GeneXpert
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Xpert® MTB/RIF Ultra

REF **GXMTB-ULTRA-MII-10**

REF **GXMTB-ULTRA-MII-50**

Instructions for Use

CE IVD

Cepheid

In Vitro Diagnostic Medical Device

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Trademark, Patents and Copyright Statements

See Section 21 Revision History for a description of changes.

Xpert® MTB/RIF Ultra

For *In Vitro* Diagnostic Use

1 Proprietary Name

Xpert® MTB/RIF Ultra

2 Common or Usual Name

Xpert MTB/RIF Ultra

3 Intended Use

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

Xpert MTB/RIF Ultra is intended for use with specimens from patients for whom there is clinical suspicion of tuberculosis (TB) and who have received no antituberculosis therapy, or less than 3 days of therapy in the last 6 months. This test is intended as an aid in the diagnosis of pulmonary tuberculosis when used in conjunction with clinical and other laboratory findings.

4 Summary and Explanation

Globally, about 1.7 billion people are infected with MTB.[1] In 2018, 10.0 million people developed active disease, and 1.45 million people lost their lives to the illness.[2] The route of transmission of pulmonary TB is through the air, which makes this a highly transmissible disease. Given the infectious nature of pulmonary TB, fast and accurate diagnosis is an important element of TB treatment and control.

Treatment involves prolonged administration of multiple drugs and is usually highly effective. However, *M. tuberculosis* strains may become resistant to one or more of the drugs, making cure much more difficult to achieve. Four common first-line drugs used in anti-tuberculosis therapy are isoniazid (INH), rifampin (also known as rifampicin, RIF), ethambutol (EMB), and pyrazinamide (PZA). As documented by World Health Organization, RIF resistance is rarely encountered by itself, and usually indicates resistance to a number of other anti-TB drugs.[3] It is most commonly seen in multi-drug resistant (MDR-TB) strains (defined as resistant to both RIF and INH) and has a reported frequency of greater than 95% in such isolates.[4,5,6] 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 TB and *rpoB* gene mutations associated with RIF resistance greatly reduces the time to diagnosis of both drug-susceptible and MDR tuberculosis. With Xpert MTB/RIF Ultra, this can be accomplished in unprocessed sputum samples and in prepared sediments in less than 80 minutes. The rapid detection of MTB and RIF resistance allows the physician to make critical patient management decisions regarding therapy during a single 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 melt peak detection. The system consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests on patient 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 minimized. For a full description of the system, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

Xpert MTB/RIF Ultra 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 for the presence of inhibitor(s) in the PCR reaction and subsequent melt peak detection. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The primers in Xpert MTB/RIF Ultra amplify a portion of the *rpoB* gene containing the 81 base pair "core" region and portions of the multi-copy *IS1081* and *IS6110* insertion elements target sequences. The melt analysis with four *rpoB* probes is able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance. The two insertion element probes enhance the detection of *Mycobacterium tuberculosis* complex due to the multi-copy insertion element target sequences in most TB strains.

6 Reagents and Instruments

6.1 Materials Provided

Xpert MTB/RIF Ultra kits contain sufficient reagents to process 10 samples or 50 samples. The kits contain the following:

Xpert MTB/RIF Ultra 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 of each per cartridge	1 of each per cartridge
• Reagent 1	4 mL per cartridge	4 mL per cartridge
• Reagent 2	4 mL per cartridge	4 mL per cartridge
Sample Reagent Bottles	**10**	**50**
• Sample Reagent	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 Files (ADF) • Instructions to import ADF into software • Instructions for Use (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) protein stabilizer 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 Xpert MTB/RIF Ultra cartridges at 2-28 °C.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use the kit after the expiration date stated on the kit box.

7 Materials Required but Not Provided

- GeneXpert Dx System, GeneXpert Infinity System or (catalog number varies by configuration): GeneXpert instrument, computer with proprietary GeneXpert Software Version 4.7b or higher (GeneXpert Dx System), Xpertise™ 6.4b or higher (GeneXpert Infinity System), barcode scanner, and operator manual
- Printer: If a printer is required, contact Cepheid Sales Representative to arrange for the purchase of a recommended printer.
- Leak-proof, sterile screw-capped collection containers
- Disposable gloves
- 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[7] and the Clinical and Laboratory Standards Institute.[8]
- Wear protective disposable gloves, laboratory coats and eye protection when handling samples and reagents. Wash hands thoroughly after handling samples and test reagents.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Do not substitute Xpert MTB/RIF Ultra reagents with other reagents.
- Do not open the Xpert MTB/RIF Ultra cartridge lid except when adding treated sample.
- Do not use a cartridge that has been dropped after removing it 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 place the Sample ID label on the cartridge lid or on the bar code label.
- 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.
- 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.
- Each Xpert MTB/RIF Ultra cartridge is used to process one test. Do not reuse processed cartridges.
- A single-use disposable pipette is used to transfer one specimen. Do not reuse disposable pipettes.
- Good laboratory practices should be followed, and gloves should be changed between handling each patient specimen in order to avoid contamination of specimens or reagents. Regularly clean the work surface/areas with 10% bleach then wipe the surface again with 70% ethanol or isopropyl alcohol before and after processing specimens.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution’s environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures. If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

8.2 Chemical Hazards[9,10]

DANGER 

Hazard Statements

- Flammable liquid and vapor
- 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.

