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APPROVAL ORDER

00M-0578

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Ms. Rhonda A. Moe
Sr. Regulatory Affairs Specialist
Gen-Probe Incorporated
10210 Genetic Center Drive
San Diego, CA 92121-4362

Food and Drug Administration
9200 Corporate Boulevard
Rockville MD 20850

SEP 30 1999

Re: P940034/S008
Device: Gen-Probe® Amplified™ Mycobacterium Tuberculosis
Direct (MTD) Test
Filed: November 12, 1998
Amended: April 5, April 12, June 15, August 24, September 9 and September 24, 1999

Dear Ms. Moe:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) supplement for the Gen-Probe® Amplified Mycobacterium Tuberculosis Direct (MTD) Test. This device, a target-amplified nucleic acid probe test, is indicated for: the *in vitro* diagnostic detection of *Mycobacterium tuberculosis* complex rRNA in acid-fast bacilli (AFB) smear positive and negative concentrated sediments prepared from sputum (induced or expectorated), bronchial specimens (e.g., bronchoalveolar lavages or bronchial aspirates) or tracheal aspirates. The MTD test is intended for use only with specimens from patients showing signs and symptoms consistent with active pulmonary tuberculosis. MTD is to be used as an adjunctive test for evaluating either AFB smear positive or negative sediments prepared using NALC-NaOH digestion-decontamination of respiratory specimens. Patients who are suspected of having pulmonary TB based on clinical evaluation and who have received no antituberculous therapy, less than 7 days of such therapy, or have not received such therapy in the last 12 months may be evaluated with this test. The MTD test should be performed only in laboratories proficient in the culture and identification of *M. tuberculosis* (Level II and III or extent 3 and 4). The MTD test must be performed in conjunction with mycobacterial culture.

The PMA supplement is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device as modified upon receipt of this letter.

The sale, distribution, and use of this device are restricted to prescription use in accordance with 21 CFR 801.109 within the meaning of section 520(e) of the Federal Food, Drug, and Cosmetic Act (the act) under the authority of section 515(d)(1)(B)(ii) of the act. FDA has also determined that to ensure the safe and effective use of the device that the device is further restricted within the meaning of section 520(e) under the authority of section 515(d)(1)(B)(ii), (1) insofar as the labeling specify the requirements that apply to the training of practitioners who may use the device as approved in this order, (2) insofar as the labeling specify the requirements that apply to laboratory

facilities where the device is to be used as approved, and (3) insofar as the sale, distribution, and use must not violate sections 502(q) and (r) of the act.

In addition to the postapproval requirements in the enclosure, postapproval reports must include the following:

1. Summary results of the Performance Evaluation Program compiled by Centers for Disease Control and Prevention (CDC) that have been made available within the preceding twelve months. The first summary should be filed in a separate periodic report within 6 months of receipt of this approval order and include Performance Evaluation reports made available within the prior 12 month period. Summaries should include an assessment by Gen-Probe Incorporated of any findings or observations that are unique or unusual and that may impact on effectiveness of the device. Thereafter, summaries should be included in the annual report.
2. Summary results of a survey of at least three laboratories for MTD testing done with at least 500 smear negative specimens. This survey should include the following information: total number of clinical specimens cultured for mycobacteria in each of the preceding 3 months, total numbers of AFB smear positive and negative specimens, total number of smear negative specimens with MTD Tests performed and reported, the mycobacterial culture results for those smear negative specimens with MTD Tests performed and reported, a coded listing of patients with one or more MTD Tests performed on smear negative specimens, the MTD Test results for each patient and culture results for those specimens, and the number of MTD inhibition tests (and those results) routinely performed for AFB smear positive and AFB smear negative specimens. MTD testing included in the survey should be part of routine clinical testing done in each laboratory (not an evaluation or directed study). The letter to accompany the survey and the survey form must be submitted to FDA in a periodic report within 1 month. The survey should be completed within 6 months of marketing the MTD Test with the new intended use and the summary submitted within 1 month of completion (by 9 months post-approval) in a periodic report. The laboratories surveyed should not be ones that participated in the clinical evaluation.
3. All educational, promotional, and advertising materials submitted in a periodic report within the first 2 months following approval and in the annual report thereafter.

Expiration dating for this device has been established and approved at 12 months.

CDRH will notify the public of its decision to approve your PMA by making available a summary of the safety and effectiveness data upon which the approval is based. The information can be found on the FDA CDRH Internet HomePage located at <http://www.fda.gov/cdrh/pmapage.html>. Written requests for this information can also be made to the Dockets Management Branch (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. The written request should include the PMA number or

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docket number. Within 30 days from the date that this information is placed on the Internet, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the act.

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

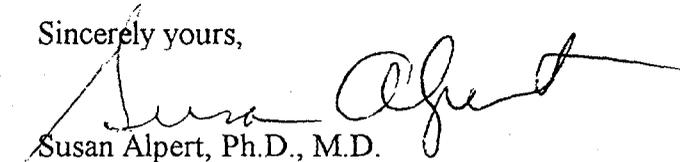
You are reminded that, as soon as possible and before commercial distribution of your device, you must submit an amendment to this PMA submission with copies of all approved labeling affected by this supplement in final printed form. As part of our reengineering effort, the Office of Device Evaluation is piloting a new process for review of final printed labeling. The labeling will not routinely be reviewed by FDA staff when PMA supplement applicants include with their submission of the final printed labeling a cover letter stating that the final printed labeling is identical to the labeling approved in draft form. If the final printed labeling is not identical, any changes from the final draft labeling should be highlighted and explained in the amendment. Please see the CDRH Pilot for Review of Final Printed Labeling document at <http://www.fda.gov/cdrh/pmat/pilotpmat.html> for further details.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Roxanne G. Shively at (301) 594-2096.

Sincerely yours,


Susan Alpert, Ph.D., M.D.
Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

Issued: 3-4-98

CONDITIONS OF APPROVAL

APPROVED LABELING. As soon as possible, and before commercial distribution of your device, submit three copies of an amendment to this PMA submission with copies of all approved labeling in final printed form to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration (FDA), 9200 Corporate Blvd., Rockville, Maryland 20850.

ADVERTISEMENT. No advertisement or other descriptive printed material issued by the applicant or private label distributor with respect to this device shall recommend or imply that the device may be used for any use that is not included in the FDA approved labeling for the device. If the FDA approval order has restricted the sale, distribution and use of the device to prescription use in accordance with 21 CFR 801.109 and specified that this restriction is being imposed in accordance with the provisions of section 520(e) of the act under the authority of section 515(d)(1)(B)(ii) of the act, all advertisements and other descriptive printed material issued by the applicant or distributor with respect to the device shall include a brief statement of the intended uses of the device and relevant warnings, precautions, side effects and contraindications.

PREMARKET APPROVAL APPLICATION (PMA) SUPPLEMENT. Before making any change affecting the safety or effectiveness of the device, submit a PMA supplement for review and approval by FDA unless the change is of a type for which a "Special PMA Supplement-Changes Being Effectuated" is permitted under 21 CFR 814.39(d) or an alternate submission is permitted in accordance with 21 CFR 814.39(e). A PMA supplement or alternate submission shall comply with applicable requirements under 21 CFR 814.39 of the final rule for Premarket Approval of Medical Devices.

All situations which require a PMA supplement cannot be briefly summarized, please consult the PMA regulation for further guidance. The guidance provided below is only for several key instances.

A PMA supplement must be submitted when unanticipated adverse effects, increases in the incidence of anticipated adverse effects, or device failures necessitate a labeling, manufacturing, or device modification.

A PMA supplement must be submitted if the device is to be modified and the modified device should be subjected to animal or laboratory or clinical testing designed to determine if the modified device remains safe and effective.

A "Special PMA Supplement - Changes Being Effectuated" is limited to the labeling, quality control and manufacturing process changes specified under 21 CFR 814.39(d)(2). It allows for the addition of, but not the replacement of previously approved, quality control specifications and test methods. These changes may be implemented before FDA approval upon acknowledgment by FDA that the submission is being processed as a "Special PMA Supplement - Changes Being Effectuated." This acknowledgment is in addition to that issued by the PMA Document Mail Center for all PMA supplements submitted. This procedure is not applicable to changes in device design, composition, specifications, circuitry, software or energy source.

Alternate submissions permitted under 21 CFR 814.39(e) apply to changes that otherwise require approval of a PMA supplement before implementation of the change and include the use of a 30-day PMA supplement or annual postapproval report. FDA must have previously indicated in an advisory opinion to the affected industry or in correspondence with the applicant that the alternate submission is permitted for the change. Before such can occur, FDA and the PMA applicant(s) involved must agree upon any needed testing protocol, test results, reporting format, information to be reported, and the alternate submission to be used.

POSTAPPROVAL REPORTS. Continued approval of this PMA is contingent upon the submission of postapproval reports required under 21 CFR 814.84 at intervals of 1 year from the date of approval of the original PMA. Postapproval reports for supplements approved under the original PMA, if applicable, are to be included in the next and subsequent annual reports for the original PMA unless specified otherwise in the approval order for the PMA supplement. Two copies identified as "Annual Report" and bearing the applicable PMA reference number are to be submitted to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850. The postapproval report shall indicate the beginning and ending date of the period covered by the report and shall include the following information required by 21 CFR 814.84:

- (1) Identification of changes described in 21 CFR 814.39(a) and changes required to be reported to FDA under 21 CFR 814.39(b).
- (2) Bibliography and summary of the following information not previously submitted as part of the PMA and that is known to or reasonably should be known to the applicant:
 - (a) unpublished reports of data from any clinical investigations or nonclinical laboratory studies involving the device or related devices ("related" devices include devices which are the same or substantially similar to the applicant's device); and
 - (b) reports in the scientific literature concerning the device.

If, after reviewing the bibliography and summary, FDA concludes that agency review of one or more of the above reports is required, the applicant shall submit two copies of each identified report when so notified by FDA.

ADVERSE REACTION AND DEVICE DEFECT REPORTING. As provided by 21 CFR 814.82(a)(9), FDA has determined that in order to provide continued reasonable assurance of the safety and effectiveness of the device, the applicant shall submit 3 copies of a written report identified, as applicable, as an "Adverse Reaction Report" or "Device Defect Report" to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850 within 10 days after the applicant receives or has knowledge of information concerning:

- (1) A mix-up of the device or its labeling with another article.
- (2) Any adverse reaction, side effect, injury, toxicity, or sensitivity reaction that is attributable to the device and
 - (a) has not been addressed by the device's labeling or

(b) has been addressed by the device's labeling, but is occurring with unexpected severity or frequency.

(3) Any significant chemical, physical or other change or deterioration in the device or any failure of the device to meet the specifications established in the approved PMA that could not cause or contribute to death or serious injury but are not correctable by adjustments or other maintenance procedures described in the approved labeling. The report shall include a discussion of the applicant's assessment of the change, deterioration or failure and any proposed or implemented corrective action by the applicant. When such events are correctable by adjustments or other maintenance procedures described in the approved labeling, all such events known to the applicant shall be included in the Annual Report described under "Postapproval Reports" above unless specified otherwise in the conditions of approval to this PMA. This postapproval report shall appropriately categorize these events and include the number of reported and otherwise known instances of each category during the reporting period. Additional information regarding the events discussed above shall be submitted by the applicant when determined by FDA to be necessary to provide continued reasonable assurance of the safety and effectiveness of the device for its intended use.

REPORTING UNDER THE MEDICAL DEVICE REPORTING (MDR) REGULATION. The Medical Device Reporting (MDR) Regulation became effective on December 13, 1984. This regulation was replaced by the reporting requirements of the Safe Medical Devices Act of 1990 which became effective July 31, 1996 and requires that all manufacturers and importers of medical devices, including in vitro diagnostic devices, report to the FDA whenever they receive or otherwise become aware of information, from any source, that reasonably suggests that a device marketed by the manufacturer or importer:

(1) May have caused or contributed to a death or serious injury; or

(2) Has malfunctioned and such device or similar device marketed by the manufacturer or importer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

The same events subject to reporting under the MDR Regulation may also be subject to the above "Adverse Reaction and Device Defect Reporting" requirements in the "Conditions of Approval" for this PMA. FDA has determined that such duplicative reporting is unnecessary. Whenever an event involving a device is subject to reporting under both the MDR Regulation and the "Conditions of Approval" for a PMA, the manufacturer shall submit the appropriate reports required by the MDR Regulation within the time frames as identified in 21 CFR 803.10(c) using FDA Form 3500A, i.e., 30 days after becoming aware of a reportable death, serious injury, or malfunction as described in 21 CFR 803.50 and 21 CFR 803.52 and 5 days after becoming aware that a reportable MDR event requires remedial action to prevent an unreasonable risk of substantial harm to the public health. The manufacturer is responsible for submitting a baseline report on FDA Form 3417 for a device when the device model is first reported under 21 CFR 803.50. This baseline report is to include the PMA reference number. Any written report and its envelope is to be specifically identified, e.g., "Manufacturer Report," "5-Day Report," "Baseline Report," etc.

Any written report is to be submitted to:

Food and Drug Administration
Center for Devices and Radiological Health
Medical Device Reporting
PO Box 3002
Rockville, Maryland 20847-3002

Copies of the MDR Regulation (FOD # 336&1336) and FDA publications entitled "An Overview of the Medical Device Reporting Regulation" (FOD # 509) and "Medical Device Reporting for Manufacturers" (FOD #987) are available on the CDRH WWW Home Page. They are also available through CDRH's Fact-On-Demand (F-O-D) at 800-899-0381. Written requests for information can be made by sending a facsimile to CDRH's Division of Small Manufacturers Assistance (DSMA) at 301-443-8818.

SUMMARY OF SAFETY AND
EFFECTIVENESS DATA (SSED)

SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. General Information

Device Generic Name: Target Amplification Test for the Direct Detection of *Mycobacterium tuberculosis*

Device Trade Name: GEN-PROBE® AMPLIFIED™ Mycobacterium Tuberculosis Direct (MTD) Test

Applicant's Name and Address: Gen-Probe® Incorporated
10210 Genetic Center Drive
San Diego, CA 92121-4362

Premarket Approval Application (PMA) Number: P940034/S008

Date of Original Panel Recommendation: May 20, 1999

Date of Notice of Approval to the Applicant: September 30, 1999

II. Intended Use and Indications for Use

The original Indications for Use of the GEN-PROBE® AMPLIFIED™ Mycobacterium Tuberculosis Direct (MTD) Test, hereinafter referred to as MTD Test, have been modified by supplement application to include testing of AFB smear negative sediments:

The GEN-PROBE® AMPLIFIED™ Mycobacterium Tuberculosis Direct (MTD) Test is a target-amplified nucleic acid probe test for the *in vitro* diagnostic detection of *Mycobacterium tuberculosis* complex rRNA in acid-fast bacilli (AFB) smear positive and negative concentrated sediments prepared from sputum (induced or expectorated), bronchial specimens (e.g., bronchoalveolar lavages or bronchial aspirates) or tracheal aspirates.

The MTD Test is intended for use only with specimens from patients showing signs and symptoms consistent with active pulmonary tuberculosis. MTD is to be used as an adjunctive test for evaluating either AFB smear positive or negative sediments prepared using NALC-NaOH digestion-decontamination of respiratory specimens. Patients who are suspected of having pulmonary TB based on clinical evaluation and who have received no antituberculous therapy, less than 7 days of such therapy, or have not received such therapy in the last 12 months may be evaluated with this test. The MTD Test should be performed only in laboratories proficient in the culture and identification of *M. tuberculosis* (Level II and III or extent 3 and 4).¹ The MTD Test must be performed in conjunction with mycobacterial culture.

III. Contraindications:

There are no known contraindications, however, the MTD Test is not to be used without AFB smear, mycobacterial culture, and patient clinical evaluation for signs and symptoms of pulmonary TB.

IV. Warnings and Precautions:

The Positive Predictive Value (PPV) of MTD results for AFB smear negative patients is lower than the PPV of MTD results for AFB smear positive patients. The average prevalence of tuberculosis in this population was 27.7% (57/206 patients). The predictive values associated with any diagnostic test are related to the prevalence of disease in a given patient group. Refer to Table 1 in the Limitations section for the hypothetical estimates of positive and negative predictive values across varying prevalence rates.

The efficacy of this test has not been demonstrated for the direct detection of *M. tuberculosis* rRNA using other clinical specimens (e.g., blood, CSF, tissue, urine, or stool). Performance of the MTD Test has not been established for sediments processed in a different fashion than described, or stored for different time periods or temperatures than specified in this Package Insert.

Sediments must be cultured to determine if *Mycobacteria* other than tuberculosis complex (MOTT) are present in addition to *M. tuberculosis* complex and to perform antimycobacterial susceptibility testing. Culture for AFB should also be performed to determine which subspecies of the *M. tuberculosis* complex (e.g., *M. bovis*) is present.

