ORIGINAL ARTICLE

A Study of the Added Value of Xpert MTB/RIF Assay for Assessment of Pulmonary Tuberculosis Transmission Risk

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ABSTRACT

Background: The elimination of TB requires early, rapid and accurate diagnosis and treatment. TB mortality is mainly due to delayed diagnosis or misdiagnosis which also increases the possibility of TB transmission. Xpert MTB/RIF assay is a new rapid point-of-care test that can in 2 hours, simultaneously detect, Mycobacterium tuberculosis and rifampicin resistance and is capable of overcoming many of the current operational problems in TB diagnosis. ***Objective:*** to evaluate the usefulness of Xpert MTB/RIF test for early, rapid, and accurate diagnosis of pulmonary tuberculosis and to determine the added value of the assay to address patients' transmission potential in a fast, accurate and reliable manner. ***Methodology:*** Spot sputum samples collected at hospital presentation from fifty patients with symptoms and signs suggestive of pulmonary TB and / or who have suggestive TB chest x-ray, during the period from August 2017 to July 2018. All microbiological analyses were performed on the same sample after splitting it into two aliquots. One aliquot was tested by ZN staining and Xpert MTB/RIF assay and the other was cultured for isolation of TB bacilli by conventional method. Diagnostic performance was done for both ZN stained smear and XPERT MTB/RIF assay using culture as a reference standard**.** ***Results:*** Compared with culture, the sensitivity and the specificity of XPERT MTB/RIF assay were 100% and 75% respectively, PPV was 95.5%, NPV was 100% and there was very good (kappa = 0.834) agreement between both methods. Two samples tested positive by XPERT MTB/RIF though negative by culture and there was significant negative correlation between semi-quantitative results of XPERT MTB/RIF test expressed in cycle threshold values against grade of smear positivity. ***Conclusion:*** Xpert MTB/RIF assay had a very high sensitivity and specificity when compared to the reference standard method. It is a single test that is more effective than smear dependent strategies for both T.B diagnosis and evaluation of patient transmission potential. It can detect M. tuberculosis complex and rifampicin resistance in two hours. The assay is rapid, easy to perform with no technical difficulties. This new diagnostic method can lead to more cases of active TB to be detected, avoiding treatment delay, reduction in the transmission risk and improvement in TB control.

INTRODUCTION

Tuberculosis (TB) is the most common infectious disease worldwide. It is caused by *Mycobacterium tuberculosis* (MTB) complex [1]. In the global TB report [2] WHO reported that in 2013, nine million people developed TB**.** In Egypt, TB is considered one of the most important public health problems after hepatitis C [3].

The elimination of TB requires early, rapid and accurate diagnosis and treatment. TB mortality is mainly due to delayed diagnosis or misdiagnosis which furthermore increases the possibility of TB transmission. Microscopy with Ziehl-Neelsen (ZN) staining is the most frequent laboratory technique used to diagnose TB in most developing countries. However, limitations associated with microscopy lead to misdiagnosis [4].

Culture is a more sensitive method for diagnosis of TB, which can also include testing for drug resistance. However, such techniques require complex laboratory infrastructure and staff, and it can take long time, weeks

or months to get results. In fact, most people who need culture to diagnose their TB will not have access to the test results in time to save their lives or to prevent transmission to others [5].

It is therefore essential to improve and probably replace microscopy with simpler and more accurate diagnostic techniques. PCR- based approaches developed over the last decades have considerably improved and enhanced diagnosis of tuberculosis; however, such approaches require specific laboratory preparations. The need for simpler PCR systems has been solved with the GeneXpert diagnostic system (Cepheid, Sunnyvale, CA, USA), which is a simple point- of-care test (POCT) that can in 2 hours, simultaneously detect Mycobacterium tuberculosis and rifampicin resistance related mutations of the rpoB gene [5].

Xpert MTB/RIF assay is a new rapid molecular test that ensured that it was able to overcome several of the current operational problems in TB diagnosis. WHO recommended Xpert MTB/RIF assay in 2010 for use in prevalent, resource limited countries as a first line test for rapid diagnosis of TB [6].

Transmission potential was assessed by smear microscopy; a positive smear result was accompanied by a high transmission risk. Patients with suspected tuberculosis, in a low-prevalence setting are isolated till three successive sputum smears are AFB negative because of the low sensitivity of this method. Such strategy can have a great influence on patient placement and can result in unnecessary prolonged stays in isolation rooms. Several studies have reported that tuberculosis transmission can happen from smear-negative patients. Thus, smear examination has a limited sensitivity and specificity for tuberculosis diagnosis[7].

