Class II Special Controls Guideline: Nucleic Acid-Based *In Vitro* Diagnostic Devices for the Detection of *Mycobacterium tuberculosis* Complex and Genetic Mutations Associated with *Mycobacterium tuberculosis* Complex Antibiotic Resistance in Respiratory Specimens

Guideline for Industry and Food and Drug Administration Staff

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health
Office of *In Vitro* Diagnostics and Radiological Health
Division of Microbiology Devices

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Preface

Public Comment

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Table of Contents

1. INTRODUCTION 1

2. BACKGROUND 2

3. SCOPE 3

4. RISKS TO HEALTH 5

5. DEVICE DESCRIPTION 7

a. Intended Use 8
b. Test Methodology 8
c. Instrumentation – Hardware and Software 9
d. Controls 12
   1) Negative Controls 12
   2) Positive Controls 12
   3) Internal Control 13
   4) Extraction Control 13
e. Ancillary Reagents 14
f. Testing Procedures Using Your Device 15
g. Interpreting and Reporting Test Results 15

6. PERFORMANCE STUDIES 16

a. General Study Recommendations 16
b. Analytical Studies 16
   1) Nucleic acid extraction 16
   3) Analytical Reactivity (Inclusivity) 18
   4) Analytical Specificity 18
   5) Precision Studies 22
   6) Specimen Collection, Storage, and Shipping Studies 23
   7) Device Shipping and Device Storage Studies 23
   8) Carry-Over and Cross-contamination Study (for multi-sample assays and devices that require instrumentation) 23
c. Clinical Studies 24
   1) Reference Method 25
   2) Study Protocols 27
   3) Study Sites 28
   4) Study Populations 28
   5) Data Analysis and Sample Size 28
   6) Electronic Data Submission 29

7. LABELING 29

a. Intended Use 30
b. Device Description 30
c. General Procedure 30

d. Directions for Use 30
e. Quality Control 31
f. Warnings, Contraindications, Precautions, and Limitations 31
g. Specimen Collection 32
h. Interpretation and Reporting of Assay Results 32
i. Performance Characteristics 32
8. REFERENCES 32

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Guideline for Industry and Food and Drug Administration Staff

1. Introduction

This document was developed to support the classification of nucleic acid-based *in vitro* diagnostic devices for the detection of *Mycobacterium tuberculosis* complex (MTB-complex) and the genetic mutations associated with MTB-complex antibiotic resistance in respiratory specimens into class II (special controls). Nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex and the genetic mutations associated with MTB-complex antibiotic resistance in respiratory specimens are qualitative nucleic acid-based devices that detect the presence of *Mycobacterium tuberculosis* complex-associated nucleic acid sequences in respiratory samples. These devices are intended to aid in the diagnosis of pulmonary tuberculosis and the selection of an initial treatment regimen when used in conjunction with clinical findings and other laboratory results. These devices do not provide confirmation of antibiotic susceptibility since other mechanisms of resistance may exist that may be associated with a lack of clinical response to treatment other than those detected by the device.

This guideline identifies measures that FDA believes will mitigate the risks to health associated with these devices and provide a reasonable assurance of safety and effectiveness. Firms submitting a 510(k) for a nucleic acid-based *in vitro* diagnostic device for the detection of MTB-complex and the genetic mutations associated with MTB-complex antibiotic resistance will need either to (1) comply with the particular mitigation measures set forth in the special controls guideline or (2) use alternative mitigation measures, but demonstrate to the Agency's satisfaction

that those alternative measures identified by the firm will provide at least equivalent assurance of safety and effectiveness.

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2. Background

Tuberculosis is a bacterial infection caused by species of MTB-complex.[1] Pulmonary tuberculosis is the most common clinical presentation of tuberculosis in adults, although extra-pulmonary disease is relatively more prevalent in children. Infection with *Mycobacterium tuberculosis* is the most common cause of pulmonary tuberculosis. Although infection with any member of the MTB-complex can lead to pulmonary tuberculosis, *M. bovis* is the cause of active pulmonary tuberculosis in less than 2% of subjects in the United States [Ref. 1], and infection due to members of MTB-complex other than *M. bovis* and *M. tuberculosis* is rare in the United States. Infection occurs by transmission of the organism to a new host through inhalation of airborne particles that contain MTB-complex released from individuals with active pulmonary disease.

Most people who are infected with *M. tuberculosis* are asymptomatic, which is known as latent tuberculosis infection; latent infections never result in clinical disease in most cases. In some people, this organism overcomes the defenses of the immune system, resulting in progression from latent tuberculosis infection to active tuberculosis disease (TB) either relatively soon after infection or after long periods of latency, usually lasting decades. Overall, there is a 5-10% lifetime risk for patients with latent infection to develop active TB disease; however, the risk varies due to many factors, and may be substantially increased by immunosuppression. [Ref.2]

The incidence of active TB in the United States has declined steadily since 1953 except for an increase in 1989–1992 attributed to an increase of active TB in HIV co-infected patients. [Ref.3] There were 9,945cases of active TB reported to the CDC in 2012; approximately 63% of the active TB cases in the United States are imported, i.e., identified among foreign-born persons who were infected prior to residency in the United States. [Ref.3]

FDA concludes that special controls, when combined with the general controls of the Federal Food, Drug & Cosmetic Act (the FD&C Act), are necessary to provide reasonable assurance of the safety and effectiveness of nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex and the genetic mutations associated with MTB-complex antibiotic resistance in respiratory specimens. A manufacturer who intends to market a device of this type must (1) conform to the general controls of the FD&C Act, including the premarket notification requirements described in 21 CFR 807 Subpart E, (2) conform to the special controls identified in 21 CFR 866.3373(b)(2), (3) address the specific issues of safety and effectiveness identified in this guideline, and (4) obtain a substantial equivalence determination from FDA prior to marketing the device.

[1] *M. tuberculosis* complex includes the following species: *M. tuberculosis, M. bovis, M. africanum, M. canetti, M. microti, M. caprae, M. pinnipedi, M. mungi and M. orygis.*

This guideline identifies the classification regulation for nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex and the genetic mutations associated with MTB-complex antibiotic resistance in respiratory specimens (refer to Section 3). In addition, other sections of this guideline list the risks to health and describe mitigation measures that, if followed by manufacturers and combined with the general controls, will address the risks associated with these devices and will generally lead to a timely premarket notification [510(k)] review. This document, will supplement other FDA documents regarding the specific content requirements of a premarket notification submission for nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex and the genetic mutations associated with MTB-complex antibiotic resistance in respiratory specimens. For additional information regarding 510(k) submissions, refer to 21 CFR 807.87 and the Center for Devices and Radiological Health (CDRH) Device Advice: Comprehensive Regulatory Assistance found at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm.

3. Scope

The scope of this document is limited to the devices identified and classified under 21 CFR 866.3373.

21 CFR 866.3373 – Nucleic acid-based *in vitro* diagnostic devices for the detection of *Mycobacterium tuberculosis* complex and the genetic mutations associated with *Mycobacterium tuberculosis* complex antibiotic resistance in respiratory specimens.

(a) *Identification*. Nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex and the genetic mutations associated with MTB-complex antibiotic resistance in respiratory specimens are qualitative nucleic acid-based devices that detect the presence of *Mycobacterium tuberculosis* complex-associated nucleic acid sequences in respiratory samples. These devices are intended to aid in the diagnosis of pulmonary tuberculosis and the selection of an initial treatment regimen when used in conjunction with clinical findings and other laboratory results. These devices do not provide confirmation of antibiotic susceptibility since other mechanisms of resistance may exist that may be associated with a lack of clinical response to treatment other than those detected by the device.