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

Specimen Collection

Follow your institution’s protocol for sample collection.

Collect sputum or aerosol-induced sputum following your institution’s standard procedures. Test unprocessed sputum or concentrated/decontaminated sputum sediment. See table below to determine adequate specimen volume.

Table 1. Required Specimen Volume

Specimen Type	Minimum Volume for One Test	Maximum sample volume	Sample to Sample Reagent (SR) Ratio
Sputum sediment	0.5 mL	2.5 mL	1.3[a]
Unprocessed sputum	1 mL	4.0 mL	1:2

[a] 1:2 sample to SR ratio should be used with sample volume of 0.7 mL or greater for one test.

Storage and Transport

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

Unprocessed sputum: Transport and store sputum at 35 °C up to 3 days, and 2 – 8 °C up to 10 days. If necessary, unprocessed sputum may be stored 35 – 45 °C up to 15 days.

10 Assay Procedure

10.1 Procedure for Decontaminated, Concentrated Sputum Sediments

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

Volume Requirements: Sputum sediments prepared according to the method of Kent and Kubica[11] and re-suspended in 67 mM Phosphate/H_2O buffer) can be tested using Xpert MTB/RIF Ultra. After resuspension, keep at least 0.5 mL of the resuspended sediment for Xpert MTB/RIF Ultra. For all volumes less than 0.7 mL perform steps 1–6. These steps require 3 parts Sample Reagent (SR) to 1 part sediment in order to generate adequate volume (~2 mL) for the optimum performance of the assay.

If the sample volume is equal to or greater than 0.7 mL, adequate test volume can be produced by adding 2 parts SR to 1 part sediment. In this example 1.4 mL of SR would be added to 0.7 mL sediment. These volumes scale at a ratio of 2 parts SR to 1 part sediment.

1. Bring the cartridge to room temperature. Label each Xpert MTB/RIF Ultra cartridge with the Sample ID. See Figure 1.

Note Write on the side of the cartridge or affix an ID label. Do not put the label on the lid of the cartridge or over the existing 2D barcode on the cartridge.

2. Mix the sediment by vortexing or use a pipette to aspirate and eject the material enough times to assure that all organisms are in suspension.
3. Transfer 0.5 mL of the total resuspended pellet to a conical, screw-capped tube for Xpert MTB/RIF Ultra using a transfer pipette.

Note Store re-suspended sediments at 2 to 8°C if they are not immediately processed. Do not run Xpert MTB/RIF Ultra on a resuspended sediment that has been refrigerated for > 7 days.

4. Transfer 1.5 mL of Xpert MTB/RIF Ultra Sample Reagent (SR) to 0.5 mL of resuspended sediment using a transfer pipette. Tighten cap securely.
5. 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 for 10 minutes at room temperature, and then shake the specimen vigorously 10 to 20 times or vortex for at least 10 seconds.
7. Incubate the sample at room temperature for an additional 5 minutes.

10.2 Procedure for Unprocessed Sputum

Volume Requirement: ≥ 1 mL of unprocessed sputum is required.

1. Bring the cartridge to room temperature. Label each Xpert MTB/RIF Ultra cartridge with the Sample ID. See Figure 1.

Note Write on the side of the cartridge or affix an ID label. Do not put the label on the lid of the cartridge or over the existing 2D barcode on the cartridge.

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

2. After receiving the sample in a leak-proof sputum collection container, carefully open the lid of the sputum collection container and examine the contents to be sure there are no food particles or other solid particles.

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

Figure 2. Opening the Sample Container

3. Pour approximately 2 times the volume of the SR into the sputum (2:1 dilution, SR:sputum).

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

Figure 3. Example of 2:1 Dilution (8 mL SR:4 mL Sputum)

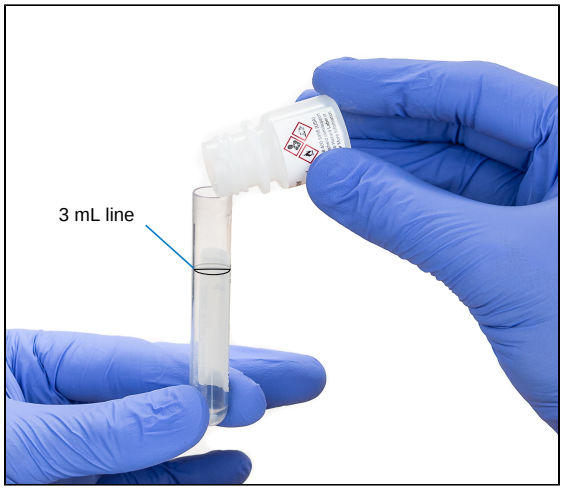

Figure 4. Example of 2:1 Dilution (2 mL SR:1 mL Sputum)

4. Replace and secure the lid. 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 the sample for 10 minutes at room temperature.
6. Shake the specimen vigorously 10 to 20 times or vortex for at least 10 seconds. Incubate the sample at room temperature for an additional 5 minutes.

Note Ensure that the specimen is liquefied completely. If specimen is not liquefied, repeat this step.

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. Once the sample is added to the cartridge, the cartridge should remain at room temperature prior to starting the test within four hours. 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 just above the line on the pipette. See Figure 5. Do not process the sample further if there is insufficient volume.

