Clinical Evaluation of the BDProbeTec ET System for Rapid Detection of *Mycobacterium tuberculosis*

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The performance of the BDProbeTec ET system (BD Biosciences, Sparks, Md.) for direct detection of *Mycobacterium tuberculosis* complex (MTBC) in respiratory specimens was evaluated by comparing results to those of conventional mycobacterial culture performed with the BACTEC 460 TB system and Middlebrook 7H11 biplates. Patients known to have been on antituberculous therapy were excluded from the analysis. Of 600 evaluable specimens (4 specimens were excluded from the analysis due to failure of the internal amplification control [IAC]) from 332 patients, 57 grew mycobacteria; 16 were MTBC (from 12 patients), and 41 were nontuberculous mycobacteria. Of the 16 MTBC culture-positive specimens, 12 were smear positive and 4 were smear negative. BDProbeTec ET detected 14 of the 16 MTBC culture-positive specimens, resulting in initial overall sensitivity, specificity, and positive and negative predictive values of 87.5, 99.0, 70.0, and 99.7%, respectively. After resolution of discrepancies by review of medical records and retesting of samples yielding discordant MTBC culture and BDProbeTec ET results, the revised overall sensitivity, specificity, and positive and negative predictive values of the BDProbeTec ET were respectively 93.8, 99.8, 93.8, and 99.8% by specimen and 91.7, 99.7, 91.7, and 99.7% by patient. The BDProbeTec ET System offers the distinct advantage of including an IAC in the specimen well. These data suggest that the test performance is very good, especially for smear-positive samples. However, the number of patients with tuberculosis in our study, especially those with smear-negative disease, was small; therefore, additional studies are needed.

Tuberculosis remains a public health problem in the United States, despite a declining incidence since 1992. One of the most important aspects of tuberculosis control is rapid identification of infectious patients, which for many years was based on staining smears for acid-fast bacilli (AFB) and culturing for mycobacteria using a liquid medium and a solid medium. AFB smear results usually are available in 24 h or less, but the smear is neither sensitive nor specific for tuberculosis. Mycobacterial culture and identification results provide a specific diagnosis but often are not available for 2 to 3 weeks or longer. In response to the need for a more rapid diagnostic test, various manufacturers have developed nucleic acid amplification tests for detection of *Mycobacterium tuberculosis* complex (MTBC) directly in respiratory specimens (1, 2, 4–7, 10, 11, 14).

A few years ago, Becton Dickinson (Sparks, Md.) developed a semiautomated system, known under the trademark name BDProbeTec, for the rapid detection of MTBC in respiratory specimens (3). The enabling chemistry utilized was a thermophilic version of strand displacement amplification (SDA) that enzymatically replicated target nucleic acid sequences exponentially to detectable levels (8, 12, 13). Becton Dickinson has now developed a new system, called the BDProbeTec ET, which couples SDA to a fluorescent energy transfer (hence the “ET” nomenclature) detection chemistry. The BDProbeTec ET system simultaneously amplifies and detects samples in a closed homogeneous assay format (8), providing higher throughput and a much more rapid assay (i.e., 94 results from processed samples in 1.6 h compared to 46 results in 4.5 h) than the original system. The purpose of this study was to evaluate the performance of the BDProbeTec ET system for

direct detection of MTBC in respiratory specimens in a clinical setting.

A maximum of three respiratory specimens (expectorated and induced sputum samples, tracheal aspirates, bronchial washings, and/or bronchoalveolar lavage fluids) per patient, submitted to the clinical microbiology laboratory at the University of Texas Medical Branch for detection of mycobacteria from July through December 1998, were included in the study. Samples from patients receiving therapy for previously diagnosed tuberculosis were excluded from the analysis.

Specimens (volume, 1 to 7 ml) were decontaminated with 1% sodium hydroxide (final concentration)–*N*-acetyl cysteine by the use of a BBL MycoPrep kit and concentrated by centrifugation at $3,000 \times g$ for 20 min, according to a standard procedure (9). To limit the potential for cross-contamination during processing, caps were removed from the tubes and replaced sequentially during the addition of reagents, and tubes were allowed to stand for a few minutes after agitation to reduce aerosols. Approximately 0.2 ml 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 total volume of 2.0 ml. For analysis by the BDProbeTec ET system, two 600- μ l aliquots were removed. For the first 187 specimens, one aliquot was held for less than 24 h at 4°C and processed for testing by the BDProbeTec ET system and the other was frozen at –20°C for later testing; thereafter, both aliquots were frozen.