This study aimed to evaluate the usefulness of Xpert MTB/RIF test for early, rapid, and accurate diagnosis of pulmonary tuberculosis and to determine the added value of the assay to address patients' transmission potential in a fast, accurate and reliable manner.

METHODOLOGY

Ethics approval

The study was approved by the local Ethics Committee of Benha faculty of medicine. Written informed consent was obtained from participants for use of their sputum in diagnostics research. Confidentiality was kept throughout the study and laboratory findings were provided to the study participants

Study participants

Fifty six patients confirmed to have pulmonary TB or with symptoms and signs suggestive of pulmonary TB and / or who have suggestive TB chest X-ray were enrolled in the study. Recruitment procedures comprised medical history, clinical examination and chest radiography. The study was conducted from August 2017 to July 2018 at Clinical Pathology Department of Benha university hospitals, and Benha chest hospital.

Sputum samples collection

Spot sputum samples collected at hospital presentation, when multiple samples were available for a certain patient, only the first sample was considered. The minimum acceptable volume of sputum was 2 mL for analysis

Six patients were excluded from the study because they were incapable to produce sputum during recruitment

Sputum sample processing

All microbiological analyses were performed on the same sample after splitting it into two aliquots. One aliquot was tested by ZN staining and Xpert MTB/RIF assay. The other was cultured by conventional method for isolation of TB bacilli.

Laboratory methods

The collected sputum specimens were tested for TB bacilli by:

Ziehl-Neelsen Staining:

The staining was performed on non-decontaminated sputum samples, purulent sputum were liquefied with the mucolytic agent N- acetyl- L- cysteine (NALC) to increase the homogeneity of the sample before smear preparation[8]**.** Smear grading was defined following International Union against Tuberculosis and Lung Disease scale**,** as follows:

Number AFB observed	Report
None	Negative for AFB
1-9/100 oil immersion field	Scanty.
10-99/100 oil immersion field	(1+)
1-10/1 oil immersion field	(2+)
> 10/1oil immersion field	(3+)

A patient was considered positive if a minimum of one smear was graded scanty or higher

Culture on Lowenstein-Jensen (LJ) medium:

Digestion decontamination of sputum was performed by NALC - NaOH method. Culture media were inoculated and incubated for up to 8 weeks using standard protocol [9]

XPERT MTB/RIF assay

The Xpert MTB/RIF assay to be used with the Cepheid GeneXpert diagnostic system is a semi-quantitative nested real-time PCR for: (1) MTB DNA detection in sputum samples of (2) finding of rifampicin resistance related mutations of the rpoB gene. The GeneXpert diagnostic system automates and integrates

sample processing, nucleic acid amplification and recognition of the target sequences. The primers in the Xpert MTB/RIF assay amplify a part of the rpoB gene containing the core {81 base pair} region. The assay uses molecular beacon technology, which are nucleic acid probes that identify and report the presence or absence of the normal, rifampicin-susceptible, (wild type) sequence of the rpoB gene of TB. Five different colored beacons are used, each covering a separate nucleic acid sequence within the amplified rpoB gene. A beacon fluoresces, when it binds to the matching sequence which points to the presence of one of the gene sequences that, is typical for rifampicin-susceptible TB. Failure of binding of the beacon or delayed binding to the matching sequence denotes potential rifampicin resistance. The number of positive beacons and timing of detection (when the fluorescent signal rises above baseline cycle threshold) as well as the results of sample processing controls allows the test to differentiate between the following:

- TB detected, rifampicin resistance is not detected as shown in figure 3.
- TB detected, rifampicin resistance detected as shown in figure 4.
- TB not detected as shown in figure 5.
- Invalid result.

A reagent buffer containing NaOH and isopropanol was added in a ratio of 2/1 to the sputum sample ensuring a final volume of at least 2 ml. After incubation for 15 min with intermittent hand mixing, 2 ml of the sample was added to the cartridge that contains the wash buffer, reagents for DNA extraction and PCR amplification, and fluorescent detection probes. After the cartridge was placed in the instrument module, the automated processes took about two hours. Results were reported as MTB negative or positive with semi-quantification (low, moderate, or high) and RIF sensitive or resistant [10].

