GeneXpert
Powered By CEPHEID INNOVATION

Xpert® MTB/XDR

REF **GXMTB/XDR-10**

In Vitro Diagnostic Medical Device

CE IVD

302-3514, Rev. C April 2021

Trademark, Patents and Copyright Statements

Cepheid®, the Cepheid logo, GeneXpert®, and Xpert® are trademarks of Cepheid, registered in the US and other countries. All other trademarks are the property of their respective owners.

THE PURCHASE OF THIS PRODUCT CONVEYS TO THE BUYER THE NON-TRANSFERABLE RIGHT TO USE IT IN ACCORDANCE WITH THIS PACKAGE INSERT. NO OTHER RIGHTS ARE CONVEYED EXPRESSLY, BY IMPLICATION OR BY ESTOPPEL. FURTHERMORE, NO RIGHTS FOR RESALE ARE CONFERRED WITH THE PURCHASE OF THIS PRODUCT.

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna,
Sweden

Xpert® MTB/XDR

For *In Vitro* Diagnostic Use

1 Proprietary Name

Xpert® MTB/XDR

2 Common or Usual Name

Xpert MTB/XDR

3 Intended Purpose

3.1 Intended Use

The Xpert MTB/XDR assay, performed on the GeneXpert Instrument Systems, is a nested real-time polymerase chain reaction (PCR) *in vitro* diagnostic test for the detection of extensively drug resistant (XDR) *Mycobacterium tuberculosis* (MTB) complex DNA in unprocessed sputum samples, concentrated sediments prepared from sputum, or BD™ Mycobacterial Growth Indicator Tube (MGIT™) culture. In specimens where MTB is detected, the Xpert MTB/XDR assay can also detect isoniazid (INH) resistance associated mutations in the *katG* and *fabG1* genes, *oxyR-ahpC* intergenic region and *inhA* promoter; ethionamide (ETH) resistance associated with *inhA* promoter mutations only; fluoroquinolone (FLQ) resistance associated mutations in the *gyrA* and *gyrB* quinolone resistance determining regions (QRDR); and second line injectable drug (SLID) associated mutations in the *rrs* gene and the *eis* promoter region.

The Xpert MTB/XDR assay is intended for use as a reflex test for a specimen (unprocessed sputum, concentrated sputum sediments, or MGIT culture) that is determined to be MTB positive. This test is intended as an aid in the diagnosis of XDR tuberculosis (TB) when used in conjunction with clinical and other laboratory findings.

3.2 Intended User/Environment

The Xpert MTB/XDR assay is intended to be performed by trained users in a laboratory setting.

4 Summary and Explanation

Tuberculosis (TB), a disease caused by *Mycobacterium tuberculosis*, remains one of the deadliest diseases in the world. In 2018, there were an estimated 10 million new cases of TB and about half a million new cases of rifampicin-resistant TB, of which 78% had multidrug-resistant TB (MDR-TB)[1]. MDR-TB, defined as resistance to isoniazid and rifampicin (two of the most effective first lines drugs), continues to be a public health threat and new treatment guidelines calling for rapid drug susceptibility testing are released by the World Health Organization (WHO)[2,3]. Nevertheless, in 2018, the global number of MDR/RR-TB cases notified was still only 39% of the estimated incident cases and the number of people enrolled in treatment was equivalent to 32%[1]. Likewise, there is also a rising concern of undiagnosed and untreated isoniazid-resistant, rifampicin-susceptible TB. Without easy access to INH-resistance testing, countries struggle to identify patients and implement the 2018 WHO treatment recommendations for Hr-TB[4]. The most worrisome cases of TB are caused by MDR MTB strains which acquired additional resistances to fluoroquinolones and any one of the second line injectable drugs, amikacin (AMK), kanamycin (KAN), or capreomycin (CAP). These highly resistant strains are termed extensively drug resistant TB (XDR-TB). XDR-TB is very difficult to treat and can lead to high rates of mortality, especially when an XDR-TB diagnosis is missed and appropriate treatment is delayed[5].

Culture and phenotypic drug susceptibility testing of MTB are time consuming, and labor-intensive and present a serious biohazard to laboratory workers, resulting in fewer accredited facilities in countries where MTB is endemic[2]. Even when available, culture-based susceptibility testing can take from weeks to months to complete. MTB may also be tested for drug resistance using fast, sensitive, and safer genotypic assays, which detect resistance by identifying mutations known to confer resistance to the first- and second-line drugs in a majority of clinical strains[2]. Genotypic testing approaches that can be reduced to a few manual steps are more amenable for near patient care, which can dramatically expand their availability to medically underserved populations in low and high endemic settings[5].

5 Principle of the Procedure

The Xpert MTB/XDR Assay is an automated *in vitro* diagnostic test for detection of XDR MTB complex DNA and resistance associated mutations. The assay is performed on Cepheid GeneXpert Instrument Systems equipped with GeneXpert 10 color modules.

The GeneXpert Instrument System integrate and automate sample processing, nucleic acid amplification, and detection of the target sequences in samples using nested real-time PCR and melt peak detection. The GeneXpert Instrument Systems consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests on collected samples and viewing the results. The system requires the use of single-use disposable Xpert cartridges that contain target specific polymerase chain reaction (PCR) reagents and hosts the PCR process and melt peak detection. Because the Xpert cartridges are self-contained, risk of cross-contamination between samples is minimized. For a full description of the system, see the *GeneXpert Dx System Operator Manual*.

The Xpert MTB/XDR Assay cartridge includes reagents for the detection of XDR MTB profile and sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The Xpert MTB/XDR Assay cartridge has all reagents on board, except sample reagent (SR) which requires the user to add the SR to the specimen prior loading the treated specimen into the cartridge. The test is intended to be run as a reflex test for MTB positive samples.

The results are interpreted by the GeneXpert software from measured fluorescent signals and embedded calculation algorithms and are shown in the View Results window in tabular and graphic formats. It also reports if the test is invalid, has encountered an error or produces no result. The Xpert MTB/XDR detects XDR MTB with resistance to INH, ETH, FLQs, and SLIDs directly from unprocessed sputum or from concentrated sediment from sputum in less than 90 minutes.

6 Reagents and Instruments

6.1 Material Provided

The Xpert MTB/XDR kit contains sufficient reagents to process 10 patient or quality-control specimens. The kit contains the following items:

Xpert MTB/XDR Cartridges with Integrated Reaction Tubes	**10 per kit**
• Bead 1, Bead 2, Bead 3, Bead 4, and Bead 5 (freeze-dried)	1 of each per cartridge
• Sample Processing Control Bead (freeze-dried)	1 of each per cartridge
• Reagent 1	4.0 mL per cartridge
• Reagent 2	4.0 mL per cartridge
Disposable transfer pipettes	**1 bag of 12 per kit**
Sample Reagent	**10 x 8 mL per bottle**
CD	**1 per kit**
• Assay Definition Files (ADF)	
• Instructions to import ADF into the GeneXpert software	
• Instructions for Use (Package Insert)	

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

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

Note The bovine serum albumin (BSA) 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.

7 Storage and Handling

- Store the Xpert MTB/XDR kit contents at 2–28°C until expiration data provided on the label.
- Do not open a cartridge lid until you are ready to perform testing.
- Start the test within 2.5 hours of adding SR to the specimen or within 4 hours if stored at 2–8°C
- Do not use reagents or cartridges that have passed the expiration date.
- Do not use a cartridge that has leaked.

8 Materials Required but Not Provided

- GeneXpert Dx system: GeneXpert instrument equipped with GeneXpert 10 color modules., computer, barcode scanner, and operator manual
  - For GeneXpert Dx system: Software version 6.2 or higher
  - Printer: If a printer is required, contact Cepheid Sales Representative to arrange for the purchase of a recommended printer.
- Sterile screw-capped sample container
- Disposable gloves
- Labels and/or indelible labeling marker
- Sterile pipettes for sample processing

9 Warnings and Precautions

9.1 General

- For *In Vitro* Diagnostic Use
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions.
- Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[3] and the Clinical and Laboratory Standards Institute.[6,7,8]
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
- 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[9].
- Sample Reagent contains sodium hydroxide (pH > 12.5) and isopropanol. Harmful if swallowed (H302), causes severe skin burns and eye damage (H314). Flammable liquid and vapor (H226).
- Performance characteristics of this test have been established with the specimen types listed in the Intended Use Section only. The performance of this assay with other specimen types or samples has not been evaluated.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.

