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Applicability of Xpert MTB/RIF assay for routine diagnosis of tuberculosis: a four-year single-center experience

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Background/aim: The aim of this study is to assess the Xpert MTB/RIF assay for diagnosis of the *Mycobacterium tuberculosis* complex in clinical samples and to compare the results by reference to the diagnostic method, Bactec MGIT 960.

Materials and methods: A total of 7407 samples were included from patients not primarily suggesting pulmonary or extrapulmonary tuberculosis (TB), collected from patients required to be screened for TB and excluding TB diagnoses since it was a differential diagnosis. Also included were a total of 411 samples from patients primarily suggesting pulmonary or extrapulmonary TB.

Results: In the first group, 152 of 7407 samples yielded positive results with the Bactec MGIT 960, 131 (1.77%) were found positive with Löwenstein–Jensen medium, and 295 (3.99%) were found positive with Ziehl–Neelsen staining. In the second group, 24 (5.8%), 17 (4.1%), and 28 (6.8%) of 411 samples were found positive. Xpert MTB/RIF [27 (6.6%) of 411 samples] detected 3 additional samples as positive, and these 3 cases were clinically compatible with TB.

Conclusion: The Xpert MTB/RIF assay shows superior performance for the diagnosis of TB. Its usefulness in culture-negative patients and the best method for integrating this diagnostic method into current tuberculosis diagnostic algorithms both need further study.

Key words: Tuberculosis diagnosis, *Mycobacterium tuberculosis* complex, Xpert MTB/RIF assay, Bactec MGIT 960

1. Introduction

Tuberculosis (TB) is one of the major global public health problems despite available potent antituberculosis drugs, causing nearly 9 million new cases and 2 million deaths per year (1,2). The emergence of multidrug-resistant tuberculosis cases, particularly in the 1990s, has become an important health problem, threatening the control of tuberculosis (3). The conventional TB diagnosis methods, including direct microscopic examination by Ehrlich–Ziehl–Neelsen staining, culture, chest radiography, and tuberculin skin testing, have limitations and are not always helpful in diagnosing TB (4). Used as a stand-alone method in some countries, smear microscopy remains the most universal diagnostic method. However, smear microscopy identifies only 45% of TB cases and does not detect drug resistance (2). Although the culture method is more sensitive, it has significant shortcomings, including a time period of 2–8 weeks required for a diagnosis and the need for biosafety precautions and experienced personnel. It has been considered that each TB patient can infect up to 10–15 persons every year (3). Therefore, delayed diagnosis prevents disease control, and each patient with a delayed diagnosis becomes a source of new cases, which further increases health costs (4).

There has been significant progress in the last decade in TB diagnostic methods. Molecular methods have been developed that can identify the *Mycobacterium tuberculosis* complex in clinical samples in as short as 2 h and can also detect drug resistance simultaneously (5,6). The Xpert MTB/RIF (Cepheid, USA) system is a molecular diagnostic method based on a real-time polymerase chain reaction (PCR) mechanism. It has been shown to have a high sensitivity and specificity for other specimens as well as for respiratory tract specimens (7–9). Starting from December 2010, the World Health Organization (WHO) has recommended the use of the Xpert MTB/RIF system as one of the onset TB diagnostic tests to diagnose suspected multidrug-resistant TB or TB infections associated with human immunodeficiency virus (HIV) (10).

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Molecular diagnostic methods are more expensive compared to other conventional methods, and this creates a challenge, particularly in low- and middle-income TB-endemic countries. Moreover, how these tests will be included into the tuberculosis diagnosis algorithm and at what level they should be performed in healthcare centers are major questions (6). This study aims primarily to compare Xpert MTB/RIF system results with BACTEC MGIT 960, Löwenstein–Jensen (LJ) medium, and direct microscopic examination methods in detecting the presence of the *M. tuberculosis* complex in specimens collected from suspected tuberculosis patients. The study also aims to seek answers to the question: should the Xpert MTB/RIF system be considered in the diagnostic algorithm of TB, which is included in the differential diagnosis of many infectious and noninfectious diseases?

