GeneXpert
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Xpert® MTB/RIF

REF **GXMTB/RIF-US-10**

Cepheid®

In Vitro Diagnostic Medical Device IVD

301-1404, Rev. F August 2019

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Xpert® MTB/RIF Assay

For In Vitro Diagnostic Use Only.

Rx Only

1. Proprietary Name

Xpert® MTB/RIF

2. Common or Usual Name

Xpert MTB/RIF Assay

3. Intended Use

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 sputum sediment 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 three 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.

An Xpert MTB/RIF Assay result of "MTB NOT DETECTED" from either one or two sputum specimens is highly predictive of the absence of *M. tuberculosis* complex bacilli on serial fluorescent acid-fast sputum smears from patients with suspected active pulmonary tuberculosis and can be used as an aid in the decision of whether continued airborne infection isolation (AII) is warranted in patients with suspected pulmonary tuberculosis. The determination of whether testing of either one or two sputum specimens is appropriate for decisions regarding removal from AII should be based on specific clinical circumstances and institutional guidelines. Clinical decisions regarding the need for continued AII should always occur in conjunction with other clinical and laboratory evaluations and Xpert MTB/RIF Assay results should not be the sole basis for infection control practices.

The Xpert MTB/RIF Assay must always be used in conjunction with mycobacterial culture to address the risk of false negative results and to recover organisms when MTB-complex is present for further characterization and drug susceptibility testing. However, decisions regarding the removal of patients from AII need not wait for culture results. Sputum specimens for TB culture, AFB smear microscopy, and Xpert MTB/RIF Assay testing should follow CDC recommendations with regard to collection methods and time frame between specimen collection.

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 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.

4. Summary and Explanation

Globally, about 2 billion people are infected with MTB.[1] In 2010, 8.8 million people developed active disease and 1.4 million people lost their lives to the illness.[2] There were 9,951 new cases of tuberculosis reported in the United States in 2012 (a rate of 3.2 cases per 100,000). In 2011, 536 U.S. deaths were attributed to tuberculosis infections.[3,4]

Standard treatment regimens for tuberculosis involve prolonged administration of multiple drugs and are usually highly effective. However, MTB-complex strains that are resistant to one or more of first line drugs require individualized treatment. Rifampin resistance is often an indication of multidrug resistance to tuberculosis (MDR TB), which is defined as resistance to at least rifampin (RIF) and isoniazid (INH). In the United States overall RIF resistance is approximately 1.8%, with approximately 90% of these strains resistant to at least RIF and INH.[5]

Active pulmonary TB is a highly infectious airborne disease. All patients in healthcare facilities with suspected TB should be maintained in airborne infection isolation (AII) according to recommended infection control guidelines.[6] The testing of either one or two sputum specimens by the Xpert MTB/RIF Assay may serve as an alternative to serial acid-fast stained sputum smears as an aid in the decision of whether continued infection control precautions are warranted in patients with suspected pulmonary tuberculosis.

Sputum specimens that are AFB smear-negative but subsequently TB culture positive have lower MTB-complex organism loads than specimens that are AFB smear-positive. Because of the greater sensitivity of the Xpert MTB/RIF Assay for the detection of MTB-complex than that of acid-fast microscopy, MTB-complex may be detected by the Xpert MTB/RIF Assay in AFB smear-negative samples.

Patients with HIV infection and pulmonary TB are known to have lower organism loads of MTB-complex in their sputum specimens relative to HIV-uninfected patients, despite more rapid disease progression if untreated. As a consequence, sputum specimens from HIV-infected patients with pulmonary TB tend to be AFB smear-negative more frequently than HIV-uninfected patients. Overall rates of detection of MTB-complex with the Xpert MTB/RIF Assay may be lower in settings with a high percentage of HIV-infected patients because these patients are more likely to produce AFB smear-negative specimens with low organism loads.

5. Principle of the Procedure

The Xpert MTB/RIF Assay is an automated *in vitro* diagnostic test using nested real-time PCR for the qualitative detection of MTB-complex and RIF resistance. 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. This assay can be performed on Cepheid GeneXpert® Instrument Systems.

GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence using real- time reverse transcriptase PCR (RT-PCR) and real-time PCR assays. The systems consist of an instrument, personal computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable GeneXpert cartridges that hold the RT-PCR and PCR reagents and host the RT-PCR and PCR processes. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, refer to the appropriate *GeneXpert Dx System Operator Manual* or *GeneXpert Infinity System Operator Manual*.

The Xpert MTB/RIF Assay includes reagents for the detection of MTB-complex and RIF resistance from raw sputum samples and in concentrated sputum sediments. 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 bacteria 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.

The Xpert MTB/RIF Assay simultaneously detects MTB-complex and RIF 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 MTB/RIF 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 condition 1) - (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 protocol and cannot be modified by the user.

6. Reagents and Instruments

6.1 Materials Provided

The Xpert MTB/RIF Assay kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert MTB/RIF Assay Cartridges with Integrated Reaction Tubes	**10**

• Bead 1 (freeze-dried)
  • Polymerase
  • dNTPs (deoxynucleoside triphosphates)
  • Probe
  • BSA (Bovine serum albumin)
	2 of each per cartridge

• Bead 2 (freeze-dried)
  • Primers
  • Probes
  • BSA (Bovine serum albumin)
	2 of each per cartridge

• Bead 3 (freeze-dried) • Sample Processing Control (SPC) ~6,000 non-infectious *B. globigii* spores	1 per cartridge

• Reagent 1
  • Tris Buffer
  • Surfactants
  • EDTA (ethylenediaminetetraacetic acid)
	4 mL per cartridge

• Reagent 2
  • Tris Buffer
  • Surfactants
  • EDTA (ethylenediaminetetraacetic acid)
	4 mL per cartridge

Sample Reagent • Sodium Hydroxide • Isopropanol	**8 mL per bottle**
Disposable Transfer Pipettes	**12**
CD	**1 per kit**

- Assay Definition File (ADF) – ADF for use with both the GeneXpert Dx and Infinity Systems
- Instructions to import ADF into GX software
- Package Insert

Note Sample Reagent (SR) can be colorless to yellow to amber. Color may intensify with time, but color has no effect on performance.

Note Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no commingling of the material with other animal materials.

Note The transfer pipettes have a single mark representing the minimum volume of sample necessary to transfer the GeneXpert cartridge. Use only for this purpose. All other pipettes must be provided by the laboratory.

6.2 Storage and Handling

- Store the Xpert MTB/RIF Assay cartridges and reagents at 2 – 28 °C.
- Do not use reagents or cartridges that have passed the expiration date.

- The cartridge is stable up to 6 weeks at 2-45°C after opening the pouch. Do not open a cartridge until ready to test.
- If using a GeneXpert Dx instrument, start the test within four hours of adding the Sample Reagent-treated sample to the cartridge.
- If using a GeneXpert Infinity system, be sure to start the test and put the cartridge on the conveyor within 30 minutes of adding the Sample Reagent-treated sample to the cartridge. Remaining shelf-life is tracked by the system by the Xpertise Software so that tests are run prior to the four hour on-board expiration.
- Do not use any reagents that have become cloudy or discolored.

6.3 Materials Required but Not Provided

- GeneXpert Instrument System (catalog number varies by configuration): 6-color GeneXpert instrument, computer with proprietary **GeneXpert Software Version 4.3 or higher,** barcode scanner, and operator manual.
- Printer: If a printer is required, contact Cepheid Technical support to arrange for the purchase of a recommended printer.
- Leak-proof, sterile, screw-capped specimen collection containers
- Disposable gloves, eye protection, laboratory coats, and labels or permanent marking pen
- Sterile, single use pipettes with dry-end barrier plugs for sample processing
- Timer

6.4 Materials Available but Not Provided

MMQCI (Maine Molecular Quality Controls, Inc.) INTROL™ External Run Control (catalog # TBNEG-04) as negative control, and MMQCI INTROL™ External Run Controls (catalog # TBWT-04 and catalog #TBMDR1-04) as RIF susceptible and RIF resistant positive controls.

7. Warnings and Precautions

- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which specimens might be infectious, all biological specimens should be treated with standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[7] and the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards).[8]
- Wear protective disposable gloves, laboratory coats, and eye protection when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Preparation of digested, decontaminated and concentrated sputum sediments, and Xpert MTB/RIF procedures should be done using Biosafety Level 2 practices.[9]
- Use only for the detection of members of the *M. tuberculosis* complex using sediments prepared following the NALC-NaOH or NaOH procedures recommended by the Centers for Disease Control and Prevention (CDC).[10] This test may only be used with raw sputum samples or concentrated sediments prepared from induced or expectorated sputa.
- When processing more than one sample at a time, open only one cartridge; add the Sample Reagent-treated sample and close the cartridge before processing the next sample. Change gloves between samples.
- Do not substitute Xpert MTB/RIF Assay reagents with other reagents.
- Do not open the Xpert MTB/RIF Assay cartridge lid except when adding the Sample Reagent-treated sample.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Do not use a cartridge that has been dropped or shaken.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert MTB/RIF Assay cartridge is used to process one test. Do not reuse spent cartridges.
- Consult your institution's environmental waste personnel regarding proper disposal of used cartridges and unused reagents. This material may contain federal EPA Resource Conservation and Recovery Act (RCRA) hazardous waste requiring specific disposal. Check state and local regulations as they may differ from federal disposal regulations. Institutions should check their country hazardous waste disposal requirements.
- Sample Reagent contains sodium hydroxide (pH > 12.5); (international chemical safety hazard codes H303, H314), which may be irritating to eyes and skin and requires eye and skin protection. Sample reagent also contains isopropanol (international chemical safety hazard code H226), which is a flammable liquid.
- The HIV/MTB co-infected population may contain an increased percentage of patients with smear-negative samples with MTB-complex levels below the assay's level of detection.
- Local, state, and federal regulations for notification of reportable diseases are continually updated and include a number of organisms for surveillance and outbreak investigations.[11,12] Additionally the Centers for Disease Control and Prevention (CDC) recommends that when pathogens from reportable diseases are detected by a culture independent diagnostic test (CIDT), the laboratory should facilitate obtaining the isolate or clinical materials for submission to the appropriate public health laboratory to aid in outbreak and epidemiological investigations. Laboratories are responsible for following their state and local regulations and should consult their state and local public health laboratories for isolate and clinical sample submission guidelines.

8. Specimen Collection, Transport and Storage

8.1 Collection

Collect raw sputum or sputum sediment samples following your institution's standard procedures. The patient should be seated or standing. See Table 1 to determine adequate specimen volume.

Table 1. Required Specimen Volume

Specimen Type	Minimum Volume for One Test	Minimum Total Volume for Test and Retest – See Section 11.2, Retest Procedure
Sputum sediment	0.5 mL	1 mL
Raw sputum	1 mL	2 mL

8.2 Storage and Transport

Sputum sediment: Store resuspended sediments at 2–8 °C for up to seven days.

Raw sputum: Transport and store specimens at 2–8 °C before processing whenever possible. If necessary, sputum specimens can be stored at a maximum of 35 °C for up to three days and then at 2–8 °C for an additional seven days.

9. Assay Procedure(s)

9.1 Concentrated Sputum Sediment Specimens

This procedure is for use with sputum sediments prepared from induced or expectorated sputa.

Note Reject samples with obvious food particles or other solid particulate.

Volume Requirements: The MTB/RIF Assay requires at least 0.5 mL of resuspended sputum sediment after digestion, decontamination and concentration. Use the method of Kent and Kubica[8] and resuspend the sediment in a 67 mM Phosphate/H_2O buffer. After resuspension, keep at least 0.5 mL of the resuspended sediment for the Xpert MTB/RIF Assay.

1. Wear protective disposable gloves.
2. Label each Xpert MTB/RIF Assay cartridge with the sample ID.

Note Write on the sides of the cartridge or affix an ID label. Do not put the label on the lid of the cartridge or cover the existing barcode on the cartridge.

3. Transfer at least 0.5 mL of the total resuspended sediment to a conical, screw-capped tube for the Xpert MTB/RIF Assay using a transfer pipette. Alternatively, the entire sediment can be processed in the original tube.

Note Store resuspended sediments at 2–8 °C for up to seven days.

4. Using a transfer pipette, transfer 1.5 mL of Sample Reagent to 0.5 mL of resuspended sediment. For larger volumes of sediment, add Sample Reagent equal to three times the volume of the resuspended sediment.
5. Recap the tube and shake vigorously 10 to 20 times or vortex for at least 10 seconds.