M. celatum and *M. terrae*-like organisms will cross-react if present at concentrations higher than 30 colony forming units (CFU) per test. However, *M. celatum* and *M. terrae*-like organisms are rarely isolated from clinical specimens.

Samples may be MTD Test negative and *M. tuberculosis* complex culture positive. This condition may be caused by inhibition of the MTD Test or the presence of low levels of the *M. tuberculosis* complex organism.

Specimens from pediatric patients have not been evaluated with the MTD Test.

The MTD Test is not indicated for use with specimens from patients being treated with antituberculous agents to determine bacteriologic cure or to monitor response to such therapy.

Specimens that are grossly bloody should not be tested with the MTD Test; blood may cause nonspecific positivity in the MTD Test.

Sediments must be resuspended in a phosphate buffer concentration of 67 mM.⁸ Concentrations substantially above 67 mM may interfere with amplification of the MTD

Test, decreasing the ability to detect *M. tuberculosis* complex in the specimen. Sediments prepared using Alpha-Tec Systems, Inc. NAC-PACT™ XPR-plus™ A. F. B. Processing Buffer have been shown to interfere with amplification.

Precautions can be found in the MTD labeling (Attachment 1).

V. Device Description

Background

1. *Mycobacterium tuberculosis* complex

M. tuberculosis complex includes the species *M. bovis*, *M. bovis* BCG, *M. microti*, *M. africanum*, and *M. canetti*. However, *M. microti* infects only animals, *M. bovis* is uncommonly transmitted from infected animals to humans, and *M. africanum* causes pulmonary disease in humans in tropical Africa.¹⁵ *M. canetti* was recently described as a member of the *M. tuberculosis* complex and is a rare cause of disease.¹⁴ *M. tuberculosis* is by far the most common member of the complex that is responsible for human disease worldwide. Nontuberculous mycobacteria (Mycobacteria other than tuberculosis, or MOTT) include *M. avium* complex (MAC) and other organisms that may also cause disease in humans.

Historically, *M. tuberculosis* complex and MOTT have been presumptively identified in slides made from clinical specimens by their acid fast nature. All mycobacteria have cell walls with a high lipid content that prevents easy decolorization once stained (acid fastness). Growth rates are slow to very slow, with some species requiring supplementation for laboratory culture.¹¹ Definitive identification of *M. tuberculosis* and MOTT requires identification by traditional methods (observation of growth rate, colonial morphology, pigmentation and biochemical profiles), chromatographic analysis of lipid composition (thin layer chromatography, capillary gas chromatography, or high pressure liquid chromatography), or hybridization assays with specific nucleic acid probes.

M. tuberculosis complex organisms are a proven hazard to laboratory personnel as well as others that may be exposed to infectious aerosols in the laboratory. The infective dose for humans is low (ID₅₀ less than 10 bacilli). Biosafety Level 2 practices are required for activities at the American Thoracic Society (ATS) laboratory Level I (preparation of AFB smears, collection and transport of mycobacterial specimens for culture). Biosafety Level 3 practices are required for laboratory activities of ATS Levels II (isolation and identification of *M. tuberculosis*) and III (additionally performing susceptibility testing of *M. tuberculosis* isolates and identification of MOTT).⁵

2. Tuberculosis

Tuberculosis is a bacterial disease caused by organisms of the *Mycobacterium tuberculosis* complex; it is transmitted primarily by airborne droplet nuclei from individuals with pulmonary or laryngeal tuberculosis.¹³

Tuberculosis (TB) can occur in any organ of the body, but only 5 to 15 percent of infected individuals will develop active disease within 2 years of primary infection. Pulmonary TB is still the primary manifestation in infected individuals who develop disease, but the incidence of extra-pulmonary TB has progressively increased. HIV-infected individuals who develop active TB have a high rate of extra-pulmonary disease. HIV-positive patients with low CD4 counts tend to have radiographic presentations markedly different from the classical upper lobe cavitory disease of immunocompetent patients. Clinical disease due to reactivation of dormant TB is influenced by predisposing factors such as AIDS, malignancy, silicosis, immunosuppressive therapy, malnourishment, and other risk factors.¹⁶

Population groups in the United States that are at increased risk for infection with *M. tuberculosis* include medically underserved, low-income populations, immigrants from countries with a high prevalence of TB, and residents of long-term-care and correctional facilities. Those at increased risk of developing disease following infection include individuals with HIV infection; close contacts of infectious cases; children less than 5 years old; patients with renal failure, silicosis, and diabetes mellitus; and individuals receiving treatment with immunosuppressive medications.¹³

The initial treatment of TB includes multiple antimicrobial agents, since administration of single drug often leads to the development of resistance. *M. tuberculosis* becomes drug resistant through random, spontaneous genetic mutation. Susceptibility testing of the first isolate from all patients should be done to provide the physician a basis for therapeutic management, to identify emerging drug resistance, and to help monitor control efforts in areas where resistance is established. If culture positive sputum continues after three months of therapy, susceptibility testing should be repeated. During the first week of therapy, few patients convert from culture positive to culture negative.⁷ Thereafter, patients responding to therapy will have significant reductions in organism loads and become culture negative. The time course until a patient becomes noninfectious is influenced by initial organism load, the presence of a drug-resistant strain, and the severity of coughing. Organism load reduction can be monitored with AFB smears when a patient has smear positive disease at the time of diagnosis. Infection control practices may vary; individuals with smear positive pulmonary disease are considered highly infectious until two weeks of treatment have been completed, or until three successive sputum specimens are AFB smear negative. Patients with AFB smear negative pulmonary TB are less infectious than those with positive smears, but can still transmit *M. tuberculosis*.² For these patients, clinical suspicion may be used as criteria to continue isolation. Isolation practices are guided by state

and local public health recommendations along with infection control practices in individual institutions.

Culturing is used to monitor bacteriologic sputum conversion, to assess response to therapy, and to monitor the emergence of resistant strains.⁶

After uniform national reporting of TB began in 1953, the number of cases reported annually declined steadily until 1985. Since that time TB has reemerged as a serious public health problem.¹³ In addition, the development of multi-drug resistant strains of *M. tuberculosis* has become a major concern. Factors contributing to the increase in TB morbidity in the United States include an increase in foreign-born cases, the HIV/AIDS epidemic, and increased active TB transmission in higher risk populations.⁴

Principles of the MTD Test Operation

The MTD Test is an *in vitro* device that utilizes Transcription Mediated Amplification (TMA) and the Hybridization Protection Assay (HPATM) to qualitatively detect *M. tuberculosis* complex ribosomal ribonucleic acid (rRNA) in respiratory specimens. The MTD was modified from its original configuration approved December 15, 1995. The following changes from the original format were made in a supplemental application (P940034/S004):

Design changes

- deleting termination reagent and hybridization controls
- reformulating specimen dilution and enzyme dilution buffers
- increasing volume of reconstitution buffer
- changing the lyophilization process for the hybridization and amplification reagents
- increasing volume of amplification reagent

Procedural changes

- increased specimen volume with accompanying decrease in added specimen dilution buffer
- decreased amplification time (from 2 hr to 30 min)
- increased selection time (from 10 min to 15 min)
- elimination of termination step and testing of hybridization controls

Initially, 450 μ L of processed (digested and decontaminated) respiratory specimen is sonicated to release cellular nucleic acids. Each intact *M. tuberculosis* cell contains approximately 2000 copies of rRNA. TMA is an RNA transcription-dependent amplification technology, in which RNA strands serve as templates for the synthesis of DNA intermediates. These DNA intermediates are then used for the transcription of multiple copies of RNA amplicon. The RNA amplicon can then serve as templates for further synthesis of DNA intermediates, which in turn are used for further transcription of additional copies of RNA amplicon.

In the HPA step of the MTD, *M. tuberculosis* complex-specific rRNA sequences within the amplicon are detected by hybridization with a chemiluminescent-labeled single-stranded DNA probe (Detector Probe) that is complementary to sequences in the amplicon. An important aspect of HPA is the steric protection of the acridinium ester chemiluminescent label that is linked to the Detection Probe from a hydrolysis reaction that destroys the chemiluminescence of the label. This protection occurs when the Detection Probe hybridizes with the complementary amplicon sequence. When Selection Reagent (containing a high pH buffer) is added to a solution containing a mixture of hybridized and unhybridized Detection Probe, only hybridized probe will retain chemiluminescent properties. Following this "differential hydrolysis" step, Detection Reagents are added to the solution and the acridinium ester molecules that are attached to the hybridized Detection Probes emit photons. The photons emitted (chemiluminescence) are measured with a luminometer (LEADER™, Gen-Probe, Incorporated) as Relative Light Units (RLU). The presence or absence of the target rRNA is determined by the level of RLU measured. The magnitude of the RLU reading is not indicative of the numbers of *M. tuberculosis* organisms present in the specimen, as saturated levels are obtained above 60 copies of *M. tuberculosis* rRNA (equivalent to 0.05 to 0.25 cells *M. tuberculosis*).

The MTD Test will detect rRNA from both cultivable and non-cultivable organisms within the *M. tuberculosis* complex and enables detection of *M. tuberculosis* complex rRNA within 2.5 to 3.5 hours after the respiratory specimen has been digested, decontaminated, and concentrated. The MTD Test does not differentiate between rRNA from viable and non-viable cells, and therefore, cannot be used for specimens from treated patients, because nonculturable organisms may be shed in the respiratory secretions during antituberculous therapy. A positive MTD result would not correlate with organism viability and would not be useful for monitoring response to therapy.

Positive and negative amplification cell controls (*M. tuberculosis* complex and MOTT cell suspensions prepared from cultures) must be used with the MTD Test to monitor the amplification efficiency and specimen processing effects. These controls are not included in the assay kit and must be prepared and evaluated for acceptability by each laboratory performing the assay.

Results for the MTD Test may be affected by inhibitory substances (exogenous or endogenous) in clinical specimens or in reagents used for decontaminating and concentrating respiratory specimens. Final concentrations of sodium hydroxide (NaOH) greater than 1.5 percent (initial 3.0 percent) used for specimen decontamination inhibit detection of *M. tuberculosis* rRNA using the MTD Test. Inappropriate phosphate buffer concentrations (greater than 67 mM) or some commercially available buffers (e.g., NAC-PAC™ XPR-plus™ A.F.B. Processing Buffer) also may inhibit or interfere with MTD amplification. Amplicon from previous MTD testing or *M. tuberculosis* in the laboratory may contaminate reagents, laboratory surfaces, and equipment resulting in false positive MTD results. Although amplification and hybridization are performed in the same tube, thereby avoiding a transfer procedure that could be an added source of contamination, the MTD Test must be performed

with appropriate precautions (separation of specimen processing and MTD testing; and uni-directional workflow), and strict adherence to the specified procedure.

VI. Alternative Practices and Procedures

The diagnosis of TB is complicated by unusual presentation of the disease in special groups (e.g., elderly and immunocompromised persons) and clinically differentiating other disease entities that may mimic TB or be present simultaneously (e.g., carcinoma of the lung). Tuberculin skin testing, radiography, assessment of physical findings, and identification of risk factors are used to determine that patients with a high index of suspicion may have TB. AFB smears and cultures of clinical material are necessary to establish a definitive diagnosis of TB although a strong presumptive diagnosis may be made on radiographic findings when the patterns are typical.¹ A proportion of patients with pulmonary TB may be culture negative. Depending on the report, the percentage of patients diagnosed with TB that are culture negative range from 13 to greater than 20 percent.^{9,10} However, the accuracy of culture-negative diagnoses have not been well-evaluated.

Because respiratory specimens contain an abundance of bacterial flora that can quickly overgrow slowly-reproducing mycobacteria, it is necessary to digest or liquefy organic material in order to expose nonmycobacterial organisms to decontaminating agents. It is also important to transport these specimens to the laboratory as quickly as possible to prevent overgrowth by bacterial flora to optimize AFB smears and mycobacterial culturing. CDC (Centers for Disease Control and Prevention) recommends rapid delivery of specimens to the laboratory to ensure arrival within 24 hours.¹³ The recommended procedure for digesting and decontaminating respiratory specimens uses N-acetyl-L-cysteine-sodium hydroxide (NALC) as a mucolytic agent, and NaOH to eliminate contaminating nonmycobacterial organisms. The method must be carefully monitored to prevent overexposure that will kill mycobacteria as well as the undesirable organisms.¹¹

While the sensitivity of the AFB smear is lower than that of culture methods, the AFB smear has an important role in early diagnosis of mycobacterial infection and the presumptive diagnosis of pulmonary TB because of the relatively long time required for mycobacteria to be detected by culture methods. Also patients with positive smears due to *M. tuberculosis* complex are considered more likely to spread TB.² A minimum of 5×10^3 to 1×10^4 bacilli per mL of sputum is required for detection by AFB smear, whereas culture detects as few as 10 to 100 viable organisms in a specimen.¹¹

Definitive diagnosis of mycobacterial disease (except leprosy), including TB, requires growth of the microorganism. Although patients will be initially treated with a predetermined therapeutic regimen, culture isolates are required for susceptibility testing to confirm the anticipated effectiveness of treatment. Culture for AFB is usually performed by inoculating several media with decontaminated sediment and incubating for up to 8 weeks. Conventional culture methodologies can detect *M. tuberculosis* growth as early as 1 week, but may take up to 8 weeks. Radiometric liquid culture requires an average of 13 days to

final culture result. Current recommendations from CDC are to inoculate both a liquid medium and a solid medium.¹³ After recovery of mycobacteria from culture media, identification of *M. tuberculosis* may be done by conventional biochemical testing, analysis of lipid content, or hybridization with specific DNA probes.

The American Thoracic Society (ATS), in collaboration with CDC, provides a classification scheme for TB that is based on pathogenesis and current treatment recommendations.¹ Patients with clinical suspicion of TB or positive AFB smears are reported to local health departments for appropriate public health management (including contact investigations).¹³ Final species identification from positive cultures and susceptibility test results are also reported to the health department.

VII. Marketing History

The MTD Test has been in distribution in the United States since 1995. MTD Tests have also been marketed internationally since 1994 (Australia, Austria, Canada, Denmark, Finland, France, Germany, Greece, Hong Kong, Italy, The Netherlands, New Zealand, Norway, South Africa, Spain, Sweden, Switzerland, Taiwan, the United Kingdom, and Japan).

VIII. Potential Adverse Effects of the Device on Health

Prompt diagnosis of TB is critical, both to initiate appropriate therapy and to institute measures to prevent further exposures and spread of the disease to uninfected individuals in the community and health care facilities. A false positive MTD Test result could lead to misdiagnosing a patient's medical status, resulting in the administration of unnecessary therapy and/or placing a patient in unwarranted isolation; the patient would be reported to the local health department for public health management, and contact investigations initiated. Additionally a false positive result that causes a misdiagnosis of TB can influence patient management so that additional diagnostic procedures needed to correctly diagnose (and treat) are delayed or not done.

A false negative MTD Test result could delay or impede the correct diagnosis of TB, initiation of appropriate therapy, and maintaining respiratory precautions (isolation). Delayed or missed diagnoses can result in rapidly progressive disease, especially in HIV-positive patients and patients infected with multi-drug resistant strains of *M. tuberculosis*. In addition, highly infectious patients may transmit TB infection to others unless respiratory isolation and appropriate therapy are initiated.

False negative results with the MTD Test can be caused by specimen inhibition, interfering substances, insufficient mixing, procedural deviations, use of the test by unqualified personnel, inappropriate test result reporting, presence of high numbers of MOTT, or presence of low numbers of *M. tuberculosis* complex in the specimen with or without the presence of inhibitors. False positive results may be caused by presence of cross-reacting

species in the specimen, procedural errors, carryover contamination, sample misidentification, or transcription errors.

IX. Summary of Studies

Analytical Studies

Some analytical studies presented in the original application were repeated for P940034/S004 when the MTD assay configuration was modified.

1. Establishment of the Cutoff Value and Validation

Originally, a total of 291 respiratory specimens negative by culture for *M. tuberculosis* and 134 specimens positive by culture for *M. tuberculosis* (including both AFB smear positive and negative samples) were tested with the MTD to determine the best cutoff value for defining specimen positivity/negativity. The cutoff was selected based on Receiver Operator Characteristic (ROC) curve analysis of the data and similar analyses. The ROC curve showed a cutoff value of 30,000 RLU represented the best balance between sensitivity (85.4 percent) and specificity (98.9 percent). Subsequently (P940034/S003, approved August 22, 1996), the cutoff was modified, to include an equivocal region of 30,000 to 500,000 RLU following field reports of MTD cross-reactivity with *M. kansasii* in respiratory specimens. Ensuing studies by the applicant resulted in changing the selection timing for the MTD assay to reduce such nonspecific reactivity.

For the modifications approved with P940034/S004 (approved May 15, 1998), modified MTD RLU results for 129 smear positive specimens from 55 patients (98 specimens positive with *M. tuberculosis* and 31 specimens with no *M. tuberculosis*) were compared to MTD RLU results using the original assay configuration. There were minimal differences between the RLU levels for the modified MTD compared to the original MTD, but test numbers were statistically insufficient for *M. tuberculosis* culture negative specimens to conclude that specificity was improved or at least comparable using the same cutoff criteria. Two specimens with *M. kansasii* were negative with the modified MTD but positive or equivocal with the original MTD.

