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Original Research

RESPIRATORY INFECTIONS

# Use of the Amplified *Mycobacterium tuberculosis* Direct Test in a Public Health Laboratory*

## Test Performance and Impact on Clinical Care

*Renata L. Guerra, MD; Nancy M. Hooper, BA; James F. Baker, BS; Roya Alborz, MS; Derek T. Armstrong, MHS; Gina Maltas, BSN; Julia A. Kiehlbauch, PhD; and Susan E. Dorman, MD*

***Background:*** **The Amplified *Mycobacterium tuberculosis* Direct Test (MTD; Gen-Probe; San Diego, CA) is a nucleic-acid amplification test for rapid pulmonary tuberculosis (PTB) diagnosis. In a routine public health setting, test accuracy and impact on clinical decisions are unknown.**
***Methods:*** **Retrospectively, we evaluated MTD accuracy and impact on clinical decisions in a public health setting. To estimate MTD accuracy, mycobacterial culture was used as the "gold standard." To evaluate MTD impact on clinical decisions, concordance of clinician presumptive diagnosis (at time of MTD and smear availability) and definitive diagnosis, and duration of nonindicated tuberculosis therapy were determined for smear-positive PTB suspects in a period of MTD availability (MTD group) and a prior period of MTD nonavailability (non-MTD group).**
***Results:*** **A total of 1,151 respiratory specimens from 638 PTB suspects were analyzed. MTD sensitivity, specificity, positive predictive value, and negative predictive value were 91.7%, 98.7%, 96.7%, and 96.5% overall, respectively; and 98.7%, 97.8%, 98.7%, and 97.8% for smear-positive patients; and 62.2%, 98.9%, 85.2%, and 96.1% for smear-negative patients. In the MTD group, concordance between definitive and clinician presumptive diagnoses was 78% (95% confidence interval [CI], 64 to 88%), similar to that for the non-MTD group (79%; 95% CI, 68.4 to 89.6%). However, concordance between definitive diagnosis and the MTD test was 98% (95% CI, 94.1 to 100%). Median duration of nonindicated tuberculosis treatment was 6 days for the MTD group vs 31 days for the non-MTD group (p = 0.002).**
***Conclusion:*** **In this public health setting, MTD was accurate and rapidly detected more than half of the smear-negative PTB cases. For smear-positive PTB suspects, MTD had excellent concordance with definitive diagnosis, but clinicians often inappropriately initiated TB therapy despite a negative MTD result.** ***(CHEST 2007; 132:946–951)***

**Key words:** *Mycobacterium tuberculosis* direct test; diagnosis; respiratory specimens; tuberculosis; amplified

**Abbreviations:** AFB = acid-fast bacilli; CI = confidence interval; DHMH = Department of Health and Mental Hygiene; MTD = Amplified *Mycobacterium tuberculosis* Direct Test; NPV = negative predictive value; NTM = nontuberculous mycobacteria; PPV = positive predictive value; PTB = pulmonary tuberculosis; RLU = relative light unit

New tools are needed to increase the efficiency of *Mycobacterium tuberculosis* detection. The conventional rapid test (smear microscopy for acid-fast bacilli [AFB]) has poor sensitivity and specificity, and its positive predictive value (PPV) for pulmonary tuberculosis (PTB) is lower in settings in which nontuberculous mycobacteria (NTM) are commonly isolated from respiratory secretions.[1] The conventional "gold standard" test, mycobacterial culture, is slow because it can take up to 6 to 8 weeks for *M tuberculosis* to grow.

The Amplified *Mycobacterium tuberculosis* Direct Test (MTD; Gen-Probe; San Diego, CA) can be used for detection of *M tuberculosis* nucleic acid directly from respiratory specimens, in approximately the same turnaround time as an AFB smear.[2] In clinical

trials, the MTD has had high specificity; and for smear-positive respiratory specimens, high sensitivity.[3,4] However, the majority of published MTD studies[5–7] were performed from the laboratory perspective. There is a need for better understanding of MTD performance under routine testing conditions.[3,8] In addition, to our knowledge, no studies have evaluated the impact of the MTD on clinical decisions during initial management of PTB suspects in routine practice.