9.2 Specimen

- Specimen collection and handling procedures require specific training and guidance.
- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 12, Procedure). Specimen stability under shipping conditions other than those recommended has not been evaluated.
- Reject specimens with obvious food particles or other solid particulates.
- Proper sample collection, storage, and transport are essential for correct results

9.3 Assay/Reagent

- Do not substitute Xpert MTB/XDR Assay reagents with other reagents.
- Do not open the Xpert MTB/XDR Assay cartridge lid except when adding sample.
- Do not use a cartridge that has been dropped after removing from the kit or shaken after the cartridge lid has been opened. 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 barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert MTB/XDR Assay cartridge is used to process one test. Do not reuse spent cartridges.
- A single-use disposable pipette is used to transfer one specimen. Do not reuse spent disposable pipettes.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens or reagents.
- In the event of a spill of specimens or controls, wear gloves and absorb the spill with paper towels. Then, thoroughly clean the contaminated area with a 1:10 dilution of freshly prepared household chlorine bleach. Final active chlorine concentration should be 0.5% regardless of the household bleach concentration in your country. Allow a minimum of two minutes of contact time. Ensure the work area is dry before using 70% denatured ethanol to remove bleach residue. Allow surface to dry completely before proceeding. Or, follow your institution's standard procedures for a contamination or spill event. For equipment, follow the manufacturer's recommendations for decontamination of equipment.
- The assay has been validated using Cepheid GeneXpert Dx software version 6.2 or higher.

10 Chemical Hazards[9,10]

Sample Reagent:

- Contains Isopropyl Alcohol
- Contains Sodium Hydroxide
- Signal Word: DANGER
- UN GHS Hazard Pictograms:

- **UN GHS Hazard Statements**
  - Flammable liquid and vapor.
  - Causes severe skin burns and eye damage.
  - Causes severe eye damage.
  - Suspected of causing genetic defects.
  - Suspected of damaging fertility or the unborn child.
  - May cause damage to organs through prolonged or repeated exposure.
- **UN GHS Precautionary Statements**
- **Prevention**
  - Obtain special instructions before use.
  - Do not handle until all safety precautions have been read and understood.
  - Keep away from heat, sparks, open flames and/or hot surfaces. - No smoking.
  - Keep container tightly closed.
  - Do not breath mists, vapours, and/or spray.
  - Wash thoroughly after handling.
  - Wear protective gloves, protective clothing, eye protection, face protection.
  - Use personal protective equipment as required.

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

11 Specimen Collection, Transport and Storage

Specimens can be collected following the user institution's standard procedures.

Proper specimen collection, storage, and transport are critical to the performance of this test. Specimen stability under shipping and storage conditions other than those listed below have not been evaluated with the Xpert MTB/XDR Assay.

11.1 Sputum Sediment Transport

Sediment specimens should be transported at 2–8 °C.

11.2 Unprocessed Sputum Transport

Unprocessed sputum specimens should be transported at 2–35°C.

11.3 Specimen Storage

Unprocessed sputum specimens can be stored at 2–35°C for 7 days (including shipping time)

Decontaminated/concentrated and resuspended sputum sediment can be stored at 2–8 °C for up to 7 days until testing is performed on the GeneXpert.

When testing unprocessed sputum or decontaminated/concentrated sputum sediment refer to Table 1 below to determine adequate specimen volume.

Table 1. Required Specimen Volume

Specimen Type	Minimum Volume for One Test	Maximum sample volume	Specimen to Sample Reagent (SR) Ratio
Sputum sediment	0.5 mL	2.5 mL	1:3[a]
Unprocessed sputum	1.0 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.

11.4 Leftover Specimens Treated with SR

The Xpert MTB/XDR assay can be used to test left over SR treated specimen from Xpert MTB/RIF or Xpert MTB/RIF Ultra assays. However, in such cases, the volume of the leftover SR treated specimen must be ≥ 2mL and the mix should be stored 2–8 °C for no longer than 4 hours or up to 35°C for no longer than 2.5 hours.

11.5 Culture isolates from a BD™ MGIT™ (Mycobacterial Growth Indicator Tube; Becton, Dickinson, and Company)

Valid results have been generated with Xpert MTB/XDR using MTB positive cultures from an MGIT. For testing MTB isolates from MGIT positive culture bottles, use at least 1.0 mL of culture material.

Note Cultures of mycobacteria from clinical specimens should be handled under appropriate biosafety containment controls.

Culture material from a positive MGIT culture bottle can either be used undiluted or diluted 100-fold with PBS or Middlebrook 7H9 media. The test can also be performed with heat inactivated cultures. For heat inactivation, it is recommended that the culture is first diluted 100-folds with PBS or Middlebrook 7H9 media and then heated at 100°C for 20 minutes.

Before starting the test, a 1:2 sample to SR ratio should be used followed by a 15 minutes incubation with 10 sec vortexing every 5 minutes to prevent settling or continuous shaking. Start the GeneXpert test run within 30 minutes of adding 2 mL of SR to the culture material.

12 Procedure

12.1 Procedure for Unprocessed Sputum

Important **Start the test within 2.5 hours of adding SR to the specimen or within 4 hours if stored at 2–8 °C.**

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

***Volume Requirements*:** ≥1mL of unprocessed sputum is required.

1. Carefully open the lid of the leak-proof sputum collection container. See Figure 1.

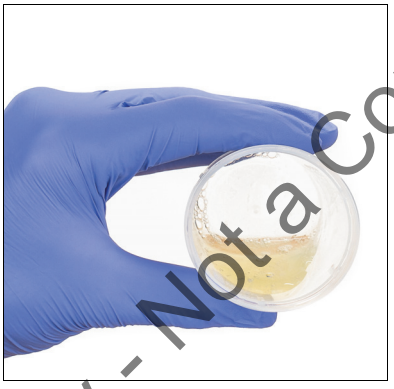


Figure 1. Opening the sputum collection container

2. Pour approximately 2 times the volume of the SR to the sputum (2:1 dilution, SR:sputum). See Figure 2.

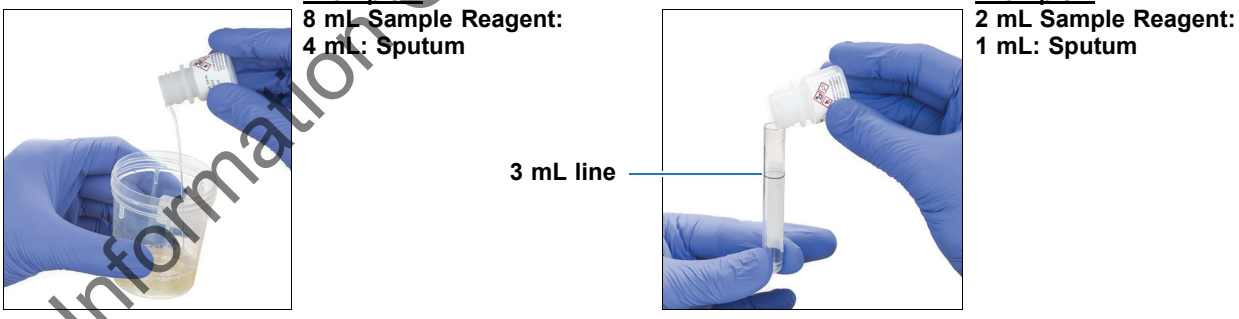


Figure 2. Examples of 2:1 dilutions

Note Discard the leftover SR and bottle in an appropriate waste container according to your institution's standard practices.

3. Secure the lid on the sample container.
4. Shake vigorously 10 to 20 times or vortex for at least 10 seconds.

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

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

12.2 Procedure for Decontaminated Concentrated Sputum Sediments

Important **Start the test within 2.5 hours of adding SR to the specimen or within 4 hours if stored at 2–8 °C.**

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

Volume Requirements: the method of Kent and Kubica[11] (Digestion-Decontamination Procedure using the NALC-NaOH method and re-suspended in 67 mM Phosphate/H2O buffer) can be tested using the Xpert MTB/XDR assay. After resuspension, keep at least 0.5 mL of the resuspended sediment for the Xpert MTB/XDR Assay. For all volumes less than 0.7 mL perform steps 1 through 5 to prepare samples. These steps require 3 parts SR to 1 part sediment in order to generate adequate volume 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. Transfer 0.5 mL of the total resuspended pellet to a conical, screw-capped tube labeled with the sample and/or patient ID using a transfer pipette.

Note Store re-suspended sediments at 2–8 °C if they are not immediately processed. Do not store for more than 7 days.

2. Add 1.5 mL of Sample Reagent (SR) to 0.5 mL of resuspended sediment.
3. Shake vigorously 10 to 20 times or vortex for at least 10 seconds.

