WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: Xpert MTB/RIF Ultra
WHO reference number: PQDx 10295-070-00

Xpert MTB/RIF Ultra with product codes GXMTB-ULTRA-HB-10 and GXMTB-ULTRA-HB-50, manufactured by Cepheid AB, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 29 November 2024. The product is the Rest-of-World version, leveraging the Class C IVD -SINGAPORE MEDICAL DEVICE REGISTER (SMDR) regulatory version of the product codes GXMTB/RIF-ULTRA-10 and GXMTB/RIF-ULTRA-50 to qualify for abridged assessment because there are no substantial differences observed between the two regulatory versions in terms of design, manufacturing, and performance.

Summary of WHO prequalification assessment for Xpert MTB/RIF Ultra

	Date	Outcome
Prequalification listing	29 November 2024	listed
Dossier assessment	25 June 2024	MR
Desk assessments of the quality management system	10-13 September 2024	MR
Product performance evaluation	28 October 2024	MR

MR: Meets Requirements

Intended use

According to the intended use claim from Cepheid AB, "*The Xpert MTB/RIF Ultra test, performed on the GeneXpert Systems is a semi-quantitative, nested realtime 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, the Xpert MTB/RIF Ultra test can also detect rifampin-resistant associated mutations of the rpoB gene.*

The Xpert MTB/RIF Ultra test is intended for use with specimens from adult 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.

The Xpert MTB/RIF Ultra test is intended to be used by laboratory professionals, trained health care professionals or other healthcare workers receiving appropriate training on the use of the test. This test may be used in laboratory or in near-patient testing environments."

Assay description

According to the claim of assay description from *Cepheid AB, "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.*

The Xpert MTB/RIF Ultra test 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 the Xpert MTB/RIF Ultra test 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."

Test kit contents:

Component	**10 tests/kit (product code GXMTB-ULTRA-HB-10)**	**50 tests/kit (product code GXMTB-ULTRA-HB-50)**
Xpert MTB/RIF Ultra Cartridges with integrated Reaction Tubes	10	50
Sample Reagent Bottles	10	50
Disposable Transfer Pipettes	12	60
CD	1	1

Items required but not provided:

- GeneXpert Dx System or GeneXpert Infinity System (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, and operator manual
  - For GeneXpert Dx System: Software version 4.7b or higher
  - For GeneXpert Infinity System: Software version 6.4b or higher
- 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.

Storage:
The test kit should be stored at 2-28 °C.

Shelf-life upon manufacture:
18 months.

Warnings/limitations:
Please refer to the current version of the manufacturer's instructions for use attached to this public report.

Prioritization for prequalification
Based on the established eligibility criteria, the Xpert MTB/RIF Ultra was given priority for the WHO prequalification assessment.

Product dossier assessment

Cepheid AB submitted a product dossier for the Xpert MTB/RIF Ultra as per the "Instructions for compilation of a product dossier" (PQDx_018). The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO. The manufacturer's responses to the nonconformities found during dossier screening and assessment findings were accepted on 25 June 2024.

Based on the product dossier screening and assessment findings, the product dossier for the Xpert MTB/RIF Ultra meets WHO prequalification requirements.

Manufacturing sites assessments

At the time of considering the product application for Prequalification, the Manufacturer of the product had a well-established quality management system and manufacturing practices in place that would support the manufacture of a product of consistent quality. Routine assessments of the Manufacturing sites will be conducted with copies of the WHO Public Inspection Reports (WHOPIR) published on the WHO Prequalification web page as per Resolution WHA57.14 of the World Health Assembly. Note that a WHOPIR reflects the information on the most current assessment performed at a manufacturing site for in vitro diagnostic products and summarises the assessment findings.

https://extranet.who.int/pqweb/vitro-diagnostics/who-public-inspection-reports

All published WHOPIRs are with the agreement of the manufacturer.

Based on the sites assessments and corrective action plan review, the quality management system for Xpert MTB/RIF Ultra meets WHO prequalification requirements.

Product performance evaluation

The Xpert MTB/RIF Ultra was evaluated by the Centre for Tuberculosis, National Institute for Communicable Diseases on behalf of WHO in the third quarter of 2024, according to protocol IVD/PR/4/P23, version 2.1, March 2024.

In accordance with the WHO procedure for prequalification assessment and given the fact that Xpert MTB/RIF Ultra is to be used as the comparator assay in the WHO evaluation protocol IVD/PR/4/P23, the clinical evaluation was waived, and the product was evaluated according to the analytical and operational characteristics part of the evaluation protocol only.

Analytical performance evaluation

Analytical performance characteristics	
Limit of detection (LoD) using the WHO International Standard for M. tuberculosis (H37Rv) DNA for NAT-based assays (NIBSC code: 20/152)	The LoD for *M. tuberculosis* detection was estimated at 143.4 IU/mL (95% CI: 79.29 – 259.3) for *M. tuberculosis* detection. The LoD for drug resistance detection was estimated at 582.6 IU/mL (95% CI: 310 - 1093).
Reproducibility	The hit rate for detection of *M. tuberculosis* (sensitive strain) at 10^2 CFU/mL was 100%. The hit rate for drug resistance detection of *M. tuberculosis* (resistant strain) at approx. 10^3 CFU/mL was 100%.
Inclusivity, exclusivity	The following mycobacteria (MTBC) were detected: *M. bovis*, *M. africanum.* The following non-tuberculous mycobacteria (NTM) were not detected: *M. avium*, *M. kansasii*, *M. intracellulare*, in agreement with manufacturer's claim.
Resistance detection	The following drug-resistance mutations were detected: *rpoB*_S450L, *rpoB*_D435V, *rpoB*_H445Y, *rpoB*_H445D, *rpoB*_D435Y, *rpoB*_S450W, *rpoB*_L452P, *rpoB*_H445L, *rpoB*_S450F, *rpoB*_L430P and *rpoB*_H445R, in agreement with manufacturer's claim. The following drug-resistance mutations were not detected: *rpoB*_V170F and *rpoB*_I491F as these regions are not targeted by the assay. One of the three *rpoB*_S450L replicates was classified as RIF resistance INDETERMINATE.
Cross-contamination / carry-over	No carry-over was observed when high positive and negative specimens were tested alternatively.

Operational characteristics and ease of use

This assay requires laboratory equipment and can be performed in laboratories with limited facilities or in non-laboratory settings. The instrument requires a stable source of electricity for processing. Furthermore, training and implementation of good laboratory practice is essential to obtaining accurate results. Adequate technical support from manufacturer or representative is necessary.

The assay was found easy to use by the operators performing the evaluation.

Key operational characteristics	
Time to result for one test	110 minutes
Operator hands-on time for one test	20 minutes
Level of automation	Full automation of specimen extraction and amplification. Specimen pre-processing step is performed by operator.
Quality controls	Each cartridge includes two internal controls (sample processing control (SPC) and probe check control (PCC)). External QC are not provided by the manufacturer and not required per IFU.
Operating temperature	15 – 30°C
Result display and connectivity	Results are displayed on the connected computer. They may be printed using a standard printer. The results can be exported to the laboratory information system and other health information systems.
Power sources	Main power The use of a UPS is recommended, as stable electricity is required.
Biosafety *(outside of infectious specimen handling)*	Operators reported no biosafety considerations. However, chemical hazards are listed in the IFU for reference. Sample reagent contains isopropyl alcohol and sodium hydroxide.
Waste	Cartridge = 42.1 g (includes ~5mL of liquid waste) SR buffer = 6.5 g - 12.5 g (varies based on the volume of SR buffer used with a minimum of 0 mL to a maximum of 6 mL liquid waste). Waste disposal does not require specific measures in addition to usual biohazard waste disposal procedures as the waste is contained within the cartridge.
Calibration	Xpert Check software and kits are provided by the manufacturer and should be purchased separately for annual calibration of the GeneXpert modules.
Maintenance	Daily maintenance is required.
Other specific requirements	The system must be placed away from heat and air conditioning ducts. The instrument must not be placed directly under an air vent or in direct sunlight.

Based on these results, the performance evaluation for Xpert MTB/RIF Ultra meets the WHO prequalification requirements.

