GeneXpert
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Xpert® MTB/RIF

REF **GXMTB/RIF-IN-10**

REF **CGXMTB/RIF-IN-50**

Instructions For Use

Cepheid *In Vitro* Diagnostic Medical Device CE IVD

302-4827, Rev. D April 2023

Trademark, Patents and Copyright Statements

See Section 21, Revision History for a description of changes.

Xpert® MTB/RIF

In Vitro Diagnostic Medical Device

1 Proprietary Name

Xpert® MTB/RIF Assay

2 Common or Usual Name

Xpert MTB/RIF Assay

3 Intended Use

The Xpert MTB/RIF Assay for use with the Cepheid GeneXpert® systems is a semi-quantitative, nested real-time PCR *in-vitro* diagnostic test for the detection of:

- *Mycobacterium tuberculosis* complex DNA in sputum samples or concentrated sediments prepared from induced or expectorated sputa that are either acid-fast bacilli (AFB) smear positive or negative
- Rifampin-resistance associated mutations of the *rpoB* gene in samples from patients at risk for rifampin resistance

The Xpert MTB/RIF Assay is intended for use with specimens from untreated patients for whom there is clinical suspicion of tuberculosis (TB). Use of the Xpert MTB/RIF Assay for the detection of *M. tuberculosis* (MTB) or determination of rifampin susceptibility has not been validated for patients who are receiving treatment for tuberculosis.

4 Summary and Explanation

Globally, about 2 billion people are infected with MTB.[1] Every year almost 9 million people develop active disease, and 2 million people die of the illness. Active MTB, which is predominantly pulmonary in nature, is a highly infectious airborne disease. Given the infectious nature of MTB, fast and accurate diagnosis is an important element of MTB treatment and control.

Treatment involves prolonged administration of multiple drugs and is usually highly effective. However, MTB strains can become resistant to one or more of the drugs, which makes cure difficult to achieve. Four common first-line drugs used in anti-tuberculosis therapy are:

- Isoniazid (INH)
- Rifampin (RIF or Rifampicin)
- Ethambutol (EMB)
- Pyrazinimide (PZA)

RIF resistance rarely occurs in isolation and usually indicates resistance to a number of other anti-TB drugs.[2] RIF resistance is most commonly seen in multi-drug resistant (MDR-TB) strains and has a reported frequency of greater than 95% in such isolates.[3, 4, 5] MDR-TB is defined as a tuberculous disease caused by a bacterial strain that is resistant to at least INH and RIF. Resistance to RIF or other first-line drugs usually indicates the need for full susceptibility testing, including testing against second-line agents.

Molecular detection of MTB and *rpoB* gene mutations associated with RIF resistance speeds the diagnosis of both drug-susceptible and MDR-TB. With the Xpert MTB/RIF Assay, this can be accomplished in fresh sputum samples and in prepared sediments in less than 2.5 hours. The rapid detection of MTB and RIF resistance allows the physician to make critical patient management decisions regarding therapy during the same medical encounter.

5 Principle of the Procedure

The GeneXpert Instrument Systems integrate and automate sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and reverse transcriptase PCR. The system consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests on collected samples and viewing the results. The system requires the use of single-use disposable GeneXpert cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is eliminated. For a full description of the system, see the *GeneXpert Dx System Operator Manual* or *GeneXpert Infinity System Operator Manual*.

Xpert MTB/RIF includes reagents for the detection of MTB and RIF resistance and a Sample Processing Control (SPC) 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 primers in the Xpert MTB/RIF Assay amplify a portion of the *rpoB* gene containing the 81 base pair "core" region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance.

6 Reagents and Instruments

6.1 Material Provided

The Xpert MTB/RIF kit (GXMTB/RIF-IN-10) contains sufficient reagents to process 10 patient or quality-control specimens, and the Xpert MTB/RIF kit (CGXMTB/RIF-IN-50) contains sufficient reagents to process 50 patient or quality-control specimens. The Xpert MTB/RIF kits contain the following items:

Xpert MTB/RIF Cartridges with Integrated Reaction Tubes	**10 per kit**	**50 per kit**
• Bead 1 and Bead 2 (freeze-dried)	2 of each per cartridge	2 of each per cartridge
• Bead 3 (freeze-dried)	1 per cartridge	1 per cartridge
• Reagent 1	4.0 mL per cartridge	4.0 mL per cartridge
• Reagent 2	4.0 mL per cartridge	4.0 mL per cartridge
Sample Reagent Bottles	**10 per kit**	**50 per kit**

• Sample Reagent (SR)
  • Sodium hydroxide
  • Isopropanol
	8 mL per bottle	8 mL per bottle

Disposable transfer pipettes	**12 per kit**	**60 per kit**
CD	**1 per kit**	**1 per kit**
• Assay Definition File (ADF) • Instruction to import ADF into software • Instructions for Use (Package Insert)		

Note The 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 mixing of the material with other animal materials.

Note The transfer pipettes have a single mark representing the minimum volume of treated sample necessary to transfer to the 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 kit contents at 2–28 °C.
- Do not use reagents or cartridges that have passed the expiration date.
- Do not open a cartridge lid until you are ready to perform testing.
- The cartridges are stable up to 6 weeks at 2 to 45 °C after opening the pouch.

7 Materials Required but Not Provided

- GeneXpert Dx System, GeneXpert Infinity System (catalog number varies by configuration):
  - For GeneXpert Dx System: GeneXpert instrument, computer with proprietary GeneXpert Software Version 4.0 or higher
  - For GeneXpert Infinity-48 system: Software version Xpertise 4.3 or higher

- For GeneXpert Infinity-80 and Infinity-48s systems: Software version Xpertise 6.0 or higher
- Printer: If a printer is required, contact a Cepheid Sales Representative to arrange for the purchase of a recommended printer.
- Leak-proof, sterile, screw-capped specimen collection containers
- Disposable gloves and eye protection
- Labels and/or indelible labeling marker
- Sterile pipettes for sample processing