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

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

12.3 Preparing the Cartridge

Important **Ensure a module is ready to accept a cartridge. Start the test as soon as possible and within 2.5 hours of adding the Sample Reagent-treated sample to the cartridge or within 4 hours if stored at 2–8 °C.**

Obtain the following items: Xpert cartridge, transfer pipette (provided), and an appropriately collected and labeled test sample.

1. Remove a cartridge from the package.
2. Inspect the cartridge for damage. If damaged, do not use it.
3. Bring the cartridge to room temperature. Label each Xpert MTB/XDR cartridge with the Sample ID. See Figure 3.

Figure 3. Write on Side of Cartridge.

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.

4. Open the cartridge lid, and then open the sample container.

5. Using the provided transfer pipette, aspirate the liquefied sample to the line on the pipette. Do not process the sample further if there is insufficient volume. See Figure 4.

Figure 4. Aspirating to the line on the pipette

6. Dispense the sample slowly to minimize the risk of aerosol formation. See Figure 5.

Figure 5. Xpert MTB/XDR Assay Cartridge

7. Close the cartridge lid.

12.4 Starting the Test

Important **Before starting the test, make sure that the Xpert MTB/XDR Assay definition file is imported into the software. This section lists the basic steps of 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 instrument:
   - If using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. The GeneXpert Dx software will launch automatically or may require double-clicking the GeneXpert Dx shortcut icon on the Windows® desktop.
2. Log on to the GeneXpert Instrument System software using your user name and password.
3. In the GeneXpert Dx System window, click **Create Test**. The Create Test window appears.

4. Scan in Patient or Sample ID or type in the Patient or Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the test results.
5. Scan the barcode on the Xpert MTB/XDR Assay cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: **Reagent Lot ID**, **Cartridge S/N**, and **Expiration Date**. See Figure 6.

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

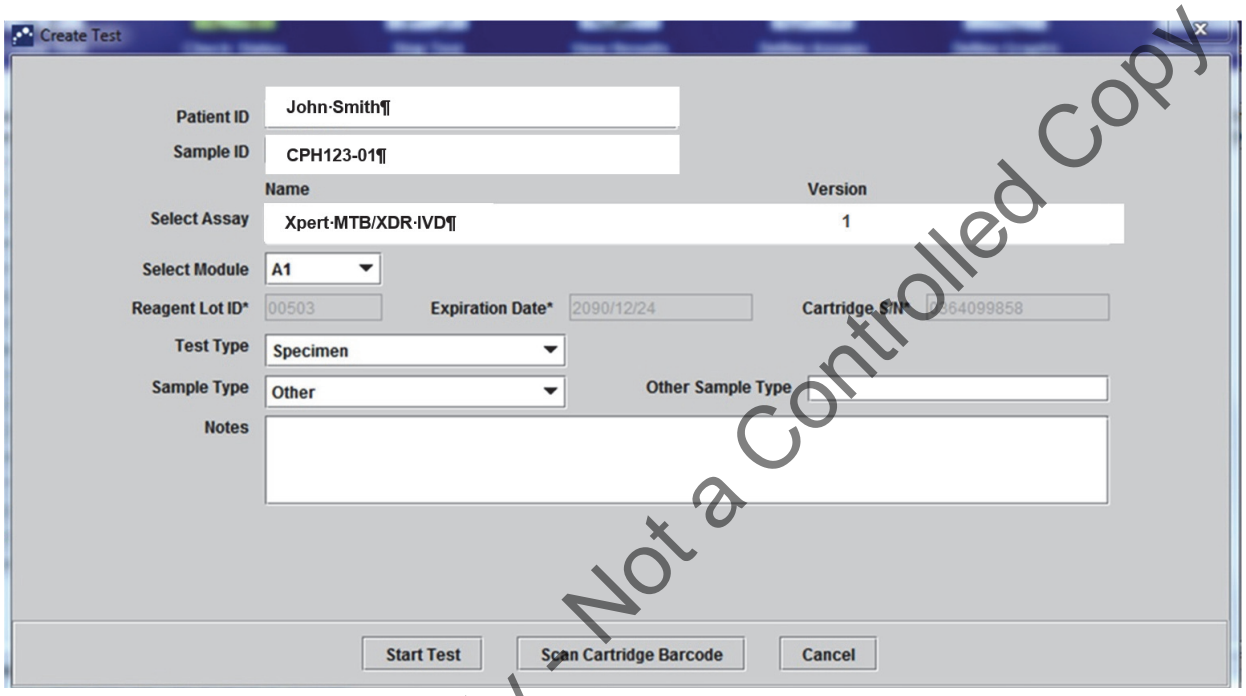


Figure 6. GX Dx Create Test Window

6. Click **Start Test**. Type your password in the dialog box that appears.
7. For the GeneXpert Dx Instrument:
   A. Open the instrument module door with the blinking green light and load the cartridge.
   B. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
   C. Wait until the system releases the door lock before opening the module door and removing the cartridge.
8. Dispose of used cartridges in the appropriate specimen waste container according to your institution's standard practices.

13 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*.

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

14 Quality Control

14.1 Built-in Quality Controls

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

- **Sample Processing Control (SPC)**— The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. 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 assigned acceptance criteria.
- **Probe Check Control (PCC)—**Before the start of the PCR reaction, the GeneXpert System 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.
- **Sample Volume Adequacy (SVA) control**—Before sample processing, the GeneXpert system measures if adequate volume of specimen is present in the sample chamber. If SVA check fails, it implies that adequate volume of sample required for testing has not been added to the sample chamber.

15 Interpretation of Results

The GeneXpert Instrument System generates the results from a combination of measured fluorescent signals and melting temperature (*Tm*) values. Mutations and wild type sequences are detected by the GeneXpert System using *Tm* values. Susceptibility or resistance determination depends on where the *Tm* values fall within the wild type or mutant window respectively for a particular analyte. Positive results for the Xpert MTB/XDR Assay can be **MTB DETECTED** and all resistance targets are **NOT DETECTED** or **MTB DETECTED** and one or more of the resistance targets is **DETECTED** or **MTB DETECTED** and/or one or more of the following resistance targets is **INDETERMINATE**. See Table 2 for a list of possible results for each target.

Table 2. Possible Test Results for Each Target in the Xpert MTB/XDR Assay

Drug Class	Result Call
N/A	INVALID/ERROR/NO RESULT
	MTB DETECTED
	MTB NOT DETECTED
Isoniazid	Low INH Resistance DETECTED
	INH Resistance DETECTED
	INH Resistance NOT DETECTED
	INH Resistance INDETERMINATE
Fluoroquinolone	Low FLQ Resistance DETECTED
	FLQ Resistance DETECTED
	FLQ Resistance NOT DETECTED
	FLQ Resistance INDETERMINATE
Amikacin	AMK Resistance DETECTED
	AMK Resistance NOT DETECTED
	AMK Resistance INDETERMINATE
Kanamycin	KAN Resistance DETECTED
	KAN Resistance NOT DETECTED
	KAN Resistance INDETERMINATE

Table 2. Possible Test Results for Each Target in the Xpert MTB/XDR Assay (Continued)

Drug Class	Result Call
Capreomycin	CAP Resistance DETECTED
	CAP Resistance NOT DETECTED
	CAP Resistance INDETERMINATE
Ethionamide[a]	ETH Resistance DETECTED
	ETH Resistance NOT DETECTED

a. Ethionamide will not provide an indeterminant by assay design.

Table 3 summarizes the genes targeted by the Xpert MTB/XDR Assay and codon region and nucleotides covered for each of genes interrogated to identify or infer drug resistance.

Table 3. IDrug Resistance Determining Regions Interrogated

Drug	Gene Target	Codon Regions	Nucleotide
Isoniazid	*inhA* promoter	NA	-1 to -32 intergenic
	katG	311-319	939-957
	fabG1	199-210	597-630
	oxyR- ahpC intergenic region	NA	-5 to -50 intergenic (or -47 to -92)[12,13]
Ethionamide	*inhA* promoter	NA	-1 to -32 intergenic
Fluoroquinolones	*gyrA*	87-95	261-285
	gyrB	531-544 (or 492-505)[12,14]	1596-1632
Amikacin, Kanamycin, Capreomycin	*rrs*	NA	1396-1417
	eis promoter	NA	-6 to -42 intergenic

See Table 4 for examples of possible results and corresponding interpretation. Figure 7 through Figure 15 are examples of possible Xpert MTB/XDR assay results.

