in Table (2).

3.1. Ziehl-Neelsen smears microscopy

In the current study, ZN smear has detected MTB in pulmonary samples more often than in extra-pulmonary specimens and the difference is statistically significant ($p < 0.001$) (Table 1). In the combined samples, the ZN smear microscopy has resulted in the detection of 106/778 (13.6 %) positive smears and 672/778 (86.4 %) negative ones. In pulmonary samples, 96/571 (16.8 %) are smear-positive and 475/571 (83.2 %) are smear-negative, while in extra-pulmonary specimens, 10/207 (4.8%) are smear-positive and 197/207 (95.2 %) are smear-negative as shown in Table (3).

3.2. Culture on Löwenstein-Jensen medium

In all the 778 specimens, 144 (18.5 %) are CP and 634 (81.5 %) are CN. Out of 571 pulmonary specimens, 108 (18.9 %) are CP for MTB, while 463 (81.1 %) are CN. On the other hand, throughout the extra-pulmonary specimens examined; 36/207 (17.4 %) are CP for MTB, whereas 171/207 (82.6 %) are CN, as demonstrated in Table (1). The difference between the detection rate of MTB in pulmonary and extra-pulmonary specimens using culture is not statistically significant ($p = 0.629$), as shown in Table (1).

3.3. GeneXpert MTB/RIF assay

Out of the 778 specimens included in this study, the GeneXpert assay detected MTB in 152 (19.5%), compared to 144 (18.5%) specimens by culture, while 10 specimens gave errors (1.3 %) (Table 1).

Table 1: Demographic, clinical and laboratory characteristics of the study groups

		Pulmonary sample		Extra-pulmonary sample		Total		<i>p</i> -value
		N	%	N	%	N	%	
Sex	Male	375	65.7	126	60.9	501	64.4	0.26
	Female	196	34.3	81	39.1	277	35.6	
Residence	Rural	343	60.1	104	50.2	447	57.4	0.004*
	Urban	218	38.2	103	49.8	321	41.3	
	Prison	10	1.8			10	1.3	
Age	<18	25	4.4	9	4.3	34	4.4	0.985
	>18 years	546	95.6	198	95.7	744	95.6	
SD	Mean±	44.1±14.8		44.8±15.2		44.3±14.9		
DM	Yes	21	3.7	17	8.2	38	4.9	0.01*
HIV	Yes	1	0.2	0	0	1	0.1	
ZN staining	Negative	475	83.2	197	95.2	672	86.4	<0.001
	Positive	96	16.8	10	4.8	106	13.6	*
Culture on LJ media	Negative	463	81.1	171	82.6	634	81.5	0.629
	Positive	108	18.9	36	17.4	144	18.5	
GeneXpert MTB/RIF assay	Negative	452	79.2	165	79.7	616	79.2	0.887
	Positive	112	19.6	40	19.3	152	19.5	
	Error	7	1.2	2	1.0	10	1.3	
CRS	Confirmed	102	17.9	38	18.4	140	18.0	<0.001
	Probable case	32	5.6	58	28.0	90	11.6	
	Possible case	12	2.1	1	0.5	13	1.7	
	No TB	425	74.4	110	53.1	535	68.8	

Where; *p* value: between pulmonary and extra-pulmonary cases; *: significant; SD: Standard deviation; DM: *Diabetes Melitus*; HIV: Human Immunodeficiency Virus; ZN: Ziehl-Neelsen; LJ: Lowenstein-Jensen; MTB/RIF: *Mycobacterium tuberculosis*/Rifampicin; CRS: Composite reference standard

Table 2: Diagnosis of Tuberculosis (TB) in different clinical specimens, by culture on Lowenstein-Jensen, ZN smear microscopy, GeneXpert MTB/RIF assay, and the Composite Reference Standard

Specimen	Frequency (%) (N= 778)	MTB detection by Culture N (%)	MTB detection by ZN smear microscopy N (%)	MTB detection by GeneXpert MTB/RIF assay N (%)	TB diagnosis by Composite Reference Standard N (%)
Sputum	571(73.4)	108(75)	96(90.6)	112(73.6)	138(56.8)
Pleural effusion	163(21.0)	31(21.5)	10(9.4)	33(21.7)	98(40.3)
CSF	10(1.3)			2(1.3)	1(0.4)
Urine	10(1.3)	3(2.1)		3(2)	4(1.6)
Stool	4(0.5)				
ascites	4(0.5)				
bone	5(0.6)	1(0.7)		1(0.7)	1(0.4)
skin	4(0.5)				
LN	7(0.9)	1(0.7)		1(0.7)	1(0.4)
Total	778(100.0)	144	106	152	243

