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Clinical Evaluation of the BDProbeTec Strand Displacement Amplification Assay for Rapid Diagnosis of Tuberculosis

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The reliability of the BDProbeTec MTB Test (Becton Dickinson, Sparks, Md.) for direct detection of *Mycobacterium tuberculosis* in respiratory specimens was evaluated by comparing results to those of conventional mycobacterial culture, with the BACTEC TB 460 and Middlebrook 7H11 biplates. Patients known to have tuberculosis were excluded from analysis. Of 523 specimens from 277 patients, 53 grew a mycobacterium: 24 specimens of *M. tuberculosis* and 29 specimens of nontuberculous mycobacteria. After initial testing, 42 specimens were positive by the BDProbeTec, for overall sensitivity, specificity, and positive and negative predictive values of 95.8, 96.2, 54.8, and 99.8%, respectively. After resolution of discrepancies, 28 specimens were positive by the BDProbeTec, for overall sensitivity, specificity, and positive and negative predictive values of 100, 99.2, 85.7, and 100%, respectively. These same values were 100, 80.8, 93.4, and 100%, respectively, for smear-positive samples and 100, 99.4, 75.0, and 100%, respectively, for smear-negative specimens.

Tuberculosis remains a public health problem in the United States, despite a declining incidence during the past several years. One of the most critical aspects of tuberculosis control is rapid identification of infectious patients, a process which for many years was based on staining smears for acid-fast bacilli (AFB) and culturing samples for mycobacteria with a liquid and a solid medium. AFB smear results generally are available in 24 h or less, but a positive result is not specific for tuberculosis. Mycobacterial culture and identification results, which provide a specific diagnosis, are not available for 2 to 3 weeks or longer. In response to the need for a more rapid diagnostic test, several manufacturers have developed nucleic acid amplification tests specific for *Mycobacterium tuberculosis* complex (MTBC) (9). Currently, two such tests (Amplified Mycobacterium tuberculosis Direct Test [Gen-Probe, Inc., San Diego, Calif.] and AMPLICOR Mycobacterium tuberculosis PCR assay [Roche Molecular Systems, Branchburg, N.J.]) are commercially available in the United States for detection of MTBC in AFB smear-positive specimens (1–6, 8, 10).

Becton Dickinson (Sparks, Md.) recently developed a semiautomated system—the BDProbeTEC—for the rapid detection of MTBC in respiratory specimens. The enabling chemistry utilized is a thermophilic version of strand displacement amplification, which enzymatically replicates target nucleic acid sequences exponentially to detectable levels. Sediments of decontaminated and concentrated clinical specimens are processed off-line, with several washes to remove inhibitors, followed by heat inactivation and mechanical agitation to lyse the mycobacteria. Processed specimens are then placed onto the BDProbeTec sample handling unit, where they are robotically introduced to the decontamination-amplification devices (DADs), which are disposable, single-use reagent cartridges that allow for amplicon decontamination followed by subsequent amplification (by strand displacement amplification) of the target and provide an internal amplification control. Once amplification is complete, the amplified material is harvested, and product is detected via target-specific sandwich hybridization assays, which occur in disposable, single-use assay devices (ADs). Amplification is demonstrated via a chemiluminescent signal that is detected in a luminometer. The purpose of this study was to evaluate the performance of the BDProbeTec MTB Test for direct detection of MTBC in respiratory specimens in a clinical setting.

Specimens. Respiratory specimens (expectorated and induced sputum samples, tracheal aspirates, bronchial washings, and bronchoalveolar lavage fluids) submitted to the clinical microbiology laboratory at the University of Texas Medical Branch for detection of mycobacteria from April through June 1997 were included in the study. Samples from patients receiving therapy for previously diagnosed tuberculosis were excluded from analysis.