8 Warnings, Precautions and Chemical Hazards

8.1 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 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[6] and the Clinical and Laboratory Standards Institute.[7]
- 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.
- The performance of Xpert MTB/RIF Assay for the detection of MTB complex has not been demonstrated from non-respiratory specimens, such as blood, CSF, stool or urine. The performance of the Xpert MTB/RIF Assay has not been evaluated with specimens processed by methods other than those described in this package insert.
- When processing more than one sample at a time, open only one cartridge; add the Sample Reagent-treated sample and close the cartridge lid before processing the next sample. Change gloves between samples.
- Do not open the Xpert MTB/RIF cartridge lid except when adding the treated sample.
- Do not use a cartridge that has been dropped after removing from the kit.
- Do not use a cartridge that has been dropped or shaken or has spilled contents of cartridge after you have added the treated sample. Shaking or dropping the cartridge after opening the lid may yield false or non-determinate results.
- 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 a damaged reaction tube.
- Each Xpert MTB/RIF cartridge is used to process one test. Do not reuse processed cartridges.
- Check your regional/country hazardous and medical waste disposal requirements. If regulations do not provide clear directions on the proper disposal of specimens or used cartridges, they should be treated as capable of transmitting infectious agents. Dispose of the used cartridges as chemical hazardous health-care waste in durable waste containers per WHO (World Health Organization) following medical waste handling and disposal guidelines.

8.2 Chemical Hazards[8,9]

Sample Reagent:

Sample Reagent contains sodium hydroxide (pH > 12.5) and isopropanol.

- UN GHS Hazard Pictograms: 
- Signal Word: DANGER
- **UN GHS Hazard Statements**
  - Flammable liquid and vapour
  - Causes severe skin burns and eye damage
  - Causes serious eye damage
  - Suspected of causing genetic defects.
  - Suspected of damaging fertility or the unborn child.
  - May cause damage to organs through prolonged or repeated exposure
- **UN GHS Precautionary Statements**
  - Prevention
    - Obtain special instructions before use.

- Do not handle until all safety precautions have been read and understood.
- Keep away from heat, sparks, open flames and/or hot surfaces. No smoking.
- Keep container tightly closed.
- Do not breathe mists, vapors, and/or spray.
- Wash thoroughly after handling.
- Wear protective gloves/protective clothing/eye protection/face protection.
- Use personal protective equipment as required.

- Response
  - In case of fire: Use appropriate media for extinction.
  - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
  - Immediately call a POISON CENTER or doctor/physician.
  - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
  - Wash contaminated clothing before reuse.
  - Specific treatment, see supplemental first aid information.
  - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  - IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
  - IF exposed or concerned: Get medical advice/attention.
  - Get medical advice/attention if you feel unwell.
- Storage/Disposal
  - Dispose of content and/or container in accordance with local, regional, national, and/or international regulations.

9 Specimen Collection, Transport and Storage

You can process resuspended sediment or fresh sputum with this assay. 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
Sputum sediment	0.5 mL	1 mL
Fresh sputum	1 mL	2 mL

Note To obtain an adequate fresh sputum specimen, follow the instructions in this section. The patient should be seated or standing.

9.1 Storage and Transport

Store and transport specimens at 2 to 8 °C prior to processing whenever possible. However, if necessary the specimens can be stored at a maximum of 35 °C for ≤ 3 days and at 2 to 8 °C for 4 to 10 days.

9.2 Specimen Collection Procedure

1. Have the patient rinse his or her mouth twice with water.
2. Open the lid on the sputum collection container.
3. Have the patient inhale deeply, cough vigorously, and expectorate the material into the container. Avoid spills or soiling the outside of the container.
4. Secure the lid on the collection device, and then send the container to the processing area.

10 Assay Procedures

10.1 Preparing the Sputum Sediments

Note Reject specimens with obvious food particles or other solid particles.

Volume Requirements: Sputum sediments prepared according to the method of Kent and Kubica[10] and re-suspended in 67mM Phosphate/H2O buffer can be tested using Xpert MTB/RIF Assay. After resuspension, keep at least 0.5 mL of the resuspended sediment for the Xpert MTB/RIF Assay.

1. Label each Xpert MTB/RIF 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 2D barcode on the cartridge.

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

Note Store re-suspended sediments at 2 to 8 °C if they are not immediately processed. Do not store for more than 12 hours.

3. Transfer 1.5 mL of Xpert MTB/RIF Sample Reagent (SR) to 0.5 mL of resuspended sediment using a transfer pipette.
4. Shake vigorously 10 to 20 times or vortex for at least 10 seconds.

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

5. Incubate for 10 minutes at room temperature, and then shake the specimen vigorously 10 to 20 times or vortex for at least 10 seconds.
6. Incubate the sample at room temperature for an additional 5 minutes.

10.2 Preparing the Expectorated Sputum Sample

Note Reject specimens with obvious food particles or other solid particles.

1. Label each Xpert MTB/RIF 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 2D barcode on the cartridge.

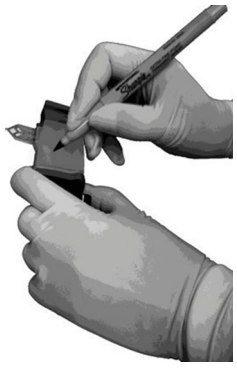

Figure 1. Writing on the Cartridge with a Permanent Marking Pen

2. In a leak-proof sputum collection container:
   A. Carefully open the lid of the sputum collection container.

Figure 2. Opening the Sample Container

B. Pour approximately 2 times the volume of the Sample Reagent to the sputum (2:1 dilution, SR:sputum).

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

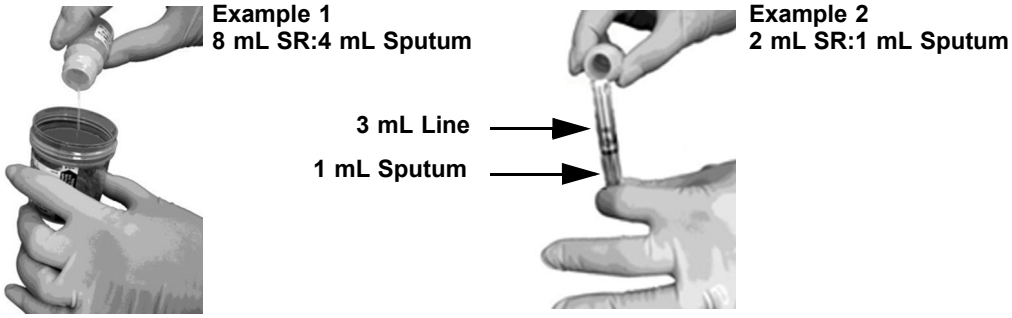

Figure 3. Examples of 2:1 Dilutions

C. Replace and secure the lid.

D. Shake vigorously 10 to 20 times or vortex for at least 10 seconds.

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

3. Incubate the sample for 10 minutes at room temperature, and then shake the specimen vigorously 10 to 20 times or vortex for at least 10 seconds.
4. Incubate the sample at room temperature for an additional 5 minutes.