Where; MTB/RIF: *Mycobacterium tuberculosis*/ Rifampicin; CSF: Cerebrospinal fluid, LN: Lymph Node; ZN: Ziehl-Neelsen

Table 3: The inter-relationship among results of the Ziehl-Neelsen smear, LJ culture medium, GeneXpert MTB/RIF assay and the CRS

		Pulmonary specimens (N= 571)		Extra-pulmonary specimens (N= 207)		Total (N= 778)	
		CRS		CRS		CRS	
		Negative	Positive	Negative	Positive	Negative	Positive
ZN smear	Negative	349	126	104	93	453	219
	Positive	76	20	6	4	82	24
LJ culture	Negative	340	123	92	79	432	202
	Positive	85	23	18	18	103	41
GeneXpert MTB/RIF assay	Negative	337	122	91	76	428	198
	Positive	88	24	19	21	107	45
		LJ Culture		LJ Culture		LJ Culture	
		Positive	Negative	Positive	Negative	Positive	Negative
ZN smear	Positive	90	6	10	0	100	6
	Negative	18	457	26	171	44	628
GeneXpert MTB/RIF assay	Positive	108	4	36	4	144	8
	Negative	0	459	0	167	0	626

Where; ZN: Ziehl-Neelsen; LJ: Lowenstein-Jensen; CRS: Composite reference standard; MTB/RIF: *Mycobacterium tuberculosis*/ Rifampicin

Rifampicin resistance is detected in 13/152 (8.6 %) of the GeneXpert assay positive specimens; 11 specimens (11/112) are pulmonary, and 2 samples (2/40) are extra-pulmonary (data not presented in

Tables). By the GeneXpert assay, 112/571 (19.6 %) of pulmonary specimens and 40/207 (19.3 %) extra-pulmonary specimens are TB-positive. The frequency of extra-pulmonary specimens detected by

the GeneXpert assay is outlined in Table (2). No statistical difference is observed for detection of MTB in PTB and EPTB specimens by the GeneXpert assay ($p=0.887$), as clear in Table (1). Out of the 152 positive cases, 100 are SP-CP specimens (90 pulmonary cases and 10 extra-pulmonary cases), 44 are SN-CP (18 pulmonary cases and 26 extra-pulmonary cases), 7 cases are SN-CN (three pulmonary cases and four extra-pulmonary cases), and only a single case of pulmonary specimens is SP-CN. Throughout the 152 GeneXpert positive cases, 144 are GeneXpert positive-CP (108 pulmonary cases and 36 extra-pulmonary cases), while no case is gene negative-CP. Eight cases are gene positive-CN (four pulmonary cases and four extra-pulmonary cases). Detailed distribution of results of the GeneXpert assay among culture and CRS positive cases is shown in Table (3).

3.4. Diagnosis of TB using a composite reference standard (CRS)

According to the combined clinical and microbiological results (CRS), 243 patients are diagnosed with tuberculosis; 140 are confirmed cases, 90 probable cases, and 13 possible cases (Table 1). Out of the 243 CRS positive cases; 146 (25.6%) are pulmonary TB; and 97 (46.9 %) are extra-pulmonary TB (Table 1). Of the CRS positive pulmonary specimens; 102 (17.9 %) are confirmed TB cases, 32 (5.6 %) probable cases, and 12 (2.1 %) are possible cases (Table 1). Out of 97 (46.9 %) patients diagnosed with EPTB according to the CRS, 38 (18.4 %) are confirmed TB, 58 (28 %) probable cases, and 1(0.5 %) are possible cases, as reported in Table (1). Results of ZN staining, culture on LJ medium, GeneXpert assay and CRS in both groups are reported in summary in Tables (1 and 3).

