Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Clinical Research

J. Exp. Clin. Med., 2020; 37(4): 105-109
doi: 10.5835/jecm.omu.37.04.001

Comparison of culture, Xpert MTB/RIF and EZN staining method to identify *Mycobacterium tuberculosis* from pulmonary and extra pulmonary specimens

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ARTICLE INFO

Article History
Received 28 / 01 / 2020
Accepted 06 / 04 / 2020
Online Published 11 / 09 / 2020

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Keywords:
Culture
EZN staining
Mycobacterium tuberculosis
Xpert MTB / RIF

ABSTRACT

Mycobacterium tuberculosis is a major health problem worldwide, especially in developing countries. The effective treatment of *M. tuberculosis* depends on early diagnosis. In this study, 1577 *M. tuberculosis* strains were evaluated retrospectively from clinical samples sent between March 2014 and June 2018. The rate of pulmonary samples was determined as 694 (44%). Positive sample rate was 74 (4.69%) in culture, 19 (1.20%) in EZN staining and 75 (4.75%) in Xpert MTB/RIF. Compared to standard culture, the sensitivity, specificity, positive and negative predictive values of the Xpert MTB / RIF system were 100%, 99.93%, 98.66% and 100%, respectively. The sensitivity, specificity, positive and negative predictive values of EZN were 57.36%, 100%, 100% and 96.46%, respectively. Early diagnosis and treatment of *M. tuberculosis* is of great importance. According to these results, it can be concluded that Xpert MTB / RIF is a fast and reliable system that can be used in the diagnosis of tuberculosis and when used together with conventional tests, it can make important contributions to the diagnosis of tuberculosis.

1. Introduction

Mycobacterium tuberculosis is still a significant health problem, particularly in developing countries (Forbes et al., 2007). Rapid and accurate diagnosis of tuberculosis (TB) is important to control the disease. Diagnosis of TB especially in developing countries continues to rely on smear microscopy, which detects 40-60% pulmonary TB and 0-60% extrapulmonary TB (EPTB) cases (Mehta et al., 2012a; Mehta et al., 2012b; Fuchs et al., 2014). In addition, in order to detect bacilli in stained preparations, there should be approximately 5.000-10.000 bacilli/mL. Therefore, negative microscopy does not exclude the presence tuberculosis (Çöplü, 2002).

The gold standard and mostly used test for diagnosis of TB is the culture method (Trent, 2005). Culture method is not straightforward because isolation and drug susceptibility testing for this bacterium on solid media can take at least four to eight weeks or even longer. Furthermore, the turnaround time of *M. tuberculosis* is high and the method is not always accessible (Soini and Musser, 2001).

Rapid, specific and sensitive methods for the detection and identification of tuberculosis is needed to avoid unnecessary delay in making appropriate decisions. Molecular methods can advantageously complement and accelerate this process by minimizing the need to wait for a culture report (Sevilla et al., 2015).

The Xpert MTB/RIF assay (Xpert) was developed to improve TB and rifampin resistance (RIF-R) detection. This was accomplished by automating most of the steps required to process clinical samples and by improving the sensitive detection of both *M. tuberculosis* and RIF-R (Helb et al., 2010). This system is based on a fully automated nucleic acid amplification and detection, in which extraction, amplification and detection takes place inside a single-use cartridge, which is inserted into the GeneXpert Instrument System (Cepheid) (Vergara Gómez et al., 2017). The technique detects a fragment of the rpoB gene, which encodes for the subunit of RNA polymerase. Five molecular beacon type genetic probes are used, each labeled with a different fluorophore. They completely cover an area of 81 base pairs, RIF resistance determining region (RDRR), between the codons 507 and 533 (Vergara Gómez et al., 2017). The technique's lower detection limit, with 95% confidence, is 5 copies of DNA or 131 CFU/mL (World Health Organization, 2011). In comparison, the microscopic examination requires at least 10.000 CFU/mL while the culture between 100 and 500 CFU/mL (Lawn and Nicol, 2011).

The assay can be performed in approximately 2 h. Pooled data have shown Xpert MTB/RIF to have an overall sensitivity and specificity of approximately 89% and 98%, respectively (Steingart et al., 2014). Xpert MTB/RIF system has many advantages but it is not cost-effective as culture method or staining.

In this study we aimed to compare the results of the direct microscopic method of EZN, culture (LJ and MGIT 960), the Xpert MTB/RIF device, retrospectively.

2. Material and methods

A total of 1577 samples (694 pulmonary samples from sputum, bronchoalveolar lavage, and tracheal aspirate and 883 extrapulmonary samples from urine, pleural fluid, aspirate, cerebrospinal fluid [CSF], etc.) sent to the Ondokuz Mayıs University Medical Faculty Tuberculosis Laboratory between March 2014 and June 2018 on suspicion of tuberculosis disease were included in the study.