10.3 Preparing the Cartridge

Note When using the GeneXpert Dx System, start the test within 4 hours of adding the 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 Xpertise Software so that tests are run prior to the 4-hour onboard expiration.

1. Open the cartridge lid, and then open the sample container.
2. Using the provided transfer pipette, aspirate the liquefied sample to the line on the pipette. Do not process the sample further if there is insufficient volume. See Figure 4.

Figure 4. Aspirating to the Line on the Pipette

3. Transfer the sample into the sample chamber of the Xpert MTB/RIF cartridge. Dispense the sample slowly to minimize the risk of aerosol formation. See Figure 5.

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

4. Close the cartridge lid firmly.

Important **If using a GeneXpert Dx System, be sure to load the cartridge into the instrument and start the test within 4 hours of preparing 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 Xpertise Software so that tests are run prior to the four hour onboard expiration.**

11 Running the Test

- For the GeneXpert Dx System, see Section 11.1.
- For the GeneXpert Infinity System, see Section 11.2.

11.1 GeneXpert Dx System

Starting the Test

Important

Before you start the test, make sure that:

- **The system is running the correct GeneXpert Dx software version shown in section - Materials Required but Not Provided.**
- **The correct 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*.

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

1. Turn on the GeneXpert Dx System, then turn on the computer and log on. The GeneXpert software will launch automatically. If it does not, double-click the GeneXpert Dx software shortcut icon on the Windows® desktop.
2. Log on using your username and password.
3. In the **GeneXpert System** window, click **Create Test**.

   The **Create Test** window displays. The **Scan Patient ID barcode** dialog box displays.
4. Scan or type in the Patient ID. If typing the Patient ID, make sure the Patient ID is typed correctly.

   The Patient ID is associated with the test results and displays in the **View Results** window and all the reports. The **Scan Sample ID barcode** dialog box displays.
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 displays in the **View Results** window and all the reports. The **Scan Cartridge Barcode** dialog box displays.
6. Scan the barcode on the cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.Viewing and Printing Results.

Note If the barcode on the cartridge does not scan, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the software and the assay definition file is not available, a screen displays indicating the assay definition file is not loaded on the system. If this screen displays, contact Cepheid Technical Support.

7. Click **Start Test**. In the dialog box that displays, type your password, if required.
8. Open the instrument module door with the blinking green light and load the cartridge.
9. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
10. Wait until the system releases the door lock before opening the module door, then remove the cartridge.
11. Dispose of the used cartridges in the appropriate specimen waste containers according to your institution's standard practices.

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*.

1. Click the **View Results** icon to view results.
2. Upon completion of the test, click the **Report** button of the **View Results** window to view and/or generate a PDF report file.

11.2 GeneXpert Infinity System

Starting the Test

Important

Before you start the test, make sure that:

- **The system is running the correct GeneXpert Dx software version shown in section - Materials Required but Not Provided.**
- **The correct assay definition file is imported into the software.**

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

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

1. Power up the instrument. The Xpertise software will launch automatically. If it does not, double-click the Xpertise software shortcut icon on the Windows® desktop.
2. Log on to the computer, then log on to the GeneXpert Xpertise software using your user name and password.
3. In the **Xpertise Software Home** workspace, click **Orders** and in the **Orders** workspace, click **Order Test**. The **Order Test - Patient ID** workspace displays.
4. Scan or type in the Patient ID. If typing the Patient ID, make sure the Patient ID is typed correctly.

   The Patient ID is associated with the test results and displays in the **View Results** window and all the reports.

5. Enter any additional information required by your institution, and click the **CONTINUE** button. The **Order Test - Sample ID** workspace displays.
6. 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 displays in the **View Results** window and all the reports.
7. Click the **CONTINUE** button.

   The **Order Test - Assay** workspace displays.
8. Scan the barcode on the cartridge. 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 cartridge does not scan, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the software and the assay definition file is not available, a screen displays indicating the assay definition file is not loaded on the system. If this screen displays, contact Cepheid Technical Support.

   After the cartridge is scanned, the **Order Test - Test Information** workspace displays.
9. Verify that the information is correct, and click **Submit**. In the dialog box that displays, type your password, if required.
10. Place the cartridge on the conveyor belt.

    The cartridge automatically loads, the test runs, and the used cartridge are placed into the waste container.

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 Infinity System Operator Manual*.

1. In the **Xpertise Software Home** workspace, click the **RESULTS** icon. The Results menu displays.
2. In the Results menu, select the **VIEW RESULTS** button. The **View Results** workspace displays showing the test results.
3. Click the **REPORT** button to view and/or generate a PDF report file.

12 Discarding Used Cartridges

1. Discard used cartridges in a hard-sided biohazard container according to your institution's standard practices. See Figure 6.

Figure 6. Dispose of Used Cartridges Properly

2. Do not burn used cartridges. See Figure 7.

Figure 7. Do Not Burn Used Cartridges

3. Do not discard used cartridges in a landfill or dump. See Figure 8.

Figure 8. Do Not Discard Used Cartridges in a Landfill

13 Quality Control

CONTROL

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

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 lysis of MTB has 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 assay.

The SPC should be positive in a 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 a negative test.

PCC: Before the start of the PCR reaction, the GeneXpert Instrument System measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. PCC passes if it meets the validated acceptance criteria.

