ELSEVIER

Diagnostic Microbiology and Infectious Disease
47 (2003) 573–578
Mycobacteriology

DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE

www.elsevier.com/locate/diagmicrobio

Evaluation of the BDProbeTec ET system as screening tool in the direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens

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Received 3 February 2003; received in revised form 2 July 2003

Abstract

We evaluated the BDProbeTec ET System (Becton Dickinson) for the routine detection of *Mycobacterium tuberculosis* complex (MTC) in respiratory specimens and pleural fluids, comparing with microscopy (Ziehl Neelsen stain, ZN) and culture in liquid (BACTEC MGIT 960, MGIT) and solid (Löwenstein Jensen, LJ) media. Five hundred and two specimens, collected from 266 patients, of which 257 with suspected tuberculosis and 9 receiving anti-tuberculosis treatment, were investigated. Thirty-nine specimens were positive by any method, including false positives. Mycobacteria were isolated from 33 specimens (32 *Mycobacterium tuberculosis* and 1 *Mycobacterium chelonae*). Thirty-six specimens were BDProbeTec ET positive, 33 specimens were MGIT positive, 27 were LJ positive and 22 were ZN positive. With BDProbeTec ET, 2 specimens were false negative (culture positive), and 2 specimens from non-treated patients were false positive (culture negative). The overall sensitivity, specificity, and positive and negative predictive values for BDProbeTec ET compared to culture were 93.7, 98.7, 83.3, and 99.5%, respectively, while with smear-positive and smear-negative specimens the sensitivities were 100% and 81.5% respectively. In five treated patients the disappearance of MTC could be monitored using BDProbeTec ET in parallel with culture. The overall inhibition rate was 0.2%. BDProbeTec ET can be very useful for rapid detection of MTC, especially in smear-negative respiratory specimens.

Keywords: SDA; Mycobacterium; Tuberculosis

1. Introduction

Tuberculosis (TB) is an increasing health problem worldwide, especially in developing countries. The spread of HIV/AIDS and the emergence of multidrug-resistant TB are contributing to the worsening impact of this disease. Up to one third of the world's population is estimated by the World Health Organization (WHO) to be infected with *Mycobacterium tuberculosis* and it is estimated that between 2002 and 2020, approximately 1 billion people will be newly infected, over 150 million people will develop the disease, and 36 million people will die of TB if control is not further strengthened (World Health Organization, 2002).

TB control is based on early detection by acid-fast bacilli (AFB) through AFB stain and culture of mycobacteria using liquid and/or solid media. AFB smear results are available in hours or less but the technique has poor sensitivity and can not distinguish among different species of mycobacteria (Peterson et al., 1999). Although culture is the gold standard with excellent sensitivity, while the liquid media have significantly reduced the detection time, it requires on the average 2-3 weeks to obtain results for slow growing mycobacteria and up to 6 weeks to finalize negative specimens. Because of the low sensitivity of AFB microscopy it is possible that in some cases tuberculosis will not be early detected, which can cause the patient to become an important source of transmission, particularly if culture is not performed. Tuberculosis transmission from smear negative patients is estimated to be approximately 17% (Behr et al., 1999).

Methods based on direct nucleic acid testing (NAT) are more sensitive than microscopy and have reduced the time to detection of *Mycobacterium tuberculosis* complex

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0732-8893/03/$ – see front matter
doi:10.1016/S0732-8893(03)00163-9

(MTC). Polymerase chain reaction (PCR), transcript-mediated assay (TMA), and ligase chain reaction (LCR) were used as molecular tools in the diagnosis of tuberculosis (Bergmann & Woods, 1996; Dalovisio et al., 1996; Ichiyama et al., 1996; Moore & Curry, 1995; Wobeser et al., 1996; Bergmann et al., 1999; Gamboa et al., 1998; Piersimoni et al., 1998; Bennedsen et al., 1996; Jonas et al., 1993; Jungkind et al., 1996; Lindbrathen et al., 1997; Pfyffer et al., 1996). Strand Displacement Amplification (SDA) is an isothermal method for the detection of IS*6110*, a specific target for MTC (Ichiyama et al., 1997; Pfyffer et al., 1999). The BD ProbeTec ET system (Becton Dickinson) uses SDA in combination with real time fluorescence detection of amplified product, thus making results available in a few hours (Little et al., 1999). The BD ProbeTec ET system is approved for direct detection of MTC DNA (DTB test) from decontaminated, digested clinical respiratory specimens such as sputa, induced sputa, bronchial washings, and other respiratory specimens. The DTB test is not available in the USA but is commercially available for diagnostic purposes in Europe and recently in Asia. We have evaluated the BD ProbeTec ET System for the direct detection of MTC in respiratory specimens from patients with suspected TB.

