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## Evaluation of the Ehrlich-Ziehl-Neelsen (EZN) and Amplified *Mycobacterium tuberculosis* Direct Test According to the BACTEC Method in Respiratory and Nonrespiratory Samples\*

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**Aim:** Tuberculosis remains a significant and threatening disease, particularly in developing countries. *Mycobacterium tuberculosis* should be detected and identified as soon as possible to ensure the prevention of the spread of the disease. For this purpose, use of fast and reliable laboratory diagnostic methods with high sensitivity and specificity was initiated in recent years.

**Materials and Methods:** In this study, 107 respiratory and 198 nonrespiratory (305 in total) samples submitted to Dicle University Faculty of Medicine Clinical Microbiology Laboratory were examined using the Ehrlich-Ziehl-Neelsen (EZN), BACTEC 460 TB (Becton and Dickinson Diagnostic Instrument System, Towson, MD), and MTD (Amplified *Mycobacterium tuberculosis* Direct Test, Gen-Probe, USA) methods.

**Results:** In respiratory samples, sensitivity of EZN was found as 83.33%, specificity as 95.04%, positive predictive value as 50%, and negative predictive value as 98.96%, whereas in nonrespiratory samples these values were 18.18%, 98.39%, 40%, and 95.37%, respectively. In respiratory samples, sensitivity of MTD was found as 83.33%, specificity as 94.05%, positive predictive value as 45.45%, and negative predictive value as 98.95%, whereas in nonrespiratory samples these values were 54.54%, 88.23%, 21.42%, and 97.05%, respectively.

**Conclusions:** In view of the above, the pre-diagnostic EZN test and the MTD method based on nucleic acid amplification should be applied together with the BACTEC 460 system, which is considered as a gold standard, and the evaluation should be made accordingly. Furthermore, MTD should not be used as a screening test due to its high cost, and should rather be preferred in smear-positive samples.

**Key Words:** Tuberculosis, Amplified *Mycobacterium tuberculosis* Direct Test, BACTEC, Ehrlich-Ziehl-Neelsen

### Solunum ve Solunum Dışı Örneklerde BACTEC Yöntemine Göre Ehrlich- Ziehl-Neelsen (EZN) ve Amplified *Mycobacterium tuberculosis* Direct Test Yöntemlerinin Değerlendirilmesi

**Amaç:** Tüberküloz özellikle gelişmekte olan ülkelerde önemli ve tehdit edici bir hastalık olarak varlığını sürdürmektedir. Hastalığın yayılımının önlenmesi bakımından *Mycobacterium tuberculosis*'in tespiti ve identifikasyonunun mümkün olan en kısa sürede yapılması gerekmektedir. Bu amaçla son yıllarda duyarlılık ve özgüllüğü yüksek, güvenilir, hızlı laboratuvar tanı yöntemleri kullanılmaya başlanmıştır.

**Yöntemler:** Bu çalışmada Dicle Üniversitesi Tıp Fakültesi Klinik Mikrobiyoloji Laboratuvarı'na gönderilen 107 respiratuvar ve 198 nonrespiratuvar olmak üzere toplam 305 örnek Ehrlich- Ziehl-Neelsen (EZN), BACTEC 460 TB (Becton and Dickinson Diagnostik Instrumant Sistem, Towson, MD), MTD (Amplified *Mycobacterium tuberculosis* Direct Test, Gen-Probe, ABD) yöntemleri ile incelenmiştir.

**Bulgular:** EZN için respiratuvar örneklerde duyarlılık %83.33, özgüllük %95.04, pozitif prediktif değer %50, negatif prediktif değer %98.96, nonrespiratuvar örneklerde ise bu değerler sırasıyla %18.18, %98.39, %40, %95.37 MTD için respiratuvar örneklerde duyarlılık %83.33, özgüllük %94.05, pozitif prediktif değer %45.45, negatif prediktif değer %98.95, nonrespiratuvar örneklerde ise bu değerler sırasıyla %54.54, %88.23, %21.42, %97.05 olarak bulunmuştur.

**Sonuç:** Ön tanı testi olan EZN ve nükleik asit amplifikasyon esasına dayanan MTD yöntemlerinin, altın standart olarak kabul edilen BACTEC TB 460 sistemiyle birlikte çalışılması ve değerlendirmelerin buna göre yapılması gerekmektedir. Ayrıca pahalı olması nedeni ile MTD, tarama testi olarak kullanılmamalı, daha çok smear pozitif örneklerde kullanılmaları tercih edilmelidir.

