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SHORT COMMUNICATION

Five Years' Evaluation of the BD ProbeTec System for the Direct Molecular Detection of *Mycobacterium tuberculosis* Complex in Respiratory and Nonrespiratory Clinical Samples

CAN BICMEN[1]*, ONUR KARAMAN[2], AYRIZ T. GUNDUZ[1], ONUR F. ERER[2], MERAL COSKUN[1], OSMAN KAFTAN[1], MAHMUT M. DEMIREL[1], GUNES SENOL[1], TULAY AKARCA[2], SEVKET DERELI[2] and AYSE OZSOZ[2]

[1] Dr Suat Seren Chest Diseases and Chest Surgery Training and Research Hospital, Medical Microbiology Laboratory, Izmir, Turkey
[2] Dr Suat Seren Chest Diseases and Chest Surgery Training and Research Hospital, Tuberculosis and Chest Diseases Service, Izmir, Turkey

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Abstract

In this study, *Mycobacterium tuberculosis* complex was detected by BD ProbeTec ET system in 4716 respiratory and 167 nonrespiratory samples [mostly (98%) smear negative]. Sensitivity, specificity, positive and negative predictive values were 81.8%, 98.3, 85.1 and 97.9 for respiratory and 100%, 96.2, 64.7 and 100, for nonrespiratory samples, respectively. Among 149 (3.1%) ProbeTec DTB positive and culture negative samples, 72 (65 respiratory and seven nonrespiratory) (48.3%) were recovered from the patients who were evaluated as having TB infection. The system has been found as useful in early diagnosis of tuberculosis infection in association with the clinical, radiological and histopathological findings.

Key words: BD ProbeTec ET, extrapulmonary, molecular detection, pulmonary, tuberculosis

According to the World Health Organization (WHO), tuberculosis (TB) has been still an important problem all over world (WHO, 2013a). The definitive TB diagnosis depends on the results of microbiological tests in addition to the clinical, radiological and histopathological data and the mycobacterial culture has been accepted as the gold standard. As the WHO started DOTS [Directly Observed Therapy Strategy (plus)] (WHO, 1999; Gupta *et al*., 2003) for treatment of TB disease, early and accurate diagnosis has become an important issue to take precautions and to initiate treatment plan in the hospital clinics for chest diseases. Although microscopic examination and culture are major microbiological tests for TB diagnosis, the low sensitivity of microscopy and the necessity of long incubation time for culture are the most significant limitations. Therefore, nucleic acid amplification (NAA) techniques have been used for early and differentiative detection of causative mycobacteria in clinical samples and also to support the clinical and radiological diagnosis in patients with presumptive *Mycobacterium tuberculosis* infection (CDC, 2009). One of the automated systems widely used is the BD ProbeTec ET which is based on the strand displacement amplification (SDA) technology. The system utilizes homogenous SDA technology as the amplification method and fluorescent energy transfer (ET) as the method of detecting the presence of *Mycobacterium tuberculosis* complex directly from clinical samples.

The aim of this study was to evaluate the performance of BD ProbeTec ET *M. tuberculosis* complex (DTB) Assay according to the laboratory data of five years in accordance with the clinical diagnosis. The clinical evaluation of patients for TB diagnosis and follow-up procedures were made by specialist physicians according to the WHO and national guidelines (WHO, 2013b).

A total of 4883 samples [4716 respiratory (4626 bronchial aspiration and 90 sputum), and 167 nonrespiratory (NR) (102 fine needle aspiration biopsy (FNAB), 27 urine, 15 cerebrospinal fluid (CSF), 11 pleural fluid, 2 gastric lavage, 2 surgical material and 8 various sterile body fluids or pus)] recovered from 4304 patients between October 2009 and October 2013 were analyzed in the Microbiology Laboratory of Izmir Training and Research Hospital for Chest Diseases and Chest

* Corresponding author: C. Bicmen, Dr Suat Seren Chest Diseases and Chest Surgery Training and Research Hospital, Medical Microbiology Laboratory, Izmir, Turkey; **e-mail: cbicmen@yahoo.com**

Surgery which has been a regional reference hospital for TB patients at the Aegean Coast of Turkey (West Anatolian Region). The patients included in the study were evaluated as having suspicious TB infection. An acid-fast smear preparation, mycobacterial cultivation, identification and molecular detection were applied to each sample.