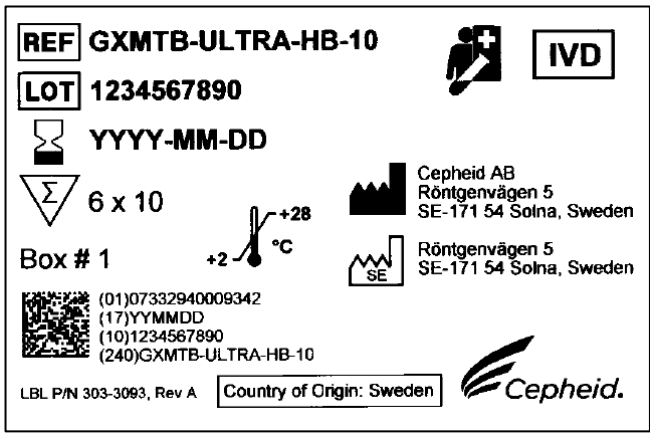
Labelling

1. **Labels**
2. **Instructions for use**

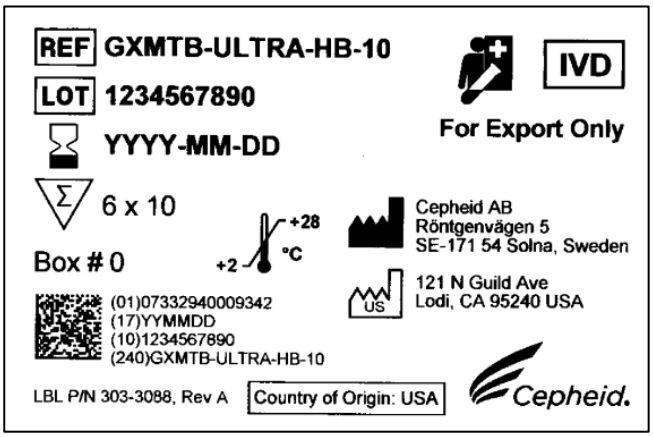
1. Labels

1.1 Shipping Box Label for GXMTB-ULTRA-HB-10

Solna Physical Manufacturer

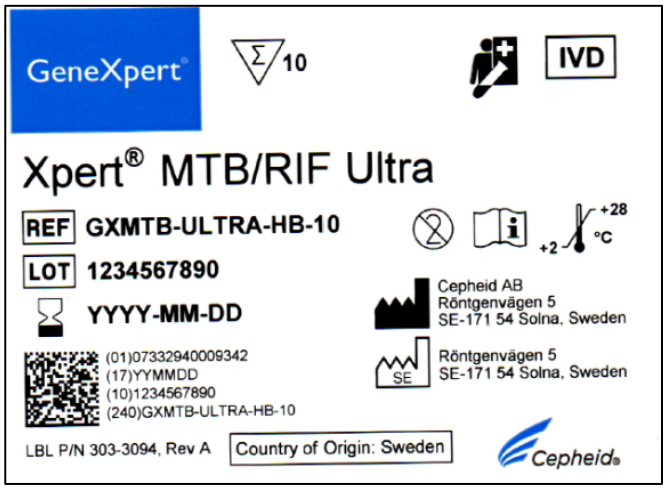


Lodi Physical Manufacturer

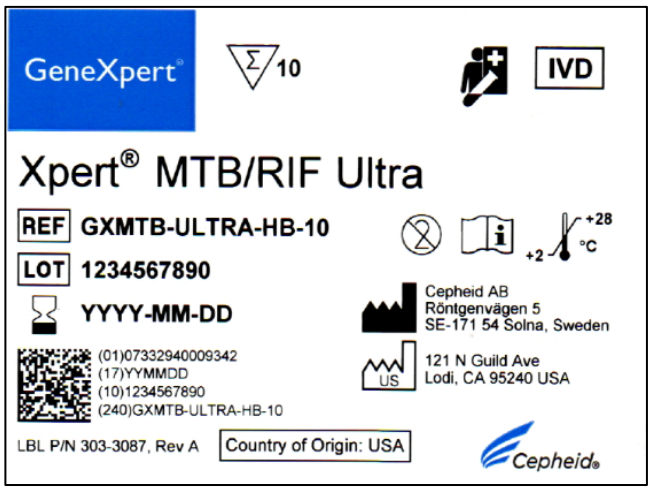


1.2 Kit Box – Top Label for GXMTB-ULTRA-HB-10

Solna Physical Manufacturer

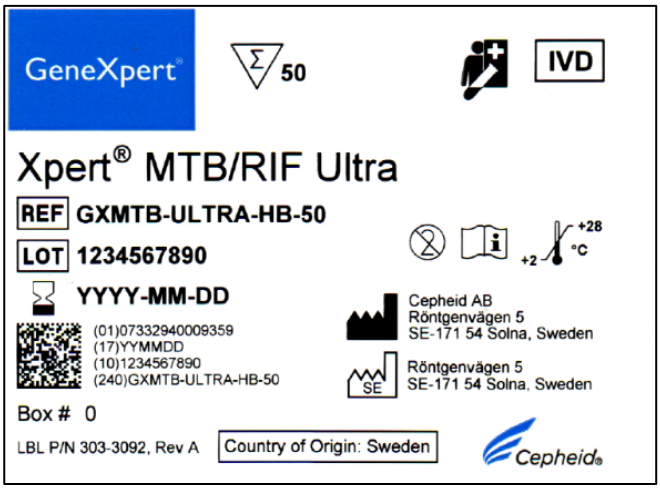


Lodi Physical Manufacturer

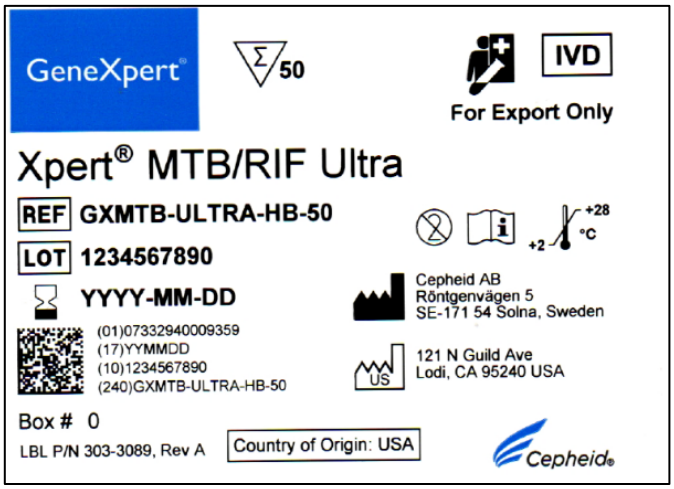


1.3 Kit Box – Label for GXMTB-ULTRA-HB-50

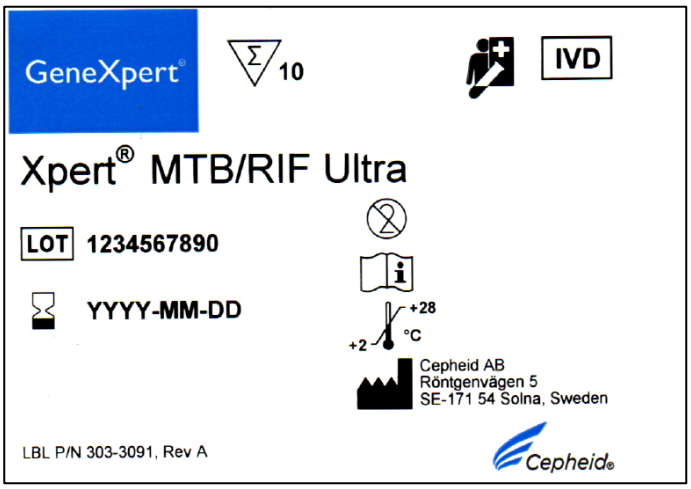
Solna Physical Manufacturer



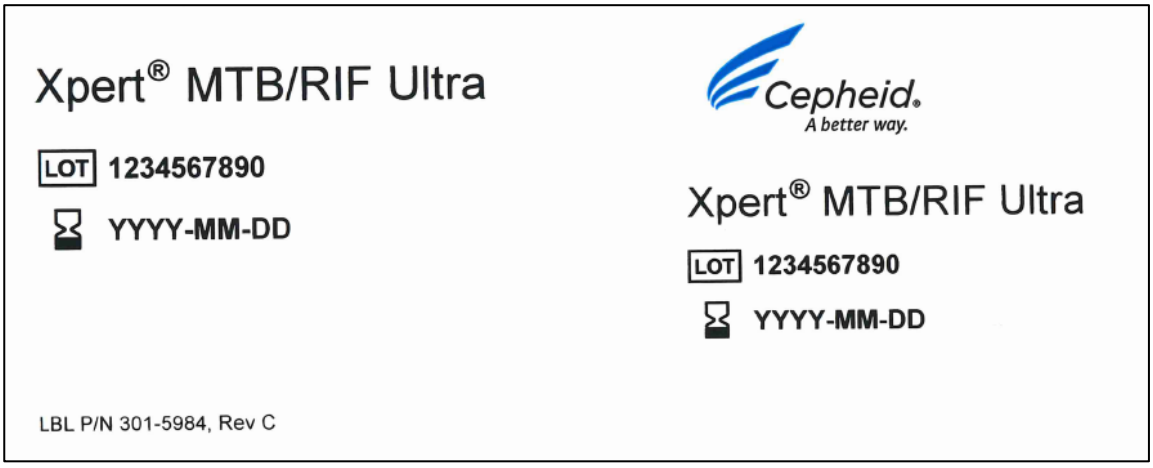
Lodi Physical Manufacturer



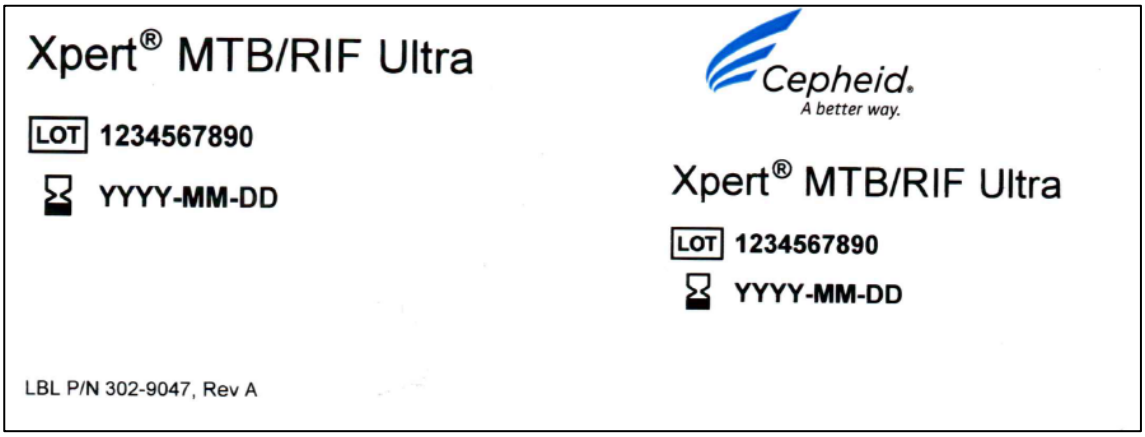
1.4 Brick Label for GXMTB-ULTRA-HB-50



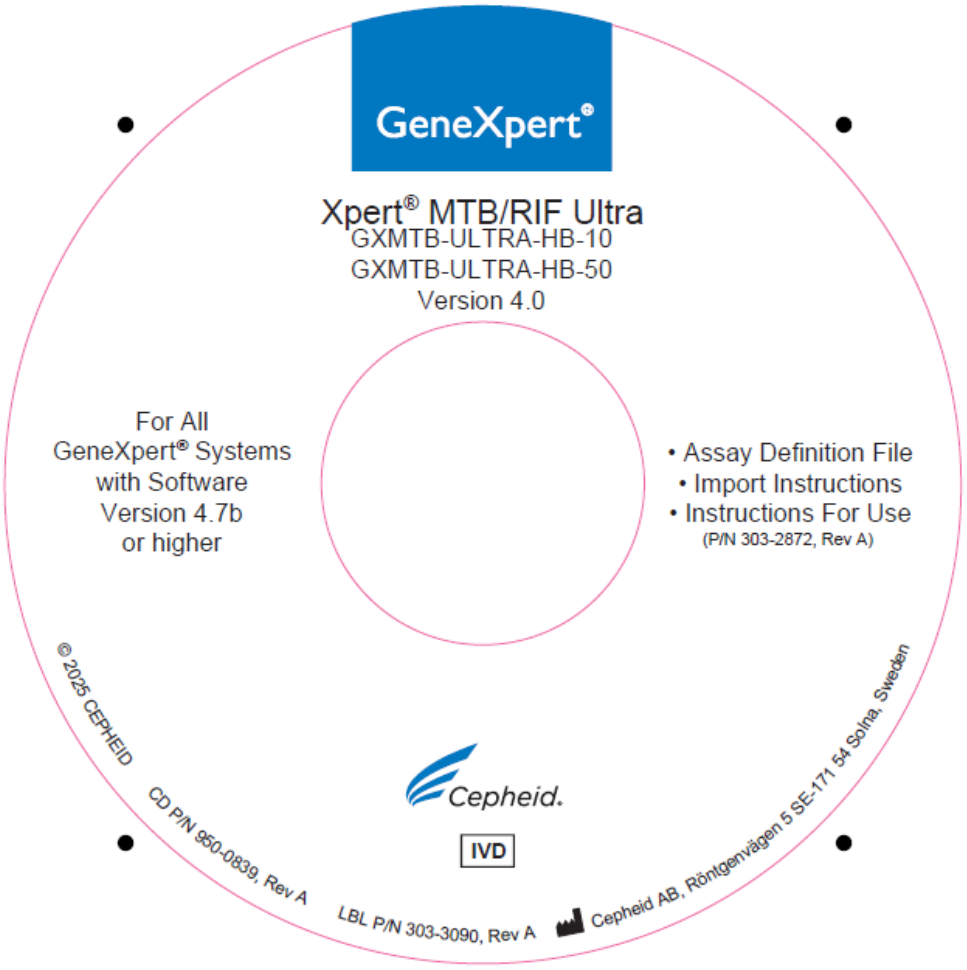
1.5 Kit Box – Side Label for GXMTB-ULTRA-HB-10 and GXMTB-ULTRA-HB-50 Solna Physical Manufacturer



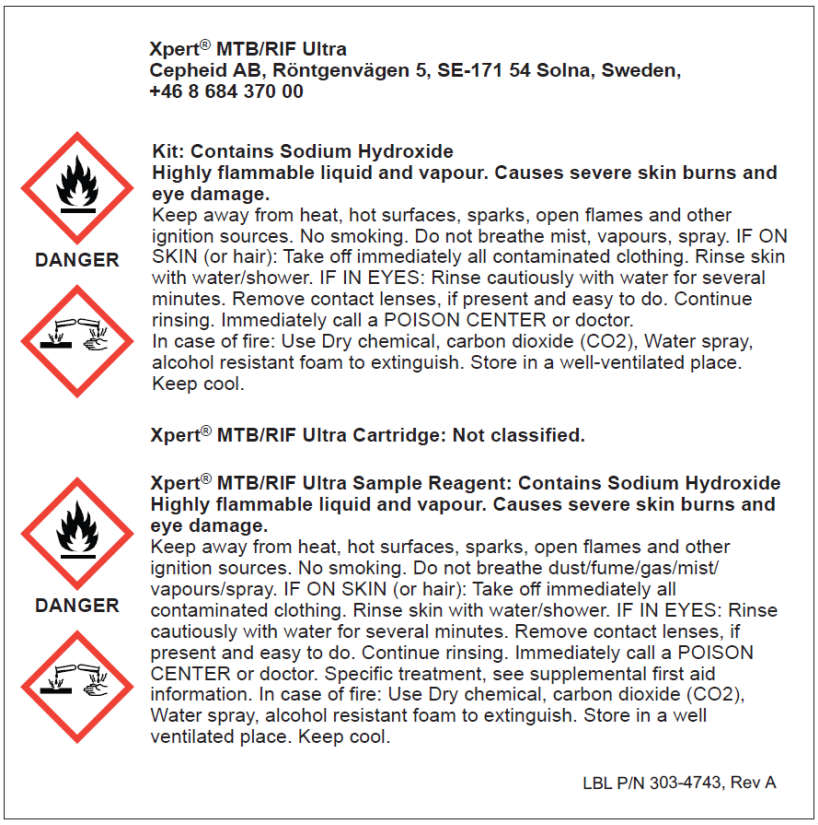
1.6 Kit Box – Side label for GXMTB-ULTRA-HB-10 and GXMTB-ULTRA-HB-50 Lodi Physical Manufacturer



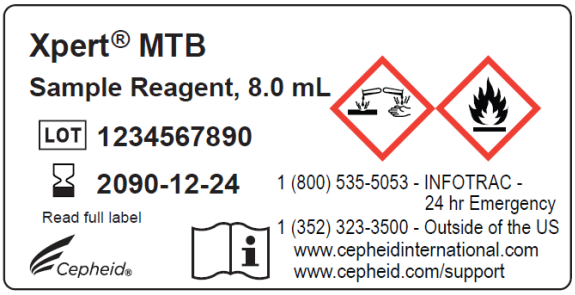
1.7 CD Label


GeneXpert®
Xpert® MTB/RIF Ultra
GXMTB-ULTRA-HB-10
GXMTB-ULTRA-HB-50
Version 4.0
For All
GeneXpert® Systems
with Software
Version 4.7b
or higher
• Assay Definition File
• Import Instructions
• Instructions For Use
(P/N 303-2872, Rev A)
© 2025 CEPHEID
CD P/N 950-0839, Rev A
LBL P/N 303-3090, Rev A
Cepheid AB, Röntgenvägen 5 SE-171 54 Solna, Sweden
Cepheid.
IVD

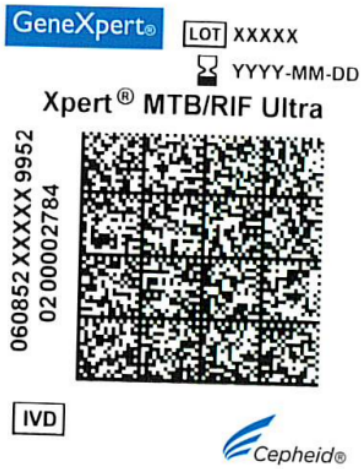
1.8 Hazard Label



1.9 Sample Reagent Label



1.10. Cartridge Label



2. Instructions for use[1]

[1] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

GeneXpert
Powered By CEPHEID INNOVATION

Xpert® MTB/RIF Ultra

REF **GXMTB-ULTRA-HB-10**

REF **GXMTB-ULTRA-HB-50**

Instructions for Use

IVD

Cepheid®

In Vitro Diagnostic Medical Device

303-2872, Rev. A
2025-02

Trademark, Patents and Copyright Statements

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

Xpert® MTB/RIF Ultra

IVD

1 Proprietary Name

Xpert® MTB/RIF Ultra

2 Common or Usual Name

Xpert MTB/RIF Ultra

3 Intended Purpose

3.1 Intended Use

The Xpert MTB/RIF Ultra test, performed on the GeneXpert® 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, the Xpert MTB/RIF Ultra test can also detect rifampin-resistance associated mutations of the *rpoB* gene.

The Xpert MTB/RIF Ultra test is intended for use with specimens from adult 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.

3.2 Intended User/Environment

The Xpert MTB/RIF Ultra test is intended to be used by laboratory professionals, trained health care professionals or other healthcare workers receiving appropriate training on the use of the test. This test may be used in laboratory or in near-patient testing environments.

4 Summary and Explanation

