510(k) DECISION SUMMARY

A. 510(k) Number:

K131706

B. Purpose for Submission:

De novo request for evaluation of automatic class III designation for the Xpert® MTB/RIF Assay for use with the GeneXpert® Instrument Systems, including the GeneXpert® Diagnostic (Dx) Systems and the GeneXpert® Infinity Systems.

C. Measurand:

M. tuberculosis complex DNA and rifampin-resistance associated mutations of the *rpoB* gene.

D. Type of Test:

Qualitative, nested real-time polymerase chain reaction (PCR)

E. Applicant:

Cepheid®

F. Proprietary and Established Names:

Trade Name: Xpert® MTB/RIF Assay

Common Name: Xpert MTB/RIF Assay

G. Regulatory Information:

1. Regulation section: 21 CFR 866.3373
2. Classification: Class II (special controls)
3. Product code: PEU
4. Panel: Microbiology (83)

H. Intended Use:

1. Intended use(s):

The Xpert® MTB/RIF Assay, performed on the GeneXpert® Instrument Systems, is a qualitative, nested real-time polymerase chain reaction (PCR) *in vitro* diagnostic test for the detection of *Mycobacterium tuberculosis* complex DNA in raw sputum or concentrated sediments prepared from induced or expectorated sputum. In specimens where *Mycobacterium tuberculosis* complex (MTB-complex) is detected, the Xpert MTB/RIF Assay also detects the rifampin-resistance associated mutations of the *rpoB* gene.

The Xpert MTB/RIF Assay is intended for use with specimens from patients for whom there is clinical suspicion of tuberculosis (TB) and who have received no antituberculosis therapy, or less than 3 days of therapy. This test is intended as an aid in the diagnosis of pulmonary tuberculosis when used in conjunction with clinical and other laboratory findings.

The Xpert MTB/RIF Assay does not provide confirmation of rifampin susceptibility since mechanisms of rifampin resistance other than those detected by this device may exist that may be associated with a lack of clinical response to treatment.

Specimens that have both MTB-complex DNA and rifampin-resistance associated mutations of the *rpoB* gene detected by the Xpert MTB/RIF Assay must have results confirmed by a reference laboratory. If the presence of rifampin-resistance associated mutations of the *rpoB* gene is confirmed, specimens should also be tested for the presence of genetic mutations associated with resistance to other drugs.

The Xpert MTB/RIF Assay must be used 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.

The Xpert MTB/RIF Assay should only be performed in laboratories that follow safety practices in accordance with the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories publication and applicable state or local regulations.

2. Indication(s) for use: Same as Intended Use

3. Special conditions for use statement(s):

The Xpert® MTB/RIF Assay is for prescription use only in accordance with 21 CFR 801.109.

4. Special instrument requirements:

The Xpert® MTB/RIF Assay is for use with GeneXpert® Instrument Systems, including the GeneXpert® Diagnostic (Dx) Systems and the GeneXpert® Infinity Systems.

I. Device Description:

The Xpert® MTB/RIF Assay is an automated *in vitro* diagnostic test for the qualitative detection of MTB-complex DNA and the genetic mutations associated with rifampin (Rif) resistance in raw sputum samples or concentrated sputum sediments from patients for whom there is clinical suspicion of TB and who have received no antituberculosis therapy, or less than 3 days of therapy. The primers in this test amplify a portion of the *rpoB* gene containing the 81 base pair *core* region. The probes are designed to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with Rif resistance. The assay is performed on Cepheid GeneXpert® Instrument Systems.

The Xpert® MTB/RIF Assay includes single-use disposable cartridges and sample reagent for sample preparation. The Xpert® MTB/RIF Assay cartridges contain reagents for the detection of MTB-complex DNA and Rif resistance associated mutations. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is present to control for adequate processing of the target microorganism and to monitor the presence of inhibitors in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability.

Sputum specimens are collected according to the institution's standard procedures and transported to the GeneXpert® Instrument System area. For raw sputum, Sample Reagent is added to the sample (2:1, v:v). Sample Reagent is added to the resuspended sputum sediment (1.5 mL Sample Reagent to 0.5 mL suspension or 3:1, v:v, for larger volumes of sediment suspension). For both specimen types, the solution is shaken vigorously to mix, and then incubated at 20-30ºC for 15 minutes. Using the transfer pipette provided, the specimen is transferred to the open port of the Xpert® MTB/RIF Assay cartridge.

The user initiates a test from the system user interface, the Xpert® MTB/RIF Assay cartridge is loaded onto the GeneXpert® Instrument System platform, which performs hands-off, automated sample processing, and real-time PCR for detection of DNA. Summary and detailed test results are obtained in approximately 2 hours and are displayed in tabular and graphic formats.

The Xpert® MTB/RIF Assay simultaneously detects MTB-complex and the genetic mutations associated with rifampin resistance by amplifying a MTB-complex specific sequence of the *rpoB* gene, which is probed with five molecular beacons (Probes A – E) for mutations within the rifampin-resistance determining region (RRDR). Each molecular beacon is labeled with a different fluorophore.

The valid maximum cycle threshold (Ct) of 39.0 for Probes A, B and C and 36.0 for Probes D and E are set for data analysis.

- "MTB DETECTED", is reported when at least two probes result in Ct values within the valid range and a delta Ct min (the smallest Ct difference between any pair of

probes) of less than 2.0.

- "Rif Resistance NOT DETECTED" is reported if the delta Ct max (the Ct difference between the earliest and latest probe) is ≤4.0.

- "Rif Resistance DETECTED" is reported if the delta Ct max is >4.0.

- "Rif Resistance INDETERMINATE" is reported when the following two conditions are met:

  1. the Ct value of any probe exceeds the valid maximum Ct (or is zero, i.e. no threshold crossing); and
  2. the earliest *rpoB* Ct value is greater than [(Valid maximum Ct of probe in condition1) - (delta Ct max cut-off of 4.0)]

- "MTB NOT DETECTED" is reported when there is only one or no positive probe.

All assay settings are included as automatic calculations in the Xpert® MTB/RIF Assay protocol and cannot be modified by the user.

J. Standards/Guidance Documents Referenced:

1. CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement
2. Methods; Approved Guideline -Second Edition. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2004
3. EN 13640, Stability Testing of in vitro Diagnostic Reagents, June 2002.
   ASTM D4169-05, Standard Practice for Performance Testing of Shipping Containers and Systems
4. CLSI MM3-A2, Molecular Diagnostic Methods for Infectious Disease; Approved Guideline- Second Edition. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2006
5. EMC (Electromagnetic Compatibility) Directive, 2004/108/EC LVD (Low Voltage Directive) 2006/95/EC
6. IEC 61010-1:2001 2nd Edition "Safety Requirements for electrical equipment for measurement, control, and laboratory use-Part 1: General Requirements"
7. EN 61010-1:2001 2nd Edition "Safety Requirements for electrical equipment for measurement, control, and laboratory use-Part 1: General Requirements"
8. UL 61010-1:2004 2nd Edition "Safety Requirements for electrical equipment for measurement, control, and laboratory use-Part 1: General Requirements"
9. EN 61010-2-101:2002 "Safety Requirements for electrical equipment for measurement, control, and laboratory use-Part 2-101: Particular Requirements for in vitro diagnostic (IVD) medical equipment"
10. CAN-CSA 22.2 No. 61010-1: 2004 2nd Edition "Safety Requirements for electrical equipment for measurement, control, and laboratory use-Part 1: General Requirements"
11. CAN-CSA 22.2 No. 61010-2-101: 2004 "Safety Requirements for electrical equipment for measurement, control, and laboratory use-Part 2-101: Particular Requirements for in vitro diagnostic (IVD) medical equipment"
12. WEEE Directive 2002/96/EC

13. EN 55011:2007 +A1:2007 "Industrial, scientific and medical (ISM) radio-frequency equipment- Electromagnetic disturbance characteristics- Limits and methods of measurements"
14. EN 61326-1:2006 "Electrical Equipment for measurement, control and laboratory use- EMC Requirements"
15. EN 61326-2-6:2006 "Electrical Equipment for measurement, control and laboratory use- EMC requirements-Part 2-6: Particular Requirement for in vitro diagnostic (IVD) medical equipment"
16. FCC Part 15 Rules and Regulations for Information Technology Equipment
17. FCC Part 18 Rules and Regulations for Information Technology Equipment
18. CISPR 11:2004 "Industrial, scientific and medical equipment- Radio-frequency disturbance characteristics - Limits and methods of measurement" (Class A Radiated Emission Requirements)
19. CISPR 22:2006 "Information technology equipment -Radio disturbance characteristics- Limits and methods of measurement" (Class A Radiated Emission Requirements)

K. Test Principle:

The primers and probes in the Xpert® MTB/RIF Assay detect the presence of a unique gene sequence in MTB-complex DNA by using fluorogenic target-specific hybridization for detection of the amplified DNA. Sputum specimens are collected from patients with clinical suspicion of tuberculosis. The specimen is mixed with Sample Reagent, shaken 10 to 20 times or vortexed for at least 10 seconds, and incubated for 15 minutes at 20-30ºC. At 5 to 10 minutes into the incubation period, the specimen is shaken or vortexed again, and incubated for the remainder of the 15 minute incubation period prior to transferring it to the assay cartridge for testing. The GeneXpert® performs sample preparation by mixing the prepared sample with the lyophilized Sample Processing Control. The cells are filtered and washed with buffer to remove inhibitors and contaminants, and lysed using glass beads and an ultrasonic horn, eluting the released DNA. The DNA is mixed with dry real-time polymerase chain reaction (PCR) reagents and transferred into the integrated PCR tube for real-time PCR amplification and detection of chromosomal DNA gene sequences for MTB-complex.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:

   External Reproducibility (Study 154A)

   The reproducibility of the Xpert® MTB/RIF Assay was evaluated at three testing sites. The test panel included five contrived panel samples, each prepared using pooled human sputa preserved with cetyl pyridinium chloride (CPC) and spiked with a cultured strain of either rifampin-resistant or rifampin-susceptible *Mycobacterium tuberculosis*. Strains

were spiked at a "*low positive*" concentration (approximately 1X Limit of Detection - LoD) and a "*moderate positive*" (approximately 2 to 3X LoD). A negative sample, prepared using only CPC preserved human sputa, was also included in the test panel (see panel listing below).