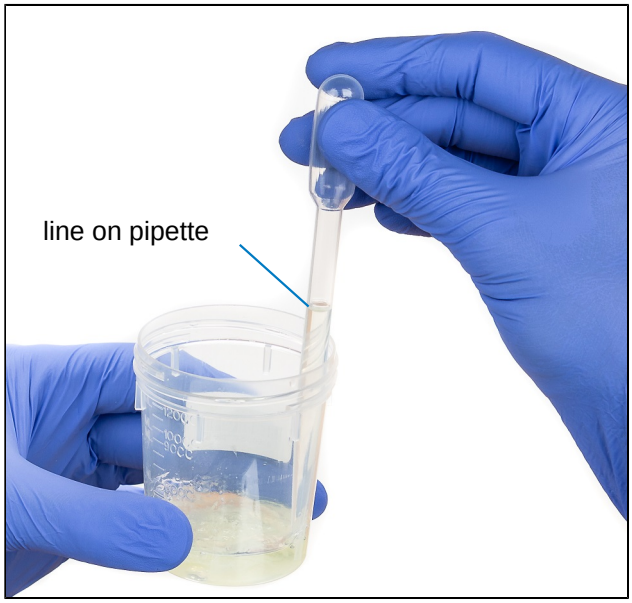

Figure 5. Aspirating to the Line on the Pipette

3. Transfer the sample into the sample chamber of the Xpert MTB/RIF Ultra cartridge. Dispense the sample slowly to minimize the risk of aerosol formation. See Figure 6.

Figure 6. Dispensing Decontaminated Liquefied Sample into the Sample Chamber of the Cartridge

4. Close the cartridge lid firmly. Remaining liquefied sample may be kept for up to 4 hours at 2 to 8 °C in case retesting is required.

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

11.1.1 Starting the Test

Before you start the test, make sure that:

Important

- 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.

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.

Note Do not turn off or unplug the instrument while a test is in progress. Turning off or unplugging the instrument or computer will stop the test.

11.1.2 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

11.2.1 Starting the Test

Before you start the test, make sure that:

Important

- The system is running the correct Xpertise 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.

Note Do not turn off or unplug the system while a test is in progress. Turning off or unplugging the GeneXpert instrument or computer will stop the test.

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

11.2.2 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 Quality Control

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

Sample Processing Control (SPC)

Ensures the sample was processed correctly. 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.

Probe Check Control (PCC)

Before the start of the PCR reaction, Xpert MTB/RIF Ultra measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the assigned acceptance criteria.

13 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 7, Figure 8, Figure 9 for specific examples, and see Table 3 for a list of all possible results.

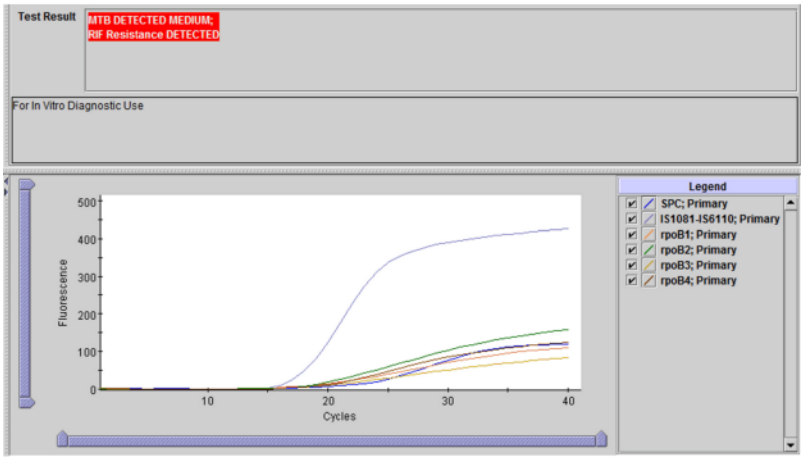

Figure 7. MTB DETECTED MEDIUM; RIF Resistance DETECTED (GeneXpert Dx Detailed User View)

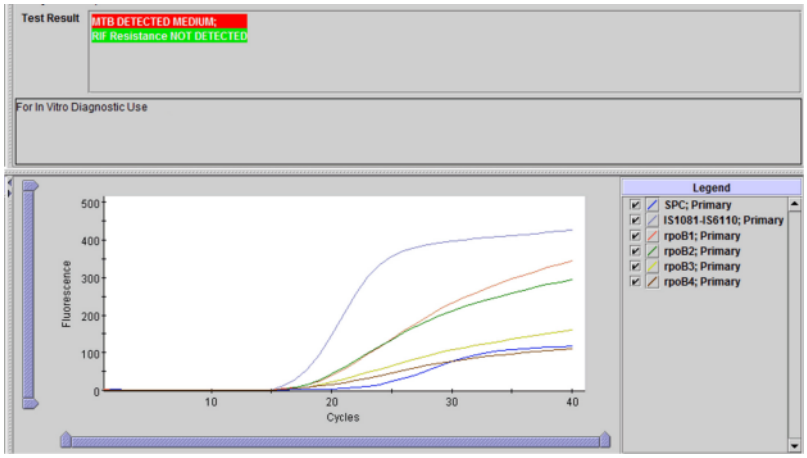

Figure 8. MTB DETECTED MEDIUM; RIF Resistance NOT DETECTED (GeneXpert Dx Detailed User View)