(b) Classification. Class II (special controls). The special controls for this device are:

1) The FDA document entitled "Class II Special Controls Guideline: Nucleic Acid-Based *In Vitro* Diagnostic Devices for the Detection of *Mycobacterium tuberculosis* Complex and Genetic Mutations Associated with *Mycobacterium tuberculosis* Complex Antibiotic Resistance in Respiratory Specimens," which addresses the mitigation of risks specific to the detection of *Mycobacterium tuberculosis* complex. For availability of the document, see §866.1(e).
2) The following items, which address the mitigation of risks specific to the detection of the genetic mutations associated with antibiotic resistance of *Mycobacterium tuberculosis* complex:

i) The device must include an external positive assay control as appropriate. Acceptable positive assay controls include *Mycobacterium tuberculosis* complex isolates containing one or more antibiotic-resistance associated target sequences detected by the device.

ii) The device must include internal controls as appropriate. An acceptable internal control may include human nucleic acid co-extracted with *Mycobacterium tuberculosis* complex containing nucleic acid sequences associated with antibiotic resistance and primers amplifying human housekeeping genes (e.g., RNaseP, β-actin).

iii) The device's intended use must include a description of the scope of antibiotic resistance targeted by the assay, i.e., the specific drugs and/or drug classes.

iv) The specific performance characteristics section of the device's labeling must include information regarding the specificity of the assay oligonucleotides for detecting mutations associated with antibiotic resistance of *Mycobacterium tuberculosis* complex, and any information indicating the potential for non-specific binding (e.g., BLAST search).

v) In demonstrating device performance you must perform:

A) Pre-analytical studies that evaluate:

1) If there is use of any frozen samples in the device performance studies, or if there is a device claim for the use of frozen samples for testing, the effect of freezing samples prior to testing and the effect of multiple freeze/thaw cycles on both antibiotic susceptible and antibiotic resistant strains of *Mycobacterium tuberculosis* complex.

2) Nucleic acid extraction methods. Extraction methods must parallel those used in devices for the detection of *Mycobacterium tuberculosis* complex nucleic acid, and confirm that the detection of the genetic mutations associated with antibiotic resistance is not affected.

B) Analytical studies that analyze:

1) Limit of Detection. Limit of Detection must be determined in the most challenging matrix (e.g., sputum) claimed for use with the device. The Limit of Detection must be determined using both antibiotic susceptible and antibiotic resistant strains of *Mycobacterium tuberculosis* complex. The antibiotic resistant strains must be those with well characterized genetic mutations associated with antibiotic resistance.

2) Analytical Reactivity (Inclusivity). Testing must be conducted to evaluate the ability of the device to detect

genetic mutations associated with antibiotic resistance in a diversity of *Mycobacterium tuberculosis complex* strains. Isolates used in testing must be well characterized. Isolate strain characterization must be determined using standardized reference methods recognized by a reputable scientific body and appropriate to the strain lineage.

3) Within-Laboratory (Repeatability) Precision Testing. Within-laboratory precision studies, if appropriate, must include at least one antibiotic resistant and one antibiotic susceptible strain of *Mycobacterium tuberculosis* complex.

4) Between Laboratory Reproducibility Testing. The protocol for the reproducibility study may vary slightly depending on the assay format; however, the panel must include at least one antibiotic resistant and one antibiotic susceptible strain of *Mycobacterium tuberculosis* complex.

C) Clinical Studies. Clinical performance of the device must be established by conducting prospective clinical studies that include subjects with culture confirmed active tuberculosis. Studies must attempt to enroll subjects at risk for antibiotic-resistant *Mycobacterium tuberculosis* complex; however, it may be necessary to include supplemental antibiotic resistant retrospective and contrived samples. Clinical studies must compare device results to both phenotypic drug susceptibility testing and genotypic reference methods. The genotypic reference method must be a polymerase chain reaction based method that uses primers different from those in the experimental device and confirmed by bi-directional sequencing.

Measures for the mitigation of risks specific to the detection of genetic mutations associated with antibiotic resistance of *Mycobacterium tuberculosis* complex associated with the device can be found in 21 CFR 866.3373(b)(2) and must be followed.

This special controls document is not intended to address devices for the detection of MTB-complex antigens, for assessment of host serological or immunological responses to MTB-complex infection, or for non-respiratory specimen types. Additionally, this guideline is not intended to address the performance of devices for other intended uses. Those seeking guidance for devices that fall outside of the scope of this special controls document may contact the Division of Microbiology Devices.

4. Risks to Health

FDA has identified the risks of false negative and false positive test results, which can lead to individual and public health consequences, as risks to health associated with this device that require special controls. In addition, FDA has identified biosafety risks to healthcare workers

handling specimens and control materials with the possibility of transmission of tuberculosis infection to healthcare workers as a risk requiring special controls. These risks, and the location of requirements for mitigating them, are summarized in Table 1 below.

Failure of nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex and the genetic mutations associated with MTB-complex antibiotic resistance in respiratory specimens to perform as indicated, or an error in the interpretation of test results may lead to misdiagnosis and improper patient management. In addition, device errors may lead to inaccurate epidemiological information that could contribute to less effective public health responses. A false positive result may lead to incorrect treatment of the individual with possible adverse effects, unnecessary patient isolation, unnecessary patient and general public distress, and unnecessary patient contact investigation. A false negative result may lead to disease progression and the risk of transmitting antibiotic resistant TB to contacts and the general public.

Under this guideline, manufacturers who intend to market a device of this type must conduct a risk analysis prior to submitting a premarket notification to identify any other risks specific to their device. The premarket notification must describe the risk analysis method used. If you elect to use an alternative approach to mitigate a particular risk identified in this guideline, or if you or others identify additional potential risks from use of a device of this type, you must provide sufficient detail regarding the approaches used to mitigate these risks and a justification for your approach.

Table 1 – Identified Risks and Mitigation Measures

Identified Risks	Mitigation Measures
False positive test results for the presence of *Mycobacterium tuberculosis* complex may lead to incorrect treatment of the individual with possible adverse effects. The patient may be subjected to unnecessary isolation. Unnecessary contact investigations may also occur.	The FDA document entitled "Class II Special Controls Guideline: Nucleic Acid-Based In Vitro Diagnostic Devices for the Detection of *Mycobacterium tuberculosis* Complex and Genetic Mutations Associated with Antibiotic Resistance in Respiratory Specimens," which addresses this risk through: Section 5 (Device Description Containing the Information Specified in the Special Control Guideline) Section 6 (Performance Studies) Section 7 (Labeling)
False negative test results for the presence of *Mycobacterium tuberculosis* complex could contribute to disease progression and increase the risk of transmitting infection to others.	The FDA document entitled "Class II Special Controls Guideline: Nucleic Acid-Based In Vitro Diagnostic Devices for the Detection of *Mycobacterium tuberculosis* Complex and Genetic Mutations Associated with Antibiotic Resistance in Respiratory Specimens," which addresses

	this risk through: Section 5 (Device Description Containing the Information Specified in the Special Control Guideline) Section 6 (Performance Studies) Section 7 (Labeling)
False positive test results for the presence of genetic mutations associated with *Mycobacterium tuberculosis* complex antibiotic resistance may lead to incorrect treatment of the individual with possible adverse effects. The patient may be subjected to unnecessary isolation. Unnecessary contact investigations may also occur.	21 CFR 866.3373(b)(2)
False negative test results for the presence of genetic mutations associated with *Mycobacterium tuberculosis* complex antibiotic resistance could contribute to disease progression and increase the risk of transmitting antibiotic resistant TB to others.	21 CFR 866.3373(b)(2)
Biosafety risks to healthcare workers handling specimens and control materials with the possibility of transmission of tuberculosis infection to healthcare workers	The FDA document entitled "Class II Special Controls Guideline: Nucleic Acid-Based In Vitro Diagnostic Devices for the Detection of *Mycobacterium tuberculosis* Complex and Genetic Mutations Associated with Antibiotic Resistance in Respiratory Specimens," which addresses this risk through: Section 7 (Labeling)

5. Device Description Containing the Information Specified in the Special Control Guideline

You must ensure your device description meets the requirements of 21 CFR 807.87. Your 510(k) submission must include proposed labels, labeling, and advertisements sufficient to describe the device, its intended use, and the directions for its use (see 21 CFR 807.87(e)). Further, the proposed labeling submitted for your device must satisfy the requirements in 21 CFR 809.10(a) and (b). In satisfying 21 CFR 807.87 (e), you must include the intended use, the specimen types that can be used with the device, the technological characteristics of the device, the relevant regulation, and the appropriate product code. In addition to information required to meet 21 CFR 809.10(a) and (b) and 21 CFR 807.87(e), a premarket submission for this type of device must include the descriptive information in the following subsections:

a. Intended Use

You must clearly state the clinical indications for which the test is to be used, as well as the specimen types and the specific population for which the test is intended (e.g., sex, age, symptoms) and any limitations on the device use. Your intended use statement must also specify that the test is qualitative, and that it is to be used as an adjunct to other laboratory tests and clinical findings. In addition, you must include the following statement immediately below the intended use statement:

> *"The [Insert name of test] test should only be performed in laboratories that follow safety practices according to the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories[2] and applicable state or local regulations. The [Insert name of test] test must be performed in conjunction with mycobacterial culture to address the risk of false negative results and to recover the organisms for further characterization and drug susceptibility testing."*

b. Test Methodology

You must include the following elements of test methodology, as applicable:

1. The specific test methodology to be used (e.g., real-time polymerase chain reaction (PCR), transcription mediated amplification, nucleic acid-based signal amplification).
2. Additional information regarding assay oligonucleotides, including:
    - Rationale for the oligonucleotides selected, including a listing of the specific sequences used.).
    - Specificity of these oligonucleotides in distinguishing MTB-complex and any information indicating the potential for non-specific binding.
    - Justifications for alignments made to generate consensus sequences, best-fit modifications, or both made to existent sequences (e.g., to permit maximum homology to several strains).
    - Information on size, guanine-cytosine content, melting temperatures, hairpin or other secondary structures if any, and the nucleotide position on the genome map of the assay oligonucleotides.
3. Assay procedural steps (e.g., pipetting, incubation, washing, and mixing).
4. Methods for collection and handling of each specimen type.
5. Reagent components provided or recommended for use, and their function within the system (e.g., solid support, buffers, fluorescent dyes, chemiluminescent reagents, substrates, conjugates, other reagents).
6. Illustrations or photographs of the device and any non-standard equipment or methods as appropriate.