14 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 9, Figure 10 and Figure 11 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 HIGH; RIF Resistance DETECTED **MTB DETECTED MEDIUM; RIF Resistance DETECTED** **MTB DETECTED LOW;** **RIF Resistance DETECTED** **MTB DETECTED VERY LOW; RIF Resistance DETECTED** See Figure 10.	The MTB target is present within the sample: • A mutation in the rpoB gene target sequence has been detected. • SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control. • Probe Check: PASS. All probe check results pass.
MTB DETECTED HIGH; **RIF Resistance NOT DETECTED** **MTB DETECTED MEDIUM; RIF Resistance NOT DETECTED** **MTB DETECTED LOW;** **RIF Resistance NOT DETECTED** **MTB DETECTED VERY LOW; RIF Resistance NOT DETECTED** See Figure 9.	The MTB target is present within the sample: • No mutation in the rpoB gene target sequence has been detected. • SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control. • Probe Check: PASS. All probe check results pass.
MTB DETECTED HIGH; **RIF Resistance INDETERMINATE** **MTB DETECTED MEDIUM;** **RIF Resistance INDETERMINATE** **MTB DETECTED LOW;** **RIF Resistance INDETERMINATE** **MTB DETECTED VERY LOW; RIF Resistance INDETERMINATE**	The MTB target is present within the sample: • RIF resistance could not be determined due to invalid melt peaks. • SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control. • Probe Check: PASS. All probe check results pass.
MTB Not Detected See Figure 11.	The MTB target is not detected within the sample: • SPC: PASS. The SPC met the acceptance criteria. • Probe Check: PASS. All probe check results pass.
INVALID	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 14.2, 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: PASS. All probe check results pass.

Table 2. Xpert MTB/RIF Assay Results and Interpretations

Result	Interpretation
ERROR	The presence or absence of MTB cannot be determined. Repeat the test. See Section 14.2, Retest Procedure. • MTB: NO RESULT • SPC: NO RESULT • Probe Check: FAIL. All or one of the probe check results failed. Note: If the probe check passed, the error is caused by a system component failure.
NO RESULT	The presence or absence of MTB cannot be determined. Repeat the test. See Section 14.2, Retest Procedure. A NO RESULT indicates that insufficient data was collected. For example, the operator stopped a test that was in progress. • MTB: NO RESULT • SPC: NO RESULT • Probe Check: NA (not applicable)

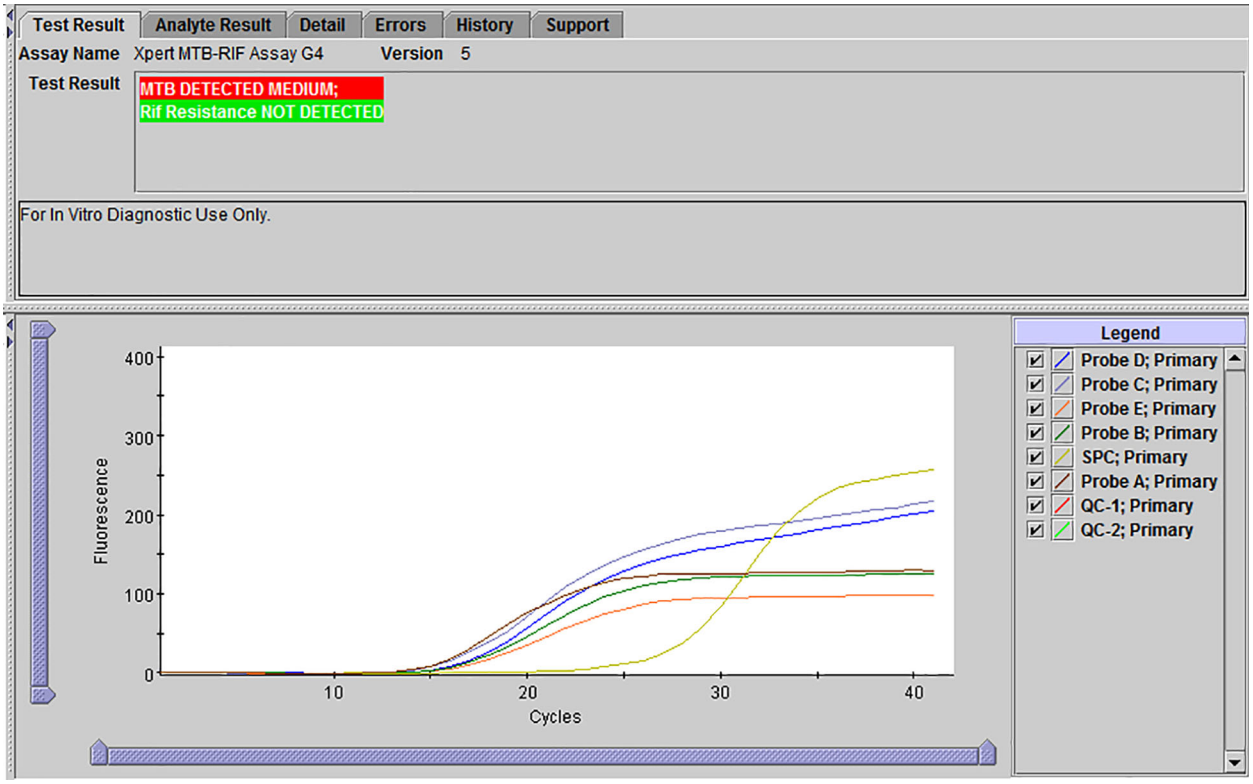

Figure 9. MTB DETECTED MEDIUM; Rif Resistance NOT DETECTED (GeneXpert Dx Detailed User View)