Specimen processing and culture. Specimens were decontaminated with 1% sodium hydroxide (final concentration)–*N*-acetylcysteine and concentrated by centrifugation at 3,000 × *g* for 20 min, according to a standard procedure (7). Approximately 0.2 μl of the sediment was used to prepare a smear for staining with auramine O. Phosphate-buffered saline was added to the remaining sediment to give a volume of 2.0 ml. For mycobacterial culture, 0.5 ml of the suspension was inoculated into a BACTEC 12B bottle and 0.2 ml was inoculated onto each side of a Middlebrook 7H11 selective biplate. The remainder of the specimen was stored at −20°C for batch testing by the BDProbeTec MTB Test. BACTEC bottles were incubated at 37°C in 8% CO_2 and monitored for growth for 8 weeks by the BACTEC 460 TB instrument according to the manufacturer's recommendations, as described in detail elsewhere (7). Plates were incubated at 37°C in 8% CO_2 and examined weekly for growth for 8 weeks. Isolates of mycobacteria were identified by DNA probes (AccuProbe [Gen-Probe, Inc.] for MTBC, *Mycobacterium avium* complex, *Mycobacterium kansasii*, and *Mycobacterium gordonae*) or by conventional biochemical tests (for rapidly growing mycobacteria), performed according to the standard protocol (7). Isolates not identified by these procedures were referred to the Texas Department of Health for identification by high-performance liquid chromatography and/or conventional biochemical tests.

BDProbeTec MTB Test. Frozen samples were thawed, vigorously agitated on a vortex mixer, and processed according to the manufacturer's directions. Briefly, 500 μl of specimen was

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added to 1.0 ml of sample diluent A (which had been heated for 1 h in a 50°C water bath), agitated on a vortex mixer, and centrifuged at 12,200 × *g* for 3 min. The supernatant was discarded, and the pellet was washed three times as follows: 1.0 ml of sample diluent B was added, the pellet was resuspended by being mixed on a vortex mixer, and the suspension was centrifuged at 12,200 × *g* for 3 min. After the third wash, the supernatant was discarded, and one sample processing capsule, one glass bead, and 400 μl of sample diluent B were added to the pellet. The mixture was vigorously agitated on a vortex mixer and then frozen at −20°C. Prior to BDProbeTec testing, samples were thawed at room temperature. Two positive and three negative controls were prepared by adding 400 μl of sample diluent B to each. Samples and controls were lysolyzed for 30 min at 105°C, and samples only were agitated in the CellPrep instrument for 45 s at 5.0 m/s. Both samples and controls were pulse-centrifuged for 15 s and then transferred to the BDProbeTec instrument, which was programmed according to the manufacturer's directions to transfer an aliquot of each patient sample and control to the DAD and then to the AD. Within 10 min after the BDProbeTec process was completed, trays containing samples and controls were manually transferred to the luminometer for reading.

Results were considered interpretable if the internal amplification control was greater than 10 relative light units (RLU) or if the MTBC result was greater than 20 RLU, regardless of the internal amplification control. In such cases, specimens with a result greater than 20 RLU were considered positive for MTBC. MTBC results less than 20 RLU were considered negative if the internal control was greater than 10 RLU. If the internal control was less than 10 RLU and the MTBC result was less than 20 RLU, the specimen result could not be interpreted, and a second aliquot of the frozen sample that had been processed for amplification was tested by the BDProbeTec after thawing at room temperature, relysolyzation, and pulse-centrifugation.

Discrepant analysis. If the culture and BDProbeTec results were discordant, a second aliquot of the frozen sample that had been processed for amplification was tested by the BDProbeTec. If the results remained discrepant, the patient's medical record was reviewed (if available).

A total of 526 specimens were included in the study. For 24 of these specimens, the initial BDProbeTec result could not be interpreted due to failure of the internal amplification control. After testing of a second aliquot from these 24 samples, 3 remained uninterpretable due to failure of the internal control. These latter three specimens were excluded from the analysis, leaving 523 evaluable specimens from 277 patients.

Fifty-three specimens (10.1%) grew a mycobacterium: 24 specimens of MTBC (from nine patients) and 29 of nontuberculous mycobacteria (NTM), including 12 of *M. avium* complex, 9 of *Mycobacterium fortuitum-chelonae* complex, 2 of *M. gordonae*, 1 of *M. kansasii*, and 5 of *Mycobacterium nonchromogenicum*. The smear for AFB was positive in 20 cases (12 patients), of which 15 were from a culture that grew MTBC and 5 were from a culture that grew NTM (two of *M. avium* complex, two of *M. fortuitum-chelonae* complex, and one of *M. nonchromogenicum*).

