

## Clinical Evaluation of the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test for Rapid Detection of *Mycobacterium tuberculosis* in Select Nonrespiratory Specimens

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**The performance of the Amplified Mycobacterium Tuberculosis Direct Test (MTD; Gen-Probe, Inc., San Diego, Calif.) for rapid diagnosis of extrapulmonary tuberculosis was evaluated by testing 178 nonrespiratory specimens from 158 patients. Criteria for specimen inclusion were (i) a positive smear for acid-fast bacilli ( $n = 54$ ) and (ii) the source if the smear was negative (tissue biopsies and aspirates and abscess material were tested;  $n = 124$ ). Results were compared to those of mycobacterial culture; clinical history was reviewed when MTD and culture results disagreed. Forty-eight specimens (27.0%) were positive for mycobacteria, including 23 *Mycobacterium tuberculosis* complex specimens; of which 21 were smear positive. Twenty-five specimens were MTD positive; 20 of these grew *M. tuberculosis* complex. All of the five MTD-positive, *M. tuberculosis* complex culture-negative specimens were considered truly positive, based on review of the medical record. Of the three MTD-negative, *M. tuberculosis* complex culture-positive specimens, two contained inhibitory substances; one of the two was smear positive. Excluding the latter specimen from analysis, after chart review, the sensitivity, specificity, and positive and negative predictive values of the MTD were 92.6, 100, 100, and 98.7%, respectively, by specimen and 89.5, 100, 100, and 98.6% by patient. Given the few smear-negative samples from patients with extrapulmonary tuberculosis in our study, additional similar studies that include more smear-negative, *M. tuberculosis* complex culture-positive specimens to confirm our data are desirable.**

Tuberculosis remains a public health problem in the United States, despite a continual decline in incidence in each of the past several years. A key aspect of tuberculosis control is rapid diagnosis, which for many years has been based on the staining of smears for acid-fast bacilli (AFB) and culturing for mycobacteria. AFB smear results should be available within 24 h (7), but the test lacks sensitivity and is not specific for tuberculosis (8). Mycobacterial culture and identification results, which provide a specific diagnosis, often are not available for 2 to 3 weeks or longer. In response to the need for a more rapid diagnostic test, nucleic acid amplification tests specific for *Mycobacterium tuberculosis* complex have been developed. Currently in the United States, two such tests are commercially available for testing of respiratory specimens: the Amplified Mycobacterium Tuberculosis Direct Test (MTD; Gen-Probe, Inc., San Diego, Calif.), which is approved for use regardless of the AFB smear result, and the AMPLICOR *Mycobacterium tuberculosis* Test (Roche Molecular Systems, Branchburg, N.J.), which is approved for smear-positive specimens only.

Despite the fact that the nucleic acid amplification tests are approved only for respiratory specimens, many investigators have used them to test specimens from other sites (2, 3, 5, 6). Most such studies, however, have included a mixture of specimen types, and in many cases the number of any one type of specimen is insufficient for test validation. In our institution, the rate of recovery of *M. tuberculosis* complex from nonrespiratory specimens is low. Therefore, testing of all such spec-

imens by nucleic acid amplification would be cost prohibitive and would contribute little to patient management. The purpose of our study, therefore, was to evaluate the performance of the MTD assay with those nonrespiratory specimens that in our institution are most likely to be culture positive for *M. tuberculosis* complex—i.e., those that are AFB smear positive and tissue biopsies, fine-needle aspirates of tissues (predominantly lymph nodes), and abscesses or wounds that are smear negative.

**Clinical specimens.** One hundred seventy-eight nonrespiratory specimens (from 158 patients) received in the laboratory for detection of mycobacteria between November 1998 and May 2000 were selected for evaluation based on two criteria: the smear result and, for AFB smear-negative specimens, the source. All smear-positive nonrespiratory specimens were tested; this included feces ( $n = 20$ ); tissue biopsies ( $n = 17$ ) and aspirates ( $n = 8$ ); wound, drainage, or abscess material ( $n = 8$ ); and pleural fluid ( $n = 1$ ). Of the smear-negative specimens, only tissue biopsies, fine-needle aspirates of tissues (predominantly lymph nodes), and those labeled abscess, drainage, or wound were tested ( $n = 124$ ). All specimens were processed within 24 h of receipt in the laboratory.

**Specimen processing and culture.** Specimens that were considered contaminated (i.e., feces and specimens labeled wound, drainage, or abscess) were decontaminated with 1% (final concentration) sodium hydroxide-N-acetylcysteine and concentrated by centrifugation at  $3,000 \times g$  for 20 min in accordance with standard procedures (4). Protocols to limit the potential for cross-contamination during processing included the use of single-use containers for phosphate buffer and digestant, removal and replacement of the cap of each specimen tube sequentially during the addition of specimens and re-

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agents, and allowing tubes to stand for a few minutes after agitation on a vortex mixer (9). Tissues were ground with a disposable sterile tissue grinder, and liquid specimens (i.e., pleural fluid and some aspirates) were concentrated prior to smear preparation and culture. Smears were stained with auramine O. For culture, 0.5 ml of the decontaminated or fresh specimen was inoculated into an ESP II bottle (Trek Diagnostics, Westlake, Ohio) or MGIT tube (BD Biosciences, Sparks, Md.) and 0.2 ml was inoculated onto each side of a Middlebrook 7H11/7H11 selective biplate (BD Biosciences). The remainder of the specimen was reserved for MTD testing: one 500- $\mu$ l aliquot was stored at 2 to 8°C and tested within 72 h of processing; if the volume was sufficient, a second 500- $\mu$ l aliquot was stored frozen at -70°C for future analysis, if necessary.