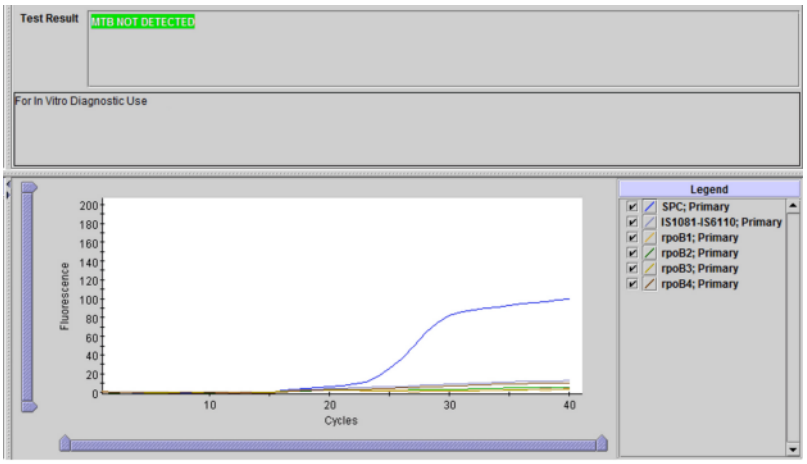

Figure 9. MTB NOT DETECTED (GeneXpert Dx Detailed User View)

Table 2. Xpert MTB/RIF Ultra Results and Interpretation

Result	Interpretation
MTB DETECTED HIGH; RIF Resistance DETECTED	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 MEDIUM; RIF Resistance DETECTED	
MTB DETECTED LOW; RIF Resistance DETECTED	
MTB DETECTED VERY LOW; RIF Resistance DETECTED	
MTB DETECTED HIGH; RIF Resistance NOT DETECTED	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 MEDIUM; RIF Resistance NOT DETECTED	
MTB DETECTED LOW; RIF Resistance NOT DETECTED	
MTB DETECTED VERY LOW; RIF Resistance NOT DETECTED	
MTB DETECTED HIGH; 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 DETECTED MEDIUM; RIF Resistance INDETERMINATE	
MTB DETECTED LOW; RIF Resistance INDETERMINATE	
MTB DETECTED VERY LOW; RIF Resistance INDETERMINATE	
MTB Trace DETECTED; RIF Resistance INDETERMINATE	The MTB target is present within the sample: ● RIF resistance cannot 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: PASS. All probe check results pass.
MTB NOT DETECTED	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.

Result	Interpretation
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 the Retest Procedure section of this document. • 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.
ERROR	The presence or absence of MTB cannot be determined. Repeat the test. See the Retest Procedure section of this document. • 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 the Retest Procedure section of this document. 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: NA (not applicable)

Table 3. Xpert MTB/RIF Ultra: All Possible Results

TB Results	RIF Results
MTB DETECTED HIGH	RIF Resistance DETECTED
MTB DETECTED HIGH	RIF Resistance NOT DETECTED
MTB DETECTED HIGH	RIF Resistance INDETERMINATE
MTB DETECTED MEDIUM	RIF Resistance DETECTED
MTB DETECTED MEDIUM	RIF Resistance NOT DETECTED
MTB DETECTED MEDIUM	RIF Resistance INDETERMINATE
MTB DETECTED LOW	RIF Resistance DETECTED
MTB DETECTED LOW	RIF Resistance NOT DETECTED
MTB DETECTED LOW	RIF Resistance INDETERMINATE
MTB DETECTED VERY LOW	RIF Resistance DETECTED
MTB DETECTED VERY LOW	RIF Resistance NOT DETECTED
MTB DETECTED VERY LOW	RIF Resistance INDETERMINATE
MTB Trace[a] DETECTED	RIF Resistance INDETERMINATE
MTB NOT DETECTED	
INVALID	
ERROR	

TB Results	RIF Results
NO RESULT	

[a] A Trace result call means that low levels of MTB are detected but no RIF resistant result is detected. This occurs due to the increased sensitivity of TB detection using multi-copy targets IS6110 and IS1081 as opposed to RIF resistance detection using the single copy rpoB gene. Therefore a RIF resistant or susceptible result cannot be determined in a Trace sample. The Trace sample is always **RIF Resistance INDETERMINATE**.

13.1 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 is 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, because 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.

13.2 Retest Procedure

If you have leftover fresh sputum or reconstituted sediment, always use new SR to decontaminate and liquefy the sputum or the sediment before running the assay. See Section 10 or Procedure for Unprocessed Sputum.

If you have a 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 and start the test immediately. See Preparing the Cartridge.

14 Limitations

Because the detection of MTB is dependent on the number of organisms present in the sample, reliable results are dependent on proper sample collection, handling, and storage. Erroneous test results might occur from improper sample collection, handling or storage, technical error, sample mix-up, or an insufficient concentration of starting material. Careful compliance to the instructions for use is necessary to avoid erroneous results.

Those individuals with results of **MTB Trace DETECTED** may require further clinical information and consideration of their clinical context for TB treatment decisions in some settings.

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

Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown MDR-MTB or rifampin resistant strains resulting in a false rifampin-sensitive result.

Xpert MTB/RIF Ultra performance has not been evaluated in patients less than eighteen years of age.

Xpert MTB/RIF Ultra 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 Xpert MTB/RIF Ultra should be considered for additional drug susceptibility testing.

The performance of Xpert MTB/RIF Ultra 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.