On initial testing, 42 of the 523 specimens from 27 patients were positive for MTBC by the BDProbeTec. Twenty-three of these were MTBC culture positive, three grew NTM, and the rest were culture negative. Based on these results, the initial overall sensitivity, specificity, and positive and negative predictive values of the BDProbeTec for diagnosis of tuberculosis were 95.8, 96.2, 54.8, and 99.8%, respectively. These same values were 100, 80.0, 93.8, and 100%, respectively, for the 20 AFB smear-positive specimens and 88.9, 96.4, 30.8, and 99.8%, respectively, for the AFB smear-negative samples. The one false-positive BDProbeTec result for smear-positive specimens was from a specimen that grew *M. fortuitum-chelonae* complex.

TABLE 1. AFB smear and MTBC culture results for BDProbeTec-positive[a] specimens

MTBC culture result for[c]:		No. of specimens with result:	
BACTEC	7H11	Smear+	Smear−
+	+	9	4
+	c	4	1
+	−	1	2
−	+	1	2
c	+	0	1
−	−	1[b]	2

[a] After discrepancy analysis.
[b] 7H11 plate positive for *M. fortuitum-chelonae* complex.
[c] +, positive; −, negative; c, contaminated with bacteria.

On testing of a second aliquot of the 20 specimens that yielded discordant BDProbeTec and culture results, one specimen failed due to fluid in the device and was excluded from analysis. Of the remaining 522 evaluable samples, 28 samples from 16 patients were BDProbeTec positive (Table 1). Based on these data, the overall revised sensitivity, specificity, and positive and negative predictive values were 100, 99.2, 85.7, and 100%, respectively. These same values were 100, 80.0, 93.4, and 100%, respectively, for AFB smear-positive specimens and 100, 99.4, 75.0, and 100%, respectively, for smear-negative samples. Of the four patients with discrepant culture and BDProbeTec results, charts of two were available for review, and both had pulmonary disease caused by *M. fortuitum-chelonae* complex.

The BDProbeTec MTB Test is the first nucleic acid amplification system using strand displacement amplification technology that has been evaluated in a clinical laboratory for direct detection of MTBC in respiratory specimens. With this assay, the time to results after the specimen has been decontaminated and concentrated varies based on the number of samples being processed, ranging from approximately 5.45 h for one patient sample (plus three controls [two positive and one negative]) to about 10 h for 45 specimens (plus three controls). The first part of the procedure, during which the specimen is washed several times and further prepared for amplification, is the most labor-intensive, requiring about 4.5 h for 45 specimens. Thereafter, the assay is nearly completely automated, with the exception of manual removal of the AD from the BDProbeTec instrument to the luminometer for reading.

Initial results for 24 (4.6%) of the 526 specimens in our study could not be interpreted due to failure of the internal amplification control. Potential reasons for this failure are the presence of inhibitory substances in the sample and a problem with the instrument, but it is not possible to determine the exact cause in each case. However, for the three samples that remained uninterpretable after the testing of a second aliquot, it is very likely that inhibitory substances were responsible for the failure, and in four cases, fluid remained in the DAD, indicating instrument failure.

After initial testing, there were 20 specimens with discordant BDProbeTec and culture results, 19 of which were false-positive BDProbeTec results. The majority of these false-positive results occurred when the BDProbeTec assay was performed by a certain technologist, suggesting technical error or failure

to pay close attention to detail. After testing of a second aliquot of samples yielding discordant results, there were four false-positive BDProbeTec results. Three of these four samples were adjacent to either the positive control or a strong-positive specimen, suggesting carryover at some time during the procedure rather than nonspecific amplification.

In summary, our data suggest that the BDProbeTec MTB Test is a very sensitive technique for detection of MTBC directly in respiratory specimens. However, the number of patients with tuberculosis in our study is small; therefore, further studies to confirm our findings are needed. The major problem with the assay in our evaluation was false-positive results, although it is likely that many of these false-positive results were related to lack of attention to detail on the part of technical personnel and, therefore, could be eliminated by more experience with the assay.

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