ESP II bottles and MGIT tubes were incubated in the ESP II and BACTEC 960 instruments, respectively, where they were continuously monitored for growth for 6 weeks. Plates were incubated at 37°C in 8% CO<sub>2</sub> and examined weekly for growth for 6 weeks: Isolates of mycobacteria were identified by using DNA probes (AccuProbe [Gen-Probe, Inc.] for *M. tuberculosis* complex, *M. avium* complex; and *M. kansasii*) or conventional biochemical tests (for rapidly growing mycobacteria) in accordance with the standard protocol (4). 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.

**MTD.** MTD was performed in accordance with the manufacturer's protocol for respiratory specimens as described in detail elsewhere (1). Interpretation of results was as follows: <30,000 relative light units (RLU), negative;  $\geq$ 500,000 RLU, positive; 30,000 to 499,999 RLU, equivocal. For samples yielding an equivocal result, a second aliquot of a decontaminated or fresh specimen was tested; if the second result was  $\geq$ 30,000 RLU, the sample was considered positive, whereas if it was <30,000 RLU, it was negative. MTD results were acceptable when the negative control was <20,000 RLU and the positive control was  $\geq$ 500,000 RLU (>1,000,000 after September 1999, at which time the manufacturer's directions changed). MTD results were not reported because the test was not approved by the Food and Drug Administration for this purpose.

**Protocol for detection of inhibitory substances.** Specimens that grew *M. tuberculosis* complex but were MTD negative were analyzed for the presence of inhibitors or interfering substances as follows. The specimen lysate was thawed, and 25  $\mu$ l was added to an amplification tube containing 50  $\mu$ l of amplification reagent and 200  $\mu$ l of oil. The tube was then seeded with 5  $\mu$ l of the positive cell control lysate, placed in a 95°C heat block for 15 min, and tested in accordance with the manufacturer's protocol. Positive and negative controls were processed and tested. Additionally, 5  $\mu$ l of the positive cell control lysate was added to a second, negative control tube to serve as a positive control for the seeded lysates. Interpretation of the results was as follows:  $\geq$ 30,000 RLU, negative for inhibitory substances that prevent amplification; <30,000 RLU, substances that inhibit amplification present.

**Resolution of discrepancies.** For specimens with discordant *M. tuberculosis* complex culture and MTD results, the medical record (including initial symptoms, Mantoux test result [if the test was performed and the result was recorded], chest radiograph findings, response to therapy, and admission and dis-

TABLE 1. Summary of *M. tuberculosis* complex culture and MTD results for smear-positive and smear-negative specimens

Smear result and specimen (no. of specimens) <sup>a</sup>	No. of patients with tuberculosis by:		
	Clinical findings	Culture	MTD
<b>Positive</b>			
Feces (20)	4	3	4
W/D/A (8)	5	3	4
Aspirate (8)	5	5	4 <sup>b</sup>
Tissue (17)	9	9	9
Pleural fluid (1)	1	1	1
<b>Negative</b>			
Tissue (60)	1	1	0 <sup>b</sup>
Aspirate (44)	2	0	2
W/D/A (20)	1	1	1

<sup>a</sup> W/D/A, wound, drainage, or abscess; aspirate, aspirate of tissue; tissue, tissue biopsy.

<sup>b</sup> Culture-positive, MTD-negative specimen contained inhibitors.

charge diagnoses) was reviewed, and laboratory records were examined to investigate the possibility of cross-contamination. For MTD-negative specimens that grew *M. tuberculosis* complex, the lysate was tested for inhibitory substances.

**Evaluation of the MTD.** Forty-eight (27.0%) of the 178 specimens were positive for mycobacteria, including 23 that were *M. tuberculosis* complex positive. Twenty-one *M. tuberculosis* complex and all 25 nontuberculous mycobacterial strains (18 *M. avium* complex, 3 *M. fortuitum-chelonae* complex, 2 *M. kansasii*, and 2 *M. simiae*) were recovered from smear-positive specimens. *M. tuberculosis* complex culture and MTD results are summarized in Table 1. On initial testing, 24 specimens were MTD positive, 1 (a pleural fluid specimen) was equivocal but yielded a positive result when retested, and the remainder were negative. Twenty of the 25 MTD-positive specimens were *M. tuberculosis* complex culture positive. Of the five specimens that were MTD positive and *M. tuberculosis* complex culture negative, four (two smear positive and two smear negative) were from patients who had been previously diagnosed with tuberculosis and were receiving appropriate therapy. The fifth specimen (smear-positive feces) grew *M. avium* complex; however, 42 days after that specimen had been collected, a sputum specimen from the patient grew *M. tuberculosis* complex. Therefore, the MTD result for each of these five specimens was considered truly positive. Two (one smear positive and one smear negative) of the three specimens that were *M. tuberculosis* complex culture positive and MTD negative contained inhibitory substances.