1. MTB/Rif-R Low Positive (~1 X LoD)
2. MTB/Rif-R Mod. Positive (~2-3 X LoD)
3. MTB/Rif-S Low Positive (~1 X LoD)
4. MTB/Rif-S Mod Positive (~2-3 X LoD)
5. Negative

Each sample was identified by an alpha/numeric identification code. Operators performing the testing were blinded to the expected results.

Testing of one assay kit lot was conducted over five days, by two operators, each conducting three panel runs per day. Table 1 below provides a summary of the reproducibility study results.

Table-1. Summary of Reproducibility Results – Percent Agreement by Study Site/Instrument

Sample	Site 1 (Infinity-80)	Site 2 (GeneXpert Dx)	Site 3 (Infinity-48)	% Total Agreement by Sample
MTB/Rif- R ~2-3X LoD (Moderate Positive)	**100.0% (30/30)**	**100.0% (30/30)**	**100.0% (30/30)**	**100.0% (90/90)**
MTB/Rif- R ~1X LoD (Low Positive)	**93.3% (28/30)**	**96.7% (29/30)**	**96.7% (29/30)**	**95.6% (86/90)**
MTB/Rif- S ~2-3X LoD (Moderate Positive)	**100.0% (30/30)**	**100.0% (30/30)**	**100.0% (30/30)**	**100.0% (90/90)**
MTB/Rif-S ~ 1X LoD (Low Positive)	**96.7% (29/30)**	**100.0% (30/30)**	**100.0% (30/30)**	**98.9% (89/90)**
Negative	**100.0% (30/30)**	**100% (29/29)** [a]	**100.0% (30/30)**	**100% (89/89)**

[a]One sample was non-determinate after the initial test and upon retest

- Of the MTB/Rif-R, Moderate Positive samples, 100% (90/90) were correctly classified as "*MTB DETECTED; Rif Resistance DETECTED*".

- Of the MTB/Rif-R, Low Positive samples, 95.6% (86/90) were correctly classified as "*MTB DETECTED; Rif Resistance DETECTED*". Four

samples were classified as *"MTB NOT DETECTED"*.

- Of the MTB/Rif-S, Moderate Positive samples 100% (90/90) were correctly classified as *"MTB DETECTED; Rif Resistance NOT DETECTED"*.

- Of the MTB/Rif-S, Low Positive samples, 98.9% (89/90) were correctly classified as "*MTB DETECTED; Rif Resistance NOT DETECTED*". One sample was classified as "*MTB NOT DETECTED*".

- Of the negative samples, 100% (89/89) were correctly classified as "*MTB NOT DETECTED*". One sample was non-determinate after the initial test and upon retest.

Comparative Precision Evaluation of the Xpert MTB/RIF Assay on the GeneXpert Dx and Infinity-80 System:

An in-house instrument precision study was conducted to compare the performance of the GeneXpert® Dx System and the Infinity-80 Instrument System. The test panel included five contrived panel samples, each prepared using pooled human sputa preserved with cetyl pyridinium chloride (CPC) and spiked with a cultured isolate of either a rifampin-resistant strain of *Mycobacterium tuberculosis* or a rifampin susceptible strain of *Mycobacterium bovis* BCG. Isolates were spiked at a "*low positive*" concentration (approximately 1X Limit of Detection - LoD) and a "*moderate positive*" (approximately 2 to 3X LoD). A negative sample, prepared using only CPC preserved human sputa only, was also included in the test panel (see panel listing below).

1. MTB/Rif-R Low Positive (~1 X LoD)
2. MTB/Rif-R Mod. Positive (~2-3 X LoD)
3. MTB/Rif-S Low Positive (~1 X LoD)
4. MTB/Rif-S Mod Positive (~2-3 X LoD)
5. Negative

Samples were blinded with a Sample ID number which was used to define the order in which the samples were tested. Samples were tested in a different order throughout the study. Testing of one assay kit lot was conducted over 12 days, by two operators, each conducting four panel runs per day, on each instrument. Table 2 below provides a summary of the instrument precision study results.

Table- 2 : Summary of Instrument Precision Results - Percent Agreement

Sample	GeneXpert Dx	Infinity-80	% Total Agreement by Sample
MTB/Rif-R ~2-3X LoD (Moderate Positive)	**100% (94/94)**	**99.0% (95/96)**	**99.5% (189/190)**
MTB/Rif-R ~1X LoD (Low Positive)	**97.9% (94/96)**	**99.0% (95/96)**	**98.4% (189/192)**
MTB/Rif-S ~2-3X LoD (Moderate Positive)	**100.0% (96/96)**	**100.0% (96/96)**	**100.0% (192/192)**
MTB/Rif-S ~1X LoD (Low Positive)	**91.6% (87/95)**	**86.5% (83/96)**	**89.0% (170/191)**
Negative	**99.0% (95/96)**	**97.9% (94/96)**	**98.4% (189/192)**

- Of the MTB/Rif-R, Moderate Positive samples, 99.5% (189/190) were correctly classified as "*MTB DETECTED; Rif Resistance DETECTED*". Two samples tested were non-determinate after the initial test and upon repeat. One sample tested was classified "*MTB NOT DETECTED"*.

- Of the MTB/Rif-R, Low Positive samples, 98.4% (189/192) were correctly classified as *"MTB DETECTED; Rif Resistance DETECTED*". Two samples were classified as "*MTB NOT DETECTED"*. One sample was classified as *"MTB DETECTED; Rif Resistance INDETERMINATE"*.

- Of the MTB/Rif-S, Moderate Positive samples 100% (192/192) were correctly classified as *"MTB DETECTED; Rif Resistance NOT DETECTED*".

- Of the MTB/Rif-S, Low Positive samples, 89.0% (170/191) were correctly classified as *"MTB DETECTED; Rif Resistance NOT DETECTED"*. Seventeen samples (11- Infinity 80; 6 GeneXpert DX) were classified as "MTB NOT DETECTED". Four samples (2-Infinity 80; 2-GeneXpert DX) were classified as "*MTB DETECTED, Rif Resistance INDETERMINATE"*. One sample tested was non-determinate after the initial test and upon repeat.

- Of the negative samples, 98.4% (189/192) were correctly classified as *"MTB NOT DETECTED"*. Three samples were classified as *MTB DETECTED; Rif Resistance NOT DETECTED"*.

b. *Linearity/ assay reportable range:*

Not applicable, the Xpert MTB/RIF Assay is a qualitative assay.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods)*

Internal Controls

Sample Processing Control (SPC): The SPC verifies that the specimen processing is adequate for cartridge tested. In addition, the SPC detects specimen-associated inhibition of the real-time PCR reactions. The SPC, which is included in each cartridge, contains non-infectious spores in the form of a dry spore cake. The SPC PASSES if a valid cycle threshold (Ct) is generated in a negative sample. A SPC result is not required in a positive sample because MTB amplification can compete with this control. If the SPC fails, the test should be repeated.

Probe Check Control (PCC): Before the start of the first and second nested real-time PCR reactions, the GeneXpert Instrument System measures the fluorescent signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity, and dye stability. These readings are then compared to default settings established by Cepheid. The PCC PASSES if the fluorescence generated meets the validated acceptance criteria. If the PCC fails, the test should be repeated.

External Controls:

A study was conducted to evaluate commercially available ready-to-use control materials for the Xpert® MTB/RIF Assay. The control material was evaluated using the GeneXpert® DX instrument in combination with the Xpert® MTB/RIF Assay. INTROL TB Controls are custom made by Main Molecular Quality Controls Inc. (MMQCI; Scarborough Maine). The controls are non-infectious and can be shipped and stored at 2-8°C. The INTROL TB controls were tested exactly as a raw sputum sample. The INTROL TB control panel included a wild-type control, (2) mutant controls and a negative control. Replicates of 20 were run per control type. Table 3 provides a detailed description of the external controls evaluated in the study.

Table 3. External Controls

External Control ID	rpoB Genotype	Probes Affected by rpoB Gene Mutations of Control	Concentration (CFU/mL)	Expected Result
INTROL TBWT-04	rpoB: wild type (H37Rv)	None	$2X10^4$	"MTB Detected, Rifampin Resistance Not Detected "
INTROL TBMDR1-04	rpoB: Mutants (F505L, L511P, D516V, H526Y)	A, B, and D	$2X10^4$	"MTB Detected, Rifampin Resistance Detected"
INTROL TBMDR2-04	rpoB: Mutants (S522L, H526D, S531L)	C, D, and E	$2X10^4$	"MTB Detected, Rifampin Resistance Detected"
INTROL TBNEG-04	rpoB: NEG	None	No Cells	"MTB Not Detected"

The results of the study are described in the following summary tables – Table 4 through Table 7.