Statistical analysis

The collected data were tabulated and analyzed using SPSS version 16 software (SPSS, Inc., Chicago, IL, USA). Categorical data were presented as number and percentages, Kappa test was used to detect the degree of agreement between different techniques. Continuous data were tested for normality using Shapiro-Wilks test, assuming normality at P>0.05. Ranked correlation was assessed by Spearman's correlation coefficient (rho) ROC curve analysis was used to assess the diagnostic performance of ZN and XPERT. The accepted level of significance in this work was stated at 0.05 (P <0.05 was considered significant). Value of K (0.2=Poor, 0.21-0.4= Fair, 0.41-0.6= Moderate, 0.61-0.8 = Good, 0.81-1.00=Very good)

RESULTS

In the current study, 50 sputum samples ordered for routine AFB smear microscopy and culture on L.J medium were included. 36 samples were obtained from male and 14 from female with male to female ratio of 2.6: 1. The age of the study participants ranged from 22 to 77 years with mean age of 44.76 ± 14.36 year

42/50 (84%) patients had microbiologically confirmed pulmonary tuberculosis with Mycobacterium TB detected in their sputum culture. When the samples examined by smear microscopy 34/50 (68%) had smear positive, culture positive (S+/C+) TB, whereas 8/50 (16%) had smear negative, culture positive (S-/C+) TB.

Eight patients out of 50 (16%) were shown to have no TB (C-).These patients were smear negative and culture negative and showed an improvement upon receiving antibiotic therapy.

Patient classification

Participants were classified into 2 groups:

Group 1: microbiologically confirmed TB

(S +/ C+) = 34

(S -/ C+) = 8

Group 2: no microbiological proof of TB no TB (C-) = 8

A confirmed positive culture was used as a gold standard. Compared with culture, the sensitivity, specificity, PPV, and NPV for Z.N smear was 81%, 100%, 100% and 50% respectively as shown in table 1 & figure 1 and there was moderate agreement (kappa = 0.576) between the two methods as shown in table 2

Performance of XPERT MTB/RIF assay

A total of 50 sputum specimens were run in XPERT MTB/RIF assay, all specimen results were obtained within two hours of starting the analysis. No technical problems were encountered with the use of the instrument that required intervention.

From the 50 sputum samples examined, 44 (88%) were positive and 6 (12%) were negative. Two false positive results were recorded in the results of XPERT MTB/RIF assay. Compared with culture, the sensitivity was 100%, specificity was 75%, PPV was 95.5% and NPV was 100% as shown in table 1 & figure 1 with very good agreement (kappa = 0.834) between both assays as shown in table 3.

Two samples tested positive by XPERT MTB/RIF but negative by culture. These samples were from patients presented with cough, dyspnea and chest pain. CT scan revealed the presence of pulmonary consolidation and cavitation and an anti-tuberculus drugs was initiated

We found that all samples with smear examination positive had a positive XPERT MTB/RIF test, thus corresponded to the overall sensitivity of the XPERT MTB/RIF to detect smear positive samples of 100% (34/34). Conversely samples with negative XPERT

MTB/RIF results were all also smear negative (NPV= 100%, 6/6) and there was moderate agreement between both methods (kappa = 0.449) as shown in table 4

The XPERT MTB/RIF provides semi quantitative results defined by the manufacturer as follows: positive very low (Ct >28), low (Ct 22 – 28), medium (Ct 16 - 22), or high (Ct <16). A Ct value of ≥ 40 denoted a negative result. In the present study, there is significant negative correlation between the semi-quantitative results of XPERT MTB/RIF method expressed in Ct values (which correlate inversely with the target DNA concentration) and the grade of smear positivity as shown in figure 2

The transmission potential of tuberculosis patients is generally evaluated on the basis of smear examination. Smear negative patients are generally considered as non-infectious or of low transmission risk. In this study, 10 patients were identified positive by XPERT MTB/RIF test but negative by smear examination. Most of these patients were symptomatic and some of them presented with pulmonary cavitation which is frequently associated with high transmission potential. This high transmission potential was ignored by the initial negative smear result

Out of 44 specimens with MTB positive XPERT test, rifampicin resistance was detected in 4 cases (9.1%). All of them were culture and smear positive and have Ct value < 16. The four patients presented with shortness of breath, cough and expectoration, 2 of them presented with hemoptysis and only one presented with chest pain.