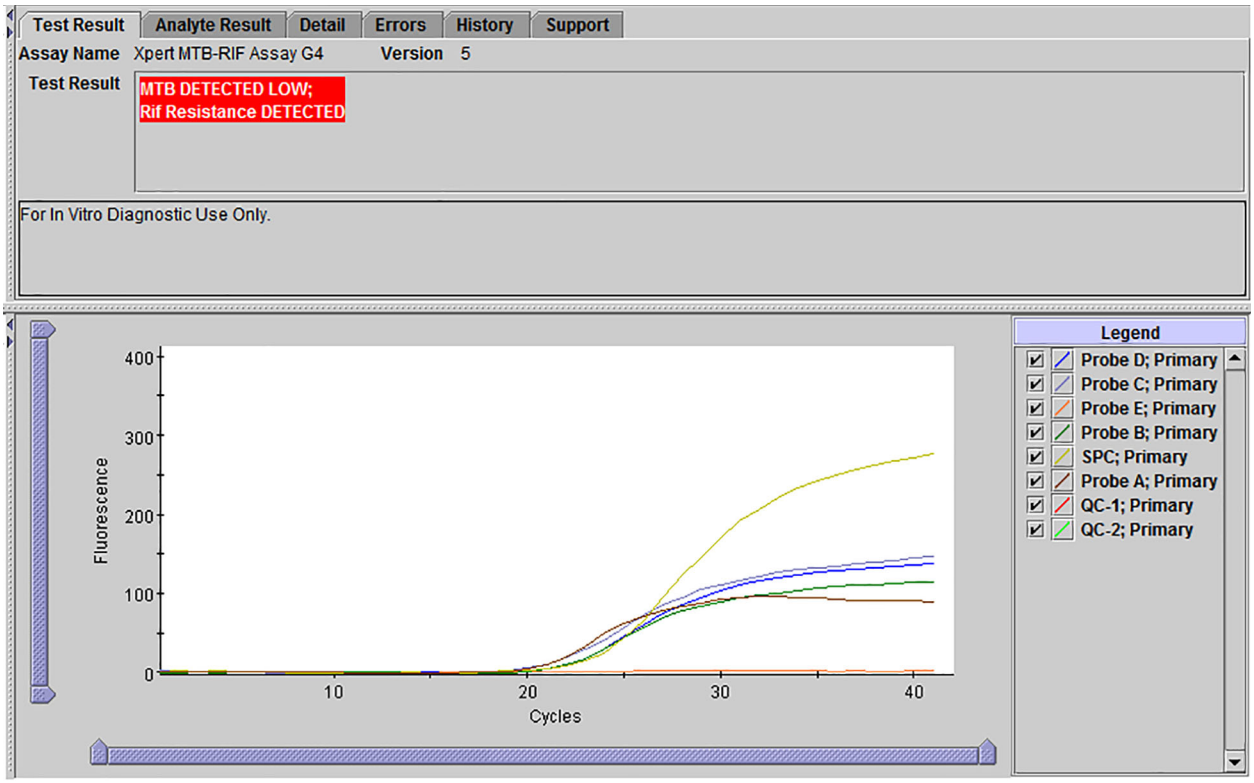

Figure 10. MTB DETECTED LOW; Rif Resistance DETECTED (GeneXpert Dx Detailed User View)

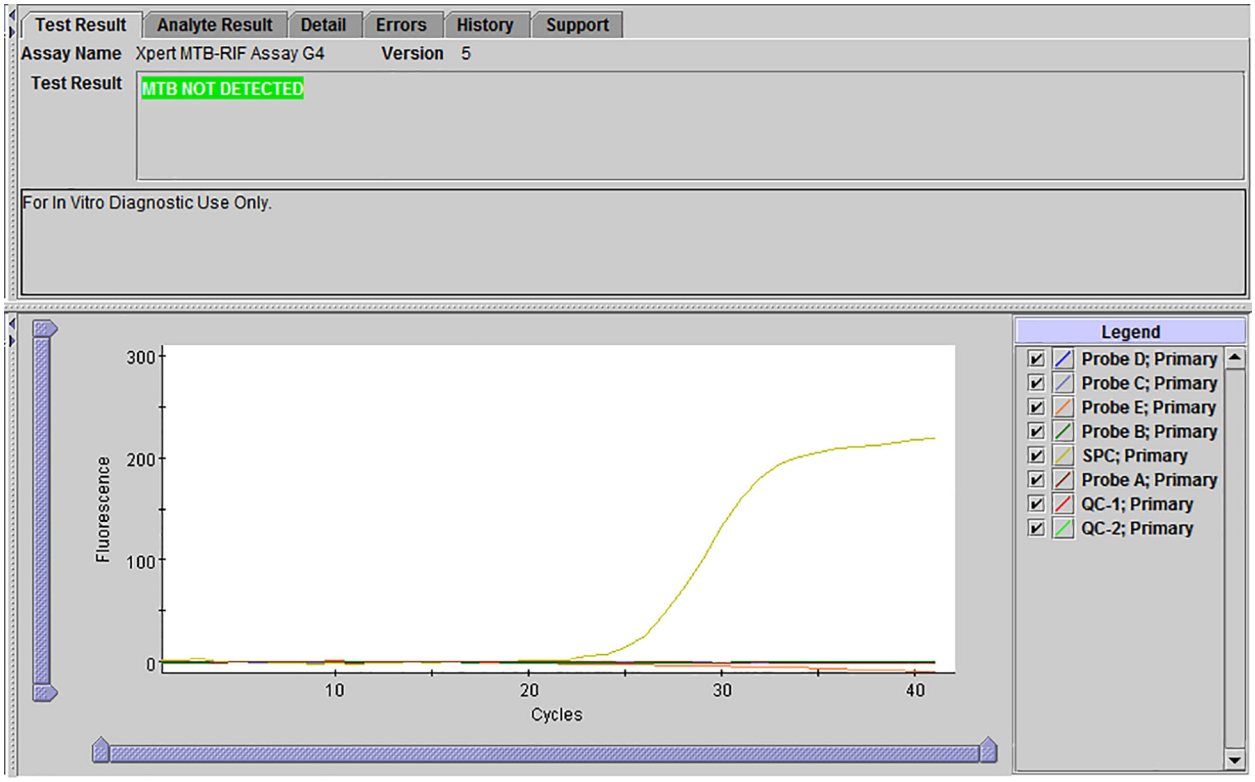

Figure 11. MTB NOT DETECTED (GeneXpert Dx Detailed User View)

14.1 Reasons to Repeat the Assay

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 failed, and the assay was aborted 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.

14.2 Retest Procedure

If you have leftover fresh sputum or reconstituted sediment, always use new Sample Reagent to decontaminate and liquefy the sputum before running the assay. See Section 10.1, Preparing the Sputum Sediments or Section 10.2, Preparing the Expectorated Sputum Sample.

If you have sufficient leftover SR-treated sample and are within 4 hours of the initial addition of SR to the sample, you can use the leftover sample to prepare and process a new cartridge. When retesting, always use a new cartridge. See Section 10.3, Preparing the Cartridge.

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

15 Limitations

The performance of the Xpert MTB/RIF was validated using the procedures provided in this package insert. Modifications to these procedures may alter the performance of the test. Results from the Xpert MTB/RIF should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

Because the detection of MTB is dependent on the number of organisms present in the sample, reliable 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, 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.

A positive test result does not necessarily indicate the presence of viable organisms. It is however, presumptive for the presence of MTB and RIF resistance.