3.5. Relative diagnostic efficiencies (overall sensitivity, specificity, PPV and NPV) of the GeneXpert assay, with reference to the LJ culture medium

With reference to the culture results, the sensitivity of the GeneXpert assay for the combined PTB and EPTB is 100 % (95 % CI. 72 %-100 %), the specificity is 98.7 (95 % CI. 97.8 %-99.6 %), PPV is 94.7 % (95 % CI. 90 %-97.3 %), and NPV is 100 % (95 % CI. 99 %-100 %). For pulmonary and extra-pulmonary samples the sensitivity, specificity, PPV, and NPV of the GeneXpert assay, using a culture as reference, is demonstrated in Table (4). The sensitivity of the GeneXpert assay is 100 % for CP-SP and CP-SN, in pulmonary and extra-pulmonary specimens (Table 4). Statistically, on using the culture results as a reference standard, no significant difference is observed in the sensitivity of the GeneXpert assay between PTB and EPTB cases ($p=1$).

3.6. Relative diagnostic efficiencies (overall sensitivity, specificity, PPV and NPV) of GeneXpert MTB/RIF assay with reference to CRS

The combined sensitivity of the GeneXpert test (for pulmonary and extra-pulmonary specimens) is 60.5 % (95 % CI. 54.3 %-67 %), the specificity is 99.1 % (95 % CI. 98 %-99.9 %), the PPV is 96.7 % (95 % CI. 92.5 %-97.5 %), and NPV is 84.7 % (95 % CI. 81.6 %-87.3 %), as demonstrated in Table (5). On comparing the sensitivity of the GeneXpert assay in PTB with that of EPTB, it showed a significant difference ($p<0.001$).

Among the extra-pulmonary specimens, the GeneXpert assay specificity is high for almost all specimens, while the sensitivity varied among different clinical specimens. The sensitivity is higher for lymph node and bones specimens; 100% (95% CI. 20.6%-100%), followed by urine; 75% (95% CI. 30%-95.4%), CSF; 66.3% (95% CI. 20.6%-100%), whereas the lowest sensitivity is recorded for pleural effusion; 33.7% (95% CI 25%-43.5%), as shown in Table (6).

Table 4: Relative diagnostic efficiencies (Overall sensitivity and specificity) of ZN microscopy staining and GeneXpert MTB/RIF assay, in reference to LJ culture (n= 778)

		GeneXpert MTB/RIF Assay					
		ZN smear	Total (N=778)	SN-CN (N=628)	SP-CN (N=6)	SN-CP (N=44)	SP-CP (N=100)
Pulmonary specimens (=571)	Sensitivity	83.3% (67.2-90.5)	100.0% (97.5-100)			100%	100%
	Specificity	98.7% (97.7-99.7)	99.1% (98.3-100)	99.3%	83.3%		
	PPV	93.8% (87-97.1)	96.4% (91-98.6)			100%	100%
	NPV	96.2% (94-97.5)	100.0% (99.4-100)	100.0%	100.0%		
	Kappa	0.857	0.977				
Extra-pulmonary specimens (=207)	Sensitivity	27.8% (12.4-43)	100.0% (90-100)		No cases	100%	100%
	Specificity	100% (97.8-100)	97.7% (95.4-99.9)	97.7%			
	PPV	100% (72-100)	96.4% (80-99)			100%	100%
	NPV	86.8% (81.6-91)	100.0% (72-100)	100.0%			
	Kappa	0.389	0.936			100%	100%
	<i>p</i> value [±]	<0.001*	1				
Total (=778)	Sensitivity	69.4% (61.8-77)	100.0% (72-100)				
	Specificity	99.1% (98.3-99.8)	98.7% (97.8-99.6)	98.9%	83.3%		
	PPV	94.3% (88-97.4)	94.7% (90-97.3)				
	NPV	93.5% (91-95)	100% (99-100)	100.0%	100.0%	100%	100%
	Kappa	0.763	0.967			100%	100%

Where; *p* value[±]: between pulmonary and extra-pulmonary cases; *: significant; ZN, Ziehl-Neelsen; MTB/RIF: *Mycobacterium tuberculosis*/ Rifampicin; CN: Culture negative, CP: Culture positive, SP: Smear positive, SN: Smear negative; PPV: Positive predictive value; NPV: Negative predictive value

Table 5: Relative diagnostic efficiencies (Overall sensitivity and specificity) of ZN microscopy staining, LJ culture medium and GeneXpert MTB/RIF assay, in reference to a Composite reference standard (n= 778)