With the evaluable data for MTD testing of clinical specimens in P040034/S008 (536 specimens from 206 patients) that included testing of smear negative specimens, nine specimens had initial RLU results in the MTD equivocal region. Of these, four were from AFB smear positive patients and five from AFB smear negative patients. All were from patients with *M. tuberculosis* recovered from culture (three specimens had MOTT in addition to *M. tuberculosis* cultured). The one false positive MTD Test observed for the evaluable MTD data had a RLU measurement greater than 1,000,000. *M. kansasii* was identified in 15 specimens (three were AFB smear

positive) from seven patients. All were negative both with the initial MTD Test and repeated MTD Test from the reserved lysate.

When MTD testing was repeated from reserved specimen lysate, thirteen specimens had results in the equivocal range. One of these lysates was from a patient not diagnosed with TB. Eleven were from specimens with initially positive or equivocal MTD Tests; all eleven patients were diagnosed with TB. The other equivocal was from a specimen initially MTD negative; four cultures for this patient were negative and none of the other three specimens from the patient had MTD positive results (initially or repeat); the patient was determined to have TB based on clinical criteria. Four additional specimens that were initially negative were MTD positive (greater than 500,000 RLU) with the repeated test from reserved lysates (two specimens had *M. tuberculosis* and one specimen had MOTT recovered by culture; the remaining specimen was culture negative and the patient was not considered to have pulmonary TB).

Although the numbers of specimens with MTD equivocal results are small, results in the equivocal RLU measurement range are often not repeatable with MTD retesting of the reserved lysate. Of the nine specimens with equivocal results, two were negative (<30,000 RLU), three were again equivocal (30,000-499,000 RLU), and four were positive (\geq 500,000 RLU) when retested from the lysate. Analytical data shows that small numbers of organisms (less than 5 CFU per test) should reproducibly produce RLU values greater than 1,000,000. A published report suggests that MTD results with RLUs less than 1,000,000 should be considered equivocal and likely false positive.³ The limited MTD data from the applicant's study do not support expanding the equivocal region. Additional information would be needed to clarify the appropriateness of the cutoff and the effects of repeat testing from reserved lysates, particularly for smear negative specimens.

2. Specificity of the MTD Test

Specificity of the MTD Test was assessed using bacteria, fungi, and viruses. For bacteria and fungi, specificity testing included 159 strains (150 species from 62 genera) of closely related mycobacteria, other organisms causing lower respiratory disease, normal respiratory flora, and organisms representing a cross-section of phylogeny. As with the original MTD Test, false positive results were observed with *M. celatum* and *M. terrae*-like organisms at greater than 30 CFU per test. *M. celatum* and *M. terrae*-like organisms have only one or two rRNA mismatches with the probes used in the MTD Test.

3. Analytical Sensitivity – Probe Detection Limits

Thirty strains of *M. tuberculosis* from a wide geographic distribution obtained from CDC, including representative drug-resistant and drug-sensitive strains, were tested

with the MTD Test. All strains were tested in triplicate and diluted in 0.02 percent (v/v) Tween. The MTD Test was positive with greater than 1,000,000 RLU for one CFU per test for all 30 strains.

4. Precision Studies

Precision panels, consisting of two negative samples, two positive samples (approximately 100 CFU per test, equivalent to approximately 4,000 CFU per mL) and two high positive samples (approximately 1000 CFU per test, equivalent to approximately 40,000 CFU/mL) were tested at Gen-Probe Incorporated, and two clinical laboratory sites. The positive samples were prepared by spiking a suspension of WBC DNA (8µg per test), to simulate inhibition, with known amounts of *M. tuberculosis*. Precision for samples with low levels of *M. tuberculosis* cells (e.g. 10-500 CFU per mL) were not assessed in the precision study. The samples were tested in triplicate twice a day for 3 days at each of the three sites. Positive and negative amplification cell controls were included in each run.

There was no significant site-to-site or day-to-day variability observed. Data from the 3 sites were combined and are shown in Table 1. The two levels of *M. tuberculosis* cells tested resulted in reproducible RLU values greater than 2,000,000 (saturated signal levels with the MTD test). Because none of the samples tested had RLU levels <2,000,000 and no samples with less than 100 CFU per test were included in these data, no conclusions can be made for precision of RLU measurements for samples with lower organism loads or for RLU measurements near the assay cutoffs (30,000 and 500,000 RLU). The data do support reproducible detection of organism loads near the minimum detection level for AFB smear (5×10^3 organisms per mL). Three out of 108 negative samples and 2 out of 54 negative controls had MTD tests falsely positive due to probable technical error (splashing). These findings suggest that specificity of the MTD would be highly dependent on operator proficiency, and that with routine testing of specimens from patients without *M. tuberculosis* present, false positive MTD results (3-4 percent with these precision data) may occur due to operator or technical error.

Table 1: Precision Study

	# Observations	% Correct	Range (RLU)	Mean (RLU)
Sample 1 High Positive	108	100%	> 2,000,000	> 2,000,000
Sample 2 Low Positive	108	100%	> 2,000,000	> 2,000,000
Sample 3 Negative	105*	100%	1,484 -13,129	2,605
Positive Cell Control	54	100%	> 2,000,000	> 2,000,000
Negative Cell Control	52**	100%	2,129 -3,525	2,542

* Three observations have been removed from final study results as a result of operator error.

**Two observations have been removed from final study results due to one operator reporting splashing during one run. One observation yielded a positive result; one observation yielded a result in the equivocal zone.

5. MTD Test Reproducibility

A reproducibility panel consisting of 25 samples with 0, 1, 10, 25, 50, or 100 colony forming units (CFU) per test was tested at Gen-Probe and two clinical laboratory sites using the modified device (P940034/S004). Each sample was replicated in the panel. The prepared samples did not include specimen matrix and samples were not processed using decontamination/concentration procedures. Amplification Cell Negative Controls (*M. terrae* cells at approximately 5 CFU per test) were interspersed throughout for a total of 50 samples tested at each site.

Data from this study are shown in Table 2. No false positive results were observed at any site. Site 1 experienced reduced RLU measurements for positive samples, including two equivocal test results for a 1 CFU per test sample. Overall, all negative samples were MTD negative (120/120) and 97.5% (78/80) of samples with *M. tuberculosis* were MTD positive; the remaining two were equivocal with the MTD Test. Both equivocals were recorded at Site 1.

Table 2: Reproducibility Testing (RLU)

Sample #	Cell Level*	Site 1	Site 2	Site 3	Site 4
1	Negative	1,199	8,599	15,804	2,489
2	1 cell	847,652	>2,000,000	>2,000,000	1,101,423
3	Negative	3,169	7,338	2,762	2,720
4	10 cells	1,408,904	>2,000,000	>2,000,000	>2,000,000
5	Negative	1,104	2,712	2,546	2,472
6	25 cells	1,489,814	>2,000,000	>2,000,000	>2,000,000
7	Negative	1,045	4,021	2,704	2,470
8	100 cells	1,310,069	>2,000,000	>2,000,000	>2,000,000
9	Negative	1,023	2,760	2,584	2,597
10	1 cell	177,659	>2,000,000	>2,000,000	819,841
11	Negative	1,123	3,744	2,756	2,639
12	0 cells	1,052	3,613	2,899	2,442
13	Negative	1,098	2,647	2,855	2,237
14	50 cells	1,456,529	>2,000,000	>2,000,000	>2,000,000
15	Negative	1,119	3,202	2,897	2,950
16	0 cells	992	3,005	2,685	2,400
17	Negative	2,061	2,749	2,659	2,800
18	10 cells	1,258,465	>2,000,000	>2,000,000	>2,000,000
19	Negative	1,104	3,106	2,657	2,404
20	50 cells	1,408,123	>2,000,000	>2,000,000	>2,000,000
21	Negative	2,085	3,035	2,580	2,496
22	25 cells	1,376,260	>2,000,000	>2,000,000	>2,000,000
23	Negative	7,271	2,997	2,831	2,358
24	100 cells	1,311,114	>2,000,000	>2,000,000	>2,000,000
25	Negative	4,154	2,738	2,643	2,561
26	Negative	8,415	2,649	2,576	2,414
27	1 cell	363,895	756,944	>2,000,000	>2,000,000
28	Negative	8,330	2,700	4,801	2,541
29	10 cells	1,236,037	>2,000,000	>2,000,000	>2,000,000
30	Negative	1,157	2,573	2,362	2,461
31	25 cells	1,309,739	>2,000,000	>2,000,000	>2,000,000
32	Negative	967	2,824	2,435	2,794
33	100 cells	1,315,542	>2,000,000	>2,000,000	>2,000,000
34	Negative	990	2,818	2,428	2,477
35	1 cell	1,064,275	>2,000,000	>2,000,000	735,721
36	Negative	1,056	2,402	2,503	2,731
37	0 cells	1,029	2,561	2,488	2,468
38	Negative	996	2,147	2,256	2,699
39	50 cells	1,220,052	>2,000,000	>2,000,000	>2,000,000
40	Negative	934	2,627	2,253	4,120
41	0 cells	976	3,185	2,371	2,346
42	Negative	984	2,904	2,382	2,551
43	10 cells	1,223,578	>2,000,000	>2,000,000	>2,000,000
44	Negative	2,851	3,316	2,359	2,617
45	50 cells	876,364	>2,000,000	>2,000,000	>2,000,000
46	Negative	1,010	2,412	2,488	2,372
47	25 cells	970,548	>2,000,000	>2,000,000	>2,000,000
48	Negative	992	3,005	2,240	2,695
49	100 cells	1,015,390	>2,000,000	>2,000,000	>2,000,000
50	Negative	1,023	2,626	2,825	2,751

* cells = CFU *M. tuberculosis* per test Negative=5 CFU *M. terrae* per test

6. Interference Studies

With the original application, the presence of 2.5 percent (v/v) blood in sputum that was digested and decontaminated did not interfere with the amplification of 0, 5, 100, or 300 CFU per test of *M. tuberculosis*. Five (5) percent and 10 percent (v/v) blood in sputum did not inhibit amplification of 100 and 300 CFU per test, but the five CFU per test sample was falsely negative in the presence of 5 and 10 percent blood with the MTD. These data demonstrated that amounts of blood greater than 2.5 percent could interfere with MTD results. Warnings and limitations to the use of the device with specimens that are bloody were included in the original labeling and remain in the revised labeling.

Testing was performed with the modified MTD to determine if human leukocyte DNA or non-target organism interferes with detection of *M. tuberculosis* rRNA. Because respiratory specimens contain human nucleic acid, MTD test results were assessed in the presence of varying amounts of human white blood cell DNA (WBC-DNA) per test. No interference with the MTD Test signal was observed at concentrations of *M. tuberculosis* rRNA ranging from 5 fg (equivalent to one cell) to 50 fg (equivalent to 10 cells) in the presence of 0.002 mg to 0.10 mg WBC-DNA. Partial interference of amplification was observed with 0.10 mg WBC DNA (the equivalent of 1×10^7 WBC) and the 5 fg rRNA control. These data support that purulent specimens (such as in highly mucopurulent specimens from cystic fibrosis patients that may contain up to 15 mg of DNA per mL) could interfere with the MTD results.

Interference from non-target organisms was assessed by testing *M. tuberculosis* rRNA (25 fg equivalent to 5 CFU) in the presence and absence of 14 species of bacteria at concentrations of 540,000 CFU per test. The species were either closely related to *M. tuberculosis* or other pathogens causing respiratory disease. Some of these (*P. aeruginosa*, *M. gordonae*, *M. avium*, *G. sputi*, *Nocardia otitidis-caviarum*, and *R. bronchialis*) had been observed to reduce RLU levels for positive samples with the original MTD configuration when testing samples with 5 fg *M. tuberculosis* rRNA (one CFU equivalent) and 290,000 CFU per test of the non-*M. tuberculosis* organisms. No reduction was seen with the modified MTD using higher concentrations of *M. tuberculosis* rRNA (25 fg).

7. Inhibition

MTD directions for use describe a procedure for assessing inhibition of individual specimens that test negative with the MTD (using an *M. tuberculosis* cell spike-in procedure). For specimens from patients tested with MTD in the most recent supplement, such testing was done at one of the clinical testing sites with 36 specimens. Of these 36, seven specimens had negative MTD test results. None of those seven specimens showed inhibition using that spike-in test.

A rRNA spike-in procedure was performed retrospectively at the manufacturer's facility or at a separate clinical laboratory not participating in the clinical evaluation with 819 specimens. The summary presented did not separate inhibition testing done for MTD negative specimens (indicated for inhibition testing) from MTD positive specimens. Of the 819 specimens with inhibition tests performed from reserved lysates, 151 or 18.4% were inhibitory. For specimens from 36 patients with smear positive specimens and from 19 patients with all smear negative specimens that were included in the clinical evaluation, all specimens from one smear positive patient (1/36 or 2.8%) and 3 smear negative patients (3/19 or 15.8%) were inhibitory.

This testing does not represent the inhibition effects for MTD testing of freshly prepared lysates according to the directions for use of the assay. Inhibition effects could be different when testing fresh lysates because amplification inhibitors have been shown to be labile in other amplification systems. Inhibition may be the cause of MTD negative results and thus a negative MTD test should not be used to rule out the presence of *M. tuberculosis*. Inhibition testing of specimens with negative MTD tests may improve the reliability of negative MTD tests, but use of the inhibition testing recommended in the directions for use (using an *M. tuberculosis* cell spike-in procedure) was not evaluated for effectiveness during the clinical study.

Clinical Study

The objective of the applicant's study was to evaluate the performance of the MTD Test for detection of *M. tuberculosis* complex rRNA in respiratory specimens from patients suspected to have active pulmonary TB. Suspicion for TB was determined at each site based on tuberculin skin test, abnormal or unstable chest x-ray, risk factors or clinical evidence. Patients were eligible for the evaluation if not currently receiving anti-tuberculous treatment for more than seven days, not previously treated within three months, and if a medical history was available. Sample size targeted was 227 patients without pulmonary TB, based on statistical power needed to show a 4% increase in specificity for the modified MTD over the original MTD (92.1% specificity estimate by a patient analysis). Each site was expected to contribute 100 evaluable patients, 20 of whom would have pulmonary TB. MTD testing was performed at seven clinical laboratory sites:

State University of New York, Health Science Center at Brooklyn
University of Zurich, Tuberculosis Laboratory and Swiss National Center for
Mycobacteria
Veterans Affairs Medical Center, Houston
University of California at San Diego Medical Center
Columbia-Presbyterian Medical Center, New York
University of Texas Medical Branch, Galveston
San Diego County Department of Health Services

These sites represented a European national mycobacteriology laboratory, 5 large U.S. metropolitan hospital centers with TB treatment centers (2 in Texas, 1 in Southern California, 2 in New York City), and one public health laboratory (Southern California). Prevalence of pulmonary TB in the populations these laboratories served was not available.

Of the 397 patients enrolled in the evaluation, 386 had culture, AFB smear and an MTD test done on at least one respiratory specimen. These 386 patients are shown in Table 3, categorized by culture and smear status. Patients without *M. tuberculosis* recovered from culture were categorized into those with pulmonary TB and those without active pulmonary TB based on other clinical findings. Specific criteria for a diagnosis of pulmonary TB or a diagnosis of no TB were not specified when cultures were negative. Patients with all specimens collected greater than 7 days after beginning therapy are listed separately without AFB smear status because AFB smear findings at that time would not necessarily represent initial AFB smear status. Patients with two to three consecutive AFB smear positive specimens followed by smear negative specimens after therapy was begun are grouped with the “All Smear Positive” category. Patients with only one specimen submitted are also noted in each patient category.

Table 3 – Enrolled Clinical Study Patients by culture and smear status

	All Smear +	All Smear -	Mixed Smear	All specimens >7d post rx	Total
<i>M. tuberculosis</i> Culture Positive Patients	33 (7)*	22 (2)*	13	2	70 (9)*
Patients with TB diagnosis and negative cultures	0	5 ** (2)*	1	2 (1)*	8 (3)*
Culture Negative Patients (no mycobacteria) with no TB diagnosed	0	223 (42)*	0	2 (1)*	225 (43)*
Patients with no TB diagnosed and MOTT from ≥ 1 cultures	10 (3)*	57 (6)*	14	2	83 (9)*
Total	43 (10)*	307 (52)*	28	8 (2)*	386 (64)*

* Patients with only one specimen collected for AFB smear and culture.

** One patient had *Mycobacterium* sp. from 1 of 3 cultures and one patient had extra-pulmonary TB (bone).

MTD tests were performed on 1259 specimens from these patients; a repeat MTD test was also done from the reserved lysate for each specimen. Of these 386 patients for whom data were described in the application, 8 patients did not have any MTD tests done within 7 days of starting therapy, one patient's MTD testing was excluded because of a lab error in

performing the MTD Test, and a final diagnosis of TB could not be determined for one patient.

