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-PAC™ 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 (ID_{50} 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 cavitary 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

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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 (HPA™) 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 µL 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 (≥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 X 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 X 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
***M. tuberculosis* 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 evaluting 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 *M. tuberculosis*) 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 *M. tuberculosis*) 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

	Smear Positive Patients			Smear Negative Patients		
MTD Performance			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

	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 + (≥30,000 RLU, including equivocal results from 30,000 to 499,999 RLU that are to be repeated, and those ≥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 -	
M. tuberculosis Culture + Specimens (n=117)	Initial MTD Test :		MTD + *or equivocal**	MTD – (<30,000 RLU)	MTD +* or equivocal**	MTD – (<30,000 RLU)
			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
M. tuberculosis Culture Negative Specimens from patients with other *M. tuberculosis* 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 *M. tuberculosis* (n=93)	Initial MTD Test		**0**	**27**	**0**	**66**
	Repeat from stored lysate	MTD+	0	0	0	1
		MTD -	0	27	0	65
M. tuberculosis Culture Negative Specimens and no other cultures *M. tuberculosis* 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 ≥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

	All Patients			Patients with TB			Patients without TB		
Site	**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 ≥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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