Globally, about 2 billion people are infected with *Mycobacterium tuberculosis* (MTB). Every year almost 10 million people develop active disease, and 1.3 million people died of the illness.[1] 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.[2] 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.[3,4,5] 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 the Xpert MTB/RIF Ultra, this can be accomplished in unprocessed sputum samples and in prepared sediments in less than 80 minutes. The detection of MTB and RIF resistance allows the physician to make critical patient management decisions regarding therapy during a single medical encounter. A number of studies have been published on the use of Xpert MTB/RIF Ultra in near patient settings. These studies demonstrate that clinical performance in a near patient testing environment compares favorably with the clinical study results obtained in a laboratory setting.[6,7]

5 Principle of the Procedure

The GeneXpert 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*.

The Xpert MTB/RIF Ultra test 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 the Xpert MTB/RIF Ultra test 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

Users must familiarize themselves with the materials provided before they begin testing.

6.1 Materials Provided

The Xpert MTB/RIF Ultra test 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 (contains sodium hydroxide and isopropyl alcohol)	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 (IFU)

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 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 the Xpert MTB/RIF Ultra test cartridges at 2-28 °C.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use reagents or cartridges that have passed the expiration date.

7 Materials Required but Not Provided

- GeneXpert Dx System or GeneXpert Infinity System (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, and operator manual
  - For GeneXpert Dx System: Software version 4.7b or higher
  - For GeneXpert Infinity System: Software version 6.4b or higher
- 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 Global Laboratory Initiative (GLI) handbook of StopTB partnership[8] and the Clinical and Laboratory Standards Institute.[9]
- Wear protective disposable gloves, laboratory coats and eye protection when handling specimens 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 the treated sample has been added. 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[10]

Kit

Contains Sodium Hydroxide

Signal Word: Danger

Hazard Statements

- Highly flammable liquid and vapor
- Causes severe skin burns and eye damage

Precautionary Statements

- Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking
- Do not breathe mist, vapours, spray.
- IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
- IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- Immediately call a POISON CENTER or doctor.
- In case of fire: Use Dry chemical, carbon dioxide (CO2), Water spray, alcohol resistant foam to extinguish.
- Store in a well-ventilated place. Keep cool.