Table 4: INTROL TBNEG-04

Analyte	Ct Range	Mean Ct Value	Result
Specimen Processing Control (SPC)	23.1-27.9	24.6	All replicates tested were correctly reported as expected - "MTB Not Detected"

Table 5: INTROL TBWT-04

Analyte	Ct Range	Mean Ct Value	Result
Specimen Processing Control (SPC)	23.1-27.9	24.6	All replicates tested were correctly reported as expected - "MTB Detected; Rifampin Resistance Not Detected"
Probe E	21.2- 24.7	22.5	
Probe D	21.2-25.4	22.8	
Probe C	19.9-23.8	21.0	
Probe B	21.2-25.3	23.0	
Probe A	19.4-23.3	20.9	

Table 6: INTROL TBMDR1-04

Analyte	Ct Range	Mean Ct Value	Result
Specimen Processing Control (SPC)	22.3-28.9	24.1	All replicates tested were correctly reported as expected - "MTB Detected; Rifampin Resistance Detected" No Ct values were obtained for Probes A, B, and D as expected using this mutant control.
Probe E	20.8-23.5	22.1	
Probe D	0-0	0	
Probe C	19.8-22.9	21.5	
Probe B	0-0	0	
Probe A	0-0	0	

Table 7: INTROL TBMDR2-04

Analyte	Ct Range	Mean Ct Value	Result
Specimen Processing Control (SPC)	21.6-34.7	24.2	All replicates tested were correctly reported as expected - "MTB Detected; Rifampin Resistance Detected"
Probe E	0-0	0	
Probe D	0-0	0	
Probe C	0-0	0	
Probe B	19.4-28.7	22.9	
Probe A	18.5-27.3	21.5	No Ct values were obtained for Probes C, D, and E as expected using this mutant control.

External controls may be used in accordance with accrediting institutions and government regulations. External controls are not provided in the assay kit; however they are available for purchase from outside sources. The outside source and the catalog numbers are provided in the Materials Available but Not Provided section of the Xpert® MTB/RIF Assay Package Insert.

During the clinical trial, three external controls (MTB NEG, MTB Positive Rif-susceptible, and MTB Positive Rif-resistant) were run each day that study specimens were tested. Study specimens were not run until correct results were obtained for both the negative and positive controls. Of the 467 external control samples that were run, 97% (453/467) gave expected results on the first attempt. Of the 14 external controls that failed to give expected results on the first attempt (i.e., a non-determinate result of INVALID, ERROR, or NO RESULT), eight gave expected results when retested. Six external control specimens which failed to give expected results on the first attempt were not repeated; no study specimens were tested on these occasions.

d. Limit of Detection:

Studies were performed to determine the limit of detection (LoD) of isolates of *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG (Bacille Calmette-Guerin) diluted in human sputum and human sputum sediment. The LoD is the lowest concentration reported in CFU/mL that can be reproducibly distinguished from negative samples with 95% confidence. Replicates of 20 were evaluated at five to eight concentrations and LoD was determined using probit analysis with the exception of testing performed with *M. tuberculosis* mutant rifampin-resistant strain TDR125 cells in sputum sediment which was performed at one concentration only, in replicates of 40. See Table 8 below.

Table 8: Probit Analysis Data and Claimed LoD in CFU/mL

Microorganism	Specimen Type	LoD Estimate	Claimed LoD (CFU/mL)
M. bovis BCG	Sputum	486	525
	Sediment	703	700
M. tuberculosis (H37Rv)	Sputum	414	600
	Sediment	2046	3000
M. tuberculosis (TDR125)	Sputum	872	1000
	Sediment	ND[a]	4000

[a]Not determined (ND) by probit analysis.

e. *Rifampin Resistance Study*

Due to the low prevalence of rifampin resistance, an additional study was conducted to evaluate the performance of the Xpert MTB/RIF Assay in detecting genetic mutations associated with rifampin resistance in well characterized rifampin-susceptible and rifampin-resistant clinical isolates spiked into pooled sputum confirmed negative for MTB-complex and mycobacteria other than MTB-complex. Fifty aliquots of pooled negative sputa were randomly intermixed for testing among the positives. Results of Xpert MTB/RIF Assay were compared to prior MTB identification, phenotypic drug susceptibility testing (agar proportion method), and bi-directional sequencing results. The results of the study are shown in the Table 9 below.

Table 9: Xpert MTB/RIF Performance vs. DST

		DST		
		Res	**Susc**	**Total**
Xpert MTB/RIF Assay	**MTB DETECTED Rif Resistance DETECTED**	85	9	94
	MTB DETECTED Rif Resistance DETECTED	2	89	91
	Total	87	98	185
	Sensitivity: 97.7% (95% CI 92.0-99.4) Specificity**:** 90.8% (95% CI 83.5-95.1)			

Of the 11 samples with rifampin discordant results, bi-directional sequencing results were concordant with the Xpert MTB/RIF Assay for 10 of 11 samples and discordant with one sample. See Table 10 below.

Table 10: Discrepant Testing Results

DST	Xpert MTB/RIF	n	Bi-directional sequencing
Rif-resistant	Rif Resistance NOT DETECTED	2	2 of 2 Wild Type
Rif-susceptible	Rif Resistance DETECTED	9	8 of 9 Rif resistance mutations present. 1 of 9 Wild Type

f. *Analytical Reactivity (Inclusivity):*

A total of 62 well characterized strains of *Mycobacterium tuberculosis* representing geographic and phenotypic diversity were evaluated with the Xpert MTB/RIF Assay. Whole organism was used for testing the majority of the strains. For 15 strains, due to lack of availability or viability of whole organism, DNA was used for testing (see footnote "a" in Table 11). All strains were tested in triplicate near the analytical limit of detection.

Table 11 below includes 26 rifampin susceptible and 36 rifampin resistant strains, as determined by phenotypic drug susceptibility testing (DST).

Xpert MTB/RIF Assay results of "MTB DETECTED; Rif Resistance NOT DETECTED" compared to DST was 87% (67/77) accurate in replicate tests using 26 strains sensitive to rifampin. One replicate in 78 tests was reported as "INVALID" and not repeated. Three of the DST susceptible strains (see footnote "c" in Table 11) that tested as "MTB DETECTED; Rif Resistance DETECTED" by the Xpert MTB/RIF Assay were found to have rifampin resistance mutations according to DNA sequence analysis. One of three replicates of the wild type strain TDR 33 was reported "MTB DETECTED; Rif Resistance INDETERMINATE".

Xpert MTB/RIF Assay results of "MTB DETECTED; Rif Resistance DETECTED" compared to DST was 100% (107/107) accurate in replicate tests using 36 strains resistant to rifampin. One replicate in 108 tests was reported as "INVALID" and not repeated. Results by strain are shown in Table 11.

Table 11: Analytical Reactivity (Inclusivity) of the Xpert MTB/RIF Assay

Strain ID	Origin	Rifampin Susceptibility by DST[d]	Rifampin Susceptibility by Xpert[e]
TDR 116	S. Korea	R	R
TDR 21	RD Congo	R	R
TDR 28[a]	Bangladesh	R	R
TDR 191[a]	Peru	R	R
TDR 125	Brazil	R	R
TDR 34	Bangladesh	R	R
TDR 73	Peru	R	R
TDR 35	Bangladesh	R	R
TDR 190[a]	Spain	R	R
TDR 117	S. Korea	R	R
TDR 129	Brazil	R	R
TDR 186[a]	Morocco	R	R
TDR 59[a]	Burundi	R	R
TDR 185[a]	Nigeria	R	R
TDR 6	Bangladesh	R	R
TDR 19	Azerbaijan	R	R
TDR 148	Nepal	R	R
TDR 13[a]	Bangladesh	R	R
TDR 12	Bangladesh	R	R
H37Rv[b]	Lab strain	S	S
TDR 22	RD Congo	S	S
TDR 29	Azerbaijan	S	S
TDR 33	Belgium	S	S
CDC 1551[a]	USA	S	S
TDR 146[a]	Nepal	S	S
TDR 78[a]	S. Korea	S	S
TDR 54[a]	Bangladesh	S	S
TDR 215[a]	Peru	S	S
TDR 158[a]	Peru	S	S
TDR 178[a]	Guinea	S	S
TDR 64[a]	S. Africa	S	S
97-05193	Peru	R	R
97-05201	Peru	R	R
97-06877	Peru	R	R
97-08341	Peru	R	R
97-12004	Peru	R	R
97-17582	Peru	R	R
97-18875	Peru	R	R
97-20784	Peru	R	R
97-20985	Peru	R	R

Strain ID	Origin	Rifampin Susceptibility by DST[d]	Rifampin Susceptibility by Xpert[e]
99-09120	Peru	R	R
99-R396	Peru	R	R
01-R0612	Beijing	R	R
02-R1141	Beijing	R	R
02-R1794	Beijing	R	R
02-R1840	Beijing	R	R
03-R1517	Beijing	R	R
TDR 0116	S. Korea	R	R
01-R1403[c]	Peru	S	R
97-15246[c]	Peru	S	R
98-R839[c]	Peru	S	R
99-R460	Peru	S	S
99-R485	Peru	S	S
00-06461	US	S	S
00-R0222	Peru	S	S
00-R0454	US	S	S
00-R0460	Peru	S	S
01-10979	US	S	S
01-R1118	Peru	S	S
02-02880	US	S	S
02-03222	Peru	S	S
02-R0040	Peru	S	S

[a]DNA used for testing; quantified cultured strains was not available..
[b]ATCC Strain H37Rv tested as both whole organism and DNA.
[c]Isolate rifampin susceptible by DST, but bi-directional sequencing and Xpert MTB/RIF Assay revealed the presence of rifampin resistance mutations.
[d]R=Rif resistant; S=Rif susceptible.
[e]R=Rif resistance mutations detected, S=Rif resistance mutations not detected.

An additional five *Mycobacterium tuberculosis* complex strains, i.e., *M. africanum M. bovis*, *M. canettii*, *M. caprae*, and *M. microti*, were not wet tested but were evaluated *in silico* to assess the analytical reactivity. The results of the *in silico* analyses predict a very high likelihood of amplification and detection using the Xpert MTB/RIF Assay. See Table 13.