Table 4. Examples of Xpert MTB/XDR Assay Results and Interpretation

Result	Interpretation
MTB DETECTED; **INH Resistance NOT DETECTED** **FLQ Resistance NOT DETECTED** **AMK Resistance NOT DETECTED** **KAN Resistance NOT DETECTED** **CAP Resistance NOT DETECTED** **ETH Resistance NOT DETECTED**	The MTB target is present within the sample: • Mutations leading to INH, FLQs, AMK, KAN, CAP, or ETH resistance are not detected. • SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control. • Probe Check: PASS. All probe check results pass.

Table 4. Examples of Xpert MTB/XDR Assay Results and Interpretation (Continued)

Result	Interpretation
MTB DETECTED; **INH Resistance DETECTED** **FLQ Resistance DETECTED** **AMK Resistance DETECTED** **KAN Resistance DETECTED** **CAP Resistance DETECTED** **ETH Resistance DETECTED**	The MTB target is present within the sample: • Mutations contributing to INH resistance have been detected in one or more of the following genes: *katG*, *fabG1*, *oxyR-ahpC* intergenic region and *inhA* promoter • Mutations contributing to FLQ resistance have been detected in one or more of the following genes: *gyrA* and *gyrB* quinolone resistance determining regions (QRDR) • Mutations contributing to AMK resistance have been detected in one or more of the following genes: *rrs* gene and *eis* promoter • Mutations contributing to KAN resistance have been detected in one or more of the following genes: *rrs* gene and *eis* promoter • Mutations contributing to CAP resistance have been detected in the following gene: *rrs* gene • Mutations contributing to ETH resistance have been detected in the following gene: *inhA* promoter • 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; **INH Resistance DETECTED** **FLQ Resistance NOT DETECTED** **AMK Resistance NOT DETECTED** **KAN Resistance NOT DETECTED** **CAP Resistance NOT DETECTED** **ETH Resistance NOT DETECTED**	The MTB target is present within the sample: • Mutations leading to FLQs, AMK, KAN, CAP, and ETH resistance are not detected. • Mutations contributing to INH resistance have been detected in one or more of the following genes: *katG*, *fabG1* and *oxyR-ahpC* intergenic region • 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; **INH Resistance DETECTED** **FLQ Resistance INDETERMINATE** **AMK Resistance NOT DETECTED** **KAN Resistance NOT DETECTED** **CAP Resistance NOT DETECTED** **ETH Resistance NOT DETECTED**	The MTB target is present within the sample: • Mutations leading to AMK, KAN, CAP, and ETH resistance are not detected. • Mutations contributing to INH resistance have been detected in one or more of the following genes: *katG*, *fabG1* and *oxyR-ahpC* intergenic region • Mutations contributing to FLQ resistance could not be determined due to the detection of only WT Tm from one or more probes and missing Tms from one or more probes targeting one or more of the following genes: *gyrA* or *gyrB*. "OR" no Tm from any of the probes targeting *gyrA* and *gyrB* genes. • 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; **Low INH Resistance DETECTED** **FLQ Resistance NOT DETECTED** **AMK Resistance NOT DETECTED** **KAN Resistance NOT DETECTED** **CAP Resistance NOT DETECTED** **ETH Resistance DETECTED**	The MTB target is present within the sample: • Mutations leading to FLQ, AMK, KAN, and CAP resistance are not detected. • Mutations contributing to low INH resistance have been detected in *inhA* promoter region • Mutations contributing to ETH resistance have been detected in the *inhA* promoter region • 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.

Table 4. Examples of Xpert MTB/XDR Assay Results and Interpretation (Continued)

Result	Interpretation
MTB DETECTED; **INH Resistance NOT DETECTED** **Low FLQ Resistance DETECTED** **AMK Resistance NOT DETECTED** **KAN Resistance NOT DETECTED** **CAP Resistance NOT DETECTED** **ETH Resistance NOT DETECTED**	The MTB target is present within the sample; low level FLQ, resistance is detected: • Mutations leading to INH, AMK, KAN, CAP and ETH resistance are not detected. • Mutations contributing to low FLQ resistance have been detected in the following genes: *gyrA* • 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; **INH Resistance DETECTED** **FLQ Resistance NOT DETECTED** **AMK Resistance DETECTED** **KAN Resistance DETECTED** **CAP Resistance DETECTED** **ETH Resistance NOT DETECTED**	The MTB target is present within the sample: • Mutations leading to FLQ and ETH resistance are not detected. • Mutations contributing to INH resistance have been detected in one or more of the following genes: *katG*, *fabG1*, *oxyR-aphC* • Mutations contributing to AMK resistance have been detected in one or more of the following genes: *rrs* gene; *eis* promoter • Mutations contributing to KAN resistance have been detected in one or more of the following genes: *rrs* gene; *eis* promoter • Mutations contributing to CAP resistance have been detected in the following gene: *rrs* gene • 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; **INH Resistance DETECTED** **Low FLQ Resistance DETECTED** **AMK Resistance NOT DETECTED** **KAN Resistance DETECTED** **CAP Resistance NOT DETECTED** **ETH Resistance NOT DETECTED**	The MTB target is present within the sample: • Mutations leading to AMK, CAP, and ETH resistance are not detected. • Mutations contributing to INH resistance have been detected in one or more of the following genes: *katG*, *fabG1*, *oxyR-ahpC* intergenic region and *inhA* promoter • Mutations contributing to Low FLQ resistance have been detected in the following gene: *gyrA* • Mutations contributing to KAN resistance have been detected in the *eis* promoter region • 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.
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 Cycle Threshold (Ct) is not within valid range. • Probe Check: PASS. All probe check results pass.

Table 4. Examples of Xpert MTB/XDR Assay Results and Interpretation (Continued)

Result	Interpretation
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 may be caused by a system component failure, operator error or cartridge integrity issue.
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 was collected. For example, the operator stopped a test that was in progress. • MTB: NO RESULT • SPC: NO RESULT • Probe Check: NA (not applicable)

Note The following figures provide representative results including melt peak tab that can be expected with the Xpert MTB/XDR assay in the GeneXpert Dx Detailed User View. Not all possible combinations of results are shown.