In December 2003, the tuberculosis laboratory of the Maryland Department of Health and Mental Hygiene (DHMH) initiated routine MTD testing of respiratory specimens submitted for AFB smear and culture. The objectives of this retrospective study were to evaluate, under routine clinical conditions, the accuracy of the MTD test and its impact on clinical decisions.

## Materials and Methods

### *Study Design and Subjects*

There were two components to this retrospective study: a laboratory study to determine MTD accuracy, and a clinical study to evaluate impact of MTD use on clinical decisions. This study was approved by institutional review boards of Johns Hopkins University, the Baltimore City Health Department, and the Maryland DHMH.

The TB Laboratory of the Maryland DHMH serves as the primary mycobacteriology laboratory for the Baltimore City Health Department as well as for all public tuberculosis control programs in Maryland. This laboratory routinely performs the MTD on respiratory specimens submitted for AFB smear and mycobacterial culture.

For the laboratory study, records were reviewed from PTB suspects in whom MTD, AFB smear, and mycobacterial culture tests were routinely performed on respiratory specimens at the TB Laboratory of the Maryland DHMH between December 2003 and March 2006. A minimum of one and a maximum of three specimens per patient were included in the study. Individual specimens were not included in the data analysis if either the culture was contaminated or the MTD result was equivocal.

*From the School of Medicine (Ms. Maltas, and Drs. Guerra and Dorman), Johns Hopkins University; and the Maryland State Department of Health and Mental Hygiene (Ms. Hooper, Mr. Baker, Ms. Alborz, Mr. Armstrong, and Dr. Kiehlbauch), Baltimore, MD.
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*Correspondence to: Susan E. Dorman, MD, School of Medicine, Johns Hopkins University, Room 1M-06, 1550 Orleans St, Baltimore, MD 21231; e-mail: dsusan1@jhmi.edu*
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To evaluate the impact of MTD use on clinical decisions, clinical records were reviewed for all smear-positive PTB suspects undergoing initial diagnostic evaluation at the Baltimore City Health Department between December 2000 and March 2006. Patients evaluated between December 2003 and March 2006 were classified as the "MTD group" because MTD testing was performed routinely during this period. Patients evaluated between December 2000 and March 2003 were classified as the "non-MTD group" because the MTD was not available during that period. Patients were excluded if they had received antituberculosis therapy for $\geq 7$ days prior to specimen collection. The definitive diagnosis was considered to be tuberculosis if culture findings were positive for *M tuberculosis*, and/or a full course of tuberculosis treatment was prescribed and the patient was reported as a tuberculosis case to the Maryland DHMH. For each group, the following were determined and compared: (1) concordance between definitive diagnosis and clinician presumptive diagnosis (at the time of availability of MTD results and positive smear results); and (2) median duration of tuberculosis treatment for patients who did not have a definitive diagnosis of tuberculosis (duration of "nonindicated" tuberculosis treatment).

### *AFB Smear*

Respiratory specimens were digested, decontaminated, and concentrated by the NALC-NaOH method.[9] A smear of the processed sediment was stained by the Truant fluorescence acid-fast staining method (auramine O-rhodamine B). Microscopy and results reporting were according to published standards.[9]

### *Culture*

A 0.5-mL portion of the decontaminated specimen was inoculated into liquid culture medium (Bactec 12B; Becton, Dickinson and Company; Franklin Lakes, NJ), and 0.2 mL was inoculated onto one Lowenstein Jensen slant. Liquid cultures were incubated at 37°C for 6 weeks. Lowenstein Jensen slants were incubated at 35°C and examined weekly for 8 weeks. Mycobacterial isolates were identified using DNA probes (Accuprobe; Gen-Probe) or by the Bactec NAP test (Becton, Dickinson and Company) or by conventional biochemical tests.[10]