After chart review, the sensitivity, specificity, and positive and negative predictive values of the MTD were 89.3, 100, 100, and 98.0%, respectively, by specimen and 85, 100, 100, and 97.9% by patient. Based on our usual laboratory practice for respiratory specimens of testing all smear-positive, MTD-negative specimens for inhibitors and reporting MTD results for those with a positive inhibitory assay as uninterpretable, deleting the smear-positive specimen with inhibitors from analysis is reasonable. Excluding that specimen, the sensitivity, specificity, and positive and negative predictive values of the MTD were 92.6, 100, 100, and 98.7%, respectively, by specimen and 89.5, 100, 100, and 98.6% by patient.

Rapid diagnosis is critical to the control of tuberculosis,

especially pulmonary disease. Rapid diagnosis of extrapulmonary tuberculosis has a greater impact on patient management than on limiting spread of the disease. It allows prompt initiation of appropriate therapy (and discontinuation of potentially toxic, unnecessary drugs) and may obviate the need for additional invasive diagnostic tests and decrease hospital stays. For many years the AFB smear was the only rapid diagnostic test available. Because the smear lacks sensitivity and specificity, a laboratory test with superior performance characteristics but a similar turnaround time is needed. Theoretically, nucleic acid amplification tests can fulfill these requirements; however, those currently available are approved for respiratory specimens only.

Studies evaluating the performance of the current version of the MTD with nonrespiratory specimens have included a mixture of specimen types, in various proportions. Sensitivity has ranged from 79 to 100%, and specificity has ranged from 99 to 100% (2, 3, 5, 6). However, because the performance of the MTD may not be the same for all types of specimens, comparison of the results of studies in which the proportion of different specimens varies considerably may not be appropriate. Our study design and results are most similar to those of Chedore and Jamieson, who reported a sensitivity and specificity of 100% for smear-positive specimens and 100% and 98.9% for smear-negative specimens (2). They included all AFB smear-positive specimens, as did we, and any smear-negative specimen when the physician specifically requested the test based on a high clinical suspicion of tuberculosis. Our goals were to optimize patient management and achieve cost effectiveness; therefore, we tested those nonrespiratory specimens that in our experience were most likely to yield *M. tuberculosis* complex. Additionally, testing of a reasonable number of specimens with similar characteristics allows test validation. To our knowledge, a universally accepted number deemed appropriate for validation does not exist. However, we believe that our study included an adequate number of tissue biopsies, fine-needle aspirates, and smear-positive specimens (assuming that it is reasonable to consider smear result a specimen characteristic).

Three specimens in our study had false-negative MTD results. Two of these contained inhibitors, which explains the negative results. The third specimen was included in a run (performed prior to September 1999) in which the result of the positive control was 690,400 RLU. This was in the acceptable range but was much lower than the typical positive control results in our laboratory, i.e.,  $\geq 3,000,000$  RLU. The kit reagents used on the day when the low acceptable positive control value was obtained were on day 30 of their 30-day expiration period. Therefore, fresh reagents from a new kit were prepared and the specimen with the false-negative result was retested and tested for inhibitors. The repeat result was positive, and no inhibitors were detected; the positive control for the run was  $>3,000,000$  RLU. Based on this observation, we modified our procedure for respiratory specimens such that if the positive control result were considerably less than the usual value, the patient results would not be reported and the run would be repeated. Then, in September 1999, when the MTD was approved for testing regardless of the AFB smear result,

the manufacturer changed the cutoff for the positive control to  $>1,000,000$ . This modification likely will decrease the possibility of false-negative patient results.

In conclusion, the performance of the MTD with nonrespiratory smear-positive specimens and smear-negative tissue biopsies, fine-needle aspirates, and abscess material was very good. Based on our results, we currently perform the MTD on any smear-positive nonrespiratory specimen if it is specifically requested by the physician and we test those with negative MTD results for inhibitors. We also test smear-negative tissue biopsies and aspirates with the approval of an infectious disease or pulmonary specialist. Testing of all smear-negative, MTD-negative specimens for inhibitors is cost prohibitive; therefore, in our institution it requires a specific request by an infectious disease or pulmonary specialist and approval of the microbiology laboratory director. Although performance of the MTD was excellent with our smear-negative tissue biopsies and aspirates and abscess material, only 4 (3.2%) of the 124 smear-negative specimens tested were from patients with tuberculosis. Therefore, additional, similar studies that include more smear-negative, *M. tuberculosis* complex culture-positive specimens should be conducted to confirm our findings.

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