An additional 170 patients had all MTD testing performed with MTD kit lots that were excluded from the study. Although MTD testing was repeated from frozen lysates for 298 specimens when one of these MTD kits was used for the initial testing, the FDA Microbiology Advisory Panel agreed with FDA that use of MTD testing of frozen lysates should not be used to represent performance under the proposed conditions for use. The 170 patients and specimens from other patients that were tested with the excluded kit lots, regardless of whether specimens were retested, were excluded from all final MTD performance analyses.

MTD testing on any specimen was also excluded from analyses if the MTD test was performed more than 7 days after specimen collection, if duplicate specimens collected on the same day were tested (only the first chronologically collected specimen was used in analyses), if six specimens had already been tested, if the specimen contained blood, and if a procedural error was documented when MTD testing was performed. After all specimen exclusions were applied by the applicant, 536 specimens from 206 patients had MTD tests that were not excluded.

MTD results for these remaining 206 patients were evaluated in performance analyses. Of these 206 patients, 57 patients had active pulmonary TB based on the site's final diagnosis (53 had *M. tuberculosis* recovered from respiratory cultures and four patients had culture negative pulmonary TB based on the site diagnosis). The four patients who had included MTD testing done within 7 days of beginning therapy and whose respiratory specimens were culture negative for *M. tuberculosis*, were diagnosed with TB based on other clinical evidence. These included one patient with pleural TB (pleural biopsy culture positive) and probable past pulmonary TB; one patient determined to have disseminated TB at autopsy; one patient with a positive skin test who had been exposed to TB and who had an RUL infiltrate that responded to therapy; and one patient with a prior history of TB and noncompliance who had one of four sputum specimens AFB smear positive (chronic TB). Five patients in the evaluated data had both *M. tuberculosis* and MOTT recovered from cultures (one smear positive, three smear negative, and one patient with mixed smear results). Of the 149 patients categorized as not having pulmonary TB, one patient had extra-pulmonary TB (bone).

The study plan intended to compare MTD test results to the patient's final diagnosis and also to culture results. A method to account for multiple MTD tests was not incorporated into the study plan (except that any one patient could have up to six specimens tested). Because many specimens were excluded from the analysis by the applied criteria and some patients had only one MTD test while others had up to six included in the data, FDA believes that using the first chronological specimen that had an included MTD result provides the least biased representation of performance when evaluating MTD results on a patient basis. Alternatively, the second specimen could also be used but such an analysis would have

fewer numbers of patients represented because 41 out of 206 patients had only one MTD Test included.

For the 206 patients, the first specimen with an included MTD test was smear negative for 167 and smear positive for 39 patients. Table 4 shows MTD Test results for the first specimen of the patients for whom this specimen was AFB smear positive (32 with TB and 7 not diagnosed with TB). Table 5 shows MTD results for patients with the first specimen AFB smear negative (25 patients with TB, 21 of whom were culture positive, and 142 patients not diagnosed with TB). These tables also show the results of the second MTD Test done when a second specimen was collected and the MTD Test was not excluded.

Table 4 – MTD Test results for first specimens evaluated; AFB Smear positive patients

SMEAR POSITIVE PATIENTS			MTD+	MTD-
Patients Diagnosed with TB (All patients had at least one positive culture result) N= 32	1st Specimen		28	4
	2nd Spec n=24	MTD+	21	3
		MTD-	0	0
Patients not Diagnosed with TB (all cultures negative for <i>M. tuberculosis</i>) N=7	1st Specimen		0	7
	2nd Spec n=6	MTD+	0	0
		MTD-	0	6

Table 5 - MTD Tests results for first specimens evaluated; AFB Smear negative patients

SMEAR NEGATIVE PATIENTS			MTD+	MTD-	
Patients Diagnosed with TB N= 25	Culture Positive N=21	1st Specimen		15	6
		2nd Spec	MTD+	7 ^a	2
			MTD-	5	4
	Culture Negative N=4 ^c	1st Specimen		1	3
		2nd Spec	MTD+	1 ^a	0
			MTD-	0	2
Patients not Diagnosed with TB (all cultures negative for <i>M. tuberculosis</i>) N=142	1st Specimen		0	142^d	
	2nd Spec	MTD+	0	1	
		MTD-	0	113 ^{b,d}	

^a One patient's 2nd specimen AFB smear +
^b 2 patients with 2nd specimen AFB smear +
^c 1 disseminated; 1 pleural; 2 response to therapy (one with chronic TB)
^d 1 extra-pulmonary TB (bone)

The performance of the MTD for identifying patients with TB and without TB for smear positive and smear negative patients (using the first specimen smear result) was estimated using the first MTD Test results and independently using the second MTD Test result when available. These performance estimates are shown in Tables 6 and 7. Estimates and confidence intervals are remarkably similar for the smear positive patients when MTD performance from either specimen was used in the analysis.

Table 6 – Performance Estimates with 1st MTD Test only

MTD Performance	Smear Positive Patients			Smear Negative Patients		
			95% CI			95% CI
Sensitivity*	28/32	87.5%	71.0-96.5%	16/25	64.0%	42.5-82.0%
Specificity	7/7	100%	59.0-100%	142/142	100%	97.4-100%
PPV	28/28	100%	87.7-100%	16/16	100%	79.4-100%
NPV	7/11	63.6%	30.8-89.1%	142/151	94.0%	89.0-97.2%

Table 7 – Performance Estimates with 2nd MTD Test only

MTD Performance	Smear Positive Patients			Smear Negative Patients		
			95% CI			95% CI
Sensitivity	21/24	87.5%	67.6-97.3%	10/21	47.6%	25.7-70.2%
Specificity	6/6	100%	54.1-100%	113/114	99.1%	95.2-100%
PPV	21/21	100%	83.9-100%	10/11	90.9%	58.7-99.8%
NPV	6/9	66.7%	29.9-92.5%	113/124	91.1%	84.7-95.5%

Because the directions for use recommend MTD testing of an additional specimen when the MTD result is negative and there is a clinical suspicion that a patient has pulmonary TB, CDRH considered performance using the first two MTD Tests despite the lack of a statistical design component for considering effects of multiple MTD Tests on the analyses. Using the first two MTD Tests in an analysis is confounding when the second MTD test disagrees with the first MTD Test and when a patient had the first MTD Test negative, but no second MTD Test collected or included. The applicant does not provide recommendations in the directions for use to verify positive MTD Test results, but does recommend repeated testing for MTD negative specimens when there is a clinical suspicion of TB. For these analyses, CDRH considered a positive first or second MTD Test to have equal weight and assumed that patients with a second available MTD test had a clinical suspicion for TB. Table 8 shows MTD performance when the first specimen was MTD positive or a second test was MTD positive (when the first MTD test was negative). No patients were excluded from this analysis. Patients with only one MTD Test that was negative are included using the one negative test result.

Table 8 – Performance for First plus Second MTD Tests for all patients

	Smear Positive Patients	Smear Negative Patients
Sensitivity	96.9% (31/32) [83.8-99.9%]	72.0% (18/25) [50.6-87.9%]
Specificity	100% (7/7) [59.0-100%]	99.1% (141/142) [96.1-100%]
PPV	100% (31/31) [88.8-100%]	94.7% (18/19) [74.0-99.9%]
NPV	87.5% (7/8) [47.3-99.7%]	95.3% (141/148) [90.5-98.1%]

Table 9 shows performance parameters using either of two MTD Tests positive, but only considering MTD results for those 165 patients (30 smear positive and 135 smear negative) that had at least two MTD Tests included in the evaluation. This analysis was chosen by the applicant to represent MTD Test performance when multiple MTD Tests are performed. Both analyses in Table 8 and 9 are imbalanced in that patients with more than one specimen tested with MTD had a greater chance for being scored MTD positive. Also, patients with only one specimen cultured would have a lower likelihood of having a diagnosis of pulmonary TB based on culture confirmation.

Table 9 – Performance for First plus Second MTD Tests for patients with 2 MTD Tests

	Smear Positive Patients	Smear Negative Patients
Sensitivity	100% (24/24) [85.8%-100%]	71.4% (15/21) [47.8%-88.7%]
Specificity	100% (6/6) [54.1%-100%]	99.1% (113/114) [95.2%-100%]
PPV	100% (24/24) [85.8%-100%]	93.8% (15/16) [69.8%-99.8%]
NPV	100% (6/6) [54.1%-100%]	95.0% (113/119) [89.3%-98.1%]

Analyses based on more than one MTD Test are conditional on assumptions in the statistical study design that were not prospectively applied in using the MTD Test to categorize patients with and without pulmonary TB at the time MTD testing was performed. These assumptions are that one positive and one negative MTD Test would have the same diagnostic utility regardless of the patient's AFB smear status, as two or more positive and two or more negative MTD tests respectively. The statistical imbalance from patients with multiple specimens tested when estimating patient-based performance of the MTD Test was also not accounted for. CDRH believes conclusions from such analyses are provisional and should be verified in an independent population. Using the first plus second MTD model only for patients with at least two acceptable MTD Tests, excludes 41 of the 206 patients remaining in the evaluable dataset. In order to support the claimed use of the assay, and to avoid loss of patients and introducing further selection bias, CDRH believes that the independent estimates of the first and second MTD Tests (and their corresponding

confidence intervals) represent the range of expected performance with the MTD Test. Those analyses avoid problems with multiple sampling and the lack of interpretive criteria when multiple specimens are tested, while maximizing the available data.

MTD results for all 536 specimens with an included MTD result (up to 6 specimens for some patients) are represented in Table 10 according to the culture status of each specimen (specimens that were *M. tuberculosis* culture positive; those that were *M. tuberculosis* culture negative, but from a patient with other cultures positive for *M. tuberculosis*; those with MOTT recovered; and for those that were culture negative and the patient had no other respiratory cultures positive for *M. tuberculosis*). The last group includes 10 specimens from the 4 patients with culture negative TB and two specimens from the one patient with extra-pulmonary TB. Specimens are separated by the smear findings for the specimen (not the smear status of the patient). MTD results are categorized by MTD - (<30,000 RLU and reported as negative) or MTD + (\geq 30,000 RLU, including equivocal results from 30,000 to 499,999 RLU that are to be repeated, and those \geq 500,000 RLU that are reported as positive). Results from testing the reserved specimen lysate, for each of these specimens is also shown. In practice, only MTD equivocal results would be retested using the lysate, or laboratories may choose to store the lysate overnight prior to MTD testing.

Table 10 – Specimen Analysis

Specimen Culture status				AFB Sm +		AFB Sm -	
		Initial MTD Test :		MTD + *or equivocal**	MTD – (<30,000 RLU)	MTD +* or equivocal**	MTD – (<30,000 RLU)
<i>M. tuberculosis</i> Culture + Specimens (n=117)				76 (4 equiv)	2	33 (5 equiv)	6
	Repeat from stored lysate	MTD+	74 (6 equiv)	1	32 (4 equiv)	1	
		MTD -	2	1	1	5	
<i>M. tuberculosis</i> Culture Negative Specimens from patients with other <i>M. tuberculosis</i> culture positive specimens (n=18)			Initial MTD Test	-	1	1	16
	Repeat from stored lysate	MTD+	-	-	1 (1 equiv)	0	
		MTD -	-	1	-	16	
MOTT Culture positive specimens; no <i>M. tuberculosis</i> (n=93)			Initial MTD Test	0	27	0	66
	Repeat from stored lysate	MTD+	0	0	0	1	
		MTD -	0	27	0	65	
<i>M. tuberculosis</i> Culture Negative Specimens and no other cultures <i>M. tuberculosis</i> positive (n=308)			Initial MTD Test	1^a	3	3^b	301^c
	Repeat from stored lysate	MTD+	1	0	3	3 (2 equiv) ^d	
		MTD -	0	3	0	298	

* MTD + are reported for tests with $\geq 500,000$ RLU

** MTD equivocal are tests with 30,000-499,999 RLU; such results are repeated before reporting

^a one specimen from patient with prior history of TB and noncompliance

^b two additional specimens from patient ^a

^c includes 7 specimens from 3 culture negative pulmonary TB patients

^d includes 1 equivocal specimen from a culture negative pulmonary TB patient

An inter-site analysis of MTD performance was not done because numbers of patients and specimens tested with the MTD were low at most sites (5 of 7 sites had fewer than 20 patients) and prevalence of TB in the sampled patient populations was high due to exclusions or patient selection criteria applied. In the population of 386 patients, 8.9 percent (27/307) of those with negative AFB smears were determined to have pulmonary TB. In the subset of patients that were not excluded from the data analyses, 15 percent (25/167) had pulmonary TB. The rate of

pulmonary TB disease in the patients included in the analysis from each site, the rate of smear positivity (using the first specimen tested with MTD) and the MTD results for these patients (using the first specimen tested) are shown in Table 11.

Table 11 – Site Patient Characterization

Site	All Patients			Patients with TB			Patients without TB		
	Total #	% TB +	% Smear +	#sm +	#sm-	MTD+	#sm +	#sm-	MTD-
31	13	92.3	69.2	9	3	12	0	1	1
32	8	75.0	62.5	4	2	2	1	1	2
35	103	16.5	7.8	6	11(3)*	13	2	84(6)*	86
33	15	20.0	33.3	3	0	3	2	10	12
37	7	/	NA	/	/	/	0	7	7
36	8	25.0	12.5	1	1	1	0	6	6
34	52	32.7	21.2	9	8 (1)*	13	2	33(2)*	35
All	206	27.7	18.9	32	25	44	7	142	149

*patients in parentheses had the first specimen smear negative and subsequent specimen(s) smear positive

Additional Information

The *M. tuberculosis* Nucleic Acid Amplification Testing Performance Evaluation Program administered by CDC has reported false positive MTD Tests (analytic specificity for samples with *M. gordonae*, *M. avium*, and *M. abscessus* was 92.1 percent, 645/700). Each sample is tested once at each of the participating laboratories using the MTD Test.¹²

X. Conclusions Drawn from the Studies

The analytical study data demonstrate that the MTD Test detects a clinically relevant level of *M. tuberculosis* complex rRNA. Although false positive MTD results were found for the modified device with *M. celatum* and *M. terrae-like* strains, these species are rarely encountered as clinical isolates and appropriate warnings are included, and culturing done in conjunction with the MTD test would detect non-tuberculous mycobacteria.

Precision testing demonstrated that with repetitive testing of samples with $\geq 4,000$ CFU *M. tuberculosis* per mL, MTD results were reproducible, but samples with no *M. tuberculosis* were subject to false positive results when procedural errors were noted. With the original MTD, a reproducibility study performed in the manner of a proficiency assessment revealed technical factors (i.e., water bath levels, pipetting, vortexing procedures, and potential amplicon contamination) that may result in false positive results with the MTD. Warnings have been incorporated into the labeling to advise users of the procedural precautions that are necessary to minimize such effects.

A reproducibility study that did not include specimen matrix factors showed variability at one of the testing sites with low-level positive samples. However, because these effects did not result in false negative results, CDRH believes that labeling warnings and procedural precautions along with considerations for interpreting MTD results will address this finding.

In interference studies, grossly bloody specimens and very high levels of WBC-DNA were shown to interfere (falsely negative results in samples with *M. tuberculosis* cells or rRNA present) using the MTD Test. Non-target organisms (e.g., MOTT) that were also amplified in the MTD Test reduced the RLU signal when present in high numbers. At the levels of organisms tested, however, no samples with *M. tuberculosis* rRNA present had false negative results in revised data (P940034/S004). Warnings and considerations for interpretation of negative MTD results address the potential for unreliable MTD results if bloody or extremely purulent specimens are tested.

Validity of Clinical Studies

The clinical study to support effectiveness included MTD testing of specimens from patients that were included in analyses based on whether specimen MTD testing was not excluded. MTD Test results for 722 specimens were not considered in performance analyses. The MTD dataset remaining after applied exclusions was also limited by the applicant's criteria for categorizing patients included in the study. The majority of patients (53/57) considered to have pulmonary TB had one or more respiratory specimens with *M. tuberculosis* recovered from culture. An expert panel reviewed patient records (patient clinical forms, laboratory testing, initial chest radiographs, and follow-up radiographs when available) of 40 patients from the 386 patients enrolled in the study whose specimens were tested with MTD. The majority of these 40 patients had a greater than 15% but less than an 80% probability of having TB. The panel disagreed with the site physician's diagnosis for 6 patients and they also believed that there was insufficient information to assess 3 other patients. CDRH believes it is appropriate to include the 4 patients that had no positive respiratory cultures in the "diagnosed with TB" patient group, but also believes that these patients were not necessarily diagnosed with active pulmonary TB (one patient had disseminated TB and another had pleural TB). It is uncertain whether criteria used in the evaluation would have detected other patients with 'culture-negative pulmonary TB' who were categorized as not having pulmonary TB, particularly for those patients who were treated and may not have had a 3-month followup radiograph available, and those patients with only one specimen submitted for culture. Current recommendations are to perform cultures on specimens collected on three consecutive days to rule out TB.