Mycobacterial cultivation was performed by MGIT 960 system (BD Biosciences, Sparks, MD, USA) and in Lowenstein-Jensen slants. Mycobacterial identification was performed by conventional methods (Koneman *et al*., 1992), BACTEC 460 p-nitro-α-acetyl amino-β-hydroxypropiophenone (NAP) or BD immunochromatographic test. Additionally, commercially available PCR based reverse hybridization (Line Probe Assay = LiPA) kits were used for further identification of nontuberculous mycobacteria in species level.

Rapid molecular detection and identification for each sample was performed by the BD ProbeTec ET *M. tuberculosis* complex (DTB) Assay on the basis of BD ProbeTec ET system according to the manufacturer's recommendations. The test system has utilized homogenous SDA technology as the amplification method and fluorescent ET as the method of detecting the presence of *M. tuberculosis* complex DNA directly from clinical specimens. All calculations were performed automatically by the instrument software. Results were reported through an algorithm as positive, negative, or indeterminate. The molecular assays with the discrepant results according to the culture and which were considered as false positive or had cross-contamination were repeated using frozen aliquots of the samples. The same result which was taken twice (either negative or positive) repeatedly was accepted as the final result in these discrepant assays. Specimens with invalid results showing inhibition of internal control (IC) and sample amplification were also retested with the dilution of 1/100. Totally, IC and sample amplification was not observed in 6 (0.12%) samples which were AFB negative and evaluated as inhibition. These samples gave valid results with the dilution of 1/100.

Among 4883 specimens tested, 4784 (98%) and 99 (2%) were smear negative and positive with acid-fast staining, respectively. ProbeTec DTB tests of 193 (4%) samples were repeated because of discrepancy. After evaluation of test results, 149 (136 respiratory, 13 NR) (3.1%) samples were culture negative and ProbeTec DTB positive, whereas 90 respiratory (1.8%) samples were culture positive and ProbeTec DTB negative. ProbeTec DTB negative and culture positive samples were also confirmed as TB by clinical evaluation. Among ProbeTec DTB positive and culture negative samples (n = 149), 72 (65 respiratory, 6 FNAB and 1 pleural fluid) (48.3%) samples were recovered from the patients who were evaluated as having TB infection and applied anti-TB treatment according to the clinical data and/or positive mycobacterial cultures taken from the other separate samples. Sixty eight of patients had TB diagnosis with clinical evaluation solely, while four patients had additional positive mycobacterial cultures taken from other separate samples. The rest of the samples (70 bronchial aspiration and 1 sputum; respiratory and 2 urine, 2 FNAB, 1 abscess and 1 gastric lavage; NR) were obtained from the patients with a diagnosis other than active TB infection [*i.e.* 69 respiratory samples obtained from patients with pneumonia (n = 20), lung malignancy (n = 20), COPD (n = 8), past TB infection (n = 6), sarcoidosis (n = 3), hemoptysis (n = 4), hydatidosis (n = 2), empyema (n = 1), asthma (n = 1), bronchiectasis (n = 1), Churg-Strauss syndrome (n = 1), interstitial lung disease (n = 1), silicosis (n = 1) and 6 NR samples obtained from patients with urinary tract infection (n = 2), lung malignancy (n = 1), sarcoidosis (n = 1), skin abscess (n = 1) and pneumonia (n = 1)]. Additionally, two bronchial aspiration which were taken from patients with diagnosis of extrapulmonary TB (pleurisy and lymphadenitis) were ProbeTec DTB positive and culture negative as well. In 13 respiratory samples, nontuberculous mycobacterial growth was positive in culture. Among these, two samples (sputum and bronchial aspiration) were found as false positive and the other 11 samples were found as negative by ProbeTec DTB test. Distribution of culture and ProbeTec DTB test results in smear negative and positive samples according to the sample type has been shown in Table I. Sensitivity, specificity, positive and negative predictive values for ProbeTec DTB test in clinical samples when compared with the culture and with the culture in combination with the clinical diagnosis have been shown in Table II.

