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Comparison of a real-time polymerase chain reaction-based system and Erlich–Ziehl–Neelsen method with culture in the identification of *Mycobacterium tuberculosis*

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Backgrund/aims: *Mycobacterium tuberculosis* is still a major health problem throughout the world, especially in developing countries. Disease control heavily depends on the establishment of early diagnosis. The aim of this study is to compare the efficacy of culture, GeneXpert MTB/RIF device, and Erlich–Ziehl–Neelsen direct microscopic method.

Materials and methods: A total of 927 samples (243 respiratory and 684 nonrespiratory), which were sent to Ondokuz Mayıs University Medical Faculty Tuberculosis Laboratory on suspicion of *M. tuberculosis*, were included in the study.

Results: When compared to standard culture, sensitivity, specificity, and positive and negative predictive values of the GeneXpert system for respiratory samples were 100%, 98.7%, 87%, and 100%, respectively; these values for nonrespiratory samples were 71%, 98.6%, 71%, and 98.6%, respectively.

Conclusion: New, reliable, rapid, and easy-to-use methods that display high specificity and sensitivity are required for an effective struggle against tuberculosis. According to these results, we suggest that GeneXpert MTB/RIF is a rapid and reliable system, and when used in company with conventional tests, it would make significant contributions to the diagnosis of tuberculosis.

Key words: *Mycobacterium tuberculosis*, GeneXpert MTB/RIF, culture

1. Introduction

Due to reasons such as health policies not giving due importance to tuberculosis, demographic changes, the human immunodeficiency virus epidemic, and deteriorating socioeconomic conditions, *Mycobacterium tuberculosis* is still a major health problem throughout the world, especially in developing countries (1,2).

Despite having a variety of methods used in the diagnosis of *M. tuberculosis*, culture (Lowenstein–Jensen [LJ]) is still the gold standard (3). For a positive result, 10–100 bacilli/mL is sufficient (4,5). However, a period of 4 to 8 weeks is required for the colonies to be visible (6).

Erlich–Ziehl–Neelsen (EZN) staining, another method used for the diagnosis of tuberculosis, is an indispensable method due to its ease of application, low cost, and ability to provide rapid results. However, factors such as the staining method used, the experience of the evaluator, the evaluation period for each sample, and the number of samples evaluated for each patient affect the sensitivity of the method (5). In addition, in order to detect bacilli in stained preparations, there should be approximately 5000–10,000 bacilli/mL. Therefore, negative microscopy does not exclude the presence tuberculosis (4).

Early diagnosis of the disease and regular monitoring of the treatment are important for an effective struggle against the disease (1,6). The main disadvantages are as follows: attainment of culture results takes a long time, and direct microscopic examination has a low sensitivity (4,5). Therefore, rapid, easy-to-use, and cost-effective laboratory methods that provide high sensitivity and specificity are required. For this purpose, nucleic acid amplification (NAA)-based methods that can identify *M. tuberculosis* through patient samples have been developed (7,8).

The aim of this study is to retrospectively compare the results of culture (LJ and MGIT 960), the GeneXpert MTB/RIF device (one of the polymerase chain reaction-based rapid diagnostic methods) (CEPHEID, USA), and the direct microscopic method of EZN.

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2. Materials and methods

A total of 927 samples (243 respiratory samples from sputum, bronchoalveolar lavage, and tracheal aspirate and 684 nonrespiratory samples from urine, pleural fluid, aspirate, cerebrospinal fluid [CSF], etc.) sent to the Ondokuz Mayıs University Medical Faculty Tuberculosis Laboratory between October 2011 and February 2014 on suspicion of *Mycobacterium tuberculosis* were included in the study.

After the decontamination process, a concentration of 200 µL of each sample, except for CSF, was taken and cultivated into LJ medium in a 0.5-mL MGIT tube and incubated. Preparations were arranged from the same sample for EZN staining and examined under a light microscope at 100× magnification (5).

In addition, all samples were studied with the GeneXpert system in accordance with the manufacturer's recommendations. For this purpose, decontaminated samples were diluted with a sample reagent solution in the ratio of 1:3 and were kept at room temperature for 15 min. In accordance with aseptic technique, 2 mL of the mixture was poured into the test cartridges and capped. The test cartridge was then inserted into the GeneXpert device and studied. The results were evaluated at the end of 2 h.

The specificity, sensitivity, and positive and negative predictive values were used for the evaluation of the performance of GeneXpert. SPSS 20.0 (IBM Corp., USA) were used for the statistical analyses.

3. Results

Of the 243 respiratory samples, 177 were sputum, 64 were bronchoalveolar lavage, and 2 were tracheal aspirate. Of the 684 nonrespiratory samples, 171 were urine, 140 were gastric fluid, 77 were pleural fluid, 63 were peritoneal fluid, 57 were surgical material, 50 were CSF, 47 were exudate, 45 were pericardial effusion, and 34 were other materials.

EZN, culture, and GeneXpert system positivity of the 927 samples were 2.7% (n = 25), 5.5% (n = 51), and 5.8% (n = 54), respectively. While culture results were positive for 9 nonrespiratory samples, the GeneXpert system and EZN test results were negative. Although 12 samples (3 respiratory samples and 9 nonrespiratory samples) had a negative culture result, the GeneXpert system was positive for these samples. Of these 12 samples, EZN test results found that 9 of the samples were negative, whereas 3 were identified as EZN-positive. Comparative results of 3 different tests are shown in Table 1.