Xpert MTB/RIF Ultra Cartridge

Not Classified

Xpert MTB/RIF Ultra Sample Reagent

Contains Sodium Hydroxide

Signal Word: Danger

Hazard Statements

- Highly flammable liquid and vapor
- Causes severe skin burns and eye damage

Precautionary Statements

- Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking
- Do not breathe mist, vapours, spray.
- IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
- IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- Immediately call a POISON CENTER or doctor.

- Specific treatment, see supplemental first aid information.
- In case of fire: Use Dry chemical, carbon dioxide (CO2), Water spray, alcohol resistant foam to extinguish.
- Store in a well-ventilated place. Keep cool.

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 to 8 °C for up to seven days.

Unprocessed sputum: Transport and store sputum at 35 °C for up to 3 days, and 2 to 8 °C up to 10 days.

10 Assay Procedure

10.1 Procedure for Unprocessed Sputum

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

1. Bring the cartridge to room temperature (20 to 28 °C). Label each Xpert MTB/RIF Ultra test 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. See Figure 2.

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). See Figure 3 and Figure 4.

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)

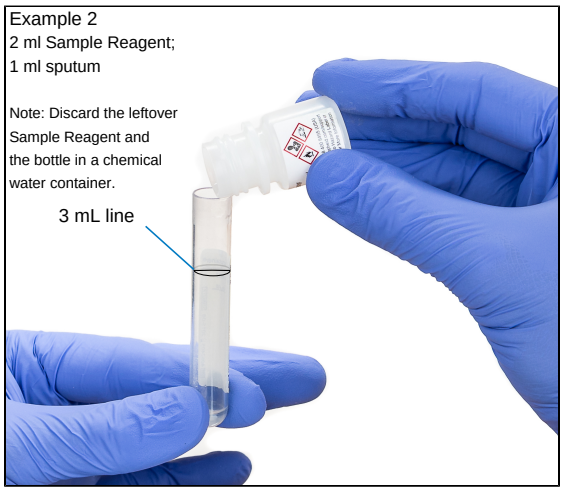


Figure 4. Example of 2:1 Dilution (2 mL SR:1 mL Sputum)

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 (20 to 28°C).
6. Shake the specimen vigorously 10 to 20 times or vortex for at least 10 seconds. Incubate the sample at room temperature (20 to 28°C) for an additional 5 minutes.

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

10.2 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 the Xpert MTB/RIF Ultra test. After resuspension, keep at least 0.5 mL of the resuspended sediment for the Xpert MTB/RIF Ultra test. For all volumes less than 0.7 mL perform steps 1 to 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 (20 to 28°C). Label each Xpert MTB/RIF Ultra test cartridge with the Sample ID.

Note Write on the side of the cartridge (Figure 5) 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 5. Writing on the Cartridge with a Permanent Marking Pen

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 the Xpert MTB/RIF Ultra test using a transfer pipette.

Note Store re-suspended sediments at 2 to 8°C if they are not immediately processed. Do not run the Xpert MTB/RIF Ultra test 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 (20 to 28°C), and then shake the specimen vigorously 10 to 20 times or vortex for at least 10 seconds.
7. Incubate the sample at room temperature (20 to 28°C) for an additional 5 minutes.

10.3 Preparing the Cartridge

Note If using a GeneXpert Dx instrument, start the test as soon as possible and within four hours of adding the Sample Reagent-treated sample to the cartridge. Once the sample is added to the cartridge, the cartridge should remain at room temperature prior to starting the test and loaded into the instrument to start a run within four hours. If using a GeneXpert Infinity System, 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 on-board 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 6. Do not process the sample further if there is insufficient volume.

Figure 6. 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 7.

Figure 7. Dispensing Decontaminated Liquefied Sample into the Sample Chamber of the Cartridge

4. Close the cartridge lid firmly.

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.

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. 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.

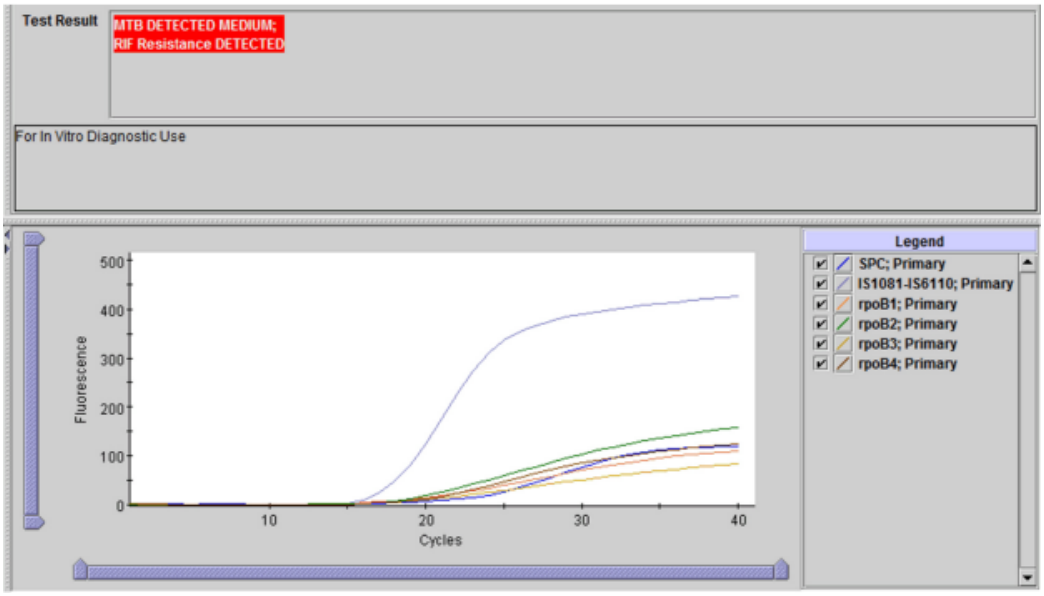
The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria. The test result will be "Invalid" if the SPC is not detected in a negative test.

Probe Check Control (PCC)

Before the start of the PCR reaction, the Xpert MTB/RIF Ultra test 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 system generates the results from measured fluorescent signals and embedded calculation algorithms. Real-time PCR amplification of MTB specific targets are used to call for detection. Melt profiles generated after real-time PCR are used to determine RIF resistance or susceptibility. The results can be seen in the **View Results** window. See Figure 8, Figure 9 and Figure 10 for specific examples, and see Table 3 for a list of all possible results.



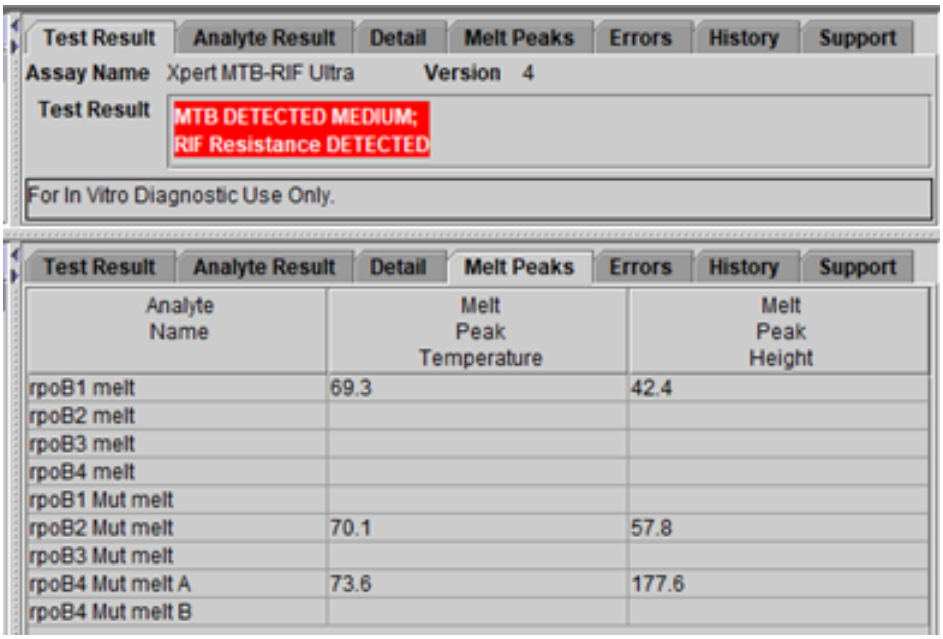
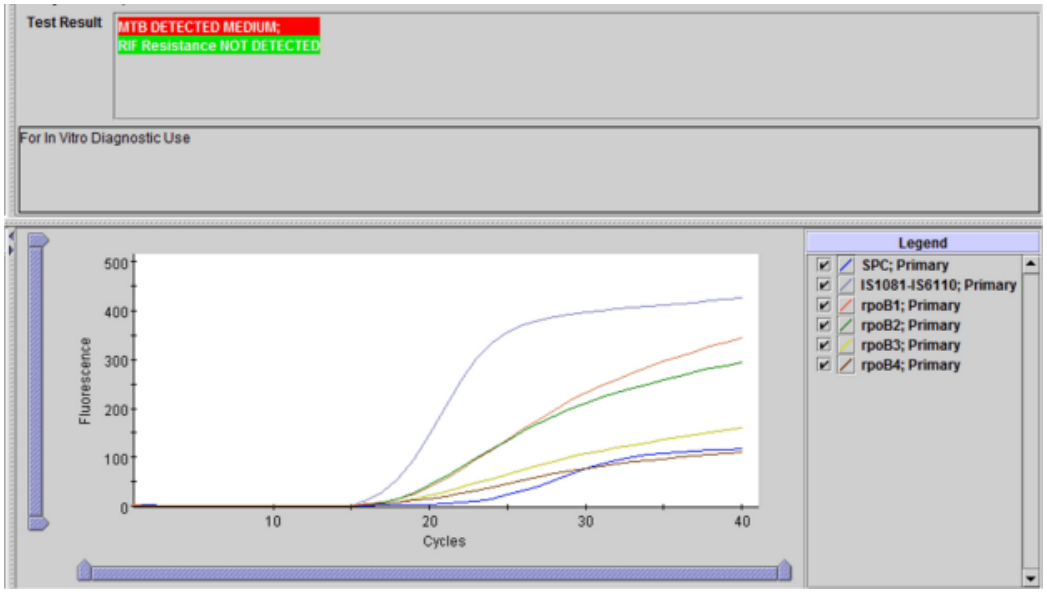