The criteria for patient selection were not standardized across sites (prevalence of TB in the evaluated population at different sites ranged from 16.5% to 92%), and specimens were tested with MTD if there was a physician determination of suspicion for TB. A standardized or weighted approach for evaluating suspicion was not applied. The final numbers of patients with smear negative pulmonary TB and smear positive non-tuberculosis disease were small with resulting wide confidence intervals for the performance estimates. The number of TB-negative patients was less than the projected sample size for supporting the applicant's hypothesis.

Safety and Effectiveness

The clinical study provides retrospective evidence that the MTD Test can detect *M. tuberculosis* in specimens from patients with active pulmonary TB. CDRH has concluded that the device is safe and effective for the stated intended use when the device procedure is performed with strict

adherence to the directions for use, and use of the MTD Test is limited to selected AFB smear negative specimens from patients who have a clinical suspicion for TB based on signs and symptoms. Because of the potential for false positive MTD results, the likelihood of a positive MTD result correlating with *M. tuberculosis* culture positivity would be expected to decrease if smear negative specimens from patients with a low *a priori* likelihood of disease are routinely tested with MTD. The smear negative patients with specimens tested with MTD and used in the analyses for clinical performance represented a uniquely high prevalence TB population (15% of these patients had pulmonary TB) compared to the expected rate of TB in unselected patients that could be suspected to have TB, and for whom mycobacterial cultures are clinically warranted.

Because of reduced sensitivity relative to culture for smear negative specimens from patients with pulmonary TB, MTD should not be used to rule out TB as a diagnostic consideration and should not replace current culturing practices. Similarly, an MTD negative result in a smear positive patient cannot be used to rule out TB because the negative MTD result may be due to inhibition when *M. tuberculosis* is present, low numbers of *M. tuberculosis* in the presence of MOTT, or MOTT alone. Furthermore, the clinical evaluation had insufficient numbers of patients that were smear positive and did not have pulmonary TB to evaluate MTD performance for this group.

A positive MTD result from a smear negative specimen would be insufficient evidence to support a definitive diagnosis of TB without culture confirmation. Such MTD results may be useful to corroborate a clinical impression for selected patients. Use of MTD Test results to guide therapy decisions and isolation practices has not been evaluated.

MTD testing of multiple patient specimens would likely increase the number of MTD-positive AFB smear-negative patients (based on projected positive predictive values and the confidence intervals for such estimates). Because the overall population evaluated in the clinical study had a high prevalence of TB and MTD performance at individual sites could not be separately evaluated due to sample size issues, the effects of prevalence on the performance of the MTD can only be postulated. Although the Microbiology Advisory Panel recommended a postapproval study to assess prevalence effects on MTD Test performance, CDRH believes that information regarding MTD false positives can be assessed by monitoring MTD user records and an available proficiency testing program.

XI. Panel Recommendation

On May 20, 1999, the FDA Microbiology Devices Panel voted to approve the modified MTD Test with conditions. The conditions specified by the Panel were not absolute, and final form was acknowledged to be subject to negotiations between the FDA and the Sponsor. The conditions were:

1. A graph showing prevalence effects on positive predictive values and guidance for interpreting be included in the package insert.
2. A warning statement to indicate that study data were based on a population with a prevalence of 11% (Note: the final evaluable dataset had a 27.7% overall prevalence; 82% for smear positive patients and 15% for smear negative patients).

3. Positive MTD results for smear negative patients must be confirmed by culture.
4. Separate performance representations for smear negative and smear positive patients.
5. Ninety-five percent confidence bands be included in the analysis of predictive value.
6. Post-approval studies should be conducted to assess the prevalence effects on test performance.
7. Interpretation of MTD results for smear negative patients should consider pretest probabilities.

XII. CDRH Action on the Supplement Application

CDRH agreed with the panel's recommendations and issued an approval order for the applicant's PMA supplement on September 30, 1999.

The applicant's manufacturing and control facilities were inspected in September of 1997 and August of 1998 and the facilities were found to be in compliance with the Good Manufacturing Practice Regulations (GMPs).

XIII. Approval Specifications

Directions for Use: See labeling.

Conditions of Approval: CDRH approval of the PMA supplement is subject to full compliance with the conditions described in the approval order. These postapproval requirements include postapproval reports for the following:

1. Summary results of the Performance Evaluation Program compiled by Centers for Disease Control and Prevention (CDC) that have been made available within the preceding twelve months. The first summary should be filed in a separate periodic report within 6 months of receipt of this approval order and include Performance Evaluation reports made available within the prior 12 month period. Summaries should include an assessment by Gen-Probe Incorporated of any findings or observations that are unique or unusual and that may impact on effectiveness of the device. Thereafter, summaries should be included in the annual report.
2. Summary results of a survey of at least three laboratories for MTD testing done with at least 500 smear negative specimens. This retrospective survey should include the following information: total number of clinical specimens cultured for mycobacteria in each of the preceding three months, total numbers of AFB smear positive and negative specimens, total number of smear negative specimens with MTD Tests performed and reported, the mycobacterial culture results for those smear negative specimens with MTD Tests performed and reported, a coded listing of patients with one or more MTD Tests performed on smear negative specimens, the MTD Test results for each patient and culture results for those specimens, and the number of MTD inhibition tests (and those results) routinely performed for AFB smear positive and AFB smear negative specimens. MTD testing included in the survey should be part of routine clinical testing done in each laboratory (not an evaluation or directed

study). The letter to accompany the survey and the survey form must be submitted to FDA in a periodic report within one month. The survey should be completed within 6 months of marketing the MTD Test with the new intended use and the summary submitted within one month of completion (by nine months post-approval) in a periodic report. The laboratories surveyed should not be ones that participated in the clinical evaluation.

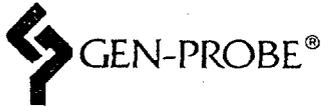
3. All educational, promotional, and advertising materials submitted in a periodic report within the first 2 months following approval and in the annual report thereafter.

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LABELING



AMPLIFIED™

AMPLIFIED MYCOBACTERIUM TUBERCULOSIS DIRECT TEST For *In Vitro* Diagnostic Use 50 Test Kit

INTENDED USE

The GEN-PROBE® AMPLIFIED™ Mycobacterium Tuberculosis Direct (MTD) Test is a target-amplified nucleic acid probe test for the *in vitro* diagnostic detection of *Mycobacterium tuberculosis* complex rRNA in acid-fast bacilli (AFB) smear positive and negative concentrated sediments prepared from sputum (induced or expectorated), bronchial specimens (e.g., bronchoalveolar lavages or bronchial aspirates) or tracheal aspirates.

The MTD test is intended for use only with specimens from patients showing signs and symptoms consistent with active pulmonary tuberculosis. MTD is to be used as an adjunctive test for evaluating either AFB smear positive or negative sediments prepared using NALC-NaOH digestion-decontamination of respiratory specimens. Patients who are suspected of having pulmonary TB based on clinical evaluation and who have received no antituberculous therapy, less than 7 days of such therapy, or have not received such therapy in the last 12 months may be evaluated with this test. The MTD test should be performed only in laboratories proficient in the culture and identification of *M. tuberculosis* (Level II and III or extent 3 and 4)¹. The MTD test must be performed in conjunction with mycobacterial culture.

WARNINGS

The Positive Predictive Value (PPV) of MTD results for AFB smear negative patients is lower than the PPV of MTD results for AFB smear positive patients. The average prevalence of tuberculosis in this population was 27.7% (57/206 patients). The predictive values associated with any diagnostic test are related to the prevalence of disease in a given patient group. Refer to Table 1 in the Limitations section for the hypothetical estimates of positive and negative predictive values across varying prevalence rates.

The efficacy of this test has not been demonstrated for the direct detection of *M. tuberculosis* rRNA using other clinical specimens (e.g., blood, CSF, tissue, urine, or stool). Performance of the MTD test has not been established for sediments processed in a different fashion than described, or stored for different time periods or temperatures than specified in this Package Insert.

Sediments must be cultured to determine if *Mycobacterium* other than tuberculosis complex (MOTT) are present in addition to *M. tuberculosis* complex and to perform antimycobacterial susceptibility testing. Culture for AFB should also be performed to determine which subspecies of the *M. tuberculosis* complex (e.g., *M. bovis*) is present.

M. celatum and *M. terrae*-like organisms will cross-react if present at concentrations higher than 30 colony forming units (CFU) per test. However, *M. celatum* and *M. terrae*-like organisms are rarely isolated from clinical specimens.

Samples may be MTD test negative and *M. tuberculosis* complex culture positive. This condition may be caused by inhibition of the MTD test or the presence of low levels of the *M. tuberculosis* complex organism.

Specimens from pediatric patients have not been evaluated with the MTD test.

The MTD test is not indicated for use with specimens from patients being treated with antituberculous agents to determine bacteriologic cure or to monitor response to such therapy.

Specimens that are grossly bloody should not be tested with the MTD test; blood may cause nonspecific positivity in the MTD test.

Sediments must be resuspended in a phosphate buffer concentration of 67 mM⁸. Concentrations substantially above 67 mM may interfere with amplification of the MTD test, decreasing the ability to detect *M. tuberculosis* complex in the specimen. Sediments prepared using Alpha-Tec Systems, Inc. NAC-PAC™ XPR-plus™ A. F. B. Processing Buffer have been shown to interfere with amplification.

PRECAUTIONS

- A. For *In Vitro* Diagnostic Use.
- B. The MTD test does not differentiate among members of the *M. tuberculosis* complex, i.e., *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. microti*, and *M. canettii*.
- C. A negative test does not exclude the possibility of isolating an *M. tuberculosis* complex organism from the specimen. Test results may be affected by specimen collection and transport, specimen sampling variability, laboratory procedural errors, sample misidentification, and transcriptional errors.
- D. Use only for the detection of members of the *M. tuberculosis* complex using sediments prepared following the NALC-NaOH or NaOH procedures recommended by the Centers for Disease Control (CDC)⁸. This test may only be used with concentrated sediments prepared from sputum (induced or expectorated), tracheal aspirates, or bronchial specimens (e.g., bronchoalveolar lavages or bronchial aspirates). Care must be taken when resuspending the sediment in phosphate buffer to ensure that the phosphate concentration is 67 mM⁸. Final specimen digestant concentrations greater than 1.5% NaOH may inhibit detection of *M. tuberculosis* complex.
- E. Avoid contact of Detection Reagents I and II with skin, eyes, and mucous membranes. Wash with water if contact with these reagents occurs. If spills of these reagents occur, dilute with water before wiping dry.
- F. Use universal precautions when performing this test⁴. Preparation of digested and decontaminated sediments, and MTD procedures, should be done using Biosafety Level 2 practices⁵.
- G. Use only supplied or specified disposable laboratory ware.

- H. Work surfaces, pipettors, and equipment must be decontaminated of rRNA amplicon with a 1:1 dilution of household bleach (1 part of bleach and 1 part of water) as described in the TEST PROCEDURE. Work surface may be wiped with water after 15 minutes to remove the bleach.
- I. Positive displacement pipettors or air displacement pipettors with hydrophobically plugged tips must be used when performing this test. When transferring lysate from Lysing Tube to Amplification Tube, extended length hydrophobically plugged tips must be used. A separate disposable tip must be used for each reaction tube. Waving of a pipette tip containing specimen over the rack of tubes should be avoided. Spent pipette tips must be immediately discarded in an appropriate biosafety waste container.
- J. When using repeat pipettors for reagent addition, after the lysate has been added to the tube, avoid touching the tube with the pipette tip in order to minimize the chance of carryover from one tube to another. The reagent stream should be aimed against the interior wall of the test tube to prevent splashing. Careful pipetting is important to avoid carryover contamination.
- K. Separate pipettors must be used for steps that precede amplification and those that follow amplification.
- L. After reading reaction tubes in the luminometer, decontaminate and carefully dispose of them as described in the TEST PROCEDURE in order to avoid contamination of the laboratory environment with amplicon.
- M. Sealing cards or snap caps should be disposed of in an appropriate biosafety waste container immediately after removing them from reaction tubes. Fresh sealing cards or snap caps should always be used to avoid cross-contamination. These materials should NEVER be reused from a previous step. Sealing cards should be firmly fixed to the top of all reaction tubes.
- N. Do not cover water bath during incubations, especially when using snap caps. (Condensation from the cover may be a possible source of contamination.)
- O. Adequate vortexing after addition of Selection Reagent is necessary to achieve accurate assay results. DO NOT VORTEX LYSATES. Please see NCCLSSMM-3A, sections 8.2, 11, and 12 for additional guidance on testing of reagents and controls, inhibition, and general laboratory procedures^{9,10}.
- P. A segregated area for the Hybridization Protection Assay (HPA) step is recommended to minimize amplicon contamination in the assay. This dedicated area should be separated from the specimen and reagent preparation and amplification areas.
- Q. To help prevent lab areas from becoming contaminated with amplicon, the laboratory area should be arranged with a uni-directional workflow. For example, proceed from specimen and reagent preparation to amplification and then to HPA areas. Specimens, equipment, and reagents should not be returned to the area where a previous step was performed. Also, personnel should not move back into previous work areas without proper anti-contamination safeguards. It is strongly recommended that the biosafety cabinet used for specimen processing not be used for performing the MTD test.

SUMMARY AND EXPLANATION OF THE TEST

The MTD test utilizes Transcription-Mediated Amplification (TMA) and the Hybridization Protection Assay (HPA²) to qualitatively detect *M. tuberculosis* complex ribosomal ribonucleic acid (rRNA). The MTD test will detect rRNA from both cultivable and non-cultivable organisms. Organisms of the *M. tuberculosis* complex include *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. microti*, and *M. canetti*^{4,15}. The MTD test can detect all organisms within the *M. tuberculosis* complex. However, *M. microti* infects only animals, *M. bovis* is uncommonly transmitted from infected animals to humans, and *M. africanum* causes pulmonary disease in humans in tropical Africa¹⁴. *M. canetti* was recently described as a member of the *M. tuberculosis* complex, although rarely described as an etiologic agent of disease¹⁴. *M. tuberculosis* is by far the most common member of the complex that is responsible for human disease worldwide. The CDC has recently reported a rise in the incidence of tuberculosis associated with AIDS, foreign-born cases, and increased transmission in higher risk populations^{7,11}. There has also been a rise in the number of *M. tuberculosis* strains that are resistant to one or more than one antituberculous drugs¹³. The public health implications of these facts are considerable.

Conventional culture methodologies can detect tuberculosis growth as early as 1 week, but may take up to 8 weeks^{8,12}. Comparatively, the MTD test provides detection of *M. tuberculosis* complex rRNA within 2.5 to 3.5 hours after beginning the test procedure. Thus, while the MTD test cannot ascertain drug susceptibility, it can result in rapid and reliable detection of *M. tuberculosis*. This could lead to more appropriate use of isolation facilities, more appropriate initiation of therapy, and earlier detection and containment of infected contacts³.

PRINCIPLES OF THE PROCEDURE

The MTD test is a two-part test in which amplification and detection take place in a single tube. Initially, nucleic acids are released from mycobacterial cells by sonication. Heat is used to denature the nucleic acids and disrupt the secondary structure of the rRNA. The Gen-Probe TMA method, using a constant 42°C temperature, then amplifies a specific mycobacterial rRNA target by transcription of DNA intermediates, resulting in multiple copies of mycobacterial RNA amplicon.

M. tuberculosis complex-specific sequences are then detected in the RNA amplicon using the Gen-Probe HPA method. The Mycobacterium Tuberculosis Hybridization Reagent contains a single-stranded DNA probe with a chemiluminescent label. This probe is complementary to *M. tuberculosis* complex-specific sequences. When stable RNA:DNA hybrids are formed between the probe and the specific sequences, hybridized probe is selected and measured in a GEN-PROBE® LEADER® luminometer.