Note One back-and-forth movement is a single shake.

6. Incubate sample for a total of 15 minutes at 20-30°C.
7. Between 5 and 10 minutes into the incubation period, shake vigorously 10 to 20 times or vortex for at least 10 seconds.

9.2 Raw Sputum Specimens

Note Reject samples with obvious food particles or other solid particulate.

1. Wear protective disposable gloves.
2. Label each Xpert MTB/RIF Assay cartridge with the sample ID. See Figure 1.

Note Write on the sides of the cartridge or affix an ID label. Do not put the label on the lid of the cartridge or cover the existing barcode on the cartridge.

Figure 1. Writing on the Cartridge with a Permanent Marker

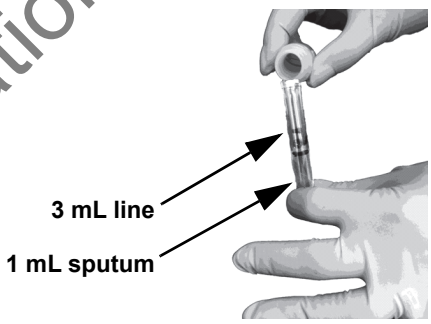
3. Carefully open the lid of the sputum collection container. See Figure 2.

Figure 2. Opening the Sample Container

4. Pour or pipette (pipette not provided) approximately 2 times the volume of the Sample Reagent into the sputum (2:1 dilution, Sample Reagent: sputum). Be careful not to allow the Sample Reagent to touch bare skin. See Figure 3.

Example 1

8 ml Sample Reagent;
4 mL sputum



Example 2

2 ml Sample Reagent;
1 mL sputum

Note: Discard the leftover Sample Reagent and the bottle in a chemical waste container.

Figure 3. Examples of 2:1 Dilutions

5. Replace and secure the lid.
6. Shake vigorously 10 to 20 times or vortex for at least 10 seconds.

Note One back-and-forth movement is a single shake.

7. Incubate sample for a total of 15 minutes at 20–30 °C.
8. Between 5 and 10 minutes into the incubation period, shake vigorously 10 to 20 times or vortex for at least 10 seconds.

9.3 Preparing the Cartridge

Important **If using a GeneXpert Dx instrument, start the test within four hours of adding the Sample Reagent-treated sample to the cartridge. If using a GeneXpert Infinity system, be sure to start the test and put the cartridge on the conveyor within 30 minutes of adding the Sample Reagent-treated sample to the cartridge. Remaining shelf-life is tracked by the system by the Xpertise Software so that tests are run prior to the four hour on-board expiration.**

Note When processing more than one sample at a time, open only one cartridge, add the Sample Reagent-treated sample and close the cartridge before moving to the next sample. Change gloves between samples.

To add the sample and reagents into the cartridge:

1. Open the cartridge lid, and then open the sample container.
2. Using the provided transfer pipette, aspirate the liquefied sample close to the line on the pipette. See Figure 4. If there is insufficient sample volume, do not proceed with testing.

Figure 4. Aspirating to the Line on the Pipette

3. Dispensing the sample slowly to minimize the risk of aerosol formation, transfer the Sample Reagent-treated sample into the sample chamber of the Xpert MTB/RIF cartridge. See Figure 5.

Figure 5. Dispensing Decontaminated Liquefied Sample into the Sample Chamber of the Cartridge

4. Close the cartridge lid firmly. Remaining Sample Reagent-treated sample can be kept for up to four hours at 2–8 ºC in case retesting is required.

9.4 Starting the Test

Important **Before you start the test, make sure that the system is running GeneXpert Software Version 4.3 or higher and that the Xpert MTB/RIF assay definition file is imported into the software.**

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual.*

Note The steps you follow may be different if the system administrator has changed the default workflow of the system.

1. Turn on the GeneXpert Instrument System.
   - If using the GeneXpert Dx instrument, first turn on the GeneXpert Dx instrument and then turn on the computer. The GeneXpert software will launch automatically or may require double-clicking on the GeneXpert Dx software shortcut icon on the Windows® desktop.

     or

   - If using the GeneXpert Infinity instrument, power up the instrument. On the Windows desktop, double-click the Xpertise software shortcut icon.
2. Log on to the GeneXpert Instrument System software using your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or **Orders** and **Order Test** (Infinity).
4. Scan or type in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and is shown in the View Results window.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is associated with the test results and is shown in the View Results window and all reports The Scan Cartridge dialog box appears.
6. Scan the barcode on the Xpert MTB/RIF Assay cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

Note If the barcode on the Xpert MTB/RIF Assay cartridge does not scan, then repeat the test with a new cartridge.

7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity)**.** Enter your password if requested.
8. For the GeneXpert Infinity System, place the cartridge on the conveyor belt. The cartridge will be automatically loaded, the test will run and the used cartridge will be placed in the waste container.

   or

   For the GeneXpert Dx Instrument:

   A. Open the instrument module door with the blinking green light and load the cartridge.

   B. Close the door. The test starts and the blinking green light changes to a solid green light. When the test is finished, the light turns off.

   C. Wait until the system releases the door lock before opening the module door and removing the cartridge.

   D. The used cartridges should be disposed of in the appropriate specimen waste containers according to your institution's standard practices.

9.5 Viewing and Printing Results

This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual.*

1. Click the **View Results** icon to view results.
2. Upon completion of the test in approximately two hours, click the **Report** button of the View Results screen to view and/or generate a pdf report file.

10. Quality Control

CONTROL

Each test includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

Sample Processing Control (SPC) – Ensures 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 MTB. The SPC verifies that conditions for lysis of MTB have occurred if the organisms are present and verifies that specimen processing is adequate. Additionally, this control detects specimen-associated inhibition of the real-time PCR reactions and acts as an internal positive control.

The SPC should be positive in a MTB-negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria. The test result will be **Invalid** if the SPC is not detected in an MTB-negative sample.

Probe Check Control (PCC, QC1, QC2) — Before the start of the first and second reactions of the nested PCR assay, the GeneXpert Instrument System measures the fluorescence signal from the QC1 and QC2 probes (reaction 1) and the *rpoB* and SPC probes (reaction 2) to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. The PCC passes if it meets the assigned acceptance criteria.

External Controls

MMQCI (Maine Molecular Quality Controls, Inc.) INTROL™ External Run Control (catalog # TBNEG-04) as negative control and MMQCI INTROL™ External Run Controls (catalog # TBWT-04 and catalog # TBMDR1-04) as RIF susceptible and RIF resistant positive controls may be used for training, proficiency testing, and external quality control. External controls should be used in accordance with local, state, and federal accrediting organizations' requirements as applicable.

11. Interpretation of Results

The GeneXpert Instrument System generates the results from measured fluorescent signals and embedded calculation algorithms. The results can be seen in the View Results window. See Figure 6, Figure 7, Figure 8, and Figure 9 for specific examples, and see Table 2 for a list of all possible results.

Table 2. Xpert MTB/RIF Assay Results and Interpretations

Result	Interpretation
MTB DETECTED; **Rif Resistance DETECTED** (Figure 6)	The MTB target is detected within the sample: • A mutation in the *rpoB* gene has been detected. • SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control. • Probe Check (QC1 and QC2): PASS. All probe check results pass.
MTB DETECTED; **Rif Resistance NOT DETECTED** (Figure 7)	The MTB target is detected within the sample: • A mutation in the *rpoB* gene has not been detected. • SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control. • Probe Check (QC1 and QC2): PASS. All probe check results pass.
MTB DETECTED; **Rif Resistance INDETERMINATE** (Figure 8)	The MTB target is detected within the sample: • A mutation in the *rpoB* gene could not be determined due to insufficient signal detection. • SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control. • Probe Check (QC1 and QC2): PASS. All probe check results pass.

Table 2. Xpert MTB/RIF Assay Results and Interpretations (Continued)

Result	Interpretation
MTB NOT DETECTED (Figure 9)	The MTB target is not detected within the sample. • SPC: PASS. The SPC met the acceptance criteria. • Probe Check (QC1 and QC2): PASS. All probe check results pass.
INVALID (Figure 10)	The presence or absence of MTB cannot be determined. The SPC does not meet the acceptance criteria, the sample was not properly processed, or PCR was inhibited. **Repeat the test**. See Section 11.2, Retest Procedure. • MTB INVALID: The presence or absence of MTB DNA cannot be determined. • SPC: FAIL. The MTB target result is negative, and the SPC Ct is not within valid range. • Probe Check (QC1 and QC2): PASS. All probe check results pass.
ERROR	The presence or absence of MTB cannot be determined. **Repeat the test**. See Section 11.2, Retest Procedure. • MTB: NO RESULT • SPC: NO RESULT • Probe Check (QC1 and QC2): PASS/FAIL. Probe check failure can be the source of error but other errors, such as system component failure, can occur even if probe check passes.
NO RESULT	The presence or absence of MTB cannot be determined. **Repeat the test**. See Section 11.2, Retest Procedure. A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • MTB: NO RESULT • SPC: NO RESULT • Probe Check (QC1 and QC2): NA (not applicable).