**Anahtar Sözcükler:** Tuberculosis, MTD, BACTEC, EZN

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## Introduction

Tuberculosis is currently the most widespread and lethal infectious disease throughout the world, and is considered a serious public health problem. One-third of the world's population is infected with *Mycobacterium tuberculosis*. The number of individuals affected by tuberculosis began to increase again after 1985. The increase was reported as due to the transmission of the disease from the homeless, drug and alcohol addicts, hostages, and individuals infected with human immunodeficiency virus (HIV) due to difficulty in its eradication to other communities, including health employees, as well as to the development of multidrug resistance in those who receive improper treatment (1,2).

The most important phase in tuberculosis control is early and correct diagnosis of active cases. Although clinical findings and traditional diagnostic methods (tuberculosis skin test, chest radiography, etc.) may be illuminating, definitive diagnosis is possible through the isolation of the bacillus from the appropriate clinical samples. However, a long period of 2 to 8 weeks is required to isolate *M. tuberculosis*. Therefore, demonstration of acid-resistant bacilli in directly stained preparations is important in pre-diagnosis (3).

Identification of mycobacteria and performance of sensitivity tests in as fast as 1 to 2 weeks is possible by using the BACTEC 460 TB system, which is recognized as the gold standard from among the radiometric methods by the National Committee for Clinical Laboratory Standards (NCCLS) (4).

The Amplified *M. tuberculosis* Direct Test (MTD–Gen-Probe, USA), which is among the tests based on nucleic acid amplification, is one of the Food and Drug Administration (FDA)-approved systems aimed at target amplification. In this system, Transcription-Mediated Amplification (TMA) and Gene Probe Hybridization Protection Assay (HPA) are combined and amplification and screening take place in two consecutive phases (5).

In this study, respiratory and nonrespiratory samples pre-diagnosed as tuberculosis sent to the Dicle University Clinical Microbiology Laboratory were examined using the conventional Ehrlich-Ziehl-Neelsen (EZN) staining, the molecular diagnostic MTD, and the BACTEC 460 TB system, which is referred to as the gold standard. The results obtained are compared based on the BACTEC 460 TB.

## Materials and Methods

A total of 107 respiratory samples consisting of 100 (93.4%) sputum and 7 (6.54%) bronchoalveolar lavage samples, and 198 nonrespiratory samples consisting of 74 (37.4%) cerebrospinal fluid, 34 (17.2%) urine, 17 (8.6%) gastric fluid, 29 (14.6%) pleural fluid, 26 (13.1%) acid fluid, 10 (5.1%) abscess, 4 (2%) pericardial fluid, 3 (1.5%) joint fluid, and 1 (0.5%) nephrostomy samples collected from patients pre-diagnosed with tuberculosis and sent to the Clinical Microbiology Laboratory of Dicle University Faculty of Medicine were examined in the study. Following decontamination (with NaOH) and homogenization of the clinical samples, preparations of samples were stained with the EZN method and examined microscopically. Cultures were started following addition of PANTA into the Middlebrook 7 TH 12 (BACTEC 12B) medium. The level of CO<sub>2</sub>, which is formed as a result of C-labeled palmitic acid utilization by mycobacteria, was measured routinely 2-3 times a week in the BACTEC 460 TB (Becton and Dickinson Diagnostic Instrument System, Towson, MD) instrument. These measured values displayed the growth index (GI). Distinction of the *M. tuberculosis* complex and mycobacteria other than tuberculosis was made by NAP test at a GI of 50-100. Mycobacterial target nucleic acid (16S rRNA) was obtained by MTD (Gen-Probe, USA); sonication method was amplified isothermally by TMA method. Amplicons were combined with acridinium ester-labeled DNA probes to form stable RNA-DNA hybrids in the HPA stage. Luminometric measurements of the labeled RNA-DNA hybrids were performed in relative light units (RLU).

In the statistical analysis, sensitivity, specificity, and positive and negative predictive values for EZN and MTD methods were calculated with methodological investigations, with BACTEC radiometric culture system taken as a reference (6).

## Results

The results of our study revealed 5.60% positivity in the respiratory samples and 5.55% in the nonrespiratory samples by BACTEC 460 TB system analysis. EZN staining method revealed rates of positivity of 9.34% and 2.52%, respectively, while these rates were found as 10.2% and 14.14%, respectively, with the MTD system.

The results for the EZN staining method and the BACTEC 460 TB system used for the laboratory diagnosis of the respiratory and non-respiratory samples are given in Table 1.

The results obtained by MTD method in the respiratory and non-respiratory samples are presented in Table 2 along with BACTEC 460 TB system results.