Culture

The *M. tuberculosis* culture and first-line phenotypic DST were performed in automated BD MGIT 960 (BD, USA) system. All sputum samples were processed using 4% sodium hydroxide (NaOH) method, and then cultured on MGIT 960 medium. *M. tuberculosis* strains grown on MGIT medium were tested for drug susceptibility in MGIT 960.

Specimens were decontaminated using sodium hydroxide (NaOH). After concentration by centrifugation at 3000 g for 15 minutes, the sediment was resuspended in 1.5 ml of 0.5 M phosphate buffer (pH 6.8) and inoculated onto Lowenstein-Jensen (LJ) medium and MGIT-7H9 broth supplemented with oleic acid-albumin-dextrose-catalase (OADC) and PANTA (BD, USA). Inoculated MGIT test tube were incubated using MGIT 960 instrument (BD, USA) and Lowenstein-Jensen (LJ) medium at 37°C in the incubator. *M. tuberculosis* strains grown on MGIT medium were tested for drug susceptibility in MGIT 960.

Microscopy

Preparations were arranged from the same sample for EZN staining and examined under the light microscope at 1000x magnification.

Procedures for Xpert testing

Xpert MTB/RIF testing was performed on samples, using version 4 cartridges, according to the manufacturer's recommendations. The Xpert assay sample reagent (containing NaOH and isopropanol) was added in a 1:3 ratio to the tubes to kill the mycobacteria and liquefy the sample. The mixture was shaken vigorously and held for 15 minutes. It was left for another 5 minutes before shaking again. Finally, 2 ml was pipetted into the Xpert assay cartridge and inserted into the Xpert MTB/RIF instrument for PCR testing. The measurement and analysis were conducted automatically and reported by the GeneXpert Dx software (version 4.0).

The specificity, sensitivity, and positive and negative predictive values were used for the evaluation of the performance of Xpert MTB/RIF.

3. Results

A total of 1577 samples with suspected tuberculosis that had been tested by three methods were evaluated. Of the 694 (44%) pulmonary samples 308 (44.4%) were sputum, 255 (36.7%) were bronchoalveolar lavage fluid, 120 (17.3%) were pleural fluid and 11 (1.6%) were endo tracheal aspirate. And of the extrapulmonary samples 294 were gastric fluid, 215 of urine, 88 were exudate, 82 were cerebrospinal fluid and 79 were surgical material.

Culture, EZN, and Xpert MTB/RIF system positivity of the 1577 samples were 4.7% (n=74), 1.2% (n=19) and 4.75% (n=75), respectively. When compared with culture results, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Xpert MTB/RIF system for pulmonary samples were 100%, 99.7%, 96%, and 100%, respectively. These values for extrapulmonary samples were 96.3%, 100%, 100%, and 96.9%, respectively. The comparison of EZN staining with culture results revealed sensitivity of 61.5%, specificity of 100%, PPV of 100%, and NPV of 95.5% for respiratory samples; these values for nonrespiratory samples were 50.9%, 100%, 100%, and 97.1%, respectively.

Three isolates were determined positive for rifampicin by both two methods Xpert MTB/RIF system and MGIT automated system.

Table 1. Sensitivity, specificity, PPV, and NPV in comparison of Xpert MTB/RIF system and EZN with culture.

			Sensitivity %	Specifity%	PPV%	NPV%
Pulmonary Samples (694)	Sputum (308)	Xpert MTB/RIF	100	98.3	78.26	100
		EZN	66.66	100	100	96.98
	BAL* (255)	Xpert MTB/RIF	93.33	100	100	99.12
		EZN	59.57	100	100	92.27
	Pleural fluid (120)	Xpert MTB/RIF	50	100	100	98.33
		EZN	50	100	100	98.33
	ETA* (11)	Xpert MTB/RIF	0	91.66	0	100
		EZN	0	100	0	100
	Gastric fluid (294)	Xpert MTB/RIF	64.28	100	100	98.27
		EZN	50	100	100	96.93
Extra Pulmonary Samples (883)	Urine (215)	Xpert MTB/RIF	100	100	100	100
		EZN	50	100	100	99.06
	Sterile body fluid (123)	Xpert MTB/RIF	100	99.18	66.66	100
		EZN	50	100	100	98.5
	Exudate (88)	Xpert MTB/RIF	100	95.4	55.55	100
		EZN	55.55	100	100	95.4
	CSF* (82)	Xpert MTB/RIF	100	100	100	100
		EZN	50	100	100	95.12
	Surgical material (81)	Xpert MTB/RIF	80	100	100	98.71
		EZN	50	100	100	95.06

*BAL: Broncho alveolar alavage, ETA: Endotracheal aspirate, CSF; cerebrospinal fluid.