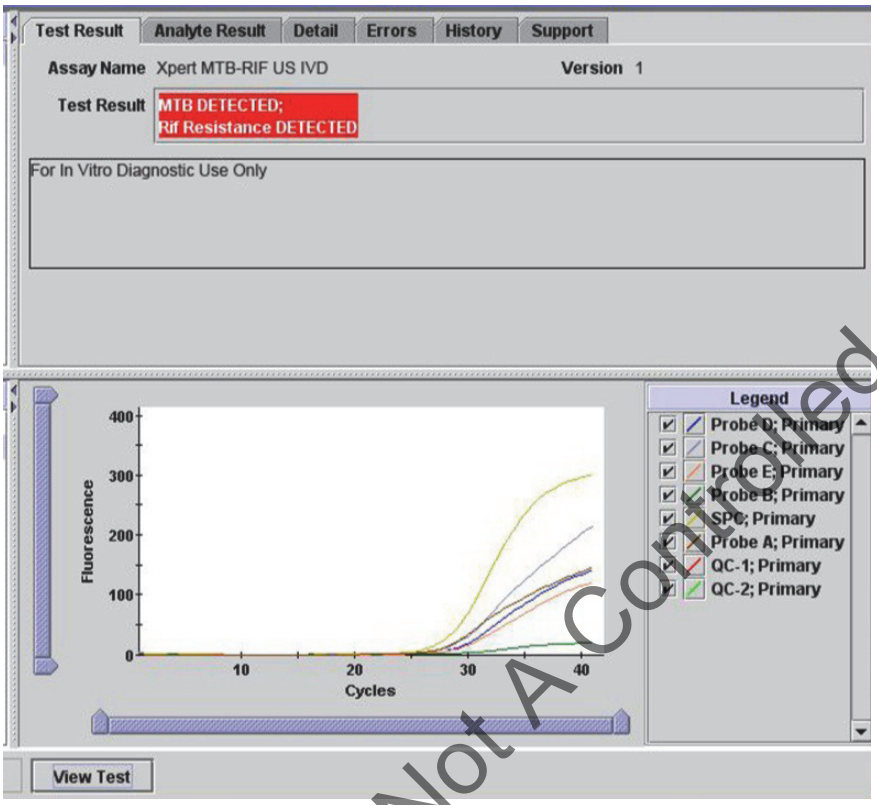


Figure 6. An Example of a MTB DETECTED; Rif Resistance DETECTED Result

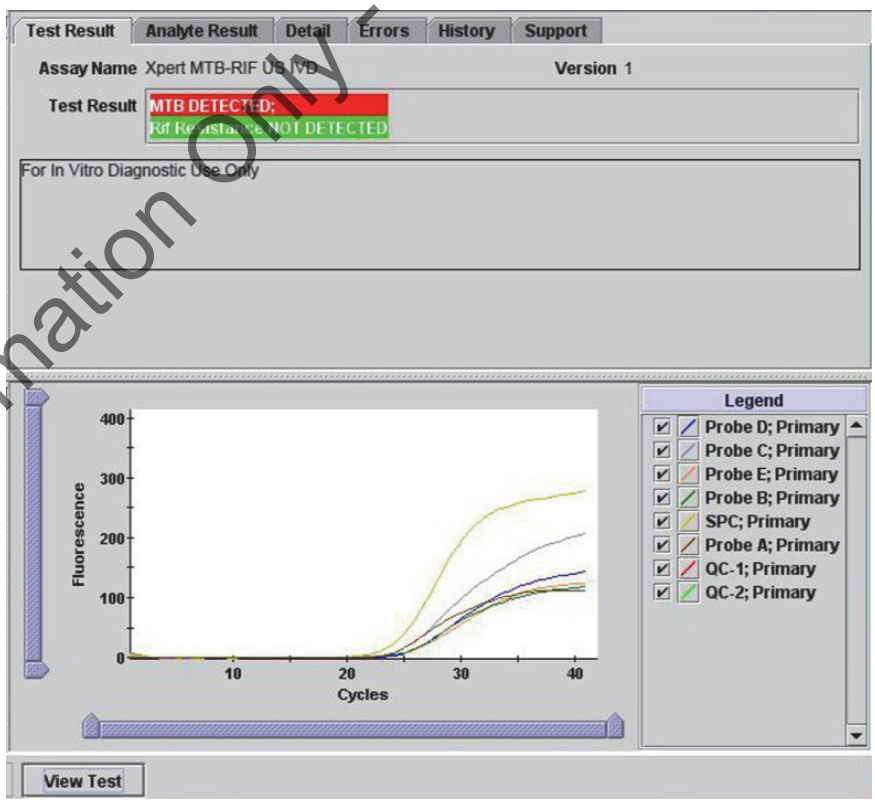


Figure 7. An Example of a MTB DETECTED; Rif Resistance NOT DETECTED Result

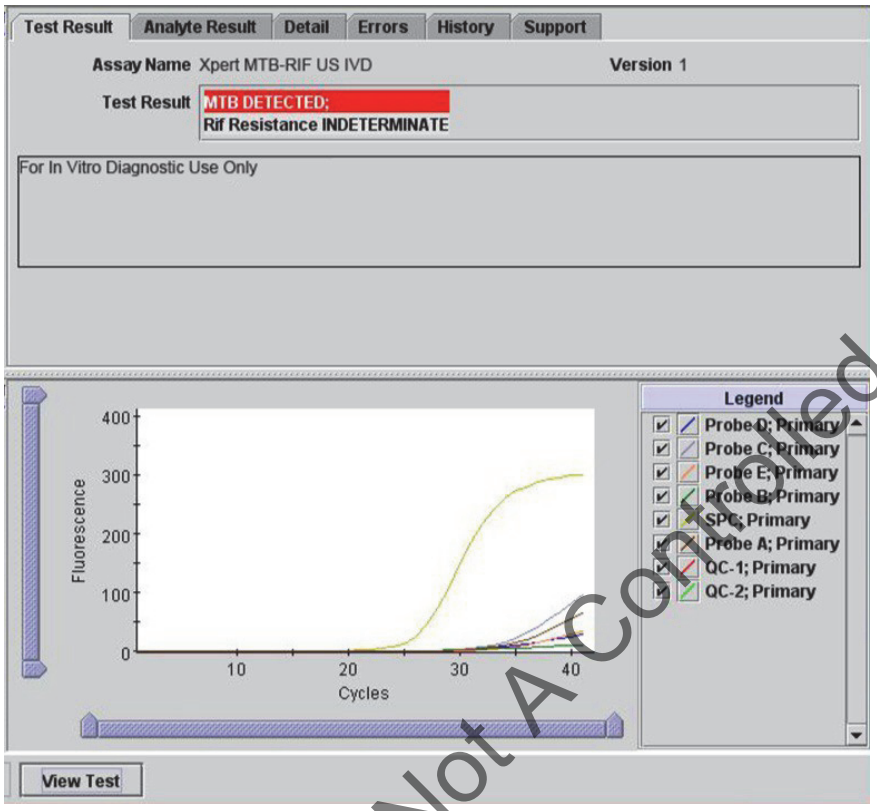


Figure 8. An Example of a MTB DETECTED; Rif Resistance INDETERMINATE Result

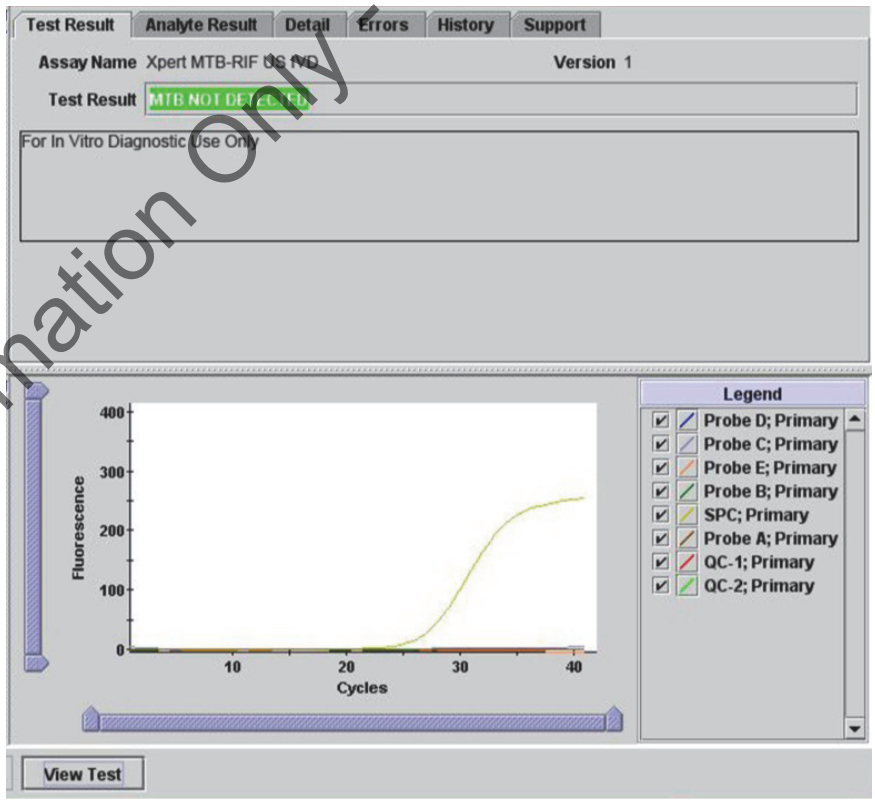


Figure 9. An Example of a MTB NOT DETECTED Result

Figure 10. An Example of an INVALID Result

11.1 Reasons to Repeat the Test

Repeat the test using a new cartridge if one of the following test results occurs:

- An **INVALID** result indicates that the SPC failed. The sample was not properly processed, or PCR was inhibited.
- An **ERROR** result indicates that the PCC (QC1 or QC2) failed or a system failure occurred, and the assay was aborted. The cause of the errors are possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, the maximum pressure limits were exceeded, or a GeneXpert module failed.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

11.2 Retest Procedure

If you have leftover reconstituted sediment or raw sputum always use new Sample Reagent to treat the sputum before running the assay. See Section 9.1, Concentrated Sputum Sediment Specimens or Section 9.2, Raw Sputum Specimens.

If you have sufficient leftover Sample Reagent-treated sample and are within four hours of sample preparation, you can use the leftover sample to prepare and process a new cartridge immediately on the GeneXpert instrument.

Note If an using an Infinity instrument, the retest should be initiated on modules that are designated as reserved STAT modules.

When retesting, always use a new cartridge. See Section 9.3, Preparing the Cartridge.

12. Limitations

- The performance of the Xpert MTB/RIF Assay was evaluated using induced or expectorated sputa. Testing of other clinical samples (e.g., blood, CSF, gastric aspirate, stool, tissue, urine) has not been evaluated and may alter test performance.
- Concentrated sputum sediments used during the performance evaluation of the Xpert MTB/RIF Assay were prepared following the NALC-NaOH or NaOH procedures recommended by the Centers for Disease Control and Prevention (CDC).[8] Use of other methods of sediment preparation may alter the performance of the test.
- The Xpert MTB/RIF Assay is not indicated for use with sputum samples from patients being treated with antituberculosis drugs either to determine bacteriologic cure or to monitor response to therapy.
- A negative test does not exclude the possibility of isolating MTB-complex from the sputum sample. 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 organism for further characterization and susceptibility testing.
- A positive test does not necessarily indicate the presence of viable organisms.
- The Xpert MTB/RIF Assay does not differentiate between the species of the MTB-complex (i.e., *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. canettii*, *M. microti*, *M. caprae*, *M. pinnipedi*, *M. mungi*, and *M. orygis*). In addition, culture must also be performed to determine if mycobacteria other than tuberculosis complex (MOTT) is present in addition to MTB-complex.
- Lower sensitivity may be observed in pediatric patients due to the diffuse nature of MTB infection in the lungs of this patient group, and difficulties encountered in obtaining adequate specimens.
- Due to the low prevalence of rifampin resistant TB in the United States and the implications of rifampin resistance for treatment, all MTB-complex strains determined to be rifampin resistant by the Xpert MTB/RIF Assay must have the presence of rifampin resistance associated mutations of the *rpoB* gene confirmed by a reference laboratory. Additional testing for the presence of mutations associated with resistance to other drugs for the treatment of TB should also be performed.
- Because the detection of MTB-complex is dependent on the number of organisms present in the sample, accurate results are dependent on proper specimen collection, handling, and storage. Erroneous test results might occur from improper specimen collection, failure to follow the recommended sample collection procedure, handling or storage problems, technical error, sample mix-up, or an insufficient concentration of starting material. Careful compliance to the instructions in this insert is necessary to avoid erroneous results.
- The performance of the Xpert MTB/RIF Assay has not been evaluated with samples from pediatric patients.
- The performance of the Xpert MTB/RIF test is dependent on operator proficiency and adherence to assay procedures. Assay 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.
- Assay interference may be observed in the presence of Lidocaine (>20% v/v), mucin (>1.5% w/v), Ethambutol (>5 µg/mL), Guaifenesin (>2.5 mg/mL), Phenylephrine (>25% v/v), or tea tree oil (>0.008% v/v).
- Studies demonstrated that *M. scrofulaceum* when tested at a concentration of 10^8 CFU/mL produced a false positive Xpert MTB/RIF Assay result.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown MDR-MTB or RIF-resistant strains resulting in a false negative result or a false resistant result in rifampin-susceptible strains.

13. Expected Values of One Xpert MTB/RIF Assay Result

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

In two multicenter prospective clinical evaluations of Xpert MTB/RIF Assay performance in subjects in the United States with suspected active TB, overall prevalence of culture-confirmed disease was 13.2%. Of subjects with culture confirmed TB, 71.6% were AFB smear-positive.

13.1 Predictive Values for One Xpert MTB/RIF Assay Result

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 3. These calculations are based on hypothetical prevalences and the overall sensitivity and specificity (when compared to culture) observed in the multi-center clinical studies. The sensitivity of the Xpert MTB/RIF Assay for AFB smear-positive specimens was 99.4% (479/482) and sensitivity for AFB smear-negative specimens was 67.2% (135/201). Overall specificity of Xpert MTB/RIF Assay was 98.7% (1355/1373). The prevalence of MTB was 11.8% in the first U.S. prospective study and 14.2% in the second prospective U.S. study.

Table 3. Hypothetical Predictive Values of One Xpert MTB/RIF Assay Result vs. MTB Culture

Prevalence of MTB Culture Positive	Probability of MTB Culture Positive Among		Probability of MTB Culture Negative Among
	Xpert MTB/RIF DETECTED AFB Smear Pos.	Xpert MTB/RIF DETECTED AFB Smear Neg.	Xpert MTB/RIF NOT DETECTED
1%	89.69%	13.67%	99.90%
2%	94.61%	24.24%	99.80%
3%	96.38%	32.65%	99.70%
4%	97.29%	39.51%	99.59%
5%	97.84%	45.20%	99.48%
10%	98.97%	63.52%	98.91%
11.8%	99.14%	67.71%	98.70%
14.2%	99.30%	72.18%	98.39%
20%	99.54%	79.67%	97.59%
40%	99.83%	91.27%	93.82%
50%	99.88%	94.00%	91.01%

13.2 Predictive Values Based on Two Xpert MTB/RIF Assay Results

Hypothetical estimated positive and negative predictive values of MTB detection for different prevalence rates for detecting MTB using two Xpert MTB/RIF Assay results are shown in Table 4. These calculations are based on hypothetical prevalence and the overall sensitivity and specificity (when compared to culture) observed in the second of the two multi-center studies where two Xpert MTB/RIF Assays were performed on each subject. The sensitivity of two Xpert MTB/RIF Assay results for AFB smear-positive specimens was 100% (133/133) and the sensitivity for AFB smear-negative specimens was 69.4% (59/85). Overall specificity of two Xpert MTB/RIF Assay results was 97.9% (746/762).