### *MTD*

The MTD assay was performed according to the protocol of the manufacturer.[11] Sample results were interpreted as follows: negative, $< 30{,}000$ relative light units (RLU); positive, $> 500{,}000$ RLU; and equivocal, 30,000 to 499,999 RLU. For samples read as equivocal, either a second specimen was tested or the first specimen was retested. If the second result was $> 30{,}000$ RLU, the test was interpreted as positive; if the second result was $< 30{,}000$ RLU, the test was interpreted as negative.[11] MTD results were reported if the negative control was $< 20{,}000$ RLU and the positive control was $> 1{,}000{,}000$ RLU. To minimize potential impact of endogenous amplification inhibitors, for each clinical respiratory specimen two tests were run in parallel: one using an undiluted aliquot of the concentrated specimen, and the other using a 1:10 dilution of the concentrated specimen.[5] If either test result was positive, the respiratory specimen was considered MTD positive.

### *Statistical Analysis*

Sensitivity, specificity, PPV, and negative predictive values (NPV) of the MTD were estimated using mycobacterial culture

as the "gold standard." Results were analyzed on a "first-specimen", "per-specimen", or "per-patient" basis. For the per-patient analysis, a patient was considered AFB smear positive if any smear finding was positive, and AFB smear negative if all smear findings were negative. $\chi^2$ test and Fisher exact test were used to analyze differences between proportions. McNemar test was used to analyze differences in paired-group proportions. Medians were compared using Mann-Whitney test; a p value of 0.05 was considered statistically significant.

## Results

### *Laboratory Performance of the MTD*

A total of 1,158 respiratory specimens from 641 PTB suspects were tested. Four specimens were excluded from the analysis: one because the culture was contaminated, and three because the MTD result was equivocal. Three patients (three specimens) were excluded due to receipt of $\geq 7$ days of tuberculosis treatment prior to specimen collection.

Characteristics of the remaining 1,151 specimens and 638 PTB suspects are shown in Table 1. Among 420 patients with two or three tested specimens, 20 patients (4.8%) had discordant MTD results between specimens; however, all 20 patients had at least one specimen that was MTD positive and culture positive for *M tuberculosis*.

**Table 1—*Specimen and Patient Characteristics (Laboratory Study Component)***

| Characteristics | No. | % |
|---|---|---|
| Specimens per patient | | |
| One | 218 | 34.2 |
| Two | 327 | 51.2 |
| Three | 93 | 14.6 |
| Total | 638 | 100 |
| Specimen type | | |
| Spontaneous sputum | 992 | 86.2 |
| Induced sputum | 82 | 7.1 |
| Invasive methods | 77 | 6.7 |
| Total | 1,151 | 100 |
| AFB smear results by specimen | | |
| Positive | 354 | 30.8 |
| Negative | 797 | 69.2 |
| Total | 1,151 | 100 |
| AFB smear results by patient | | |
| Positive | 272 | 42.6 |
| Negative | 366 | 57.4 |
| Total | 638 | 100 |
| Culture results by specimen | | |
| *M tuberculosis* | 279 | 24.3 |
| NTM | 173 | 15.0 |
| Negative | 699 | 60.7 |
| Total | 1,151 | 100 |
| Culture results by patient | | |
| *M tuberculosis* | 207 | 32.4 |
| NTM | 118 | 18.5 |
| Negative | 313 | 49.1 |
| Total | 638 | 100 |

Since the majority of tuberculosis programs perform MTD testing only on the first submitted respiratory specimen, we show in Table 2 the performance characteristics of the MTD test on the first submitted respiratory specimen for each PTB suspect. MTD performance characteristics were similar when analyzed on the basis of first submitted specimen, per specimen, or per patient. On a per-specimen basis, sensitivity and specificity were 90.0 (95% confidence interval [CI], 85.8 to 93.2) and 98.7 (95% CI, 97.8 to 99.4) overall, respectively; 98.1 (95% CI, 95.3 to 99.5) and 97.9 (95% CI, 93.9 to 99.6) for smear-positive specimens; and 63.1 (95% CI, 50.2 to 74.7) and 98.9 (95% CI, 97.9 to 99.5) for smear-negative specimens. On a per-patient basis, sensitivity and specificity were 92.3 (95% CI, 87.8 to 95.5) and 99.8 (95% CI, 98.7 to 100) overall, respectively; 98.8 (95% CI, 95.8 to 99.9) and 99.0 (95% CI, 94.7 to 100) for smear-positive patients; and 63.2 (95% CI, 46.0 to 78.2) and 100 (95% CI, 98.9 to 100) for smear-negative patients.