Table 1: Diagnostic performance test for ZN stained smear and XPERT MTB/RIF using culture as a gold standard

	TP	FN	TN	FP	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC
Z.N stained smear	34	8	8	0	81%	100%	100%	50%	84%	0.901
XPERT MTB/RIF	42	0	6	2	100%	75%	95.5%	100%	96%	0.879

TP =true positive TN= true negative FP = false positive FN = false negative PPV = positive predicted value NPV = negative predicted value.

Table 2: Comparison between the results of Lowenstein Jensen medium & ZN stained smear in diagnosis of pulmonary TB

		Lowenstein Jensen medium		Kappa value P	Degree of agreement
		Positive (n=42)	Negative (n=8)		
Ziehl Neelsen stained smear	Positive (n=34)	34(81%)	0 (0%)	0.576 <0.001 (HS)	84%
	Negative (n=16)	8 (19%)	8 (100%)		

Table 3: Comparison between the results of LJ medium and XPERT MTB/RIF in diagnosis of pulmonary TB

		Lowenstein Jensen medium		KappaValue	Degree of agreement
		Positive(n=42)	Negative(n=8)		
XPERT MTB/RIF	Positive(n=44)	42 (100%)	2 (25%)	0.834 <0.001 (HS)	96%
	Negative(n=6)	0 (0%)	6 (75%)		

Table 4: Comparison between the results of XPERT MTB/RIF & ZN stained smear in diagnosis of pulmonary TB

		Ziehl Neelsen stained smear		KappaValue	Degree of agreement
		Positive (n=34)	Negative (n=16)		
XPERT MTB/RIF	positive (n=44)	34 (100%)	10 (62.5%)	0.449 =0.001 (HS)	80%
	Negative (n=6)	0 (0%)	6 (37.5%)		

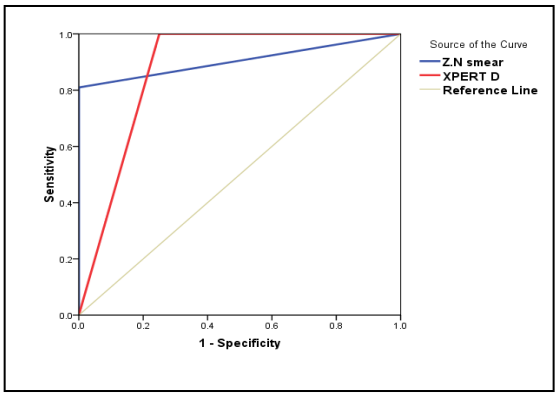

Fig. 1: Diagnostic performance test for ZN stained smear and XPERT MTB/RIF using culture as a gold standard

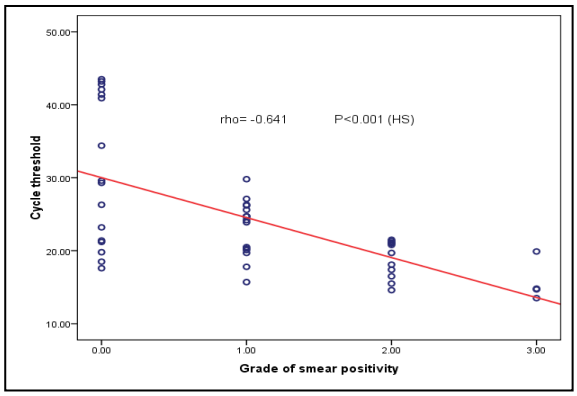

Fig. 2: Scatter graph showing significant negative correlation between cycle threshold values versus grade of smear positivity

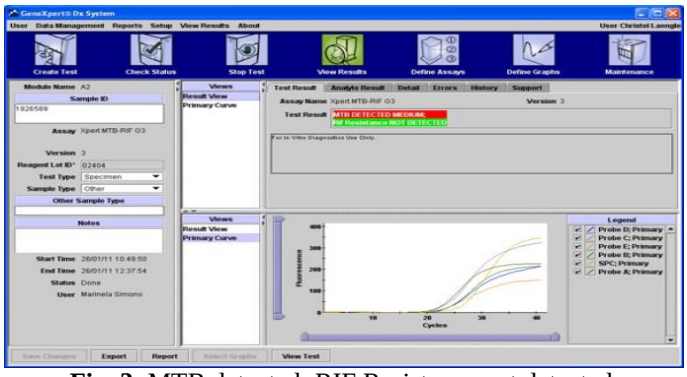

Fig. 3: MTB detected; RIF Resistance not detected.