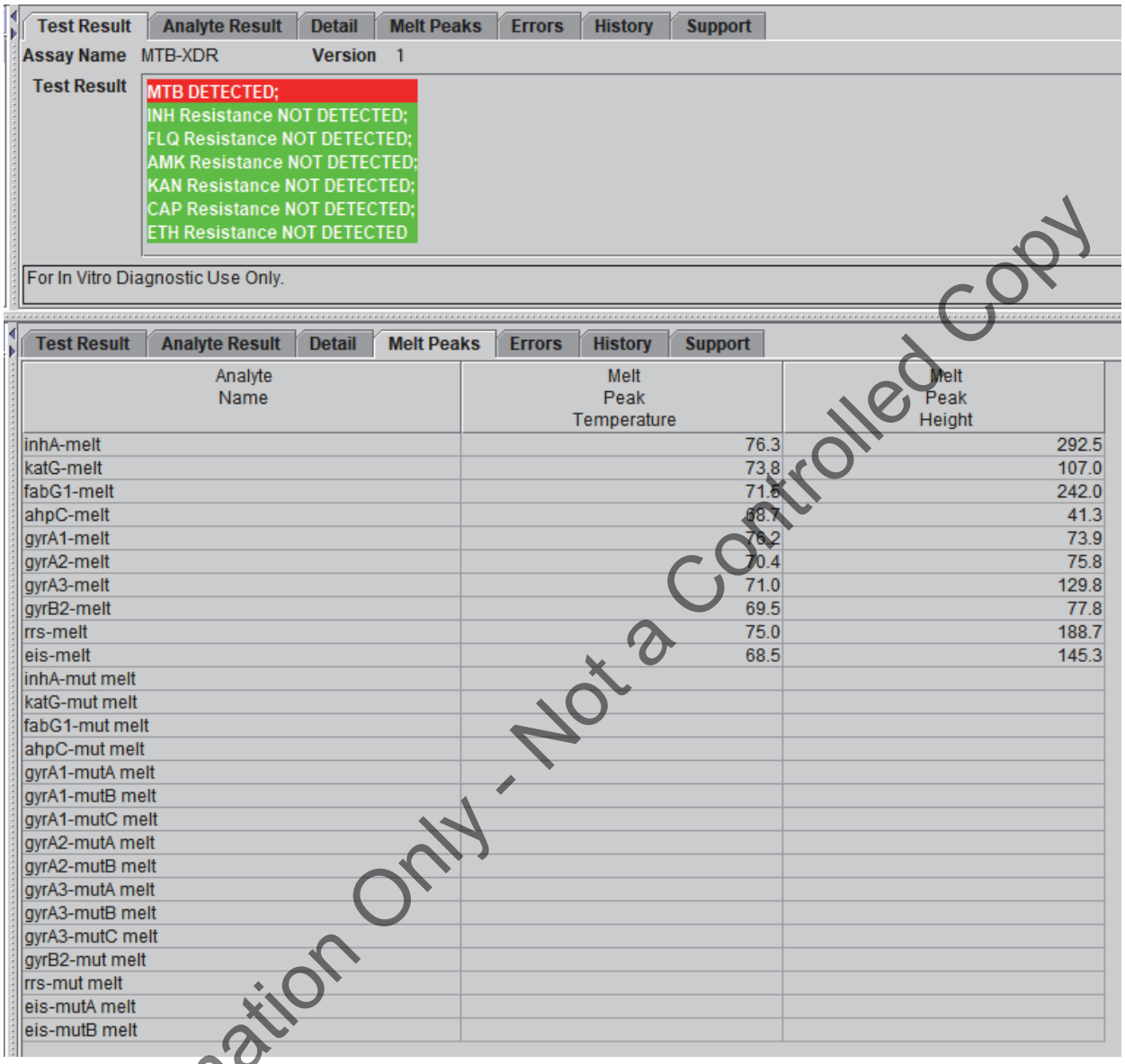
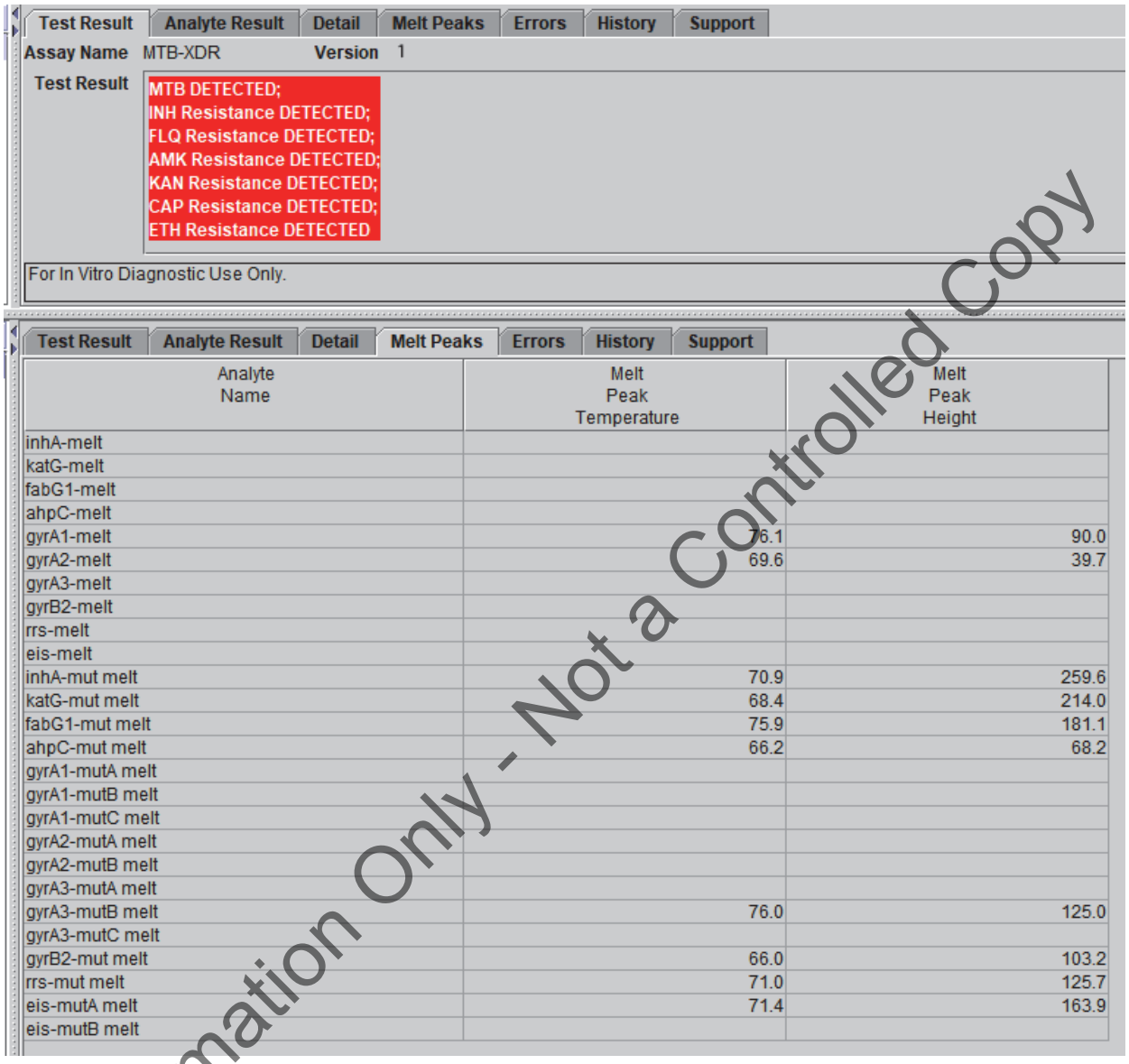
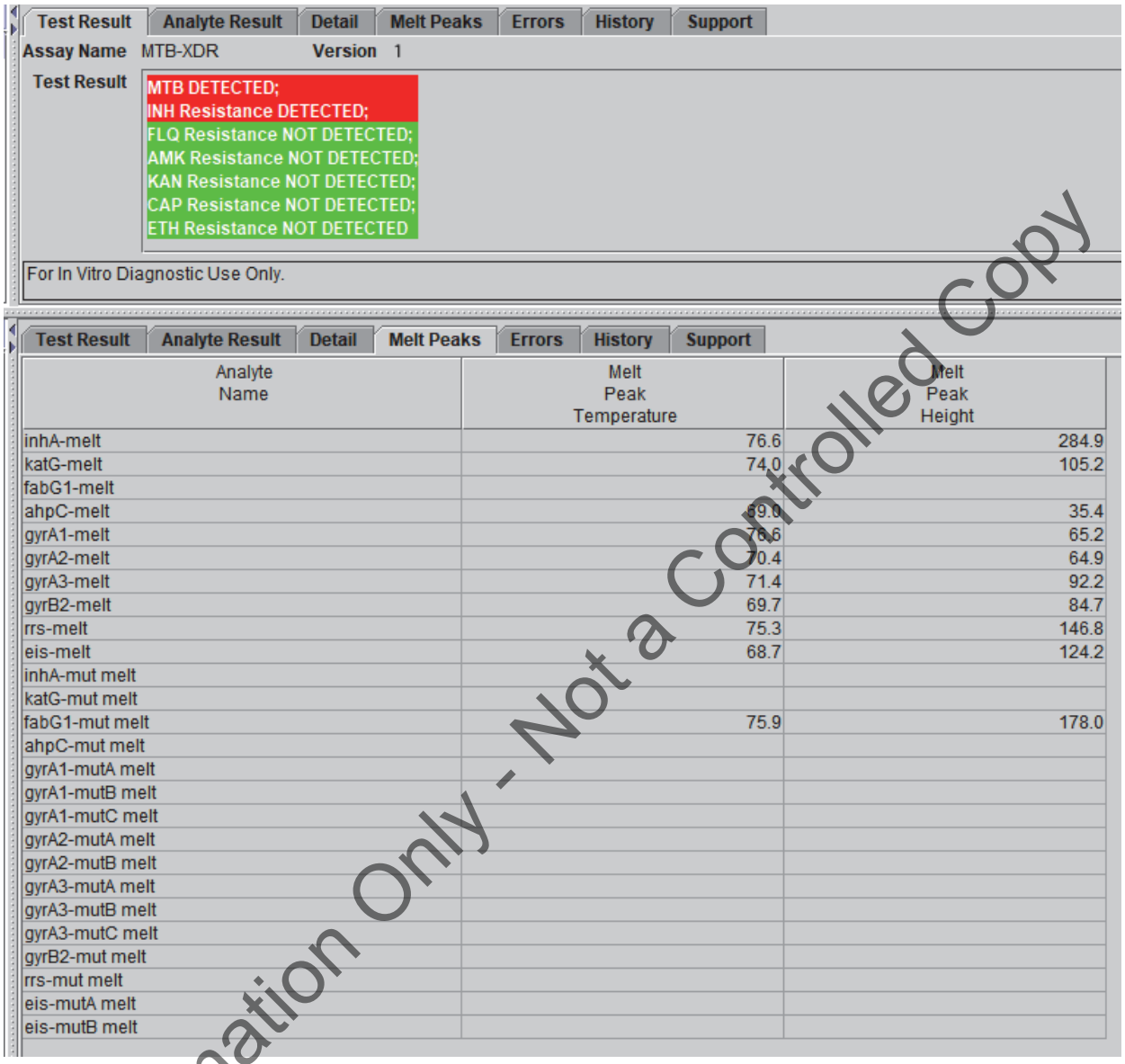