Interestingly, eight smear-negative/MTD-positive specimens classified as "not tuberculosis" based on culture results (five negative culture findings and three with NTM growth) on the per-specimen analysis were from patients with additional specimens, untested by MTD, which showed *M tuberculosis* growth on culture. To further characterize the performance of MTD for discrimination of "tuberculosis" vs "not tuberculosis" for smear-positive specimens and patients, we analyzed the subgroup of smear-positive specimens that were culture positive for NTM (*ie*, specimens having no growth in culture were excluded from this analysis). MTD specificity was 98.8% (95% CI, 95.3 to 99.8) when the first specimen from each patient was considered.

### *Impact of MTD Test on Clinical Decisions*

During the study period, 107 smear-positive PTB suspects were evaluated at the Baltimore City Health Department. Fifty PTB suspects were included in the MTD group, and 57 were included in the non-MTD group. Patient characteristics are shown in Table 3. In the non-MTD group, 47 of 57 patients (82.5%) had a definitive diagnosis of tuberculosis, including 46 patients who were culture positive for *M tuberculosis,* and 1 patient with clinical tuberculosis diagnosis in the setting of negative culture findings. In the MTD group, 30 of 50 patients (60%) had a definitive diagnosis of tuberculosis, all of whom had at least one positive culture finding for *M tuberculosis*.

Table 4 shows the clinician's presumptive diagnosis (tuberculosis or not tuberculosis) at the time of availability of AFB smear result (and MTD result for the MTD group), and the definitive diagnosis. Also shown are concordance between presumptive and

**Table 2—*Performance Characteristics of the MTD Performed on the First Respiratory Specimen for Each Tuberculosis Suspect (Laboratory Study Component)***

| MTD Results | Culture Results, No. | | Total | Sensitivity, % (95% CI) | Specificity, % (95% CI) | PPV, % (95% CI) | NPV, % (95% CI) |
|---|---|---|---|---|---|---|---|
| | TB | Not TB* | | | | | |
| Overall | | | | 91.7 (89.0–93.3) | 98.7 (97.5–99.3) | 96.7 (93.9–98.4) | 96.5 (95.4–97.1) |
| Positive | 177 | 6 | 183 | | | | |
| Negative | 16 | 439 | 455 | | | | |
| Total | 193 | 445 | 638 | | | | |
| AFB smear positive | | | | 98.7 (96.8–99.5) | 97.8 (94.5–99.2) | 98.7 (96.8–99.5) | 97.8 (94.5–99.2) |
| Positive | 154 | 2 | 156 | | | | |
| Negative | 2 | 90 | 92 | | | | |
| Total | 156 | 92 | 248 | | | | |
| AFB smear negative | | | | 62.2 (51.2–68.4) | 98.9 (97.7–99.5) | 85.2 (70.2–93.7) | 96.1 (95.0–96.8) |
| Positive | 23 | 4 | 27 | | | | |
| Negative | 14 | 349 | 363 | | | | |
| Total | 37 | 353 | 390 | | | | |

*Not tuberculosis (Not TB) is either no growth or growth of nontuberculous mycobacteria on culture.

definitive diagnoses, and concordance between the MTD test and the definitive diagnosis. In the non-MTD group, concordance between clinician presumptive and definitive diagnoses was 79%. That is, for 79% of patients the correct treatment decision was made at the time of availability of smear results. In the MTD group, despite excellent concordance between MTD results and definitive diagnosis (98%), concordance between clinician presumptive and definitive diagnoses was only 78%. For almost half of smear-positive but MTD-negative suspects in the MTD group (10 of 19 patients), the clinician elected to treat for tuberculosis until culture results were available; none of these 19 patients had a definitive diagnosis of tuberculosis.