2. Materials and methods

This study includes clinical specimens (sputum, bronchoalveolar lavage, gastric lavage aspirate, urine, cerebrospinal fluid, and others) tested for the *M. tuberculosis* complex at the clinical microbiology laboratories of the Training and Research Hospital of Cumhuriyet University between January 2009 and December 2012. Submitted specimens were classified into two groups. The first group included 7407 specimens that did not primarily suggest pulmonary or extrapulmonary TB. The specimens were collected from patients required to be screened for TB, excluding TB diagnosis, since it was a differential diagnosis. The second group included 411 specimens collected from patients with pulmonary or extrapulmonary TB due to the presence of at least one of the following (together with the clinical findings of the patients): findings of chest X-ray, history of previous TB, family history of TB, or history of contact with a confirmed TB patient. Tests in the first group were performed with conventional methods (smear microscopy, culture by LJ medium, and Bactec MGIT 960) commonly used to diagnose tuberculosis in the specimens. In the second group, tuberculosis DNA was studied using the Xpert MTB/RIF (Cepheid GeneXpert System, USA) system in specimens, in addition to the above methods. The Xpert MTB/RIF system was not used for specimens in the first group due to its high cost. Repeat specimens from the same patient were excluded from the study.

No homogenization or decontamination procedures were performed on the specimens for molecular studies. The specimens and transfer solution (Pour Sample Reagent) were transferred to 1.5-mL sterile centrifuge tubes at a ratio of 1/1 and were then incubated at room temperature for 15 min. A sufficient amount of the mixture was collected using a sterile Pasteur pipette included in the kit; this was slowly transferred into the testing cartridge and the lid was closed. The cartridge was then placed into the Xpert MTB/RIF device and the system was switched on. The results were available after 2 h.

The specimens were first homogenized and decontaminated before being taken into the MGIT 960 system (Becton Dickinson, USA) and the LJ medium. N-acetyl- L-cysteine and sodium hydroxide (NALC and NaOH) were used in the homogenization and decontamination procedures. The specimens were placed on slides, dry-fixed, stained according to the Ehrlich–Ziehl–Neelsen (EZN) method, and examined under a microscope (10 × 100). Tuberculosis–*Mycobacterium tuberculosis* complex identification and antimicrobial sensitivity tests were performed using the BACTEC MGIT 960 system according to the manufacturer's operational guidelines. Growth control was done by visual inspection and by Ziehl–Neelsen staining over the agar surface for LJ medium isolates.

This study was approved by the Medical Ethics Committee of Cumhuriyet University. The Youden index was used for the comparison of the performance of the diagnostic tests.

3. Results

The distributions of 7407 specimens from group 1 and 411 specimens from group 2, which had been sent to our laboratory for *M. tuberculosis* complex tests, are presented in Table 1.

Table 1. The distribution of clinical specimens in group 1 (n = 7407) and group 2 (n = 411).

Sample	Group 1, n (%)	Group 2, n (%)
Sputum	1521 (20.5)	76 (18.5)
Bronchial lavage	582 (7.9)	23 (5.6)
Urine	1404(18.9)	72 (17.5)
Cerebrospinal fluid	427 (5.8)	38 (9.2)
Gastric lavage	1754 (23.7)	145 (35.3)
Other	1719 (23.2)	57 (13.9)
Total	7407 (100)	411 (100)

Currently, the gold standard method recognized in the diagnosis of tuberculosis is the culture method. Automated culture systems, which are more sensitive and give quicker results compared to classical culture methods, are preferred (11–13). The BACTEC MGIT 960 system was used as the reference method in this study. Although 152 (2.05%) specimens were found to be positive in the first group, growth was found in 24 (6.5%) clinical specimens in the second group. Comparisons of clinical specimens in both groups for methods used and positivity rates are given in Table 2. Nine of the 24 specimens in group 2 cultured using BACTEC MGIT 960 that yielded growth were sputum, 5 were gastric lavage, 4 were bronchial lavage, 3 were pleural fluid, 2 were urine, and one was an abscess specimen. The Xpert MTB/RIF system also found positive results in all 24 specimens, which consisted of different clinical specimens. Furthermore, whereas the BACTEC MGIT 960 culture system, LJ medium, and EZN methods found negative results in 3 specimens consisting of one sputum, one abscess, and one urine specimen, the results were positive with the Xpert MTB/RIF molecular system. Cases from which these 3 specimens were collected were also found to be clinically consistent with tuberculosis. Antituberculosis treatment was started in these 3 patients according to positive test results with the Xpert MTB/RIF molecular system, and the patients were reported as proven tuberculosis-infected because their symptoms improved after tuberculosis treatment.