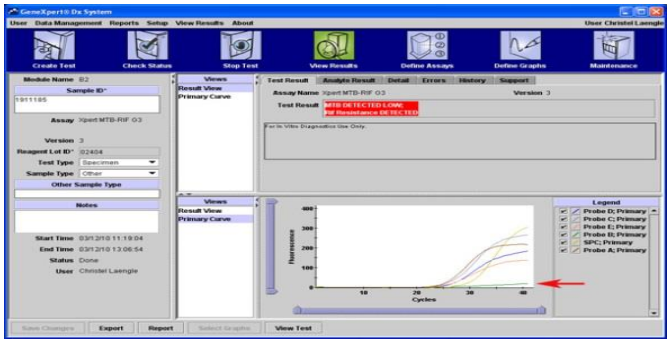

Fig. 4: MTB detected, RIF Resistance detected.

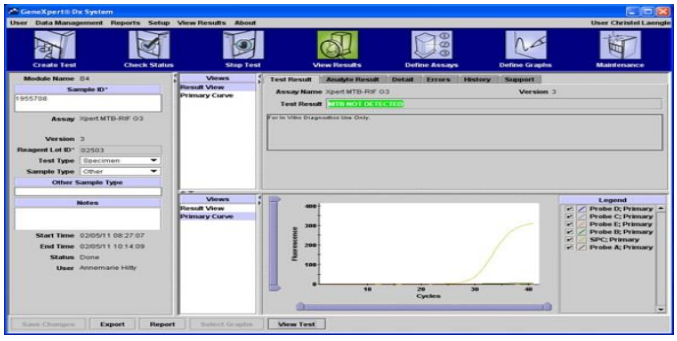

Fig. 5: MTB target DNA was not detected.

DISCUSSION

Early diagnosis of Tuberculosis is important for patient management and successful outcomes. False-negative results and misdiagnosis of TB are common in developing countries, as the majority of TB control programs use ZN smear microscopy [11].

Ziehl- Neelsen smear microscopy remains the most frequent laboratory technique both for tuberculosis diagnosis and evaluation of patient infectiousness; however, it has many disadvantages, it is labor intensive, requires specialized technicians, has a limited sensitivity and multiple visits are required leading to higher default. Also, smear microscopy cannot differentiate between MTB and NTM because it has limited specificity [12].

Sputum culture for mycobacteria, although considered the gold standard, it is slow and takes 6-8 weeks to yield a final result. In addition it requires proper infrastructure and technical expertise [12].

The WHO endorsed the Xpert MTB/RIF test for use with the GeneXpert diagnostic system which is a semi-quantitative nested real-time PCR for the diagnosis of TB. The GeneXpert uses a DNA PCR technique for concurrent detection of MTB and Rifampicin resistance related mutations. It is the first totally automated bench top cartridge based nucleic acid amplification test (CB-NAAT) for TB detection that comprises the whole necessary steps of DNA PCR**.** The GeneXpert provides results within 2 hours with high diagnostic accuracy [13, 14].

This study aimed to evaluate the usefulness of Xpert MTB/RIF test for early, rapid, and accurate diagnosis of pulmonary tuberculosis and to determine the added value of the assay to address patients' transmission potential in a fast, accurate and reliable manner.

In the current study, 36 sputum samples were obtained from male and 14 from female with male to female ratio of 2.6: 1. The age of the study participants ranged from 22 to 77 years with mean age of 44.76 ± 14.36 year

In the study carried out by Ganguly et al. male subjects accounted for 85.71% against 14.29 % for females [15]. This may be due to the fact that male subjects were more exposed to risk factors of TB than female**.**

In the present study, high prevalence of culture positive TB 84% (42/50) was detected among the study participants, ZN smear microscopy diagnosed 68% (34/50) of cases, while Xpert MTB/RIF detected 88% (44/50) of cases.

Taking conventional culture as a reference method, the sensitivity of Xpert MTB/RIF was 100% which is comparable to Willamson et al [16] and Carriquitry et al.[17] they reported 96.7% and 100% in Pero and in New-Zealand respectively and there is very good agreement {kappa=0.834, P<0.001 (HS)} between conventional culture on L.J medium and Xpert MTB/RIF methods for detection of pulmonary TB.