REAGENTS

Reagents for the MTD test are provided as follows:

(50 Test Kit)

<u>Reagent Name</u>	<u>Volume</u>
MYCOBACTERIUM TUBERCULOSIS AMPLIFICATION TRAY	
Mycobacterium Specimen Dilution Buffer (SDB) <i>Tris buffered solution containing < 3% detergent</i>	1 x 2.5 mL
Mycobacterium Tuberculosis Amplification Reagent (A) <i>Nucleic acids lyophilized in tris buffered solution containing 5% bulking agent</i>	1 x 3 mL (when reconstituted)
Mycobacterium Amplification Buffer (AB) <i>Aqueous solution containing preservatives</i>	1 x 3 mL
Mycobacterium Oil Reagent (O) <i>Silicone Oil</i>	1 x 10 mL
Mycobacterium Enzyme Reagent (E) <i>Reverse transcriptase and RNA polymerase lyophilized in HEPES buffered solution containing < 10% bulking agent and ≥ 15 mM N-acetyl-L-cysteine</i>	1 x 1.5 mL (when reconstituted)
Mycobacterium Enzyme Dilution Buffer (EDB) <i>Tris buffered solution containing a surfactant and glycerol</i>	1 x 1.5 mL

MYCOBACTERIUM TUBERCULOSIS HYBRIDIZATION TRAY

Mycobacterium Tuberculosis Hybridization Reagent (H) <i>< 100 ng/vial non-infectious DNA probe with a chemiluminescent label lyophilized in succinate buffered solution containing bulking agent and detergent</i>	1 x 6 mL (when reconstituted)
Mycobacterium Hybridization Buffer (HB) <i>Succinate buffered solution containing < 4% detergent</i>	1 x 6 mL
Mycobacterium Selection Reagent (S) <i>Borate buffered solution containing surfactant</i>	1 x 15 mL
Mycobacterium Lysing Tubes <i>Glass Beads, Bulking Agent</i>	2 x 25 Tubes

STORAGE AND HANDLING REQUIREMENTS

A. The following liquid or unreconstituted components must be stored at 2° to 8°C and are stable until the expiration date indicated:

- Mycobacterium Specimen Dilution Buffer (SDB)
- Mycobacterium Tuberculosis Amplification Reagent (A)
- Mycobacterium Amplification Buffer (AB)
- Mycobacterium Enzyme Reagent (E)
- Mycobacterium Enzyme Dilution Buffer (EDB)
- Mycobacterium Tuberculosis Hybridization Reagent (H)

The *reconstituted* Mycobacterium Tuberculosis Amplification Reagent (A) is stable for 2 months at 2° to 8°C. The Mycobacterium Tuberculosis Hybridization Reagent (H) and the Mycobacterium Enzyme Reagent (E) are stable for 1 month at 2° to 8°C after reconstitution. Once reconstituted, seal stoppered vials with parafilm to prevent exposure to air and store in an upright position to prevent spillage.

B. The following kit components are stable when stored at 2° to 25°C until the expiration date indicated.

- Mycobacterium Oil Reagent (O)
- Mycobacterium Hybridization Buffer (HB)
- Mycobacterium Selection Reagent (S)
- Mycobacterium Lysing Tubes

SPECIMEN COLLECTION, STORAGE, TRANSPORT, AND PROCESSING

Specimen Collection and Storage:

Specimens must be collected in sterile plastic containers, and stored at 2° to 8°C until transported or processed. Specimens included in the clinical trial were stored for no more than 7 days (generally less than 24 hours) prior to processing.

Transport:

Transport specimens to the laboratory as soon as possible. Federal requirements for packaging must be met when specimens are transported by common land and air carriers. Refer to 42 CFR, Part 72. The most current requirements may be obtained from the Centers for Disease Control and Prevention, Office of Health and Safety in Atlanta, Georgia at (404) 639-3883.

Processing (Decontamination and Concentration):

Specimens that are grossly bloody should not be tested with the MTD test. The MTD test is designed to detect rRNA of members of the *M. tuberculosis* complex using respiratory sediments prepared from generally accepted current adaptations of the NALC-NaOH or NaOH decontamination protocols described by the CDC using 1% to 1.5% NaOH for 15 to 20 minutes and centrifugation at $\geq 3,000 \times g^8$. Resuspension fluids other than phosphate buffer (67 mM) or bovine serum albumin should not be used. Final specimen concentrations of NaOH other than 1 to 1.5% should not be used for processing specimens to be tested.

Processed Sediment Storage:

Sediments may be stored at 2° to 8°C for up to 3 days prior to testing.

MATERIALS

Materials Provided

Cat. No. 1001	50 tests
MYCOBACTERIUM TUBERCULOSIS AMPLIFICATION TRAY	
Mycobacterium Specimen Dilution Buffer (SDB)	1 x 2.5 mL
Mycobacterium Tuberculosis Amplification Reagent (A)	1 x 3 mL (when reconstituted)
Mycobacterium Amplification Buffer (AB)	1 x 3 mL
Mycobacterium Oil Reagent (O)	1 x 10 mL
Mycobacterium Enzyme Reagent (E)	1 x 1.5 mL (when reconstituted)
Mycobacterium Enzyme Dilution Buffer (EDB)	1 x 1.5 mL
MYCOBACTERIUM TUBERCULOSIS HYBRIDIZATION TRAY	
Mycobacterium Tuberculosis Hybridization Reagent (H)	1 x 6 mL (when reconstituted)
Mycobacterium Hybridization Buffer (HB)	1 x 6 mL
Mycobacterium Selection Reagent (S)	1 x 15 mL
Mycobacterium Lysing Tubes	2 x 25 Tubes
Sealing Cards	1 package

Materials Required But Not Provided

Micropipettes capable of dispensing 25 μ L, 50 μ L, 100 μ L, 200 μ L, and 300 μ L
Vortex mixer
Sterile water (filtered or autoclaved)
Culture tubes
Sterile 3 mm glass beads
Screw cap microcentrifuge tubes
Amplification Positive Cell Controls (e.g., *M. tuberculosis*, ATCC 25177 or ATCC 27294)
Amplification Negative Cell Controls (e.g., *M. gordonae*, ATCC 14470, or *M. terrae*, ATCC 15755)
Household bleach (5.25% hypochlorite solution)
Plastic-backed laboratory bench covers

Additional Materials Available From Gen-Probe:

GEN-PROBE[®] LEADER[®] Luminometer
GEN-PROBE[®] Sonicator
GEN-PROBE[®] Detection Reagent Kit
GEN-PROBE[®] Water Bath (42° \pm 1°C and 60° \pm 1°C)
GEN-PROBE[®] Dry Heat Bath* (42° \pm 1°C, 60° \pm 1°C, and 95° \pm 5°C)
GEN-PROBE[®] Sonicator Rack
Test tube racks
Pipette tips with hydrophobic plugs
Extended length pipette tips with hydrophobic plugs
Tubes, polypropylene, 12 x 75 mm
Micropipettes capable of dispensing 25 μ L, 50 μ L, 100 μ L, 200 μ L, and 300 μ L
Snap top polypropylene caps for 12 x 75 mm tubes
Repeat pipettors

TEST PROCEDURE

Controls

1. Lysis and Amplification Controls

Cells used for the Amplification Cell Positive Control should be a member of the *M. tuberculosis* complex, such as avirulent H37Ra (ATCC 25177) or virulent H37Rv (ATCC 27294). Cells used for the Amplification Cell Negative Control should be MOTT, such as *M. gordonae* (ATCC 14470) or *M. terrae* (ATCC 15755). Controls must be prepared prior to sample testing.

Preparation and Storage of Amplification Controls

- a. Place 3 to 5 sterile 3 mm glass beads in a clean culture tube.
- b. Add 1-2 mL sterile water. Add several 1 μ L loopfuls of growth from the appropriate culture. Cap the tube and vortex repeatedly at high speed.
- c. Allow the suspension to settle for 15 minutes.
- d. Transfer the supernatant to a clean culture tube. Adjust turbidity to the equivalent of a #1 McFarland nephelometer standard using a McFarland reference.
- e. Make a 1:100 dilution (Dilution 1) of the suspension by placing 100 μ L of the #1 McFarland suspension into 10 mL sterile water. Cap and vortex.
- f. Make a second 1:100 dilution (Dilution 2) by placing 100 μ L of Dilution 1 into 10 mL sterile water. Cap and vortex.
- g. Make a third 1:60 dilution (Dilution 3) by placing 100 μ L of Dilution 2 into 6 mL of sterile water. Cap and vortex. Dilution 3 contains approximately 25 CFU per 50 μ L.
- h. Plate 50 μ L each of Dilution 2 and Dilution 3 onto culture medium and freeze remaining stock at -20°C or -70°C (both dilutions are plated due to variability in determining turbidity equivalence of a #1 McFarland).
- i. Determine which dilution provides between 25 and 150 CFU per 50 μ L on the plated culture medium.
- j. To represent the final test volume of 450 μ L, make a final 1:10 dilution (Dilution 4) by thawing the appropriate dilution as determined in the previous step (Dilution 2 or 3). Place 3 mL of this dilution into 27 mL sterile water (Dilution 4 represents the final control at a concentration of 25 to 150 CFU per 450 μ L). Cap and vortex.
- k. Test Dilution 4 with the MTD test. If Dilution 4 performs as expected with the MTD test it should be aliquotted for use as controls.
- l. The dilutions must be aliquotted into clean 1.5 mL screw cap microcentrifuge tubes as single use aliquots (500 μ L) and stored frozen at -20°C for 6 months or -70°C for 1 year. Frost-free freezers must not be used.

Testing of the recommended *M. tuberculosis* cell positive control will monitor for substantial reagent failure only. The positive control is designed to monitor effect of reagents used during processing for interference from excess NaOH and phosphate buffer. Procedural variations in timing or temperatures that may affect efficiency of amplification or adequacy of selection time may not be detected using the recommended cell controls. These recommended controls may be used for internal quality control or users may develop their own internal quality control material, as defined by NCCLS C24-A2. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. Please refer to NCCLS C24-A2 and to NCCLS MM-3A for additional guidance on appropriate internal quality control testing practices.

2. Specimen Inhibition Controls

When the AFB smear is positive and the MTD test is negative for untreated patient specimens, there are 3 conditions that might exist:
(1) the specimen is inhibitory, or

* Heating blocks must have wells properly sized for 12 x 75 mm tubes. Use of GEN-PROBE dry heat bath is recommended.

- (2) the specimen contains *Mycobacterium* other than tuberculosis complex (MOTT), or
- (3) the specimen contains a mixture of large numbers of MOTT and a low number of *M. tuberculosis* complex organisms.

When the AFB smear is negative and the MTD test is negative, there are 3 conditions that might exist:

- (1) the specimen is inhibitory,
- (2) the specimen contains *Mycobacterium* other than tuberculosis complex (MOTT), or
- (3) the specimen does not contain *Mycobacterium tuberculosis* or MOTT.

When the AFB smear is negative and the MTD test is negative, testing may also be performed to establish whether the specimen is inhibitory.

To test for specimen inhibition, the following procedure may be performed:

- a. Place 50 μ L Specimen Dilution Buffer into 2 Mycobacterium Lysing Tubes (seeded and unseeded).
- b. Add 50 μ L Amplification Positive Cell Control and 450 μ L sediment to 1 tube (seeded). Add 450 μ L sediment to the second tube (unseeded). Proceed with the testing as usual.

Interpretation

If the RLU value of the seeded tube is $\geq 30,000$, then the specimen does not contain inhibitory substances that prevent amplification. If the RLU value of the seeded tube is below 30,000, the specimen inhibits amplification and another sample should be evaluated. If the repeat testing of the unseeded specimen is positive, random sampling variability may have occurred (i.e., the first aliquot did not contain target for amplification, while the second aliquot did). Contamination may also be a source of such discrepant results. If inhibitors are present, negative results in the unseeded tube cannot be reliably interpreted. The use of this inhibition control procedure was not evaluated during clinical trials.

3. Laboratory Contamination Monitoring Control

To monitor for laboratory contamination with amplicon or *M. tuberculosis* cells, the following procedure can be performed:

- a. Place 1 mL of sterile water in a clean tube. Wet a sterile polyester or dacron swab with sterile water.
- b. Wipe area of bench or equipment to be tested.
- c. Place the swab in the water and swirl gently. Remove the swab while expressing it along the side of the tube. Discard the swab into a container containing a 1:1 dilution of household bleach.
- d. Add 25 μ L of the water containing the expressed swab material into an Amplification Tube containing 50 μ L Amplification Reagent and 200 μ L Oil Reagent.
- e. Follow the TEST PROCEDURE for amplification and detection.

Interpretation

If the results are $\geq 30,000$ RLU, the surface is contaminated and should be decontaminated by treating with bleach as recommended in TEST PROCEDURE, Equipment Preparation. If contamination of the water bath is suspected, 25 μ L of water bath water can be amplified as described for the expressed swab material providing no antimicrobials are used in the water bath.

Equipment Preparation

1. For optimal transfer of sonic energy in a sonicator, water must be thoroughly degassed according to the following procedure prior to each run:
 - a. Add enough ambient temperature tap water to fill the sonicator bath to within 1/2 inch of the top of the tank.
 - b. Run the sonicator for 15 minutes to thoroughly degas the water.
2. Adjust 1 dry heat bath to 95°C, 1 dry heat bath or water bath to 60°C and another dry heat bath or water bath to 42° \pm 1°C.
3. Wipe down work surfaces, equipment, and pipettors with a 1:1 dilution of household bleach prior to starting. Bleach must be in contact with the surface for at least 15 minutes. Work surfaces may be wiped with water to remove the bleach. Cover the surface on which the test will be performed with plastic-backed laboratory bench covers.
4. Prepare the GEN-PROBE LEADER Luminometer for operation. Make sure there are sufficient volumes of Detection Reagents I and II to complete the tests and ensure that the reagent lines are primed. Refer to the Instrument Operator's Manual for further instructions on loading of Detection Reagents. (Detection Reagents are sold separately.)

Reagent Preparation

This step should be performed prior to beginning specimen preparation. The pipettors used for this step should be dedicated for use in the REAGENT PREPARATION, SPECIMEN PREPARATION, and AMPLIFICATION steps. Do not use for adding *reconstituted* Hybridization Reagent or Selection Reagent to specimen tubes.

Reconstitute the vial (50 tests) of lyophilized Mycobacterium Tuberculosis Amplification Reagent (A) with 3.0 mL Mycobacterium Amplification Buffer (AB). Vortex until the solution is mixed. Let *reconstituted* reagent sit at room temperature until clear. The *reconstituted* Mycobacterium Tuberculosis Amplification Reagent may be stored at 2° to 8°C for 2 months. The *reconstituted* Mycobacterium Tuberculosis Amplification Reagent should be allowed to come to room temperature before use.

Sample Preparation

1. Label a sufficient number of Mycobacterium Lysing Tubes to test the samples and 1 each of the Amplification Cell Positive and Negative Controls. Remove and retain the caps.
2. Pipette 50 μ L Mycobacterium Specimen Dilution Buffer (SDB) into all Mycobacterium Lysing Tubes.
3. Cell Controls: Addition of Specimen Processing Reagents
 - A. Add 1 mL of the NALC/NaOH solution and 3 mL of phosphate buffer used to process sputum with 1 mL of sterile water to a sample processing tube.
 - B. Vortex to mix.
 - C. Transfer 450 μ L of the NALC/NaOH/phosphate buffer solution and 50 μ L Amplification Positive Cell Control dilution to the correspondingly labeled Mycobacterium Lysing Tube.
4. Specimen: Transfer 450 μ L decontaminated well-vortexed specimen from its container to the correspondingly labeled Mycobacterium Lysing Tube.

5. Recap the Mycobacterium Lysing Tubes after addition of each sample.
6. Vortex 3 seconds.

Sample Lysis

1. Push the Mycobacterium Lysing Tubes through the Sonicator Rack so that the reaction mixture in the bottom of the tube is submerged but the caps are above water. Place Sonicator Rack on water bath sonicator. DO NOT ALLOW THE TUBES TO TOUCH THE BOTTOM OR SIDES OF THE SONICATOR.
2. Sonicate for 15 minutes but no more than 20 minutes. Samples and controls that have been sonicated are now referred to as "lysates".

Amplification

The pipettors used for this step must be dedicated for use in the REAGENT PREPARATION, SPECIMEN PREPARATION, and AMPLIFICATION steps (see PRECAUTIONS).

1. Label amplification tubes (12 x 75 mm polypropylene tubes) near the top of the tube with numbers that correspond to those used on the Mycobacterium Lysing Tubes. Also label amplification tubes for each of the Amplification Cell Positive and Negative Controls.
2. Add 50 μL *reconstituted* Mycobacterium Tuberculosis Amplification Reagent to the bottom of each amplification tube using a repeat pipettor. Add 200 μL Mycobacterium Oil Reagent (O) to each amplification tube using a repeat pipettor.
3. DO NOT VORTEX LYSATE. Transfer 25 μL lysate to the bottom of the appropriately labeled amplification tube using a separate extended length hydrophobically plugged pipette tip for each transfer. Remaining lysate may be stored at 2° to 8°C for up to 7 days or stored frozen at -20°C or below for up to 1 month. Frost-free freezers must not be used. For repeat testing of lysates, bring stored lysate to room temperature.
4. Incubate the tubes at 95°C for 15 minutes, but no more than 20 minutes, in the dry heat bath.
5. Prepare the enzyme mix by adding 1.5 mL Mycobacterium Enzyme Dilution Buffer (EDB) to the lyophilized Mycobacterium Enzyme Reagent (E). Swirl to mix. Do not vortex.
6. Transfer the tubes to the 42° \pm 1°C dry heat bath or water bath and allow them to cool for 5 minutes. DO NOT ALLOW THE TUBES TO COOL AT ROOM TEMPERATURE. DO NOT COVER THE WATER BATH.
7. Add 25 μL enzyme mix to each amplification tube using a repeat pipettor while tubes are at 42° \pm 1°C. Shake to mix. Incubate at 42°C for 30 minutes, but no more than 60 minutes. Sealing cards or snap caps should be used during this incubation step. DO NOT COVER THE WATER BATH.