Table 13: Alignments of Primers and Probes to the *rpoB* sequences of *M. africanum, M. bovis, M. canettii, M. caprae and M. microti*

MTB complex organism	Sequence Alignment (number of identical residues) of Xpert MTB/RIF Assay Primer/Probe to MTB-complex Strains							
	RpoB For1	RpoB For2	RpoB Rev	RpoB Probe A[b]	RpoB Probe B[b]	RpoB Probe C[b]	RpoB Probe D[b]	RpoB Probe E[b]
Mycobacterium africanum (taxid:33894)[a]	24/24	24/24	23/24	17/17	24/24	17/17	18/18	18/18
Mycobacterium bovis (taxid:1765)[a]	24/24	24/24	23/24	17/17	24/24	17/17	18/18	18/18
Mycobacterium canetti (taxid:78331)[a]	24/24	24/24	23/24	17/17	24/24	17/17	18/18	18/18
Mycobacterium caprae (taxid:115862)[a]	24/24	24/24	23/24	17/17	24/24	17/17	18/18	18/18
Mycobacterium microti (taxid:1806)[a]	24/24	24/24	23/24	17/17	24/24	17/17	18/18	18/18

[a]taxid – unique identifier for an organism in the NCBI taxonomy database.

[b]Sequence alignment of probes done with no stem sequences.

g. Analytical Exclusivity (Cross Reactivity):

The Xpert MTB/RIF Assay was evaluated by testing a panel of 132 microorganisms (24 non-tuberculosis mycobacteria, 87 bacteria, 7 fungi and 14 viruses) representing common respiratory pathogens potentially encountered in the oral/respiratory tract. See Table 14. Nucleic acid (DNA or RNA) was used in place of whole organisms for 9 bacteria, 1 fungus, and 6 viral strains due to lack of availability or due to biosafety restrictions. All microorganisms were tested in triplicate, at a concentration of at least:

- 10^8 CFU/mL (or DNA at $1x10^7$ copies/mL) for bacteria and fungi;
- 10^5 $TCID_{50}$/mL (or nucleic acid at $2x10^9$ copies/mL) for viruses;
- 10^6 elementary bodies (EB) per mL for Chlamydia; and
- 10^6 CFU/mL for two nontuberculous mycobacteria (*M. genavense* and *M. smegmatis*)

There was no cross-reactivity observed with 131 of the 132 microorganisms tested. Cross-reactivity was observed in one of three replicates of *M. scrofulaceum* at a concentration of 10^8 CFU/mL, however, no cross-reactivity was observed when tested at a concentration of 10^7 CFU/mL.

Table 14: Microorganisms Tested for Analytical Specificity

Acinetobacter baumannii	Human influenza virus B[c]	*Neisseria lactamica*
Acinetobacter calcoaceticus	Human Metapneumovirus	*Neisseria meningitidis*
Actinomyces israelii[a]	Human parainfluenzae Type 1	*Neisseria mucosa*
Actinomyces odontolyticus	Human parainfluenzae Type 2	*Neisseria sicca*
Adenovirus	Human parainfluenzae Type 3	*Nocardia asteroides*[a]
Aspergillus fumigatus[b]	Human respiratory syncytial virus A[c]	*Nocardia cyriacigeorgica*[a]
Bacillus cereus	Human respiratory syncytial virusB[c]	*Pasteurella multocida* subsp. *tigris*
Bacillus subtilis subsp. *subtilis*	*Kingella kingae*	*Pediococcus pentosaceus*[a]
Bacteroides fragilis	*Klebsiella oxytoca*	*Peptostreptococcus anaerobius*
Bordetella parapertussis[a]	*Klebsiella pneumoniae* producing KPC-3 carbapenemase	*Porphyromonas asaccharolytica*
Bordetella pertussis	*Klebsiella pneumoniae* subsp. *pneumoniae*	*Prevotella melaninogenica*
Burkholderia cepacia	*Lactobacillus acidophilus*	*Propionibacterium acnes*
Campylobacter jejuni subsp. *jejuni*[a]	*Lactobacillus casei*	*Proteus mirabilis*
Candida albicans	*Legionella pneumophila* subsp. *pneumophila*	*Proteus vulgaris*
Candida glabrata	*Leuconostoc mesenteroides* subsp. *mesenteroides*	*Providencia stuartii*
Candida krusei	*Listeria monocytogenes*	*Pseudomonas aeruginosa*
Candida parapsilosis	*Moraxella catarrhalis*	Rhinovirus Strain 1A
Candida tropicalis	*Morganella morganii* subsp. *morganii*	*Rhodococcus equi*
Chlamydia trachomatis	*Mycobacterium abscessus*	*Salmonella enterica* subsp. *enterica serovar Dublin*
Chlamydophila pneumoniae[a]	*Mycobacterium asiaticum*	*Salmonella enterica* subsp. *enterica serovar typhimurium*
Citrobacter freundii	*Mycobacterium avium* subsp. *avium*	*Serratia marcescens* subsp. *marcescens*
Clostridium perfringens	*Mycobacterium celatum*	*Shigella flexneri*
Corynebacterium diphtheriae[a]	*Mycobacterium chelonae*	*Shigella sonnei*
Corynebacterium jeikeium	*Mycobacterium flavescens*	*Staphylococcus aureus* subsp. *aureus*
Corynebacterium pseudodiphtheriticum	*Mycobacterium fortuitum* subsp. *fortuitum*	*Staphylococcus capitis* subsp. *capitis*
Corynebacterium xerosis	*Mycobacterium gastri*	*Staphylococcus epidermidis*
Cryptococcus neoformans	*Mycobacterium genavense*	*Staphylococcus haemolyticus*

Cytomegalovirus	*Mycobacterium gordonae*	*Staphylococcus hominis* subsp. *hominis*
Eikenella corrodens	*Mycobacterium haemophilum*	*Staphylococcus lugdunensis*
Enterobacter aerogenes	*Mycobacterium intracellulare*	*Stenotrophomonas maltophilia*
Enterobacter cloacae subsp. *cloacae*	*Mycobacterium kansasii*	*Streptococcus agalactiae*
Enterococcus avium	*Mycobacterium malmoense*	*Streptococcus constellatus* subsp. *constellatus*
Enterococcus faecalis	*Mycobacterium marinum*	*Streptococcus equi* subsp. *equi*
Enterococcus faecium	*Mycobacterium scrofulaceum*	*Streptococcus mitis*
Enterovirus Type 71/NY	*Mycobacterium simiae*	*Streptococcus mutans*
Escherichia coli	*Mycobacterium smegmatis*	*Streptococcus parasanguinis*
Escherichia coli producing CTX-M-15 ESBL	*Mycobacterium szulgai*	*Streptococcus pneumoniae*
Fusobacterium nucleatum subsp. *nucleatum*	*Mycobacterium terrae*	*Streptococcus pyogenes*
Haemophilus influenzae	*Mycobacterium thermoresistibile*	*Streptococcus salivarius* subsp. *salivarius*
Haemophilus parahaemolyticus	*Mycobacterium triviale*	*Streptococcus sanguinis*
Haemophilus parainfluenzae	*Mycobacterium vaccae*	*Streptococcus uberis*
Herpes simplex virus Type 1[c]	*Mycobacterium xenopi*	*Veillonella parvula*
Herpes simplex virus Type 2[c]	*Mycoplasma pneumoniae*[a]	*Weissella paramesenteroides*
Human influenza virus A[c]	*Neisseria gonorrhoeae*	*Yersinia enterocolitica* subsp. *enterocolitica*

[a] Genomic DNA used; concentrations tested ranged from $1x10^7$ to $1x10^{10}$ copies/mL.
[b] Genomic DNA used; concentration tested at 3.2×10^8 copies/mL.
[c] Genomic DNA or RNA used; concentrations tested ranged from 3.1×10^9 to 1.2×10^{11} copies/mL.

Potential cross-reactivity of 18 microorganisms that could not be wet tested using whole organisms or nucleic acid was evaluated by *in silico* analysis. Thirteen of the 18 microorganisms tested revealed no potential for cross-reactivity. See Table 15.
Five isolates demonstrated a slight potential for cross reactivity which may result in false positive results with the Xpert MTB/RIF Assay. See Table 16.

Table 15: Microorganisms Predicted to be Non-cross Reactive by *in silico* Analysis

Bacteria	Fungi	Virus
Kingella oralis	*Blastomyces dermatitidis* (*Ajellomyces dermatitidis*)	Rubella virus
Legionella micdadei	*Penicillium* spp.	Rubeola virus
Nocardia brasiliensis	*Rhizopus* spp.	Rubulavirus
Streptomyces anulatus	*Scedosporium* spp.	Varicella Zoster Virus
	Histoplasma capsulatum	

Table 16. Microorganisms Predicted to be Potentially Cross Reactive by *in silico* Analysis

Mycobacterium kumamontonense
Mycobacterium leprae
Mycobacterium mucogenicum
Tsukamurella spp.
Nocardia otitidiscaviarum

h. Interfering Substances:

Performance of the Xpert MTB/RIF Assay was evaluated in the presence of 32 potentially interfering substances. These substances are listed in Table 17 along with the active ingredients and concentrations tested. Positive and negative samples were included in this study. Positive samples were tested near the analytical limit of detection using one inactivated rifampin-susceptible strain of *M. tuberculosis* (H37Rv) and one inactivated rifampin-resistant strain of *M. tuberculosis* TDR6 (probe E mutant). Both strains were tested in replicates of eight. Negative samples, which consisted of the interfering substance without the MTB strain, were tested in replicates of eight to determine the effect on the performance of the sample processing control (SPC). Inhibition of the Xpert MTB/RIF Assay was observed in the presence of Lidocaine at 30%; mucin at 5% and 2.5%; Ethambutol at 50 µg/mL, 25 µg/mL, and 10 µg/mL; Guaifenesin at 5 mg/mL; Phenylephrine at 100% and 50%; and tea tree oil at 0.5% to 0.015%, resulting in a false negative result "MTB NOT DETECTED" or a "Rif Resistance INDETERMINATE" result.