The MTD finding was false positive in one smear-positive AIDS patient whose cultures grew *Mycobacterium celatum.*[12] In the non-MTD group, two smear-positive patients had an incorrect presumptive diagnosis of not tuberculosis that which was later corrected when cultures grew *M tuberculosis*.

To further characterize the observed and the potential impact of MTD on clinical care, we determined the duration of tuberculosis treatment prescribed to each patient whose definitive diagnosis was not tuberculosis. The median duration of nonindicated TB treatment was 6 days for the MTD group, vs 31 days for the non-MTD group ($p = 0.002$).

## Discussion

Under routine testing conditions, the MTD had high accuracy and rapidly detected more than half of smear-negative PTB cases. For smear-positive patients, the potential impact of MTD testing on

**Table 3—*Patient Characteristics (Clinical Study Component)**

| Characteristics | MTD Group (n = 50) | Non-MTD Group (n = 57) | p Value |
|---|---|---|---|
| Median age, yr | 46.5 | 47.0 | 0.40 |
| Male gender | 30 (60.0) | 46 (80.7) | 0.02 |
| Race/ethnicity | | | |
| White | 9 (18.0) | 8 (14.0) | 0.58 |
| African-American | 28 (56.0) | 38 (66.7) | 0.26 |
| Hispanic | 4 (8.0) | 5 (8.8) | 1.00 |
| African | 3 (6.0) | 2 (3.5) | 0.66 |
| Asian | 4 (8.0) | 1 (1.8) | 0.18 |
| Unknown | 2 (4.0) | 3 (5.3) | 0.76 |
| HIV status | | | |
| Negative | 22 (44.0) | 28 (49.1) | 0.60 |
| Positive | 18 (36.0) | 10 (33.3) | 0.77 |
| Unknown | 10 (20.0) | 10 (17.5) | 0.75 |
| Culture positive for tuberculosis | 30 (60.0) | 46 (81.0) | 0.02 |
| Definitive diagnosis of tuberculosis | 30 (60.0) | 47 (82.5) | 0.01 |

*Data are presented as No. (%) unless otherwise indicated.

**Table 4—*Concordance Between Presumptive and Definitive Diagnoses of PTB for Suspects With Smear-Positive Results for AFB in Respiratory Specimens***

| Variables | Definitive Diagnosis, No. | | Total | Concordance, % | 95% CI |
|---|---|---|---|---|---|
| | TB | Not TB* | | | |
| MTD group | | | | | |
| Overall | | | | | |
| Presumptive diagnosis by clinician | | | | 78.0 | 66.5–89.5 |
| Tuberculosis | 30 | 11 | 41 | | |
| Not tuberculosis | 0 | 9 | 9 | | |
| Total | 30 | 20 | 50 | | |
| MTD positive | | | | | |
| Presumptive diagnosis by clinician | | | | 96.8 | 90.6–100 |
| Tuberculosis | 30 | 1 | 31 | | |
| Not tuberculosis | 0 | 0 | 0 | | |
| Total | 30 | 1 | 31 | | |
| MTD negative | | | | | |
| Presumptive diagnosis by clinician | | | | 47.4 | 25.0–69.9 |
| Tuberculosis | 0 | 10 | 10 | | |
| Not tuberculosis | 0 | 9 | 9 | | |
| Total | 0 | 19 | 19 | | |
| Overall | | | | | |
| Presumptive diagnosis by MTD result | | | | 98.0 | 94.1–100 |
| Tuberculosis | 30 | 1 | 31 | | |
| Not tuberculosis | 0 | 19 | 19 | | |
| Total | 30 | 20 | 50 | | |
| Non-MTD group | | | | | |
| Overall | | | | | |
| Presumptive diagnosis by clinician | | | | 79.0 | 68.4–89.6 |
| Tuberculosis | 45 | 10 | 55 | | |
| Not tuberculosis | 2 | 0 | 2 | | |
| Total | 47 | 10 | 57 | | |