Figure 8. MTB DETECTED MEDIUM; RIF Resistance DETECTED (GeneXpert Dx Detailed User View)



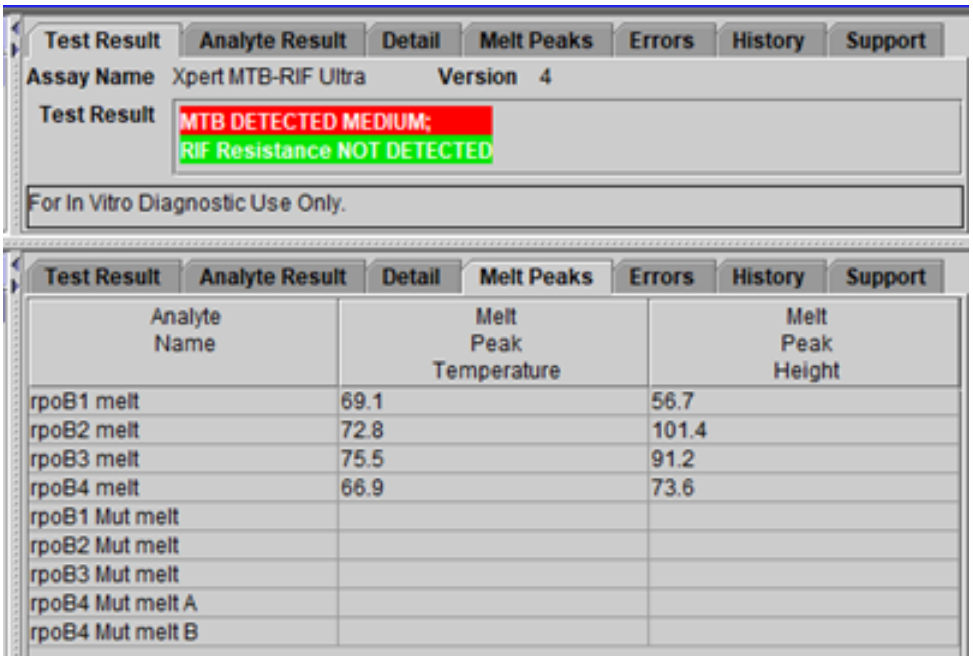
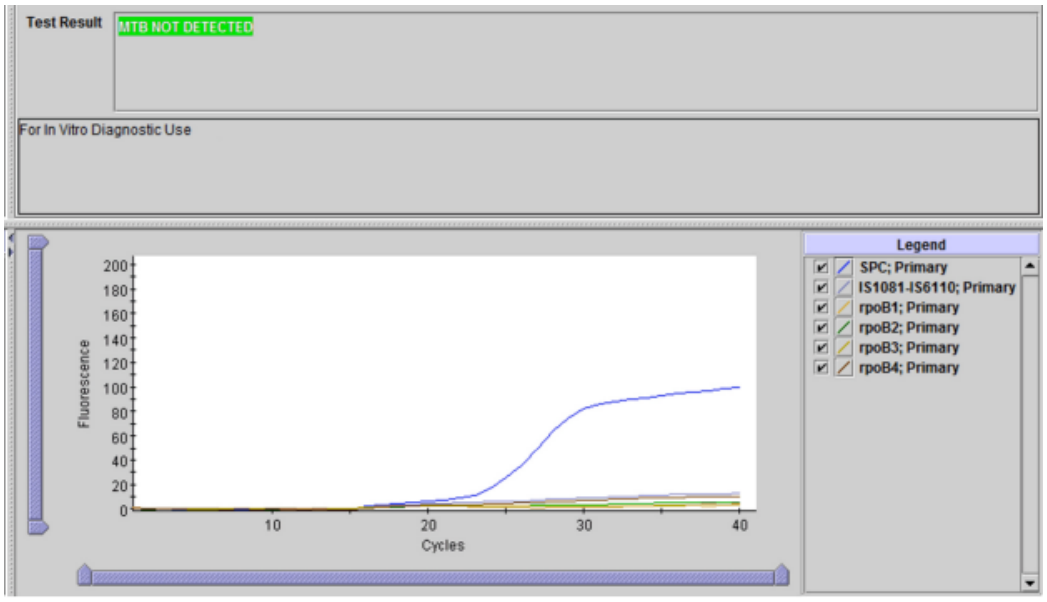


Figure 9. MTB DETECTED MEDIUM; RIF Resistance NOT DETECTED (GeneXpert Dx Detailed User View)



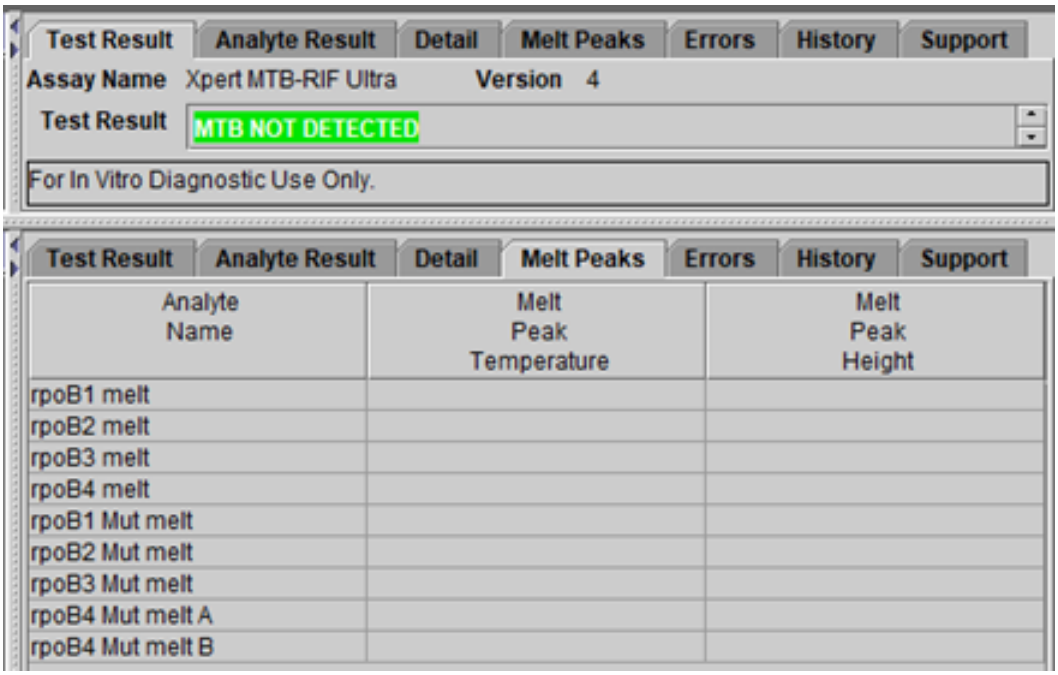


Figure 10. MTB NOT DETECTED (GeneXpert Dx Detailed User View)

Table 2. Xpert MTB/RIF Ultra Test Results and Interpretation

Result	Interpretation
MTB DETECTED HIGH; RIF Resistance DETECTED	The MTB target is present within the sample: ● The valid melt signature of the *rpoB* gene indicates mutations are present and corresponds to RIF resistance. ● 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: ● The valid melt signature of the *rpoB* gene does not indicate mutations and corresponds to RIF susceptibility. ● 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 missing or 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 of *rpoB* amplification. ● 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 fresh sputum or reconstituted sediment is left over, always use new SR to decontaminate and liquefy the sputum or the sediment before running the assay. See Procedure for Unprocessed Sputum or Section 10.2.

If sufficient SR-treated sample is left over and within 4 hours of the initial addition of SR to the sample, the leftover sample can be used to prepare and process a new cartridge. When retesting, always use a new cartridge and start the test immediately. See Section 10.3.

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.

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

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

Specimens that have both MTB-complex DNA and rifampin-resistance associated mutations of the *rpoB* gene detected by the Xpert MTB/RIF Ultra test should be considered for additional drug susceptibility testing.

The performance of the Xpert MTB/RIF Ultra test is dependent on operator proficiency and adherence to assay procedures. Assay procedural errors may cause false positive or false negative results. All device operators should have appropriate device training.

All specimens should have albuterol levels of no greater than 100 µg/mL.

15 Clinical Performance

15.1 Clinical Study Design

The performance characteristics of the Xpert MTB/RIF Ultra test were 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 the Xpert MTB/RIF Ultra test 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 subjects who were ≥18 years old, 59% male (n=1175), 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 countries outside the US (Belarus, Brazil, China, Georgia, Germany, India, Italy, South Africa, Kenya, Peru, Vietnam and Uganda).

15.2 Xpert MTB/RIF Ultra Test 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 the Xpert MTB/RIF Ultra test 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 test 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 testings; 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 test 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 the Xpert MTB/RIF Ultra test 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 the Xpert MTB/RIF Ultra test regardless of AFB smear was 95.5% (1222/1280), 95% CI: 94.2, 96.5. See tables below.

Table 4. Xpert MTB/RIF Ultra Test 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 the Xpert MTB/RIF Ultra test 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 Test 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 Test Performance vs Culture by Smear Type

The performance of the Xpert MTB/RIF Ultra test 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 Test 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 Test Performance vs. Culture by Specimen Type

The performance of the Xpert MTB/RIF Ultra test 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 Test 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 Test 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 the Xpert MTB/RIF Ultra test 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 the Xpert MTB/RIF Ultra test 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 Test 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 Test Performance vs. the Xpert MTB/RIF Assay

One thousand five hundred ninety-four (1594) specimens were tested by both the Xpert MTB/RIF Ultra test and the Xpert MTB/RIF Assay. 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 Analytical Sensitivity (Limit of Detection)

16.1.1 Mycobacteria Species

Studies were performed to determine the analytical sensitivity or Limit of Detection (LoD) of the Xpert MTB/RIF Ultra test 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 the Xpert MTB/RIF Ultra test 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 9. Probit Analysis Data and Claimed LoD in CFU/mL

Mycobacteria species	Specimen Type	Claimed LoD
M. bovis (BCG)	Sputum	30
	Sputum Sediment	33
M. tuberculosis (H37Rv)	Sputum	12
	Sputum Sediment	11

Table 10. 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

16.1.2 First WHO International Standard for *Mycobacterium tuberculosis* (H37Rv) DNA (NIBSC code 20/152)[12]

Studies were performed to determine the analytical limit of detection (LoD) of the Xpert MTB/RIF Ultra test with two lots of reagents across three testing days. An MTB positive result is based on the detection of the single copy ISinhA target. The higher LoD observed per specimen type and per lot as determined by probit analysis was selected for verification with a unique third lot. Verification of the estimated LoD claim was performed on one reagent lot across a minimum of three testing days. The LoD was established using the 1st WHO International Standard for Mycobacterium tuberculosis (H37Rv) DNA for NAT-based assays (NIBSC code 20/152) spiked into an MTB negative, unprocessed sputum and into an MTB negative, concentrated sputum sediment (i.e. sputum was processed and sedimented after cells were spiked into verification concentration).

