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Evaluation of Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test by Using Respiratory and Nonrespiratory Specimens in a Tertiary Care Center Laboratory

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The performance of the Amplified Mycobacterium Tuberculosis Direct (AMTD) test (Gen-Probe Inc., San Diego, Calif.) was assessed in a large tertiary care mycobacteriology laboratory. Both acid-fast smear-positive and smear-negative respiratory and nonrespiratory clinical specimens were analyzed. From February 1998 to 4 October 2001, AMTD assays were performed on 391 respiratory specimens and 164 nonrespiratory specimens. The AMTD assay was compared to the "gold standard" of combined culture and clinical diagnosis. The overall sensitivity for all specimens, including those for which no smear result was available, was 91.2%. The overall sensitivities of the assay, including acid-fast smear-positive and -negative specimens, were 97.8 and 77.3% for respiratory and nonrespiratory specimens, respectively. The corresponding specificities for respiratory and nonrespiratory specimens were 99.1 and 98.5%, respectively. The overall specificity for all specimens was 98.9%. Positive and negative predictive values were 93.9 and 99.7% and 91.7 and 96.4% for respiratory and nonrespiratory specimens, respectively. The time saved by using the AMTD test for making a diagnosis of tuberculosis instead of using culture was 8.99 days. Inhibitors to the AMTD assay were found in 3.1% of respiratory specimens and 3.1% of nonrespiratory specimens. The assay, used in a general mycobacteriology laboratory setting, represents an important advance in improving the speed and accuracy of diagnosis in the management of patients with tuberculosis.

Tuberculosis continues to be a significant cause of morbidity and mortality worldwide, with more than 3 million deaths reported during the past year (4). A particular concern is the spread of multidrug-resistant tuberculosis among the population (12). Since the route of transmission is the aerosolization of *Mycobacterium tuberculosis*, a key approach to the control of tuberculosis remains the rapid identification, isolation, and treatment of patients with active pulmonary tuberculosis.

The sputum acid-fast stain and culture remain the cornerstone for the diagnosis of active respiratory tuberculosis around the world; however, the former has been shown repeatedly to lack both sensitivity and, to some extent, specificity (7). Since the mid 1990s a few commercially available molecular amplification assays have been introduced in an attempt to improve the accuracy and speed of detecting *M. tuberculosis* in clinical specimens (8, 9). The Amplified Mycobacterium Tuberculosis Direct (AMTD) test (Gen-Probe Inc., San Diego, Calif.) was initially approved by the Food and Drug Administration, Washington, D.C., in 1995. Subsequently a modification of AMTD was introduced in 1998, and it was approved by the Food and Drug Administration in September 1999 (2).

The AMTD test is an isothermal transcription-mediated amplification system based on the reverse transcription of mycobacterial-specific rRNA targets, followed by transcription of the DNA intermediate template. Subsequent detection of RNA amplicons is through a hybridization protection assay using an acridinium ester-labeled *M. tuberculosis* complex-specific DNA probe.

The Mayo Clinic clinical mycobacteriology laboratory is a full-service laboratory that also receives requests from throughout the United States for the detection and identification of mycobacteria from a wide variety of clinical specimens. The laboratory processes in excess of 13,800 specimens per year. The AMTD test has been performed at the Mayo Clinic since 1996. We report our experience with the AMTD assay since its modification and introduction in 1998. Since this time, the assay has been utilized for both acid-fast smear-positive and smear-negative respiratory and nonrespiratory tract specimens. All specimens, regardless of their acid-fast smear status, are included to reflect what is tested in a high-volume clinical mycobacteriology laboratory.

From a clinical standpoint, the key aspect of any new assay that detects *M. tuberculosis* is the assay's negative predictive value. In the case of respiratory tract disease, it is critical to identify all cases of active tuberculosis and thereby interrupt the dissemination and transmission of the organism and, in extrapulmonary tuberculosis, to ensure that a readily treatable infection is not overlooked. Ideally, any test should be sensitive and specific, and it must save a significant amount of time in making a diagnosis compared to conventional methods.