Figure 7. MTB DETECTED; INH, FLQ, AMK, KAN, CAP, and ETH Resistance NOT DETECTED



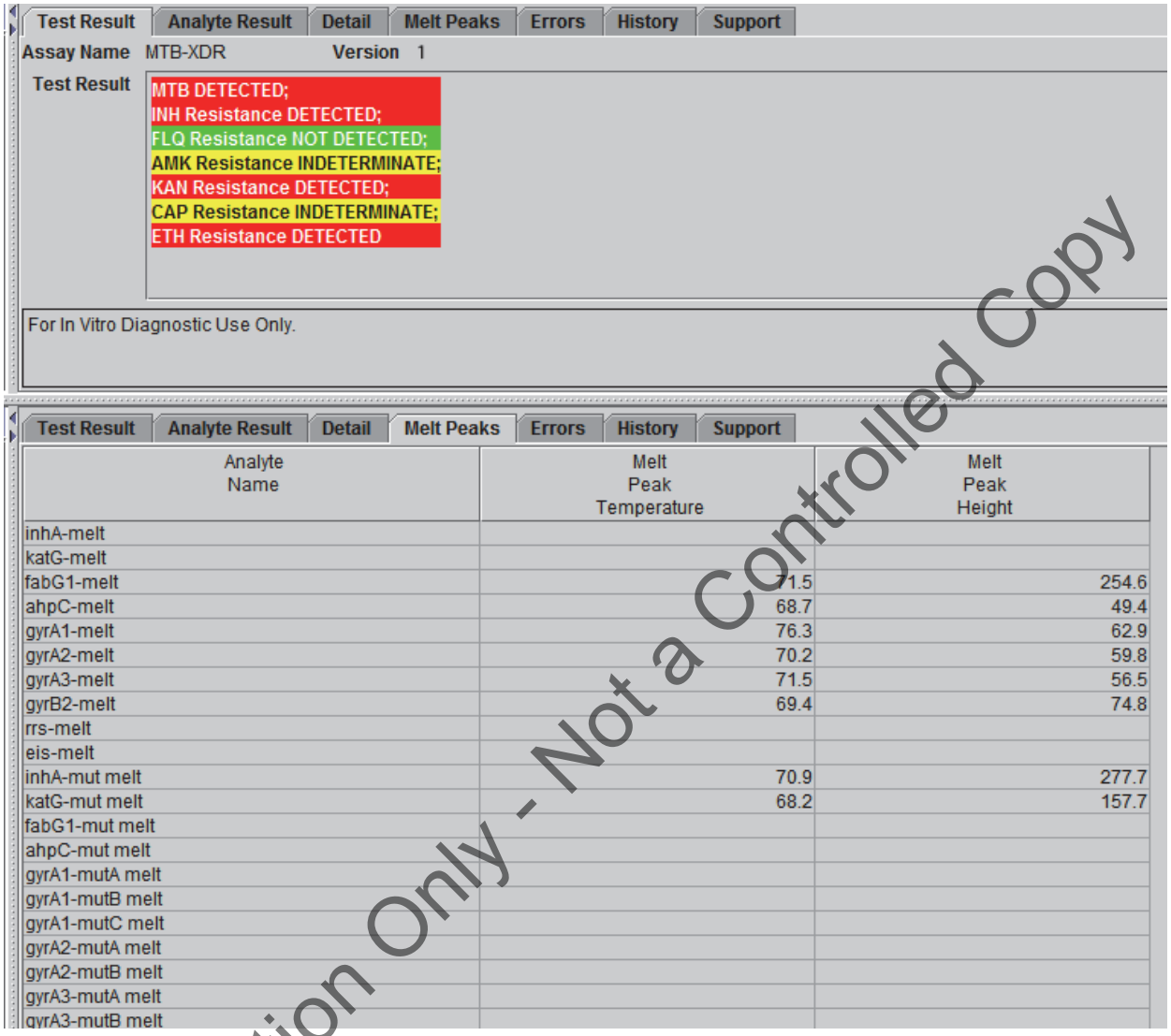
Analyte Name	Melt Peak Temperature	Melt Peak Height
inhA-melt		
katG-melt		
fabG1-melt		
ahpC-melt		
gyrA1-melt	76.1	90.0
gyrA2-melt	69.6	39.7
gyrA3-melt		
gyrB2-melt		
rrs-melt		
eis-melt		
inhA-mut melt	70.9	259.6
katG-mut melt	68.4	214.0
fabG1-mut melt	75.9	181.1
ahpC-mut melt	66.2	68.2
gyrA1-mutA melt		
gyrA1-mutB melt		
gyrA1-mutC melt		
gyrA2-mutA melt		
gyrA2-mutB melt		
gyrA3-mutA melt		
gyrA3-mutB melt	76.0	125.0
gyrA3-mutC melt		
gyrB2-mut melt	66.0	103.2
rrs-mut melt	71.0	125.7
eis-mutA melt	71.4	163.9
eis-mutB melt		

Figure 8. MTB DETECTED; INH, FLQ, AMK, KAN, CAP, and ETH Resistance DETECTED



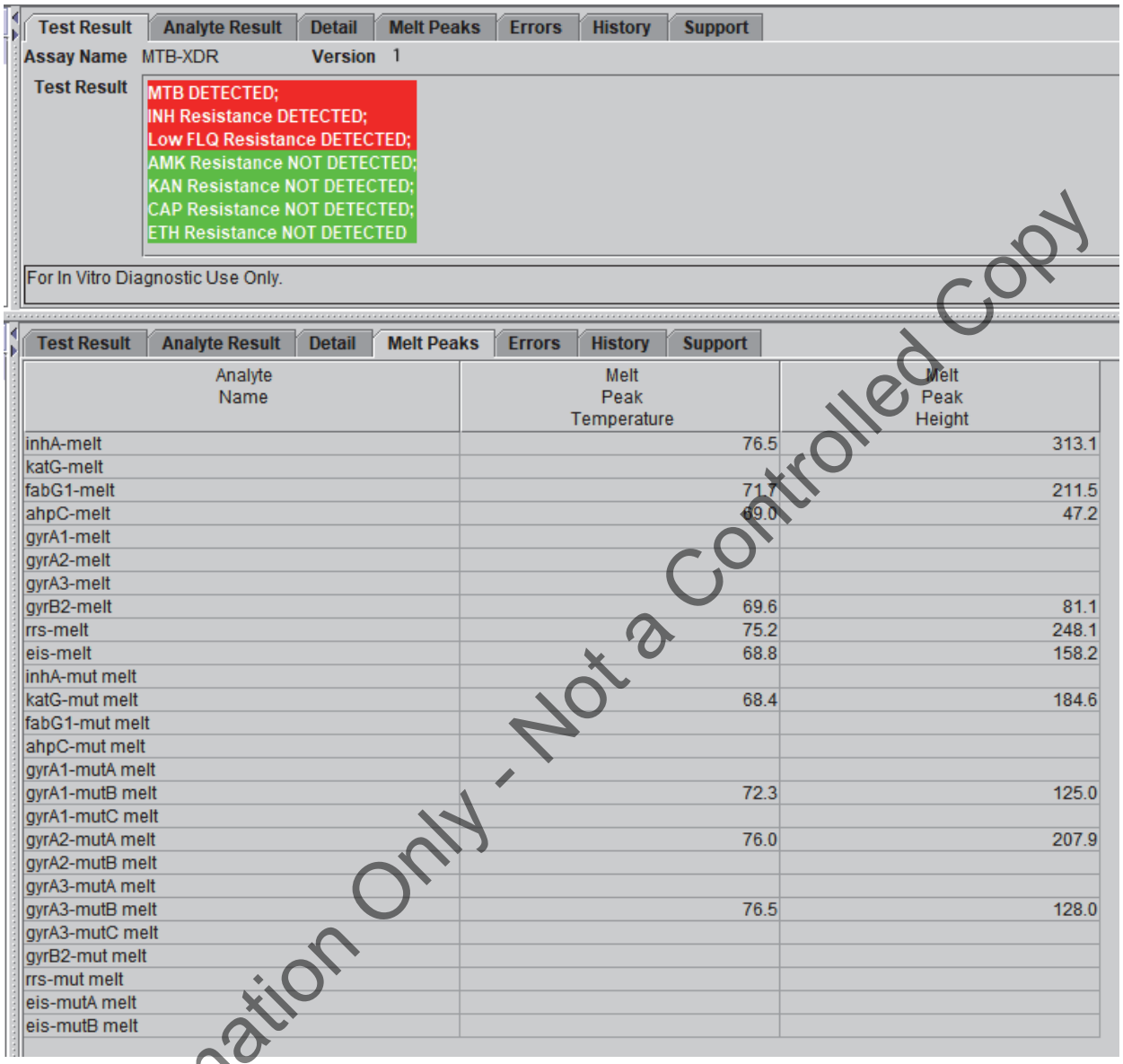
Analyte Name	Melt Peak Temperature	Melt Peak Height
inhA-melt	76.6	284.9
katG-melt	74.0	105.2
fabG1-melt		
ahpC-melt	59.0	35.4
gyrA1-melt	76.6	65.2
gyrA2-melt	70.4	64.9
gyrA3-melt	71.4	92.2
gyrB2-melt	69.7	84.7
rrs-melt	75.3	146.8
eis-melt	68.7	124.2
inhA-mut melt		
katG-mut melt		
fabG1-mut melt	75.9	178.0
ahpC-mut melt		
gyrA1-mutA melt		
gyrA1-mutB melt		
gyrA1-mutC melt		
gyrA2-mutA melt		
gyrA2-mutB melt		
gyrA3-mutA melt		
gyrA3-mutB melt		
gyrA3-mutC melt		
gyrB2-mut melt		
rrs-mut melt		
eis-mutA melt		
eis-mutB melt		