[2] http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.

When applicable, your submission must describe the device design control specifications that address or mitigate risks, such as false positive results due to sample contamination, associated with nucleic acid-based procedures for detecting MTB-complex. Design control specifications must include the following:

1. Positive, negative, and inhibition controls to ensure accurate test results.
2. Validated methods for nucleic acid extraction to be used with the reagents specified for the test system. These extraction method(s) must be described for each of the different specimen types included in the device intended use statement.
3. Methods for optimizing the reagents and test procedure for recommended instruments.
4. Design features for minimizing risks of exposure to health care personnel.

Your 510(k) submission must provide performance information that supports the conclusion that device design requirements have been met.

c. Instrumentation – Hardware and Software

In your 510(k) submission, you must provide software documentation including:

- A clear description of how raw signals are converted into a result, including adjustment to the background signal for normalization. In addition, you must describe software controls for identifying and managing anticipated problems.

- Information dependent on the level of concern for your type of software (Minor, Moderate, or Major). The level of concern must be driven by a hazard analysis in the absence of mitigations, i.e., the hazard analysis must be performed as though none of the individual hazards mitigations were present. The level of concern determined for the software device must be stated in the submission. The level of concern for these types of devices is expected to typically be moderate. Software flaws could potentially result in patient injury if inaccurate test result information is given to the healthcare provider and the patient. The level of concern is based on how the operation of the software associated with the functioning of the device could affect the patient or operator and is defined below.

    - **Major -** The level of concern is Major if a failure or latent flaw could directly result in death or serious injury to the patient or operator. The level of concern is also Major if a failure or latent flaw could indirectly result in death or serious injury of the patient or operator through incorrect or delayed information or through the action of a care provider.

    - **Moderate –** The level of concern is Moderate if a failure or latent design flaw could directly result in minor injury to the patient or operator. The level of concern is also Moderate if a failure or latent flaw could indirectly result in minor injury to the patient or operator through incorrect or delayed information or through the action of a care provider.

- **Minor -** The level of concern is Minor if failures or latent design flaws are unlikely to cause any injury to the patient or operator.

See Table 2 below for the software documentation required to meet this bullet dependent on the level of concern associated with the device's type of software.

Table 2 - Required Documentation Based on Level of Concern

SOFTWARE DOCUMENTATION	MINOR CONCERN	MODERATE CONCERN	MAJOR CONCERN
Level of Concern	A statement indicating the Level of Concern and a description of the rationale for that level.		
Software Description	A summary overview of the features and software operating environment.		
Device Hazard Analysis	Tabular description of identified hardware and software hazards, including severity assessment and mitigations.		
Software Requirements Specification (SRS)	Summary of functional requirements from SRS.	The complete SRS document.	
Architecture Design Chart	No documentation is necessary in the submission.	Detailed depiction of functional units and software modules. May include state diagrams as well as flow charts.	
Software Design Specification (SDS)	No documentation is necessary in the submission.	Software design specification document.	
Traceability Analysis	Traceability among requirements, specifications, identified hazards and mitigations, and Verification and Validation (V&V) testing.		
Software Development Environment Description	No documentation is necessary in the submission.	Summary of software life cycle development plan, including a summary of the configuration management and maintenance activities.	Summary of software life cycle development plan. Annotated list of control documents generated during development process. Include the configuration management and maintenance plan documents.

Verification and Validation Documentation	Software functional test plan, pass / fail criteria, and results.	Description of V&V activities at the unit, integration, and system level. System level test protocol, including pass/fail criteria, and tests results.	Description of V&V activities at the unit, integration, and system level. Unit, integration and system level test protocols, including pass/fail criteria, test report, summary, and tests results.
Revision Level History	Revision history log, including release version number and date.		
Unresolved Anomalies (Bugs or Defects)	No documentation is necessary in the submission.	List of remaining software anomalies, annotated with an explanation of the impact on safety or effectiveness, including operator usage and human factors.	

- Configuration of the hardware and software components must be very similar or identical to that anticipated for the final version of the device before beginning clinical studies. If any significant changes are made to the hardware or software after the completion of the clinical studies but before the clearance and distribution of the device, you must perform a risk assessment and include it in your 510(k) submission.

The following references may aid in the development and maintenance of a new device under good software life cycle practices consistent with FDA regulations:

- The guidance entitled "General Principles of Software Validation; Final Guidance for Industry and FDA Staff" found at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm085281.htm;
- The guidance entitled "Off-the-Shelf Software Use in Medical Devices" found at http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm073779.pdf;
- The guidance entitled "Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices" found at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089543.htm;
- 21 CFR 820.30 Subpart C – Design Controls of the Quality System Regulation;

- ISO 14971-1; Medical devices - Risk Management - Part 1: Application of Risk Analysis; and
- AAMI SW68:2001; Medical Device Software - Software Life Cycle Processes

For instruments and systems that measure multiple signals, and other complex laboratory instrumentation, please consult with the Division of Microbiology Devices.

d. Controls

For your device, you must include the controls described below in your 510(k) submission, if applicable for your device. Dependent on the test methodology, you may not need to include every type of control recommended in this section. You must define the supplier(s) of the controls and have a plan to provide for their continued availability. When conducting the performance studies described (see Section 6), you must run external controls, using the appropriate specimen type, every day of testing for the duration of the analytical and clinical studies.

1) Negative Controls

Blank or no template control

The blank, or no-template control, contains buffer or specimen transport media and all of the assay components except target nucleic acid. This control is used to rule out contamination with target nucleic acid or increased background in the amplification reaction. This control may not be necessary for assays performed in single test disposable cartridges or tubes.

Negative control

The negative sample control is used to evaluate the complete assay procedure, including extraction. Negative results for this control confirm that signals are not obtained in the absence of target sequences (e.g., due to non-specific priming or detection). Examples of acceptable negative sample control materials include:

- Pooled specimens from MTB-complex nucleic acid negative individuals.
- Pooled specimens containing a non-target mycobacterial organism (e.g., MTB-complex nucleic acid negative specimens spiked with *Mycobacterium gordonae*).
- Surrogate negative control (e.g., simulated sputum with non-target encapsulated nucleic acid).

2) Positive Controls

Positive control for complete assay

The positive control contains target nucleic acids, and is used to test the entire assay process, including sample lysis, nucleic acid extraction, amplification, and detection. It is designed to mimic a patient specimen and is run as a separate assay, concurrently with patient specimens, at

a frequency determined by a laboratory's quality system. Acceptable positive assay controls include MTB-complex isolates containing target sequences detected by the device.

Positive control for amplification and detection

The positive control for amplification and detection contains purified target nucleic acid at or near the limit of detection (LoD) for a qualitative assay and is not usually taken through the sample lysis or nucleic acid extraction process. It verifies the integrity of the reaction components and instrument when negative results are obtained. It also indicates that the target can be detected if it is present in the sample lysates or extracted sample. Examples of this type of control include a non-infectious DNA plasmid containing the target sequence, or purified full length double stranded genomic DNA from an MTB-complex isolate. Packaged RNA would also be an appropriate control for an assay that targets ribosomal RNA.

In some cases where a positive control for the complete assay is included, a separate positive control for amplification and detection would not be needed.