Table 4. Hypothetical Predictive Values of Two Xpert MTB/RIF Assay Results vs. MTB Culture [a]

Prevalence of MTB Culture Positive	Probability of MTB Culture Positive Among		Probability of MTB Culture Negative Among
	Two Xpert MTB/RIF DETECTED, AFB Smear Pos.	Two Xpert MTB/RIF DETECTED AFB Smear Neg.	Two Xpert MTB/RIF NOT DETECTED
1%	84.40%	9.19%	99.91%
2%	91.62%	16.98%	99.82%
3%	94.31%	23.66%	99.72%
4%	95.71%	29.46%	99.63%
5%	96.57%	34.54%	99.53%
10%	98.35%	52.69%	99.02%
11.8%	98.62%	57.28%	98.82%
14.2%	98.88%	62.39%	98.54%
20%	99.26%	71.48%	97.81%
40%	99.72%	86.98%	94.37%
50%	99.81%	90.93%	91.79%

[a] Sensitivity of 100% for two Xpert MTB/RIF assay results for AFB smear positive subjects was considered as 99.9% in this table.

13.3 Predictive Values for the Result MTB DETECTED, RIF Resistance DETECTED of One Xpert MTB/RIF Assay Result

Hypothetical estimated predictive values for the 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 5. These calculations are based on hypothetical prevalences and the overall sensitivity and specificity (compared to phenotypic drug susceptibility testing (DST)) observed during the first of two multi-center clinical studies. The sensitivity of the one Xpert MTB/RIF Assay result for the detection of RIF resistance was 94.7% (18/19) and the specificity was 99.0% (404/408). The prevalence of TB in this U.S. prospective study was 11.8%. In the U.S. population with TB the prevalence of rifampin resistance is approximately 1.8%.[5]

Table 5. Hypothetical Predictive Values of One Xpert MTB/RIF Assay Result 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%

14. Performance Characteristics—Clinical Performance

14.1 Study 1

14.1.1 Study Design

Performance characteristics of the Xpert MTB/RIF Assay for detection of MTB-complex DNA and for detection of RIF resistance in sputum samples relative to results from culture (solid and/or liquid) followed by drug susceptibility testing (DST) were determined in a multi-center study (Study 1) using prospective and archived sputum specimens collected from both U.S. and non-U.S. populations. A single leftover standard of care sputum sample or concentrated sediment prepared from induced or expectorated sputa were tested by the Xpert MTB/RIF Assay from study subjects suspected of tuberculosis. All AFB smears were performed on concentrated sediments.

Specimens from subjects 18 years or older were eligible for Study 1 if they were suspected of pulmonary tuberculosis, on no TB treatment or with less than three 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. Of the 1,126 specimens eligible and tested by the Xpert MTB/RIF Assay, 1,096 were used in the analysis. Thirty specimens were excluded from the analysis; 13 specimens due to Xpert MTB/RIF Assay non-determinate results (i.e., INVALID, ERROR or NO RESULT) and 17 specimens due to MTB culture contamination.

The specimens came from study subjects who were ≥18 years old, 62% (n=679) male, 36% female (n=396); for 1.9% (n=21) gender was unknown. They were from geographically diverse regions: 49% (n=542) were from the U.S. (California, New York and Florida) and 51% (n=554) were from outside the U.S. (Vietnam, Peru, South Africa, Mexico and Bangladesh). Of the 542 U.S. specimens, 450 were prospectively collected and 92 were from an archived specimen bank; of the 554 non-U.S. specimens, 23 were prospectively collected and 531 were from an archived specimen bank.

14.1.2 Performance of One Xpert MTB/RIF Assay Result 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 22.0% 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 MTB culture result of all specimens for this subject.

The performance of the Xpert MTB/RIF Assay for detection of MTB relative to MTB culture, stratified by AFB smear status is shown in Table 6 and Table 7. Discordant results for MTB culture positive and Xpert MTB/RIF Assay results of **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 6. Performance of One Xpert MTB/RIF Assay Result vs. MTB Culture for AFB Smear-Positive Subjects

		Culture		
		+	-	Total
Xpert MTB/ RIF Assay	MTB DETECTED	350	1[a]	351
	MTB NOT DETECTED	1[b]	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] 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.

[b] 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.

Table 7. Performance of One Xpert MTB/RIF Assay Result vs. MTB Culture for AFB Smear-Negative Subjects

		Culture		
		+	-	Total
Xpert MTB/ RIF Assay	MTB DETECTED	89	7[a]	96
	MTB NOT DETECTED	28[b]	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] 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.

[b] 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.

Overall sensitivity depends on the percent of AFB smear positive subjects among the subjects with positive MTB culture. For the prospectively collected specimens from the U.S. subjects of Study 1, 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 tested 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.

14.1.3 Performance of One Xpert MTB/RIF Assay Result vs. MTB Culture by Collection Method

The performance of the Xpert MTB/RIF Assay for detection of MTB was determined relative to MTB culture in expectorated and induced sputum specimens. Results are shown in Table 8 and Table 9. Of the 1,096 specimens, 535 were expectorated specimens, 234 induced specimens, and 327 with unknown collection method.

Table 8. Performance of One Xpert MTB/RIF Assay Result 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 9. Performance of One Xpert MTB/RIF Assay Result 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%	

14.1.4 Performance of One Xpert MTB/RIF Assay Result vs. Culture by Specimen Type

The performance of the Xpert MTB/RIF Assay for detection of MTB was determined relative to MTB culture in raw sputum and concentrated sputum sediment specimens. Results are shown in Table 10 and Table 11. Among 1,096 specimens, there were 606 raw sputum specimens and 490 concentrated sputum sediment specimens.

Table 10. Performance of One Xpert MTB/RIF Assay Result 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 11. Performance of One Xpert MTB/RIF Assay Result 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%	

14.1.5 Performance of One Xpert MTB/RIF Assay Result vs. Drug Susceptibility Testing for RIF

MTB positive culture isolates were tested for drug susceptibility (DST) to rifampin using the 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 detection of genetic mutations associated with RIF resistance was determined relative to the DST results of the MTB culture isolates. Of the 1,096 subjects eligible and tested by the Xpert MTB/RIF Assay, 1,082 were used in the analysis. Fourteen subjects were excluded from the analysis; six subjects had an Xpert MTB/RIF Assay result of **MTB DETECTED**, **Rif Resistance INDETERMINATE** and eight subjects with MTB positive cultures did not have DST results.

Results for the detection of RIF resistance associated mutations are reported by the Xpert MTB/RIF Assay only when MTB-complex was 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 12.

Table 12. Performance of One Xpert MTB/RIF Assay Result vs. DST

		DST			
		RIF Resistant	**RIF Susceptible**	**DST Not Done TB Culture Negative**	**Total**
Xpert MTB/ RIF Assay	**MTB DETECTED, Rif Resistance DETECTED**	18	4[a]	0	22
	MTB DETECTED, RifResistance NOT DETECTED	1[b]	404	7	412
	MTB NOT DETECTED[c]	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] Of the four discordant specimens determined to be RIF susceptible by DST and **RIF resistance DETECTED** by the Xpert MTB/RIF Assay, one was shown to be RIF susceptible and three were RIF resistant by bi-directional sequencing.
[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] MTB was not detected and therefore detection of RIF resistance associated mutations could not be determined.

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% (1/351, 95% CI: 0.01% to 1.58%) of AFB-smear positive specimens and 5.21% (5/96; 95% CI: 2.24% to 11.62%) of AFB-smear negative specimens.

14.2 Study 2

14.2.1 Study Design

A prospective multi-center study (Study 2) was conducted at multiple sites in the United States, as well as South Africa and Brazil. Performance of the MTB/RIF Assay was assessed as an alternative to fluorescent stained AFB-smear microscopy as an aid in determining the need for continued airborne infection isolation in patients with suspected active pulmonary tuberculosis. Results from Xpert MTB/RIF Assay testing of two serial sputum specimens in study subjects with suspected active pulmonary tuberculosis were compared to results of fluorescent stained AFB smears of the same specimens; a subset of subjects had a third sputum specimen tested by AFB-smear but not by the Xpert MTB/RIF Assay. Each specimen was cultured for MTB-complex using liquid and solid culture media, with mycobacterial growth confirmed for MTB-complex and rifampin drug susceptibility testing performed via the Middlebrook agar proportion method. Study 2 was also designed to evaluate the clinical performance of the Xpert MTB/RIF Assay using prospectively collected sputum specimens in HIV-infected and HIV-uninfected populations.

Study subjects 18 years or older were eligible for enrollment if they were suspected of pulmonary tuberculosis, on no treatment or had fewer than 48 hours of TB treatment within 180 days prior to collection of the first sputum specimen, and had determination/documentation of HIV status. Subjects were included in the analysis if they produced at least two sputum specimens collected in sufficient volume for testing by Xpert MTB/RIF Assay, AFB smear, and MTB culture, and interpretable results were available for all three methods. A third specimen for analysis was collected at some sites based on standard of care protocols. Of 992 eligible and tested subjects, thirty-two subjects (3.2%) were excluded from the analysis: 7 due to absence of culture results and 3 due to MTB culture contamination. Twenty-two subjects (2.2%) were excluded due to Xpert MTB/RIF Assay non-determinate results (i.e., **INVALID**, **ERROR**, or **NO RESULT**). Therefore, 960 subjects were used in the analysis based on the first Xpert MTB/RIF Assay result. Twenty of the 22 subjects excluded from analysis based on the first Xpert MTB/RIF Assay result gave valid results for the second Xpert MTB/RIF Assay test specimen, therefore, analysis based on two Xpert MTB/RIF Assay test specimens included a total of 980 subjects.

Study subjects were 62% male, 38% female. Sixty-five (65%) percent of subjects were from the U.S., and 35% were from non-U.S. sites. Forty-five (45%) percent of study subjects were HIV-infected and 55% were HIV-uninfected subjects. Expectorated and induced sputa represented 59.6% and 33.6% of specimens respectively; 7% of sputum specimens were unspecified. Twenty-eight percent of specimens were raw sputa and 72% were concentrated sputum sediments.

14.2.2 Xpert MTB/RIF Assay Performance as Predictive of Results of Serial Fluorescent Stained AFB Smears

Of 215 study subjects with culture confirmed MTB-complex (14.2% [88/618] of U.S. subjects and 37.1% [127/342] of non-U.S. subjects), 99% of subjects (97% of U.S. subjects and 100% of non-U.S. subjects) with suspected pulmonary tuberculosis where MTB-complex was detected by acid-fast microscopy of two or three serial sputum specimens also had MTB-complex detected by testing of a single sputum by the Xpert MTB/RIF Assay. Results from testing of two serial sputum specimens by the Xpert MTB/RIF Assay detected MTB-complex in all AFB-smear-positive subjects (100% in the U.S. subjects and 100% in non-U.S. subjects).

A single negative Xpert MTB/RIF Assay result predicted the absence of AFB smear-positive pulmonary tuberculosis with an overall negative predictive value (NPV) of 99.7% (99.6% in the U.S. and 100% in non-U.S.). Two serial negative Xpert MTB/RIF Assay results predicted the absence of AFB smear-positive pulmonary tuberculosis with an overall NPV of 100%.

14.2.3 One Xpert MTB/RIF Assay Result as Predictive of Results of Serial Fluorescent Stained AFB Smears

Table 13 and Table 14 present the overall performance of one Xpert MTB/RIF Assay result compared to the results of MTB culture, stratified by AFB smear result (Table 13). Table 14 is a side-by-side comparison of the performance of one Xpert MTB/RIF Assay result versus the composite result of two AFB smears in U.S. and non-U.S. subjects (N=960).

Overall sensitivity of one Xpert MTB/RIF Assay in AFB smear-positive and AFB smear-negative subjects (based on two AFB smears) was 98.5% (95% CI: 94.6%,99.6%) and 54.8% (95% CI: 44.1%, 65.0%) respectively, and overall specificity was 98.7% (95% CI: 97.5%, 99.3%). One Xpert MTB/RIF Assay result of "MTB Not Detected" was associated with a probability of MTB culture-positive/AFB smear-positive results of 0.4% for U.S. subjects and 0.0% for non-U.S. subjects.