For mycobacterial culture, 0.5 ml of the suspension was inoculated into a BACTEC 12B bottle and 0.2 ml was inoculated to each side of a Middlebrook 7H11 selective biplate (Becton Dickinson). BACTEC bottles were incubated at 37°C in an atmosphere of 8% CO₂ and monitored for growth for 6 weeks by use of a BACTEC 460 TB instrument according to the manufacturer's recommendations, as described in detail elsewhere (9). Plates were incubated at 37°C in an atmosphere

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of 8% CO₂ and examined for growth weekly for 8 weeks. Isolates of mycobacteria were identified by the use of DNA probes (AccuProbe; Gen-Probe, Inc., San Diego, Calif.; for MTBC, *Mycobacterium avium* complex, *Mycobacterium kansasii*, and *Mycobacterium gordonae*) or by conventional biochemical tests (for rapidly growing mycobacteria), performed according to standard protocols (9). 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.

The BDProbeTec ET Direct TB Assay was performed according to the manufacturer's directions. For fresh specimens, 500 µl was added to 1.0 ml of sample wash buffer, vortex mixed for 5 s, and centrifuged at 12,200 × *g* for 3 min. The supernatant was discarded, and the pellet was heated for 30 min at 105°C to render the organisms nonviable and then pulse centrifuged for 10 s. The pellet was resuspended in 100 µl of sample lysis buffer, vortex mixed for 5 s, and placed in a 65°C sonic water bath (Branson Ultrasonic Corp., Danbury, Conn.) for 45 min. The sample was pulse centrifuged for 10 s, and then 600 µl of sample neutralization buffer was added. The mixture was vortex mixed for 5 s, pulse centrifuged for 10 s, and assayed immediately or frozen at -20°C. Frozen specimens were thawed at room temperature, heated for 30 min at 105°C, pulse centrifuged for 10 s, and then processed as described for fresh specimens.

For each BDProbeTec ET assay, one positive and three negative controls (provided with the kit) were tested. To each control, 600 µl of neutralization buffer was added; the mixture was vortex mixed for 5 s and pulse centrifuged for 10 s. Samples and controls were randomly distributed into the sample rack. Corresponding numbers of priming and amplification microwells were placed into their respective plates. Using a programmable eight-channel pipettor and aerosol-resistant tips (provided as part of the system), 150 µl of each sample or control was dispensed into a priming microwell. The priming microwell plate was covered and incubated at room temperature for 20 min, after which the cover was discarded and the plate was placed into a 71.5°C heating block. The amplification microwell plate was then placed into a 53.5°C heating block for prewarming. After 10 min, 100 µl from each priming microwell was transferred to the corresponding amplification microwell. Amplification microwells were sealed, and the plate was immediately placed in the BDProbeTec ET instrument. Once samples were introduced to the instrument, amplification and detection occurred in 1 h. The remaining BDProbeTec ET-processed sample was frozen at -20°C.

Samples with MTBC MOTA (metric other than acceleration) values greater than 3,400 were considered positive for MTBC regardless of the internal amplification control (IAC) MOTA. If the MTBC MOTA was less than 3,400 and the IAC MOTA was greater than 5,000, the specimen was considered negative for MTBC. If the MTBC MOTA was less than 3,400 and the IAC MOTA was less than 5,000, the result was considered indeterminate and the processed sample was retested.

If the culture and BDProbeTec ET System results were discordant, the frozen aliquot of the discrepant sample was reassayed on the BDProbeTec ET instrument after being thawed at room temperature, heated in the BDProbeTec ET oven at 105°C for 30 min, and pulse centrifuged. Additionally, the patient's medical records were reviewed.

Of the 187 specimens for which both fresh and frozen aliquots were tested by BDProbeTec ET, mycobacteria were isolated from 18 (9.6%); 6 were MTBC, 5 were *M. avium* complex, 3 were *M. fortuitum-chelonae* complex, 3 were *M. gordonae*, and 1 was a pigmented, rapidly growing mycobacterium that could

not be identified to the species level. BDProbeTec ET results were positive for 6 of the fresh aliquots, all of which grew MTBC, and negative for the remaining 181 specimens (sensitivity and specificity, 100%). For the corresponding frozen specimens, BDProbeTec ET results were positive for 7, 6 of which grew MTBC, and negative for the remaining 180 specimens (sensitivity 100%, specificity 99.4%). The overall level of agreement between fresh and frozen samples was 99.5%. Based on this level of agreement, only frozen samples were tested thereafter, to optimize labor efficiency.

A total of 604 frozen specimens from 335 patients were included in the study. For 12 (2.0%) of these specimens, the initial BDProbeTec ET result could not be interpreted due to failure of the IAC to amplify the nucleic acid in the specimens. After testing a second aliquot from these 12 processed samples, 4 specimens (from 3 patients) remained indeterminate due to failure of the IAC to amplify the nucleic acid. These samples were considered to have contained inhibitory material that prevented the SDA reaction from occurring and were excluded from the analysis, leaving 600 evaluable specimens from 332 patients. Only one specimen was collected from each of 153 patients, two specimens were collected from each of 90 patients, and three specimens were collected from each of 89 patients.