As a result of statistical analyses performed in the second group by evaluating the test results of the clinical specimens, the BACTEC MGIT 960 system was used as the reference method, which showed that the sensitivity and specificity of the Xpert MTB/RIF system was very high (Youden index: 0.99). The specificity of the Xpert MTB/RIF test was found at 0.99 (95% [CL], 0.98–1.00; 384 of 387), with a sensitivity of 1.00. Positive and negative predictive values of the Xpert MTB/RIF system were found to be 0.88 and 1.00, respectively.

4. Discussion

The *M. tuberculosis* complex can affect many organs and systems. It most commonly causes pulmonary tuberculosis, constituting the main source of infection in the community until these patients are diagnosed and treated (14). Clinical presentations of extrapulmonary tuberculosis should be considered in the differential diagnosis of many infectious and noninfectious diseases (15). It can cause acute conditions such as meningitis and endocarditis, which have high morbidity and mortality rates, as well as infections with delayed diagnoses such as chronic osteomyelitis (16–18). In a study conducted in Turkey, tuberculosis was found to be the most common cause of infectious reasons for fever of unknown origin (19). Tuberculosis has even been considered in the differential diagnosis of arthritis and of malignancies due to *M. tuberculosis* complex, which causes granulomatous lesions (15,20–22). In light of these data, pulmonary tuberculosis patients, who are the main source of new pulmonary or extrapulmonary tuberculosis cases, should be diagnosed immediately. Moreover, early diagnosis is also important in extrapulmonary TB clinical presentations, which create no risks for the transmission of the disease but can cause high morbidity and mortality.

Many nucleic acid amplification test methods require experienced technicians to perform the tests. Moreover, these methods require a system where many specimens are analyzed together and a certain number of specimens is collected (23). However, the Xpert MTB/RIF system is simple and can be applied by personnel with little training. Each specimen can be tested as soon as it arrives to the laboratory, requiring no pool method where the specimens are collected. This allows for a quick diagnosis of tuberculosis, an ideal time for respiratory isolation

Table 2. The comparison of the results of Xpert MTB/RIF assay, Löwenstein–Jensen medium, and Ehrlich–Ziehl–Neelsen BACTEC using MGIT 960.

			Number of positive samples (%)		
			Xpert MTB/RIF	LJ	EZN
BACTEC MGIT 960	Group 1	Positive (n = 152)	-	118 (77.63)	101 (66.44)
		Negative (n = 7255)	-	13 (0.18)	194 (2.61)
		Total (n = 7407)	-	131 (1.77)	295 (3.99)
	Group 2	Positive (n = 24)	24 (100)	17 (70.8)	15 (62.5)
		Negative (n = 387)	3 (0.7)	0 (0)	13 (3.3)
		Total (n = 411)	27 (6.6)	17 (4.1)	28 (6.8)

LJ: Löwenstein–Jensen, EZN: Ehrlich–Ziehl–Neelsen.

decision-making, and immediate treatment without delay (6,23,24). The Xpert MTB/RIF test, which contains probes targeting the 81-bp regions on the *rpoB* gene in *M. tuberculosis*, is a molecular system used to simultaneously identify *M. tuberculosis* and rifampicin resistance. Using this system, the *M. tuberculosis* complex and rifampicin resistance can be identified with a single test in 2 h (6). Blakemore et al., in their study of 79 different *M. tuberculosis* and 89 different non-*M. tuberculosis* strains, reported that the Xpert MTB/RIF system accurately identified all 79 *M. tuberculosis* strains (100%). Helb et al. reported the system sensitivity as 100% for positive microscopy and culture specimens, and as 84.6% for negative microscopy and positive culture specimens (23). High rates of sensitivity and specificity have been reported for different clinical specimens (25–27). We reidentified all *M. tuberculosis* strains (24 strains) that we identified using our reference method, using the Xpert MTB/RIF system. We found high sensitivity and specificity rates in this study where we attempted to find out the performance of the Xpert MTB/RIF system.

