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Performance Characteristics of the BDProbeTec System for Direct Detection of *Mycobacterium tuberculosis* Complex in Respiratory Specimens

GABY E. PFYFFER,[1]* PASCALE FUNKE-KISSLING,[1] EVA RUNDLER,[1] AND RAINER WEBER[2]

Swiss National Center for Mycobacteria, Department of Medical Microbiology, University of Zurich, CH-8028 Zurich,[1] and Division of Infectious Disease, Department of Internal Medicine, University Hospital, CH-8091 Zurich,[2] Switzerland

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Strand displacement amplification (SDA) technology has been established in a fully automated system known as BDProbeTec. Target sequences of the insertion sequence IS*6110* and the 16S rRNA gene are simultaneously amplified, which thus allows the detection of *Mycobacterium tuberculosis* complex and, as an additional option, of most *Mycobacterium* species. Detection occurs via a chemiluminescent microwell assay that employs the simultaneous hybridization and capture of SDA products with a biotinylated capture probe and an alkaline phosphatase detector probe. We have evaluated the performance of the BDProbeTec system in detecting *M. tuberculosis* complex by testing 799 respiratory specimens and comparing the results to those obtained by conventional diagnostic techniques, i.e., microscopy and culture (solid and radiometric media). *M. tuberculosis* was cultivated from 41 specimens, of which 28 (68.4%) were smear positive and 13 (31.6%) were smear negative. The overall sensitivity of the SDA assay was 97.6% (for smear-positive specimens, 100%; for smear-negative specimens, 92.3%), and specificity was 95.0%. After resolution of the discrepancies by studying the patients' clinical data, sensitivity and specificity were 97.9 and 96.5%, respectively, and positive and negative predictive values were 63.9 and 99.9%, respectively. These preliminary data demonstrate that the BDProbeTec system has promising performance characteristics with respiratory specimens and that it allows the detection of *M. tuberculosis* complex within hours.

Nucleic acid amplification (NAA)-based techniques have the potential to increase the sensitivity for detecting mycobacteria as well as to dramatically reduce the time usually necessary to detect and identify these organisms in clinical specimens. To guarantee a high degree of reproducibility and to facilitate their application in a clinical mycobacteriology laboratory, several important techniques have been developed in kit-based formats for rapid detection of *Mycobacterium tuberculosis* complex, among them PCR (1, 7) and transcription-mediated amplification (6, 13), which have recently been approved by the Food and Drug Administration for use on smear-positive respiratory specimens in the United States. Various other techniques designed for the same purpose are now being clinically evaluated and include ligase chain reaction (10, 18) and, most recently, strand displacement amplification (SDA).

By using initially the restriction enzyme *Hin*cII and the exonuclease-deficient Klenow fragment of *Escherichia coli* polymerase I, a 10^8-fold amplification in 2 h could be achieved by SDA (17). By employing a new thermophilic restriction endonuclease (*Bso*B1) from *Bacillus stearothermophilus* and a new DNA polymerase (exo-*Bca*), undesirable features such as the relatively slow doubling time of 3.5 min and the high level of nonspecific background reactions could be overcome (15). In the present kit-based format, SDA allows isothermal coamplification and detection of segments of the insertion sequence IS*6110* (specific to *M. tuberculosis* complex) and of a sequence of the 16S rRNA gene (common to most mycobacterial species). The amplified target is then rendered single stranded and simultaneously hybridized with a biotinylated capture probe and an alkaline phosphatase detector probe (2, 15, 17).

Developed in the research laboratory, SDA technology is, as a fully automated system (BDProbeTec), under development by Becton Dickinson Diagnostic Instrument Systems (Sparks, Md.). Recently, Ichiyama et al. reported their data on 530 respiratory specimens (5) and Fuller et al. demonstrated the BDProbeTec's ability to detect mycobacteria from liquid medium (3). We report here on the performance characteristics of BDProbeTec in a clinical mycobacteriology laboratory where approximately 800 respiratory specimens were consecutively analyzed for acid-fast bacilli.

