Enhanced Amplified Mycobacterium Tuberculosis Direct Test for Detection of *Mycobacterium tuberculosis* Complex in Positive BACTEC 12B Broth Cultures of Respiratory Specimens

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The reliability of the Gen-Probe enhanced Amplified Mycobacterium Tuberculosis Direct Test (MTD) for identification of *Mycobacterium tuberculosis* complex (MTBC) in BACTEC 12B broth cultures of respiratory specimens was evaluated by testing aliquots from 268 bottles with a growth index of ≥50. MTD results were compared to those obtained by usual laboratory protocol, whereby MTBC was identified by DNA probe (Gen-Probe, Inc.) testing sediment from broth samples or colonies on a solid medium. For the first 134 cultures, from which 68 mycobacterial isolates (including 27 MTBC isolates) were recovered, both fresh and frozen aliquots were tested. MTD results for the frozen aliquots agreed with the identification by usual protocol in all cases, whereas there was one false-negative MTD result with fresh aliquots. For the remaining 134 cultures, only frozen aliquots were tested. Of the total 268 broth cultures (from 210 patients) evaluated, 137 (51.1%) grew mycobacteria, including 60 MTBC isolates. All 60 isolates were MTD positive, as was one additional culture that grew *Mycobacterium gordonae*. The latter culture was from a patient who was diagnosed with tuberculosis a few months earlier and was on therapy; therefore, the MTD result was considered a true positive. Sensitivity, specificity, and positive and negative predictive values of MTD were 100%. The mean times from specimen receipt to identification of MTBC were 15 (±1) days (range, 4 to 27 days) for BACTEC plus MTD and 19 (±1) days (range, 6 to 36 days) for the usual protocol (P < 0.001). These data indicate that the MTD is a rapid, reliable method for identification of MTBC in fresh or frozen aliquots of broth from positive BACTEC 12B cultures of respiratory specimens.

To help reverse the recent resurgence of tuberculosis in the United States, use of the most sensitive and rapid diagnostic laboratory tests available that provide reliable results is recommended (1, 8). Currently, these include a liquid and a solid medium for mycobacterial isolation and nucleic acid probes, a chromatographic method, or the BACTEC TB NAP (p-nitroacetyl-aminob-hydroxypropionophenone) test (Becton Dickinson, Sparks, Md.) for identification of *Mycobacterium tuberculosis* complex (MTBC). In addition, two commercially available nucleic acid amplification tests for direct detection of MTBC are the enhanced Amplified Mycobacterium Tuberculosis Direct Test (MTD; Gen-Probe, Inc., San Diego, Calif.) and the AMPLICOR *Mycobacterium tuberculosis* Test (Roche Diagnostic Systems, Inc., Somerville, N.J.), but their use has been limited by the Food and Drug Administration to testing respiratory specimens that are smear positive for acid-fast bacilli (AFB) and which have been collected from untreated patients. The target turnaround time suggested by the Centers for Disease Control and Prevention for detection and identification of MTBC is 21 days or less from receipt of a specimen in the laboratory (8).

Currently the 21-day target for detection and identification of MTBC can only be achieved by use of a nucleic acid amplification test directly from AFB smear-positive respiratory samples or by performing one of the rapid identification tests on growth-positive broth cultures. The latter method, however, occasionally yields false-negative results; i.e., the test is negative for MTBC, which then is later identified by testing colonies on a solid medium (5). When this occurs, the identification of MTBC may be delayed beyond 21 days. An alternative way in which the 21-day target for identification can be met is by performing nucleic acid amplification on aliquots of broth from cultures in which mycobacterial growth has been detected. The AMPLICOR assay has been shown to be a reliable method for identification of MTBC in BACTEC 12B (Becton Dickinson, Sparks, Md.) and ESP II (Trek Diagnostics [formerly AccuMed International], Westlake, Ohio) broth samples (2, 3, 6, 7), but to our knowledge the performance of the MTD has not been evaluated for this purpose. The intent of this study was to examine the reliability of the enhanced MTD, introduced in 1998, for detection of MTBC in positive BACTEC 12B broth cultures.

**Culture and identification.** Respiratory specimens submitted for mycobacterial culture were decontaminated with 1% sodium hydroxide (final concentration)—N-acetylcysteine and concentrated by centrifugation at 3,000 × g for 20 min, according to standard procedures (4). Approximately 0.2 ml of the sediment was used to prepare a smear for staining with auramine O. To the remaining sediment, phosphate buffer was added to give a final volume of 2.0 ml. For 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 biplate (Becton Dickinson).