15 Clinical Performance

15.1 Clinical Study Design

The clinical performance of Xpert MTB/RIF Ultra was evaluated for the detection of MTB-complex DNA and for the detection of RIF-resistance associated mutations in sputum specimens relative to results from culture (solid and/or liquid media) and drug susceptibility testing (DST), respectively. This multi-center study used prospective and archived direct (raw) sputum or concentrated sediment specimens collected from subjects 18 years or older. Subjects included pulmonary TB suspects on no TB treatment or less than 3 days of treatment within 6 months of the study start (TB suspects) as well as previously TB treated subjects who were suspected of multi-drug resistant TB (MDR TB suspects). The study was conducted worldwide (Belarus, Brazil, China, Georgia, Germany, India, Italy, Kenya, Peru, South Africa, Uganda, Vietnam and the United States). The sensitivity and specificity of Xpert MTB/RIF Ultra for MTB detection were evaluated using data from only the TB suspects; whereas the data from the MDR TB suspects were combined to evaluate the performance of RIF resistance.

Among the 1985 specimens included in the primary data analyses, the specimens came from study participants who were ≥18 years old, 59% (n=1175) male, 37% female (n=734) and for 4% (n=76) gender was unknown or not available. They were from geographically diverse regions: 11% (n=217) were from the US (California, New York and Florida) and 89% (n=1768) were from outside the US (Belarus, Brazil, China, Georgia, Germany, Italy, India, Kenya, South Africa, Peru, Vietnam and Uganda).

15.2 Xpert MTB/RIF Ultra Performance vs. MTB Culture

Up to three sputum specimens were collected from each study subject for use in the clinical study. For prospective specimens, the first sputum specimen was tested by Xpert MTB/RIF Ultra and the second two specimens were used for TB culture. For archived specimens, culture results were available from the standard of care method and Xpert MTB/RIF Ultra was performed using the first specimen with sufficient volume. If the assay result was non-determinate (**ERROR**, **INVALID** or **NO RESULT**), the specimen was retested if there was sufficient volume. MTB Ultra Assays for 96.8% (1939/2004) specimens were successful on the first attempt (initial ND rate = 3.2%). Forty-six of the 65 non-determinate cases were retested, all of which yielded valid results upon repeat testing; 19 specimens were not retested. The overall rate of assay success was 99.1% (1985/2004). The overall non-determinate rate was 0.9% (19/2004) The acid fast bacilli (AFB) smear status for a subject was determined by Auramine-O (AO) fluorescent or Ziehl-Neelsen (ZN) smear stain from the specimen with the corresponding Xpert MTB/RIF Ultra result. The MTB culture status for all subjects was defined based on the MTB culture result of all specimens collected within a seven-day period for that subject.

The performance of Xpert MTB/RIF Ultra for detection of MTB relative to MTB culture, stratified by AFB smear status, is shown in the table below. The sensitivity in smear positive and smear negative specimens was 99.5% (426/428), 95% CI: 98.3, 99.9 and 73.3% (200/273), 95% CI: 67.7, 78.2, respectively. The overall specificity of Xpert MTB/RIF Ultra regardless of AFB smear was 95.5% (1222/1280), 95% CI: 94.2, 96.5. See tables below.

Table 4. Xpert MTB/RIF Ultra Performance vs. MTB Culture

		Smear/Culture				
		Positive			Negative	Total
		AFB Smear +	AFB Smear -	Overall Culture +	Overall Culture -	
Xpert MTB/ RIF Ultra	MTB DETECTED	426	200	630[a]	58	688
	MTB NOT DETECTED	2	73	75	1222	1297
	Total	428	273	705	1280	1985

	Smear/Culture				
	Positive			Negative	Total
	AFB Smear +	AFB Smear -	Overall Culture +	Overall Culture -	
Performance in Smear Positive: Sensitivity: 99.5% (426/428), 95% CI: 98.3, 99.9					
Performance in Smear Negative: Sensitivity: 73.3% (200/273), 95% CI: 67.7, 78.2					
Performance Overall: Sensitivity: 89.4% (630/705), 95% CI: 86.9, 91.4					
Specificity: 95.5% (1222/1280), 95% CI: 94.2, 96.5					

[a] Smear results were not available for 4 culture positive specimens.

The performance of Xpert MTB/RIF Ultra for detection of MTB relative to MTB culture, stratified by Non-US vs. US sites is shown in the table below. Among 1985 specimens, there were 1768 specimens from Non-US sites and 217 from US sites.

Table 5. Xpert MTB/RIF Ultra vs. MTB Culture by Non-US vs. US Sites

	Non-US		US	
	N	Percent 95% CI)	N	Percent 95% CI)
Sensitivity Smear Pos	380/382	99.5% (98.1,99.9)	46/46	100.0% (92.3, 100)
Sensitivity Smear Neg	180/245	73.5% (67.6, 78.6)	20/28	71.4% (52.9, 84.7)
Overall Sensitivity	564/631[a]	89.4% (86.7, 91.6)	66/74	89.2% (80.1, 94.4)
Overall Specificity	1080/1137	95.0% (93.6, 96.1)	142/143	99.3% (96.1, 99.9)

[a] Smear results were not available for 4 culture positive specimens.

15.3 Xpert MTB/RIF Ultra Performance vs Culture by Smear Type

The performance of Xpert MTB/RIF Ultra for detection of MTB was determined relative to MTB culture in specimens with AFB smear performed by AO and ZN. Results are shown in the table below. Among 1985 specimens, there were 1810 specimens with AO smears and 175 with ZN smears.