4. Discussion

Rapid and accurate identification of mycobacterial infections is important to initiate appropriate treatment, contact precautions and prophylaxis (Kox et al., 1994; Field and Cowie, 2006; Mokaddas and Ahmad, 2007). Conventional diagnostic methods for detection of Mycobacterial infections are microscopic examination of samples with EZN staining and culturing on LJ medium (Mokaddas and Ahmad, 2007; Aryan et al., 2010).

Microscopic smear examination is a low-cost, rapid and easy to perform method while it has poor sensitivity and no distinctive specificity (Ritis et al., 2000). It is known that 5.000 - 10.000 bacteria per mL are required for staining and smear microscopy (Çöplü, 2002; Forbes et al., 2007; Babacan and Hasdemir, 2008). The gold standart for diagnosis of mycobacterial infections are culture method. It has high specificity and its sensitivity is considered to be about 100 folds more than that of microscopic examination. But there are some disadvantages of the culture method, such as its prolonged hands-on time and the need for good and specific laboratory infrastructure, which is limited to reference centers (Aryan et al., 2004).

PCR-based assays have been used to detect Mycobacterial DNA with high sensitivity and specificity for rapid detection of mycobacterial infections (Kox et al., 1994; Soini and Musser, 2001; Espasa et al., 2005; Mokaddas and Ahmad, 2007; Elbir et al., 2008; Davis et al., 2009; Gopinath and Singh, 2009; Coelho et al., 2010; Arjomandzadegan et al., 2011). In our study, we aimed to compare the EZN staining and culture with Xpert MTB/RIF system. Culture method was regarded as the gold standard of TB diagnosis.

The Xpert MTB/RIF system provides determination of *M. tuberculosis* complex and rifampin resistance in a single test in a short time (less than 2h) directly from clinical sample 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 clinical samples (Durmaz, 2010).

In the study by Bunsow et al. sensitivity, specificity, PPV, and NPV of the GeneXpert system for pulmonary samples and extrapulmonary 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 (Bunsow et al., 2014). In their study of 521 extrapulmonary samples, Hillemann et al. compared the results of the Xpert MTB/RIF 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 Xpert MTB/RIF system is a rapid and useful technique in the identification of extrapulmonary tuberculosis (Hillemann et al., 2014).

Ioannidis et al. compared the results of culture methods (LJ and MGIT 960) with those of the, Xpert MTB /RIF 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 (Ioannidis et al., 2011).

Özkütük and Sürücüoğlu 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 (Özkütük and Sürücüoğlu, 2014).

In a meta-analysis about accuracy of Xpert MTB/RIF assay for extrapulmonary tuberculosis, thirty-six studies were identified, with a pooled sensitivity and specificity of respectively 77% (95% CI 66-85) and 97% (95% CI 94-98). Among site specific estimates for lymph, pleural fluid, cerebrospinal fluid, gastrointestinal and urinary samples, the pooled sensitivity was lower in pleural fluid (37%, 95% CI 26-50, meta-regression p, 0.001) and higher in lymph node samples (87%, 95% CI 75-95, meta-regression P¼ 0.03). And they reported that Xpert MTB/RIF has high specificity but limited sensitivity for the detection of extrapulmonary tuberculosis. In conclusion they reported that positive Xpert MTB/RIF test results may be useful in rapidly identifying the extrapulmonary tuberculosis, while negative test results provide less certainty for ruling out disease (Penz et al., 2015).

In a study by Luetkemeyer et al. Xpert MTB/RIF was compared with acid-fast bacilli (AFB) smear and mycobacterial culture using liquid and solid culture media, from participants with suspected pulmonary tuberculosis from the United States, Brazil, and South Africa. They found the sensitivity of the Xpert MTB/RIF result was 81.4% and sensitivity was 98.5% in AFB positive and 54.8% in AFB negative participants. Also they reported that the diagnostic performance of Xpert MTB/RIF in the United States was similar to higher-tuberculosis prevalence sites in Brazil and South Africa, and was comparable to other studies in higher-tuberculosis prevalence locations (Luetkemeyer et al., 2016).

In our study, sensitivity of Xpert MTB/RIF for pulmonary samples is higher than extrapulmonary samples but both of them have high sensitivity and specificity. The sensitivity of Xpert MTB/RIF for pulmonary samples is higher than a extrapulmonary samplesdue to the higher bacterial load in pulmonary samples. Also it was reported that sensitivity at extrapulmonary samples has differences among sample types, it is about 80% in lymph nodes however does not reach 50% at pleural fluids (Vergara Gomez et al., 2017). And in our result, one sample was positive for Xpert MTB/RIF but it was negative in culture. Sometimes due to the antituberculosis treatment growth on the culture cannot be detected but pcr assays can detect the bacterial DNA.

Our study have some limitations such as we only evaluated the microbiological findings, but not radiological, histological and clinical findings for the diagnosis of tuberculosis.

In summary, Xpert 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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