The LoD is the lowest concentration reported in IU/mL that can be reproducibly distinguished from negative samples with ≥ 95% confidence. Replicates of 8 were evaluated at eight concentrations (i.e. 0.5log spacing) with two different reagent lots over 3 days, and then LoD was determined using Probit analysis.

The higher LoD estimate observed for each specimen type and lot as determined by probit analysis was selected for verification. Verification of the estimated LoD claim was performed on one reagent lot across three testing days. The LoD point estimates in IU/mL are provided in Table 11.

Table 11. Analytical Sensitivity (Limit of Detection in IU/mL)

Target Gene	Sample Type	LoD Estimates (Probit) IU/mL		LoD Verified Claim IU/mL
		Lot 1	Lot 2	Lot 3
IS*6110* and IS*1081*	Direct Sputum	**34.7** 95% CI: 22.3-73.9	**75.3** 95% CI: 45.5-169.1	**200**
IS*6110* and IS*1081*	Sputum Sediment	**415.8** 95% CI: 225.4-1042.5	**372.2** 95% CI: 180.3-768.1	**1100**

16.2 Analytical Specificity/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 the Xpert MTB/RIF Ultra test. 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.

16.3 Analytical Reactivity (Exclusivity)

The analytical specificity of Xpert MTB/RIF Ultra was evaluated through a combination of *in silico* analysis and wet testing.

In silico analysis was evaluated by analyzing the reference genomes of 127 microorganisms consisting of 65 bacteria, 12 fungi, 15 virus and 35 non-tuberculous Mycobacterium (NTMs) and aligning their sequences with the Xpert MTB/RIF Ultra primers and probes using NCBI BLAST (Basic Local Alignment Search Tool) to find potential regions of local similarity between sequences. Significant sequent matches are defined as a match of >80% homology with the IS1081-6110 primers and probe and a microorganism genome sequence. 80% homology is defined as follows: for an oligonucleotide of length N, the cutoff of 80% similarity is a number of identical nucleotides equal to, or greater than, the integer ceiling of 0.8 x N. None of the organisms analyzed resulted in a match of >80% homology with the IS1081-6110 primers and probes. Wet testing was evaluated by testing a panel of 94 organisms consisting of 45 bacteria, 6 fungi, 11 viruses and 32 non-tuberculous Mycobacterium (NTMs) representing common respiratory pathogens or those potentially encountered in the non-tuberculous tract and/or oropharyngeal flora in either ASM or TET buffer using the Xpert MTB/RIF Ultra test

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). For cells tested in TET buffer the testing controls were as follows: The bacterial testing controls were Positive Control: BCG (*Mycobacterium bovis* vaccine strain - Bacille Calmette-Guerin) and Negative Control: TET buffer, NTM testing controls were *Mycobacterium tuberculosis* (H37Rv) (Positive control) and TET buffer (Negative control), and virus testing controls were *Mycobacterium bovis* (Positive control) and TET buffer (Negative control). For cells tested in ASM the controls consisted of external controls ran as follows on each day of testing: operators tested three controls: 1 MTB WT, either a TB MDR1 or a TB MDR2, and 1 MTB negative control, alternating between TB MDR1 and TB MDR2 on each day of testing so that mutations present across the rpoB gene are interrogated throughout the study. Additionally, to ensure a clean matrix ASM was tested as an additional negative control on each day of testing.

The total 141 organisms tested (with the various analysis methods described above including in-silico[b] and cells [c]) are presented in the table below. No cross-reactivity was observed when testing cells in ASM[c] or TET [a] buffer for all 96 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. All in-silico[b] analysis data showed no oligos present in the Xpert MTB/ RIF Ultra will produce any false positive results against the 127 microorganisms listed in the table below.

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

Bacteria	NTM	Viruses	Fungi
Acinetobacter baumannii[ab]	*Mycobacterium avium subsp. Avium*[abc]	*Coronavirus*[a]	*Aspergillus fumigatus*[abd]
Chlamydophila pneumoniae[abd]	*Mycobacterium celatum*[abc]	*Human metapneumovirus*(hMPV)16 Type A1[ab]	*Candidaalbicans*[ab]
Citrobacter freundii[ab]	*Mycobacterium chelonae*[ab]	*Parainfluenza Virus* Type 1[ab]	*Candida glabrata*[bc]

Bacteria	NTM	Viruses	Fungi
Coryne bacteriumxerosis[a]	*Mycobacterium gordonae*[abc]	*Parainfluenza Virus* Type 2[ab]	*Cryptococcus neoformans*[bc]
Enterobacter cloacae[abc]	*Mycobacterium haemophilum*[ab]	*Parainfluenza Virus* Type 3[ab]	*Candida parapsilosis*[bc]
Escherichia coli[ab]	*Mycobacterium abscessus*[abc]	*Respiratory Syncytial Virus* Type A[ab]	*Candida krusei*[b]
Haemophilus influenzae[abd]	*Mycobacterium asiaticum*[abc]	*Respiratory Syncytial Virus* Type B[a]	*Blastomyces dermatitidis*[b]
Klebsiella pneumoniae[abc]	*Mycobacterium flavescens*[abc]	*Rhinovirus*[ab]	*Histoplasma capsulatum*[b]
Moraxella catarrhalis[abc]	*Mycobacterium fortuitum subsp. fortuitum*[abc]	*Human Influenza Virus (Types A)*[b]	*PPenicillium chrysogenum*[b]
Neisseria meningitidis[ab]	*Mycobacterium gastri*[abc]	*HIV*[b]	*Rhizopus arrhizus*[b]
Neisseria mucosa[ab]	*Mycobacterium genavense*[ab]	*Rubella Virus (Measles morbillivirus)*[b]	*Scedosporium apiospermum.*[b]
Nocardia asteroides[a]	*Mycobacterium intracellulare*[abc]	*Rubeola Virus*[b]	*Candida tropicalis*[bc]
Pseudomonas aeruginosa[abc]	*Mycobacterium kansasii*[abc]	*Mumps Virus (Mumps orthorubulavirus)*[b]	
Staphylococcus aureus[ab]	*Mycobacterium malmoense*[abc]	*Varicella Zoster Virus*[b]	
Staphylococcus epidermidis[ab]	*Mycobacterium marinum*[ab]	*Parainfluenza Virus Type 4*[b]	
Stenotrophomonas maltophilia[abc]	*Mycobacterium scrofulaceum*[ab]	*Human Influenza Virus (Types B)*[bc] *Adenovirus*[bc]	
Streptococcus agalactiae[ab]	*Mycobacterium simiae*[abc]	*Adenovirus*[bc]	
Streptococcus mitis[ac]	*Mycobacterium szulgai*[ab]		
Streptococcus mutans[abc]	*Mycobacterium thermoresistibile*[ab]		
Streptococcus pneumoniae[ab]	*Mycobacterium triviale*[abc]		
Streptococcuspyogenes[ab]	*Mycobacterium vaccae*[a]		
Acinetobacter calcoaceticus[bc]	*Mycobacterium xenopi*[abc]		
Bacteroides fragilis[bc]	*Mycobacterium smegmatis*[abc]		
Clostridium difficile[c]	*Mycobacterium interjectum*[a]		
Corynebacterium diptheriae[bc]	*Mycobacterium peregrinum*[a]		

Bacteria	NTM	Viruses	Fungi
Corynebacterium jeikeium[bc]	*Mycobacterium mucogenicum*[ab]		
Eikenella corrodens[b]	*Mycobacterium goodii*[ab]		
Enterobacter aerogenes[bc]	*Mycobacterium shimodei*[ab]		
Enterococcus faecalis[bc]	*Mycobacterium phlei*[abc]		
Enterococcus faecium[bc]	*Mycobacterium terrae*[abc]		
Fusobacterium necrophorum[c]	*Mycobacterium chimaera*[bc]		
	Mycobacterium bohemicum[bb]		
Kingella kingae[bc]	*Mycobacterium immunogenum*[b]		
Klebsiella oxytoca[bc]	*Mycobacterium kumamotonense*[b]		
Leuconostoc paramensenteroides[c]	*Mycobacterium leprae*[b]		
Clostridium perfringens[b]	*Mycobacterium lentiflavum*[b]		
Neisseria gonorrheae[bc]	*Mycobacterium massiliense*[b]		
Neisseria lactamica[bc]	*Mycobacterium ulcerans*[bc]		
Neisseria sicca[bc]			
Peptostreptococcus anaerobius[c]			
Proteus mirabilis[bc]			
Proteus vulgaris[bc]			
Serratia marcescens[bc]			
Streptococcus salivarius[bc]			
Veillonella parvula[bc]			
Yersinia enterocolitica[bc]			
Streptococcus sanguinis[c]			
Finegoldia magna[b]			
Actinomyces israelii[b]			
Bacillus cereus[b]			
Bacillus subtilis [b]			
Burkholderia cepacia[b]			
Corynebacterium pseudodiphtheriticum[b]			

Bacteria	NTM	Viruses	Fungi
Fusobacterium nucleatum[b]			
Haemophilus parahaemolyticus[b]			
Haemophilus parainfluenzae[b]			
Lactobacillus iners[b]			
Legionella micdadei[b]			
Legionella pneumophila[b]			
Leuconostoc mesenteroides[b]			
Listeria monocytogenes[b]			
Mycoplasma pneumoniae[b]			
Nocardia brasiliensis[b]			
Nocardia farcinica[b]			
Nocardia otitidiscaviarum[b]			
Pediococcus damnosus[b]			
Rhodococcus equi[b]			
Staphylococcus haemolyticus[b]			
Staphylococcus lugdunensis[b]			
Streptococcus dysgalactiae[b]			
Streptococcus equi[b]			
Streptococcus viridans[b]			
Streptomyces anulatus[b]			
Tsukamurella tyrosinosolvens[b]			

[a] Cells tested in TET buffer
[b] In silico testing performed
[c] Cells tested in ASM
[d] Genomic DNA used; concentration expressed as copies/mL

16.4 Microbial Interference of non-tuberculous Mycobacteria (NTM)

Microbial interference of the Xpert MTB/RIF Ultra test was evaluated by testing high concentrations of several strains of non-tuberculous Mycobacterium (NTM) in the presence of two different *Mycobacterium tuberculosis* (MTB) strains (H37Rv-mc2 6030 and MTB-BCG cells) at a higher concentration in a simulated background matrix.