Test results might be affected by antecedent or concurrent antibiotic therapy. Therefore, therapeutic success or failure cannot be assessed using this test because DNA might persist following antimicrobial therapy.

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.

A semi-quantitative result of VERY LOW indicates bacterial load very close to the limit of detection and close clinical follow up is recommended.

Semi-quantitative results of **MTB DETECTED LOW** or **MTB DETECTED VERY LOW** with **RIF Resistance DETECTED** may require confirmation of rifampin resistance results by DST or other method, particularly when the clinical suspicion of MDR-TB is low.

16 Performance Characteristics

This section lists the performance characteristics and limitations of the Xpert MTB/RIF Assay.

16.1 Performance Testing - Clinical

Performance characteristics of the Xpert MTB/RIF Assay for TB and rifampin detection were evaluated at five institutions in Asia, Europe, Africa and South America.[11]

The study was performed according to the Foundation for Innovative New Diagnostics (FIND) protocol *Xpert MTB Evaluation Study: Evaluation of the FIND/Cepheid Xpert MTB assay for the detection of pulmonary TB in sputum of symptomatic adults*.

Subjects included individuals with symptoms of pulmonary TB and at risk of multi-drug resistance. For eligible subjects, three sputum samples were obtained for testing with the Xpert MTB/RIF Assay and reference testing.

The Xpert MTB/RIF Assay performance was compared to:

- ZN smear microscopy[12]
- Liquid (Becton Dickinson BACTEC™ 960 MGIT™) and solid (Löwenstein-Jenson) culture[13]
- Drug susceptibility testing (DST) on L-J proportion or on MGIT covering at least four first-line drugs[14]

- Standard NAAT tests (Gen-Probe Amplified Mycobacterium TB Direct Test and Roche AMPLICOR® MTB Test) when performed

Samples included sputum specimens collected for routine testing from patients suspected of tuberculosis infection and at risk for multi-drug resistant TB.

16.2 Overall Results

A total of 1448 sputum specimens were tested for MTB and RIF resistance by the Xpert MTB/RIF Assay, and smear microscopy and bacterial culture. Specimens at three of the participating sites were also assessed using either the AMPLICOR MTB Test (UCT, South Africa & India) or the Amplified Mycobacterium TB Direct Test (Azerbaijan). Of the 1448 participants, 563 had smear and culture positive TB (S+C+), 170 had smear negative, culture positive TB (S-C+), and TB was excluded in 618. The remaining 97 patients were treated for TB based on clinical symptoms and improved under TB treatment, but were not tested by smear microscopy or culture; these patients are not included in the data analyses presented in the tables.

16.3 MTB Detection Results

Overall, when considering a composite of the results from three sputum samples per patient, the Xpert MTB/RIF Assay demonstrated a sensitivity among culture positive specimens of 97.3% (713/733). In S+C+ patients, the Xpert MTB/RIF Assay sensitivity was 99.5% (560/563); in S-C+ patients, the sensitivity was 90.0% (153/170). The Xpert MTB/RIF Assay specificity in non-TB patients was 97.9% (605/618). See Table 3.

Table 3. Xpert MTB/RIF Assay Performance on Sputum Specimens[a,b]

Site	Sensitivity S+C+	Sensitivity S-C+	Specificity
Peru	100% (199/199) [98.1%-100%]	83.3% (10/12) [55.2%-95.3%]	100% (102/102) [96.4%-100%]
Azerbaijan	100% (76/76) [95.2%-100%]	92.3% (60/65) [83.2%-96.7%]	95.8% (69/72) [88.5%-98.6%]
South Africa-1	99.0% (95/96) [94.3%-99.8%]	90.4% (47/52) [79.4%-95.8%]	98.4% (186/189) [95.4%-99.5%]
South Africa-2	100% (30/30) [88.6%-100%]	86.7% (13/15) [62.1%-96.3%]	97.3% (213/219) [94.2%-98.7%]
India	98.8% (160/162) [95.6%-99.7%]	88.5% (23/26) [71.0%-96.0%]	97.2% (35/36) [85.8%-99.5%]
Overall	99.5% (560/563) [98.4%-99.8%]	90.0% (153/170) [84.6%-93.7%]	97.9% (605/618) [96.4%-98.8%]

a. Represents results of 3 Xpert tests, 3 smears, and 4 cultures.
b. S=smear; C=culture

When considering only a single direct sputum sample, the Xpert MTB/RIF Assay sensitivity was 97.8% (545/557) in S+C+ patients and 73.1% (122/167) in S-C+ patients. The specificity was 99.0% (605/611) in non-TB patients.

16.4 RIF Resistance

Overall, when considering a composite of the results from three sputum samples per patient, the Xpert MTB/RIF Assay demonstrated sensitivity for RIF resistance detection among phenotypic RIF resistant patients of 96.1% (195/203). The Xpert MTB/RIF Assay specificity in phenotypic RIF sensitive patients was 98.6% (502/509). See Table 4.

Table 4. Xpert MTB/RIF Assay Performance on Sputum Specimens[a]

Site	Sensitivity in RIF-resistant Cases	Specificity in RIF-sensitive Cases
Peru	100% (16/16) [80.6%-100%]	98.4% (190/193) [95.5%-99.5%]
Azerbaijan	95.5% (42/44) [84.9%-98.7%]	98.9% (90/91) [94.0%-99.8%]
South Africa-1	93.8% (15/16) [71.7%-98.9%]	100% (126/126) [97.0%-100%]
South Africa-2	100% (3/3) [43.8%-100%]	100% (38/38) [90.8%-100%]
India	96.0% (119/124) [90.9%-98.3%]	95.1% (58/61) [86.5%-98.3%]
Overall	96.1% (195/203) [92.4%-98.0%]	98.6% (502/509) [97.2%-99.3%]

a. Represents results of 3 Xpert tests, 3 smears, and 4 cultures.

When considering only a single direct sputum sample, the Xpert MTB/RIF Assay sensitivity for RIF resistance detection was 97.2% (141/145) in RIF-resistant patients. The specificity in RIF sensitive cases was 98.3% (412/419). See Table 5.

