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NOTES

Performance of the Gen-Probe Amplified *Mycobacterium tuberculosis* Direct Test in a Laboratory that Infrequently Isolates *Mycobacterium tuberculosis*

Karen Welch, Gloria Brown, Vivian Jonas, and Mary Jane Ferraro

The performance of the Gen-Probe Mycobacterium tuberculosis *direct (MTD) test was assessed in a laboratory whose specimens were derived from a population with a low prevalence (1.3%) of tuberculosis. A total of 339 specimens from 113 patients were included in the study. Nine of 10 MTD positive samples were culture positive (smear positive, n = 7; smear negative, n = 1; smear not ordered, n = 2). The 10th MTD-positive sample, which was smear and culture negative, was from a patient whose two other study specimens were smear and culture positive and who had a clinical history consistent with tuberculosis. Prior to and following resolution of discrepant results, the sensitivities and specificities of the MTD test relative to culture were 100 and 99.7% and 100 and 100%, respectively.*

Rapid diagnosis of patients who have disease caused by *Mycobacterium tuberculosis* is necessary to initiate adequate therapy and prevent spread of the disease. Recently, several reports in the literature have described rapid methods of detecting *M. tuberculosis* directly from decontaminated lower respiratory samples using target amplification methods (Abe et al., 1993; Ehlers et al., 1994; Jonas et al., 1993; LaRocco et al., 1994; Miller et al., 1994; Pfyffer et al., 1994; Shinnick and Jonas, 1994; Zwadyk et al., 1994). The use of these rapid target amplification systems generally has been described by laboratories that isolate *M. tuberculosis* with high frequency (anywhere from 5%–20%). The positive predictive value and performance of a target amplification assay in a laboratory that infrequently isolates *M. tuberculosis* is also of concern. Our laboratory isolated mycobacteria from 6.2% of 4861 submitted specimens in 1993. However, *M. tuberculosis* accounted for only 21% of the *Mycobacterium* spp. isolated.

We describe our experience with the Gen-Probe Amplified *Mycobacterium tuberculosis* direct (MTD) test in this low prevalence population. The MTD test targets rRNA, which is amplified via the transcription mediated amplification (TMA) system designed specifically to be used with the hybridization protection assay (HPA) form of detection (Arnold et al., 1989). TMA is an isothermal transcription-based amplification method that is capable of using two primers, one of which contains a promoter for RNA transcription, and two enzymes (reverse transcriptase and RNA polymerase) to generate approximately a billion copies of RNA amplicon per template in <2 h. The amplification and detection portions of the assay take place in the same reaction tube, making the test easier to perform and minimizing the chance for carryover contamination.

All patients who had three sputum and/or in-

From the Microbiology Laboratories, Massachusetts General Hospital (K.W., G.B., M.J.F.), and Harvard Medical School (M.J.F.), Boston, Massachusetts, Gen-Probe Incorporated, San Diego, California (V.J.), USA.

Address reprint requests to Dr. M.J. Ferraro, Clinical Microbiology Laboratories Gray B526, Massachusetts General Hospital, Boston, MA 02114, USA.

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655 Avenue of the Americas, New York, NY 10010

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TABLE 1 Comparison of Culture Results with Those of *Mycobacterium tuberculosis* Direct (MTD) test

		Mycobacterial Culture Results (No. of Specimens)		
		Positive for *M. tuberculosis*	Positive for MOTT[a]	Negative for Mycobacteria
Genprobe MTD	Positive	9	0	1[b]
	Negative	0	22	307

[a]MOTT: *Mycobacteria* other than *M. tuberculosis*, including *M. avium* complex (17), *M. gordonae* (two), *M. terrae* (one), group IV (one), and *Mycobacterium* species not *M. tuberculosis* (one).
[b]Specimen from patient with two other specimens that were smear and culture positive for *M. tuberculosis*.

duced sputum specimens submitted to our laboratory for mycobacterial culture over a 6-month period were eligible for the study. Lower respiratory samples were decontaminated and digested as described previously (Kent and Kubica, 1985). Specimens were cultured for 8 weeks using conventional methods (Kent and Kubica, 1985) on Lowenstein–Jensen, Mycobactosel, and Middlebrook 7H-11 agar slants (BBL, Cockeysville, MD, USA), and a portion of the sediment was retained at −70°C for the MTD test.

Training of personnel using the MTD test took place prior to initiating the study. Each technologist was required to test a proficiency panel consisting of 20 interspersed positive and negative samples as well as six controls. Perfect results had to be obtained twice successively prior to performance of the test on clinical samples. Each of two technologists passed the proficiency training within the first three runs.

Three sediments from each of 114 patients were tested using the MTD test following the package insert protocol as previously described (Jonas et al., 1993). One patient (three samples) was eliminated from the study after testing began because the specimens were observed to be grossly bloody and were contraindicated for testing in the package insert protocol.

All of the testing was performed in the corner of one room containing a biological safety cabinet, open bench, and chemical fume hood. A total of 11 runs consisting of 17–48 patient samples were required to complete the study. Two positive and two negative amplification controls as well as one positive and one negative hybridization control were performed with each run. Positive patient samples were detected in runs 2, 5, and 6. The total amount of time to complete a run of 50 samples was 5 h, including a 2-h incubation when the technologist was free to perform other duties.

Table 1 displays the results by specimen before discrepant resolution. Nine of the 10 MTD positive samples were culture positive. Seven of nine culture-positive samples were smear positive [abundant (n = 3), few (n = 3), rare (n = 1)]. Two of the culture-positive specimens did not have a smear performed, although other specimens collected during the same time period were smear negative. The 10th sample, which was smear and culture negative for *M. tuberculosis*, came from a patient whose other two study specimens were smear and culture positive and who had a clinical history consistent with tuberculosis. There were no false-negative MTD tests as compared to culture or recent clinical history. The MTD positive–culture-negative specimen was considered as a true positive; therefore, after resolution of discrepant results, the sensitivity and specificity were 100%. Mycobacteria other than *M. tuberculosis* were isolated from 22 of the 339 study specimens (15 patients) as detailed in Table 1. None of these specimens was positive in the MTD test.

These data demonstrate that the Gen-Probe MTD test performs with reliability in a setting where *M. tuberculosis* is infrequently isolated, even when other species are commonly encountered. Although others have shown the MTD test to have relatively high specificity, the actual specificity rates ranged from 96%–100% (Abe et al., 1993; Ehlers et al., 1994; LaRocco et al., 1994; Pfyffer et al., 1994). Given our prevalence of 1.3% *M. tuberculosis* isolated from all specimens submitted to our laboratory in 1993, specificities in this range could result in anywhere from 48–192 false-positive MTD results. A predictive positive value this low would not be acceptable for our patient population. However, our experience indicates that when properly and carefully performed, this assay has the potential for nearly perfect specificity. The high predictive values in our clinical setting would allow rapid results from an MTD test to guide therapy or infection control procedures in patients with positive smears for acid-fast bacilli or symptoms consistent with a high clinical suspicion for active tuberculosis.

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