MATERIALS AND METHODS

Clinical specimens. Respiratory specimens (n = 799) included sputum (expectorated, n = 475; induced, n = 6), bronchial aspirate (n = 258), tracheal aspirate (n = 8), and bronchoalveolar lavage (n = 52) specimens. They were consecutively collected from September to December 1996 and originated from patients admitted to the University Hospital of Zurich, Zurich, Switzerland, and other hospitals in the Zurich metropolitan area as well as from patients consulting private physicians. The study cohort included 538 patients. Patients were eligible for the study (i) if there was a suspicion of tuberculosis (TB) on account of chest X ray, clinical symptoms consistent with TB, laboratory results, and/or risk factors for TB; (ii) if they had not been treated for TB (i.e., never treated, treated for 7 days at the maximum, or not treated within the past 12 months); and (iii) if current and past medical histories of the patients were available. Specimens were not acceptable (i) if specimen volume was less than 1.0 ml and (ii) if transport occurred in cetylpyridium chloride. In parallel with SDA analysis all specimens underwent conventional mycobacteriological procedures (i.e., microscopy, culture, identification, and susceptibility testing).

Pretreatment of specimens. Upon receipt, all specimens were kept at 4°C prior to being processed (two workups per day). Specimens had been pretreated by the *N*-acetyl-L-cysteine (NALC)-NaOH procedure (8) with BBL MycoPrep kits (Becton Dickinson Microbiology Systems, Cockeysville, Md.).

Culture. Processed specimens were inoculated onto a Löwenstein-Jensen slant, onto a Middlebrook 7H10 plus selective 7H11 agar biplate (Becton Dick-

* Corresponding author. Mailing address: Swiss National Center for Mycobacteria, Department of Medical Microbiology, University of Zurich, Gloriastrasse 30, 8028 Zurich, Switzerland. Phone: 41 1 634 27 86. Fax: 41 1 634 49 18. E-mail: pfyffer@immv.unizh.ch.

TABLE 1. Analysis of discrepant results (SDA versus conventional diagnostics) in conjunction with clinical data

No. of specimens[a]	Culture result (*M. tuberculosis*)	SDA result		Clinical diagnosis or comments	Final interpretation of SDA results[b]
		First run	Repeat run		
1	+[c]	−	−	Active TB	FN
6	−	+	+	Previous TB, treatment completed >12 months prior to testing	TP
23[d]	−	+	−	Various clinical diagnoses[e]	FP
3[f]	−	+	−	MAC infection	FP
6	−	+	+	Patient 1 (33, F)[g]: bronchial asthma, grew up in sub-Saharan Africa, mother was African	IC
	−	+	+	Patient 2 (56, M): cough, smoker	IC
	−	+	+	Patient 3 (41, F): pneumococcal pneumonia, alcohol abuse	IC
	−	+	+	Patient 4 (77, M): histologically confirmed bronchial carcinoma	IC
	−	+	+	Patient 5 (89, M): histologically confirmed bronchial carcinoma, unexplained sterile pleural empyema	IC
	−	+	+	Patient 6 (80, M): histologically confirmed bronchial carcinoma	IC

[a] All were smear negative.
[b] FN, false negative; TP, true positive; FP, false positive; IC, inconclusive case.
[c] Culture positive after 22 days (BACTEC 12B medium only).
[d] Cutoff was between 1.0 and 2.0 RLU for 9 of 23 specimens.
[e] Various diagnoses included bronchitis, chronic obstructive pulmonary disease, a large variety of pulmonary and extrapulmonary carcinomas, human immunodeficiency virus infection, etc.
[f] Cutoff was between 1.0 and 2.0 RLU for one of three specimens.
[g] Age (years), gender (F, female; M, male). Patients 1 to 6 had no clinical evidence for active TB disease but possible risk factors for a TB infection such as age, origin (area of endemicity), or behavior (alcohol abuse).

inson Microbiology Systems), and into BACTEC 12B medium as described previously (13). Solid media were read weekly, and BACTEC cultures were read twice weekly for the first 2 weeks and once weekly thereafter. Culture media were incubated for 8 weeks; in case of discrepant results (i.e., SDA positive but culture negative), they were kept for an additional 4 weeks.

