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BALKAN MEDICAL JOURNAL
THE OFFICIAL JOURNAL OF TRAKYA UNIVERSITY FACULTY OF MEDICINE

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Balkan Med J 2013; 30: 13-5 • DOI: 10.5152/balkanmedj.2012.061

Original Article

Comparison of Culture, Real-Time DNA Amplification Assay and Ehrlich-Ziehl-Neelsen for Detection of *Mycobacterium tuberculosis*

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ABSTRACT

Objective: *Mycobacterium tuberculosis* is still a substantial health problem universally. Although culture is the gold standard method, reliable, rapid and new methods are required for effective struggle with disease. We retrospectively compared the results of Ehrlich-Ziehl-Neelsen (EZN) stain and real-time DNA amplification assay (BD ProbeTec ET system) with culture.

Study Design: Retrospective study.

Material and Methods: A total of 703 samples, 182 pulmonary and 521 extra pulmonary, collected from 630 patients between May 2008 and February 2011 were evaluated. Culture was considered the gold standard.

Results: For pulmonary specimens, sensitivity, specificity, positive predictive and negative predictive values of BD ProbeTec ET and EZN were calculated to be 100%, 98.8%, 87.5%, 100% and 71.4%, 98.8%, 83.3%, 97.6%, respectively. For extra pulmonary specimens, sensitivity, specificity, positive predictive and negative predictive values of BD ProbeTec ET and EZN were calculated to be 80%, 98.7%, 76.9%, 98.9% and 24%, 98.3%, 42.8%, 96.2%, respectively.

Conclusion: According to these results, we suggest that the BD ProbeTec ET system is more reliable than EZN. In addition, the BD ProbeTec ET system produces faster results. Based upon these results, we consider that the BD ProbeTec ET system may be employed in the diagnosis of *M. tuberculosis*.

Key Words: *Mycobacterium tuberculosis*, BD ProbeTec ET, culture

Received: *11.05.2012* ***Accepted:*** *26.06.2012*

Introduction

Mycobacterium tuberculosis is still a significant health problem, particularly in developing countries (1). On the other hand, because of the Human immunodeficiency virus infection/Acquired immunodeficiency syndrome (HIV/AIDS) epidemic which occured in the 1980's and the spread of infections caused by multi-drug-resistant tuberculosis (MDR-TB), this organism has been reevaluated (2, 3).

It is important to provide rapid and reliable detection of *M. tuberculosis* to prevent the spread of the disease. Therefore, highly sensitive and specific, easy to apply, quick and cost effective methods are needed in the diagnosis of tuberculosis (4).

Although several methods can be used for the detection of *M. tuberculosis,* culture (Lowenstein-Jensen and Mycobacteria Growth Indicator Tube [MGIT]) remains the gold standard (2). Because mycobacteria are slow-growing microorganisms, these tests take approximately 3-8 weeks (1). The length of conventional tests has led to new searches for rapid diagnosis in recent years. For this purpose, nucleic acid amplification (NAA)-based systems have been developed to show the presence of *M. tuberculosis* directly from the patient samples (3, 5).

In our study, it is aimed to compare the retrospective results of culture, Erlich-Ziehl-Neelsen (EZN) and real-time DNA amplification assay (BD ProbeTec ET).

Material and Methods

Specimen Collection

The results of the samples with suspected tuberculosis that had been sent to the microbiology laboratory in Ondokuz Mayis University Faculty of Medicine were analyzed retrospectively. Seven hundred and three specimens in the study were collected from 630 patients between May 2008 and February 2011. Of these specimens, 182 were pulmonary (sputum, tracheal aspirate, bronchoalveolar lavage), 521 were extra-pulmonary (sterile body fluid, gastric lavage, urine, puncture fluid, cerebrospinal fluid (CSF), others).

Processing

Samples submitted to the laboratory were included in the process of decontamination. Then, 150 µL of the processed sample was inoculated onto Lowenstein-Jensen medium and incubated at 37°C for 4-8 weeks (6). Also, 0.5 mL of the same sample was inoculated into MGIT tubes and incubated in the Bactec MGIT 960 system. Smear was prepared from the processed sample, EZN staining was performed and smears were investigated

The 4th Eurasia Congress of Infectious Diseases, Sarajevo, Bosnia & Herzegovina,1-5 June 2011

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with a light microscope (6). Also, all samples were processed in the BD ProbeTec ET system with the steps of removing inhibitors, heating, extraction of DNA, neutralization and amplification according to the recommendations of the manufacturer.