Figure 9. MTB DETECTED; INH Resistance DETECTED



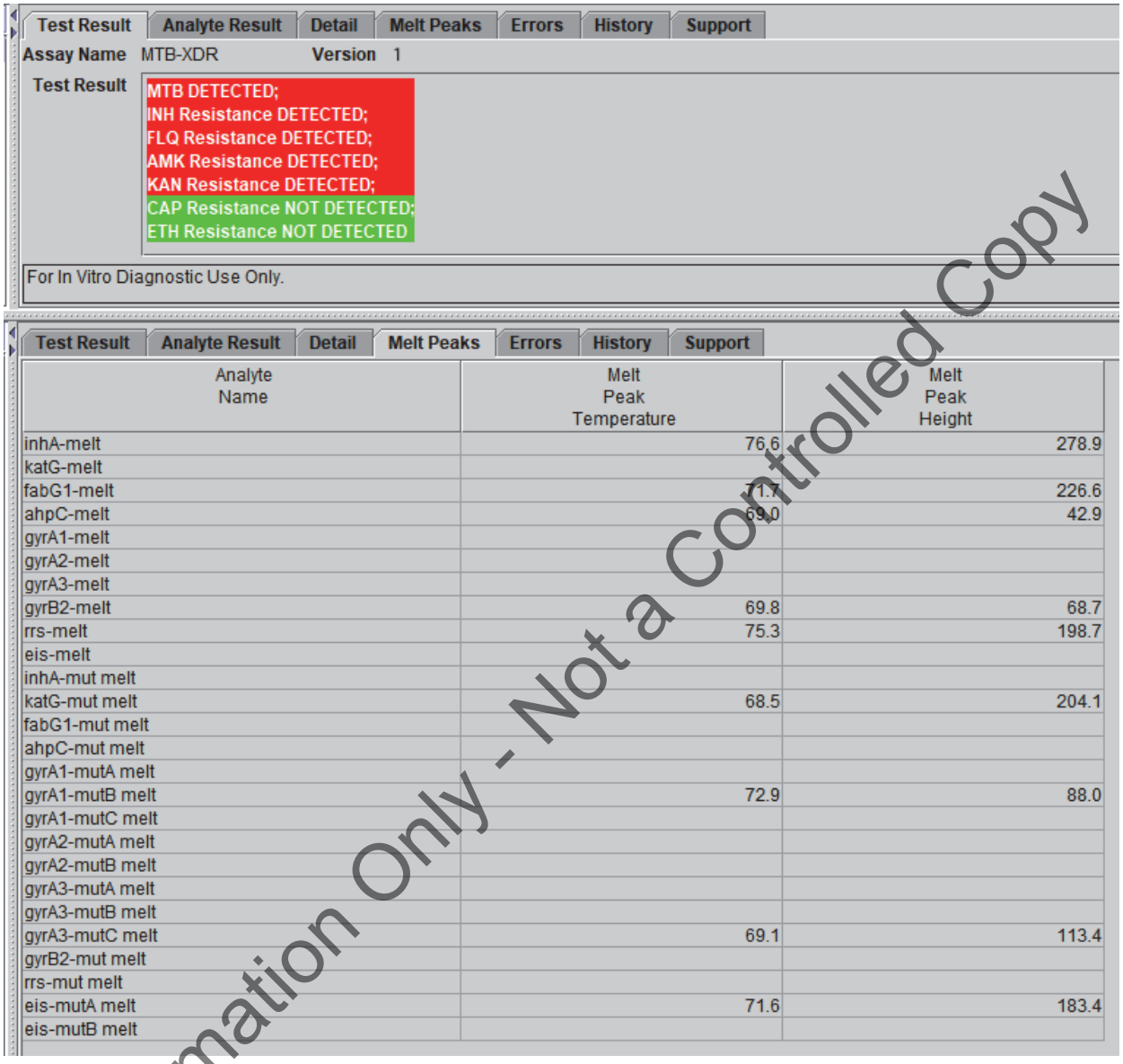
Analyte Name	Melt Peak Temperature	Melt Peak Height
inhA-melt		
katG-melt		
fabG1-melt	71.5	254.6
ahpC-melt	68.7	49.4
gyrA1-melt	76.3	62.9
gyrA2-melt	70.2	59.8
gyrA3-melt	71.5	56.5
gyrB2-melt	69.4	74.8
rrs-melt		
eis-melt		
inhA-mut melt	70.9	277.7
katG-mut melt	68.2	157.7
fabG1-mut melt		
ahpC-mut melt		
gyrA1-mutA melt		
gyrA1-mutB melt		
gyrA1-mutC melt		
gyrA2-mutA melt		
gyrA2-mutB melt		
gyrA3-mutA melt		
gyrA3-mutB melt		

Figure 10. MTB DETECTED; INH and KAN Resistance DETECTED; AMK and CAP INDETERMINATE



Analyte Name	Melt Peak Temperature	Melt Peak Height
inhA-melt	76.5	313.1
katG-melt		
fabG1-melt	71.7	211.5
ahpC-melt	69.0	47.2
gyrA1-melt		
gyrA2-melt		
gyrA3-melt		
gyrB2-melt	69.6	81.1
rrs-melt	75.2	248.1
eis-melt	68.8	158.2
inhA-mut melt		
katG-mut melt	68.4	184.6
fabG1-mut melt		
ahpC-mut melt		
gyrA1-mutA melt		
gyrA1-mutB melt	72.3	125.0
gyrA1-mutC melt		
gyrA2-mutA melt	76.0	207.9
gyrA2-mutB melt		
gyrA3-mutA melt		
gyrA3-mutB melt	76.5	128.0
gyrA3-mutC melt		
gyrB2-mut melt		
rrs-mut melt		
eis-mutA melt		
eis-mutB melt		

Figure 11. MTB DETECTED; INH and Low FLQ Resistance DETECTED



Analyte Name	Melt Peak Temperature	Melt Peak Height
inhA-melt	76.6	278.9
katG-melt		
fabG1-melt	71.7	226.6
ahpC-melt	69.0	42.9
gyrA1-melt		
gyrA2-melt		
gyrA3-melt		
gyrB2-melt	69.8	68.7
rrs-melt	75.3	198.7
eis-melt		
inhA-mut melt		
katG-mut melt	68.5	204.1
fabG1-mut melt		
ahpC-mut melt		
gyrA1-mutA melt		
gyrA1-mutB melt	72.9	88.0
gyrA1-mutC melt		
gyrA2-mutA melt		
gyrA2-mutB melt		
gyrA3-mutA melt		
gyrA3-mutB melt		
gyrA3-mutC melt	69.1	113.4
gyrB2-mut melt		
rrs-mut melt		
eis-mutA melt	71.6	183.4
eis-mutB melt		