3) Internal Control

The internal control is a non-target nucleic acid sequence that is co-extracted and/or co-amplified with the target nucleic acid. It controls for integrity of the reagents (e.g., polymerase, primers, etc.), equipment function (e.g., thermal cycler), and the presence of inhibitors in the sample. Examples of acceptable internal control materials include:

- Human nucleic acid co-extracted with MTB-complex and primers amplifying human housekeeping genes (e.g., RNaseP, β-actin).
- A non-infectious DNA plasmid containing the non-target nucleic acid that is added to the sample either prior to or after sample lysis and extraction.
- A complete non-MTB complex organism that is added to the sample prior to sample lysis and extraction.
- Packaged RNA for assays that target ribosomal RNA.

The need for this control is determined on a case-by-case basis for each specific device. You may refer to Clinical Laboratory Standards Institute (CLSI) document MM3-A2, *Molecular Diagnostic Methods for Infectious Disease* [Ref. 4], for additional information.

4) Extraction Control

The extraction control is an external control that verifies that lysis of MTB-complex isolates and subsequent nucleic acid isolation has occurred efficiently. Examples of extraction controls include a strain of MTB-complex containing the target sequences, or a known MTB-complex positive clinical specimen. The positive control for the complete assay, or the internal control, may serve as an extraction control.

e. Ancillary Reagents

Ancillary reagents are those reagents that an assay manufacturer specifies in device labeling as "required but not provided" in order to carry out the assay as indicated in its instructions for use. Ancillary reagents of concern for this category of device are those that you specify by the catalog or product number, or other specific designation, in order for the device to achieve its labeled performance characteristics. For example, if the device labeling specifies the use of Brand X nucleic acid amplification enzyme, and use of any other nucleic acid amplification enzyme may alter the performance characteristics of the device from that reported in the labeling, then Brand X nucleic acid amplification enzyme is an ancillary reagent of concern. In contrast, if the device involves the use of 95% ethanol, and any brand of 95% ethanol will allow the device to achieve the performance characteristics provided in the labeling, then 95% ethanol is not an ancillary reagent of concern for the purposes of this document.

If the instructions for use of the device specify ancillary reagents of concern, you must include in your submission a description of how you will ensure that the results obtained by testing with the device and these ancillary reagents will be consistent with the performance described in the premarket submission. Every effort must be made to bring the ancillary reagents under your quality system by recommending use of only those ancillary reagents that have been determined to meet your quality standards for the test. This may include application of quality systems approaches, product labeling, and other measures. Your 510(k) submission must address the elements described below:

(1) Use of ancillary reagents in the device risk assessment, including risks associated with the management of reagent quality and variability, risks associated with any inconsistency between instructions for use provided directly with the ancillary reagent, and those supplied with the device, and any other issues that could present a risk of obtaining incorrect results with the device.

(2) As part of the risk assessment, your submission must describe how you intend to mitigate risks through implementation of any necessary controls over ancillary reagents. These may include, where applicable:

- User labeling to assure appropriate use of ancillary reagents (see **Section 7.d. - Directions for Use** for further discussion);
- Plans for assessing user compliance with labeling instructions regarding ancillary reagents;
- Material specifications for ancillary reagents;
- Identification of reagent lots that will allow appropriate performance of your device (e.g., if only certain lots of a named ancillary reagents are appropriate for use, these lots must be identified in the labeling);
- Stability testing;
- Plans for Addressing User Technical Questions or Concerns (e.g., telephone Help-Line);
- Corrective and preventive actions;

- Plans for alerting users in the event of an issue involving ancillary reagents that would impact the performance of the device; and
- Any other issues that must be addressed in order to assure safe and effective use of your test in combination with named ancillary reagents, in accordance with the device's instructions for use.

In addition, you must provide testing data to establish that the quality controls you supply or recommend are adequate to detect performance or stability problems with the ancillary reagents.

f. Testing Procedures Using Your Device

You must describe in detail the principles of operation of the device applicable to its intended use. You must specifically describe testing conditions, procedures and controls designed to provide safeguards for conditions that can cause false positive and false negative results, or present a biosafety risk. These include, but are not limited to, procedures, methods, and practices incorporated into the directions for use to mitigate risks associated with testing (see **Section 7 - Labeling**).

g. Interpreting and Reporting Test Results

Your 510(k) submission must describe how positive, negative, equivocal (if applicable), or invalid results are determined and how they should be interpreted. This description must indicate the cut-off values for all outputs of the assay and include the following information:

- In particular, you must provide the cut-off value for defining a negative result of the assay. If the assay has only two output results (e.g., detected and not detected), this cut-off also defines a positive result of the assay.
- If the assay has an equivocal zone, you must provide cut-off values (limits) for the equivocal zone.
- If the assay has an invalid result, you must describe how an invalid result is defined.

If proper operation of your device involves retesting of equivocal results, you must provide in the device labeling: (1) a recommendation regarding whether retesting should be repeated from the same nucleic acid preparation, a new extraction, or a new patient specimen, and (2) an algorithm for defining a final result by combining the initial equivocal result and the results after retesting. This algorithm must be developed before the pivotal clinical study that evaluates the clinical performance of the assay.

The device labeling must provide recommendations that describe how the laboratory should follow up an invalid result (i.e., whether the result should be reported as invalid, or whether re-testing is recommended). If re-testing is recommended, information similar to that for the re-testing of equivocal results (i.e., whether re-testing should be repeated from the same nucleic acid preparation, a new extraction, or a new patient specimen) must be included in the device labeling.

6. Performance Studies

a. General Study Recommendations

510(k) submissions for this device category must include complete descriptions of the protocols used during assay development in order for FDA to accurately interpret acceptance criteria and data summaries (in tabular form, where applicable) contained in your application. When referring to Clinical and Laboratory Standards Institute (CLSI) protocols or guidelines, you must indicate which specific aspects of the protocols or guidelines were followed. Relevant findings in published literature may also be cited.

Contact the Division of Microbiology Devices prior to initiating clinical studies to obtain feedback regarding planned studies and to confirm that these studies will support the proposed intended use for the device. Additionally, if you are considering device clearance for use in point-of-care settings, contact the Division of Microbiology Devices early during device development.

b. Analytical Studies

The analytical studies appropriate for a device of this type will depend on the underlying technology, principles of operation, and available scientific evidence specific to the new device. The following analytical studies are always required; however, please note that additional analytical studies may be required depending on the specific device characteristics:

1) Nucleic acid extraction

Different extraction methods may yield MTB-complex nucleic acid of varying quantity and quality. Thus, the extraction method used with (or as part of) a device can be crucial for successful test performance. Purification of MTB-complex nucleic acid from clinical samples can be challenging because samples may contain low mycobacterial loads in the background of human genomic DNA and non-targeted organisms, as well as high levels of proteins and other contaminants.

For these reasons, you must evaluate possible effects of different extraction methods on the performance of the assay if more than one possible extraction method is recommended for use with your device. This must include demonstrating the LoD and reproducibility of your assay with each extraction procedure (see **Section 6(b)(2) -Analytical Sensitivity (Limit of Detection)**). Studies that include external sites (including reproducibility and clinical studies) must employ the extraction procedures intended for inclusion in the marketed product labeling. If different sample types are to be studied and included in the intended use of the device, then LoD and reproducibility studies for each extraction method must be conducted on the most challenging sample type.

You must perform these evaluations regardless of whether you intend to actually provide reagents for extraction and preparation of nucleic acid in your test kit or you simply recommend the use of the appropriate reagents.

Different extraction methods may be studied at different clinical sites. If you intend to use different extraction methods in your studies, each different extraction method must be evaluated analytically (i.e., by LoD and reproducibility testing) to demonstrate that device performance is similar with the different extraction methods. For example, if three different extraction methods are being studied, the reproducibility study can be designed to evaluate a single extraction method at a single testing site (i.e., extraction method A at site 1, extraction method B at site 2, and extraction method C at site 3). If results do not show equivalence between extraction methods from each clinical testing site (using a different nucleic acid extraction method), the results must be discussed with the Division of Microbiology Devices prior to initiating clinical trials.

If different extraction methods are proposed for use with your device, each method must be performed by at least one clinical site during the clinical performance trials.

2) Analytical Sensitivity (*Limit of Detection)*

You must determine the LoD of your device using serial dilutions of clinical isolates of *M. tuberculosis* and *M. bovis.* The isolates must be well characterized strains from resources such as the U.S. Centers for Disease Control and Prevention (CDC), American Type Culture Collection (ATCC), and German Collection of Microorganisms and Cell Cultures (DSMZ), among others. Testing must be performed for each specimen type included in the intended use.