Table 6. Performance of Xpert MTB/RIF Ultra vs. MTB Culture by Auramine O (AO) and Ziehl-Neelsen (ZN) Staining Methods

	Auramine O Method		Ziehl-Neelsen Method	
	N	Percent (95% CI)	N	Percent (95% CI)
Sensitivity Smear Pos	386/388	99.5% (98.1,99.9)	40/40	100% (91.2, 100)
Sensitivity Smear Neg	153/219	69.9% (63.5, 75.6)	47/54	87.0% (75.6, 93.6)
Overall Sensitivity	543/611[a]	88.9% (86.1, 91.1)	87/94	92.6% (85.4, 96.3)
Overall Specificity	1145/1199	95.5% (94.2, 96.5)	77/81	95.1% (88.0, 98.1)

[a] Smear results were not available for 4 culture positive specimens.

15.4 Xpert MTB/RIF Ultra Performance vs. Culture by Specimen Type

The performance of Xpert MTB/RIF Ultra for detection of MTB was determined relative to MTB culture in unprocessed sputum and concentrated sputum sediment specimens. Results are shown in the table below. Among 1985 specimens, there were 1543 unprocessed sputum specimens and 442 concentrated sputum sediment specimens.

Table 7. Xpert MTB/RIF Ultra vs. MTB Culture by Specimen Type

	Direct Sputum		Sputum Sediments	
	N	% (95% CI)	N	% (95% CI)
Sensitivity Smear Pos	323/324	99.7% (98.3, 99.9)	103/104	99.0% (94.8, 99.8)
Sensitivity Smear Neg	168/229	73.4% (67.3, 78.7)	32/44	72.7% (58.2, 83.7)
Overall Sensitivity	495/557[a]	88.9% (86.0, 91.2)	135/148	91.2% (85.6, 94.8)
Overall Specificity	937/986	95.0% (93.5, 96.2)	285/294	96.9% (94.3, 98.4)

[a] Smear results were not available for 4 culture positive specimens.

15.5 Xpert MTB/RIF Ultra Performance vs. Drug Susceptibility Testing for RIF

MTB positive culture isolates were tested for drug susceptibility (DST) to rifampin using the agar proportion method with Middlebrook or Lowenstein-Jensen media, the Thermo Scientific Sensititre™ Mycobacterium tuberculosis MIC Plate or the BD BACTEC™MGIT™ 960 SIRE assay. The performance of Xpert MTB/RIF Ultra for detection of RIF-resistance associated mutations was determined relative to the DST results of the MTB culture isolates.

Results for the detection of RIF resistance associated mutations are reported by Xpert MTB/RIF Ultra only when the *rpo*B gene sequence of MTB-complex was detected by the device. The performance of RIF susceptibility/resistance are reported in the table below. Specimens with DST not done, **MTB NOT DETECTED** and **MTB DETECTED; RIF Resistance INDETERMINATE** were excluded from the analysis. Sixty-three (63) of 67 specimens with RIF indeterminate results were **MTB Trace DETECTED; RIF Resistance INDETERMINATE**.

Table 8. Xpert MTB/RIF Ultra Performance vs. DST

		Drug Susceptibility Test		
		RIF Resistant	**RIF Susceptible**	**Total**
Xpert MTB/ RIF Ultra	**MTB DETECTED; RIF Resistance DETECTED**	128	12[a]	140
	MTB DETECTED; RIF Resistance NOT DETECTED	5[b]	314	319
	Total	133	326	459
		Sensitivity: 96.2% (128/133), 95% CI: 91.5, 98.4 Specificity: 96.3% (314/326), 95% CI: 93.7, 97.9		

[a] Discrepant sequencing results: 11 of 12 RIF resistant, 1 of 12 not available.

[b] Discrepant sequencing results: 4 of 5 RIF susceptible, 1 of 5 not available.

15.6 Xpert MTB/RIF Ultra Performance vs. the Xpert MTB/RIF Assay

One thousand five hundred ninety-four (1594) specimens were tested by both Xpert MTB/RIF Ultra and Xpert MTB/RIF. The overall percent agreement between the assays was 96.5% [(1538/1594) 95% CI: 95.5, 97.3]. The positive percent agreement and the negative percent agreement were 99.2% [(491/495) 95% CI: 97.9, 99.7] and 95.3%[(1047/1099) 95% CI: 93.8, 96.4], respectively.

16 Analytical Performance Characteristics

16.1 Interfering Substances

A study was performed in artificial sputum matrix to assess the effects of potential interfering substances with Xpert MTB/RIF Ultra. A total of 32 potentially interfering substances were evaluated. 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, *Pneumocystis 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 the table below with active ingredients and concentrations tested shown. Positive and negative samples were included in this study. Positive samples were tested near at 3 times the analytical limit of detection using BCG cells 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).

No inhibitory effect was observed for any of the 32 potentially interfering substances tested.