MATERIALS AND METHODS

Clinical specimens submitted to the mycobacteriology laboratory included those collected from Mayo Clinic inpatients and outpatients and those referred from outside laboratories to Mayo Medical Laboratories. A total of 391 respi-

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TABLE 1. AMTD and culture correlation for respiratory specimens

Smear result (no. of specimens)	No. of specimens with indicated results (AMTD/culture)				Sensitivity/specificity (%)	Predictive values (%)	
	Positive/positive	Positive/negative	Negative/positive	Negative/negative		Positive	Negative
Positive (92)	30	1	0	61	100/98.4		
Negative (244)	3	2	1	238	75/99.2		
Unknown (43)	12	0	0	31	100/100		
Total[a] (379)	45	3	1	330	97.8/99.1	93.9	99.7

[a] Includes specimens with no smear result available and does not include inhibitory specimens.

ratory specimens (Table 1) and 164 nonrespiratory specimens (see Table 4) were included in this evaluation.

Specimens were treated with an equal volume of BBL MycoPrep Reagent (BD Diagnostic Systems, Sparks, Md.) containing 2% NaOH and *N*-acetyl-L-cysteine mixed and incubated at 25°C for 15 min. MycoPrep buffer was added up to a total volume of 50 ml, and the suspension was mixed and centrifuged for 15 min at 3,000 × *g* at 10°C. Following centrifugation, the supernatant was removed and the sediment was suspended in 3 ml of MycoPrep phosphate buffer and vigorously mixed.

A 50-μl aliquot of sediment was placed on a glass microscope slide and allowed to air dry followed by heat fixation. The auramine-rhodamine stain was performed as discussed previously (K. L. Mork-Lewis, L. Stockman, and G. D. Roberts, Abstr. 98th Gen. Meet. Am. Soc. Microbiol., abstr. 180, 1998).

Cultures were performed by inoculating 0.5 ml of sediment into a MGIT tube and onto each side of a biplate that contained Middlebrook 7H10 agar and Middlebrook 7H11 selective agar with antimicrobials (Remel, Lexena, Kans.). All MGIT tubes were supplemented with the antimicrobial solution provided by the manufacturer. After inoculation, tubes were placed into the BACTEC MGIT 960 System and were incubated at 35 to 37°C for 6 weeks. Biplates were sealed in polyethylene bags and incubated at 37°C in the presence of 5 to 7% CO_2 and observed weekly for the presence of growth over an 8-week incubation period. Cultures that signaled positive in the MGIT system had a 50-μl aliquot removed and inoculated onto the surface of a sheep blood agar plate to determine if bacteria contaminated the culture. In addition, 0.15 ml of the medium in the MGIT tube was gently injected into a cytospin funnel containing 0.15 ml of sodium hypochlorite. This mixture was allowed to stand for 5 min to inactivate any viable mycobacteria. The funnel assembly was placed into a carrier, and slides were centrifuged in a Shandon Cytospin 3 (ThermoShandon, Pittsburgh, Pa.) at 451.6 × *g* for 15 min. All slides were stained with a carbol-fuchsin acid-fast stain and observed for the presence of mycobacteria.

A 1.5-ml aliquot was removed from each positive MGIT tube and centrifuged for 15 min at 20,800 × *g* in an Eppendorf 5417C centrifuge (Hamburg, Germany), and the supernatant was discarded. The pellet was used for extraction of nucleic acids, and Accuprobe testing for *M. tuberculosis* and *Mycobacterium avium-intracellulare* was performed. Procedures for testing and interpretation of results were per manufacturer's instructions. The identification of organisms other than *M. avium-intracellulare* and *M. tuberculosis* was made by biochemical profiles, other Accuprobes, or by nucleic acid sequencing.

Identification of cultures recovered from solid media was made by biochemical profiles and by selecting the most appropriate Accuprobes based on phenotypic characters or by nucleic acid sequencing if probe results were negative, as described previously (L. Stockman and G. D. Roberts, Abstr. 96th Gen. Meet. Am. Soc. Microbiol. 1996, abstr. C-14, 1996).

The AMTD test was performed according to the manufacturer's instructions. A portion (450 μl) of the sediment from the sodium hydroxide-treated specimen was placed in a tube containing lysing solution, vortexed, and sonicated for 15 min. A 0.25-μl volume of lysate was transferred into an amplification tube containing amplification reagent. Tubes were incubated for 15 min at 95°C in a water bath. This entire process was conducted in a Biosafety Level III laboratory.

