Evaluation of the diagnostic performance of the Xpert® MTB/RIF assay in pulmonary and extrapulmonary samples

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ABSTRACT

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Introduction: *This study aimed to evaluate the performance of the Xpert MTB/RIF assay in the identification of M. tuberculosis in pulmonary and extrapulmonary clinical samples by taking the results of the BACTEC MGIT 960TB culture system as a reference.*

Materials and Methods: *A total of 11,341 specimens sent to Sivas Cumhuriyet University Application and Research Hospital Tuberculosis Laboratory for microbiological examination with suspicion of tuberculosis infection between January 2013 and December 2019 were examined, and 6847 clinical specimens that underwent culture (BACTEC MGIT 960TB), Xpert MTB/RIF and AFB (Acid-fast bacilli) testing were selected and included in our study. Of the samples included in the study, 5096 samples were pulmonary, and 1751 were extrapulmonary samples.*

Results: *In our study, sensitivity, specificity, PPV and NPV values of Xpert MTB/RIF and AFB were calculated by taking TB culture test as reference test. The sensitivity of the Xpert MTB/RIF assay was calculated as 96.1%, specificity as 99.7%, positive predictive value (PPV) as 88.2%, and negative predictive value (NPV) as 99.9%. These values for pulmonary samples were determined as 98.3%, 99.7%, 89.9%, and 99.9%, respectively. For extrapulmonary samples, the sensitivity of the assay was found as 89.4%, specificity as 99.5%, PPV as 82.9%, and NPV as 99.7%. The sensitivity and PPV values for AFB-positive samples were found to be 99.0% and 97.1%, respectively. For AFB negative samples, the sensitivity, specificity, PPV, and NPV values were determined as 90.5%, 99.7%, 73.8%, and 99.9%, respectively.*

Conclusion: *A large number of clinical samples were studied with the Xpert MTB/RIF test in our study. It can be a guide in determining the performance of the test under the conditions of our country. Especially in the diagnosis of extrapulmonary TB, the effectiveness of the Xpert MTB/RIF assay has not been certainly proven in countries having a moderate prevalence of TB, such as*

Turkey. In most of the published studies, only a small part of the samples is extrapulmonary samples. So, our study provides valuable results in terms of evaluating a large number of extrapulmonary samples.

Key words: *GeneXpert MTB/RIF; BACTEC MGIT 960 TB; Mycobacterium tuberculosis; rifampicin resistance*

ÖZ

Xpert® MTB/RIF testinin pulmoner ve ekstrapulmoner örneklerde tanısal performansının değerlendirilmesi

Giriş: *Bu çalışmanın amacı BACTEC MGIT 960TB kültür sistemin sonuçları referans alınarak Xpert MTB/RIF testi'nin pulmoner ve ekstrapulmoner klinik örneklerde M. tuberculosis tanımlanmasındaki performansının değerlendirilmesidir.*

Materyal ve Metod: *Çalışmaya TB şüphesiyle veya TB infeksiyonunu destekleyen bulgularla Sivas Cumhuriyet Üniversitesi Uygulama ve Araştırma Hastanesi Tüberküloz Laboratuvarı'na gönderilen toplam 11.341 örnekten, aynı anda kültür (BACTEC MGIT 960TB), Xpert MTB/RIF (Cepheid, Sunnyvale, CA, ABD) ve AFB testi istemi yapılan 6847 klinik örnek dahil edilmiştir. Çalışmaya alınan örneklerin 5096 tanesi pulmoner, 1751 tanesi ise ekstrapulmoner örneklerden oluşmuştur.*

Bulgular: *Xpert MTB/RIF testinin duyarlılığı %96,1; özgüllüğü %99,7; pozitif prediktif değeri (PPD) %88,2; negatif prediktif değeri (NPD) ise %99,9 olarak hesaplanmıştır. Pulmoner örnekleri için bu değerler sırasıyla %98,3; %99,7; %89,9 ve %99,9 olarak belirlenmiştir. Ekstrapulmoner için ise testin duyarlılığı %89,4; özgüllüğü %99,5; PPD %82,9 ve NPD %99,7 olarak bulunmuştur. AFB pozitif örnekler için duyarlılık ve PPD değeri sırasıyla %99,0 ve %97,1 olarak bulunmuştur. AFB negatif örnekler için ise duyarlılık, özgüllük, PPD ve NPD değerleri sırasıyla; %90,5; %99,7; %73,8 ve %99,9 olarak belirlenmiştir.*