Table 9. Interfering Substances

Substance	Description/Active Ingredient	Concentration Tested
Blood	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%	30% (v/v)
Nebulizing solutions	NaCl 5% (w/v)	5% (w/v)
Mucin	Mucin 5% (w/v)	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	75 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	50 µg/mL
Anti-tuberculosis drugs	Isoniazid 1 mg/mL	50 µg/mL
Oral expectorants	Guaifenesin (400mg/tablet)	5 mg/mL
Anti-tuberculosis drugs	Pyrazinamide 10 mg/mL	10 µg/mL
Nasal gel (Homeopathic)	Zicam gel	50% (w/v)
Nasal spray	Phenylephrine 0.5%	1% (v/v)
Anti-tuberculosis drugs	Rifampicin 1mg/mL	25 µg/mL
Allergy relief medicine (Homeopathic)	Tea tree oil (<5% Cineole, >35% Terpinen- 4-01)	0.5% (v/v)
Live intranasal influenza virus vaccine	Live influenza virus vaccine FluMist	5% (v/v)

Substance	Description/Active Ingredient	Concentration Tested
Pneumocystis jiroveci medication	Pentamidine	300 ng/mL (v/v)
Bronchodilator	Epinephrine (injectable formulation)	1 mg/mL
Anti-tuberculosis drugs	Amoxicillin	25 µL

16.2 Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the analytical sensitivity or Limit of Detection (LoD) of Xpert MTB/RIF Ultra using Mycobacterium *tuberculosis* strain H37Rv and Mycobacterium *bovis* BCG (Bacille Calmette-Guerin) diluted in human sputum and human sputum sediment. An MTB positive result is based on the detection of the IS1081/IS6110 targets.

Studies were also performed to determine the analytical sensitivity or Limit of Detection of Xpert MTB/RIF Ultra for detection of RIF resistance using a well characterized clinical Mycobacterium tuberculosis rifampin-resistant strain (TDR125) bearing a D516V mutation in the 81-base pair “core” region of the rpoB gene 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 at least 20 for two strains were evaluated at five to eight concentrations over 3 days and the LoD was determined using probit analysis. The claimed LoD are summarized in the table below.

Table 10. Probit Analysis Data and Claimed LoD in CFU/mL

Mycobacteria species	Specimen Type	Claimed LoD
M. bovis (BCG)	Sputum	30
	Sputum Sediment	21
M. tuberculosis (H37Rv)	Sputum	12
	Sputum Sediment	25

Table 11. Probit Analysis Data and Claimed RIF Resistance LoD in CFU/mL

Mycobacteria species	Specimen Type	Claimed LoD
M. tuberculosis (TDR125)	Sputum	1093
	Sputum Sediment	4000

16.3 Analytical Specificity (Exclusivity)

The analytical specificity of Xpert MTB/RIF Ultra was evaluated by testing a panel of 61 organisms consisting of 21 bacteria, 2 fungi, 8 viruses and 30 non-tuberculosis Mycobacterium (NTMs) representing common respiratory pathogens or those potentially encountered in the respiratory tract and/or oropharyngeal flora in Xpert MTB/RIF Ultra.

All bacterial and fungi were tested at $\geq 10^6$ CFU/mL. All viruses were tested at $\geq 1x10^5$ $TCID_{50}$/mL. DNA or RNA was tested for 1 bacterial strain at concentrations of $\geq 10^6$ copies/mL, as whole organisms were not available or could not be accessed due to biosafety restrictions.

The organisms and nucleic acids used in this study originated from the American Type Culture Collection (ATCC, Manassas, Virginia, USA) and Hardy Diagnostics (Santa Maria, CA, USA). The bacterial testing controls were Positive Control: BCG (*Mycobacterium bovis* vaccine strain - Bacille Calmette-Guerin) and Negative Control: TET buffer. The NTM testing controls were *Mycobacterium tuberculosis* (H37Rv) (Positive control) and TET buffer (Negative control). The virus testing controls were *Mycobacterium bovis* (Positive control) and TET buffer (Negative control).

The 61 organisms tested are presented in the table below. No cross-reactivity was observed when testing the 61 organisms at a minimum concentration of $>10^6$ CFU/mL or $>10^6$ copies/mL for genomic DNA for bacteria and fungi, $\geq 10^5$ $TCID_{50}$/mL for viruses and $\geq 10^6$ CFU/mL NTMs.

Table 12. Analytical Specificity Determination for Xpert MTB/RIF Ultra

Bacteria	NTM	Viruses	Fungi
Acinetobacter baumannii	*Mycobacterium avium subsp. avium*	*Coronavirus*	*Aspergillus fumigatus*
Chlamydophila pneumoniae[a]	*Mycobacterium celatum*	*Human metapneumovirus* (hMPV) 16 Type A1	*Candida albicans*
Citrobacter freundii	*Mycobacterium chelonae*	*Parainfluenza Virus* Type 1	
Corynebacterium xerosis	*Mycobacterium gordonae*	*Parainfluenza Virus* Type 2	
Enterobacter cloacae	*Mycobacterium haemophilum*	*Parainfluenza Virus* Type 3	
Escherichia coli	*Mycobacterium abscessus*	*Respiratory Syncytial Virus* Type A	
Haemophilus influenzae[a]	*Mycobacterium asiaticum*	*Respiratory Syncytial Virus* Type B	
Klebsiella pneumoniae	*Mycobacterium flavescens*	*Rhinovirus*	
Moraxella catarrhalis	*Mycobacterium fortuitum subsp. fortuitum*		
Neisseria meningitidis	*Mycobacterium gastri*		
Neisseria mucosa	*Mycobacterium genavense*		
Nocardia asteroides	*Mycobacterium intracellulare*		
Pseudomonas aeruginosa	*Mycobacterium kansasii*		
Staphylococcus aureus	*Mycobacterium malmoense*		
Staphylococcus epidermidis	*Mycobacterium marinum*		
Stenotrophomonas maltophilia	*Mycobacterium scrofulaceum*		
Streptococcus agalactiae	*Mycobacterium simiae*		
Streptococcus mitis	*Mycobacterium szulgai*		
Streptococcus mutans	*Mycobacterium thermoresistibile*		
Streptococcus pneumoniae	*Mycobacterium triviale*		
Streptococcus pyogenes	*Mycobacterium vaccae*		
	Mycobacterium xenopi		
	Mycobacterium smegmatis		