		ZN Smear	LJ Culture	GeneXpert MTB/RIF Assay		
				Total	Smear positive	Smear negative
Pulmonary specimens (=571)	Sensitivity	66.7% (58.7-74.6)	75.4% (68.1-82.6)	78.3% (71-85)	94.6% (90-99)	45.7% (31-61)
	Specificity	99.1% (98-100)	99.1% (98.2-100)	99.1% (97-100)	0	100.0%
	PPV	95.8% (89.7-98)	96.3% (90.5-98.5)	96.4% (91-98)	95.6%	100.0%
	NPV	90.39% (87-93)	92.7% (90-95)	93.5% (90.8-95)	0	94.5%
	Kappa	0.733	0.804	0.826	0	0.603
Extra-pulmonary specimens (=207)	Sensitivity	9.5% (3.8-15.2)	34.3% (25-43.5)	37.1% (28-46.5)	100% (71-100)	30.5% (21-40)
	Specificity	100.0% (90-100)	100.0% (90-100)	99% (98.2-100)		99.0% (97-100)
	PPV	100.0% (72-100)	100.0% (91-100)	97.5% (87-99.5)	100.0%	96.7%
	NPV	51.8% (44.8-58)	59.6% (52-66.7)	60.5% (53-67.5)		60.5%
	Kappa	0.00	0.340	0.358		0.303
	<i>p</i> value [±]	<0.001*	<0.001*	<0.001*		
Total (=778)	Sensitivity	42% (35.7-48.2)	57.6% (51.4-64)	60.5% (54.3-67)	95.1% (90-99.4)	35.5% (27.7-43.5)
	Specificity	99.3% (98-100)	99.3% (98.5-100)	99.1% (98-99.9)	0	99.8% (99.4-100)
	PPV	96.2% (91-98.5)	83.8 (80.6-86.4)	96.7% (92.5-97.5)	96	98
	NPPV	79% (75.8-81.9)	97.2% (93-99)	84.7% (81.6-87.3)	0	95.3
	Kappa	0.487	0.640	0.663	0.0	0.461

Where; *p* value[±]: between pulmonary and extra-pulmonary cases; *: significant; ZN: Ziehl-Neelsen; LJ: Lowenstein-Jensen medium; MTB/RIF: *Mycobacterium tuberculosis*/ Rifampicin; PPV: Positive predictive value; NPV: Negative predictive value

Table 6: Relative diagnostic efficiencies (Overall sensitivity and specificity) of GeneXpert MTB/RIF assay, in reference to a Composite reference standard in diagnosis of extrapulmonary tuberculosis (EPTB) (n=207)

Extra-pulmonary specimens (n=207)	Sensitivity	Specificity	PPV	NPV
Pleural effusion (n=163)	33.7% (25-43.5)	100%	100%	50%
CSF (n=10)	66.3% (20.6-100)	88.9%	50.0%	100%
Urine (n=10)	75.0% (30-95.4)	100.0%		85.7%
Stool (n=4)		100%		100%
Ascites (n=4)		100%		100%
Bone (n=5)	100% (20.6-100)	100%	100%	100%
Skin (n=4)		100%		100%
LN (n=7)	100% (20.6-100)	100%	100%	100%

Where; CSF: Cerebrospinal fluid, LN: Lymph Node; PPV: Positive predictive value; NPV: Negative predictive value

3.7. The inter-rater agreement of the GeneXpert assay and other tests for PTB and EPTB

The GeneXpert assay showed almost perfect agreement with culture on LJ medium for both pulmonary ($\kappa=0.977$) and extra-pulmonary specimens ($\kappa=0.936$), and for the combined samples ($\kappa=0.967$), as demonstrated in Table (4). The agreement between the smear and culture is strong ($\kappa=0.857$) for pulmonary specimens, minimum ($\kappa=0.389$) for extra-pulmonary specimens, and moderate ($\kappa=0.763$) for the combined samples. According to the results presented in Table (5), the agreement between the GeneXpert assay and the culture on LJ medium, with the CRS for pulmonary specimens is strong ($\kappa=0.826$ and 0.804); respectively, although it

is moderate for ZN staining ($\kappa=0.733$). This is remarkably better than those for extra-pulmonary specimens; where the GeneXpert assay and culture on LJ medium showed minimal agreement with the CRS ($\kappa=0.358$ and 0.340); respectively, while ZN staining showed no agreement with the CRS ($\kappa=0.0$).