Sonuç: *Çalışmamızda Xpert MTB/RIF testi ile çok sayıda klinik örneğin çalışılmış olması, testin ülkemiz koşullarında performansının belirlenmesinde yol gösterici olabilir. Özellikle ekstrapulmoner TB tanısında, Xpert MTB/RIF testinin etkinliği, Türkiye gibi orta derecede TB prevalansına sahip ülkelerde kesin olarak gösterilememiştir. Çalışmamız, çok sayıda ekstrapulmoner örneğin değerlendirilmesi açısından değerli sonuçlar vermektedir.*

Anahtar kelimeler: *GeneXpert MTB/RIF; BACTEC MGIT 960 TB; Mycobacterium tuberculosis; rifampisin direnci*

INTRODUCTION

Tuberculosis (TB) has been one of the most important infectious diseases throughout human history and is still the cause of high morbidity and mortality worldwide. Until it was brought under control in the 19th century, it continued in the form of large epidemics. While there was an idea that it was almost eradicated in the 1980s, in 1993, the World Health Organization included the disease into the 'global emergency health' problems (1). In order to successfully treat and control the disease, it is necessary to determine the microbial agent and drug sensitivity quickly. Especially if multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB patients cannot be quickly diagnosed and treated, morbidity and mortality increase, and resistance against new TB drugs may also develop (2).

AFB (Acid-fast bacilli) smear microscopy and culture methods are still essential in detecting the agent and diagnosing TB. Although culture is the gold standard method, it is time-consuming and results in around six weeks. Likewise, although microscopy is fast and cheap, its sensitivity varies (20-80%). The bacilli detection limit is 104-105 AFB/ml. The diagnosis is difficult because the bacilli load is low, especially at the beginning of the disease (3-5).

The limiting factors of conventional methods have led researchers to simple and rapid diagnostic tests. Nowadays, there are molecular techniques targeting and amplifying different genes on mycobacterial genome for rapid TB diagnosis. One of these, the GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA) system is an automated, integrated, real-time PCR system developed for the rapid detection of rifampin (RIF) resistance of *Mycobacterium tuberculosis* (MTB) (6,7).

As in other diagnostic tests, the performance of the Xpert MTB/RIF assay also varies depending on the prevalence of TB disease, rifampicin resistance in the tested population and the reference test used. WHO reports that the Xpert MTB/RIF assay should be used as the first diagnostic test, especially in adults and children who are expected to have MDR-TB or HIV-associated TB (8).

The BACTEC MGIT 960 culture system (Becton Dickinson Microbiology System, Sparks, MD, USA) is a culture method that used for diagnostic purposes in routine TB laboratories nowadays. WHO also recommends the use of conventional culture methods in routine TB laboratories, reports that the liquid culture is the gold standard in the diagnosis of TB and that it gives results faster in comparison with the solid cul-

ture. In particular, culture is required to monitor the response of MDR-TB patients to anti-TB treatment (8). This study aimed to evaluate the performance of the XPERT MTB/RIF assay in the identification of M. tuberculosis in pulmonary and extrapulmonary clinical samples by taking the results of this system, which is also used in our laboratory.

MATERIALS and METHODS

Clinical Samples

Among the 11,341 samples sent to Sivas Cumhuriyet University Application and Research Hospital Tuberculosis Laboratory with the suspicion of TB or findings supporting TB infection between January 2013 and December 2019, 6847 clinical samples, for which culture (BACTEC MGIT 960TB), Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA), and AFB testing were requested simultaneously, were included in the study. Of the samples included in the study, 5096 were pulmonary [(3856 sputum, 1050 bronchoalveolar lavage (BAL) and bronchial aspirate (BA), 116 fasting gastric fluid (FGF), 77 endotracheal aspirates (ETA) and transtracheal aspirates (TTA)], and 1751 were extrapulmonary [116 urine, 350 pleural fluid, 155 tissue, 155 cerebrospinal fluid (CSF), 817 abscesses, 116 peritoneal fluid, 39 joint fluid] samples. Repeat specimens from the same patient were excluded from the study.