Your LoD studies must examine possible device variability by testing three to five samples over three to five days for each serial dilution. The LoD of the device must be estimated as the level of each MTB-complex strain that gives a 95% detection rate. The estimated LoD must be confirmed by preparing at least 20 additional replicates at the LoD concentration and demonstrating that the MTB-complex strain was detected 95% of the time. The required reference method for LoD determination is plating and counting bacterial colony forming units (CFU). CFU must be based on colony counts from actual plating and counting of bacteria rather than a theoretical calculation deduced from an estimated cells/mL number (i.e., McFarland units). LoD may also be presented as genomic DNA copy numbers/mL for each dilution; however, whole organisms are still necessary for performing LoD studies. Consult with the Division of Microbiology Devices early during device development if you intend to use genome equivalents/mL for describing the results for your LoD studies.

For studies with respiratory specimens we require the use of either MTB-complex negative respiratory samples or simulated respiratory samples with serial dilutions of each MTB-complex strain to be studied. A simulated sputum sample can be contrived using saline solution, mucin, and human cells. If you choose to use a simulated sputum sample in the LoD study, an analytical sample equivalency study must be carried out to demonstrate that your assay will generate equivalent results using both the natural sample and the simulated sample.

You may refer to CLSI document EP17-A, *Protocols for Determination of Limits of Detection and Limits of Quantitation* [Ref. 5], when designing your LoD studies.

3) Analytical Reactivity (Inclusivity)

You must demonstrate that your device can detect MTB-complex strains representing the global genetic diversity of MTB-complex. The concentration of MTB-complex isolates used in inclusivity studies must be at levels at or near the specific LoD or cut-off value and must be confirmed by plating and counting bacterial CFUs. Isolate strain characterization must be determined using standardized reference methods recognized by a reputable scientific body. Your submission must also cite literature evidence that all species contain the target of interest.

You must demonstrate that your device can detect isolates of MTB-complex to include: *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. microti*, *and M. caprae*.

In addition you must demonstrate that your device can detect isolates representing the *M. tuberculosis* strain families. *In silico* testing may also be acceptable as an alternative for inclusivity testing for select strains that may be difficult to study. Consult with the Division of Microbiology Devices early during device development regarding the applicability of *in silico* testing to your device.

4) Analytical Specificity

(a) Cross-Reactivity

Listed below are microorganisms for testing in cross-reactivity studies. Cross-reactivity studies must include mycobacterial species known to cross react with certain MTB-complex targets (e.g., *M. celatum and M. kumamotonense*), other mycobacterial species related to MTB-complex species, common oral and respiratory tract commensals and pathogens, selected fungi, and selected viruses. You must conduct cross-reactivity studies using a concentration of a minimum 10^6 CFU/mL for mycobacteria, fungi and bacteria; a minimum of 10^5 plaque forming units (PFU)/mL for viruses; and a minimum of 10^6 elementary bodies (EB) or inclusion forming units (IFU)/mL for *Chlamydophila (Chlamydia).* You must confirm the microorganism identities, and must base the concentrations tested on actual counts rather than a theoretical calculation deduced from estimation (e.g., McFarland units or an absorbance reading).

*In silico t*esting may also be acceptable as an alternative for cross reactivity testing for select microorganisms that may be difficult to study. Consult with the Division of Microbiology Devices early during device development regarding the applicability of *in silico* testing to your device.

Category 1: Mycobacteria

M. abscessus	*M. leprae*
M. asiaticum	*M. malmoense*
M. avium	*M. marinum*
M. celatum	*M. mucogenicum*
M. chelonae	*M. scrofulaceum*
M. flavescens	*M. simiae*
M. fortuitum	*M. smegmatis*

M. gastri	*M. szulgai*
M. gordonae	*M. terrae complex*
M. intracellulare	*M. thermoresistibile*
M. kansasii	*M. triviale*
M. kumamotonense	*M. xenopi*

Category 2: Fungi

Candida albicans	*Aspergillus fumigatus*
Candida glabrata	*Blastomyces dermatitidis*
Candida krusei	*Histoplasma capsulatum*
Cryptococcus neoformans	*Penicillium spp.*
Candida parapsilosis	*Rhizopus spp.*
Candida tropicalis	*Scedosporium spp.*

Category 3: Viruses

Adenovirus	Rhinovirus
Human Immunodeficiency Virus	Rubella Virus
Human InfluenzaVirus (Types A and B)	Rubeola Virus
Human Metapneumovirus	Mumps Virus
Human ParainfluenzaVirus (Types 1, 2, 3, 4)	Varicella Zoster Virus
Respiratory Syncytial Virus	

Category 4: Bacteria

Actinomyces israelii	*Kingella kingae*	*Staphylococcus lugdunensis*
Acinetobacter baumannii	*Klebsiella pneumoniae*	*Streptococcus equi*
Acinetobacter calcoaceticus	*Klebsiella oxytoca*	*Streptococcus pneumoniae*
Bacteroides fragilis	*Lactobacillus spp.*	*Streptococcus pyogenes*
Bacillus cereus	*Legionella pneumophila*	*Streptococcus agalactiae*
Bacillus subtilis	*Legionella micdadei*	*Streptococcus salivarius*
Burkholderia cepacia	*Leuconostoc spp.*	*Stenotrophomonas maltophilia*
Chlamydophila (Chlamydia) pneumoniae	*Listeria monocytogenes*	*Streptomyces anulatus*
Citrobacter freundii	*Moraxella catarrhalis*	*Veillonella spp.*
Clostridium spp.	*Mycoplasma pneumoniae*	*Viridans Group Streptococcus (a minimum of 5 different species)*
Corynebacterium diptheriae	*Neisseria sicca*	*Yersinia enterocolitica*
Corynebacterium jeikeium	*Neisseria meningitidis*	*Nocardia farcinica*
Corynebacterium pseudodiptheriticum	*Neisseria gonorrheae*	*Nocardia brasiliensis*
Eikenella corrodens	*Neisseria mucosa*	*Nocardia otitidiscaviarum*
Enterobacteriaceae (including ESBL and KPC producers)	*Neisseria lactamica*	*Rhodococcus equi*
Enterobacter aerogenes	*Pediococcus spp.*	*Tsukamurella spp.*
Enterobacter cloacae	*Peptostreptococcus spp.*	
Enterococcus faecalis	*Proteus mirabilis*	
Enterococcus faecium	*Proteus vulgaris*	
Escherichia coli	*Pseudomonas aeruginosa*	
Fusobacterium spp	*Serratia marcescens*	
Haemophilus influenzae	*Staphylococcus aureus*	
Haemophilus parainfluenzae	*Staphylococcus epidermidis*	
Haemophilus parahemolyticus	*Staphylococcus haemolyticus*	

(b) Interfering Substances

You must conduct a comprehensive interference study. Relevant interfering substances include, but are not limited to, endogenous substances such as blood and mucus, and exogenous substances such as topical nasal and throat medications and oral medications that may be secreted into respiratory secretions. Potentially interfering substances are presented in Table 3 below. Your interference studies must include, at a minimum, a representative of each listed substance or class.

You must evaluate each interfering substance at its highest medically-relevant concentration ("the worst case") in a simulated matrix with a target concentration close to the assay cut-off; if

no significant effect is observed, testing at lower concentrations of the interferent is not necessary.

You may refer to the CLSI document EP07-A2, *Interference Testing in Clinical Chemistry* [Ref. 6], for additional information.

Table 3. Substances and Classes for Interference Testing

Substance and Class	Active Ingredient
Anesthetics (endotracheal intubation)	Lidocaine
Antibacterial, systemic	Tobramycin, Amoxicillin, Levofloxacin
Antibiotic, nasal ointment	Mupirocin
Anti-tuberculosis drugs	Isoniazid, Rifampin, Pyrazinamide, Ethambutol, Streptomycin
Anti-viral drugs	Zanamivir
Blood (human)	
Bronchodilators	Ephedrine hydrochloride, Epinephrine, Theophylline
Gastric Acid	
Homeopathic allergy relief medicine	Stinging Nettle, Goldenseal, Butterbur, Tea Tree Oil
Human DNA	
Inhaled bronchodilators	Albuterol sulfate, Formoterol, Budesonide
Live intranasal influenza virus vaccine (FluMist®)	Live influenza virus vaccine
Mouthwash and Gargle Solutions*	Eucalyptol, Methyl, Salicylate, Thymol, Cetylpyridinium Chloride, Denatured Alcohol
Mucin: Bovine submaxillary gland, type I-S	Purified mucin protein
Nasal corticosteroids	Beclomethasone, Dexamethasone, Flunisolide, Triamcinolone, Budesonide, Mometasone, Fluticasone
Nasal gel (homeopathic)	Luffa opperculata, Sulfur
Nasal sprays or drops	Phenylephrine, Oxymetazoline, Sodium chloride with preservatives
Nebulizing solutions (hypertonic saline)	NaCl (3-5%)
Oral anesthetic/ and Analgesic	Benzocaine, Menthol
Oral Expectorant	Guaifenesin
Physiologic Saline	NaCl (0.9%)
Pneumocystis jiroveci medications	Pentamidine
Specimen Processing Reagents	Cetylpyridinium Chloride (CPC), Oxalic Acid, Neutralization Buffer
Nicotine*	
White Blood Cells (human)	

*Concentrations approximating those in commercial products or literature references.