Bacteria	NTM	Viruses	Fungi
	Mycobacterium interjectum		
	Mycobacterium peregrinum		
	Mycobacterium mucogenicum		
	Mycobacterium goodii		
	Mycobacterium shimodei		
	Mycobacterium phlei		
	Mycobacterium terrae		

16.4 Species/Strains Tested for Specificity

The microorganisms in the table below including Gram-negative bacteria, Gram-positive bacteria, fungal organisms, viruses and yeast were tested for false positivity in Xpert MTB/RIF Ultra. The replicates of each isolate were spiked onto buffer and tested at a concentration of $\geq 10^7$ CFU/mL (bacteria and fungal strains) or $\geq 10^6$ copies/mL (genomic DNA for bacteria and fungi) and $\geq 10^5$ $TCID_{50}$ /mL (virus strains).

Table 13. Species and Strains

Acinetobacter baumannii	***Klebsiella pneumoniae***	***Respiratory Syncytial Virus Type B***
Aspergillus fumigatus	***Moraxella catarrhalis***	***Rhinovirus***
Candida albicans	***Neisseria meningitidis***	***Staphylococcus aureus***
Chlamydophila pneumoniae	***Neisseria mucosa***	***Staphylococcus epidermidis***
Citrobacter freundii	***Nocardia asteroides***	***Stenotrophomonas maltophilia***
Corynebacterium xerosis	***Parainfluenza Virus Type 1***	***Streptococcus agalactiae***
Coronavirus	***Parainfluenza Virus Type 2***	***Streptococcus mitis***
Enterobacter cloacae	***Parainfluenza Virus Type 3***	***Streptococcus mutans***
Escherichia coli	***Pseudomonas aeruginosa***	***Streptococcus pneumoniae***
Haemophilus influenzae	***Respiratory Syncytial Virus Type A***	***Streptococcus pyogenes***
Human metapneumovirus (hMPV) 16 Type A1		

Under the conditions of the study, all of the microorganisms tested were reported as **MTB NOT DETECTED**. Positive and negative controls were included in the study. The specificity was 100%.

16.5 Analytical Reactivity/Inclusivity

Forty-one MTB-complex strains consisting of 20 rifampin-susceptible strains with a wild-type *rpoB* core region and 21 rifampin-resistant strains with single nucleotide polymorphisms (SNPs) in the rpoB core region were tested in quadruplet using Xpert MTB/RIF Ultra. DNA samples from a total of 41 MTB strains were tested on the GeneXpert using an Xpert MTB/RIF Ultra protocol modified for DNA testing. The final reaction components and PCR cycling conditions were unchanged from the protocol designed for patient sample testing. The mutant strain thermolysates (heat treated cells) and nucleic acids (DNA) were obtained from Belgium and Rutgers New Jersey and originated from the Centers for Disease Control and Prevention (CDC), American Type Culture Collection (ATCC), and the United Nations Children's Fund/UNDP/

World Bank/WHO Special Program for Research and Training in Tropical Diseases collection. The assay correctly identified all 20 wild-type strains and correctly identified rifampin resistance in all 21 strains resistant to rifampin with mutations in the *rpoB* core region.

16.6 Analytical Inactivation of Mycobacteria in Sputum Samples

The disinfection capability of the Xpert MTB/RIF Ultra sample reagent was determined using a standardized quantitative tuberculocidal culture method.[12] 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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19 Technical Assistance

Before Contacting Us

Collect the following information before contacting Cepheid Technical Support:

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

United States Technical Support

Telephone: + 1 888 838 3222
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Telephone: + 33 563 825 319
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Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/support/contact-us.

20 Table of Symbols

Symbol	Meaning
	Catalog number
	CE marking – European Conformity
	*In vitro* diagnostic medical device
	Authorized Representative in the European Community
	Do not reuse
	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Manufacture date
	Contains sufficient for *n* tests
	Control
	Expiration date
	Temperature limitation
	Biological risks
	Flammable liquids
	Skin corrosion
	Reproductive and organ toxicity

Cepheid India Private Limited
Plot No-37/2, Survey No 198, Block Nos-55,56,57,60 and 61
Hitech Defence and Aerospace Park Industrial area
Mahadevakodigehalli Village, Hobli Jala Taluka
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EC REP

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CE IVD

21 Revision History

Description of Changes: 302-5776, Rev. C to Rev. D

Purpose: Update per core team revisions

Section	Description of Change
Trademark, Patents and Copyright Statements	Added license statement.
Numerous	Updated references to the product to match the officially registered product name ("Xpert MTB/RIF Ultra").
Materials Provided	Note about BSA edited to include name and description: "BSA protein stabilizer".
Starting a Test	Note about not turning off instrument during a test added.