Processing of the Samples and Culture

Decontamination and homogenization of the samples were performed with a standard 4% N-acetyl-L-cysteine + sodium hydroxide (NALC-NaOH), and they were neutralized with phosphate buffer. Afterward, they were concentrated by centrifugation at 3000 rpm for 15 minutes. Samples considered sterile were used directly after being centrifuged without being decontaminated. Tissue samples were cut into pieces under sterile conditions, and they were vortexed and homogenized in tubes containing sterile saline and glass beads. All the processed samples were taken into the study for microscopic examination and culture. The Ehrlich Ziehl-Neelsen (EZN) staining method was used in the microscopic examination.

The samples processed for the culture were inoculated into the liquid medium containing Middlebrook 7H9 broth to incubate in the BACTEC Mycobacterial Growth Indicator Tube (MGIT) 960TB (BD, Sparks MD, USA) device. A sample tube that gave a positive

signal while incubation with MGIT for six weeks was taken into the identification and antimycobacterial sensitivity test study. Both the NAP/PNBA (ρ-nitro-α-acetylamino-β-hydroxypropiophenone/ρ-nitro benzoic acid) assay and the Microflex LT MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) system were used for identification.

Xpert MTB/RIF Assay

The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) was carried out in accordance with the manufacturer's recommendations. One volume (0.5 ml) of the processed sample and three volumes (1.5 ml) of the sample reagent were mixed in a sterile centrifuge tube. They were incubated for 15 minutes at room temperature. The mixture was then slowly transferred to the test cartridge, and the cartridge was placed in the Xpert MTB/RIF device. The results from the fully automated system were obtained after two hours and evaluated.

Statistical Analysis

Sensitivity, specificity, positive and negative predictive values were used to evaluate the performance of the Xpert MTB/RIF assay and AFB microscopy. These values were calculated by comparing the Xpert MTB/RIF assay and AFB results with the culture results, the recommended standard method. All analyses were performed using SPSS version 25.0 (SPSS Inc, USA).

Each stage of the research was conducted in accordance with ethical principles. Written permission was obtained from Sivas Cumhuriyet University Non-invasive Clinical Research Ethics Committee before starting the application (Approval number: 2020-01/22, Date: 15.01.2020).

RESULTS

A total of 6847 clinical samples [5096 (74.4%) pulmonary] (Sputum, BAL, BA, AMS, ETA, and TTA), 1751 (25.6%) extrapulmonary (urine, pleural fluid, tissue, CSF, abscess, peritoneal fluid, and joint fluid) samples] were evaluated retrospectively in the study. A total of 176 samples were found to be positive in at least one of the XPERT MTB/RIF or culture methods. In 156 (2.27%) of these samples, *M. tuberculosis* grew in the culture, and the Xpert MTB/RIF assay was found to be positive in 170 (2.48%) of the samples. Of the 170 samples with positive Xpert MTB/RIF assay, 150 (85.2%) were determined to be positive in the culture, and 20 (11.4%) were determined to be

negative in the culture. Six (3.4%) samples that were negative in the Xpert MTB/RIF assay were found to be positive in the culture. AFB was found to be positive in 106 (1.54%) of the samples. Of the 70 (39.7%) AFB negative samples, 48 (27.2) were determined to be positive both in the culture and Xpert MTB/RIF assay. Five (2.8%) of them were found to be positive in the culture and negative in the Xpert MTB/RIF assay, and 17 (9.7%) of them were found to be positive in the Xpert MTB/RIF assay and negative in the culture (Figure 1).

Of the 1751 extrapulmonary samples included in the study, 34 (1.94%) were found to be positive in both the culture and Xpert MTB/RIF assay. Of them, seven (0.4%) were determined to be positive in the Xpert MTB/RIF assay and negative in the culture, while four (0.2%) of them were determined to be negative in the Xpert MTB/RIF assay and positive in the culture (Table 1).