(c) Cut-off and Equivocal Zone

Your submission must describe how the assay cut-off was determined and validated. Assay cut-off must be determined using appropriate statistical methods. For example, you may provide a result distribution, 95th and 99th percentiles, percentages of the non-negative (positive or equivocal) results, and other descriptive statistics for the clinical samples without MTB-complex in your pilot studies. Selection of the appropriate cut-off can be justified by the relevant levels of sensitivity and specificity based on Receiver Operating Curve (ROC) analysis of the pilot studies with clinical samples. For details about ROC analysis, see CLSI document GP10-A, *Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristics (ROC) Plots*). [Ref. 7] If the assay has an equivocal zone, you must explain how you determined the limits of the equivocal zone. You must validate the performance of your device using the pre-determined cut-off (and equivocal zone, if applicable) in an independent population consistent with the defined intended use of your device.

5) Precision Studies

(a) Within-Laboratory Precision (Repeatability)

Dependent on your device methodology, precision studies may or may not be required; please contact the Division of Microbiology Devices regarding the need for precision studies for your device. If precision studies are required, you must conduct these studies using the instruments and/or automated components anticipated for use in your clinical study. You may perform these studies in-house (i.e., within your own company facility).

You must evaluate sources of variability (such as operators, days, and assay runs) by testing your device for a minimum of 12 days (not necessarily consecutive), with two runs per day and at least two replicates of each sample per run. In some situations, test days spanning at least two calibration cycles may be necessary. The test panel must consist of at least two strains of *M. tuberculosis* spiked in sample matrix or simulated sample matrix (provided that you can demonstrate that your device will generate equivalent results using both the relevant sample matrix and the simulated sample matrix) at three concentration levels that include:

- Negative sample: a sample with no analyte such that results of repeated tests of this sample are negative 100% of the time.
- "Low positive" sample (C 95 concentration): a sample with a concentration of analyte just above the clinical cut-off such that results of repeated tests of this sample are positive approximately 95% of the time.
- "Moderate positive" sample (e.g., approximately two to three times the concentration of the clinical cut-off): sample with a concentration at which one can anticipate positive results approximately 100% of the time.

CLSI documents EP05-A2, *Evaluation of Precision Performance of Quantitative Measurement Methods* [Ref. 8], and EP12-A2, *User Protocol for Evaluation of Qualitative Test Performance* [Ref. 9], contain further information about designing and performing repeatability studies.

(b) Between Laboratory Reproducibility

The protocol for the reproducibility study may vary slightly depending on the device format. In general, the protocol must:

- Evaluate the reproducibility of your device at a minimum of three testing sites; this may include one in-house site. At least two of the sites must be domestic sites.
- Use the same sample panel as described in the within-laboratory precision study above (i.e., negative, low positive, and moderate positive samples).
- Use a five day testing protocol (not necessarily consecutive days), including a minimum of two runs per day (unless the instrument throughput precludes multiple runs per day), with three replicates of each panel sample per run. A minimum number of 90 observations must be provided for each test panel sample across the three sites (with at least 30 observations per site for each test panel sample).
- Have at least two operators at each facility perform the test each day.

CLSI document EP15-A2, *User Verification of Performance for Precision and Trueness* [Ref. 10], contains additional information on reproducibility study design.

6) Specimen Collection, Storage, and Shipping Studies

Unless all specimens are expected to be processed as fresh samples within a specified time frame, you must study device performance under different storage conditions. You must demonstrate that your device generates similar results for the stored specimens at several time points throughout the duration of the recommended storage period and at both ends of your recommended temperature range. The specimen storage studies must include samples close to the cut-off. In addition to the analysis of qualitative results, you must provide analysis of the raw signals, such as cycle threshold (CT), if applicable.

CLSI document MM13-A, *Collection, Transport, Preparation and Storage of Specimens for Molecular Methods* [Ref. 11], contains additional information specific to this topic.

7) Device Shipping and Device Storage Studies

You must evaluate the performance of your device after exposing the device to various shipping and storage conditions that are similar to shipping and storage conditions that you intend to include in your product labeling.

8) Carry-Over and Cross-contamination Study (for multi-sample assays and devices that require instrumentation)

For multi-sample assays and devices that require instrumentation, you must demonstrate that carry-over and cross-contamination does not occur with your device. In a carry-over and cross-contamination study, you must perform an assay run with high positive samples alternating with negative samples in patterns dependent on the operational function of the device. You must perform at least five runs alternating high positive and negative samples. Analyte concentrations in high positive samples must be high enough to exceed 95% or more of the results obtained from specimens of infected patients in the intended use population provide a positive result.

Negative samples must be samples with no analyte such that results of repeated tests of this sample are negative 100% of the time.

The carry-over and cross-contamination effect can then be estimated by the percent of negative results for the negative samples that are adjacent to high positive samples in the carry-over study compared to the percent of negative results in the absence of adjacent high positive samples. In addition, you must provide the following information: an analysis of the raw signals (if applicable) in terms of the median of the raw signal of negative samples, the median of the raw signal of negative samples in the carry-over study, a cross-contamination study and difference in the medians along with 95% confidence intervals.

c. Clinical Studies

The clinical performance (i.e., sensitivity and specificity) of your device must be established from a prospective clinical study (or studies) that includes subjects being evaluated for suspected active tuberculosis. Consult with the Division of Microbiology Devices prior to study initiation regarding proposed studies that may include retrospective or banked specimens to supplement specimens from prospectively enrolled subjects**.**

It is anticipated that sputum will be the most common respiratory specimen type tested with these devices; accordingly your study must be statistically powered (see Section 6.c.(5)) to demonstrate acceptable device performance for sputum samples. It is acceptable to combine induced and expectorated sputum samples for analysis purposes. If bronchial specimens are also intended for use with the device, discuss your proposed studies with Division of Microbiology Devices.[3] Tracheal aspirates are considered comparable to sputum and do not need to be studied independently.

You must establish early during device development whether your device is intended to test respiratory specimens directly or after undergoing specimen processing[4] (i.e., digestion-decontamination-concentration) and clearly state this information in your study protocol. If your device is intended for use both with specimens tested directly and with specimens that have undergone processing, your clinical studies must include both direct and processed specimens.

The specimen processing method(s) intended for use with your device (e.g., NALC-NaOH, Oxalic Acid, Cetylpyridinium Chloride) must be established early during device development and clearly stated in your study protocol. If more than one method of specimen processing is intended for use with your device, each of those methods must be evaluated during your clinical studies.

There must be at least two specimens tested by the investigational device and reference method per subject. This may be achieved by aliquoting two separate subject samples or collecting four separate specimens from each subject. If both unprocessed and processed respiratory specimens

[3] Describe in a Pre-submission the number of patients with non-sputum specimens that will be enrolled in the clinical studies. The use of non-sputum specimen types must be supported by analytical studies demonstrating that the different matrix has no effect on device performance.

[4] Throughout this special controls guideline, the term “processed” or “processing” is used to describe a combination of digestion, decontamination, and centrifugation to pellet of respiratory specimens.

will be tested in the same study, one specimen must be tested directly with your device and the other must be tested with your device as a processed specimen. These respiratory specimens must either be randomized by method (i.e., direct or processed), within subjects, or follow a set sequence (e.g., testing of direct specimen followed by processed specimen for all subjects).

Your clinical study protocol must also clearly describe whether patient specimens will be split for testing between both your device and the reference method, or if multiple patient specimens will be used. Both methods may be permissible within a study depending on specific specimen volume.