Tubes may be covered and placed at 2° to 8°C for up to 2 hours or at -20°C overnight after the 30 minute incubation. If stored at -20°C overnight, tubes must be completely thawed at room temperature or no greater than 60°C prior to the Hybridization step. If held overnight, snap caps rather than sealing cards should be used.

Hybridization Protection Assay (HPA)

The repeat pipettor used for this step must be dedicated for use in this step only. Perform this step in the dedicated HPA area (see PRECAUTIONS).

1. Reconstitute lyophilized Mycobacterium Tuberculosis Hybridization Reagent (H) with 6 mL Mycobacterium Hybridization Buffer (HB). Mycobacterium Tuberculosis Hybridization Reagent (H) and Mycobacterium Hybridization Buffer (HB) must be at room temperature prior to reconstitution. If Mycobacterium Hybridization Buffer (HB) has been refrigerated, warm at 60°C while swirling gently to ensure that all the components are in solution. Vortex until the solution is clear (this could take up to 1 minute). The *reconstituted* Hybridization Reagent is stable for 1 month at 2° to 8°C after reconstitution. If the *reconstituted* Hybridization Reagent has been refrigerated, warm at 60°C while swirling gently to ensure that all components are in solution.
2. Add 100 μL *reconstituted* Hybridization Reagent to each tube using a repeat pipettor. Cover tubes with sealing cards or snap caps. Vortex 3 times for at least 1 full second each time* at medium speed. To achieve proper mixing in reaction tube(s), maintain tubes in an upright position and allow reaction mixture to reach upper half of tube wall throughout vortexing procedure. (To avoid possible contamination do not allow reaction mixture to come in contact with sealing cards or caps.) After adequate vortexing the reaction mixture should be uniformly yellow.
3. Incubate at 60°C for 15 minutes, but no more than 20 minutes, in a dry heat bath or water bath.

Selection

1. Mycobacterium Selection Reagent (S) must be at room temperature prior to starting the test. Remove tubes from the 60°C water bath or dry heat bath and add 300 μL Mycobacterium Selection Reagent (S) using a repeat pipettor. Cover tubes with sealing cards or snap caps. Vortex 3 times for at least 1 full second each time* at medium speed. To achieve proper mixing in reaction tube(s), maintain tubes in an upright position and allow reaction mixture to reach upper half of tube wall throughout vortexing procedure. (To avoid possible contamination do not allow reaction mixture to come in contact with sealing cards or caps.) After adequate vortexing the reaction mixture should be uniformly pink.
2. Incubate tubes at 60°C for 15 minutes, but no more than 16 minutes, in a dry heat bath or water bath.
3. Remove tubes from the water bath or dry heat bath. Cool tubes at room temperature for at least 5 minutes but not more than 1 hour. Remove sealing cards or caps just prior to detection.

Detection

1. Select the appropriate protocol from the menu of the luminometer software. Use a 2 second read time.
2. Using a damp tissue or lint-free paper towel, wipe each tube to ensure that no residue is present on the outside of the tube, and insert the tube into the luminometer according to the instrument directions. Tubes must be read within 1 hour of Selection Step 3.
3. When the analysis is complete, remove the tube(s) from the luminometer.
4. After reading the reaction tubes, carefully fill them to the top with a 1:9 dilution of household bleach using a squirt bottle. Allow tubes to sit with bleach for a minimum of 1 hour before discarding. This will help to prevent contamination of the laboratory environment with amplicon.

*Due to vortexing equipment differences and set speed variations, a longer vortex time may be required depending on individual vortexing equipment. Adjust vortexer speed and follow vortex handling procedures as described under PROCEDURAL NOTES, Section E, to allow reaction mixture to reach and maintain a height within the upper half of the tube. Adequate vortexing as described is necessary to achieve accurate assay results. Times may be increased up to a total of 15 seconds without affecting assay results.

5. Test tube racks, including racks used for the specimens and tests, should be decontaminated by complete immersion in a 1:1 dilution of household bleach with water for a minimum of 15 minutes. The bleach should then be rinsed off with water and the racks should be wiped dry or allowed to air dry.
6. Decontaminate the laboratory surfaces and equipment using a 1:1 dilution of household bleach.

Repeat Testing

1. To repeat patient specimen testing (e.g. if controls are unacceptable or initially equivocal results are repeated), bring prepared lysate to room temperature. **DO NOT VORTEX LYSATE.**
2. Follow TEST PROCEDURE protocol as outlined, beginning with the Amplification step.

PROCEDURAL NOTES

A. Reagents

1. Enzyme Reagent should not be held at room temperature for more than 15 minutes after it is reconstituted.
2. Mycobacterium Hybridization Buffer (HB) may precipitate. Warming and mixing the Mycobacterium Hybridization Buffer (HB) or reconstituted Hybridization Reagent at 60°C will dissolve the precipitate.
3. Seal reconstituted stoppered vials with parafilm to prevent exposure to air and store in an upright position to prevent spillage.

B. Temperature

1. The amplification, hybridization and selection reactions are temperature dependent; ensure that the water bath or dry heat bath is maintained within the specified temperature range.
2. The tubes must be cooled at 42°C for 5 minutes before addition of enzyme mix for optimal amplification performance.
3. The temperature is critical for the amplification (42° ± 1°C).

C. Time

It is critical that the time limits specified in the TEST PROCEDURE be followed.

D. Water Bath

1. The level of water in the water bath should be maintained to ensure that the entire liquid reagent volume in the reaction tubes is submerged, but the level must not be so high that water gets into the tubes.
2. During the Amplification step, water bath covers should not be used to ensure that condensate cannot drip into or onto the tubes.

E. Vortexing

It is important to have a homogeneous mixture during the Hybridization and Selection steps, specifically after the addition of the *reconstituted* Mycobacterium Tuberculosis Hybridization Reagent (H) (the reaction mixture will be uniformly yellow) and Mycobacterium Selection Reagent (S) (the reaction mixture will be uniformly pink).

Vortexing is the manipulation of a solution to produce a uniform suspension. When the reagents are placed into a test tube and supplied with an external energy source, a rapid rotation of the solution about the tube axis is produced. The output of this rapid rotation is the production of a uniform test suspension. During vortexing the tubes should be held in an upright, vertical position and supported by the top portion of the tube to ensure that adequate vortexing is achieved. If an adequate vortexing motion is achieved, the suspension rotates in a circular motion at a rate capable of lifting the solution to a height within the upper half of the tube. During the Hybridization and Selection steps, this manipulation is applied sequentially 3 times and the vortex maintained for at least 1 full second each time.

TEST INTERPRETATION

The specimen result when tested using the GEN-PROBE AMPLIFIED Mycobacterium Tuberculosis Direct (MTD) Test is interpreted based on an initial negative result (< 30,000 RLU), an initial positive result (≥ 500,000 RLU), or an initial equivocal result (30,000 to 499,999 RLU). The MTD test should be repeated from the reserved lysate when an initial test result is equivocal. A repeat result from the lysate greater than 30,000 is considered positive.

A. Quality Control Results and Acceptability

The Amplification Cell Negative Control and Amplification Cell Positive Control should produce the following values:

Amplification Cell Negative Control < 20,000 RLU
 Amplification Cell Positive Control ≥ 1,000,000 RLU

Patient test results must not be reported if the MTD test control values do not meet the criteria above or limits established in each laboratory. See TROUBLESHOOTING section for further information.

Target values or limits for Amplification Cell Positive and Amplification Cell Negative controls should be determined in each laboratory using test results for each batch of prepared controls. See NCCLS-recommended procedures for using target values and limits, C24-A, Internal Quality Control Testing: Principles and Definitions⁹.

B. Patient Test Results

If the controls do not yield the expected results, test results on patient specimens in the same run must not be reported.

Results:

≥ 500,000 RLU	positive for <i>M. tuberculosis</i> complex rRNA
< 30,000 RLU	negative for <i>M. tuberculosis</i> complex rRNA
30,000 to 499,999 RLU	probable <i>M. tuberculosis</i> complex rRNA positive; repeat to verify results:
	Repeat ≥ 30,000 RLU positive for <i>M. tuberculosis</i> complex rRNA
	Repeat < 30,000 RLU negative for <i>M. tuberculosis</i> complex rRNA

C. Reporting of Results

Results from the MTD test should be interpreted in conjunction with other laboratory and clinical data available to the clinician. Based upon the degree of clinical suspicion, testing of an additional specimen should be considered.

If the initial MTD test result is positive at $\geq 500,000$ RLU, or the repeat MTD test result is positive at $\geq 30,000$ RLU, then report the following:

Report:	<i>Mycobacterium tuberculosis</i> complex rRNA detected. AFB smear (positive or negative).
Additional Information:	AFB culture pending. Specimen may contain both MOTT and <i>M. tuberculosis</i> or <i>M. tuberculosis</i> alone. This test should not be the sole basis for diagnosing tuberculosis. The positive predictive value for a smear negative patient is lower than for a smear positive patient. This is particularly important in test populations where the prevalence of tuberculosis is low and the positive predictive values of diagnostic methods are correspondingly reduced.

If the initial or the repeat MTD test result is negative at $< 30,000$ RLU, then report the following:

Report:	No <i>Mycobacterium tuberculosis</i> complex rRNA detected. AFB smear (positive or negative).
Additional Information:	AFB culture pending. Specimen may not contain <i>M. tuberculosis</i> , the result may be falsely negative due to low numbers of <i>M. tuberculosis</i> in the presence or absence of MOTT, or the result may be falsely negative due to assay interference by specimen inhibitors. Testing of another patient specimen is recommended if active tuberculosis is clinically suspected or specimen inhibition is suspected.

LIMITATIONS

Use only for the detection of members of the *M. tuberculosis* complex using sediments prepared following the NALC-NaOH or NaOH (<1.5%) procedures recommended by the CDC⁷. This test may only be used with sediments prepared from sputum (induced or expectorated), tracheal aspirates, or bronchial specimens (e.g., bronchoalveolar lavages and bronchial aspirates). Other specimen types have not been evaluated using MTD.

The MTD test does not differentiate among members of the *M. tuberculosis* complex, i.e., *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. microti*, and *M. canetti*. Culture recovery is necessary to distinguish *M. bovis* from *M. tuberculosis*. *M. celatum* and *M. terrae*-like organisms will cross-react if present at concentrations higher than 30 CFU per test. However, *M. celatum* and *M. terrae*-like organisms are rare clinical isolates. Culture procedures are also necessary for susceptibility testing.

Test results may be affected by specimen collection and transport, specimen sampling variability, laboratory procedural errors, inhibitors, sample misidentification, and transcriptional errors. A negative test does not exclude the possibility of isolating a *M. tuberculosis* complex organism from the specimen. Performance of the MTD is dependent on operator proficiency and adherence to procedural directions. Laboratory procedural errors may cause false positive or false negative results. Testing should be performed by properly trained personnel.

Table 1 below demonstrates the predictive values associated with the MTD test for a group of patients suspected of having pulmonary tuberculosis based on clinical evaluation. As an example, in a smear negative patient group with a prevalence of 5% the positive and negative predictive values are 84.3% and 98.5% with confidence intervals of 42.1% to 99.6% and 95.6% to 99.8%, respectively. In a smear positive patient group with a prevalence of 20% the positive and negative predictive values are 100% and 99.2% with confidence intervals of 63.1% to 100% and 88.8% to 100%, respectively. These data provide the physician additional information to aid in the diagnosis of tuberculosis. The predictive values associated with any diagnostic test are related to the prevalence of the disease in a given patient group. Positive Predictive Values (PPV) generally increase as the prevalence of disease in the group increases. Negative Predictive Values (NPV) generally decrease as the prevalence of disease in the group increases.

The average prevalence of tuberculosis in the clinical trial for patients suspected of having active pulmonary tuberculosis was 27.7% (57/206). MTD performance in this patient group of 206 patients is presented in the following table as Positive Predictive Value (PPV) and Negative Predictive Value (NPV). The predictive values are shown as a function of prevalence using the sensitivity and specificity generated from the clinical trial data. Data are shown by AFB smear status; predictive value ranges are presented for smear negative patients from 1% to 20% prevalence where the expected disease prevalence is lower than that for smear positive patients. The predictive values for smear positive patients are represented at a range of 20% to 80% prevalence. The sensitivity/specificity were 72.0%/99.3% for smear negative patients and 96.9%/100% for smear positive patients, respectively.

Table 1
Hypothetical Predictive Values as a Function of Prevalence

Patient Status	Predictive Value	Prevalence				
		20%	30%	40%	60%	80%
Smear Positive	PPV	100% (63.1%-100%)	100% (71.5%-100%)	100% (78.2%-100%)	100% (85.2%-100%)	100% (88.4%-100%)
	NPV	99.2% (88.8%-100%)	99.0% (81.7%-99.9%)	98.0% (78.9%-99.9%)	95.5% (79.4%-100%)	88.9% (51.8%-99.7%)
		1%	5%	10%	15%	20%
Smear Negative	PPV	50.8% (1.3%-98.7%)	84.3% (42.1%-99.6)	91.9% (64.0%-99.8%)	94.7% (74.0%-99.9%)	96.2% (79.6%-99.9%)
	NPV	99.7% (96.7%-100%)	98.5% (95.6%-99.8%)	97.0% (92.6%-98.9%)	95.3% (90.5%-98.1%)	93.4% (88.3%-97.1%)

EXPECTED VALUES

A. Range of Control Values Observed in the Clinical Studies
 The RLU range for the controls observed in a 7 site clinical study was:

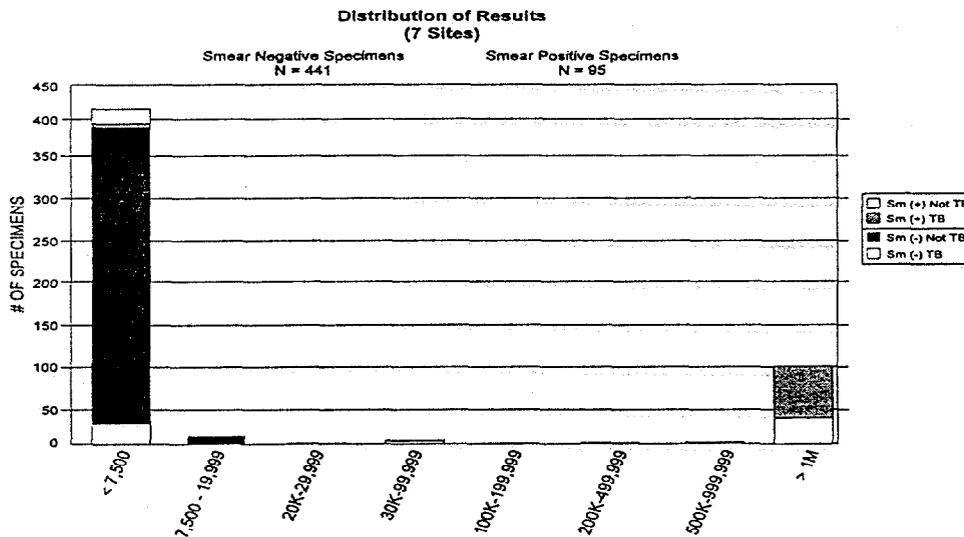
	RLU (N= 339)	
	Range	RLU Values
Amplification Cell Positive Control	556,000 to > 2,000,000*	> 2,000,000
Amplification Cell Negative Control	1300 to 18,800**	mean =3500

*336/339 positive control results were > 1,000,000 RLU.
 **331/339 negative control results were < 7,500 RLU.

B. Range of RLU Values for Clinical Specimens
 A frequency distribution of the RLU values for the 536 clinical specimens from the 206 patients, enrolled in the clinical trial whose MTD results are based on testing of fresh specimens are shown below. Results are presented versus patient diagnosis and are further defined based on the smear status of each specimen.

The range of RLU values for the 104 specimens that were MTD test positive from patients diagnosed with tuberculosis was 541,882 to > 2,000,000 RLU. Nine (9) clinical specimens from patients diagnosed with tuberculosis fell within the equivocal zone on initial testing. Of these equivocal specimens 7 were positive after repeat testing from the reserved lysate.

The range of RLU values for the 389 clinical specimens that were MTD test negative from patients not diagnosed with tuberculosis was 1,335 to 25,146 RLU. No results fell within the equivocal zone from these patients.