4. Discussion

This study aimed to assess and compare the diagnostic precision of the GeneXpert assay for the PTB and EPTB. Overall, in the current study, the GeneXpert assay diagnosed more EPTB cases than the bacterial culture did; however, this difference was not statistically significant. In contrast, [Bankar *et al.*, \(2018\)](#) recently reported a marked difference

in MTB detection between the bacterial cultures vs. the GeneXpert assay. A previous study conducted by [Vadwai *et al.*, \(2011\)](#) attributed this lower positivity to the paucibacillary character of EPTB samples, and the tendency of MTB to form clumps with uneven distribution of bacilli cells. Meanwhile, [Denkinger *et al.*, \(2014\)](#) reported that in diagnostic accuracy studies, an imperfect reference standard may lead to misclassification of samples. This agrees with the current study, where an assessment of the diagnostic accuracy according to two reference standards namely; the culture and a CRS, has provided a reasonable range for the sensitivity and specificity of the GeneXpert assay.

On using culture as the reference standard, results of the present study showed that the overall sensitivity, specificity, PPV, and NPV of the GeneXpert assay for the pulmonary samples were higher than other previous studies from China ([Ou *et al.*, 2015](#)) and Ethiopia ([Geleta *et al.*, 2015](#)). Moreover, the sensitivity for CP-SN was also higher than that recently reported by the study of [Rasheed *et al.*, \(2019\)](#). This detected variation in sensitivities between the different studies could be attributed to the population studied, genetic differences, and the relative different prevalence of TB among the various populations.

Results of this study revealed that the GeneXpert assay is highly specific for the PTB diagnosis, in accordance with other earlier studies that have also reported this high specificity ([Scott *et al.*, 2011](#); [Geleta *et al.*, 2015](#)). On the other hand, we currently recorded high specificity of the GeneXpert with regard to CN-SN pulmonary samples, in contrast to several recent studies which reported lower specificity among smear-negative PTB cases ([Kawkitinarong *et al.*, 2017](#); [Ullah *et al.*, 2017](#); [Rasheed *et al.*, 2019](#)). This high specificity of GeneXpert assay suggests that it acts as a rapid test for the diagnosis of PTB in SN specimens, compared to the traditional methods in resource-limited settings.

In this study, with reference to the bacterial culture, the sensitivity of the GeneXpert assay was 100 % for SP-CP and SN-CP pulmonary samples. However, previous studies conducted by [Boehme *et al.*, \(2010\)](#); [Helb *et al.*, \(2010\)](#); [Zeka *et al.*, \(2011\)](#); [Armand *et al.*, \(2011\)](#) have reported similar sensitivity for SP-CP pulmonary specimens and lower sensitivity with SN-CP pulmonary ones. This difference between studies may be due to the quality of samples, and differences in the diagnostic gold standard.

According to [Kohli *et al.*, \(2018\)](#), there is no specific recommendation for the use of the GeneXpert assay in specimens other than sputum. The sensitivity of the GeneXpert assay in EPTB observed in this study is similar to other previously published studies of [Tortoli *et al.*, \(2012\)](#); [Zmak *et al.*, \(2013\)](#); [Sharma *et al.*, \(2014\)](#); [Singh *et al.*, \(2016\)](#), which measured its sensitivity with reference to a bacterial culture. Among the CP specimens detected in this study, using the bacterial culture as the reference standard, the GeneXpert assay exhibited excellent sensitivity (100 %) for SP-CP and SN-CP extra-pulmonary specimens. These results correlate well with the previously published studies conducted by [Zmak *et al.*, \(2013\)](#); [Bankar *et al.*, \(2018\)](#).

In the current study, with reference to the CRS, the GeneXpert assay had high specificity, but limited sensitivity for MTB detection in extra-pulmonary specimens. Although the positive results can be of use in rapid identification of the disease; however, negative results offer less confidence for excluding EPTB. The GeneXpert assay had different sensitivities (33 % to 100 %) for MTB detection in EPTB in different types of specimens, similar to the recent study of [Allahyartorkaman *et al.*, \(2019\)](#). Furthermore, with the CRS as the reference standard, the GeneXpert assay had high sensitivity for SP and low sensitivity for SN extra-pulmonary specimens, similar to the previous report of [Armand *et al.*, \(2011\)](#) but different from the findings of [Zeka *et al.*, \(2011\)](#).