Table 17. Potential Interfering Substances in Xpert MTB/RIF Assay

Substance	Description/Active Ingredient	Concentration Tested
Blood (human)		5% (v/v)
Germicidal Mouthwash	Chlorhexidine gluconate (0.12%), 20%	20% (v/v)
Specimen Processing Reagents	Cetylpyridinium chloride, 1% in 2% NaCl	0.5% (v/v) in 1% NaCl
Specimen Processing Reagents	Cetylpyridinium chloride, 1% in 2% NALC	0.5% (v/v) in 1% NALC
Specimen Processing Reagents	Cetylpyridinium chloride, 1% in 2% NALC plus 25 mM Citrate	0.5% (v/v) in 1% NALC plus 12.5 mM Citrate
Gastric Acid	pH 3 to 4 solution in water, neutralized	100% (v/v)
Human DNA/Cells	HELA 229	10^6 cells/mL
Antimycotic; Antibiotic	Nystatin oral suspension, 20%	20% (v/v)
White Blood Cells (human)	WBC/Pus matrix (30% buffy coat; 30% plasma; 40% PBS)	100% (v/v)
Anesthetics (endotracheal intubation)	Lidocaine HCl 4%	20% to 30% (v/v)
Nebulizing solutions	NaCl 5% (w/v)	5% (w/v)
Mucin	Mucin 5% (w/v)	1.5% to 5% (w/v)
Antibacterial, systemic	Levofloxacin 25 mg/mL	5 mg/mL
Nasal corticosteroids	Fluticasone 500 mcg/spray	5 µg/mL
Inhaled bronchodilators	Albuterol Sulfate 2.5 mg/3mL	50 µg/mL; 100 µg/mL
Oral anesthetics	Orajel (20% Benzocaine)	5% (w/v)
Anti-viral drugs	Acyclovir, IV 50 mg/mL	50 µg/mL
Antibiotic, nasal ointment	Neosporin (400U Bacitracin, 3.5 mg Neomycin, 5000U Polymyxin B)	5% (w/v)
Tobacco	Nicogel (40% tobacco extract)	0.5% (w/v)
Anti-tuberculosis drugs	Streptomycin 1mg/mL	25 µg/mL
Anti-tuberculosis drugs	Ethambutol 1 mg/mL	5 µg/mL to 50 µg/mL
Anti-tuberculosis drugs	Isoniazid 1 mg/mL	50 µg/mL
Oral expectorants	Guaifenesin (400mg/tablet)	2.5 mg/mL; 5 mg/mL
Anti-tuberculosis drugs	Pyrazinamide 10 mg/mL	100 µg/mL
Nasal gel (Homeopathic)	Zicam gel	50% (w/v)
Nasal spray	Phenylephrine 0.5%	25% to 100% (v/v)
Anti-tuberculosis drugs	Rifampicin 1mg/mL	25 µg/mL
Allergy relief medicine (Homeopathic)	Tea tree oil (<5% Cineole, >35% Terpinen-4-01)	0.008% to 0.5% (v/v)
Live intranasal influenza virus vaccine	Live influenza virus vaccine	5% (v/v)
Pneumocystis jiroveci medication	Pentamidine	300 ng/mL
Bronchodilator	Epinephrine (injectable formulation)	1mg/mL
Neutralizing buffer	XPR-*plus* Neutralizing Buffer	>67 mM phosphate

i. Carry-over Contamination:

A study was conducted to demonstrate whether potential carry-over and cross-contamination occurs when using the single-use, self-contained Xpert MTB/RIF Assay cartridges. In the study, a negative sample (TET buffer) was processed in the same GeneXpert module immediately following a very high positive sample containing *M. bovis* BCG at a concentration of approximately $1x10^6$ CFU/mL spiked into TET buffer. This testing scheme was repeated 20 times on two GeneXpert modules for a total of 42 runs resulting in 20 positive and 22 negative specimens. All 20 positive samples were correctly reported as "MTB DETECTED; Rif Resistance NOT DETECTED" and all 22 negative samples were correctly reported as "MTB NOT DETECTED".

j. Assay cut-off:

The valid minimum cycle threshold (Ct) setting for all *rpoB* probes A through E in the MTB/RIF Assay is 3 because in practice, a Ct cannot be calculated before the end of the minimum background subtraction range, which is two cycles. A minimum Ct setting of 3 is well below the earliest Cts reported for all targets.

The valid maximum cycle threshold (Ct) setting for probes A, B, and C is 39. The valid maximum cycle threshold setting for probes D and E is 36. These settings were established to mitigate potential false rifampin resistant results due to very late probe D and E Cts. By evaluating three sets of pre-clinical data in which all maximum valid *rpoB* Cts were set to 39, three of six false "Rif Resistance DETECTED" results were reported as "Rif Resistance INDETERMINATE" by setting the valid maximum cycle for Probe D and Probe E to 36. To further justify these setting, analytical testing with *M. bovis* BCG demonstrated that in a total of 55 positive results, two false "Rif Resistance DETECTED" results were reported as "Rif Resistance INDETERMINATE" when the probe D and E maximum valid Ct settings were 36 relative to 39.

2. <u>Comparison studies:</u>

a. Method comparison with predicate device:

Not Applicable. Refer to Clinical Studies section.

b. Matrix comparison:

Not applicable.

3. Clinical studies:

Performance characteristics of the Xpert MTB/RIF Assay for detection of MTB-complex DNA and for detection of Rif resistance associated mutations in sputum samples relative to results from culture (solid and/or liquid) followed by phenotypic drug susceptibility testing (DST) were determined in a multi-center study using prospective and archived sputum specimens collected from both US and non-US populations. Leftover standard of care sputum specimens or concentrated sediment prepared from induced or expectorated sputa were tested by the Xpert MTB/RIF Assay from study subjects suspected of tuberculosis (TB). All AFB smears were performed on concentrated sediments.

Specimens from subjects 18 years or older were eligible for the multi-center study if they were suspected of pulmonary tuberculosis, on no TB treatment or with less than 3 days of TB treatment, had sufficient volume for testing on the Xpert MTB/RIF Assay, and had AFB smear, MTB culture and phenotypic drug susceptibility testing (DST) results. There were 1,126 subjects eligible and tested by the Xpert MTB/RIF Assay (Table 18).

Table 18. Accountability of Eligible Study Subjects for Evaluation of Xpert MTB/RIF Assay for the Detection of MTB-complex vs. MTB Culture

		Culture			
		+	-	Not Determined	Total
Xpert MTB/RIF Assay	MTB DETECTED	439	8	2	449
	MTB NOT DETECTED	29	620	15	664
	Non-Determinate	9	4	0	13
	Total	477	632	17	1126

Thirteen subjects (1.2%) had Xpert MTB/RIF Assay non-determinate results (i.e., INVALID, ERROR or NO RESULT) and were excluded from the analysis. Seventeen (1.5%) subjects had MTB culture contamination. Analysis of missing values with regard to potential biases was performed (i.e., analysis of covariate distributions and logistic regression) and the 17 subjects were excluded from the analysis. One thousand ninety six (1,096) subjects were used in the analysis of the performance of the Xpert MTB/RIF Assay relative to the detection of MTB-complex. The specimens came from study subjects who were ≥18 years old, 62% (n=679) male, 36% female (n=396); and for 1.9% (n=21) gender was unknown. Subjects were from geographically diverse regions: 49% (n=542) were from the United States (California, New York and Florida) and 51% (n=554) were from outside the United States (Vietnam, Peru, South Africa, Mexico and Bangladesh). Of the 542 US specimens, 450 were prospectively collected and 92 were from an archived specimen bank. Of the 554 non-US specimens, 23 were prospectively collected and 531 were archived from a specimen bank.

Xpert MTB/RIF Assay Performance vs. MTB Culture

One to three sputum specimens were collected from each study subject for use in the clinical study (33.9% of study subjects had 1 sputum specimen collected, 44.2% had 2 sputum specimens, and 22% had 3 sputum specimens). If more than one specimen was collected from a subject, the first sample with sufficient volume was tested by the Xpert MTB/RIF Assay. If the assay result was non-determinate (ERROR, INVALID or NO RESULT), the same specimen was retested if there was sufficient volume. Overall, 1.2% of tested samples (13/1,126; 95% CI: 0.7% to 2.0%) were non-determinate. Among 1,096 subjects with MTB culture results, an Xpert MTB/RIF Assay result was obtained with the first specimen for 85.5% of subjects, with the second specimen for 11.2% of subjects, and with the third specimen for 0.3% of subjects. AFB smear status for a subject was determined by the AFB smear result from the specimen with a corresponding Xpert MTB/RIF Assay result. The MTB culture status for a subject was defined based on the evaluation of the MTB culture results of all specimens collected for use in the study for this subject.

The performance of the Xpert MTB/RIF Assay for detection of MTB-complex relative to MTB culture, stratified by AFB smear status is shown in Table 19 and Table 20. Discordant results for MTB culture positive and Xpert MTB/RIF Assay "MTB NOT DETECTED" were further evaluated using bi-directional sequencing of the *rpoB* region of the MTB genome. No discordant analysis was performed on MTB culture negative specimens.

Table 19: Xpert MTB/RIF Assay Performance vs. MTB Culture for AFB Smear-Positive Subjects

		Culture		
		+	-	Total
Xpert MTB/RIF Assay	MTB DETECTED	350	1[b]	351
	MTB NOT DETECTED	1[a]	65	66
	Total	351	66	417
Sensitivity = 99.7% (350/351) with 95% CI: 98.4% - 99.9% Specificity =98.5% (65/66) with 95% CI: 91.9% – 99.7%				

[a]One MTB culture positive specimen was not detected by the Xpert MTB/RIF Assay. This culture isolate was determined to be MTB by bi-directional sequencing analysis.
[b]The Xpert MTB/RIF Assay detected MTB in one specimen that was MTB culture negative. The culture result was based on one sputum specimen for this subject.