Patient Diagnosis	< 7,500	7,500 - 19,999	20K-29,999	30K-99,999	100K-199,999	200K-499,999	500K-999,999	> 1M
Sm (+) Not TB	18	0	0	0	0	0	0	0
Sm (+) TB	5	0	0	2	0	2	2	66
Sm (-) Not TB	363	7	1	0	0	0	0	1
Sm (-) TB	28	0	0	3	1	1	2	34

PERFORMANCE CHARACTERISTICS

A. Clinical Evaluation

1. Study Design

The MTD test was evaluated at 7 geographically diverse clinical sites: a European national mycobacteriology laboratory, a public health laboratory and 5 large metropolitan hospital centers with TB treatment centers. The trial was performed prospectively by enrolling patients suspected of having active pulmonary tuberculosis based on clinical evaluation. Patients enrolled had been on multi-drug anti-tuberculous therapy for 7 days or less within the 3 months prior to study participation. MTD results were compared to the patient's final diagnosis as determined by physician(s) at each site.

Of the 206 patients enrolled in the study whose MTD results were based on testing of fresh specimens, 57 patients were diagnosed with pulmonary tuberculosis (of which 4 patients were culture negative and 4 patients had both MTBC and MOTT recovered); 1 patient was diagnosed with having extra-pulmonary tuberculosis; and 148 patients were diagnosed as not having tuberculosis. The 206 patients that were enrolled in the study contributed 536 specimens that were tested with MTD (a repeat MTD test was also performed from the reserved lysate for each specimen). A patient having one or more positive MTD results was defined as an MTD positive patient.

2. MTD Performance Using Patient Diagnosis as the Endpoint (1st Specimen Analysis)

For the following tables, patient smear status was determined by the smear result from the first specimen collected. Of the 206 patients, the first specimen from 167 patients was smear negative and the first specimen from 39 patients was smear positive. Patients contributed 1 to 6 specimens, with an average of 2.6 specimens per patient. A portion of these 206 patients had no 2nd specimen collected or the 2nd specimen was not available for testing.

Tables A.2-a and A.2-b provide MTD test results (sensitivity, specificity, positive predictive value, negative predictive value and the corresponding 95% confidence intervals) using patient diagnosis as the endpoint, stratified by smear status. Confidence intervals are presented in brackets. Data are presented for the MTD test results from the first specimen collected from all patients as Table A.2-a; data are presented for the cumulative MTD test results from the first two specimens from those patients that contributed two or more specimens as Table A.2-b. For those patients that contributed at least two specimens, if either or both of those specimens are MTD positive, then the patient is defined as MTD positive. A patient whose first specimen evaluated as smear positive is defined as a smear positive patient. A patient whose first specimen evaluated as smear negative is defined as a smear negative patient.

Table A.2-a
MTD Performance Using Patient Diagnosis as the Endpoint
1st Specimen
N=206

	Smear Positive Patient	Smear Negative Patient
Sensitivity	87.5% (28/32) [71.0%-96.5%]	64.0% (16/25) [42.5%-82.0%]
Specificity	100% (7/7) [59.0%-100%]	100% (142/142) [97.4%-100%]
PPV	100% (28/28) [87.7%-100%]	100% (16/16) [79.4%-100%]
NPV	63.6% (7/11) [30.8%-89.1%]	94.0% (142/149) [90.6%-98.1%]

Table A.2-b
MTD Performance Using Patient Diagnosis as the Endpoint
1st and 2nd Specimen
N=165

	Smear Positive Patient	Smear Negative Patient
Sensitivity	100% (24/24) [85.8%-100%]	71.4% (15/21) [47.8%-88.7%]
Specificity	100% (6/6) [54.1%-100%]	99.1% (113/114) [95.2%-100%]
PPV	100% (24/24) [85.8%-100%]	93.8% (15/16) [69.8%-99.8%]
NPV	100% (6/6) [54.1%-100%]	95.0% (113/119) [89.3%-98.1%]

3. MTD and Other Laboratory Test Performance Using Patient Diagnosis as the Endpoint

The following tables present MTD test results as well as results collected from the other laboratory methods used in the study for the 206 patients evaluated. Results of each method are presented using patient diagnosis as the endpoint. This is a by patient analysis and all specimens from a patient are utilized in categorizing that patient. The number of specimens per patient ranges from 1 to 6, with an average of 2.6 specimens per patient. A patient contributing one or more positive MTD results is defined as an MTD positive patient. A patient contributing one or more BACTEC (or LJ or 7H10/7H11) culture positive results for MTBC is defined as a BACTEC (or LJ or 7H10/7H11) positive patient. Data are presented overall in Table A.3-a as well as by patient smear status in Tables A.3-b and A.3-c.

Table A.3-a
MTD and Other Laboratory Test Performance Using Patient Diagnosis as the Endpoint

Test	Sensitivity	Specificity	PPV	NPV	# Patients
MTD	86.0% (49/57) [74.2%-93.7%]	99.3% (148/149) [96.3%-100%]	98.0% (49/50) [89.4%-99.9%]	94.9% (148/156) [90.1%-97.8%]	206
Smear	64.9% (37/57) [51.1%-77.1%]	89.3% (133/149) [83.1%-93.7%]	69.8% (37/53) [55.7%-81.7%]	86.9% (133/153) [80.5%-91.8%]	206
BACTEC	84.2% (48/57) [72.1%-92.5%]	100% (149/149) [97.6%-100%]	100% (48/48) [92.6%-100%]	94.3% (149/158) [89.5%-97.4%]	206
LJ	80.7% (46/57) [68.1%-90.0%]	100% (142/142) [97.4%-100%]	100% (46/46) [92.3%-100%]	92.8% (142/153) [87.5%-96.4%]	199
7H10/7H11	80.0% (44/55) [67.0%-89.6%]	100% (149/149) [97.6%-100%]	100% (44/44) [92.0%-100%]	93.1% (149/160) [88.0%-96.5%]	204

The following tables, Tables A.3-b and A.3-c, are derived from Table A.3-a and are stratified by the patients' smear status. A patient whose first specimen evaluated as smear positive is defined as a smear positive patient. A patient whose first specimen evaluated as smear negative is defined as a smear negative patient.

Table A.3-b
MTD and Other Laboratory Test Performance Using Patient Diagnosis as the Endpoint
Smear Positive Patients

Test	Sensitivity	Specificity	PPV	NPV	# Patients
MTD	96.9% (31/32) [83.8%-99.9%]	100% (7/7) [59.0%-100%]	100% (31/31) [88.8%-100%]	87.5% (7/8) [47.3%-99.7%]	39
BACTEC	96.9% (31/32) [83.8%-99.9%]	100% (7/7) [59.0%-100%]	100% (31/31) [88.8%-100%]	87.5% (7/8) [47.3%-99.7%]	39
LJ	87.5% (28/32) [71.0%-96.5%]	100% (7/7) [59.0%-100%]	100% (28/28) [87.7%-100%]	63.6% (7/11) [30.8%-89.1%]	39
7H10/7H11	96.7% (29/30) [82.8%-99.9%]	100% (7/7) [59.0%-100%]	100% (29/29) [88.1%-100%]	87.5% (7/8) [47.3%-99.7%]	37

Table A.3-c
MTD and Other Laboratory Test Performance Using Patient Diagnosis as the Endpoint
Smear Negative Patients

Test	Sensitivity	Specificity	PPV	NPV	# Patients
MTD	72.0% (18/25) [50.6%-87.9%]	99.3% (141/142) [96.1%-100%]	94.7% (18/19) [74.0%-99.9%]	95.3% (141/148) [90.5%-98.1%]	167
BACTEC	68.0% (17/25) [46.5%-85.1%]	100% (142/142) [97.4%-100%]	100% (17/17) [80.5%-100%]	94.7% (142/150) [89.8%-97.7%]	167
LJ	72.0% (18/25) [50.6%-87.9%]	100% (135/135) [97.3%-100%]	100% (18/18) [81.5%-100%]	95.1% (135/142) [90.1%-98.0%]	160
7H10/7H11	60.0% (15/25) [38.7%-78.9%]	100% (142/142) [97.4%-100%]	100% (15/15) [78.2%-100%]	93.4% (142/152) [88.2%-96.8%]	167

Sensitivity was decreased for all tests in smear negative patients, whereas specificity remained high. There were 25 smear negative patients who were diagnosed with TB. MTD correctly identified 72.0% (18/25) of the 25 patients which smear missed completely. The MTD test was comparable to the individual culture media whose sensitivity in smear negative patients ranged from 60.0% to 72.0%.

4. MTD vs. Culture Status

The following table A.4-a is a by specimen presentation of the MTD result versus the final culture result stratified by that specimen's smear status. An MTBC culture specimen is defined as positive if any of the three culture media are positive for MTBC. An MTBC culture negative specimen is defined as having all culture media negative for MTBC. The number of MTD results that were initially in the equivocal zone are presented in parentheses, e.g., (1 equiv).

Table A.4-a
MTD vs. Culture Status

	AFB Sm (+)		AFB Sm (-)		Total
	MTD+	MTD-	MTD+	MTD-	
MTBC Culture Positive	75 (3 equiv)	3 (1 equiv)	32 (4 equiv)	7 (1 equiv)	117
MTBC Culture Negative from patients with other MTBC culture positive specimens	0	1	1	16	18
MTBC Culture Negative/MOTT recovered	0	27	0	66	93
MTBC Culture Negative/no MOTT recovered	1*	3	3(2)*	301**	308
Total	76	34	36	390	536

* specimen(s) from patients diagnosed with culture negative TB

** includes 7 specimens from patients diagnosed with culture negative TB

B. Precision Studies

Precision panels, consisting of 2 negative samples, 2 low positive samples (= 100 CFU/test) and 2 moderately high positive samples (= 1000 CFU/test) were tested at 3 sites. The positive samples were prepared by spiking a contrived moderately inhibitory sediment pool with known amounts of *M. tuberculosis*. The samples were tested in triplicate twice a day for 3 days at the 3 sites. Positive and negative amplification controls were included in each run.

Because there was no significant site-to-site or day-to-day variability observed; the data from all 3 sites were combined and are presented below. The RLU values measured are limited by the luminometer photomultiplier tube. Therefore, values greater than 2,000,000 RLU are truncated.

Table B.1
Precision Studies

	# Observations	% Correct	Range (RLU)	Mean (RLU)
Sample 1 High Positive	108	100%	> 2,000,000	> 2,000,000
Sample 2 Low Positive	108	100%	> 2,000,000	> 2,000,000
Sample 3 Negative	105*	100%	1,484 -13,129	2,605
Positive Cell Control	54	100%	> 2,000,000	> 2,000,000
Negative Cell Control	52**	100%	2,129 -3,525	2,542

* Three observations have been removed from final study results as a result of operator error.

**Two observations have been removed from final study results due to one operator reporting splashing during one run. One observation yielded a positive result; one observation yielded a result in the equivocal zone.

C. Reproducibility

Reproducibility Panel

The reproducibility panel consisted of 25 samples with Amplification Cell Negative Controls interspersed between each sample for a total of 50 samples. The Reproducibility Panel was tested at 4 sites.

Overall, 100% (120/120) of the negative samples yielded the expected results and 97.5% (78/80) of the positive samples yielded the expected results (two equivocal results were obtained from the low positive sample at one study site).

Clinical Specimen Lysate Reproducibility

Specimens in the clinical study were repeat tested from the reserved specimen lysate. Data below represents the 536 specimens tested with MTD from fresh specimens.

There were 104 specimens from patients diagnosed with tuberculosis whose initial test results were > 500,000 RLU. Upon repeat testing, 103 of 104 yielded values greater than 30,000 RLU; 95 RLU values were > 500,000 RLU, and 8 values ranged from 30,208 to 489,836 RLU.

The initial and repeat test result of 1 specimen with > 500,000 RLU was from a patient diagnosed without tuberculosis.

There were 9 specimens from patients diagnosed with TB and no specimens from patients not diagnosed with tuberculosis that were initially in the equivocal zone. Upon retesting, 7 specimens were greater than 30,000 RLU, of which 4 were greater than 500,000 RLU.

There were 389 specimens with initial test results < 30,000 RLU. Upon repeat testing, 386 yielded values < 30,000 RLU, 1 was in the equivocal zone, and 2 were > 500,000 RLU.

Thirty-three (33) specimens from patients diagnosed with tuberculosis had initial MTD results of < 30,000 RLU. Upon repeat testing of the reserved lysate, 30 were < 30,000 RLU, 1 was in the equivocal zone and 2 were > 500,000 RLU.

No specimens were originally in the equivocal zone from patients not diagnosed with tuberculosis.

D. Analytical Specificity

Specificity of the MTD test was assessed using bacteria, fungi, and viruses. For bacteria and fungi, specificity testing included 159 strains (150 species from 62 genera) of closely related mycobacteria, other organisms causing respiratory disease, and normal respiratory flora or organisms representing a cross-section of phylogeny. Type strains were obtained from the American Type Culture Collection (ATCC), and 5 isolates were obtained from clinical laboratories. Lysates prepared from actively growing cultures (or rRNA in 3 cases) were evaluated in the MTD test according to the TEST PROCEDURE. Approximately 5 x 10⁷ CFU per reaction were tested. Only strains of the *M. tuberculosis* complex yielded positive results, with the exception of the spiked samples of *M. celatum* and *M. terrae*-like strains.

At concentrations higher than 30 CFU per test, *M. celatum* and some *M. terrae*-like strains will yield positive MTD test results. At a level of 30 CFU per test, *M. celatum* yielded 26,772 RLU and *M. terrae*-like ranged from 19,470 to 49,976 RLU.

E. Inhibition Testing

There were 57 patients diagnosed with active pulmonary tuberculosis in this study. Specimens from 55 patients were available and tested for inhibition. Of these 55 patients, 36 patients contributed smear positive specimens. All specimens from 1 patient were inhibitory, yielding an inhibition rate of 2.8% (1/36). Nineteen (19) patients contributed all smear negative specimens. All specimens from 3 patients were inhibitory, yielding an inhibition rate of 15.8% (3/19).

There were 8 patients who were MTD false negative and specimens from 7 patients were available and tested for inhibition. For 3 of these patients, all specimens contributed were inhibitory to amplification. These patients contributed 1, 3, and 3 specimens, respectively.

F. Limits of Detection

Thirty (30) strains of *M. tuberculosis* from a wide geographic distribution, including representative drug-resistant and drug-sensitive strains, were detected with the MTD test. The MTD test was positive with >1,000,000 RLU for 1 CFU per test of all 30 strains.

G. Recovery

Mycobacterium tuberculosis rRNA at a concentration equivalent to 5 CFU per test (25 fg) was tested in the presence of approximately 540,000 CFU per test (450 µL) of the following relevant non-target organisms: *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Mycobacterium gordonae*, *M. avium*, *M. kansasii*, *M. terrae*, *Nocardia asteroides*, *N. otitidis-caviarum*, *Corynebacterium pseudotuberculosis*, *C. diphtheriae*, *Gordona sputi*, and *Rhodococcus bronchialis*. All test results were positive for *M. tuberculosis* rRNA in the presence of these non-target organisms.

TROUBLE SHOOTING

OBSERVATION	POSSIBLE CAUSES	RECOMMENDED ACTIONS
Elevated Amplification Cell Negative Controls	<ul style="list-style-type: none">• Insufficient mixing or volume added after addition of the Mycobacterium Selection Reagent (S).• Insufficient care taken during set up of the reactions and the resultant amplification of contaminating materials introduced at that time.• Contamination of lab surface or reagents.• Skipped 5 minute cool down step.• Failure to wipe tubes prior to reading in the luminometer.	<p>Achieve complete mixing. Ensure correct volume is added. Visually verify a uniformly pink solution after vortexing.</p> <p>Exercise extreme care when pipetting. The spent reaction tubes must be decontaminated with a 1:9 dilution of household bleach as described in the TEST PROCEDURE section. Laboratory bench surfaces, dry heat bath, water baths and pipettors must be decontaminated with a 1:1 dilution of household bleach as described in the TEST PROCEDURE.</p> <p>Tubes must be wiped with a damp tissue or lint-free paper towel prior to reading in the luminometer.</p>
Low Amplification Cell Positive Controls	<ul style="list-style-type: none">• Performed the amplification step outside the recommended temperature range.• Added Amplification reagent to the side instead of to the bottom of the tube.• Insufficient mixing after addition of the <i>reconstituted</i> Mycobacterium Tuberculosis Hybridization Reagent.• Added too much Selection Reagent.• Allowed the Selection step to go over the recommended time limit.• Allowed the tubes to cool down below 42°C after the 95°C incubation.• Detection Reagent lines clogged.	<p>Check water bath and/or dry heat bath temperature and adjust as necessary to achieve the temperature ranges specified in procedure.</p> <p>Carefully vortex as specified. (See Hybridization, Step 2.) Visually verify solution is yellow after vortexing.</p> <p>Check pipettor volume setting.</p> <p>Carefully time the 60°C incubation in the Selection step to be 15 minutes.</p> <p>Transfer tubes directly from the 95°C dry heat bath to the 42°C water bath/dry heat bath.</p> <p>Perform warm water flushes as described in the Instrument Operator's Manual.</p>

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