Table 5. Xpert MTB/RIF Assay Performance on Sputum Specimens[a]

Site	Sensitivity in RIF-resistant Cases	Specificity in RIF-sensitive Cases
Peru	100% (16/16) [80.6%-100%]	98.4% (180/183) [95.3%-99.4%]
Azerbaijan	97.4% (38/39) [86.8%-99.5%]	98.7% (74/75) [92.8%-99.8%]
South Africa-1	90.9% (10/11) [62.3%-98.4%]	98.1% (102/104) [93.3%-99.5%]
South Africa-2	100% (1/1) [20.7%-100%]	100% (23/23) [85.7%-100%]
India	97.4% (76/78) [91.1%-99.3%]	97.1% (33/34) [85.1%-99.5%]
Overall	97.2% (141/145) [93.1%-98.9%]	98.3% (412/419) [96.6%-99.2%]

a. Represents results of 1 direct Xpert test, 3 smears, and 4 cultures.

The Xpert MTB/RIF Assay results on specimens from those sites where a NAAT test was also performed are shown in Table 6. The NAAT test result is shown for comparison.

Table 6. Comparison of Xpert MTB/RIF Assay and Alternative NAAT Performance on Sputum Specimens

Statistic	Test[a]	Azerbaijan	South Africa-1	India	Overall
Sensitivity S+C+	Xpert(3)	100% (76/76) [95.2%-100%]	99.0% (95/96) [94.3%-99.8%]	98.8% (160/162) [95.6%-99.7%]	99.1% (331/334) [97.4%-99.8%]
	Xpert(1)	97.3% (73/75) [90.8%-99.3%]	96.8% (92/95) [91.1%-98.9%]	98.8% (159/161) [95.6%-99.7%]	97.9% (324/331) [95.7%-99.2%]
	NAAT	100% (76/76) [95.2%-100%]	93.7% (89/95) [86.9%-97.1%]	94.2% (147/156) [89.4%-96.9%]	95.4% (312/327) [92.3%-97.4%]
Sensitivity S-C+	Xpert(3)	92.3% (60/65) [83.2%-96.7%]	90.4% (47/52) [79.4%-95.8%]	88.5% (23/26) [71.0%-96.0%]	90.9% (130/143) [85.0%-95.1%]
	Xpert(1)	68.8% (44/64) [56.6%-78.8%]	86.3% (44/51) [74.3%-93.2%]	69.2% (18/26) [50.0%-83.5%]	75.2% (106/141) [67.2%-82.1%]
	NAAT	66.2% (43/65) [54.0%-76.5%]	45.7% (16/35) [30.5%-61.8%]	72.0% (18/25) [52.4%-85.7%]	61.6% (77/125) [52.5%-70.2%]
Specificity	Xpert(3)	95.8% (69/72) [88.5%-98.6%]	98.4% (186/189) [95.4%-99.5%]	97.2% (35/36) [85.8%-99.5%]	97.6% (290/297) [95.2%-99.1%]
	Xpert(1)	97.2% (69/71) [90.3%-99.2%]	99.5% (185/186) [97.0%-99.9%]	100% (35/35) [90.1%-100%]	99.0% (289/292) [97.0%-99.8%]
	NAAT	95.8% (69/72) [88.5%-98.6%]	100% (187/187) [98.0%-100%]	100% (36/36) [90.4%-100%]	99.0% (292/295) [97.1%-99.8%]

a. Xpert(3) = results of 3 Xpert tests, 3 smears, and 4 cultures; Xpert(1) = results of 1 direct Xpert test, 3 smears, and 4 cultures; NAAT = ProbeTec (Azerbaijan), and AMPLICOR (South Africa and India); NAAT "borderline" treated as negative.

Of the Xpert MTB/RIF Assays runs performed in conjunction with this study, 96.5% (4327/4484) were successful on the first attempt. The remaining 157 gave indeterminate results on the first attempt. One hundred eight of the 157 specimens yielded valid results with retest. The overall assay success rate was 98.9% (4435/4484).

16.5 Interfering Substances

A study was performed to assess the potential inhibitory effects of substances that may be present in sputum processed with the Xpert MTB/RIF assay. These include, but are not limited to: blood, pus, mammalian cells and hemoglobin. These substances were tested at 5% final sample concentration (blood, pus, mammalian cells) or 0.2% (hemoglobin) to determine an effect, if any, on the performance of the Xpert MTB/RIF. Each substance was added to a sample containing approximately 5 times the limit of detection (LoD) of target BCG cells and was tested in duplicate.

No inhibitory effect was observed for any of the above potentially interfering substances.

16.6 Analytical Sensitivity

Additional studies were performed to determine the 95% confidence interval for the analytical limit of detection (LoD) of this assay. The limit of detection is defined as the lowest number of colony forming units (CFU) per sample that can be reproducibly distinguished from negative samples with 95% confidence. The analytical LoD was determined by testing 20 replicates of different concentrations of *M. tuberculosis* cells spiked into negative clinical sputum samples. Under the conditions of the study, results indicate that the LoD point estimate for *M. tuberculosis* is 131 CFU/mL with a 95% confidence interval ranging from 106.2 CFU to 176.4 CFU. The estimate and confidence levels were determined using logistic regression with data (number of positives per number of tests at each level) taken at different concentrations.

The confidence intervals were determined using the maximum likelihood estimates on the logistic model parameters using the large sample variance-covariance matrix.

16.7 Analytical Specificity (Exclusivity)

Cultures of 18 nontuberculosis mycobacteria, NTM (formerly MOTT), strains were tested with the Xpert MTB/RIF assay. Two or more replicates of each isolate were spiked into negative sputum samples and tested at a concentration of 10^6 CFU/mL. See Table 7.

Table 7. NTM Strains Tested for Specificity

NTM Strains Tested (10^6 CFU/mL)			
1	*M. avium*, SmT Mc2, 2500	10	*M. genevense*s, #51233
2	*M. avium*, SmD Mc2, 2501	11	*M. xenopi*, #2278
3	*M. intracellulare*, #35790	12	*M. szulgai*, Cap E9-1997
4	*M. intracellulare*, #35771	13	*M. celatum*, #51131
5	*M. kansasii*, #12478	14	*M. marinum*, Cap E10
6	*M. scrofulaceum*, Cap E5-1985	15	*M. simiae*, #25275
7	*M. malmoense*, #29571	16	*M. asiaticum*, E1-1985
8	*M. fortuitum*, #35754	17	*M. thermoresistable*, e22-1985
9	*M. chelonae*, #35749	18	*M. flavescens*, PoH 193D

Under the conditions of the study, all of the NTM isolates were reported MTB negative.