Table 20: Xpert MTB/RIF Assay Performance vs. MTB Culture for AFB Smear-Negative Subjects

		Culture		
		+	-	Total
Xpert MTB/RIF Assay	MTB DETECTED	89	7[b]	96
	MTB NOT DETECTED	28[a]	555	583
	Total	117	562	679
Sensitivity = 76.1% (89/117) with 95% CI: 67.6% - 82.9% Specificity =98.8% (555/562) with 95% CI: 97.5% – 99.4%				

[a]Twenty-eight MTB culture positive specimens were not detected by the Xpert MTB/RIF Assay. These culture isolates were determined to be MTB by bi-directional sequencing analysis.

[b]The Xpert MTB/RIF Assay detected MTB in seven specimens that were MTB culture negative. The culture results were based on one sputum specimen for three subjects, two sputum specimens for two subjects, and three sputum specimens for two subjects.

Overall sensitivity depends on the percent of AFB smear positive subjects among the subjects with positive MTB culture. In the US prospective study, this percent was 75.5% and the overall sensitivity was 93.8%. The overall specificity was 98.7% (95% CI: 97.5% - 99.4%).

In clinical use, overall sensitivity will vary depending on the percentage of patients with AFB-smear positive tuberculosis in the population being tested; overall sensitivity will be lower in a population where the probability of having AFB-smear positive tuberculosis is lower, e.g., a patient population with a higher prevalence of HIV co-infection.

Xpert MTB/RIF Assay Performance vs. MTB Culture by Collection Method

The performance of the Xpert MTB/RIF Assay for detection of MTB-complex was determined relative to MTB culture in expectorated and induced sputum specimens. Results are shown in Table 21 and Table 22. Of the 1,096 specimens, 535 were expectorated specimens, 234 were induced specimens, and 327 were of unknown collection method.

Table 21: Xpert MTB/RIF Assay Performance vs. MTB Culture (Expectorated)

	AFB Smear-Positive Subjects	AFB Smear-Negative Subjects
Sensitivity	99.6% (271/272) 95% CI: 97.9% - 99.9%	79.0% (75/95) 95% CI: 69.7% - 85.9%
Specificity	97.6% (164/168) 95% CI: 94.0% - 99.1%	

Table 22: Xpert MTB/RIF Assay Performance vs. MTB Culture (Induced)

	AFB Smear-Positive Subjects	AFB Smear-Negative Subjects
Sensitivity	100% (15/15) 95% CI: 79.6% - 100%	40.0% (4/10) 95% CI: 16.8% - 68.7%
Specificity	99.0% (207/209) 95% CI: 96.6% - 99.7%	

Xpert MTB/RIF Assay Performance vs. Culture by Specimen Type

The performance of the Xpert MTB/RIF Assay for detection of MTB-complex was determined relative to MTB culture in raw sputum and concentrated sputum sediment. Results are shown in Table 23 and Table 24. Among 1,096 specimens, there were 606 raw sputum specimens and 490 concentrated sputum sediment specimens.

Table 23: Xpert MTB/RIF Assay Performance vs. MTB Culture (Raw Sputum)

	AFB Smear Positive Subjects	AFB Smear Negative Subjects
Sensitivity	99.7% (285/286) 95% CI: 98.0% - 99.9%	79.4% (77/97) 95% CI: 70.3% - 86.2%
Specificity	97.8% (218/223) 95% CI: 94.9% -99.0%	

Table 24: Xpert MTB/RIF Assay Performance vs. MTB Culture (Concentrated Sediment)

	AFB Smear Positive Subjects	AFB Smear Negative Subjects
Sensitivity	100% (65/65) 95% CI: 94.4% - 100%	60.0% (12/20) 95% CI: 38.7% - 78.1%
Specificity	99.3% (402/405) 95% CI: 97.8% - 99.7%	

Xpert MTB/RIF Assay Performance vs. Drug Susceptibility Testing for Rifampin (Rif)

MTB positive culture isolates underwent phenotypic drug susceptibility testing (DST) to Rif using agar proportions methods with Middlebrook or Lowenstein-Jensen media or the BD BACTEC™ MGIT™ 960 SIRE assay. The performance of the Xpert MTB/RIF Assay for the detection of genetic mutations associated with Rif resistance was determined relative to the DST results of the MTB culture isolates. There were 1,096 subjects eligible, with both Xpert MTB/RIF Assay and MTB culture results.

Table 25: Accountability of Eligible Study Subjects for Evaluation of Xpert MTB/RIF Assay for the Detection of Rif Resistance vs. DST

		DST				Total
		Rif Res	Rif Susc	DST Not Done, TB Culture Positive	DST Not Done, TB Culture Negative	
Xpert MTB/RIF Assay	**MTB DETECTED, Rif Resistance DETECTED**	18	4	0	0	22
	MTB DETECTED, Rif Resistance NOT DETECTED	1	404	7	7	419
	MTB DETECTED, Rif Resistance INDETERMINATE	0	4	1	1	6
	MTB NOT DETECTED	2	26	1	620	649
Total		21	447	9	628	1096

There were 447 subjects with an Xpert MTB/RIF Assay result "MTB DETECTED"; 6 out of 447 (1.3%) subjects had an Xpert MTB/RIF Assay result of "MTB DETECTED, Rif Resistance INDETERMINATE" and were excluded from the analysis of the performance of the Xpert MTB/RIF Assay with regard to Rif resistance. Eight subjects with MTB positive cultures did not have DST results. Analysis of missing values with regard to potential biases was performed (i.e., analysis of covariate distributions and logistic regression) and the eight subjects were excluded from the analysis. In the analysis of the performance of the Xpert MTB/RIF Assay with regard to the detection of Rif resistance associated mutations, 1,082 subjects were used.

Results for the detection of Rif resistance associated mutations are reported by the Xpert MTB/RIF Assay only when MTB-complex is detected by the device. Discordant results were further evaluated using bi-directional sequencing of the *rpoB* region of the MTB genome. Overall results are reported in Table 26.

Table 26: Xpert MTB/RIF Assay Performance vs. DST

		DST			
		Rif Res	**Rif Susc**	**DST Not Done TB Culture Negative**	**Total**
Xpert MTB/RIF Assay	**MTB DETECTED, Rif Resistance DETECTED**	18	4[c]	0	22
	MTB DETECTED, Rif Resistance NOT DETECTED	1[b]	404	7	412
	MTB NOT DETECTED[a]	2	26	620	648
	Total	21	434	627	1,082
	Sensitivity: 94.7% (18/19) with 95% CI: 75.4%-99.1% Specificity: 99.0% (404/408) with 95% CI: 97.5%-99.6%				

[a]MTB was not detected and therefore, detection of Rif resistance associated mutations could not be determined.

[b]One discordant specimen determined to be Rif resistant by DST and "Rif resistance NOT DETECTED" by the Xpert MTB/RIF Assay was determined to be Rif resistant by bi-directional sequencing.

[c]Of the four discordant specimens determined to be Rif susceptible by DST and "Rif resistance NOT DETECTED" by the Xpert MTB/RIF Assay, one was shown to be Rif susceptible (Wild Type) and three were Rif resistant by bi-directional sequencing.

"Rif Resistance INDETERMINATE" results were reported for 1.3% (6/447, 95% CI: 0.6% - 2.9%) of the Xpert MTB/RIF Assay MTB-detected specimens overall; 0.28% (1/353, 95% CI: 0.05% to 1.59%) of AFB-smear positive specimens and 5.21% (5/96; 95% CI: 2.24% to 11.62%) of AFB-smear negative specimens.

4. Clinical cut-off**:**

Not Applicable.

5. Expected values/Reference range:

The likelihood that a positive test result is a true positive will vary based on the prevalence of tuberculosis in the population being tested and whether the AFB smear is positive or negative.

The prospective clinical evaluation of the Xpert MTB/RIF Assay in patients suspected

of active TB in the United States resulted in a prevalence of 11.8%, and a percentage of AFB smear-positive smears among MTB culture positive subjects of 75.5%.

Xpert MTB/RIF Assay Predictive Values for the Result "MTB DETECTED"

Hypothetical estimated positive and negative predictive values of MTB detection for different prevalence rates for detecting MTB using the Xpert MTB/RIF Assay are shown in Table 27. These calculations are based on hypothetical prevalences and the overall sensitivity and specificity (compared to culture) observed during the Xpert MTB/RIF Assay multi-center clinical studies. The sensitivity of the Xpert MTB/RIF Assay for AFB smear-positive specimens was 99.7% and sensitivity for AFB smear-negative specimens was 76.1%. Overall specificity of Xpert MTB/RIF Assay was 98.7%. The prevalence of MTB was 11.8% in the US prospective study.

Table 27: Hypothetical Predictive Values of the Xpert MTB/RIF Assay Performance vs. MTB Culture

Prevalence of MTB Culture Positive	Probability of MTB Culture Positive Among		Probability of MTB Culture Negative Among Xpert MTB NOT DETECTED
	Xpert MTB DETECTED AFB Smear Pos.	Xpert MTB DETECTED AFB Smear Neg.	
1%	82.9	14.4	99.9
2%	90.8	25.4	99.9
3%	93.7	34.1	99.8
4%	95.2	41.0	99.7
5%	96.2	46.8	99.7
10%	98.2	65.0	99.3
11.8%	98.5	69.1	99.2
20%	99.2	80.7	98.5
40%	99.7	91.8	96.1
50%	99.8	94.4	94.2

Xpert MTB/RIF Assay Predictive Values for the Result "MTB DETECTED, Rif Resistance DETECTED"

Hypothetical estimated predictive values for the Xpert MTB/RIF Assay result "MTB Detected, Rif Resistance DETECTED" for different prevalence rates of MTB culture positive subjects and different prevalence rates of Rif resistance among MTB culture positive subjects are shown in Table 28. These calculations are based on hypothetical prevalences and the overall sensitivity and specificity (compared to phenotypic drug susceptibility testing (DST)) observed during the multi-center clinical studies. The sensitivity of the Xpert MTB/RIF Assay for the detection of rifampin resistance associated mutations was 94.7% and the specificity was 99.0%. The prevalence of TB in the US prospective study was 11.8%. In the US population with TB, the prevalence of rifampin resistance is approximately 1.8%.