## Discussion

In spite of the developments in diagnosis and treatment, tuberculosis is still a globally widespread infection today, especially in developing countries, due to the increase in immune system suppressing infections and malignancies, and multidrug resistance. The treatment rate is a significant issue in tuberculosis, and diagnosis has to be made before initiation of the treatment. The routinely required processes for the laboratory diagnosis of tuberculosis were defined in three stages by the World Health Organization (WHO): direct microscopic

examination, culturing, and identification, followed by susceptibility tests to primary anti-tuberculosis drugs. Various staining methods are applied to the preparations of clinical samples for microscopic examination (1,2).

The most widely used staining method is EZN, which is also the first process we perform in our laboratory for detection of tuberculosis bacilli in the samples. Ease of application, quickness, and cost effectiveness are the advantages of the microscopic examination. Nonetheless, the bacilli can only be detected in direct microscopic examination if there are 5000-10000 bacilli per milliliter of the sample, and various studies have revealed that acid resistance staining was positive in only 50-80% of the untreated pulmonary tuberculosis patients (7).

In this study, the sensitivity, specificity, and positive and negative predictive values of the EZN staining method used in microscopic diagnosis were detected as 83.33%, 95.04%, 50%, and 98.96%, respectively, compared to the BACTEC 460 TB system, which is considered as the gold standard. Piersimoni et al. (8), in their study on

Table 1. Results obtained by BACTEC and EZN methods in the respiratory samples.

EZN	BACTEC		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative				
Nonrespiratory (n=198)			%18.18	%98.39	%40	%95.33
Positive	2	3				
Negative	9	184				
Respiratory (n=107)			%83.33	%95.04	%50	%98.96
Positive	5	5				
Negative	1	96				

EZN: Ehrlich-Ziehl-Neelsen. PPV: Positive predictive value. NPV: Negative predictive value.

Table 2. Results obtained by BACTEC and MTD methods in the non-respiratory samples.

MTD	BACTEC		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative				
Nonrespiratory (n=198)			%54.54	%88.23	%21.42	%97.05
Positive	6	22				
Negative	5	165				
Respiratory (n=107)			%83.33	%94.05	%45.45	%98.95
Positive	5	6				
Negative	1	95				

MTD: Amplified M. tuberculosis direct test. PPV: Positive predictive value. NPV: Negative predictive value.

respiratory samples, reported sensitivity of the method as 77%, whereas they declared its specificity and positive and negative predictive values to be 100%, 100%, and 95%, respectively. High values reported in the study by Presimoni et al. (8) are due to the patient group selected by the investigators including individuals with definite diagnosis of tuberculosis.

In this study, these values were found as 18.18%, 98.39%, 40%, and 95.37%, respectively, for the nonrespiratory samples. Pinar et al. (12) found a higher degree of sensitivity in the clinical samples with values of 48.8%, 92.9%, 27.2%, and 97.1%, without distinction between the respiratory and nonrespiratory samples.

Techniques based on amplification were developed for tuberculosis disease, to quicken the isolation of the bacillus and to increase sensitivity. In this study, sensitivity of the MTD method was found to be 83.33% in the respiratory samples and 54.54% in the nonrespiratory samples. The sensitivity value for MTD (detected as 83.33% in respiratory samples) is in accordance with the values found by Pfyffer et al. (9) (86.6%) and Gamboa et al. (10) (86.8%). However, Vlaspolder et al. (11) (98.4%) and Piersimoni et al. (8) (95%) detected high values, while a low sensitivity was reported by Pinar et al. (12) (67.6%). The high specificity detected for MTD in the respiratory samples in our study (94.05%) is in accordance with the values detected by other investigators (between 96.4% and 100%). Sensitivity and specificity values detected in the nonrespiratory samples in our study were 54.54% and 88.23%, respectively. These values were reported by Pfyffer et al. (9) as 93.1% and 97.7%, by Vlaspolder et al. (11) as 100% and 95%, by Woods et al. (13) as 87.5% and 100%, and by Gamboa et al. (10) as 93.1% and 97.7%, respectively. The different results reported

in the literature by Pfyffer et al. (9) for MTD sensitivity were related with different technicians performing the tests and with the large variety of samples tested.

While MTD is a sensitive, easy to apply and quick test, it requires a good quality procedure and a controlled study. Various factors have been related to the false-positive and false-negative results in the MTD method in the literature. For instance, Vlaspolder et al. (11) stated that excessive contamination with blood accounted for the false-positive results obtained in respiratory samples. The same study stated that MTD-positive and culture-negative results were common in patients receiving treatment. MTD was observed to turn negative during the therapy. It was claimed that MTD could be used for the follow-up of the therapy. Some studies recommend the use of the MTD test in smear-negative cases, to provide early initiation of the specific therapy (14). Other investigators suggest that MTD should only be preferred in smear-positive clinical samples, due to higher sensitivity and higher cost in smear-positive samples (5,15).