In general, the following clinical studies principles must be followed:

- Clinical samples must be collected from a minimum of three geographically diverse locations, one of which must be in the United States.
- Laboratory testing using your device must be performed at a minimum of three different sites representing settings where the device is intended for use. Laboratory testing sites may be the same as the clinical enrollment sites. One laboratory testing site may be the manufacturer's laboratory.
- Reference method testing may be conducted at a centralized laboratory.
- The collection, transport, and testing of specimens to be tested with the investigational device must be performed by individuals with training equivalent to that anticipated for users of the marketed device.
- Clinical enrollment sites and laboratory testing sites must be supervised by qualified principal investigators

1) Reference Method

Your clinical studies must compare the performance of your device to a composite reference method derived from the results of culture and identification, and direct specimen nucleic acid amplification. More specifically, the composite reference method is defined as testing for MTB-complex by:

i. Mycobacterial culture and isolate identification **AND**

ii. Direct specimen testing using a FDA cleared or approved nucleic acid amplification based diagnostic device or a non-FDA cleared or approved validated direct nucleic acid PCR amplification test followed by bi-directional sequencing.

If you use a non-FDA cleared or approved validated direct nucleic acid PCR amplification test followed by bi-directional sequencing, then you must provide additional information regarding the safety and effectiveness of the test to support a determination by the Agency that the test is appropriate for use.

A positive result is defined as a sample which tests positive by **either** i **or** ii above. A negative result is defined as a sample which tests negative by **both** i **and** ii above.

Table 4: Composite Reference Method Result Interpretation for Each Specimen

Composite Reference:		Composite Reference Method Result Interpretation
Direct Specimen Culture	**Direct Specimen PCR**	
+	-	+
-	+*	+
+	+	+
-	-	-

*If a non-FDA cleared or approved nucleic-acid PCR amplification test is used as part of the composite reference for direct specimen testing, a positive PCR result must be confirmed by bi-directional sequencing.

Mycobacterial culture refers to isolation of the organism using liquid media, solid media, or both, followed by identification of the cultured isolates using FDA-cleared molecular probes, high-performance liquid chromatography, mass spectrometry, or sequencing of DNA from isolates.

If non-FDA cleared or approved PCR tests are used as part of the reference method, you must provide information about the targets and primers used, any literature references describing their use, and validation of the PCR method used. The PCR method must use alternative primers than your device, and must be followed by bi-directional sequencing of all PCR positive specimens.

You may refer to CLSI document MM18-A, *Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing* [Ref. 12], for guidelines specific to target sequencing of MTB-complex.

Your study protocol must include a description of the specific tests used in the composite reference method determination. If composite reference method testing is done at multiple sites rather than a single central laboratory, your study protocol must describe any differences in the methods used across testing sites. You must describe all quality control measures used for your device and for the tests included in the composite reference method.

While not directly included as part of reference method testing, you must perform acid-fast smears using fluorochrome staining on all respiratory specimens included in the clinical study. These data must be included in your submission for subgroup analysis of device performance based on acid-fast smear status.

Due to the nature of TB disease, we recognize that it is possible that a small percentage of samples may be positive by your device but negative by the reference method. This may be due

to inhibition of growth of the organism in culture due to the effect of processing on the respiratory specimen or low numbers of organisms in the sample, rather than the result being an actual false positive. This could be addressed in your study design by specifying a definition of clinical tuberculosis that includes response to treatment during follow-up. [Ref. 13] This may be included as a secondary analysis of test performance which may improve the specificity of your investigational device, particularly for devices with high sensitivity.

2) Study Protocols

Your clinical study protocol(s) must be finalized prior to study initiation. The protocol(s) must include complete patient inclusion and exclusion criteria, study procedures, a description of where the tests will be performed, a detailed statistical analysis plan that includes the statistical analysis methods to be used, justification of the study sample size, and other components as appropriate. The study protocol must be explicit regarding whether results from your device will be used for patient management; if so, an Investigational Device Exemption (IDE) may be required for study sites in the United States (see Section 6(c)(3) below). You must use FDA data standards for capturing clinical trial data in case report forms and data analysis sets, as appropriate; see Study Data Standards Resources found at http://www.fda.gov/ForIndustry/DataStandards/StudyDataStandards/default.htm for current CDRH data standards.

In the protocol(s) you must describe blinding procedures, for both clinicians and laboratory personnel, used to ensure that operators of your device are not aware of reference assay results or the results from other diagnostic evaluations (e.g., acid-fast smear results) and laboratory personnel performing reference method testing are not aware of results from your device.

Any planned interim analyses of the study data or examinations of study progress must be described in the clinical protocol and the statistical analysis plan, and submitted to the Division of Microbiology Devices for review prior to the start of your clinical studies. In addition, any changes to the study protocol based on examination of the study data, (e.g., addition of high prevalence sites due to a lower than expected prevalence of tuberculosis), must be submitted to the Division of Microbiology Devices for review with a revised protocol.

You must include copies of the original study protocols, and all protocol modifications in your 510(k) submission with any other relevant study information.

Contact the Division of Microbiology Devices to request a review of your proposed clinical study protocols while in the development stage as part of the pre-submission review process. For guidance related to the clinical protocol, you may also refer to the guidance entitled “Design Considerations for Pivotal Clinical Investigations for Medical Devices” found at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm373750.htm.

In addition, your clinical protocol must also specify acceptable specimen transport methods, storage conditions, and maximum storage times (if appropriate). Study case report forms must capture any time-sensitive steps (e.g., the amount of time a specimen is stored if not tested immediately). The protocol must also describe safety precautions for the collection, handling, processing, and testing of specimens that will be tested during the study.

3) Study Sites

You must collect respiratory samples from at least three different clinical sites in geographically separate locations. At least one of the sites must be located in the United States.

Your device must be tested at a minimum of three different testing sites representing environments where the device would be used (e.g., clinical laboratories), and by laboratory personnel with training similar to those likely to perform the test in laboratory settings. Testing sites must document all quality control results and all repeat tests for runs with out-of-range quality control values.

Clinical investigations of FDA unapproved and uncleared *in vitro* diagnostic devices, including these devices, are subject to the IDE provisions of section 520(g) of the FD&C Act (21 U.S.C. 360j(g)) and the implementing regulations. You must consider how 21 CFR part 812 (IDEs) applies to your particular study and refer to 21 CFR part 50 (informed consent) and 21 CFR part 56 (institutional review board review) for other applicable requirements.

4) Study Populations

Subjects enrolled in your clinical studies must be patients suspected of having active tuberculosis who meet study inclusion and exclusion criteria. Patients who have been treated for tuberculosis for less than seven days in the past six months can be enrolled. The study inclusion and exclusion criteria must match the intended use population of your device. A minimum set of demographic characteristics, including age, sex, HIV status (including CD4 count and viral load if available), the presence of other relevant medical conditions and/or medications (including tobacco use), signs and symptoms of tuberculosis (with date of onset), and radiographic results, must be captured. You must also record the results of the tuberculin skin testing (TST) and interferon gamma release assay (IGRAs), if available.

Due to the unique aspects of studying tuberculosis in children, including the possible use of gastric aspirates for diagnosis, manufacturers who wish to include children in their device clinical studies must contact the Division of Microbiology Devices early in device development.

5) Data Analysis and Sample Size

The data analysis plan must clearly specify the primary analysis, which must be based on the specimens collected during the clinical trials. Results must be based on a minimum of two separate specimens from each patient. The reference method result must be based on results from all specimens combined (i.e., if any specimen is positive using Table 4 above, then reference testing must considered positive for that patient). Results for your device must be analyzed serially (i.e., sensitivity and specificity for the first specimen against the overall reference result must be determined, followed by sensitivity and specificity of serial specimens combined against the reference method).

You must include in the data analysis plan a mechanism to account for all subjects enrolled and for all specimens collected. This information must be provided in the 510(k) submission as well as explanations of all specimens and subjects not included in analysis of the device performance. The 510(k) submission must also include comparisons of device performance against the

composite reference method in an appropriate tabular format, and additional analyses for pre-defined patient sub-populations such as those infected with HIV.

The sample size for your study must be based on the number of subjects providing respiratory specimens and not on the number of respiratory specimens collected per patient. Studies must be powered to evaluate the intended use of your device.

The sensitivity of your device must be presented both as an overall result and separately for smear positive and smear negative patients enrolled. In general, approximately 30% of the enrolled subjects must be acid-fast smear negative, and approximately 70% must be acid-fast smear positive. It is anticipated that the point estimate for sensitivity will be approximately 99% in acid-fast smear positive TB subjects and no less than 72% in acid-fast smear negative subjects. Point estimates of device performance (i.e., sensitivity and specificity) for respiratory specimen types other than sputum must be similar or superior to results for sputum. All results must include 95% two-sided confidence intervals; for methods of calculation of confidence intervals, see CLSI EP12-A2. [Ref. 9]

If both processed and unprocessed specimens were tested you must evaluate whether results for processed and unprocessed specimens are sufficiently similar for the two specimen types to be combined in your analyses.