Table 28: Hypothetical Predictive Values of the Xpert MTB/RIF Assay Performance vs. DST

Prevalence of MTB Culture Positive	Prevalence of Rif Resistance Among MTB Culture Positive	Probability of Rif Resistance among Xpert results "MTB DETECTED Rif Resistance DETECTED"	Percent of Xpert results "MTB DETECTED Rif Resistance DETECTED" in the population	Probability of Rif Resistance among Xpert results "MTB DETECTED, Rif Resistance NOT DETECTED"
5%	1.0%	48.4%	0.09%	0.04%
	1.5%	58.6%	0.11%	0.06%
	2.0%	65.5%	0.13%	0.08%
	10%	91.2%	0.47%	0.45%
	50%	98.9%	2.17%	3.39%
11.8%	1.0%	48.4%	0.21%	0.05%
	1.5%	58.6%	0.26%	0.07%
	2.0%	65.5%	0.31%	0.10%
	10%	91.2%	1.11%	0.51%
	50%	98.9%	5.11%	4.16%
20%	1.0%	48.4%	0.35%	0.05%
	1.5%	58.6%	0.44%	0.07%
	2.0%	65.5%	0.52%	0.10%
	10%	91.2%	1.88%	0.54%
	50%	98.9%	8.66%	4.46%

M. Instrument Names:

GeneXpert Instrument Systems to include the following instruments:

- GeneXpert Dx
- GeneXpert Infinity-48
- GeneXpert Infinity-48s
- GeneXpert Infinity-80

N. System Descriptions:

1. Modes of Operation:
   The GeneXpert Instrument System family comprises GeneXpert Dx Systems and GeneXpert Infinity Systems. The GeneXpert Dx Systems include GeneXpert Dx R1 and Dx R2 versions in GX-I, GX-II (R2 version only) GX-IV, GX-XVI instruments. The GX-I is available with 1 module, the GX-II with 2 modules, the GX-IV with up to 4 modules and the GX-XVI is available with 4, 8, 12 or 16 modules. The GeneXpert Infinity Systems comprise the GeneXpert Infinity-48, available with 16, 24, 32, 40, or 48 modules; a GeneXpert Infinity-80 available with 16, 24, 32, 40, 48, 56, 64, 72, or 80 modules, and a 48 module version of the Infinity-80, called the Infinity-48s, available with 16, 24, 32, 40 or 48 modules.

The Xpert MTB/RIF Assay is performed on the Cepheid GeneXpert Instrument Systems, including the GeneXpert Diagnostic (Dx) Systems and the GeneXpert Infinity Systems. The GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification and detection of the target sequences in samples using real-time PCR. The systems consist of an instrument, personal computer, and preloaded software for running the tests and viewing the results. The GeneXpert Instrument Systems require the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process. The cartridges are self-contained and therefore cross-contamination between samples is reduced. In these platforms, additional sample preparation, amplification, and real-time detection are fully-automated. Results of the Xpert MTB/RIF Assay are displayed in tabular and graphic formats.

2. Software:
FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ___X___ or No _____

The GeneXpert Instrument Systems consist of random access, closed-system, computer-based software and embedded firmware running dedicated microprocessor-controlled modules to integrate sample preparation, amplification and real-time detection in a single system. The software assigns access by predefined user types – basic, detail, and administrative. The user interface software is designed with a graphical user interface consisting of a monitor with a pointing device (mouse or touchpad) and keyboard. The GeneXpert Infinity also provides touch screen capability.

Once the Xpert MTB/RIF Assay cartridge is loaded into the instrument system, a computer system prepares the instructions to run a test (assay profile) and then downloads the assay profile to the GeneXpert module. The system integrates sample processing and real-time PCR amplification and detection in a single Xpert MTB/RIF cartridge. The Xpert MTB/RIF Assay completes sample preparation and real-time PCR in approximately two hours. A test result is provided when the test is completed. During the test, the software collects test data from the GeneXpert module periodically, analyzes the optical data, and computes the test result. After the test is completed, the result is shown on the user interface and a report can be generated.

The Xpertise software is the user interface for the Cepheid Infinity System which provides functionality for ordering tests as well as automation of loading and unloading of cartridges into GeneXpert modules within the system. The Xpertise user interface builds upon the existing core software functionality for handling GeneXpert modules for cartridge fluidics control, temperature control, optics control, and data analysis by the addition of automation handling for the robotic arm. Each of the GeneXpert Instrument Systems process data in the same manner using the same optics, are calibrated the same, and process signal the same.

The Xpert MTB/RIF Assay cartridges and the GeneXpert modules are the same for all the GeneXpert Instrument Systems and the Xpert MTB/RIF Assay is designed to perform on any of the GeneXpert Instrument System family models. Each GeneXpert module, regardless of the Instrument System, processes one sample at a time. Each module contains a syringe drive for dispensing fluids, an ultrasonic horn for lysing cells or spores, a valve drive for sample movement, and I-CORE thermocycler for performing real-time PCR and detection.

3. Specimen Identification:

   Barcode

4. Specimen Sampling and Handling:

   The test must be started within four hours of adding the sample reagent-treated specimen to the cartridge. For the Infinity Systems, the user is instructed to place the cartridge on the system within 30 minutes of adding the sample reagent-treated specimen to the cartridge. Once the cartridge is placed onto the conveyor belt to be automatically loaded into an empty GeneXpert module, the instrument monitors the cartridge hold time and rejects the cartridge if it exceeds the maximum hold time of four hours.

5. Calibration:

   Optical and thermal calibration of the GeneXpert Instrument Systems is performed by the firm at the time of manufacture (prior to installation). Alternatively, the laboratory can perform optical re-calibration using the Cepheid Xpert Calibration kit, with performance verification by the firm. Thermal and optical calibration studies were conducted to determine calibration stability of the GeneXpert Instrumentation Systems. The data from the studies demonstrated that the instruments could support 2,000 runs or one year of use, whichever comes first, for a scheduled thermal and optical recalibration.

6. Quality Control:

   Xpert MTB/RIF Assay includes two system controls, referred to as the Probe Check Control and a Sample Processing Control. These internal controls function the same for the MTB/RIF assay target and are described below:

   Sample Processing Control
   The Sample Processing Control (SPC) ensures that the sample was correctly processed. The SPC contains non-infectious spores in the form of a dry spore cake that is included in each cartridge to verify adequate processing of DNA extracted from the sample bacteria. The SPC verifies that binding and elution of MTB-complex bacterial DNA have occurred if the organisms are present and verifies that sample processing is adequate. Additionally this control detects sample-associated inhibition of the real-time PCR assay. The SPC should be positive in an analyte negative sample and can be negative or positive in an analyte positive sample.

The Probe Check Control
The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

External Controls
External positive and negative controls are available but not provided with the Xpert MTB/RIF Assay.

O. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

Specimen Stability Study:

A study was conducted to establish specimen transport and storage claims for sputum specimens for use in the Xpert MTB/RIF Assay. Positive and negative sputum samples were tested. Positive samples consisted of a strain of *M. bovis* BCG at a concentration near the assay LoD spiked in a background matrix of human sputum. Negative samples consisted of human sputum only. The recommended storage conditions for sputum are 35°C for up to three days and then refrigerated at 2-8ºC for an additional 7 days until sample testing. The study supported this claim.

A second study was conducted to establish specimen transport and storage claims for sputum sediment specimens after sputum digestion, decontamination (using NALC-NaOH) and concentration and resuspension in phosphate buffer, for use in the Xpert MTB/RIF Assay. Positive and negative sputum sediment samples were tested. Positive samples consisted of a strain of *M. bovis* BCG at a concentration near the LoD spiked in human sputum. The spiked samples were then digested, decontaminated (using NALC-NaOH), concentrated and resuspended in phosphate buffer. Negative samples consisted of human sputum only. The recommended storage condition for sputum sediment is 2-8°C for up to 7 days until sample testing. The study supported this claim.

Cartridge Hold Time*:*

A study was conducted to establish the cartridge hold time for sputum specimens. Cartridge hold time is defined as interval from the time the sputum sample is added to the assay cartridge until the time processing of the cartridge begins. Positive and negative sputum samples were tested. Positive samples consisted of a strain of *M. bovis* BCG at a concentration near the assay LoD spiked into a background matrix of human sputum. Negative samples consisted of human sputum only. The recommended cartridge hold time for sputum is 4 hours. The study supported this claim.

A second study was conducted to establish the cartridge hold time for sputum sediment specimens. Cartridge hold time is defined as interval from the time the sputum sediment sample is added to the assay cartridge until the time processing of the cartridge begins. Positive and negative sputum samples were tested. Positive samples consisted of a strain of

M. bovis BCG at a concentration near the assay LoD spiked into a background matrix of human sputum. The spiked samples were then digested, decontaminated (using NALC-NaOH), concentrated and resuspended in phosphate buffer. Negative samples consisted of human sputum only. The recommended cartridge hold time for sputum sediment is 4 hours. The study supported this claim.

Shelf-Life:

The Xpert MTB/RIF Assay shelf-life was demonstrated in stability studies using real-time stability results and linear regression analysis to support a shelf-life of 18 months when reagents and cartridges are stored at 2-28°C; and an open package for up to 6 weeks at 2-45ºC.

The shelf-life stability of the product was evaluated at four temperatures (5°± 3°C, 25° ± 3°C, 35º ± 3°C, and 45º ± 3°C) at predefined time points up to 36 months, following the study plan described in the 510k submission.

The open package stability of the product was evaluated at three temperatures (5°± 3°C, 25° ± 3°C/75% relative humidity, and 45º ± 3°C/75% relative humidity) at predefined time points up to 6 weeks, following the study plan described in the 510k submission.

Failure Mode Testing*:*

Failure Mode Testing was performed to determine the effect of failure modes that might occur with the Xpert MTB/RIF Assay. Failures may be due to operator errors, manufacturing errors or instrument malfunction.