Fifty-seven specimens (9.5%) grew mycobacteria; there were 16 MTBC isolates (from 12 patients) and 41 isolates of nontuberculous mycobacteria, including 13 *M. avium* complex isolates (from 11 patients), 14 *M. fortuitum-chelonae* complex isolates (from 11 patients), 9 *M. gordonae* isolates (from 6 patients), 2 *M. kansasii* isolates (from 1 patient), 1 *M. terrae* isolate, and two pigmented rapidly growing mycobacterial isolates (from 2 patients) that could not be identified to the species level. Twenty-three specimens (from 18 patients) were AFB smear positive; 12 of these grew MTBC, 8 grew nontuberculous mycobacteria (6 *M. avium* complex, 1 *M. kansasii*, and 1 *M. gordonae*), and 3 were culture negative.

On initial testing of the 600 frozen specimens (including retesting of those samples that originally gave indeterminate results), 20 samples from 16 patients were positive for MTBC by the BDProbeTec ET. Fourteen of these specimens were MTBC culture positive, and the rest were culture negative. Review of the medical records of the six patients who were MTBC positive by the BDProbeTec ET but negative by culture showed that none had evidence of tuberculosis. Based on these results, the initial overall sensitivity, specificity, and positive and negative predictive values of the BDProbeTec ET for diagnosis of tuberculosis were 87.5, 99.0, 70.0, and 99.7%, respectively, by specimen and 83.3, 98.1, 62.5, and 99.4%, respectively, by patient. These values were 100, 100, 100, and 100%, respectively, for the 23 AFB smear-positive specimens and 50.0, 99.0, 25.0, and 99.6%, respectively, for the AFB smear-negative samples.

On retesting of the six processed specimens that were MTBC positive by BDProbeTec ET but culture negative, five were found to be negative by the BDProbeTec ET system. Two of these specimens yielding false-positive results by the BDProbeTec ET were located adjacent to MTBC culture-positive specimens during loading of the priming and amplification wells prior to analysis by the BDProbeTec ET system, suggesting possible cross-contamination. For the one specimen that remained positive by the BDProbeTec ET, the companion fresh aliquot was BDProbeTec ET negative. The patient from whom the specimen was collected had two other specimens tested; fresh and frozen aliquots of both were negative for MTBC by both culture and BDProbeTec ET. The fact that this sample remained positive by the BDProbeTec ET system whereas the others were negative suggests that MTBC

DNA actually was present and that an error (either cross-contamination or labeling) occurred during initial decontamination, concentration, and aliquoting of the specimen; this, however, cannot be proven. Upon retesting of the two AFB smear-negative specimens that were MTBC culture positive but negative by BD-ProbeTec ET, one became BDProbeTec ET positive while the other remained negative. This change from negative to positive suggests that the sample contained small numbers of tubercle bacilli and that the initial false-negative result was due to a sampling or distribution error. Based on these data, the revised sensitivity, specificity, and positive and negative predictive values were respectively 93.8, 99.8, 93.8, and 99.8% by specimen and 91.7, 99.7, 91.7, and 99.7% by patient.

The BDProbeTec ET system is the first nucleic acid amplification system using SDA technology and fluorescent energy transfer detection 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 depending on the number of samples being processed, ranging from approximately 3 h for 5 patient samples (plus 2 controls) to about 3.5 h for 15 specimens and 5 h for 40 specimens. The first part of the procedure, during which the specimen is prepared for amplification, is the most labor-intensive; thereafter, the assay is nearly completely automated.

The BDProbeTec ET System offers several advantages for laboratories performing nucleic acid amplification testing for direct detection of MTBC. In our opinion, the most important is the inclusion of an IAC in the same well as the patient specimen. Second, amplicon contamination is minimized because the sealed microwells in which amplification occurs are never reopened. This, however, does not eliminate the potential for cross-contamination during initial specimen processing or preparation of samples for amplification. Because organisms are heat killed early in the specimen preparation process, the remainder of the procedure may be performed on the countertop; it does not have to be done in a biological safety cabinet. Initial specimen processing (i.e., decontamination and concentration) and amplification can be performed in the same room. The manufacturer provides positive and negative controls; laboratory personnel do not have to prepare their own. Finally, all materials may be stored at room temperature; no refrigeration, freezing, or preparation of reagents is required.

In summary, our data suggest that the BDProbeTec ET system is a reliable means of direct detection of MTBC in respiratory specimens. However, the number of patients with tuberculosis in our evaluation, especially those with AFB smear-negative disease, was small; therefore, further studies to confirm our findings are needed.

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