ProbeTec ET is one of the widely used semi-automated NAA systems based on SDA technology. The semi-automated systems like ProbeTec ET, Cobas Taqman MTB (Roche, Germany), Gen-Probe Amplified MTB Direct Test (Gene-Probe, USA) and recently, some other technologies such as RT-PCR based assays have the advantage of processing larger batch of samples at once. In low-prevalence settings, use of these systems may be more preferable to make a differential diagnosis for TB from many other clinical pictures. Patient population selected for NAA testing in TB can be variable according to the clinical findings, stage of the disease (*i.e.* anti-TB treatment), the incidence of mycobacteria in that region and the experience of the laboratory. Each TB control or treatment program should evaluate the overall costs and benefits of NAA testing in deciding the value and optimal use of the test in their setting. As the incidence of *M. tuberculosis* complex has been reported to be low (app. 20%) in our region (WHO, 2013a), it has been considered that

Table I
Distribution of culture* and ProbeTec DTB test results in smear negative and positive samples according to sample type

Sample type		Smear negative (n = 4784) Culture / BD ProbeTec				Smear positive (n = 99) Culture / BD ProbeTec			
		+/–†	–/+‡	+/+	–/–	+/–†	–/+‡	+/+	–/–
Respiratory (n = 4716)	BA / BAL (n = 4626)	81	130	237	4085	3	1	86	3
	Sputum (n = 90)	6	3	14	61	0	2	3	1
NR (n = 167)	Biopsy§ (n = 104)	0	8	0	96	0	0	0	0
	Urine (n = 27)	0	2	1	24	0	0	0	0
	CSF (n = 15)	0	0	2	13	0	0	0	0
	PF (n = 11)	0	1	0	10	0	0	0	0
	SBF‖ (n = 8)	0	1	1	6	0	0	0	0
	GL (n = 2)	0	1	0	1	0	0	0	0
Total (n = 4883)		87	146	255	4296	3	3	89	4

Abbreviations: NR: Nonrespiratory, BA: Bronchial aspiration, BAL: bronchoalveolar lavage, CSF: cerebrospinal fluid, PF: Pleural fluid, SBF: Sterile body fluids, GL: Gastric lavage, –: negative, +: positive

* Culture was done by BACTEC 960 system and in Lowenstein Jensen medium.

† BD ProbeTec ET DTB negative and culture positive samples were also confirmed as TB by clinical evaluation.

‡ Among BD ProbeTec ET DTB positive and culture negative samples, 72 (48.3%) samples (65 respiratory, 7 nonrespiratory) were recovered from the patients who were evaluated as having TB infection and applied anti-TB treatment according to the clinical data and/or positive mycobacterial cultures taken from the other separate samples.

§ Biopsy (*i.e.* 102 fine needle aspiration biopsy (FNAB) and two surgical materials).

‖ Sterile body fluids (*e.g.* pericardial fluid, wound, ascites)

Table II
Sensitivity, specificity, positive and negative predictive values for ProbeTec DTB test in clinical samples*