Tubes were transferred to a 42°C water bath and left to cool for 5 min. Enzyme reagent (25 μl) was added to each tube and mixed, and the mixture was incubated for 30 min at 42°C. This portion of the assay was completed in a general Biosafety Level II laboratory.

Hybridization reagent (100 μl) was added to each tube, vortexed, and incubated for 15 min at 60°C in a water bath. Tubes were removed, 300 μl of selection reagent was added, and the mixture was vortexed and incubated for 15 min at 60°C in a water bath. Tubes were cooled and placed in a luminometer to determine the number of relative light units (RLU) produced by the reaction.

Interpretation of the AMTD results was as follows. Quality control was acceptable if the negative and positive controls were <20,000 and >1,000,000 RLU, respectively. Those specimens with values of ≤30,000 RLU were considered negative and those with ≥500,000 RLU were considered positive. All specimens were tested in duplicate; the duplicate specimen was spiked with a known quantity of *M. tuberculosis* to detect inhibition. Specimens having values of 30,000 to 4,999,999 RLU were considered indeterminate. If the repeat value was >30,000 RLU, the result was considered positive. Those specimens that were negative and had a duplicate spiked specimen reported as negative were reported as inhibitory specimens.

RESULTS

Mycobacteria were recovered from 31.1% (173 of 555) of specimens and from 33.2% (130 of 391) of respiratory specimens; mycobacteria recovered from the latter included *M. avium-intracellulare* (*n* = 46), *M. gordonae* (*n* = 11), *M. chelonae* (*n* = 9), *M. fortuitum* (*n* = 3), *M. kansasii* (*n* = 3), and *M. xenopi* (*n* = 1). For nonrespiratory specimens, 26.2% (43 of 164) had positive cultures, which included *M. avium-intracellulare* (*n* = 8), *M. chelonae* (*n* = 5), *M. gordonae* (*n* = 5), and *M. kansasii* (*n* = 1). *M. tuberculosis* was recovered from 46 (11.8%) and 22 (13.4%) respiratory and nonrespiratory specimens, respectively.

The overall sensitivity of the AMTD assay was 97.8% among all respiratory specimens and 77.3% for all nonrespiratory specimens. The assay had sensitivities of 100 and 75% for acid-fast smear-positive and -negative respiratory specimens, respectively (Table 1). The sensitivities were 90 and 63.6% for smear-positive and smear-negative nonrespiratory specimens, respectively (Table 2).

The specificity of the assay was 99.1% among all respiratory specimens; there were three false-positive tests. The specificities were 98.4 and 99.2% for smear-positive and -negative respiratory specimens, respectively. There were two false-positive results when AMTD was compared to culture in the nonrespiratory group. The overall specificity for respiratory and nonrespiratory specimens was 98.5%. The specificities for smear-positive and -negative nonrespiratory specimens were 100 and 99.1%, respectively

Our overall experience for all specimens submitted to the laboratory, regardless of smear status, showed a sensitivity of 91.2% and a specificity of 98.9%.

There were 11 discrepant results between cultures and AMTD (five false-positive and six false-negative results) (Table 3). Clinical histories of patients were reviewed, and seven had either clinical or cultural evidence of tuberculosis. Of the five discrepant false-positive test results, two were for patients who had received antituberculous medications and two were for patients whose cultures showed growth of *Mycobacterium xenopi* for one and *Mycobacterium chelonae* for the other. The

TABLE 2. AMTD and culture correlation for nonrespiratory specimens

Smear result (no. of specimens)	No. of specimens with indicated results (AMTD/culture)				Sensitivity/specificity (%)	Predictive values (%)	
	Positive/positive	Positive/negative	Negative/positive	Negative/negative		Positive	Negative
Positive (21)	9	0	1	11	90/100		
Negative (117)	7	1	4	105	63.6/99.1		
Unknown (21)	1	1	0	19	100/100		
Total[a] (159)	17	2	5	135	77.3/98.5	91.7	96.4

[a] Includes specimens with no smear result available and does not include inhibitory specimens.

latter two discrepancies could not be resolved. Perhaps the organisms could have caused the false-positive results. The remaining false-positive result was related to possible contamination from a positive specimen in an adjacent test position; however, no residual specimen was available for retesting, and the discrepancy was unresolved.