For all the samples examined, the sensitivity of the Xpert MTB/RIF assay was calculated as 96.1%, specificity as 99.7%, positive predictive value (PPV) as 88.2%, and negative predictive value (NPV) as 99.9%. For pulmonary samples, these values were

determined as 98.3%, 99.7%, 89.9%, and 99.9%, respectively. For extrapulmonary samples, the sensitivity of the assay was found as 89.4%, specificity as 99.5%, PPV as 82.9%, and NPV as 99.7%. The sensitivity and PPV values for AFB-positive samples were found to be 99.0% and 97.1%, respectively. The specificity and NPV values could not be calculated. For AFB negative samples, the sensitivity, specificity, PPV, and NPV values were determined as 90.5%, 99.7%, 73.8%, and 99.9%, respectively (Table 1).

DISCUSSION

The Xpert MTB/RIF assay is an essential automated system that can yield results in a much shorter time (two hours) than other conventional methods in detecting TB agents and can reveal RIF resistance simultaneously. In our study, the performance of the Xpert MTB/RIF assay in pulmonary and extrapulmonary clinical samples was evaluated according to the culture method which is still accepted as the gold standard in the diagnosis of TB.

In this study, the Xpert MTB/RIF sensitivity, specificity, PPV, and NPV values were calculated by adhering to the culture and found to be 96.1%, 99.7%, 88.2%, and 99.9%, respectively. In our study, sensitivity,
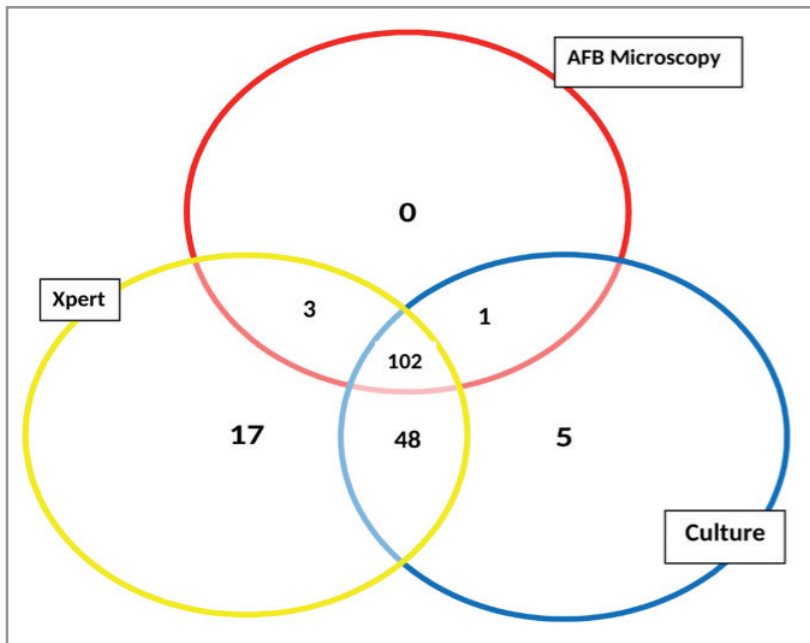

Figure 1. Venn diagram showing the distribution of all samples (pulmonary and extrapulmonary specimen) for detection of *M. tuberculosis* by using Xpert, AFB microscopy and culture. Among 176 samples from TB patients, total 170 were positive for *M. tuberculosis* by Xpert assay, whereas, 106 and 156 samples were positive by AFB microscopy and culture, respectively.