BACTEC 12B bottles were incubated at 37°C in 8% CO₂ and monitored for growth for 5 weeks by the BACTEC 460 TB instrument according to the manufacturer’s instructions, as described in detail elsewhere (4). When the growth index (GI) of a 12B bottle reached ≥50, two 500-μl aliquots were removed for analysis by MTD. Plates were incubated at 37°C in 8% CO₂ and examined weekly for growth for 6 weeks. Isolates of mycobacteria were identified by DNA probes (AccuProbe;
Enhanced MTD. For each of the first 134 specimens, one aliquot was refrigerated and tested by MTD within 72 h and one aliquot was stored for up to 2 months at −70°C for batch testing. Thereafter, both aliquots were stored at −70°C for batch analysis to optimize labor efficiency for the purpose of this evaluation. MTD was performed and interpreted according to the manufacturer’s protocol for processed respiratory specimens. Frozen aliquots were first thawed and vigorously agitated with a vortex mixer. Briefly, 50 μl of Specimen Dilution Buffer was added to each Lysing Tube, after which 450 μl of broth specimen, positive cell control, or negative cell control was added to the correspondingly labeled tube. Tubes were vigorously agitated on a vortex mixer for 3 s and then sonicated for 15 min at ambient temperature in a water bath sonicator (Lab-Line Instruments, Melrose Park, Ill.). To labeled amplification tubes, 50 μl of Mycobacterium Tuberculosis Amplification Reagent and then 200 μl of Mycobacterium Oil Reagent were added. Using an extended-length hydrophobically plugged pipette tip, 25 μl of both the sample and control lysates were transferred to the appropriate tube (the remaining lysate was stored at −70°C for further testing, if necessary). Tubes were incubated at 95°C for 15 min in a dry heat block and then transferred to a 42°C water bath for 5 min. To each tube 25 μl of Mycobacterium Enzyme Reagent was added; tubes were capped, gently shaken, and incubated in a 42°C water bath for 30 min. Tubes were carefully uncapped, and 100 μl of Hybridization Reagent was added. Tubes were covered with new caps, vigorously agitated on a multitube vortex mixer (VWR Scientific Products, West Chester, Pa.) for at least 30 s and incubated in a 60°C water bath for 5 min. After hybridization, 300 μl of Selection Reagent was added. Tubes were covered with new caps, vigorously agitated on a multitube vortex mixer for at least 30 s, incubated in a 60°C water bath for 15 min, allowed to cool at ambient temperature for 5 min, and read in relative light units [RLU] in a luminometer (Leader 50; Gen-Probe, Inc.). Interpretation of results was as follows: <30,000 RLU, negative; ≥500,000 RLU, positive; 30,000 to 500,000 RLU, equivocal. For samples yielding an equivocal result, a second aliquot was tested; if the second result was ≥30,000 RLU, the sample was considered positive, whereas if it was <30,000 RLU, it was considered negative. Results of the enhanced MTD were considered acceptable when the negative cell control was <20,000 RLU and the positive cell control was ≥500,000 RLU. If the MTD result was discordant from the result obtained by the usual laboratory protocol, the MTD was repeated with the second aliquot and the patient’s medical record was reviewed.

Statistical analysis. The Student’s t test was used to compare times to identification of MTBC in 12B broth cultures by MTD (i.e., time from bottle inoculation until the culture reached a GI of ≥50 and the aliquots of broth were removed for MTD analysis) and by the usual laboratory protocol. For the usual laboratory protocol, the interval was time from inoculation of a 12B bottle until the GI reached 999, if the probe result for the broth sediment was positive, and time from inoculation until colonies on the original solid medium or on the solid medium to which broth from a positive 12B bottle was subcultured were identified, if the probe result from the broth sediment was negative.

Aliquots from a total of 268 12B broth cultures (210 patients) were collected over 10 months for analysis by MTD. Results are summarized in Tables 1 and 2. From the 134 cultures for which both fresh (≥72 h) and frozen aliquots were tested, mycobacteria ultimately were isolated from 68 (50.7%) cultures as follows: 27 MTBC isolates, 25 M. avium complex isolates, 3 M. gordonae isolates, 5 Mycobacterium fortuitum-chelonae complex isolates, 4 Mycobacterium nonchromogenicum isolates, 3 M. kansasi isolates, and 1 Mycobacterium mucogenicum isolates. MTD results for the fresh aliquots were positive for 26 of the 27 isolates that were culture positive for MTBC and negative for the 107 isolates from which MTBC was not recovered (sensitivity, 96.3%; specificity, 100%; positive predictive value [PPV], 100%; negative predictive value [NPV], 99.1%). For the frozen aliquots, the correlation between MTD and culture results was 100%. Based on these data, only frozen aliquots were tested thereafter to optimize labor efficiency.