Certain concentrations of bacteria should be found in specimens in order to be able to detect positive results using microscopic and culture methods (28). Rachow et al. reported that all clinical specimens in which growth was detected using the culture method were also found positive with the Xpert MTB/RIF system; in addition, they found positive results with the Xpert MTB/RIF system in 4 culture-negative clinical specimens (29). In our study, 3 specimens found to be negative with the BACTEC MGIT 960 system and 10 specimens that were negative with LJ medium were found to be positive using the Xpert MTB/RIF system. We consider that the Xpert MTB/RIF system detects bacteria with lower concentrations in clinical specimens, yielding more positive results than other methods used in our study.

More than 95% of new tuberculosis cases and most mortalities associated with tuberculosis are seen in developing countries (2,23). The diagnosis, treatment, follow-up, and care of many patients in these countries are carried out in health centers depending on a microscopy center including staining with the EZN method to detect acid-resistant bacilli (ARB) (6,23,30). Although direct microscopy is the most common method worldwide, only 45% of tuberculosis cases were found positive in 2009 (6,31). In our study, LJ staining was positive in only 76.7% (135/176) of all culture-positive cases. There are also bacteria with positive ARB microscopy other than mycobacteria (*Nocardia*, etc.) (32,33). In our study, 207 (2.7%) specimens that were found positive with the staining method were not found positive with any other methods we used. We attribute this to ARB-positive-stained bacteria other than mycobacteria.

The need for more secure and quicker diagnostic tests to control tuberculosis globally is of key importance, especially in countries with highly endemic TB and high HIV seroprevalence (34). The high cost of the Xpert MTB/RIF system ultimately leads to its limited use (23,34). Tuberculosis is a disease more commonly seen in developing countries. This system should be applicable in these countries at a special price, which should be competitive enough to keep pace with classical culture and conventional methods (23). However, the classical diagnostic methods used at these centers are not sufficiently sensitive, and microscopic examination is very dependent on the performance of the person in question, and this results in inability to diagnose the case. This is one of the most important factors contributing to the inability to control tuberculosis worldwide. It has been argued that the Xpert MTB/RIF system's applicability at these centers within the bounds of possibility could be a very important opportunity to control the disease (6,23). It has also been reported that in order to eliminate tuberculosis by the year 2050, its incidence should be reduced by an average of 16% every year. It has also been argued that a single method cannot achieve this target, and that new diagnostic methods, particularly nucleic acid amplification methods, could contribute greatly to its elimination (6,35). In our study, we included the Xpert MTB/RIF system into the onset diagnostic tests only in the presence of findings and risk factors that would support a TB diagnosis, due to its high costs. We consider that the patients should be diagnosed within 2 h, which will ensure the immediate start of effective treatment for the patients; it will also ensure that the decision of isolation, which plays a very important role in controlling tuberculosis, can be taken during the early period. We also think that TB diagnosis in culture-negative patients could play an effective role in reducing morbidity and mortality rates associated with TB, and in preventing the spread of TB worldwide.

In conclusion, although the Xpert MTB/RIF system is costly, certain points should be considered in cost-effectiveness studies. It will certainly increase the costs of tuberculosis diagnosis. However, the reduction in morbidity and mortality with early diagnosis and especially the reduced spread of tuberculosis should be considered. Diagnosis algorithms should also be considered in cases with risk factors such as a history of previous TB, family history of TB, and history of contact with a confirmed TB patient, or in suspected cases as a factor of a possible infection developing in immunosuppressive patients or that can present with high mortality. We consider that molecular diagnostic methods can be better appreciated with long-term cost-effectiveness studies, which consider potential long-term issues, instead of short-term cost-effectiveness studies.

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