Table 1. Comparison of Xpert MTB/RIF assay performance with culture results

	Positive MTBC culture		Negative MTBC culture						
	Xpert MTB/RIF+ (TP)	Xpert MTB/RIF- (FN)	Xpert MTB/RIF+(FP)	Xpert MTB/RIF- (TN)	Total number	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Positive AFB Smear	102	1	3	-	106	99.0	-	97.1	-
Negative AFB Smear	48	5	17	6777	6847	90.5	99.7	73.8	99.9
Pulmonary	**116**	**2**	**13**	**4965**	**5096**	**98.3**	**99.7**	**89.9**	**99.9**
Sputum	93	2	4	3757	3856	97.8	99.8	95.8	99.9
BAL/BA	18	-	9	1023	1050	100	99.1	66.6	100
Gastric aspirate	3	-	-	113	116	100	100	100	100
ETA/TTA	2	-	-	75	77	100	100	100	100
Extrapulmonary	**34**	**4**	**7**	**1706**	**1751**	**89.4**	**99.5**	**82.9**	**99.7**
Urine	1	1	1	113	116	50	99.1	50	99.1
Tissue	3	-	1	151	155	100	99.3	75	100
CSF	3	1	-	151	155	75	100	100	99.3
Abscess	16	1	4	796	817	94.1	99.5	80	99.8
Plural fluid	9	-	-	341	350	100	100	100	100
Peritoneal fluid	2	1	-	113	116	66.6	100	100	99.1
Synovial fluid	-	-	1	38	39	-	97.4	-	100
Total Specimen	**150**	**6**	**20**	**6671**	**6847**	**96.1**	**99.7**	**88.2**	**99.9**

MTBC: *M. tuberculosis* complex, RIF: Rifampin, NPV: Negative predictive value, PPV: Positive predictive value, TP: True positive, FN: False negative, FP: False Positive, TN: True negative, BAL: Bronchoalveolar lavage, BA: Bronchial aspirate, ETA: Endotracheal aspirate, TTA: Transtracheal aspirate, CSF: Cerebrospinal fluid.

specificity, PPV and NPV values of Xpert MTB/RIF and AFB were calculated. These values of Xpert MTB/RIF were 96.1%, 99.7%, 88.2% and 99.9%, respectively. Similar results were also obtained in some studies conducted in Turkey and across the world (9-12).

In a study conducted by Rice et al., the researchers have found the sensitivity, specificity, PPV, and NPV values of the Xpert MTB/RIF assay to be 89.6%, 97.2%, 87.6%, and 97.7%, respectively. Metcalf et al. have found in their study that the sensitivity, specificity, and NPV values of the Xpert MTB/RIF assay were 86%, 97%, and 97%, respectively. In their study, Özkütük et al. have found that the sensitivity and specificity values of the Xpert MTB/RIF assay were 73.9% and 98.2%, respectively. Compared to these studies, the sensitivity of the Xpert MTB/RIF assay was observed to be higher in our study (13-15).

In our study, the sensitivity and specificity ratios of the Xpert assay in pulmonary samples were found to be 98.3% and 99.7%, respectively, and in extrapulmonary samples, they were found to be 89.4% and 99.5%, respectively. It has also been demonstrated in many studies that the Xpert assay is more sensitive in pulmonary samples than extrapulmonary samples (3,9,16,17).

It is stated that differences in the sensitivities reported in studies may vary depending on the number of samples, the incidence of TB in the region, the type of the sample (pulmonary or extrapulmonary), and the bacilli load (18).

Since the current Xpert MTB/RIF buffer has been developed for sputum samples, the Xpert MTB/RIF sensitivity decreases in non-sputum samples. For this reason, it may be recommended to process extrapulmonary samples effectively and develop particular protocols for this. Furthermore, the scarcity of bacilli density in extrapulmonary samples and the presence of PCR inhibitors are also other factors that affect sensitivity. Therefore, in such samples, special processes are needed to increase the bacilli density and eliminate PCR inhibitors. For example, it has been stated that washing the pellet formed after the first centrifugation in cerebrospinal fluid samples and resuspending in buffer solution can eliminate PCR inhibitors and increase sensitivity (3,19). In addition,

it can be predicted that more careful processing of tissue samples that require fragmentation and homogenization will increase sensitivity in samples with low bacillus load. Homogenization of samples with dense viscosity should also be done carefully to eliminate the effect of PCR inhibitors.

In addition to the detection of *M. tuberculosis*, determining rifampicin sensitivity is also very important for the successful treatment of patients. In all of the samples examined in our study, rifampicin resistance was not observed either in the conventional drug susceptibility test or in the Xpert MTB/RIF assay.