Microbial interference of Non-tuberculous Mycobacteria (NTM) in the presence of H37Rv-mc2 6030 was assessed using six representative NTM strains (*M. avium, M. intracellulare, M. kansasii, M. celatum, M. abscessus 19977*, and *M. gordonae*). Replicates of 5 were tested for each target strain and each competitive strain combination. The 5 replicate samples at 3x LoD yielded 5 of 5 valid results for all 6 combination mixtures at CFU/mL of $36 \geq 1 \times 10^6$ of H37Rv-mc^2 6030. Under the conditions of this study, high concentrations of NTM did not inhibit the detection of low levels of *Mycobacterium tuberculosis* using the Xpert MTB/RIF Ultra test. No competitive inhibitory effects were observed.

Microbial interference of Non-tuberculous Mycobacteria (NTM) in the presence of MTB-BCG cells were assessed using eight representative NTM strains (*M. aviumn 19250, M. intracellulare 35771, M. kansasii 12478, M. kansasii 35776, M. celatum 51131, M. abscessus 700868, and M. gordonae 14470, M. gordonae 35760*). Replicates of 5 were tested for each target strain and each competitive strain combination. The 5 replicate samples yielded 5 of 5 valid results for all 8 combination mixtures at CFU/mL of $90 \geq 1 \times 10^6$ of MTB-BCG cells. Under the conditions of this study, high concentrations of NTM did not inhibit the detection of low levels of *Mycobacterium tuberculosis* using the Xpert MTB/RIF Ultra test. No competitive inhibitory effects were observed.

16.5 Potentially Interfering Substances

A study was performed in artificial sputum matrix to assess the effects of potential interfering substances with the Xpert MTB/RIF Ultra test. 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 or 9. 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. See table below.

Table 13. Potentially Interfering Substances Tested

Substance/Replicates	Description/Active Ingredient	Concentration(s) Tested
Blood	Blood 5% v/v (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 S3 1×10^7	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)

Substance/Replicates	Description/Active Ingredient	Concentration(s) Tested
Mucin	Mucin 5% (w/v)	5% (w/v)
Antibacterial, systemic	Levofloxacin 25 mg/mL	5 mg/mL (w/v)
Nasal corticosteroids	Fluticasone 500 mcg/spray	5 µg/mL (w/v)
Inhaled bronchodilators	Albuterol Sulfate 2.5 mg/5mL	75 µg/mL (w/v)
Oral anesthetics	Orajel (20% Benzocaine)	5% (w/v)
Anti-viral drugs	Acyclovir, IV 50 mg/mL	50 µg/mL (w/v)
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 1 mg/mL	25 µg/mL (w/v)
Anti-tuberculosis drugs	Ethambutol 1 mg/mL	50 µg/mL (w/v)
Anti-tuberculosis drugs	Isoniazid 1 mg/mL	50 µg/mL (w/v)
Oral expectorants	Guaifenesin (400mg/tablet)	5 mg/mL (w/v)
Anti-tuberculosis drugs	Pyrazinamide 10 mg/mL	10 µg/mL (w/v)
Nasal gel (Homeopathic)	Zicam gel	50% (w/v)
Nasal spray	Phenylephrine 1%	0.5% (v/v)
Anti-tuberculosis drugs	Rifampicin 1 mg/mL	25 µg/mL (w/v)
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)
Pneumocystis jiroveci medication	Pentamidine	300 ng/mL (v/v)
Bronchodilator	Epinephrine (injectable formulation)	1 mg/mL (w/v)
Anti-tuberculosis drugs	Amoxicillin	25 µg/mL (w/v)

16.6 Assessment of Carry-over Contamination

A study was conducted to demonstrate whether single–use, self–contained Xpert MTB/RIF Ultra test cartridges prevent specimen and amplicon carry–over contamination into negative samples when these are run following very high positive samples in the same GeneXpert® module.

Negative and positive samples were used in this study. The negative sample consisted of 7H9 media and the positive sample was prepared by spiking Bacille Calmette Guerin (*M. bovis* vaccine strain) BCG cells spiked into 7H9 media at a concentration of $1x10^6$ CFU/mL.

The negative sample was tested on each module prior to the start of testing (NEG 1). Following the initial negative sample testing, the high positive sample was processed in the same GeneXpert module immediately followed by another negative sample. This was repeated 20 times on 1 GeneXpert module for a total of 41 runs (10 high positive samples and 11 negative samples).

Additional data was obtained from an exact same set up run on another module starting with a negative sample (NEG 1-1) to account for rare chances of module failure during the study. This process was repeated 20 times with a second GeneXpert module.

Under the conditions of this study, no evidence of specimen or amplicon carry–over contamination was observed for the high positive samples and negative samples tested with the two GeneXpert modules.

16.7 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.[13] 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.

16.8 Precision (Repeatability and Reproducibility)

16.8.1 Repeatability

Repeatability was established in a single center, blinded study using a 7-member panel. Testing was performed at a single internal site using the GeneXpert Dx System.

H37Ra-1 cells (ZeptoMetrix Mycobacterium tuberculosis H37Ra-1, Titered Catalog No: 22-156-844) were used to create the MTB Positive RIF, sensitive panels. TDR-86 cells (characterized mutant strain of *Mycobacterium* tuberculosis, heat inactivated and procured from Rutgers) were used to create the MTB Positive RIF, resistant panels. Artificial sputum matrix was used as the background matrix for all panels and was run as the MTB Negative panel.

The 7 panels were tested with one lot, with a single operator, across 20 non-consecutive days, with one run per day, consisting of two replicates, for a total of 40 results per panel member.

Table 14. Summary of Repeatability Results

Panel Member	Level	Agreement	% Agreement (95% CI)
MTB Positive, RIF sensitive	Low Positive	40/40	100% (95.0%-100%)
MTB Positive, RIF sensitive	Medium Positive Specimen Set 1	40/40	100% (95.0%-100%)
MTB Positive, RIF sensitive	Medium Positive Specimen Set 2	40/40	100% (95.0%-100%)
MTB Positive, RIF resistant	Low Positive	40/40	100% (95.0%-100%)
MTB Positive, RIF resistant	Medium Positive Specimen Set 1	40/40	100% (95.0%-100%)
MTB Positive, RIF resistant	Medium Positive Specimen Set 2	40/40	100% (95.0%-100%)
MTB Negative	No Organism	40/40	100% (95.0%-100%)

All replicates from the seven panels produced their expected result call and met their expected % agreement.

The mean, standard deviation (SD), and coefficient of variation (CV) between days and within test was analyzed and results are presented in Table 15.

Table 15. ANOVA Summary of Repeatability Data by the Coefficient of Variance

Panel	Target	N	Average Ct	Between Days		Within Test	
				SD	CV%	SD	CV%
No Organism	SPC	40	24.86	0.69	2.8%	0.85	3.4%
MTB Low Pos, RIF sensitive	SPC	40	24.24	0.96	4.0%	0.95	3.9%
	IS1081-IS6110	40	22.48	0.70	3.1%	0.77	3.4%
	rpoB1	40	29.67	1.56	5.3%	1.42	4.8%
	rpoB2	40	29.84	1.59	5.3%	1.41	4.7%
	rpoB3	40	31.42	1.67	5.3%	1.48	4.7%
	rpoB4	40	33.31	1.61	4.8%	1.40	4.2%
MTB Med Pos, RIF sensitive Specimen Set 1	SPC	41	24.58	0.73	3.0%	0.75	3.1%
	IS1081-IS6110	41	21.80	0.83	3.8%	0.79	3.6%
	rpoB1	41	29.79	1.73	5.8%	1.63	5.5%
	rpoB2	41	30.00	1.55	5.2%	1.59	5.3%
	rpoB3	41	31.51	1.56	5.0%	1.50	4.8%
	rpoB4	41	33.36	1.51	4.5%	1.38	4.1%
MTB Med Pos, RIF sensitive Specimen Set 2	SPC	41	24.56	0.87	3.5%	0.79	3.2%
	IS1081-IS6110	41	21.78	0.70	3.2%	0.80	3.7%
	rpoB1	41	30.27	2.54	8.4%	2.57	8.5%
	rpoB2	41	30.06	1.82	6.0%	1.74	5.8%
	rpoB3	41	31.59	1.77	5.6%	1.72	5.5%
	rpoB4	41	33.47	1.77	5.3%	1.62	4.9%
MTB Low Pos, RIF resistant	SPC	40	24.48	0.73	3.0%	0.93	3.8%
	IS1081-IS6110	40	17.70	0.45	2.6%	0.45	2.6%
	rpoB1	40	23.55	0.60	2.6%	0.80	3.4%
	rpoB2	40	26.21	0.66	2.5%	0.80	3.0%
	rpoB3	40	25.16	0.63	2.5%	0.79	3.2%
	rpoB4	40	27.38	0.74	2.7%	0.92	3.4%
MTB Med Pos, RIF resistant Specimen Set 1	SPC	40	24.73	0.79	3.2%	0.75	3.0%
	IS1081-IS6110	40	17.08	0.29	1.7%	0.32	1.9%
	rpoB1	40	22.54	0.75	3.3%	0.64	2.8%
	rpoB2	40	25.02	0.89	3.6%	0.79	3.1%
	rpoB3	40	24.07	0.77	3.2%	0.69	2.9%
	rpoB4	40	26.25	0.97	3.7%	0.87	3.3%
MTB Med Pos, RIF resistant Specimen Set 2	SPC	40	24.55	1.01	4.1%	0.95	3.9%
	IS1081-IS6110	40	17.15	0.36	2.1%	0.33	1.9%