Sample Type		Sensitivity (%)		Specificity (%)		PPD (%)		NPD (%)	
		A	B	A	B	A	B	A	B
Smear negative	Respiratory	74.3	78.4	96.9	98.3	65.4	82.3	97.9	97.9
	Nonrespiratory	100.0	100.0	92	96.2	23.5	64.7	100.0	100.0
	Total	74.6	78.8	96.7	98.5	63.6	80.8	98	98
Smear positive	Respiratory	96.7	96.8	N/A	N/A	96.7	100.0	N/A	N/A
All Samples	Respiratory	79.1	81.8	96.8	98.3	71.4	85.1	97.9	97.9
	Nonrespiratory	100.0	100.0	92	96.2	23.5	64.7	100.0	100.0
	Total	79.3	82.2	96.7	98.2	69.8	84.4	97.9	97.9

* Column A indicates the sensitivity, specificity, positive and negative predictive values compared with the culture, whereas column B indicates the values compared with the culture in combination with the clinical diagnosis.

a NAA testing in addition to the conventional culture and automated cultivation systems which is available for batch processing and random access under the same platform would be an ideal solution for rapid and accurate diagnosis and treatment planning.

In previous studies, the sensitivity, the specificity, positive and negative predictive levels of the BD ProbeTec ET were reported within the ranges of 56.7–100, 95.3–100, 59.6–100 and 96–100%, for respiratory, 50–100 and 93.3–98.7, 33.3–80 and 97.8–100%, for nonrespiratory samples, respectively (Abdel-Aziz *et al*., 2011; Antonenka *et al*., 2013; Barber, 2008; Barrett *et al*., 2002; Bergmann and Woods, 1998; Bergmann *et al*., 2000; Johansen *et al*., 2002; Karadag *et al*., 2013; Maugein *et al*., 2002; Mazzarelli *et al*., 2003; Pfyffer *et al*., 1999; Piersimoni *et al*., 2002; Rüsch-Gerdes and Richter, 2004; Tu *et al*., 2011; Wang *et al*., 2004; Wang *et al*., 2006). In the present study, the corresponding performance values were found as 81.8, 98.3, 85.1 and 97.9%, for respiratory, and 100, 96.2, 64.7 and 100%, for nonrespiratory samples, respectively. The evaluation of discrepant results in accordance with the other positive cultures and clinical outcome made a low level of increase in the sensitivity and specificity values, but a high level of increase was observed in positive predictive values in respiratory and nonrespiratory samples (Table II). Sensitivity was evaluated as low as with the other studies using different NAA methods in smear negative and nonrespiratory samples whilst the specificity was high. However, this study differs from other studies using BD ProbeTec ET system which reported low sensitivity (25–60%) (Barber, 2008; Barrett *et al*.,

2002; Johansen *et al*., 2002; Wang *et al*., 2004; Wang *et al*., 2006) and positive predictive values (40.9% and 44.7%) (Pfyffer *et al*., 1999; Wang *et al*., 2004) for smear negative respiratory samples and higher values of positive predictive values (70–80%) (Abdel-Aziz *et al*., 2011; Karadag *et al*., 2013; Rüsch-Gerdes and Richter, 2004) for nonrespiratory samples. The performance values of this system might have been misevaluated due to the low sample population which has been included in these studies. As this study was undertaken with high number of sample population for a long period of time, it was thought that a more precise evaluation was achieved for the diagnostic performance of BD ProbeTec ET in smear negative respiratory samples. In nonrespiratory samples, less number of samples (n = 167) were submitted to our laboratory and a low positivity (n = 17) was found as a natural feature of these samples due to the difficulty in laboratory as well as clinical diagnosis. Thus, in two patients who was evaluated as TB clinically, FNAB samples were negative by both culture and BD ProbeTec. Among thirteen BD ProbeTec positive and culture negative nonrespiratory samples, six fine needle aspiration biopsy and a pleural fluid were recovered from the patients who were evaluated as tuberculosis clinically.

In conclusion, in terms of performance values, the ProbeTec ET system has been found as useful in early diagnosis of pulmonary and extrapulmonary TB infection in association with the clinical, radiological and histopathological findings. Nevertheless, the low positive predictive values also address the need for advanced technologies in addition to the conventional methodologies to provide more precise diagnosis in extrapulmonary TB cases.

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