Table 13. Performance of One Xpert MTB/RIF Assay Result Stratified by Two AFB Smears Relative to MTB Culture in U.S. and non-U.S. Subjects

		Culture						
		Positive			Negative			
		AFB Smear +	AFB Smear -	Overall Culture +	AFB Smear +	AFB Smear -	Overall Culture -	Total
Xpert MTB/ RIF Assay	Positive	129	46	175	1	9	10[a]	185
	Negative	2	38	40	17	718	735	775
	Total	131	84	215	18[b]	727	745	960

Performance of Xpert MTB/RIF Assay for Smear Positive:
Sensitivity: 98.5% (129/131), 95% CI: 94.6%,99.6%
Specificity: 94.4% (17/18), 95% CI: 74.2%, 99.0%

Performance of Xpert MTB/RIF Assay for Smear Negative:
Sensitivity: 54.8% (46/84), 95% CI: 44.1%, 65.0%
Specificity: 98.8% (718/727), 95% CI: 97.7%, 99.4%

Prevalence of MTB Culture Positive: 22.4% (215/960)
Prevalence of MTB Culture Positive in U.S. subjects: 14.2% (88/618)
Prevalence of MTB Culture positive in non-U.S. subjects: 37.1% (127/342)

Percent of AFB smear positive subjects among subjects with MTB Culture Positive: 60.9% (131/215)

Overall Probability of MTB Culture Positive among subjects with an Xpert MTB/RIF Negative Result: 5.2% (40/775), 95% CI: 3.8%, 7.0%

Probability of MTB Culture Positive among subjects with an Xpert MTB/RIF Negative Result (U.S. subjects): 2.4% (13/539), 95% CI: 1.4%, 4.1%

Probability of MTB Culture Positive among subjects with an Xpert MTB/RIF Negative Result (non-U.S. subjects): 11.4% (27/236), 95% CI: 8.0%, 16.1%

Overall Probability of MTB Culture Positive and AFB smear positive among subjects with an Xpert MTB/RIF Negative Result: 0.3% (2/775), 95% CI: <0.1%, 0.9%

Probability of MTB Culture Positive and AFB smear positive among subjects with an Xpert MTB/RIF Negative Result (U.S. subjects): 0.4% (2/539), 95% CI: 0.1%, 1.3%

Probability of MTB Culture Positive and AFB smear positive among subjects with an Xpert MTB/RIF Negative Result non-U.S.) subjects: 0.0% (0/236), 95% CI: 0.0%, 1.6%

[a] Of the 10 MTB culture-negative specimens that were positive by Xpert MTB/RIF Assay, 5 grew non-tuberculosis mycobacteria (NTM). MTB-complex was isolated and identified using standard of care methods not associated with the study protocol in 4 of the 5 specimens.
[b] Of the 18 MTB culture-negative/AFB smear-positive specimens, 14 grew NTM.

One Xpert MTB/RIF Assay was associated with a sensitivity of 81.4% (95% CI: 75.7%, 86.0%) for identifying MTB culture-positive subjects compared to a sensitivity of 60.9% (95% CI: 54.3%, 67.2%) for two AFB smears.

Table 14. Comparison of Performance of One Xpert MTB/RIF Assay Result vs Two AFB Smears Each Versus MTB Culture in U.S. and non-U.S. Subjects

One Xpert MTB/RIF Assay Result		Culture			Two AFB Smears		Culture		
		Positive	Negative	Total			Positive	Negative	Total
Xpert	Positive	175	10	185	AFB Smear	Positive	131	18	149
	Negative	40	735	775		Negative	84	727	811
	Total	215	745	960		Total	215	745	960
Sensitivity:		81.4% (95% CI: 75.7, 86.0)			Sensitivity:		60.9% (95% CI: 54.1, 67.5)		
Specificity:		98.7% (95% CI: 97.5, 99.3)			Specificity:		97.6% (95% CI: 96.2, 98.6)		
U.S. prevalence		14.2% (95% CI: 11.7, 17.2)			U.S. prevalence		14.2% (95% CI: 11.7, 17.2)		
PPV:		94.9% (95% CI: 87.7, 98.0)			PPV:		77.2% (95% CI: 66.8, 85.1)		
NPV:		97.6% (95% CI: 95.9, 98.6)			NPV:		95.0% (95% CI: 92.8, 96.5)		
Non-U.S. prevalence		37.1% (95% CI: 32.2, 42.4)			Non-U.S. Prevalence:		37.1% (95% CI: 32.2, 42.4)		
PPV		94.3% (95% CI: 88.2, 97.4)			PPV		100% (95% CI: 94.8, 100)		
NPV		88.6 (95% CI: 83.9, 92.0)			NPV		79.0% (95% CI: 73.8, 83.5)		

In U.S. subjects, the NPV for one Xpert MTB/RIF Assay result was 97.6% (95% CI: 95.9%, 98.6%) while the NPV for two AFB smears results was 95.0% (95% CI: 92.8%, 96.5%) with a prevalence of TB in U.S. subjects of 14.2%. The difference in NPVs was 2.6% with 95% CI: 1.2%, 4.2%.

14.2.4 Two Xpert MTB/RIF Assay Results as Predictive of Results of Serial Fluorescent Stained AFB Smears

Table 15 and Table 16 present the overall performance of two Xpert MTB/RIF Assay results compared to the results of MTB culture, stratified by AFB smear result (Table 15). Table 16 compares the performance of two Xpert MTB/RIF Assays versus the composite result of two AFB smears in U.S. and non-U.S. subjects (N=980).

Overall sensitivity of two Xpert MTB/RIF Assay results in AFB smear-positive and AFB smear-negative subjects based on two AFB smears was 100.0% (95% CI: 97.2%, 100.0%) and 69.4% (95% CI: 59.0%,78.2%) respectively, and the overall specificity was 97.9% (95% CI: 96.6%, 98.7%). No MTB culture-positive/AFB smear-positive results were observed in subjects with two serial negative Xpert MTB/RIF Assay results.

Table 15. Performance of Two Xpert MTB/RIF Assay Results Stratified by Two AFB Smears Relative to MTB Culture in U.S. and non-U.S. Subjects

		Culture						
		Positive			Negative			
		AFB Smear +	AFB Smear -	Overall Culture +	AFB Smear +	AFB Smear -	Overall Culture -	Total
Xpert MTB/ RIF Assay	Positive	133	59	192	1	15	16[a]	208
	Negative	0	26	26	17	729	746	772
	Total	133	85	218	18[b]	744	762	980

Performance of Xpert MTB/RIF Assay for Smear Positive:

Sensitivity: 100% (133/133), 95% CI: 97.2%, 100%
Specificity: 94.4% (17/18), 95% CI: 74.2%, 99.0%

Performance of Xpert MTB/RIF Assay for Smear Negative:

Sensitivity: 69.4% (59/85), 95% CI: 59.0%, 78.2%
Specificity: 98.0% (729/744), 95% CI: 96.7%, 98.8%

Prevalence of MTB Culture Positive: 22.2% (218/980)
Prevalence of MTB Culture Positive in U.S. subjects: 14.4% (91/633)
Prevalence of MTB Culture positive in non-U.S. subjects: 36.6% (127/347)

Percent of AFB smear positive subjects among subjects with an MTB Culture Positive Result: 61.0% (133/218)

Probability of MTB Culture Positive among subjects with Xpert MTB/RIF Negative Results: 3.4% (26/772), 95% CI: 2.3%, 4.9%
Probability of MTB Culture Positive among subjects with Xpert MTB/RIF Negative Results (U.S. subjects): 1.5% (8/544), 95% CI: 0.7%, 2.9%
Probability of MTB Culture Positive among subjects with Xpert MTB/RIF Negative Results (non-U.S. subjects): 7.9% (18/228), 95% CI: 5.1%, 12.1%

Probability of MTB Culture Positive and AFB smear positive among subjects with Xpert MTB/RIF Negative Results: 0.0% (0/772), 95% CI: 0.0%, 0.5%
Probability of MTB Culture Positive and AFB smear positive among subjects with Xpert MTB/RIF Negative Results (U.S. subjects): 0.0% (0/544), 95% CI: 0.0%, 0.7%
Probability of MTB Culture Positive and AFB smear positive among subjects with Xpert MTB/RIF Negative Results (non-U.S. subjects): 0.0% (0/228), 95% CI:0.0%, 1.7%

[a] Of the 16 MTB culture-negative specimens that were positive by Xpert MTB/RIF Assay, 6 grew non-tuberculosis mycobacteria (NTM). MTB-complex was isolated and identified using standard of care methods not associated with the study protocol in 4 of the 6 specimens.
[b] Of the 18 MTB culture-negative/AFB smear-positive specimens, 14 grew NTM.

Table 16 compares performance of two Xpert MTB/RIF Assay Results and two AFB Smears to MTB Culture. Xpert MTB/RIF Assay results identified 88.1% (95% CI: 83.1%, 91.7%) of MTB culture-positive subjects compared to 61.0% (95% CI:54.4%, 67.2%) two AFB smears.

Table 16. Comparison of Performance of Two Xpert MTB/RIF Assay Results vs Two AFB Smears Each Versus MTB Culture in U.S. and non-U.S. Subjects

One Xpert MTB/RIF Assay Result		Culture			Two AFB Smears		Culture		
		Positive	Negative	Total			Positive	Negative	Total
Xpert	Positive	192	16	208	AFB Smear	Positive	133	18	151
	Negative	26	746	772		Negative	85	744	829
	Total	218	762	980		Total	218	762	980
Sensitivity:		88.1% (95% CI: 83.1, 91.7)			Sensitivity:		61.0% (95% CI: 54.4, 67.2)		
Specificity:		97.9% (95% CI: 96.6, 98.7)			Specificity:		97.6% (95% CI: 96.3, 98.5)		
U.S. prevalence		14.4% (95% CI: 11.9, 17.3)			U.S. prevalence		14.4% (95% CI: 11.9, 17.3)		
PPV:		93.3% (95% CI: 86.1, 96.9)			PPV:		77.8% (95% CI: 67.6, 85.5)		
NPV:		98.5% (95% CI: 97.1, 99.3)			NPV:		94.9% (95% CI: 92.8, 96.5)		
Non-U.S. prevalence		36.6% (95% CI: 31.7, 41.8)			Non-U.S. Prevalence:		36.6% (95% CI: 31.7, 41.8)		
PPV		91.6% (95% CI: 85.2, 95.4)			PPV		100% (95% CI: 94.8, 100)		
NPV		92.1 (95% CI: 87.9, 94.9)			NPV		79.4% (95% CI: 74.3, 83.8)		

In U.S. subjects, the NPV for two Xpert MTB/RIF Assay results was 98.5% (95% CI: 97.1%, 99.3%) while the NPV for two AFB smears results was 94.9% (95% CI: 92.8%, 96.5%) when the prevalence of TB in the U.S. subjects was 14.4%.

Detailed information of Xpert MTB/RIF Assay performance as compared to AFB smears with regard to time between collection of sputum specimens in U.S. subjects is presented in Table 17.