Quality control. The organisms used for quality control of mycobacterial media were *M. tuberculosis* H37Ra ATCC 25177, *Mycobacterium kansasii* ATCC 12478, *Mycobacterium scrofulaceum* ATCC 19981, *Mycobacterium intracellulare* ATCC 13950, and *Mycobacterium fortuitum* ATCC 6841. *E. coli* ATCC 25922 was used to show either partial or complete inhibition on nonselective and selective media. In addition, 20 sputum specimens obtained from patients diagnosed with other nonmycobacterial pulmonary diseases (bacterial or fungal infections) were tested. Isolates consisted of members of the family *Enterobacteriaceae* as well as *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Pseudomonas*, and *Haemophilus* species. None of them grew on the mycobacterial media used in this study.

Microscopy. Smears were stained with auramine-rhodamine fluorochrome. Positive slides were confirmed by Ziehl-Neelsen staining (8).

Identification of mycobacteria. Routine biochemical methods (8, 12) and Accuprobe culture confirmation kits (Gen-Probe, San Diego, Calif.) were employed for the identification of isolates. For identification of some of the nontuberculous mycobacterium (NTM) isolates, we used additional techniques, such as (i) analysis of cellular fatty acids by gas-liquid chromatography in conjunction with the Microbial Identification System (Microbial ID Inc., Newark, Del.) (14), (ii) PCR-restriction enzyme analysis of the 65-kDa heat shock protein gene (16), and (iii) sequence analysis of the 16S rRNA gene (9).

BDProbeTec. The BDProbeTec technology basically consists of the following steps: (i) heating of the NALC-NaOH-treated samples, (ii) decontamination, (iii) amplification (SDA), (iv) hybridization, and (v) detection. The protocol of the manufacturer was strictly followed. Briefly, 250 μl of a NALC-NaOH-treated specimen was transferred to a sample-processing tube containing 1.0 ml of sample diluent. After being mixed and centrifuged (12,000 × *g*, 3 min, 4°C), a sample-processing capsule containing zirconium and silica for mechanical disruption of the mycobacterial cell wall and 1.0 ml of sample diluent were added to the pellet, which was mixed (vortexed) for 5 s. Positive and negative controls were prepared accordingly (see below). The tube was incubated at 105°C for 1 h with a Lysolyzer instrument (Becton Dickinson). Subsequent breakage of the capsule occurred by agitation (45 s) in a FastPrep apparatus (Bio 101, Vista, Calif.). If the capsule failed to break, the FastPrep step was repeated. Specimens were then ready for analysis with the fully automated BDProbeTec instrument, i.e., for amplification and hybridization. Detection of the products was done in a spectrophotometer provided with the BDProbeTec equipment. To minimize carryover of previously amplified products, decontamination with uracil DNA glycosylase was performed and an internal control for monitoring SDA inhibition was included, in addition to the positive and negative controls. Samples containing *M. tuberculosis* complex were IS*6110* positive and 16S rRNA gene positive. Up to 48 specimens could be analyzed per run, which required less than 6 h. Quality control for the BDProbeTec assay per the manufacturer's recommendations consisted of inclusion of three *M. tuberculosis*-negative controls and two positive multiplex controls (provided by Becton Dickinson) in each assay run.

Interpretation of BDProbeTec results. The cutoff value of the method was set at 1.0 relative light units (RLU); i.e., values of ≥1.0 were considered positive and values of <1.0 were considered negative.