However, reports indicate that the Xpert MTB/RIF test sensitivity is as low as 62.6% in South Africa and 67.6% in Adama, Ethiopia [18, 19] The higher sensitivity detected in the present study may be explained by the use of samples under more selective conditions than other studies which used samples from consecutive patients without any previous selection, another cause for this difference is the use of fresh samples in our study, while they use frozen samples which may cause some degradation of the TB DNA. This difference may also be due to different sample type.

In the current study, the specificity of the evaluated assay was 75%, higher specificity was found by other studies[19,20] which have shown a specificity ranging from 94.1% to 100%. This difference may be explained by the presence of two false positive results detected by Xpert MTB/RIF from two patients whose sputum was culture negative, these positive results may be true positive due to high sensitivity of the assay, presence of residual DNA of old dead organisms in patient with previous history of TB or a sub- clinical relapse of the disease.

In the present study, we found that the sensitivity and specificity of Xpert MTB/RIF assay were higher than those of ZN smear microscopy and there was moderate (kappa=0.449, P=0.001) agreement between the two methods. These results are in harmony with most of the studies conducted before for evaluation of the performance of Xpert MTB/RIF[21,22]. In addition the sensitivity of ZN smear may vary between different geographical regions and within the same regions between different laboratories, which is unlikely to occur with nucleic acid- based assays methods.

ZN smear microscopy is still done to assess the degree of patients' infectivity; the tuberculosis infectious dose is lower than ten bacilli, whereas the sensitivity of ZN smear microscopy ranges from 5000 to 10000 AFB/mL, this means that microscopy would miss many potentially infectious patients [13].

The present study reported an added value for the Xpert MTB/RIF over smear microscopy in evaluating the transmission potential of patients with pulmonary tuberculosis because it is negative when the smear microscopy is negative suggesting no or low patient infectiousness and when positive, the semi-quantitative result can help to distinguish patients with high risk of being infectious (high or medium positive results) from those with low risk (low or very low positive results). In addition, our study reported that 22.7% (10/44) of

patients with Xpert MTB/RIF positive results had negative smear microscopy although they had a clinical presentation suggestive of high transmission potential; this means that microscopy would miss many potentially infectious cases.

Comparing the semi-quantitative results of the Xpert test with those of smear microscopy using international smear grading system, there is significant negative correlation between cycle threshold values versus grade of smear positivity. We found that all cases with negative results by Xpert MTB/RIF were also negative by smear examination (NPV = 100%).

Among 44 specimens with MTB positive result by XPERT, 4 rifampicin resistant samples were detected (9.09%). The four samples were culture and smear positive and have Ct value < 16. The four patients presented with shortness of breath, cough and, expectoration, 2 of them presented with hemoptysis and only one case presented with chest pain. In slightly lower frequency than the present study, Khalil et al.[22] found that 6 out of 93 isolates (6.5%) showed rifampicin resistance, while 87 isolates (93.5%) were susceptible strains.

The additional cost made by Xpert MTB/RIF assay must be compared to the benefit from avoiding poor sensitivity and specificity of a smear based strategy. To minimize the relative high cost of the assay, it is important to decrease the chance of getting failed or invalid test result. The present study found that an Xpert MTB/RIF based strategy is more effective than smear based for both detection of pulmonary tuberculosis and evaluation of transmission potential for infected patients.

One of the limitations in our study is that Xpert MTB/RIF was done for patients with increased probability of pulmonary tuberculosis and the second limitation is the reliance on smear microscopy to evaluate transmission potential of patients.

CONCLUSION

Xpert MTB/RIF assay had a very high sensitivity and specificity when compared to the reference standard method. in addition it was positive in two patients who were classified as smear negative, culture negative but with strong clinical data suggestive of pulmonary tuberculosis. The assay was rapid, easy to perform and no technical difficulties were encountered during use of the instrument.

Our study also found that one single sputum sample tested by Xpert MTB/RIF is synonymous to three microscopic smear examinations with better sensitivity than smear microscopy and the results could be obtained on the day of sample collection. Thus this new diagnostic method (point of care test) can lead to more cases of active TB to be detected, avoiding treatment delay, reduction in the transmission potential (tuberculosis transmission) and improvement in TB control

For future studies, it is important to ascertain that those patients reported positive by Xpert MTB/RIF test only and negative by smear microscopy and culture are true TB cases.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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