*Among 20 MTD group patients with a definitive diagnosis of not tuberculosis, culture findings were positive for *Mycobacterium kansasii* in 8 patients, *Mycobacterium avium* in 7 patients, *Mycobacterium gordonae* in 1 patient, *Mycobacterium abscessus* in 1 patient, and *M celatum* in 1 patient; culture findings were negative in for 2 patients. In the non-MTD group, among 10 patients with a definitive diagnosis of not tuberculosis, culture findings were positive for *M avium* in 6 patients, and *M kansasii* in 4 patients. See Table 2 for definition of abbreviation.

clinical decision making was high, given 98% concordance between MTD results and definitive diagnoses. However, there was no observed impact: clinicians made correct initial treatment decisions for approximately 80% of patients whether or not MTD was available. Failure of clinicians to correctly use negative MTD results for clinical decision making led to inaccurate initial diagnosis and treatment decisions in approximately half of patients with a final diagnosis of not tuberculosis.

Our study provides much-needed information about MTD performance under routine conditions in a public health laboratory. To date, most published information about MTD performance was obtained in the context of prospective laboratory studies designed to determine assay performance, and performed mostly in academic medical centers. Information obtained under such circumstances may not be reproducible under nonstudy testing conditions due to differences in staff workload, specimen selection, and other factors. Interestingly, however, MTD performance in our study was remarkably similar to that reported in other studies,[3,6,7,13] supporting the robustness of this test and feasibility in routine laboratory settings.

The MTD was able to rapidly detect *M tuberculosis* in approximately 60% of patients for whom all smear findings were negative. Prompt confirmation of tuberculosis can benefit the individual patient because treatment can be initiated. While it can be argued that many smear-negative PTB suspects are correctly initiated on antituberculosis treatment based on factors such as chest radiograph appearance and/or clinical suspicion, early microbiologic confirmation of a tuberculosis diagnosis can be helpful in cases in which drug toxicity or drug-drug interactions arise. Prompt confirmation of PTB can also have public health benefits because steps to minimize transmission can be taken.

A potential benefit of the MTD (with a demonstrated high NPV) would be to avert tuberculosis treatment in smear-positive individuals who do not

have tuberculosis. Unexpectedly, tuberculosis treatment was initiated for approximately half of smear-positive PTB suspects in whom a negative MTD result was available to the clinician; none of these patients had a final diagnosis of tuberculosis. Since MTD results were reported on the same forms as smear results, it is unlikely that problems with result receipt by clinicians played a role. For MTD, the NPV was 98% when all 272 smear-positive TB suspects were considered, and 100% when the 50 MTD group patients were considered. In this situation in which the NPV of the MTD is high but not perfect, the potential risks and costs to the patient (of initiation of treatment that is highly likely to be not indicated) must be weighed against the potential consequences of failure to promptly treat or isolate the very small proportion of smear-positive, MTD-negative tuberculosis patients.

Along these lines, in our study approximately 35% of mycobacterial isolates from smear-positive patients were NTM during the period 2003 to 2006. Although in the United States NTM disease is not reportable, its frequency appears to be increasing over time, with coincident decreases in tuberculosis rates.[14,15] Therefore, tools for rapid discrimination between tuberculosis and NTM pulmonary disease, especially in smear-positive patients, are increasingly needed in order to facilitate accurate decisions about individual treatment and public health activities.

Our retrospective study has important limitations. The technologists performing the MTD tests were not blinded as to the clinical status of patients, but rather were in communication with tuberculosis clinicians as per routine. While we do not believe that this influenced MTD results, it may have influenced numbers of specimens submitted/tested for individual patients. The MTD group and the non-MTD group were comprised of tuberculosis suspects from different time periods; although all attended the same tuberculosis clinic, there may have been unrecognized differences in clinical suspicion for tuberculosis or clinical decision making (not related to MTD).

In conclusion, in this routine public health setting, MTD performance characteristics were similar to those previously reported under "study" conditions. Incorporation of MTD testing into routine public health laboratory algorithms could aid in the initial management of PTB suspects.

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