Patients' clinical data. For each specimen included in the study, the clinical data of the patient was available and evaluated. Clinical assessment included a patient's past and present history, clinical signs and symptoms, chest X ray, skin test, laboratory results, and follow-up observation as well as the results obtained with additional specimens that were sent to the mycobacteriology laboratory. In addition, sociodemographic data were also available.

Statistical analyses. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of SDA were calculated in comparison with smear and culture results and, subsequently, in comparison with smear and culture results plus the patients' clinical data.

RESULTS AND DISCUSSION

Of the 799 specimens from 538 patients, 6 specimens yielded contaminated cultures on all media, despite a second pretreatment with NALC-NaOH. Eventually, they were exempted from the study. Of the remaining 793 specimens, 63 were culture positive for acid-fast bacilli. Twenty-two yielded NTM (among them 3 smear positive specimens), including the following species (number of isolates in parentheses): *Mycobacterium avium* complex (MAC; $n = 9$), *Mycobacterium xenopi* ($n = 4$), *Mycobacterium gordonae* ($n = 4$), *Mycobacterium aurum* ($n = 1$), *Mycobacterium malmoense* ($n = 1$), *Mycobacterium abscessus* ($n = 1$), *Mycobacterium flavescens* ($n = 1$), and *M. fortuitum* ($n = 1$). In these specimens the BDProbeTec result was negative for *M. tuberculosis* complex, except with three specimens which grew MAC. RLU values of these three specimens were 1.5, 3.6, and 12.5. Upon repetition, all three values were negative (Table 1).

Of all clinical specimens tested, 40 (28 smear-positive and 12 smear-negative specimens) were SDA positive and yielded *M. tuberculosis* in culture. In addition to the above-mentioned three discordant results (SDA-positive and culture-positive with MAC), there were 36 discrepant results (Table 1): one smear-negative specimen was SDA negative but grew *M. tuberculosis* in culture, and 35 specimens were SDA positive but culture negative. The remaining 695 specimens were negative by both methods. The overall sensitivity, specificity, PPV, and NPV of SDA were 97.6, 95.0, 51.3, and 99.9%, respectively. After a review of discrepant results by studying the clinical data of the patients, these values were adjusted to 97.9, 96.5, 63.9, and 99.9%, respectively. These data as well as the corresponding values for smear-positive and smear-negative specimens are given in Table 2.

TABLE 2. Results of SDA compared with results of culture and clinical assessment of patients[a]

Smear result (n^b)	SDA result	No. of specimens with clinical TB diagnosis:		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		Positive	Negative				
+ and − (787[c])	+	46	26	97.9	96.5	63.9	99.9
	−	1	714				
+ (31)	+	28		100	100	100	100
	−		3[d]				
− (756)	+	18	26[e]	94.7	96.5	40.9	99.9
	−	1	711[f]				

[a] Values in this table are based on data after resolution of discrepant results.
[b] Number of specimens.
[c] In total, 799 specimens were included in the study. Six specimens were lost due to contaminated cultures (all media). The six inconclusive specimens (Table 1) were excluded from the calculations as well.
[d] MAC ($n = 1$), *M. gordonae* ($n = 1$), *M. malmoense* ($n = 1$).
[e] Including three specimens which grew MAC in culture.
[f] Including 16 specimens which grew NTM other than MAC.