ProbeTec assay

Clinical specimens were treated according to the manufacturer's recommendations. Decontaminated specimens (100-500 µL) were washed with wash buffer (1 mL) and centifugated at 12.200 g for 3 min. The supernatant was removed and mycobacteria were killed by heating the pellet at 105˚C for 30 min. After sonication, DNA was extracted from the cells by using 100 µL lysis buffer. Then, after neutralization with BD-SDA neutralization buffer, DNA extracts were inoculated into wells. The plate was incubated at room temperature for 20 min, and then the priming mix was re-incubated at 72.5˚C for 10 min. After activating enzymes, dNTPs and buffer in a separate plate at 54˚C for 10 min, they were added with 100 µL priming mix. Plates were inoculated to the ProbeTec analyser. For each run, positive and negative controls were added and each test well included an internal control. Samples which had fluorescence >3400 Method Other Than Acceleration (MOTA) were considered as positive.

Results

A total of 703 samples with suspected tuberculosis that had been tested by three methods were evaluated. Distribution of pulmonary (n=182) and extrapulmonary (n=521) samples is shown in the Figure 1.

According to culture results, 39 specimens were positive and 664 were determined to be negative. Out of the culture positive 39 specimens, 34 were detected to be positive and 5 negative with BD ProbeTec ET, and with EZN 16 were positive and 23 were determined to be negative. The ProbeTec ET system gave 5 false-negative and 8 false-positive results compared to culture. All 5 false negative specimens were extrapulmonary (pleural fluid, peritoneal fluid and puncture fluid). EZN results of these specimens were detected to be negative also. Four out of 8 false positive patients were detected to be diagnosed with tuberculosis and treated within the last five years. EZN evaluation results were detected to be negative in 6 patients and positive in 2 patients. The results of culture, EZN and BD ProbeTec ET are shown in Table 1.

In the comparison of culture EZN results, sensitivity was determined to be 100%, and specificity 98.8%, positive predictive value 87.5% and negative predictive values 100% for pulmonary specimens, while for extra pulmonary specimens, sensitivity has been determined to be 80%, specificity 98.7%, positive predictive value 76.9% and negative predictive values 98.9% as well. Upon comparison of culture and EZN results for pulmonary specimens, sensitivity was determined to be 71.4%, 98.8%, positive predictive value 83.3% and negative predictive values 97.6%, while for extra pulmonary specimens sensitivity 24%, specificity 98.3%, positive predictive value 42.8% and negative predictive values 96.2% were determined. The comparison of specificity, sensitivity, positive predictive and negative predictive values of EZN and BD ProbeTec ET are given in Table 2.

Discussion

Recent and various methods have been developed for the diagnosis of tuberculosis, however EZN is still considered to be rapid, cheap and the first applied method in staining the tubercle bacilli (2). However, 5000-10.000 bacteria per mL are required for staining and smear microscopy (2, 3, 6). The gold standard for diagnosis of mycobacterial infections is the classical diagnostic culture method (2). However, new methods that can give rapid results are needed due to the length of culture period.

In the study by Wang et al. (7) the authors compared the BD ProbeTec ET system with culture. Sensitivity, specificity, positive and negative predictive values of the respiratory samples in this study were as follows; 77.5%, 98.0%, 75.6%

Table 1. The results of culture, EZN and BD ProbeTec ET

			EZN		BD Probe Tec ET	
			Positive	Negative	Positive	Negative
Pulmonary (182)	Culture	Positive (14)	10	4	14	-
		Negative (168)	2	166	2	166
Extrapulmonary (521)	Culture	Positive (25)	6	19	20	5
		Negative (496)	8	488	6	496

EZN: Erlich-Ziehl-Neelsen

Table 2. Comparison of specificity, sensitivity, positive predictive and negative predictive values of EZN and BD ProbeTec ET

		Specificity (%)	Sensitivity (%)	Positive predictive values (%)	Negative predictive values (%)
Pulmonary	BD Probe Tec ET	98.8	100	87.5	100
	EZN	98.8	71.4	83.3	97.6
Extrapulmonary	BD Probe Tec ET	98.7	80	76.9	98.9
	EZN	98.3	24	42.8	96.2