2. Materials and Methods

A total of 502 clinical specimens (435 sputa, 39 broncheoalveolar lavages (BAL), 27 pleural fluids and one pulmonar aspirate) from 266 patients (257 with suspected tuberculosis and 9 receiving anti-tuberculosis treatment), were included in the study. A variable number of specimens per patient was received: more than three per patient in 13 cases, three in 72 cases, two in 43 cases, and only one in 138 cases. Specimens were tested at the Clinical Microbiology Laboratory, Puerto Real University Hospital, Cádiz, Spain, the same day they were collected (refrigerated transport and storage).

All specimens were processed following conventional methods for mycobacterial isolation. Specimens were decontaminated with 1% NaOH/*N*-acetyl-L-cysteine (BBL MycoPrep, Becton Dickinson, Sparks, MD) and concentrated by centrifugation at 3,000× *g* for 20 min. The sediment was used for AFB microscopy (Ziehl-Neelsen stain, which is the routine method in our laboratory) and liquid (BACTEC MGIT 960, Becton Dickinson) and solid (Löwenstein-Jensen, Becton Dickinson) culture. Two 500 μL aliquots of the sediment were stored at −20°C for SDA testing. Löwenstein-Jensen cultures were examined twice per week, whereas the Bactec MGIT 960 is an automated system that provides continuous monitoring (Kanchana et al., 2000). Cultures were reported negative if no growth was observed after 8 weeks of incubation. All mycobacterial isolates were identified using commercially available molecular tests (AccuProbe, Gen-Probe, San Diego), except *Mycobacterium chelonae* that was identified using conventional methods (growth characteristics, growth on McConkey Agar, nitrate reduction, and 3-day arylsulphatase test).

BDProbeTec ET testing was done according to the manufacturer's recommendations, and is described elsewhere (Bergmann et al., 2000). Briefly, a 500 μL aliquot of treated sediment was added to 1 mL of de sample wash buffer and centrifuged for 3 min at 12,200× *g*. The supernatant was discarded and the pellet was heated at 105°C for 30 min and then resuspended in 100 μL of sample lysis buffer. This mixture was sonicated for 45 min at 65°C. Finally, 600 μL of sample neutralization buffer was added. For each run, one positive and one negative control were prepared. Samples and controls were distributed randomly in the sample rack. Inhibition of amplification is monitored by the internal amplification control (IAC), which runs as a diplex test along with the target in the same microwell.

2.1. Consumables and workflow

The BDProbeTec ET DTB assay uses two types of microwells. The priming microwells contain primers, nucleotides, and detector probes, while the amplification microwells contain nucleotides and the SDA enzymes (polymerase and exonuclease). All pipetting is done using a programmable 8-channel pipettor. Lysed specimens are transferred to the priming wells, which are then kept at RT for 20 min (or up to 6 h) and consequently heated for 10 min at 72.5°C. Primed specimens are transferred to the amplification wells, which are immediately placed in the BDProbeTec ET instrument for homogenous amplification and detection at 52.5°C. After 1 h, results are automatically calculated and reported as MOTA values, and interpreted as positive, negative, or inconclusive. MOTA values (Metric Other Than Accelaration) represent the area under the relative fluorescence versus time curve. Samples with MOTA value >3,400 were positive for MTC DNA, samples with MOTA value <3,400 and for which the IAC MOTA value was >5,000 were negative for MTC DNA, and samples with MOTA value <3,400 and for which the IAC MOTA value was <5,000 were considered inhibited; the latter were inconclusive, and therefore, retested.