Our data demonstrate that the BDProbeTec System exhibits excellent sensitivity in that it missed only one smear-negative TB specimen of 46 bacteriologically and/or clinically confirmed specimens. Upon repetition of SDA, the single false-negative specimen remained negative. From this, we assumed that the false negativity was more likely due to the presence of inhibitors in that particular specimen rather than to a sampling error. These figures are in line with those of the first large-scale demonstration of *M. tuberculosis* complex by SDA in clinical sputum specimens by Down et al. (2), who reported a sensitivity of 100% for smear-positive specimens and of 95.0% for smear-negative specimens. As commonly observed with NAA-based direct tests, SDA technology did not show absolute specificity either. Discrepant results (SDA positive and *M. tuberculosis* culture negative, $n = 38$; Table 1) were observed with (i) 26 specimens (three of which grew MAC in culture) originating from patients with no clinical signs of tuberculous disease, either pulmonary or extrapulmonary (considered false positive); (ii) 6 specimens originating from former TB patients (whose ends of treatment were >12 months prior to testing; considered true positive); and (iii) 6 specimens (SDA positive in the first and repeat runs) originating from patients for whom an infection with *M. tuberculosis* could not safely be excluded because of persisting clinical symptoms and common risk factors for TB (having grown up in a high-incidence country, alcohol abuse, or advanced age; considered inconclusive). It is well known that positive amplification results may arise for patients in the last two categories of risk factors even though their cultures for *M. tuberculosis* remain negative. Even if anti-TB therapy has been completed and patients are considered clinically cured, positive amplification results are occasionally observed, regardless of whether the NAA-based technology targets DNA or RNA. In line with our results, Hellyer et al., for instance, have demonstrated that after more than 1 year, as much as 22% of the specimens that they tested by PCR and SDA were still positive but that the cultures had long since converted to negative (4). Similarly, Moore et al. have reported that by using transcription-mediated amplification, 56% of their patients had a period of shedding of noncultivable *M. tuberculosis* which lasted 7 to 245 days (11). Hence, both studies demonstrated that NAA-based methods should not be used as a tool to monitor the efficacy of antituberculous therapy.

The sensitivity of nearly 98% found in our study is higher than that reported by Ichiyama et al. (94.7% [5]); on the other hand, those authors attained a higher specificity (99.8 versus 96.5%). The latter is, however, not surprising since 54 of their 55 SDA-positive and culture-negative specimens had originated from TB patients during or after anti-TB therapy and were, as a whole, considered true-positive specimens. Also, it is not clear whether the Japanese study had been conducted by including consecutively enrolled or selected specimens, since as much as 24% of the specimens yielded *M. tuberculosis* in culture. For comparison, the proportion of *M. tuberculosis* cultures amounted to 5% in the present evaluation, which perfectly matches the annual isolation rate of *M. tuberculosis* observed in our laboratory.

Disregarding the six inconclusive situations and the six former TB patients (who completed TB therapy more than 12 months prior to testing), as many as 26 false-positive results were generated by BDProbeTec. In this context, the question of assessing the cutoff value of this method has to be carefully addressed by the manufacturer. Given that the cutoff value had been set at 2.0 instead of 1.0 (as defined by the study protocol), 10 of the 26 false-positive specimens would not have been diagnosed as such because their RLU values were between 1.0 and 2.0; thus, specificity would have increased from 96.5 to 97.8% without a loss in sensitivity (97.9% [cutoff value of 1.0] versus 97.9% [cutoff value of 2.0]).

Undoubtedly, the major drawback of present-day SDA probe technology is the fact that sample preparation (i.e., centrifugation, lysolyzation, and bead beating) is not an integral part of automatization yet. Although the different steps in sample preparation are easy to perform, a sample preparation time of at least 2 h is necessary before the instrument can be loaded. In contrast, handling of the highly sophisticated BDProbeTec instrument is very easy and user-friendly, which is crucial for any application of such technology in a clinical mycobacteriology laboratory. With the exception of two minor accidents (due to loose pipette tips), which caused the instrument to stop in the middle of a run, the automated instrument ran safely and efficiently overnight. The two unsuccessful runs were easily repeated the next day without the precious clinical samples being lost.

In conclusion, BDProbeTec technology offers several advantages for direct detection of *M. tuberculosis* complex in clinical specimens. Once the specimens have been prepared, all subsequent steps involving amplification and hybridization are performed automatically in a fully self-contained system. With its high sensitivity, BDProbeTec technology looks, at this stage of development, very promising for allowing more hands-free time in clinical mycobacteriology.

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