AFB smear microscopy is a simple and inexpensive scanning tool. It provides clinicians with preliminary information for diagnosis. Nevertheless, its sensitivity is low. In the sample examined, there should be 5.000-10.000 bacilli per ml. In contrast, 10-100 organisms are sufficient for culture. It cannot distinguish MTB from other mycobacteria (atypical) that do not cause tuberculosis. It cannot also discriminate between living or inanimate, and, therefore, the false positivity rate is high, and the PPV value is generally low (20-22).

In our study, the sensitivity and PPV values for AFB positive samples were found to be 99.0% and 97.1%, respectively. The specificity and NPV values could not be calculated. For AFB negative samples, the sensitivity, specificity, PPV, and NPV values were determined to be 90.5%, 99.7%, 73.8%, and 99.9%, respectively. In the study conducted by Rice et al., they have found the sensitivity, specificity, PPV and NPV values to be 97.7%, 90.5%, 86.7%, and 98.4%, respectively, in smear-positive samples and 74.5%, 99.2%, 89.7%, and 97.5%, respectively, in smear-negative samples. In our study, sensitivity was higher in smear-negative samples, while the PPV value was found to be lower. Furthermore, 53 (34.0%) of 156 culture-positive samples were found to be smear-negative. Forty-eight of these samples (30.7%) were determined to be positive in the Xpert MTB/RIF assay (13).

In our study, the Xpert MTB/RIF assay gave six false-negative and 20 false-positive results when we accepted the culture as a reference test. While the detection limit reported for sputum samples in the Xpert MTB/RIF assay is 131 CFU/ml, this rate in the liquid culture is 1-50 CFU/ml. Therefore, the low bacilli density may be indicated as the probable reason for six Xpert MTB/RIF negative samples were positive in the culture (20,23).

Another result we encountered in our study was that the TBC DNA amount of the Xpert MTB/RIF report of 20 samples, which were positive in the Xpert MTB/RIF assay and negative in the culture, were found as either low (4) or very low (14). Only two specimens were observed to have a moderate bacterial load. Moreover, it is crucial to determine whether patient groups having 20 Xpert positive, culture-negative samples use anti-TB drugs because, even if there are organisms that are not living in the samples taken from these patient groups, the Xpert MTB/RIF assay may give false results since it is a molecular test for detecting genetic material. Therefore, this test is not designed to be used for monitoring response to therapy (8,24).

Although the use of the Xpert MTB/RIF assay is recommended for rapid diagnosis and ease of application, traditional microscopy is required to monitor the patient during treatment and to detect resistance to anti-TB agents other than rifampicin does not eliminate the need for culture (21).

In our study, patients with positive Xpert result but negative culture result draw attention. The evaluation of the clinical data of these patients will also shed light on how effective the Xpert MTB/RIF test can be in patients who cannot be diagnosed by conventional method. However, due to the retrospective nature of the study, clinical data of this patient group could not be reached or very limited information was obtained. In order not to mislead the reader, these limited data, which were deemed insufficient for analysis, were not included in the article. This situation is the most important limiting factor in our study.

In conclusion, the fact that a large number of clinical samples were studied with the Xpert MTB/RIF assay in our study may be a guide in determining the performance of the test under the conditions of our country. Especially in the diagnosis of extrapulmonary TB, the effectiveness of the Xpert MTB/RIF assay has not been certainly proved in countries having a moderate prevalence of TB, such as Turkey. Most published studies have a low number of extrapulmonary samples. Our study also provides valuable results in terms of evaluating a large number of extrapulmonary samples.

Ethical Committee Approval: The approval for this study was obtained from Sivas Cumhuriyet University Non-invasive Clinical Research Ethics Committee (Decision No: 2020-01/22, Date: 15.01.2020).

CONFLICT of INTEREST

The authors of this meta-analysis declare that they have no conflict of interest.

AUTHORSHIP CONTRIBUTIONS

Concept/Design: MH, CÇ

Analysis/Interpretation: MH, AHTK

Data acqusition: MH

Writing: MH, AHTK

Clinical Revision: MH, CÇ

Final Approval: MH, AHTK

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