Six false-negative test results were seen during the study; five of the six specimens tested grew *M. tuberculosis*. The one remaining specimen represented a mislabeling of a patient's specimen; three AMTD tests were performed, and the patient had no clinical evidence of tuberculosis.

Tables 3 and 4 present information related to the number of specimens tested and the total number of AMTD assays performed on additional specimens. It is of interest that 63.6% of the discrepant specimens had no additional specimens submitted for testing; only two were acid-fast smear positive.

A comparison of the times required for test performance and reporting of the AMTD and culture methods was made. The average time for AMTD was 1.52 days, while cultures required an average of 10.5 days for detection and identification. Its use in our laboratory provided a diagnosis of tuberculosis an average of 8.99 days earlier than the culture.

DISCUSSION

We have assessed the sensitivity and specificity of the AMTD test relative to clinical tuberculosis and a positive culture for *M. tuberculosis* in the setting of a routine tertiary care center and reference clinical laboratory. Much of the data relating to the performance of the AMTD assay have been generated with respect to distinct specimen types, e.g., acid-fast smear-positive or -negative specimens. The original AMTD assay was assessed in smear-positive respiratory specimens. More recent AMTD assay performance assessments have used similar approaches (10, 13). While such data are valuable, they are not always readily applicable to a routine clinical setting. Given that the AMTD assay is offered for potentially all clinical specimens, irrespective of either smear positivity or type of specimen, we were particularly interested in assessing the sensitivity and specificity of the assay in the broad setting that included all assay results. We therefore included those specimens for which we did not have acid-fast smear data available to represent our overall experience. These were specimens that were sent via Mayo Medical Laboratories and that had only enough volume to perform AMTD and culture. In this way we hope to provide infectious diseases and pulmonary physicians with data immediately relevant to their everyday practice.

The assay had a sensitivity of 97.8% and a specificity of 99.1% when respiratory specimens were tested (however, when clinical history was reviewed, it was apparent that a mix-up in patient specimens [bronchoalveolar lavage fluid] had occurred, and this increased the sensitivity to 100%). A number of other laboratories have reported sensitivities ranging from 85.7 to 100% for respiratory specimens (1, 3, 5, 10, 11). As would be expected, there was a higher sensitivity for smear-positive than for smear-negative respiratory specimens, 100 and 75%, respectively, a finding similar to those of most previous studies (5, 10, 11). However, there were only three smear-negative specimens in this study and the lower sensitivity might be misleading.

TABLE 3. Distribution, smear result, and *M. tuberculosis* culture of nonrespiratory specimens tested

AMTD test result	Acid-fast smear result[a]	Specimen source	No. of specimens	No. of AMTD tests performed[b]	Resolution of discrepant result
False positive	−	Urine	1	1	Clinical diagnosis of tuberculosis; patient responded to therapy
False positive	−	Gastric washing	1	1	Clinical diagnosis of tuberculosis; patient received therapy
False positive	+	Induced sputum	1	1	Culture grew *M. xenopi*
False positive	−	Induced sputum	1	2	Culture grew *M. chelonae*
False positive	−	Bronchial washing	1	1	Could not resolve; possible contamination in test run
False negative	−	Cerebrospinal fluid	1	2	Culture grew *M. tuberculosis*
False negative	+	Lymph node	1	1	Culture grew *M. tuberculosis*
False negative	−	Lymph node	1	1	Culture grew *M. tuberculosis*
False negative	−	Lymph node	1	2	Possible sampling error of tissue; second AMTD on same day was positive
False negative	−	Spine	1	1	Culture grew *M. tuberculosis*
False negative	−	Bronchoalveolar lavage fluid	1	≥3	Determined to be a mix-up in patient specimens; result was incorrect

[a] −, negative; +, positive.
[b] Includes initial AMTD and any additional tests performed on other specimens from the same patient.