Panel	Target	N	Average Ct	Between Days		Within Test	
				SD	CV%	SD	CV%
	rpoB1	40	22.56	0.60	2.6%	0.59	2.6%
	rpoB2	40	25.14	0.65	2.6%	0.68	2.7%
	rpoB3	40	24.17	0.67	2.8%	0.72	3.0%
	rpoB4	40	26.27	0.51	2.0%	0.62	2.4%

CV % = SD/Mean Ct * 100

16.8.2 Reproducibility

Reproducibility was established in a multicenter, blinded study using a 5-member specimen panel. Testing was performed at three sites (one internal, two external) using the GeneXpert Dx System, the Infinity-48 system, and the Infinity-80 system. One panel of five samples using *Mycobacterium tuberculosis bovis* (BCG) for the rifampin susceptible organism and NATtrol *Mycobacterium tuberculosis* (REF: 0601954NTS) for rifampin resistance were tested (see table below). Both organisms were tested at 1x LoD (low positive) and 2-3x LoD (moderate positive). A negative sample containing no organism was also prepared. The panel was prepared using cultured material in simulated sputum matrix. Each site tested the 5-member panel across 3 lots, 2 operators, 2 days per lot, 2 runs, and 2 replicates per run for a total of 144 results per panel member.

Table 16. Summary of Reproducibility Results

Sample	Site 1			Site 2			Site 3			% Total Agreement by Sample
	Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
Negative	96%	100%	98%	100%	100%	100%	100%	100%	100%	99.3%
	(23/24)	(24/24)	(47/48)	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(143/144)
MTB Low Positive, RIF Resistant	92%	100%	96%	96%	96%	96%	96%	100%	98%	96.5%
	(22/24)	(24/24)	(46/48)	(23/24)	(23/24)	(46/48)	(23/24)	(24/24)	(47/48)	(139/144)
MTB Moderate Positive, RIF Resistant	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(144/144)
MTB Low Positive, RIF Sensitive	100%	100%	100%	100%	100%	100%	96%	100%	98%	99.3%
	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(23/24)	(24/24)	(47/48)	(143/144)
MTB Moderate Positive, RIF Sensitive	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
	(23/23)	(24/24)	(47/47)	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(143/143)

Among the negative samples, 99.3% (143/144) were classified as negative. All of the MTB moderate positive samples were classified as **MTB DETECTED** with the correct Rifampin result.

The MTB low positive samples were prepared to target a concentration at the limit of detection. At this concentration we would expect approximately 95% of samples to be positive. Overall 99.3% (143/144) of the MTB Low Positive/RIF Susceptible samples and 96.5% (139/144) of the MTB Low Positive/RIF Resistant samples were classified as detected.

The mean, standard deviation (SD), and coefficient of variation (CV) between-sites, between-lots, between- days, between-operators, and within-run/assay for each panel member was analyzed and results are presented in Table 17.

Table 17. ANOVA Summary of Reproducibility Data by the Coefficient of Variance

Sample		N	Mean Ct	Variance											
				Between-Sites		Between-Lots		Between-Days		Between-Operators		Within-Run/Assay		Total	
				SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
Negative	**SPCCt**	144	25.7	0.00	0.0	0.30	1.1	0.00	0.0	0.70	2.8	1.40	5.5	1.60	6.3
MTB Low Positive, RIF Resistant	**ICCt**	144	20.0	0.00	0.0	0.20	1.1	0.00	0.0	0.40	2.0	0.90	4.6	1.00	5.1
	rpo1C	141	31.0	0.00	0.0	0.50	1.6	0.00	0.0	0.60	2.0	2.20	7.2	2.40	7.7
	rpo2C	141	29.8	0.20	0.7	0.40	1.4	0.00	0.0	0.80	2.5	2.10	7.1	2.30	7.7
	rpo3C	139	33.8	0.20	0.6	0.60	1.9	0.00	0.0	0.70	2.0	2.00	5.9	2.20	6.5
	rpo4C	141	30.4	0.80	2.5	0.50	1.7	0.00	0.0	0.80	2.5	2.50	8.4	2.80	9.2
MTB Moderate Positive, RIF Resistant	**ICCt**	144	18.4	0.30	1.4	0.00	0.0	0.10	0.5	0.10	0.3	0.70	3.7	0.80	4.1
	rpo1C	143	28.3	0.40	1.5	0.00	0.0	0.50	1.8	0.00	0.0	1.80	6.4	1.90	6.8
	rpo2C	144	27.2	0.50	1.8	0.00	0.0	0.50	1.8	0.00	0.0	1.80	6.7	1.90	7.1
	rpo3C	143	31.1	0.10	0.4	0.00	0.0	0.50	1.6	0.00	0.0	1.70	5.6	1.80	5.8
	rpo4C	144	27.2	0.80	3.1	0.00	0.0	0.70	2.4	0.00	0.0	2.20	8.0	2.40	8.9
MTB Low Positive, RIF Sensitive	**ICCt**	143	23.7	0.00	0.0	0.20	0.6	0.40	1.6	0.00	0.0	1.70	7.4	1.80	7.6
	rpo1C	130	30.2	0.10	0.3	0.00	0.0	0.90	3.0	0.00	0.0	2.60	8.4	2.70	9.0
	rpo2C	130	29.3	0.00	0.0	0.00	0.0	0.80	2.6	0.00	0.0	2.40	8.1	2.50	8.5
	rpo3C	130	31.5	0.00	0.0	0.00	0.0	0.80	2.6	0.20	0.7	2.30	7.4	2.50	7.8
	rpo4C	120	36.1	0.30	0.9	0.40	1.1	0.00	0.0	0.50	1.4	2.10	5.7	2.20	6.1
MTB Moderate Positive, RIF Sensitive	**ICCt**	143	21.8	0.10	0.6	0.00	0.0	0.20	1.1	0.00	0.0	1.20	5.4	1.20	5.5
	rpo1C	142	27.6	0.20	0.7	0.00	0.0	0.30	1.2	0.00	0.0	2.00	7.2	2.00	7.3
	rpo2C	141	26.7	0.00	0.0	0.40	1.4	0.00	0.0	0.10	0.2	1.60	5.9	1.60	6.1
	rpo3C	141	28.9	0.00	0.0	0.30	1.1	0.00	0.0	0.50	1.7	1.70	5.7	1.70	6.0
	rpo4C	140	33.9	0.70	2.0	0.60	1.7	0.00	0.0	0.00	0.0	2.00	5.9	2.20	6.5

17 References

1. Global tuberculosis report 2023. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO.
2. Anti-tuberculosis resistance in the world: fourth global report. WHO/HTM/TB/2008.394.
3. Morris SL, Bai GH, Suffys P, Portillo-Gomez L, Fairchok M, Rouse D. Molecular mechanisms of multidrug resistance in clinical isolates of Mycobacterium tuberculosis. J Infect Dis. 1995. 171:954-60.
4. Rattan A, Kalia A, Ahmad N. 1998. Multidrug-Resistant Mycobacterium tuberculosis: Molecular Perspectives, Emerging Infectious Diseases, Vol.4 No.2, http://www.cdc.gov/ncidod/EID/vol4no2/rattan.htm
5. Francis J. Curry National Tuberculosis Center and California Department of Public Health, 2008: Drug-Resistant Tuberculosis, A Survival Guide for Clinicians, Second Edition.
6. Evrevin, et al. Improving tuberculosis management in prisons: Impact of a rapid molecular point-of-care test. Journal of Infection, December 2020.
7. Chakravorty, et al. The New Xpert MTB/RIF Ultra: Improving Detection of Mycobacterium tuberculosis and Resistance to Rifampin in an Assay Suitable for Point-of-Care Testing. mBio 2017, Aug 29.
8. Global edition, publication of the Global Laboratory Initiative, a Working Group of the StopTB Partnership. 2019. ISBN: 978-0-6486845-1-0 (PDF) https://stoptb.org/wg/gli/assets/documents/TB%20Safety_RGB_lo_res%20%20pdf%20FINAL.pdf

9. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (refer to latest edition).
10. Globally Harmonized System of Classification and Labelling of Chemicals
11. Kent PT, Kubica GP. 1985. Public Health Mycobacteriology—A Guide for Level III Laboratory, Centers of Disease Control, Atlanta, Publication no. PB 86-216546.
12. Banada, P. et al. Containment of Bioaerosol Infection Risk by the Xpert MTB/RIF Assay and Its Applicability to Point-of-Care Settings. Journal of Clinical Microbiology. 2010. 48:10. 3551-3557.
13. WHO International Standard 1st IS for Mycobacterium tuberculosis (H37Rv) DNA for NATbased assays NIBSC code: 20/152 Instructions for use (Version 3.0, Dated 05/11/2021)

18 Cepheid Headquarters Locations

Corporate Headquarters

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA

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 Us

Collect the following information before contacting Cepheid Technical Support:

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United States Technical Support

Telephone: + 1 888 838 3222

Email: techsupport@cepheid.com

France Technical Support

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/support/contact-us.

20 Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	Do not reuse
	Batch code
	Consult instructions for use
	Manufacturer
	Country of manufacture
	Contains sufficient for *n* tests
	Expiration date
	Temperature limitation
	Biological risks
	Skin corrosion
	Flammable liquids
	Near patient testing
Country of Origin: Sweden	Country of Origin: Sweden
Country of Origin: USA	Country of Origin: United States of America

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna
Sweden

21 Revision History

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