As the MTD method is applied according to certain protocols and is a standardized method, the likelihood of application errors is quite low. A low positive predictive value and a high negative predictive value suggest a careful evaluation of the positive results obtained by MTD, and a higher reliability of the negative results (12).

In conclusion, the pre-diagnostic EZN test and the MTD method based on nucleic acid amplification should be applied together with the BACTEC 460 system, which is considered as a gold standard, and the evaluation should be made accordingly. Furthermore, MTD should not be used as a screening test due to the high cost, and should rather be preferred in smear-positive samples.

## References

1. Forbes AB, Sahm FD, Weissfeld SA. Diagnostic microbiology. 11th ed. St. Louis: Mosby, Inc; 2002. p. 538-9.
2. Murray RP, Rosenthal SK, Kobayashi SG, Pfaller AM. Medical microbiology. 4th ed. St. Louis: Mosby, Inc; 2002. p. 369.
3. Ba F, Rieder HL. Comparison of fluorescence microscopy with the Ziehl-Neelsen technique in the examination of sputum for acid-fast bacilli. *Int J Tuberc Lung Dis* 1999; 3: 1101-1105.
4. Piersimoni C, Scarparo C. Relevance of commercial amplification methods for direct detection of *Mycobacterium tuberculosis* complex in clinical samples. *J Clin Microbiol* 2003; 41: 5355-5365.
5. Siddiqi SH. Procedure for primary isolation of mycobacteria from clinical specimens. P.II- II.13. In BACTEC TB System Product and Procedure Manual, Becton Dickinson, Maryland 1989.
6. Beaglehok R, Bonita R, Kjellström T. Epidemiology and Prevention. *Basic Epidemiology*. WHO, Geneva 1993; 83-96.
7. Huggett FJ, McHugh DT, Zumla A. Tuberculosis: amplification-based clinical diagnostic techniques. Available online 8 April 2003.
8. Piersimoni C, Callegaro A, Nista D, Bornigia S, De Conti F, Santini G et al. Comparative amplification assays for direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens. *J Clin Microbiol* 1997; 37: 193-196.

9. Pfyffer GE, Kissling P, Jahn EM, Welscher HM, Salfinger M, Weber R. Diagnostic performance of amplified *Mycobacterium tuberculosis* direct test with cerebrospinal fluid, other nonrespiratory, and respiratory specimens. *J Clin Microbiol* 1996; 34: 834-841.
10. Gamboa F, Manterola JM, Lonca J, Vinado B, Matas L, Gimenez M et al. Rapid detection of *Mycobacterium tuberculosis* in respiratory specimens, blood and other non-respiratory specimens by amplification of rRNA. *J Tuberc Lung Dis* 1997; 1: 542-555.
11. Vlasploder F, Singer P, Roggeveen C. Diagnostic value of an amplification method (Gen-Probe) compared with that of culture for diagnosis of tuberculosis. *J Clin Microbiol* 1995; 33: 2699-2703.
12. Pınar A, Ercis S, Haşçelik G. Evaluation of Ziehl-Neelsen staining and amplified *Mycobacterium tuberculosis* direct test methods in comparison with Bactec system for the diagnosis of tuberculosis in various clinical samples submitted to clinical microbiology laboratory. *Turkish J Infection* 2004; 18: 429-432.
13. Woods GL, Bergmann JS, Williams-Bouyer N. Clinical evaluation of the Gen-Probe amplified mycobacterium tuberculosis direct test for rapid detection of *Mycobacterium tuberculosis* in select nonrespiratory specimens. *J Clin Microbiol* 2001; 39: 747-749.
14. Bergmann JS, Yuoh G, Fish G, Woods GL. Clinical evaluation of the enhanced Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test for rapid diagnosis of tuberculosis in prison inmates. *J Clin Microbiol* 1999; 37: 1419-1425.
15. Alcalá L, Ruiz-Serrano MJ, Hernangomez S, Marin M, Viedma DG, San Juan R et al. Evaluation of the upgraded amplified *Mycobacterium tuberculosis* direct test (Gen-Probe) for direct detection of *Mycobacterium tuberculosis* in respiratory and non-respiratory specimens. *Diagn Microbiol Infect Dis* 2001; 41: 51-56.