Table 1
Experimentally encountered combinations of positivity in the various mycobacterial detection systems included in this study

BDProbeTec ET	Z-N Microscopy	MGIT culture	L-J culture	N° specimens
POS	POS	POS	POS	18[a]
POS	NEG	POS	POS	7[a]
POS	POS	POS	NEG	3[a]
POS	NEG	POS	NEG	2[a]
POS	NEG	NEG	NEG	6[a]
NEG	POS	POS	POS	1[b]
NEG	NEG	POS	POS	1[a]
NEG	NEG	POS	NEG	1[a]

[a] *Mycobacterium tuberculosis*; [b] *Mycobacterium chelonae.*

Table 2
Sensitivity, specificity, and positive and negative predictive values for BDProbeTec ET SDA assay compared to MGIT culture for all specimens and smear-negative specimens respectively, excluding patients which received antimycobaterial treatment

Microscopy	ProbeTec ET result	MGIT culture		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		MTB Pos	MTB Neg				
All	+	29	2	93.5	99.5	93.5	99.5
	−	2	452				
Negative	+	9	2	81.8	99.5	81.8	99.5
	−	2	442				

Retesting and discrepancy resolution were done following the manufacturer's instructions (processed specimens were stored at −20°C for no longer than 14 weeks, thawed and re-lysed before re-testing). To minimize carry-over of previously amplified products, we used disposable gloves and worked in a class 2 biologic safety cabinet following GLP procedures.

3. Results

Thirty-nine from 502 respiratory specimens collected from 23 patients were positive with any method (ZN microscopy, culture on LJ slant, culture in MGIT broth, and/or BDProbeTec ET). Only one specimen, which was negative in all methods, was inhibited in BDProbeTec ET. Results are summarized in Table 1. Twenty-two specimens were ZN positive. Mycobacteria grew from 33 specimens (32 *Mycobacterium tuberculosis* and 1 *Mycobacterium chelonae*); 32 MTC isolates grew in MGIT liquid medium and 26 grew on LJ slant). Thirty-six specimens were BDProbeTec ET SDA positive (32 sputa and 4 BAL). Excluding the *Mycobacterium chelonae* isolate, discrepant results were obtained with 8 specimens: two specimens were MGIT culture positive and BDProbeTEC ET negative, and 6 specimens were MGIT culture negative and BDProbeTec ET positive. Discrepant results were confirmed after retesting; MOTA values were significantly lower for culture negative specimens than for culture positive specimens. Excluding the patients receiving antimycobacterial treatment, the overall sensitivity, specificity, positive, and negative predictive values of BDProbeTec ET compared to culture were 93.5, 99.5, 81.8, and 99.5%, respectively. For microscopy positive specimens, the sensitivity was 100% compared to culture, and 81.8% for microscopy negative specimens (Table 2).

Of the total of 257 patients with suspected tuberculosis 13 were diagnosed positive by BDProbeTec ET. Four of these 13 patients could be monitored till MTB negative using BDProbeTec ET in parallel with culture (Table 3). Four of the 9 patients that received antimycobacterial treatment before the start of the study, remained BDProbeTec ET and culture negative, while the five others were BDProbeTec ET positive with only one culture positive.

The MOTA values for true positives ranged from 6,624 to 103,818 with median values of 60,711 and 34,520 for microscopy positive and negative specimens respectively. MOTA values for the 6 false-positive specimens ranged from 3,555 to76,614 with a median value of 17,676 (Fig. 1).

4. Discussion

SDA is a molecular technique based on isothermal amplification of DNA, using a two-enzyme system (restriction enzyme and DNA polymerase) (Walker et al., 1992). More recently, new thermophilic restriction endonuclease (BsoBI) and DNA polymerase (exo'Bca) were incorporated (Spargo et al., 1996), as well as fluorescence polarization

Table 3
TB diagnosed patients whose treatment was monitored using culture, AFB stain and SDA

	N° specimens	Therapy (days)	BD Probe Tec ET SDA	Ziehl-Neelsen stain	MGIT 960 culture	Lowenstein Jensen culture
Patient 1	2	0	+	+	+	−
	4	150	−	−	−	−
Patient 2	3	0	+	−	+	±[a]
	3	49	−	−	−	−
Patient 3	2	0	+	+	+	+
	2	37	−	−	−	−
Patient 4	3	0	+	±[a]	+	±[b]
	2	62	−	−	−	−
Patient 5	1	46	+	+	+	−
	3	71	−	−	−	−

[a] Only one specimen positive; [b] only two specimens positive.