Operator error might include failing to add the sample to the cartridge or adding an insufficient amount of sample to the cartridge.

Manufacturing error might result in beads being loaded incorrectly into the cartridge, liquid reagent loaded incorrectly, or cartridges being assembled incorrectly.

Instrument malfunction examples include, motion of the syringe drive not detected, syringe pressure reading exceeding protocol limit, system fails to find the plunger home position, detection of a valve positioning error, digital temperature reading of the thermistor(s) not within acceptable range, and the optical signal from the detectors failing to reach the expected value. Because the software performs self-check procedures prior to the start of each test, if any of the instrument malfunctions described above occur the test is aborted, and no assay results are reported.

Replicates of four MTB negative and four MTB positive samples were tested per condition. Positive samples contained MMQCI control material (representing the wild-type *rpoB* gene sequence) at a concentration near the LoD, in TET buffer. Negative samples contained TET buffer only. Results were compared to positive and negative controls in which all cartridges were assembled correctly and all samples were added correctly and completely.

Tables 29 and 30 below include the results of the studies describing operator error and manufacturing error, respectively.

Table 29. Xpert MTB/RIF Assay Test Results – Possible Operator Errors

Condition	Addition of Liquid Reagents	Results	
		Pos[1]	Neg[2]
A*	Control – All reagents added correctly and completely	Pos	Neg
B^	No addition of sample to cartridge	Neg	
C	Addition of 90% recommended sample volume (1.8ml)	Pos	Neg
D	Addition of 80% recommended sample volume (1.6ml)	Pos	Neg
E	Addition of 70% recommended sample volume (1.4ml)	Pos	Neg

(1) Positive results reported as "MTB DETECTED; Rif Resistance NOT DETECTED"
(2) Negative results reported as "MTB NOT DETECTED".
(*) Replicates of 8 positive and 8 negative were run under the control condition.

Table 30. Xpert MTB/RIF Assay Test Results – Possible Manufacturing Errors

	Cartridge Assembly										
Condition	Rgnt 1	Rgnt 2	EZR1	EZR2	TSR1	TSR2	SPC	Pre-Filter	Capture Filter	Pos[1]	Neg[2]
A	+	+	1	1	1	1	1	+	+	Pos	Neg
F	+	+	1	1	1	1	1	-	+	Pos	Neg
G	+	+	0	1	1	1	1	+	+	*Error*	*Error*
H	+	+	1	0	1	1	1	+	+	*Error*	*Error*
I	+	+	1	1	0	1	1	+	+	*Error*	*Error*
J	+	+	1	1	1	0	1	+	+	*Error*	*Error*
K	+	+	1	1	1	1	0	+	+	Pos	*Invalid*
L*	+	+	1	1	1	1	1	+	+	*Error*	*Error*
M^	+	+	1	1	1	1	1	+	+	*Error*	*Error*
N	+	-	1	1	1	1	1	+	+	*Error*	*Error*
O	-	+	1	1	1	1	1	+	+	Pos	Neg
P	-	-	1	1	1	1	1	+	+	*Error*	*Error*
Q	+	+	1	1	1	1	1	+	-	*Invalid*	*Invalid*

(1) Positive results reported as "MTB DETECTED; Rif Resistance NOT DETECTEI
(2) Negative results reported as "MTB NOT DETECTED".
(*) EZR 1 and 2 bead chamber locations are switched.
(^) TSR 1 and 2 bead chamber locations are switched.

P. Proposed Labeling:

The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10, 21 CFR 801.109, and the special controls.

Q. Risks to Health and Mitigations:

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 device description, performance studies, and labeling.
False negative test results for the presence of *Mycobacterium tuberculosis* complex could contribute to disease progression and increase the risk of transmitting disease 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 device description, performance studies, and labeling.
False positive test results for the presence of the 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.	The following items: 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.
False negative test results for the presence of the genetic mutations associated with *Mycobacterium tuberculosis* complex antibiotic resistance could contribute to disease progression and increase the risk of transmitting disease to others.	The following items: 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.
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 labeling.

R. Benefit/Risk Analysis

The primary benefit from this device is more rapid detection of active *M. tuberculosis* complex pulmonary infection and the ability to detect mutations associated with rifampin resistance when *M. tuberculosis* complex is detected. There are FDA-approved devices for rapid detection of *M. tuberculosis* complex*;* however, the design characteristics of this device (i.e., a closed system with internal controls) are a significant advance in this area, and availability of this device is likely to increase the adoption of nucleic-acid based testing for the detection of *M. tuberculosis* complex. The benefits of local testing to patients and practitioners include more rapid detection of disease and potentially shorter periods of respiratory isolation for patients suspected of tuberculosis. More rapid detection of *M. tuberculosis* may decrease transmission to uninfected individuals, although in practice this effect may be limited.

The detection of mutations associated with resistance to rifampin may more rapidly identify individuals who should be treated with alternative regimens and/or identify patients with multidrug resistant tuberculosis, an important public health objective.

The benefits of closed system testing are substantial but are far greater in settings with limited clinical laboratory infrastructure. Benefits from this (and similar) devices are likely to be far greater in areas with limited laboratory capability, higher rates of tuberculosis, and higher rates of drug resistance.

Current accepted practice for the diagnosis of tuberculosis is performance of an AFB smear with conventional microbiological cultures. FDA analysis of the sponsor's data suggests that AFB smear may be unnecessary in the setting of Xpert® MTB/RIF Assay results where *M. tuberculosis* is detected given the high sensitivity of the assay for AFB smear-positive samples and the high likelihood that an AFB smear positive that is Xpert® MTB/RIF Assay negative is far more likely to have an alternative diagnosis. The ability to obviate AFB-smear testing is another potential benefit from device use; however, AFB smear testing may still be useful for the diagnosis of mycobacterial diseases other than tuberculosis, and testing is likely to continue to be recommended as other mycobacterial diseases can have a clinical presentation similar to pulmonary tuberculosis. (AFB testing (i.e., smear and culture) may be unnecessary when active tuberculosis is the sole diagnosis under consideration and other diseases [e.g., other

mycobacterial diseases] are not a concern; this is likely to be a relatively uncommon circumstance.)

Risk of a false positive result increases as prior probability of disease decreases. As discussed earlier, however, there are several mitigations: a false positive result in the setting of an AFB smear positive sample is extremely unlikely; an AFB smear-negative specimen in the setting of an Xpert® MTB/RIF Assay positive result would decrease the PPV and raise clinical concern for a false positive result, although a major benefit of the device is the ability to detect *M. tuberculosis* in 60-70% of AFB-smear negative specimens). Conventional culture will always be performed in the setting of Xpert® MTB/RIF Assay testing, and a negative result, although delayed, strongly suggests a false positive result. Clinical resolution without treatment for tuberculosis obviously also suggests a false positive result.

Although a false positive result would have a substantial impact on the individual and contacts (many of whom may require testing for tuberculosis exposure), the worst possible scenario whereby a patient would be treated for presumed tuberculosis would not pose an acceptably high risk to health as patients are carefully monitored and the risks of treatment are well recognized. There is also the risk that a false positive result for tuberculosis in the setting of active pulmonary disease (the rationale for the original testing) would limit further investigation of the true cause of illness; however, it is difficult to assess the true morbidity from this risk as tuberculosis testing usually occurs in the setting of simultaneous workup for other possible causes of illnesses, and tuberculosis may coexist with other causes of pulmonary disease. It is also important to recognize that this is not a new risk intrinsic to this device as a similar (although perhaps slightly lower) risk of a false positive result is present with existing nucleic-acid based devices for the detection of *M. tuberculosis* complex and lower than an AFB smear test.

False positive results for the detection of mutations associated with rifampin resistance would likely be mitigated by testing at a reference laboratory given the implications for treatment and the need for testing for susceptibility to other drugs.

False negative test results for the presence of *Mycobacterium tuberculosis* complex pose risks to patients and potential contacts; however, a false negative result in the setting of an AFB smear-positive sample is unlikely, and therefore the risk of spread to uninfected contacts is low. The device is more sensitive than AFB smear testing, and although there is an overall 10 – 40% risk of false negative results, nucleic acid-based tests for tuberculosis are currently the most sensitive methods available for the rapid diagnosis of tuberculosis. AFB culture and smear should always be performed in the setting of testing for tuberculosis, which would mitigate a false negative Xpert® MTB/RIF Assay result (although the culture result will be delayed). In addition, in the setting where tuberculosis is strongly suspected and rapid testing is negative, a more invasive test such as bronchoscopy is likely to be performed.

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. A fixed, relatively low incidence of false negative results for the detection of mutations associated with rifampin resistance is anticipated

as mechanisms of phenotypic resistance to rifampin exist separate from the genetic mutations; these would be in addition to false negative device errors from other causes. False-negative results would be detected when the results of standard phenotypic drug susceptibility testing become available, approximately two - three months after obtaining respiratory samples. Overall, given the relatively low prevalence of rifampin resistance in patients in the US with tuberculosis (< 2%), risk from a false negative result is relatively low and poses little patient risk given the current standard of care in the absence of rapid testing.

Tuberculosis remains a significant public health concern in the US despite decreasing prevalence; similarly, although drug resistant and multidrug resistant strains are less prevalent in the US relative to endemic areas, drug resistant tuberculosis represents a potentially significant public health issue and can be transmitted to contacts. Devices with high accuracy that can expedite testing for tuberculosis are viewed very favorably by public health officials. The Centers for Disease Control has recommended nucleic-acid based testing of all tuberculosis suspects, a goal likely to be enhanced by clearance of the Xpert® MTB/RIF Assay.

S. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.3373. FDA believes that special controls, along with the applicable general controls, provide reasonable assurance of the safety and effectiveness of the device type. The special controls for this device, and similar devices, 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 Antibiotic Resistance in Respiratory Specimens," which addresses the mitigation of risks specific to the detection of *M. tuberculosis* complex.

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.