When compared with culture results, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the GeneXpert system for respiratory samples were 100%, 98.7%, 87%, and 100%, respectively. These values for nonrespiratory samples were 71%, 98.6%, 71%, and 98.6%, respectively.

Comparison of EZN staining with culture results revealed sensitivity of 60%, specificity of 100%, PPV of 100%, and NPV of 96.5% for respiratory samples; these values for nonrespiratory samples were 32.3%, 99.5%, 76.9%, and 96.9%, respectively. The comparative results of EZN and GeneXpert with culture are shown in Table 2.

4. Discussion

The need for rapid and reliable methods for the diagnosis of tuberculosis has led to molecular diagnostic methods becoming widespread and taking a strong and complementary role along with conventional tests (9).

In the GeneXpert MTB/RIF system, *M. tuberculosis* complex and rifampin resistance can be determined in a single test in a short time (less than 2 h) directly from patient material via a semiquantitative nested real-time PCR method. Since all reagents required for the test are kept in a closed cartridge, there is no cross-contamination possibility between samples (10). It was reported that NAA testing alone is not sufficient for the rapid diagnosis of tuberculosis from suspicious clinical samples in routine practice and that these test should not be used for screening purposes. However, along with conventional tests, they

Table 1. Comparison of the GeneXpert system and EZN results with the results of culture.

		TBC culture			
		Respiratory samples (n = 243 [26.2%])		Nonrespiratory samples (n = 684 [73.8%])	
		Positive (n = 20)	Negative (n = 223)	Positive (n = 31)	Negative (n = 653)
EZN	Positive	12	0	10	3
	Negative	8	223	21	650
GeneXpert PCR	Positive	20	3	22	9
	Negative	0	220	9	644

Table 2. Sensitivity, specificity, PPV, and NPV in comparison of GeneXpert system and EZN with culture.

		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Respiratory samples	EZN	60	100	100	96.5
	GeneXpert	100	98.7	87	100
Nonrespiratory samples	EZN	32.3	99.5	76.9	96.9
	GeneXpert	71	98.6	71	98.6

are considered to be quite valuable in supporting clinical findings (9).

In the study by Bunsow et al., sensitivity, specificity, PPV, and NPV of the GeneXpert system for respiratory samples and nonrespiratory samples were found to be 97.1%, 98.6%, 95.7%, and 99.1% and 33.3%, 99.7%, 80.0%, and 97.3% respectively. The GeneXpert system was reported to be a rapid and easy-to-use test giving accurate results in identifying *M. tuberculosis*, particularly in smear-positive respiratory samples (11).

In their study of 521 nonrespiratory samples, Hillemann et al. compared the results of the GeneXpert system with those of conventional liquid (MGIT 960) and solid (LJ) culture methods and found sensitivity and specificity as 77.3% and 98.2%, respectively. They expressed that the GeneXpert system is a rapid and useful technique in the identification of nonrespiratory tuberculosis (12).

Ioannidis et al. compared the results of culture methods (LJ and MGIT 960) with those of the GeneXpert system and found sensitivity, specificity, PPV, and NPV as 90.6%, 94.3%, 93.5%, and 91.7% in respiratory samples and 100%, 91.6%, 50%, and 100% in nonrespiratory samples, respectively. At the end of the study, they concluded that the GeneXpert system, a NAA-based method, would be beneficial in treating tuberculosis (13).

In a study conducted in Turkey, Çiftçi et al. compared the performance of the Xpert MTB/RIF system with those of the BACTEC 460TB 12B (BD Diagnostic, USA), the LJ culture, and the Ziehl–Neelsen direct microscopic examination method. When BACTEC 460TB results were taken as the reference, the sensitivity, specificity, PPV, and NPV of the Xpert MTB/RIF system were found to be 96%, 98%, 96%, and 98%, respectively (14).

In their study, Özkütük et al. compared Xpert MTB/RIF test results with culture results (BACTEC MGIT 960 and LJ medium). For pulmonary samples, specificity, sensitivity, PPV, and NPV were found to be 80.8%, 98.8%, 84.9%, and 98.4%, respectively. These values for nonpulmonary samples were 58.2%, 98.4%, 66.7%, and 97.7%, respectively. They suggested that Xpert MTB/RIF is a useful method for the diagnosis of tuberculosis (15).

In our study, we had 9 false negative results from the GeneXpert system; all were nonrespiratory tract samples. There were twelve false positive results; 3 were respiratory samples and 9 were nonrespiratory samples. It was understood that 100% of false negative and 75% of false positive results occurred in nonrespiratory samples. Since live and dead bacilli cannot be distinguished by PCR methods, it is known that false positivity can be seen in patients with a history of tuberculosis (5).

In accordance with our results obtained from the comparison of the GeneXpert system, EZN staining, and culture in terms of sensitivity, specificity, PPV, and NPV, we found that the literature shows that the GeneXpert system has higher sensitivity rates for both respiratory and nonrespiratory samples and that these results were similar with respect to other values.

In conclusion, early diagnosis is of great importance for the treatment of tuberculosis. For this purpose, easy-to-use new methods that can provide reliable and fast results with high specificity and sensitivity are being sought. According to these results, we can conclude that the GeneXpert MTB/RIF is a rapid and reliable system that can be employed in the diagnosis of tuberculosis, and when utilized together with conventional tests, it can make significant contributions to tuberculosis diagnosis.

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