Table 17. Xpert MTB/RIF Assay Performance vs AFB Smear Microscopy Relative to Collection Time Between Sputum Specimens

Xpert Results	AFB Smear Results
One Xpert Result **Sensitivity = 85.2% (75/88)** Specificity = 99.2%(526/530) Prevalence = 14.2% (88/618) NPV = 97.6% (526/539) Probability of MTB culture-positive AFB smear-positive subjects among Xpert MTB/RIF negative results = 0.4% (2/539)	Data not analyzed for one AFB smear
Two Xpert Results **Sensitivity = 91.2% (83/91)** Specificity = 98.9% (536/542) Prevalence = 14.4% (91/633) NPV = 98.5% (536/544) Probability of MTB culture-positive/AFB smear-positive subjects among Xpert MTB/RIF Assay negative results = 0.0% (0/544)	**Two AFB Smear Results** **Sensitivity = 69.2% (63/91)** Specificity = 96.7% (524/542) Prevalence = 14.4% (91/633) NPV = 94.9% (524/552)

Table 17. Xpert MTB/RIF Assay Performance vs AFB Smear Microscopy Relative to Collection Time Between Sputum Specimens (Continued)

Xpert Results	AFB Smear Results
Two Xpert Results with two sputum specimens collected ≥8 hours apart[a] **Sensitivity = 92.5% (49/53)** Specificity = 98.9% (342/346) Prevalence = 13.3% (53/399) NPV = 98.9% (342/346) Probability of MTB culture-positive /AFB smear-positive subjects among Xpert MTB/RIF Assay negative results = 0.0%	**Two AFB Smear Results with two specimens collected ≥8 hours apart[a]** **Sensitivity = 71.7% (38/53)** Specificity = 98.0% (339/346) Prevalence = 13.3% (53/399) NPV = 95.8% (339/354)
Two Xpert Results with two specimens collected <8 hours apart[b] **Sensitivity = 89.5% (34/38)** Specificity = 99.0% (194/196) Prevalence = 16.2% (38/234) NPV = 98.0% (194/198) Probability of MTB culture-positive/AFB smear-positive patients among Xpert MTB/RIF Assay negative results = 0.0%	**Two AFB Smear Results with two specimens collected <8 hours apart[b]** **Sensitivity = 65.8% (25/38)** Specificity = 99.4% (185/196) Prevalence = 16.2% (38/234) NPV = 93.4% (185/198)
No subjects had three specimens tested by the Xpert MTB/RIF Assay	**Three AFB Smear Results** **Sensitivity = 60.4% (29/48)** Specificity = 96.6% (284/294) Prevalence = 14.0% (48/342) NPV = 93.7% (284/303)
	Three AFB Smear Results with three specimens collected ≥8 hours apart[c] **Sensitivity = 69.2% (9/13)** Specificity = 95.7% (112/117) Prevalence = 10.0% (13/130) NPV = 96.6% (112/116)
	Three AFB Smear Results with three specimens collected <8 hours apart[d] **Sensitivity = 57.1% (20/35)** Specificity = 97.2% (172/177) Prevalence = 16.5% (35/212) NPV = 92.0% (172/187)

[a] The time frame between the collection of the first sputum specimen and the second sputum specimen is greater than or equal to 8 hours.

[b] The time frame between the collection of the first sputum specimen and the second sputum specimen is less than 8 hours.

[c] The time frame between the collection of the first sputum specimen and the second sputum specimen was greater than or equal to 8 hours, and the time frame between the collection of the second sputum specimen and the third sputum specimen was greater than or equal to 8 hours.

[d] Three AFB smears with less than 8 hours means that at least one of the time intervals between specimen collection was less than 8 hours apart.

Table 13, Table 14, Table 15, Table 16, and Table 17 present data in which results for raw sputum and concentrated sputum sediments are combined. Table 18 is a summary of the NPV exclusively for U.S. subjects delineated by raw sputum and concentrated sputum sediments.

Table 18. Summary of the NPV for Raw Sputum and Concentrated Sputum Sediments in U.S. Subjects[a, b]

		Raw Sputum (%) [95% CI]	Concentrated Sputum Sediments (%) [95%CI]
One Xpert Result	Probability of MTB culture positive among subjects with an Xpert MTB/RIF negative result	3.7% (9/242) [1.7%, 6.9%]	1.3% (4/297) [0.4%, 3.4%]
	Probability of MTB culture Positive and AFB smear positive among subjects with an Xpert MTB/RIF negative result	0.8% (2/242) [0.1%, 3.0%]	0.0% (0/297) [0.0%, 1.2%]
Two Xpert Results	Probability of MTB culture positive among subjects with Xpert MTB/RIF negative results	2.5% (6/239) [0.9%, 5.4%]	0.7% (2/283) [0.1%, 2.5%]
	Probability of MTB culture Positive and AFB smear positive among subjects with Xpert MTB/RIF negative results	0.0% (0/239) [0.0%, 1.5%]	0.0% (0/283) [0.0%, 1.3%]

[a] Negative Predictive Value (NPV) = (1 – %Probability)
[b] Prevalence of MTB culture positive among U.S. study subjects = 14.3%

Note For two Xpert Results the table above only includes specimen pairs where both the first and second specimens were the same type. For 239 specimens, both the first and second tests were on raw sputum specimens; for 283 specimens, both the first and second tests were on concentrated sputum sediments; for 2 pairs, the first specimen was raw sputum and second was concentrated sputum sediment (data not included in table above, but specimen pairs showed 100% concordant results); finally, for 20 pairs, the first specimen was a concentrated sputum sediment and second was raw sputum (data not included in table above, but specimen pairs showed 100% concordant results).

14.3 Xpert MTB/RIF Assay Performance in an HIV Population

To compare performance of the Xpert MTB/RIF Assay in HIV-infected and HIV-uninfected subjects, data from Study 2 were analyzed by smear status of specimens and HIV status of the population. Table 19 and Table 20 compare the sensitivities and specificities of one Xpert MTB/RIF Assay result in specimens obtained from HIV-infected and HIV-uninfected subjects stratified by AFB smear-positive and AFB smear-negative results, respectively. For both HIV-infected and HIV-uninfected subjects, the sensitivity of the Xpert MTB/RIF Assay for detection of MTB-complex was higher in AFB smear-positive specimens (100.0% and 97.8%, respectively) than in AFB smear-negative specimens (52.1% and 58.3%, respectively). These data are summarized in Table 20.

Table 19. Comparison of Sensitivity and Specificity of One Xpert MTB/RIF Assay Result in HIV-Infected and HIV-Uninfected Subjects—AFB Smear Positive Only

Xpert MTB/RIF	Overall	HIV-infected	HIV-uninfected	Difference (95% CI)
Sensitivity	98.5% (129/131)	100% (39/39)	97.8% (90/92)	2.2% (-0.8%, 5.2%)
Specificity	94.4% (17/18)	100% (7/7)	90.9% (10/11)	9.1% (-7.9%, 26.1%)

Table 20. Comparison of Sensitivity and Specificity of One Xpert MTB/RIF Assay Result in HIV-Infected and HIV-Uninfected Subjects—AFB Smear Negative Only

Xpert MTB/RIF	Overall	HIV-infected	HIV-uninfected	Difference (95% CI)
Sensitivity	54.8% (46/84)	52.1% (25/48)	58.3% (21/36)	-6.3% (-27.7%, 15.2%)
Specificity	98.8% (718/721)	98.2% (332/338)	99.2% (386/389)	-1.0% (-2.7%, 0.7%)

15. Performance Characteristics—Analytical Performance

15.1 Analytical Reactivity (Inclusivity)

The analytical reactivity of the Xpert MTB/RIF Assay was evaluated against 62 well characterized *Mycobacterium tuberculosis* isolates representing geographic and phenotypic diversity. Table 21 lists 26 strains sensitive to rifampin and 36 strains resistant to rifampin 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 valid replicate tests using 26 strains sensitive to rifampin which were tested in triplicate. One replicate in 78 tests was not valid and not repeated. Three of the DST susceptible strains (see footnote "c" in Table 21) that tested rifampin resistant 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 valid replicate tests using 36 strains resistant to rifampin which were tested in triplicate. One replicate in 108 tests was not valid and not repeated. Results by strain are shown in Table 21.

Table 21. Analytical Reactivity (Inclusivity) of the Xpert MTB/RIF Assay

Strain ID	Origin	Susceptibility by DST[a]	Susceptibility by Xpert[b]
TDR 116	S. Korea	R	R
TDR 21	RD Congo	R	R
TDR 28[c]	Bangladesh	R	R
TDR 191[c]	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[c]	Spain	R	R
TDR 117	S. Korea	R	R
TDR 129	Brazil	R	R
TDR 186[c]	Morocco	R	R
TDR 59[c]	Burundi	R	R
TDR 185[c]	Nigeria	R	R
TDR 6	Bangladesh	R	R
TDR 19	Azerbaijan	R	R
TDR 148	Nepal	R	R
TDR 13[c]	Bangladesh	R	R
TDR 12	Bangladesh	R	R
H37Rv[d]	Lab strain	S	S

Table 21. Analytical Reactivity (Inclusivity) of the Xpert MTB/RIF Assay (Continued)

Strain ID	Origin	Susceptibility by DST[a]	Susceptibility by Xpert[b]
TDR 22	RD Congo	S	S
TDR 29	Azerbaijan	S	S
TDR 33	Belgium	S	S
CDC 1551[c]	USA	S	S
TDR 146[c]	Nepal	S	S
TDR 78[c]	S. Korea	S	S
TDR 54[c]	Bangladesh	S	S
TDR 215[c]	Peru	S	S
TDR 158[c]	Peru	S	S
TDR 178[c]	Guinea	S	S
TDR 64[c]	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
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[e]	Peru	S	R
97-15246[e]	Peru	S	R
98-R839[e]	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
01-1118	Peru	S	S
02-02880	US	S	S

Table 21. Analytical Reactivity (Inclusivity) of the Xpert MTB/RIF Assay (Continued)

Strain ID	Origin	Susceptibility by DST[a]	Susceptibility by Xpert[b]
02-03222	Peru	S	S
02-R0040	Peru	S	S

[a] R=RIF resistant, S=RIF susceptible
[b] R= RIF resistance mutations detected, S=RIF resistance mutations not detected
[c] DNA was used; quantified cultured strains were not available.
[d] The reference ATCC strain H37Rv was tested as both cells and DNA.
[e] Isolate rifampin susceptible by DST, but rifampin resistant by DNA sequence and Xpert MTB/RIF Assay.

An additional 5 *Mycobacterium tuberculosis* complex strains, i.e., *M. africanum* (taxid:33894), *M. bovis* (taxid:1765), *M. canettii* (taxid:78331), *M. caprae* (taxid:115862), and *M. microti* (taxid:1806), were not wet tested but were evaluated *in silico* to assess the analytical reactivity or inclusivity. The results of the *in silico* analyses predict a very high likelihood of amplification and detection using the Xpert MTB/RIF Assay. See Table 22.

Table 22. 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 organisms							
	RpoB For1	RpoB For2	RpoB Rev	RpoB Probe A[a]	RpoB Probe B[a]	RpoB Probe C[a]	RpoB Probe D[a]	RpoB Probe E[a]
Mycobacterium africanum (taxid:33894)[b]	24/24	24/24	23/24	17/17	24/24	17/17	18/18	18/18
Mycobacterium bovis (taxid:1765) [b]	24/24	24/24	23/24	17/17	24/24	17/17	18/18	18/18
Mycobacterium canettii (taxid:78331) [b]	24/24	24/24	23/24	17/17	24/24	17/17	18/18	18/18
Mycobacterium caprae (taxid:115862) [b]	24/24	24/24	23/24	17/17	24/24	17/17	18/18	18/18
Mycobacterium microti (taxid:1806) [b]	24/24	24/24	23/24	17/17	24/24	17/17	18/18	18/18

[a] Sequence alignment of probes done with no stem sequences.
[b] taxid – unique identifier for an organism in the NCBI taxonomy database.

15.2 Analytical Specificity (Exclusivity)

One hundred and thirty-two (132) different microorganisms, representing common respiratory pathogens potentially encountered in the oral/respiratory tract, were tested at a concentration of at least 10^8 CFU/mL (or DNA at $1x10^7$ copies/mL) for bacteria and fungi; a concentration of 10^5 TCID50/mL (or nucleic acid at $2x10^9$ copies/mL) for viruses; a concentration of 10^6 elementary bodies (EB) per mL for Chlamydia; and a concentration of 10^6 CFU/mL for two nontuberculous mycobacteria. See Table 23. The analytical specificity of the Xpert MTB/RIF Assay is 100% at a concentration of 10^8 CFU/mL (or DNA at $1x10^7$ copies/mL) for bacteria and fungi; a concentration of 10^5 TCID50/mL (or nucleic acid at $2x10^9$ copies/mL) for viruses; and a concentration of 10^6 elementary bodies (EB) per mL for Chlamydia. The analytical specificity of the Xpert MTB/RIF Assay is 100% at a concentration of 10^8 CFU/mL for 21 of 24 nontuberculous mycobacteria (NTM) tested. Cross-reactivity was observed in one of three replicates using *M. scrofulaceum* at 10^8 CFU/mL, however, no cross-reactivity was observed at 10^7 CFU/mL. Two NTMs (*M. genavense* and *M. smegmatis*) were not tested above 10^6 CFU/mL due to poor growth. The analytical specificity of the Xpert MTB/RIF Assay is 100% at a concentration of 10^6 CFU/mL for these 2 organisms. Positive and negative controls were included in the study.