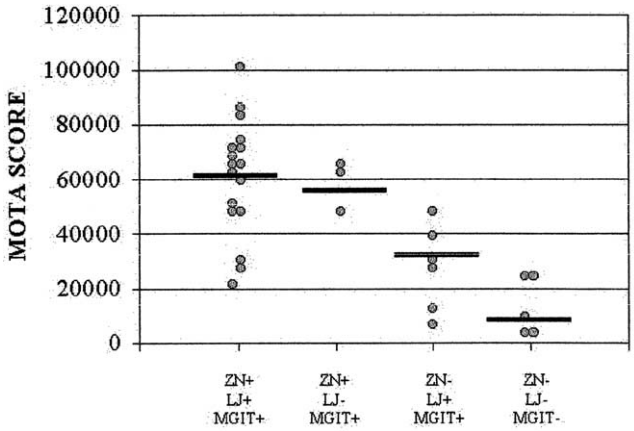

Fig. 1. Correlation between MOTA value and detection of MTC by microscopy and culture in BDProbeTec ET positive specimens (circles are MOTA value of positive specimens by BDProbeTec, and bars represent median values).

detection (Walker et al., 1996) which was applied to the diagnosis of *Mycobacterium tuberculosis* using the IS6110 insertion sequence (Walker & Linn, 1996). Down et al. (Down et al., 1996) compared an early predecessor of the current SDA technology with culture and clinical patterns, thus establishing a threshold value for SDA positivity. Later, a semi-automated BDProbeTec SDA system was evaluated with excellent sensitivity (from 94.7-97.6%) and negative predictive values (Ichiyama et al., 1997; Bergmann & Woods, 1998; Pfyffer et al., 1999) (Table 4).

Further technical modifications led to the introduction of a new automated instrument, BDProbeTec ET, which detects DNA in real-time using exponential SDA. In this study we evaluated this system and found a sensitivity of 93.5%. Using the same technology, Bergmann et al. (Bergmann et al., 2000) and Johansen et al. (Johansen et al., 2002) found lower sensitivities (87.5% and 83.0% respectively), while Piersimoni et al. (2002) reported a sensitivity of 94.5%.

The early detection of smear negative/culture positive specimens is very important because of potential tuberculosis transmission from these patients (Behr et al., 1999). In their study, Johansen et al. (2002) included 65 smear negative specimens, of which 39 were detected as true positives by using BDProbe Tec ET (sensitivity = 60%). In our study we found a sensitivity of 81.8% (9 out of 11 smear negative specimens were detected as true positives by BDProbetec ET). These results, combined with an excellent negative predictive value (99.5% in our study), make BDProbeTec ET a powerful tool to exclude tuberculosis in respiratory specimens.

Two specimens were BDProbeTec ET false negative (culture positive), of which one was a very cellular pleural fluid and one was a BAL; upon retesting both specimens remained negative, while the IAC didn't reveal inhibition.

In our study a very high specificity was obtained with

Table 4
Comparative results of several NAAT methods for direct detection of *Mycobacterium tuberculosis* complex

References (N° specimens/N° patients)	Sensitivity (%)	Specificity (%)	PPV[a] (%)	NPV[b] (%)	Smear negative specimens	
					NAAT[c] positive/N° specimens	Specificity (%)[d]
SDA (BDProbeTec, Becton Dickinson)						
(Bergmann & Woods, 1998) (523/277)	95.8	96.2	54.8	99.8	10/503	88.9
(Pfyffer et al, 1999) (799/538)	97.6	95.0	51.3	99.9	18/756	94.7
(Ichiyama et al, 1997) (530/299)	94.7	86.3	62.0	98.5	NA°	NA
SDA (BDProbeTec ET, Becton Dickinson)						
(Bergmann et al, 2000) (600/332)	87.5	99.0	70.0	99.7	3/577	50.0
(Johansen et al, 2002) (351/247)	83.0	98.5	97.6	86.8	39/239	60.0
(Piersimoni et al, 2002) (331/402)	94.5	99.0	98.8	98.0	11/223	73.3
PCR (Cobas Amplicor, Roche)						
(Ichiyama et al, 1996) (422/170)	97.7	86.9	78.1	98.8	49/330	94.5
(Bergmann & Woods, 1996) (956/502)	78.7	99.3	88.8	98.5	8/904	40.0
(Ichiyama et al, 1997) (530/299)	89.5	87.5	62.9	97.2	NA	NA
(Bennedsen et al, 1996) (7,194/3,794)	81.9	96.1	67.8	98.1	NA	NA
(Rajalahti et al, 1998) (324/151)	83.0	99.0	97.5	95.0	12/269	68.0
(Reischl et al, 1998) (643/807)	84.2	99.1	90.5	98.4	6/588	50.0
(Scarparo et al, 2000) (296/323)	94.2	100	100	96.0	7/180	75.0
(Bogard et al, 2001) (5,221/2,373)	85.2	99.7	96.4	92.8	69/4,828	71.7
TMA (AMTD, GenProbe)						
(Ichiyama et al, 1996) (422/170)	100	90.1	81.2	100	47/325	100
(Pfyffer et al, 1996)(1,117/998)	86.6	96.4	76.8	98.1	NA	NA
(Piersimoni et al, 1998)(457/357)	92.8	99.4	98.5	97.0	24/198	85.3
(Scarparo et al, 2000) (296/323)	85.7	100	100	90.4	7/180	65.6
(Bergmann et al, 1999)(1,004/489)	77.8	98.8	70.0	99.2	15/982	65.2
(Piersimoni et al, 2002) (331/402)	88.5	100	100	90.8	10/223	66.6