With reference to the CRS, the sensitivity of the GeneXpert assay varied considerably between the extra-pulmonary specimen types. Its sensitivity for pleural effusion specimens was markedly lower than that for other extra-pulmonary specimens. Suboptimal performance of the assay was observed for body fluids; pleural effusion, CSF and urine, while its sensitivity was better with bone and LN biopsy, which is consistent with several previous studies of [Tortoli *et al.*, \(2012\)](#); [Patel *et al.*, \(2013\)](#); [Sharma *et al.*, \(2014\)](#). As the available GeneXpert assay buffer has been assigned for sputum, the sensitivity for specimens other than sputum could give rise to many false-negative results, as reported by [Theron *et al.*, \(2014\)](#).

The limited diagnostic utility of the GeneXpert assay in pleural fluid is attributed to the poor sensitivity of the assay in pleural fluid ([Porcel, 2009](#); [Ahmed *et al.*, 2020](#)). However, in the investigations of pleural TB, with available resources, the GeneXpert assay should be considered, since it has better sensitivity than staining and is more rapid than histology and bacterial culture, as highlighted by [Denkinger *et al.*, \(2014\)](#). A recent work conducted by [Tadesse *et al.*, \(2019\)](#) reported that we can't rely on a negative result of GeneXpert assay for the exclusion of the diagnosis of EPTB in fluid specimens, and thus ATT should be started in patients with a high clinical possibility of EPTB.

According to a meta-analysis study conducted by [Denkinger *et al.*, \(2014\)](#) that assessed the diagnostic precision of the assay versus a CRS, the pooled sensitivity of GeneXpert with pleural fluid samples was 21.4 % versus 33.7 % in the present study. However, the GeneXpert sensitivity for CSF was similar to ours. Furthermore, and parallel to the current findings, [Vadwai *et al.*, \(2011\)](#) reported low sensitivity of the GeneXpert assay for detecting the MTB in CSF. According to [Denkinger *et al.*, \(2014\)](#), the WHO recommends the GeneXpert assay as the preferred initial test for the diagnosis of tuberculous meningitis.

Limited numbers of Egyptian studies have evaluated the diagnostic ability of the GeneXpert assay for PTB diagnosis, with reference to the bacterial culture ([Moussa *et al.*, 2016](#); [Omar *et al.*, 2019a](#); [Tag Eldin *et al.*, 2019](#)). However, the tuberculosis pleural effusion was the only EPTB specimen tested by the GeneXpert assay in two recent Egyptian studies conducted by [Omar *et al.*, \(2019b\)](#); [Ahmed *et al.*, \(2020\)](#). Both studies have reported poor sensitivity but good specificity of the assay. The GeneXpert assay offers rapid detection of rifampicin resistance with reasonable precision ([Singh *et al.*, 2016](#)). Rifampicin resistance was detected in only 13/152 (8.6 %) samples in this study, which was lower than that detected by [Tag Eldin *et al.*, \(2019\)](#), but similar to that recorded by [Omar *et al.*, \(2019a\)](#). In this study, the GeneXpert assay had a strong inter-rater agreement with the bacterial culture for both PTB and EPTB; respectively, which differs from the findings of [Allahyartorkaman *et al.*, \(2019\)](#).

Finally, lack of studying the diagnostic precision of the GeneXpert assay on samples other than those assessed in this study (e.g., blood), in addition to shortage of studying the impact of the assay on patient's outcomes; are the most recognized limitations of this study.

Conclusion

The GeneXpert assay showed better sensitivity for the diagnosis of the PTB than the EPTB. Diagnosis of EPTB should be based on combining many tests such as bacterial culture and the GeneXpert assay. The diagnostic accuracy of this assay was high in ruling-in, but not in ruling-out of EPTB. The manufacturer or the WHO should provide standard recommendations for the non-respiratory sample preparation.

Conflict of interest

All authors report no conflict of interests relevant to this article.

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Ethical approval

The Ethics Committee of the Faculty of Medicine, Fayoum University had approved this study according to the relevant guidelines. The patient's consents and statement of protection of the patient's privacy are provided.

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