Table 23. Microorganisms Tested for Analytical Specificity

Acinetobacter baumannii	Human influenza virus B[a]	*Neisseria lactamica*
Acinetobacter calcoaceticus	Human Metapneumovirus	*Neisseria meningitidis*
Actinomyces israelii[b]	Human parainfluenzae Type 1	*Neisseria mucosa*
Actinomyces odontolyticus	Human parainfluenzae Type 2	*Neisseria sicca*
Adenovirus	Human parainfluenzae Type 3	*Nocardia asteroides*[b]
Aspergillus fumigatus[c]	Human respiratory syncytial virus A[a]	*Nocardia cyriacigeorgica*[b]
Bacillus cereus	Human respiratory syncytial virusB[a]	*Pasteurella multocida* subsp. *tigris*
Bacillus subtilis subsp. *subtilis*	*Kingella kingae*	*Pediococcus pentosaceus*[b]
Bacteroides fragilis	*Klebsiella oxytoca*	*Peptostreptococcus anaerobius*
Bordetella parapertussis[b]	*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*[b]	*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[b]	*Mycobacterium asiaticum*	*Salmonella enterica* subsp. *enterica serovar typhimurium*

Table 23. Microorganisms Tested for Analytical Specificity (Continued)

Citrobacter freundii	*Mycobacterium avium* subsp. *avium*	*Serratia marcescens* subsp. *marcescens*
Clostridium perfringens	*Mycobacterium celatum*	*Shigella flexneri*
Corynebacterium diphtheriae[b]	*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[a]	*Mycobacterium xenopi*	*Veillonella parvula*
Herpes simplex virus Type 2[a]	*Mycoplasma pneumoniae*[b]	*Weissella paramesenteroides*
Human influenza virus A[a]	*Neisseria gonorrhoeae*	*Yersinia enterocolitica* subsp. *enterocolitica*

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

Potential cross-reactivity of 25 microorganisms that could not be wet tested using whole organisms or nucleic acid was evaluated by *in silico* analysis. Twenty of the 25 microorganisms tested revealed no potential for cross-reactivity. See Table 24, Table 25, and Table 26.

Five isolates demonstrated a slight potential for cross reactivity which may result in false positive results with the Xpert MTB/RIF Assay. See Table 27.

Table 24. Microorganisms Predicted to be Non-cross Reactive by *in silico* Analysis (RpoB For 1 + RpoB Rev Probe)

Organism	Accession	Max Score	Query Cov	E Value	Identity
Kingella oralis Taxid:505	GU561427.1	16.4	69%	20	100%
Legionella micdadei Taxid:451	NR_041791.1	18.3	34%	3.6	100%
Norcardia brasiliensis Taxid:37326	JN215639.1	40.1	73%	0.000003	100%
Streptomyces anulatus Taxid:1892	AB431435.1	28.2	61%	0.026	100%
Histoplasma capsulatum Taxid:339724	XM_001536322.1	28.2	28%	1.5	100%
Blastomyces dermatitidis (Ajellomyces dermatitidis) Taxid:559298	XM_002624017.1	28.2	48%	1.5	100%
Penicillium spp. Taxid:500485	XM_002566094.1	30.2	38%	2	95%
Rhizopus spp. Taxid:4847	AY847625.1	22.3	28%	8.1	93%
Scedosporium spp. Taxid:563467	HQ231813.1	18.3	57%	23	100%
Rubella virus Taxid:111041	AB588191.1	24.3	97%	2.4	100%
Rubeola virus Taxid:884098	JN635408.1	22.3	22%	36	100%
Rubula virus Taxid:11161	EU606317.1	22.3	22%	13	100%
Varicella Zoster Virus Taxid:10335	JQ972914.1	22.3	89%	45	100%
Mycobacterium franklinii Taxid:948102	HQ662080.1	22.3	83%	0.36	100%
M. massiliense, M. bolletii, M. abscessus subsp. bolletii Taxid:319705	NC_018150.2	32.2	100%	0.21	95%

Table 24. Microorganisms Predicted to be Non-cross Reactive by *in silico* Analysis (RpoB For 1 + RpoB Rev Probe) (Continued)

Organism	Accession	Max Score	Query Cov	E Value	Identity
Mycobacterium chimaera Taxid:222805	AY943187.1	26.3	83%	0.016	90%
Mycobacterium avium subsp. paratuberculosis Taxid:1770	AF057479.1	36.2	85%	0.005	100%
Mycobacterium avium subsp. silvaticum Taxid:44282	AY544889.1	28.2	85%	0.004	94%
Mycobacterium avium subsp. hominissuis Taxid:439334	AP012555.1	30.2	100%	0.15	100%
Mycobacterium immunogenum Taxid:83262	HM454251.1	32.2	48%	5E-04	95%

Table 25. Microorganisms Predicted to be Non-cross Reactive by *in silico* Analysis (RpoB For2 + RpoB Rev Probe)

Organism	Accession	Max Score	Query Cov	E Value	Identity
Kingella oralis Taxid:505	GU561427.1	14.4	57%	79	100%
Legionella micdadei Taxid:451	X57520.1	18.3	55%	3.6	100%
Norcardia brasiliensis Taxid:37326	DQ085110.1	38.2	46%	0.00001	100%
Streptomyces anulatus Taxid:1892	AB431435.1	28.2	71%	0.026	100%
Histoplasma capsulatum Taxid:339724	XM_001536322.1	28.2	28%	1.5	100%
Blastomyces dermatitidis (Ajellomyces dermatitidis) Taxid:559298	XM_002625196.1	30.2	30%	0.38	100%
Penicillium spp. Taxid:500485	XM_002565312.1	30.2	44%	2	91%
Rhizopus spp. Taxid:4847	HM130700.1	20.3	73%	32	100%
Scedosporium spp. Taxid:563467	AY625497.1	20.3	44%	5.7	100%
Rubella virus Taxid:111041	AB588191.1	24.3	81%	2.4	100%

Table 25. Microorganisms Predicted to be Non-cross Reactive by *in silico* Analysis (RpoB For2 + RpoB Rev Probe) (Continued)

Organism	Accession	Max Score	Query Cov	E Value	Identity
Rubeola virus Taxid:884098	JN635410.1	24.3	38%	9.1	100%
Rubula virus Taxid:11161	BK005918.1	24.3	85%	3.2	100%
Varicella Zoster Virus Taxid:10335	JQ972914.1	20.3	91%	177	100%
Mycobacterium franklinii Taxid:948102	HQ662038.1	22.3	83%	0.36	100%
M. massiliense, M. bolletii, M. abscessus subsp. bolletii Taxid:319705	NC_018150.2	32.2	100%	0.21	100%
Mycobacterium chimaera Taxid:222805	AY943187.1	40.1	91%	1E-06	96%
Mycobacterium avium subsp. paratuberculosis Taxid:1770	AF057479.1	36.2	59%	0.005	100%
Mycobacterium avium subsp. silvaticum Taxid:44282	AY544889.1	28.2	79%	0.004	94%
Mycobacterium avium subsp. hominissuis Taxid:439334	AP012555.1	32.2	100%	0.38	100%
Mycobacterium immunogenum Taxid:83262	HQ662101.1	24.3	36%	0.13	100%

Table 26. Microorganisms Predicted to be Non-cross Reactive by *in silico* Analysis (All RpoB Probes with no stem sequences)

Organism	Accession	Max Score	Query Cov	E value	Identity
Kingella oralis Taxid:505	0	0	0%		0
Legionella micdadei Taxid:451	AF367743.1	33.7	72%	0.0001	72%
Norcardia brasiliensis Taxid:37326	DQ085110.1	77	100%	4E-17	81%
Streptomyces anulatus Taxid:1892	U64692.1	26.5	27%	0.14	86%

Table 26. Microorganisms Predicted to be Non-cross Reactive by *in silico* Analysis (All RpoB Probes with no stem sequences) (Continued)

Organism	Accession	Max Score	Query Cov	E value	Identity
Histoplasma capsulatum Taxid:339724	XM_001538897.1	30.1	27%	0.63	91%
Blastomyces dermatitidis (Ajellomyces dermatitidis) Taxid:559298	XM_002623914.1	28.3	18%	2.3	100%
Penicillium spp. Taxid:500485	XM_002557696.1	30.1	20%	3.5	100%
Rhizopus spp. Taxid:4847	AY147870.1	22.9	15%	8.8	0
Scedosporium spp. Taxid:563467	0	0	0%		0
Rubella virus Taxid:111041	0	0	0%		0
Rubeola virus Taxid:884098	0	0	0%		0
Rubula virus Taxid:11161	0	0	0%		0
Varicella Zoster Virus Taxid:10335	0	0	0%		0
Mycobacterium franklinii Taxid:948102	HQ662092.1	24.3	88%	0.16	100%
M. massiliense, M. bolletii, M. abscessus subsp. bolletii Taxid:319705	DQ987717.1	75.8	75%	3E-14	92%
Mycobacterium chimaera Taxid:222805	AY943187.1	91.7	100%	6E-22	90%
Mycobacterium avium subsp. paratuberculosis Taxid:1770	CP005928.1	83.8	100%	4E-17	89%
Mycobacterium avium subsp. silvaticum Taxid:44282	AY544889.1	107	100%	8E-27	90%
Mycobacterium avium subsp. hominissuis Taxid:439334	AP012555.1	107	100%	1E-24	90%
Mycobacterium immunogenum Taxid:83262	HM454251.1	95.1	97%	1E-22	87%

Table 27. Microorganisms Predicted to be Potentially Cross Reactive by *in silico* Analysis

Mycobacterium kumamontonense
Mycobacterium leprae
Mycobacterium mucogenicum
Tsukamurella spp.
Nocardia otitidiscaviarum

15.3 Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the limit of detection (LoD) of human 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 limit of detection 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 28.

Table 28. Probit Analysis Data and Claimed LoD in CFU/mL

Microorganism (Strain)	Specimen Type	LoD Estimate	Claimed LoD
***M. bovis* (BCG)**	Sputum	486	525
	Sputum Sediment	703	700
***M. tuberculosis* (H37Rv)**	Sputum	414	600
	Sputum Sediment	2,046	3,000
***M. tuberculosis* (TDR125)**	Sputum	872	1,000
	Sputum Sediment	ND[a]	4,000

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

15.4 Interfering Substances Study

Performance of the Xpert MTB/RIF Assay was evaluated in the presence of 32 potentially interfering substances. Potentially endogenous interfering substances may include, but are not limited, to blood, pus (white blood cells), cells from the respiratory tract, mucin, human DNA, and gastric acid from the stomach. Other potentially interfering substances may include anesthetics, antibiotics, antibacterial, anti-tuberculosis drugs, anti-viral drugs, bronchodilators, inhaled bronchodilators, live intranasal influenza virus vaccine, germicidal mouthwash, specimen processing reagents, P*neumocystis jiroveci* medication, homeopathic allergy relief medications, nasal corticosteroids, nasal gels, nasal sprays, oral anesthetics, oral expectorants, neutralizing buffers, and tobacco. These substances are listed in Table 29 with active ingredients and concentrations tested shown. 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 H37Rv and one inactivated rifampin-resistant strain TDR6 (probe E mutant). Both strains were tested in replicates of 8. Negative samples, comprised of the substance absent the MTB strain, were tested per substance in replicates of 8 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 29. Potentially 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% solution	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 with sodium bicarbonate	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	>67mM phosphate

15.5 Carry-Over Contamination Study

A study was conducted to demonstrate that potential carry-over and cross-contamination does not occur when using the single-use, self-contained Xpert MTB/RIF Assay cartridges. The study consisted of a negative sample 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**.

15.6 RIF Resistance Study

Due to the low prevalence of RIF resistance, an additional non-clinical study was conducted to assess the performance of the Xpert MTB/RIF Assay for the detection of genetic mutations associated with RIF resistance in well characterized RIF susceptible and resistant clinical isolates spiked into a pool of known MTB negative sputum. Fifty aliquots of pooled MTB-negative human sputum negatives were randomly intermixed for testing among the positives.The sensitivity and specificity of the Xpert MTB/RIF Assay for detection of mutations associated with RIF resistance, relative to drug susceptibility testing using Middlebrook agar proportions methods, were 97.7% (85/87) with 95% CI: 92.0%-99.4%; and 90.8% (89/98) with 95% CI: 83.5%-95.1%, respectively.