For device specificity the lower limit of a 95% two-sided confidence interval must be approximately 96%.

6) Electronic Data Submission

All study data must be included in your 510(k) submission in an acceptable electronic format; this must include individual patient level data. Data files must include all primary and derived variables, with appropriate annotations or separate codebooks as necessary. The data files must include the results of applying the clinical reference algorithm for determining the presence of MTB-complex at both specimen and patient levels. Separate data files for the analytical studies must also be included in the 510(k) submission. Description of the statistical methods applied to the data set must be sufficiently detailed to allow the Agency to reproduce the results reported in the submission. Manufacturers may refer to the guidance entitled "Providing Regulatory Submissions in Electronic Format – General Considerations" found at http://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm124751.pdf for guidance related to electronic data submissions.

7. Labeling

All *in vitro* diagnostic devices, including these devices, are subject to statutory requirements for labeling (sections 201(n) and 502(a) of the FD&C Act (21 USC § 321(n) and 352(a))), including adequate directions for use and adequate warnings and precautions (section 502(f) of the FD&C Act (21 USC § 352(f))). Labeling requirements for all IVD devices are set forth in 21 CFR Parts 801 and 809.

Labeling for your device must include information similar to that described below to aid in mitigating the risks identified in Section 4 of this guideline document to ensure safe and effective use of these devices.

The labeling for your devices must include the information described below:

a. Intended Use

The intended use statement must clearly specify the intended use of the device, the population in whom the test should be used, and other significant aspects of use as appropriate (e.g., whether the test must be used in conjunction with mycobacterial culture and/or acid-fast smear). The intended use must clearly state that the device is to serve only as an aid in diagnosis and that false negative test results may occur. Additional qualifications may be appropriate based on the results of the clinical studies.

The following statement must be included immediately below the intended use statement:

"The [Insert Test Name] *test should only be performed in laboratories that follow safety practices according to the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories*[5]*and applicable state or local regulations. The* [Insert Test Name] *test must be performed in conjunction with mycobacterial culture to address the risk of false negative results and to recover the organisms for further characterization and drug susceptibility testing."*

b. Device Description

The Device Description must briefly describe the test methodology used in your device.

c. General Procedure

The General Procedure must include a description of the entire testing process from obtaining patient specimens to result reporting.

d. Directions for Use

The Directions for Use must present clear instructions that systematically describe the procedures for using the device and the types of control measures that will minimize risks of inaccurate results. This section of the labeling must include guidance for biosafety precautions with specimen handling and testing procedures and must clearly specify at which procedural step the test is rendered non-infectious.

Device handling and storage instructions must be included as well as a description of the expiration dating for both open and closed storage conditions for your device and any reagents or other components.

For devices that involve the use of ancillary reagents of concern (see also Section 5(e) of this document), you must:

[5] http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.

- Emphasize prominently that proper device performance involves use of specific ancillary reagents as directed. This may include warnings against device use if specified ancillary reagents are not available.
- Ensure that users can clearly identify which ancillary reagents are suitable for use with your device.
- Ensure that users of your device will understand which instructions for use they should follow when using ancillary reagents that are supplied with instructions for use or other warnings or limitations by the ancillary reagent manufacturer. If there is a conflict between the directions and warnings provided by the manufacturer of the ancillary reagents and the instructions for use that you supply with your device, you must assess and address the risk that users may mistakenly follow the labeling provided directly with the ancillary reagent manufacturer and possibly obtain invalid or inaccurate test results with your device. We note that in some circumstances, statements in the labeling of your device may not be sufficient to address the risks created by this conflict.

e. Quality Control

Your quality control recommendations in the package insert must include a clear explanation of what controls should be used with the assay and the expected results for the control material. If controls are included with your device, the 510(k) submission must include the specifications for control materials.

f. Warnings, Contraindications, Precautions, and Limitations

All warnings, contraindications, precautions, and limitations relevant to the specific device must be included in the device labeling. At a minimum, you must include a discussion of certain populations where device performance may differ or where the device has not been studied (e.g., pediatrics). Specific precautions regarding the use of specimen types other than respiratory specimens must be included if these other specimen types have not been studied.

If positive and negative interference has been detected or reported for any commonly used collection materials or substances that may be endogenously or exogenously introduced into a specimen prior to testing, you must advise of the possibility of false negative or false positive results as a limitation of your device.

In addition, the following statements must be included as a limitation:

> "The performance of the [Insert Test Name] test is dependent on operator proficiency and adherence to procedural directions. Laboratory procedural errors may cause false positive or false negative results. All device operators should have appropriate device training.
>
> A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests."

g. Specimen Collection

Your label must state how specimens should be collected, stored, and transported, and that inadequate or inappropriate specimen collection, storage, number of freeze/thaw cycles, and transport are likely to yield false negative test results. Labeling must also state that specimens should be collected as soon as possible after symptom onset and that ongoing treatment may affect device performance.

h. Interpretation and Reporting of Assay Results

You must describe how the operator should interpret each of the possible device results (e.g., positive, equivocal, and negative). You must also describe the recommendations for retesting or reporting of specimens that are equivocal (if this is a possible device output), or where specimen processing fails (e.g., whether another aliquot of the same specimen or a fresh specimen is necessary). See also Section 5(g) of this document entitled "Interpreting and Reporting Test Results." In addition, you must include the clinical circumstances under which immediate or delayed retesting is indicated.

If appropriate, you must include photographs and/or diagrams to indicate how to interpret results for tests with a qualitative result.

Your labeling must include a statement that tuberculosis is a notifiable disease that must be reported to public health authorities in accordance with state and local law. Additionally, you must indicate that users should verify reporting requirements for their institution, and notify appropriate agencies (e.g., state or local public health departments, the Centers for Disease Control and Prevention) as specified by applicable local and state regulations, if MTB-complex is detected or tuberculosis is suspected.

i. Performance Characteristics

Your labeling must include a summary of the study designs and study results described in Sections 5 and 6 of this document that would aid the user in interpreting test results and understanding device performance. This section must include descriptions of both clinical and analytical study results.

8. References

1. Centers for Disease Control and Prevention Fact Sheets - *Mycobacterium bovis* (Bovine Tuberculosis) in Humans. Division of Tuberculosis Elimination. September 9, 2011. http://www.cdc.gov/tb/publications/factsheets/general/mbovis.htm.

2. Recommendations for Use of an Isoniazid–Rifapentine Regimen with Direct Observation to Treat Latent *Mycobacterium tuberculosis* Infection. *MMWR* 2011;60:1650–1653 and

Targeted tuberculin testing and treatment of latent tuberculosis infection. MMWR 2000;49(No. RR-6).

3. CDC MMWR, Vol. 62, No. 11; 201-205. *Reported Tuberculosis in the United States, 2012*.Atlanta, GA: U.S. Department of Health and Human Services, CDC, March, 2013.

4. Clinical and Laboratory Standards Institute. 2006 Molecular Diagnostic Methods for Infectious Disease; Proposed Guideline-Second Edition. MM3-A2. Clinical and Laboratory Standards Institute, Wayne PA.

5. Clinical and Laboratory Standards Institute. 2004. Protocol for Determination of Limits of Detection and Limits Quantitation; Approved Guideline. EP17-A. Clinical and Laboratory Standards Institute, Wayne PA.

6. Clinical and Laboratory Standards Institute. 2005. Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition. EP07-A2. Clinical and Laboratory Standards Institute, Wayne PA.

7. Clinical and Laboratory Standards Institute. 1995. Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristics (ROC) Plots; Approved Guideline. GP10-A. Clinical and Laboratory Standards Institute, Wayne PA.

8. Clinical and Laboratory Standards Institute. 2004. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition. EP05-A2. Clinical and Laboratory Standards Institute, Wayne PA.

9. Clinical and Laboratory Standards Institute. 2008. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline-Second Edition. EP12-A2. Clinical and Laboratory Standards Institute, Wayne PA.

10. Clinical and Laboratory Standards Institute. 2005. User Verification of Performance for Precision and Trueness; Approved Guideline-Second Edition. EP15-A2. Clinical and Laboratory Standards Institute, Wayne PA.

11. Clinical and Laboratory Standards Institute. 2005. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline. MM13-A. Clinical and Laboratory Standards Institute, Wayne PA.

12. Clinical and Laboratory Standards Institute. 2008. Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline. MM18-A. Clinical and Laboratory Standards Institute, Wayne PA.

13. American Thoracic Society, CDC, and Infectious Diseases Society of America. Treatment of Tuberculosis. American Journal of Respiratory and Critical Care Medicine (2003) 167:603 - 62.