Additionally, in order to determine if high concentrations of NTM would interfere with the detection of low levels of TB, the strains listed in Table 7 were mixed with the TB strain H37Rv in sputum to a final concentration of 10^6 CFU/mL NTM and 200 CFU/mL H37Rv.

NTM strains tested for ability to interfere with TB detection included:

- *M. avium*, SmT Mc2, 2500
- *M. avium*, SmD Mc2, 2501
- *M. intracellulare*, #35790
- *M. intracellulare*, #35771
- *M. kansasii*, #12478
- *M. malmoense*, #29571

Five of the six strains did not interfere in the detection of 200 CFU/mL of *M. tuberculosis*; thus, the signals were the same as H37Rv alone. The sixth, *M. malmoense*, produced a weak interference at 10^6 CFU/mL but none at 10^5 CFU/mL or lower. Therefore, there is no interference in the detection of *M. tuberculosis* even with 10^5 CFU/mL of NTM.

Non-mycobacterial organisms (n = 59) that represent a wide-range of pathogens, common contaminants and microflora commonly present in sputum or the mouth were tested at a concentration of 10^6 copies of DNA per final reaction volume. All organisms were correctly identified as MTB-negative by the Xpert MTB/RIF assay. Positive and negative controls were included in the study. The specificity was 100%.

16.8 Species/Strains tested for Specificity

Table 8 shows species and strains tested for specificity.

Table 8. Species/Strains Tested for Specificity

Acinetobacter baumanii	*Haemophilus influenzae*	*Salmonella typhi*
Acinetobacter calcoaceticus	*Haemophilus parahemolyticus*	*Serratia marcescens*
Actinomyces meyeri	*Haemophilus parainfluenzae*	*Shigella boydii*
Bacillus cereus	*Klebsiella pneumoniae*	*Shigella flexneri*
Bacillus subtilis	*Legionella pneumophila*	*Staphylococcus aureus*
Bordetella parapertussis	*Leuconostoc mesenteroides*	*Staphylococcus capitis*
Campylobacter jejuni	*Listeria grayi*	*Staphylococcus epidermidis*
Candida albicans	*Moraxella catarrhalis*	*Staphylococcus haemolyticus*
Citrobacter freundii	*Morganella morganii*	*Staphylococcus hominis*
Corynebacterium pseudodiptheriticum	*Mycoplasma pneumoniae*	*Stenotrophomonas maltophilia*
Corynebacterium xerosis	*Neisseria gonorrhoeae*	*Streptococcus equi*
Cryptococcus neoformans	*Neisseria lactamica*	*Streptococcus pyogenes*
Enterobacter aerogenes	*Neisseria meningitidis*	*Streptococcus agalactiae*
Enterobacter cloacae	*Neisseria mucosa*	*Streptococcus constellatus*
Enterococcus avium	*Peptostreptococcus anaerobius*	*Streptococcus mitis*
Enterococcus faecalis	*Porphyromonas gingivalis*	*Streptococcus mutans*
Enterococcus faecium	*Prevotella melaninogenica*	*Streptococcus pneumoniae*
Escherichia coli (Strain type 2)	*Propionibacterium acnes*	*Streptococcus uberis*
Escherichia coli O157H7 (Strain type 1)	*Proteus mirabilis*	*Veillonella parvula*
Fusobacterium nucleatum	*Pseudomonas aeruginosa*	

16.9 Analytical Inclusivity

DNA samples from a total of 79 MTB strains were tested on the GX using an Xpert MTB/RIF protocol modified for DNA testing. The final reaction components and PCR cycling conditions were unchanged from the protocol designed for patient sample testing. Seventy of the strains were from the WHO/TDR collection and 9 from the laboratory collection at the University of Medicine and Dentistry of New Jersey (UMDNJ). Collectively these strains represent isolates from 31 countries and contained 37 RIF-resistant isolates comprised of 13 unique *rpoB* core region mutations. These include every unique *rpoB* mutation found in the TDR database. The negative reactions used water as the sample.

The final reaction mixture contained 90 genomic copies of the isolates in 100 µL total volume.

Table 9 shows that the Xpert MTB/RIF correctly detected all MTB strains and correctly identified the RIF-resistant isolates.

Table 9. Detection of MTB Strains and RIF-resistant Isolates

			GeneXpert Result		
			MTB Positive		MTB Negative
			RIF Detected	RIF Not Detected	
Reference	MTB +	RIF Resistance	37	0	0
		RIF Sensitive	0	42	0
	MTB –		0	0	52

16.10 Analytical Inactivation of Mycobacteria in Sputum Samples

The disinfection capability of the Xpert MTB/RIF sample reagent was determined using a standardized quantitative tuberculocidal culture method.[15] Samples of sputum were spiked with a high concentration of viable *M. bovis*, mixed with sample reagent at a ratio of 2:1, and incubated for 15 minutes. Following incubation the sample reagent/sputum mixture was neutralized by dilution and filtration and then cultured. The viability of the *M. bovis* organisms from the treated sputum was reduced by at least 6 logs relative to the un-treated control.

Each laboratory must determine the effectiveness of the sample reagent disinfection properties using their own standardized methods and must adhere to recommended biosafety regulations.

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18 Cepheid Headquarters Locations

Corporate Headquarters

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
United States

Telephone: + 1 408 541 4191

Fax: + 1 408 541 4192

www.cepheid.com

European Headquarters

Cepheid Europe SAS
Vira Solelh
81470 Maurens-Scopont
France

Telephone: + 33 563 825 300

Fax: + 33 563 825 301

www.cepheidinternational.com

19 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

Contact Information

United States
Telephone: + 1 888 838 3222
Email: techsupport@cepheid.com

France
Telephone: + 33 563 825 319
Email: support@cepheideurope.com

Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/CustomerSupport.

20 Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	CE marking – European Conformity
	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 Liquids
	Skin Corrosion

	Reproductive and Organ Toxicity
	Warning

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna
Sweden
Tel: + 46 8 6843 7000
Fax: + 46 8 6843 7010

21 Revision History

Description of Changes: 302-4827, Rev. C to Rev. D
Purpose: Deleted information about GeneXpert Edge System

Section	Description of Change
5, 7, 10.3, 11, 14	Deleted information about GeneXpert Edge System.