Of the 11 specimens with RIF discordant results, bi-directional sequencing was concordant with the Xpert MTB/RIF Assay for 10 of the 11 specimens and discordant with 1 of the 11 specimens.

16. Reproducibility

Reproducibility of the Xpert MTB/RIF Assay was evaluated at three sites using specimens comprised of cultured strains of *M. tuberculosis* spiked into a pool of MTB-negative human sputum. The specimens were prepared at concentration levels representing low positive (~1X LoD) and moderate positive (2-3X LoD) for both RIF susceptible and RIF resistant strains. Negative panel members were also included, and were comprised of pooled MTB-negative human sputum. A panel of five specimens was tested on five different days by two different operators three times per day at three sites (30 tests at each site =2 operators x 5 days x 3 replicates per day). One reagent kit lot of the Xpert MTB/RIF Assay was used in the study. The percent agreement for each panel member is presented by site in Table 30.

Table 30. Summary of Reproducibility Results – Agreement by Study Site/Instrument

Sample	Site 1 (Infinity-80)	Site 2 (GeneXpert Dx)	Site 3 (Infinity-48)	% Total Agreement by Sample
MTB/RIF resistant 2-3X LoD	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)
MTB/RIF resistant 1X LoD	93.3% (28/30)	96.7% (29/30)	96.7% (29/30)	95.6% (86/90)
MTB/RIF sensitive 2-3X LoD	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)
MTB/RIF sensitive 1X LoD	96.7% (29/30)	100.0% (30/30)	100.0% (30/30)	98.9% (89/90)
Negative	100.0% (30/30)	100.0% (29/29)	100.0% (30/30)	100.0% (89/89)[a]

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

The reproducibility of the Xpert MTB/RIF Assay was also evaluated in terms of the fluorescence signal expressed in cycle threshold (Ct) values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-site, between- day, between-operator and within-run components for each panel member are presented in Table 31. A run is defined as the three samples per panel member tested by one operator at one site on one day.

Table 31. Summary of Reproducibility Data[a]

Probe	Target		Agreement /Total Number (%)	Mean Ct	Between-Site		Between-Day		Between-Operator		Within-Run		Total	
	MTB/ RIF	Conc (LoD)			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
A	POS/R	2-3X	90/90 (100)	26.23	0.19	0.7	0.09	0.4	0.00	0.0	1.36	5.2	1.37	5.2
	POS/R	1X	86/90 (95.6)	27.13	0.00	0.0	0.78	2.9	0.00	0.0	1.74	6.4	1.91	7.0
	POS/S	2-3X	90/90 (100)	25.89	0.00	0.0	0.38	1.5	0.00	0.0	1.43	5.5	1.48	5.7
	POS/S	1X	89/90 (89.9)	27.25	0.00	0.0	0.00	0.0	0.36	1.3	1.28	4.7	1.33	4.9
	NEG	NEG	89/89 (100)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
B	POS/R	2-3X	90/90 (100)	38.41	0.26	0.7	0.72	1.9	0.00	0.0	1.59	4.1	1.76	4.6
	POS/R	1X	86/90 (95.6)	38.47	0.00	0.0	0.82	2.1	0.16	0.4	1.68	4.4	1.88	4.9
	POS/S	2-3X	90/90 (100)	27.01	0.00	0.0	0.33	1.2	0.00	0.0	1.42	5.3	1.46	5.4
	POS/S	1X	89/90 (89.9)	28.21	0.03	0.1	0.00	0.0	0.34	1.2	1.25	4.4	1.29	4.6
	NEG	NEG	89/89 (100)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C	POS/R	2-3X	90/90 (100)	26.52	0.21	0.8	0.00	0.0	0.00	0.0	1.30	4.9	1.31	4.9
	POS/R	1X	86/90 (95.6)	27.37	0.00	0.0	0.79	2.9	0.00	0.0	1.66	6.1	1.84	6.7
	POS/S	2-3X	90/90 (100)	26.13	0.00	0.0	0.36	1.4	0.00	0.0	1.44	5.5	1.49	5.7
	POS/S	1X	89/90 (89.9)	27.44	0.00	0.0	0.00	0.0	0.35	1.3	1.25	4.6	1.30	4.7
	NEG	NEG	89/89 (100)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
D	POS/R	2-3X	90/90 (100)	27.65	0.18	0.6	0.00	0.0	0.00	0.0	1.33	4.8	1.35	4.9
	POS/R	1X	86/90 (95.6)	28.51	0.00	0.0	0.81	2.9	0.00	0.0	1.63	5.7	1.82	6.4
	POS/S	2-3X	90/90 (100)	27.35	0.00	0.0	0.36	1.3	0.00	0.0	1.36	5.0	1.41	5.2
	POS/S	1X	89/90 (89.9)	28.62	0.00	0.0	0.00	0.0	0.26	0.9	1.16	4.1	1.19	4.2
	NEG	NEG	89/89 (100)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
E	POS/R	2-3X	90/90 (100)	27.77	0.27	1.0	0.13	0.5	0.00	0.0	1.50	5.4	1.53	5.5
	POS/R	1X	86/90 (95.6)	28.72	0.22	0.8	0.79	2.8	0.00	0.0	1.84	6.4	2.01	7.0
	POS/S	2-3X	90/90 (100)	27.47	0.23	0.8	0.34	1.2	0.00	0.0	1.52	5.5	1.57	5.7
	POS/S	1X	89/90 (89.9)	28.86	0.00	0.0	0.00	0.0	0.51	1.8	1.37	4.8	1.46	5.1
	NEG	NEG	89/89 (100)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[a] Conc=concentration, Ct=cycle threshold, CV=coefficient of variation, LoD=limit of detection, N=number, N/A=Not Applicable for negative samples, SD=standard deviation; R=RIF resistant; S=RIF susceptible

17. Instrument System Precision

An in-house precision study was conducted to compare the performance of the GeneXpert Dx and the Infinity-80 Instrument Systems using specimens comprised of cultured strains of *M. tuberculosis* spiked into a pool of MTB-negative human sputum. The specimens were prepared at concentration levels representing low positive (~1X LoD) and moderate positive (2-3X LoD) for both RIF susceptible and RIF resistant strains. Negative panel members were also included and were comprised of MTB-negative human sputum. A panel of five specimens was tested on 12 different days by two operators. Each operator conducted four runs of each panel specimen per day on each of the two instrument systems (96 tests at each instrument =4 tests x12 days x 2 operators). One reagent kit lot of the Xpert MTB/RIF Assay was used for the study. The percent agreement for each panel member is presented by instrument in Table 32.

Table 32. Summary of Instrument Precision Results; Percent Agreement

Sample	GeneXpert Dx	Infinity-80	% Total Agreement by Sample
MTB/RIF resistant 2-3X LoD	100.0% (94/94)	99.0% (95/96)	99.5% (189/190)[a,b]
MTB/RIF resistant 1X LoD	97.9% (94/96)	99.0% (95/96)	98.4% (189/192)[c]
MTB/RIF sensitive 2-3X LoD	100.0% (96/96)	100.0% (96/96)	100.0% (192/192)
MTB/RIF sensitive 1X LoD	91.6% (87/95)	86.5% (83/96)	89.0% (170/191)[a,d]
Negative	99.0% (95/96)	97.9% (94/96)	98.4% (189/192)[e]

[a] 2 MTB/RIF resistant moderate pos and 1 MTB/RIF sensitive low pos specimens were non-determinate by Xpert MTB/RIF Assay upon initial and retest.
[b] 1 specimen **MTB NOT DETECTED**.
[c] 2 specimens **MTB NOT DETECTED** and 1 specimen **MTB DETECTED; Rif Resistance INDETERMINATE**.
[d] 17 specimens **MTB NOT DETECTED** and 4 specimens **MTB DETECTED; Rif Resistance INDETERMINATE**.
[e] 3 specimens **MTB DETECTED; Rif Resistance NOT DETECTED**.

The precision of the Xpert MTB/RIF Assay was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-instrument, between-day, between-operator, and within-run components for each panel member are presented in Table 33. A run is defined as the four samples per panel member tested by one operator on one instrument on one day.

Table 33. Summary of Instrument Precision Data[a]

Probe	Target		Agreement/ Total Number (%)	Mean Ct	Between-Instrument		Between-Day		Between-Operators		Within-Run		Total	
	MTB/ RIF	Conc (LoD)			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
A	POS/R	2-3X	189/190 (99.5)	25.52	0.00	0.0	0.62	2.4	0.00	0.0	1.23	4.8	1.37	5.4
	POS/R	1X	189/192 (98.4)	27.03	0.00	0.0	0.00	0.0	0.59	2.2	1.36	5.0	1.48	5.5
	POS/S	2-3X	192/192 (100)	25.43	0.23	0.9	0.41	1.6	0.00	0.0	1.28	5.0	1.36	5.4
	POS/S	1X	170/191 (89.0)	27.44	0.00	0.0	1.24	4.5	1.16	4.2	1.79	6.5	2.46	9.0
	NEG	NEG	189/192 (98.4)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
B	POS/R	2-3X	189/190 (99.5)	37.45	0.00	0.0	0.00	0.0	0.00	0.0	2.00	5.3	2.00	5.3
	POS/R	1X	189/192 (98.4)	38.06	0.00	0.0	0.00	0.0	0.66	1.7	1.76	4.6	1.88	4.9
	POS/S	2-3X	192/192 (100)	26.44	0.30	1.1	0.25	0.9	0.00	0.0	1.31	5.0	1.37	5.2
	POS/S	1X	170/191 (89.0)	28.16	0.00	0.0	0.96	3.4	1.01	3.6	1.79	6.4	2.26	8.0
	NEG	NEG	189/192 (98.4)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C	POS/R	2-3X	189/190 (99.5)	25.77	0.00	0.0	0.62	2.4	0.00	0.0	1.19	4.6	1.34	5.2
	POS/R	1X	189/192 (98.4)	27.27	0.00	0.0	0.00	0.0	0.54	2.0	1.32	4.8	1.42	5.2
	POS/S	2-3X	192/192 (100)	25.63	0.23	0.9	0.36	1.4	0.00	0.0	1.29	5.0	1.36	5.3
	POS/S	1X	170/191 (89.0)	27.62	0.00	0.0	1.01	3.6	1.09	4.0	2.06	7.4	2.54	9.2
	NEG	NEG	189/192 (98.4)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
D	POS/R	2-3X	189/190 (99.5)	27.00	0.00	0.0	0.57	2.1	0.00	0.0	1.17	4.3	1.30	4.8
	POS/R	1X	189/192 (98.4)	28.44	0.10	0.3	0.00	0.0	0.53	1.9	1.31	4.6	1.42	5.0
	POS/S	2-3X	192/192 (100)	26.98	0.24	0.9	0.34	1.3	0.00	0.0	1.26	4.7	1.33	4.9
	POS/S	1X	170/191 (89.0)	28.79	0.00	0.0	1.22	4.2	1.11	3.8	1.64	5.7	2.33	8.1
	NEG	NEG	189/192 (98.4)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
E	POS/R	2-3X	189/190 (99.5)	28.84	0.00	0.0	0.61	2.3	0.00	0.0	1.20	4.5	1.35	5.0
	POS/R	1X	189/192 (98.4)	28.44	0.00	0.0	0.00	0.0	0.63	2.2	1.38	4.8	1.52	5.3
	POS/S	2-3X	192/192 (100)	26.89	0.18	0.7	0.42	1.6	0.00	0.0	1.31	4.9	1.39	5.2
	POS/S	1X	170/191 (89.0)	28.86	0.00	0.0	1.41	4.9	0.81	2.8	1.74	6.0	2.38	8.2
	NEG	NEG	189/192 (98.4)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[a] Conc=concentration, Ct=cycle threshold, CV=coefficient of variation, LoD=limit of detection, N=number, N/A=Not Applicable for negative samples, SD=standard deviation; R=RIF resistant; S=RIF susceptible

18. References

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19. Cepheid Headquarters Locations

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20. Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

Region	Telephone	Email
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21. Table of Symbols

Symbol	Meaning
	Catalog number
	In vitro diagnostic medical device
	Do not reuse
	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Contains sufficient for <n> tests
	Control
	Expiration date
	Temperature limitation
	Biological risks
	Flammable Liquid and Vapor
	Causes severe skin burns and eye damage

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