[a] Positive predictive value; [b] negative predictive value; [c] nucleic acid amplification test; [d] Specificity for culture positive specimens, NA not avalaible.

BDProbeTec ET (99.5%), in line with previous papers (98.5-99.0%; Johansen et al., 2002; Bergmann et al., 2000; Piersimoni et al., 2002). One specimen from a patient with tuberculosis compatible X-ray result appeared false positive (all other tests negative). One specimen from another patient was confirmed false positive after retesting with two different lots of SDA reagents.

Although the BDProbeTec ET SDA technology is not validated to monitor the outcome of antimycobaterial treatment, this study revealed that the results become quickly negative with specimens from patients which were diagnosed TB positive during the study and for which the result of treatment could be evaluated. Combined with the high negative predictive value of this technology, this observation suggests its possible use as a prognostic marker which would allow to predict complete recovery without having to wait an additional 40 days for the results of mycobacterial culture. Yet, with four of the nine patients which received antimycobacterial treatment, BDProbeTec ET SDA was the only test which remained positive, possibly because of the detection of DNA from non-viable *Mycobacterium tuberculosis.*

Table 4 summarizes a comparison with other NAAT methods (PCR and TMA), based on a literature overview. Such comparative evaluation is not always straight forward because of different study designs and specimen types, patients from various clinical situations, and some occasional modifications to the manufacturer's recommended procedures.

The manufacturer doesn't recommend the BDProbeTec ET SDA system as a quantitative method. However we found a close relationship between the MOTA value and the result obtained from culture and microscopy. Figure 1 illustrates a possible relation between the SDA MOTA value, growth on LJ and/or in MGIT broth, and smear result. Although not claimed by the manufacturer, possibly, like with other NAAT (Thomsen et al., 1999; Rajalahti et al., 1998), the SDA MOTA values can be used to assess the efficacy of antituberculous treatment.

In our study, SDA was inhibited in only one specimen, which was culture and AFB stain negative (inhibition rate of only 0.2%). Similar results were obtained by Johansen et al. (2002). SDA seems to be less sensitive to inhibition compared to PCR and TMA, which show inhibition rates above 2% (Thomsen et al., 1999; Reischl et al., 1998; Scarparo et al., 2000). No cross-reaction with other mycobacteria was observed, however, we isolated only one strain of *Mycobacterium chelonae*.

In conclusion, the BDProbeTec ET SDA system is a rapid and specific method for direct detection of MTC in respiratory specimens. The procedure was easy to perform and fitted well in our laboratory work flow. The high level of automation and containment contributed to avoid contamination. The BDProbeTec ET SDA system is a useful and important additional laboratory method for the rapid detection of MTC, especially in patients with smear-negative respiratory specimens.

Acknowledgments

We are indebted to André De Bock, Yolanda Gonzalo, and Fernando García (Becton Dickinson) for their valuable help in the preparation of the manuscript. We thank Becton Dickinson (Madrid, Spain) for providing reagents and instrumentation. This study was partially presented at the 42nd ICAAC, San Diego, CA, 2002.

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