For the 268 frozen aliquots tested, 137 (51.1%) of the corresponding cultures grew mycobacterial isolates as follows: 60 MTBC (34 patients), 38 M. avium complex, 15 M. fortuitum-chelonae complex, 9 M. gordonae, 5 M. kansasi, 4 M. nonchromogenicum, 1 each M. mucogenicum, M. smegmatis, M. szulgai.

### Table 1. Summary of MTD results for testing positive BACTEC 12B cultures

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Initial RLU</th>
<th>n</th>
<th>Mean RLU (±SEM)</th>
<th>RLU range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>&lt;30,000</td>
<td>108</td>
<td>3,931 (±181)</td>
<td>2,130-14,593</td>
</tr>
<tr>
<td></td>
<td>30,000-499,999</td>
<td>0</td>
<td>2.84 × 10^6 (±0.07 × 10^6)</td>
<td>2.12 × 10^6-3.41 × 10^6</td>
</tr>
<tr>
<td></td>
<td>≥500,000</td>
<td>26</td>
<td>1.414 (±161)</td>
<td>1.744-17,399</td>
</tr>
<tr>
<td>Frozen</td>
<td>&lt;30,000</td>
<td>207</td>
<td>459,404^a</td>
<td>1.05 × 10^6-3.58 × 10^6</td>
</tr>
<tr>
<td></td>
<td>30,000-499,999</td>
<td>1</td>
<td>2.92 × 10^6 (±0.06 × 10^6)</td>
<td>1.05 × 10^6-3.58 × 10^6</td>
</tr>
<tr>
<td></td>
<td>≥500,000</td>
<td>59</td>
<td>500,000</td>
<td>500,000</td>
</tr>
</tbody>
</table>

^a Value for repeat testing was 43,058 RLU (positive by MTD and culture).

### Table 2. Performance of MTD for identification of MTBC in positive BACTEC 12B cultures

<table>
<thead>
<tr>
<th>Culture result</th>
<th>Fresh samples</th>
<th>Frozen samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>107</td>
</tr>
</tbody>
</table>

^a Specimen was from a patient known to have tuberculosis and to be on therapy (true-positive MTD result).
and *M. simiae*, and in 2 cases an unequivocal identification was not possible (*M. vaccae* versus *M. neoaurum* was reported by the Texas Department of Health). MTD results were positive for 61 of the 268 aliquots, including all those for which the culture grew MTBC and for one culture which grew *M. gordonaee*. For the latter culture, the second aliquot was tested, and it, too, was MTD positive. Review of this patient's medical record showed that he had been diagnosed with tuberculosis several weeks earlier and was receiving antituberculous therapy. Therefore, the MTD result was considered a true positive. The sensitivity, specificity, PPV, and NPV of the MTD for identification of MTBC were 100%.

The mean times (± standard errors of the means) from receipt of the specimen in the laboratory to identification of MTBC were 15 ± 1 days (range, 4 to 27 days) for BACTEC 12B plus MTD and 19 ± 1 days (range, 6 to 36 days) for the usual laboratory protocol, as described above (*P* < 0.001).

The exact cause of the initial incorrect MTD test result is unknown. However, we suspect that for the culture for which the MTD results for the fresh and frozen aliquots were discordant, the error occurred during collection and labeling of the aliquots. This emphasizes the need for close attention to detail and for taking care to avoid distractions not only while performing the MTD assay but also during initial preparation of the specimens for testing.

In summary, our data indicate that the enhanced MTD is a reliable method for rapid detection of MTBC in positive (GI ≥50) BACTEC 12B broth cultures of respiratory specimens. The performance of the MTD was comparable for fresh versus frozen samples. In addition to reliability, use of the MTD allows identification of MTBC in positive 12B cultures, tested as soon as the GI reaches 50 or greater, in a significantly shorter period of time than is possible by nucleic acid probe.

Enhanced MTD kits for this study were kindly provided by Gen-Probe, Inc. We appreciate the expertise provided by the University of Texas Medical Branch medical technologists who work in the mycobacteriology laboratory.

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REFERENCES