EZN: Erlich-Ziehl-Neelsen

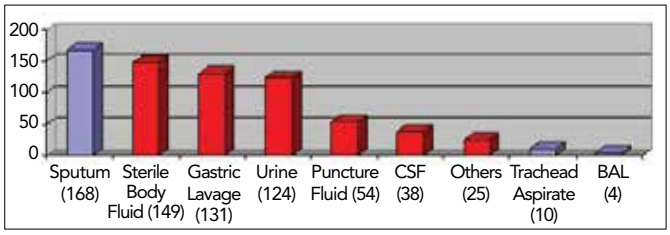

Figure 1. Distribution of pulmonary and extrapulmonary samples (Pulmonary samples are depicted as blue columns and extrapulmonary samples are red)

and 98.2%, respectively. We had false positive results in 15 specimens evaluated in this study. These 5 isolates were determined to be non-tuberculosis mycobacteria and the false positivity ratio was high in these specimens, as well.

In another study conducted by Wang et al. (8) *M. tuberculosis* determination of the BD ProbeTec ET system was evaluated in 600 clinical specimens. Of these specimens, 552 were respiratory and 48 were non-respiratory. When the BD ProbeTec ET system results were compared with culture results, sensitivity, specificity, positive predictive values and negative predictive results of respiratory and non-respiratory specimens were detected to be 73.3%, 93.3%, 38.6%, 98.4%; 0%, 93.6%, 0% and 97.8% respectively. 23 false positive results were obtained. Eleven of these patients were either former tuberculosis patients who were not being given any treatment or patients with active tuberculosis who were being given treatment.

According to the study by Barrett et al. (9) the BD ProbeTec ET system provided positive results in 101 out of culture positive 109 isolates. In this study, when the BD ProbeTec ET system results were compared with culture, sensitivity was determined to be 92.7%, specificity 96.0%, positive predictive value 97%, and negative predictive values 90%.

In the study by Wang et al. (10) for comparing the BD ProbeTect with NAP test to differentiate *M. tuberculosis* complex from non-tuberculosis myobacteria, the sensitivity of the ProbeTec system in determining *M. tuberculosis* complex was reported as 97.8%.

In a study by McHugh et al. (11) they compared the BD ProbeTec ET system with culture. Sensitivity, specificity, positive and negative predictive values of the respiratory samples in this study were as follows; 98%, 89%, 73%, 99%, respectively. Also, sensitivity, specificity, positive and negative predictive values of non-respiratory samples, cerebrospinal fluid, were as follows; 100%, 95%, 29%, 100%, respectively.

In our study, when the gold standard culture and BD ProbeTec ET system results were compared, sensitivity was determined to be 100%, and specificity 98.8%, positive predictive value 87.5% and negative predictive values 100% in the pulmonary specimens, while sensitivity was determined to be 80%, specificity 98.7%, positive predictive value 76.9% and negative predictive values 98.9% in extra pulmonary specimens.

When EZN, a cheap and rapid method for detection of acid-fast bacilli, was compared with the ProbeTec ET system, it is observed that sensitivity and positive predictive values, in particular, are quite low.

Of the 5 samples which were detected negative by the BD ProbeTec ET system and positive by culture, none was a pulmonary material (four were sterile body fluid and 1 was puncture fluid).

Four of the eight false-positive samples detected by BD ProbeTec ET were isolated from patients who had previously received tuberculosis diagnosis and treatment in the last five years.

Although various methods are used for the determination of *M. tuberculosis*, we are seeking to find out better methods (12).

Culture maintains its importance in the detection of tuberculosis bacilli, but we also suggest that BD ProbeTec ET is a reliable system that can give rapid results with high specificity and sensitivity.

Ethics Committee Approval: *Ethics committee approval was received for this study.*

Informed Consent: *N/A.*

Peer-review: *Externally peer-reviewed.*

Author contributions: *Concept - A.K., E.U., K.B.; Design - A.K., E.U., K.B.; Supervision - A.K., C.E., M.G.; Resource - A.K., E.U., K.B.; Materials - A.K., E.U., K.B.; Data Collection&/or Processing - A.K., E.U., K.B., A.K.G.; Analysis&/or Interpretation - C.E., A.K., M.G.; Literature Search - A.K., K.B., A.K.G.; Writing - A.K., A.K.G., K.B.; Critical Reviews - C.E., M.G.*

Conflict of Interest: *No conflict of interest was declared by the authors.*

Financial Disclosure: *No financial disclosure was declared by the authors.*

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