TABLE 4. Distribution, smear result and MTB culture of nonrespiratory specimens tested

Source of specimen	Smear result		
	Negative	Positive	Unknown
Gastric washing	22	2	1
Lung tissue	16	5	
Pleural fluid	5		7
Pericardial tissue	3	2	
Lymph node	8	3	
Synovial fluid	1		
Synovial tissue		1	
Urine	7	2	
Neck abscess	1		
Neck mass	3	1	
Liver biopsy	3		
Vertebrae	12	1	
Sacral mass	1		
Cerebrospinal fluid	22	1	9
Ventricular fluid	2		
Brain tissue	4		
Skin	3	1	
Abdominal fluid	1		
Bone marrow	2		
Chest wall	1		
Colon	1		
Stool	1	1	
Duodenal tissue	1		
Thoracentesis	1		1
Ileum	1		
Penis	1		
Nasal sinus	1	2	

The assay's sensitivity declined to 77.3% overall when used for nonrespiratory specimens. Reduced sensitivity was seen among acid-fast smear-negative specimens (63.9%) when compared to smear-positive specimens (90%). This result is very similar to that of Piersimoni et al. (10), but this value was lower than those reported by Chedore and Jamieson (3) and others (5, 6, 11, 13), the former group reporting a sensitivity of 100% among smear-negative nonrespiratory specimens. The overall sensitivity for all specimens in our study was 90.7%. Importantly, the assay was 100% specific for nonrespiratory specimens when compared to a clinical diagnosis of tuberculosis. The specificity for respiratory specimens was 99.1%, and the three false-positive AMTD assays could not be resolved.

Interestingly, the type of specimens tested may explain the reduced sensitivity observed with the nonrespiratory specimens. Four of the five discrepant specimens were tissues, including three lymph node specimens and one vertebral tissue. It is well known that sampling error occurs with tissues and that several sites should be tested. In our study, only one AMTD was performed on three of the specimens; the one remaining specimen had another portion of the tissue sample tested on the same day, which yielded a positive result. The single cerebrospinal fluid had two AMTD tests performed; both were negative. It is also well known that few organisms are present in the cerebrospinal fluid of patients with tuberculosis meningitis. These problems are difficult to surmount; however, perhaps if additional AMTD testing were done, the sensitivity might be greater. Only 4 of 11 specimens having discrepant results had more than one AMTD test performed on additional specimens; one was previously described (bronchoalveolar lavage fluid) and was truly negative since the clinical history showed that the positive culture result was incorrect; the laboratory determined it to be a mix-up in patient specimens. The second discrepant result had two AMTD tests performed, and the second specimen was positive. The cerebrospinal fluid previously described accounted for the third specimen, and the fourth discrepant result could not be resolved. The overall sensitivity for all specimens could be increased from 91.2 to 93.9% if the bronchoalveolar lavage fluid and tissue specimen were not considered to have discrepant results.

In the general setting of a large mycobacteriology laboratory where all specimen sources are tested, the AMTD assay is extremely sensitive and specific for detecting *M. tuberculosis*. The recommendations by the Centers for Disease Control and Prevention (2) suggest that three specimens collected on different days be submitted for testing. If the specimen is smear positive and yields a positive AMTD result, no further testing needs to be done. If the specimen is acid-fast smear positive and AMTD negative, additional specimens should be tested. If the sputum is smear negative and AMTD positive, additional specimens should be tested. If a subsequent specimen is positive, the patient may be presumed to have tuberculosis. If the patient has a specimen that is acid-fast smear negative, and the AMTD assay is negative, and another specimen is also negative, clinical judgment must be made to exclude the possibility of active tuberculosis. If inhibitors are detected in the specimen, the test is of no diagnostic value; additional specimens must be submitted. More data regarding nonrespiratory specimens must be analyzed to determine if these criteria may be applied to this group of specimens.

Given the public health concerns and very high morbidity and mortality of tuberculosis, the key aspect of AMTD performance is the negative predictive value; i.e., it is critically important that any patients with tuberculosis not be overlooked. Such values are dependent, however, on the prevalence of each type of infection in a given population. In this study, the unadjusted positive and negative predictive values for respiratory and nonrespiratory specimens are 93.9 and 99.7% and 91.7 and 96.4%, respectively. Considering the high specificity of the assay, irrespective of specimen type, it is reasonable to deduce that the assay is highly useful for detecting *M. tuberculosis* in all specimen sources regardless of the acid-fast smear result, if representative samples are submitted to the laboratory. It offers the greatest potential benefit to patients with acid-fast smear-negative results (13). In addition to being a sensitive and specific assay, it provides results approximately 9 days earlier than culture. The sensitivity and specificity of this assay can be greatly enhanced if specimens are submitted only from patients for whom there is a high index of suspicion for tuberculosis.

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