Figure 12. MTB DETECTED; INH, FLQ, AMK, and KAN Resistance DETECTED

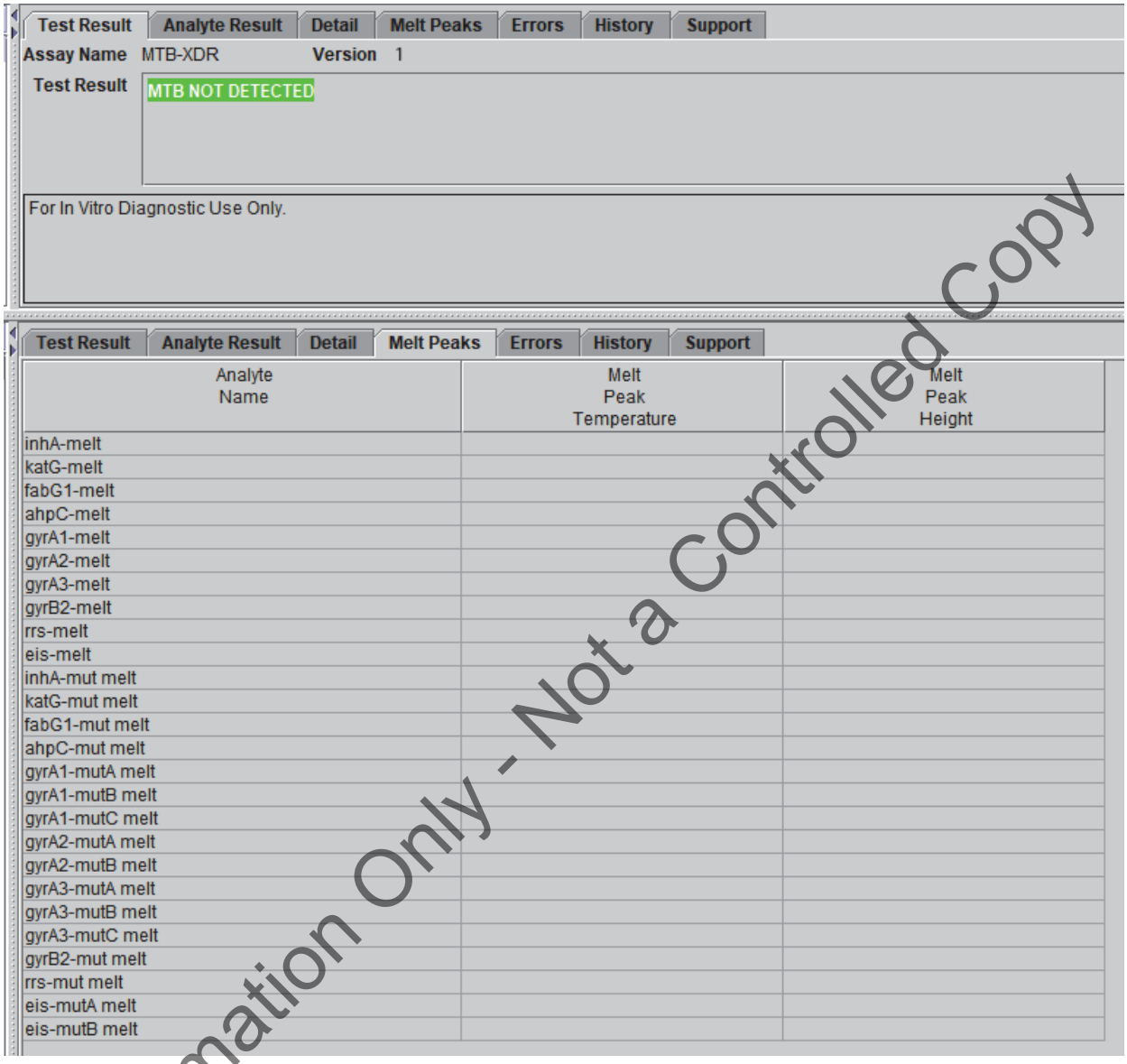
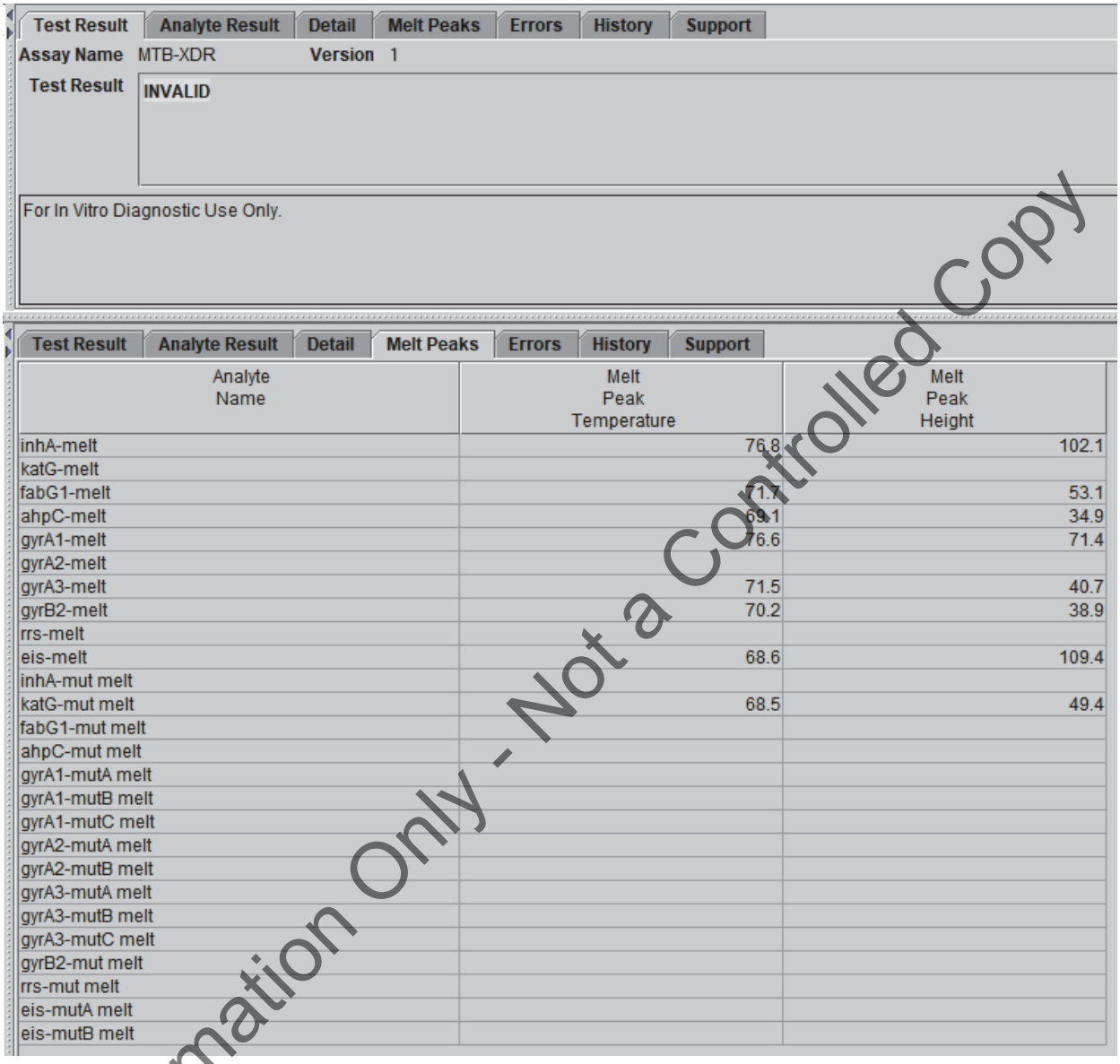


Figure 13. MTB NOT DETECTED



Analyte Name	Melt Peak Temperature	Melt Peak Height
inhA-melt	76.8	102.1
katG-melt		
fabG1-melt	71.7	53.1
ahpC-melt	69.1	34.9
gyrA1-melt	76.6	71.4
gyrA2-melt		
gyrA3-melt	71.5	40.7
gyrB2-melt	70.2	38.9
rrs-melt		
eis-melt	68.6	109.4
inhA-mut melt		
katG-mut melt	68.5	49.4
fabG1-mut melt		
ahpC-mut melt		
gyrA1-mutA melt		
gyrA1-mutB melt		
gyrA1-mutC melt		
gyrA2-mutA melt		
gyrA2-mutB melt		
gyrA3-mutA melt		
gyrA3-mutB melt		
gyrA3-mutC melt		
gyrB2-mut melt		
rrs-mut melt		
eis-mutA melt		
eis-mutB melt		

Figure 14. INVALID

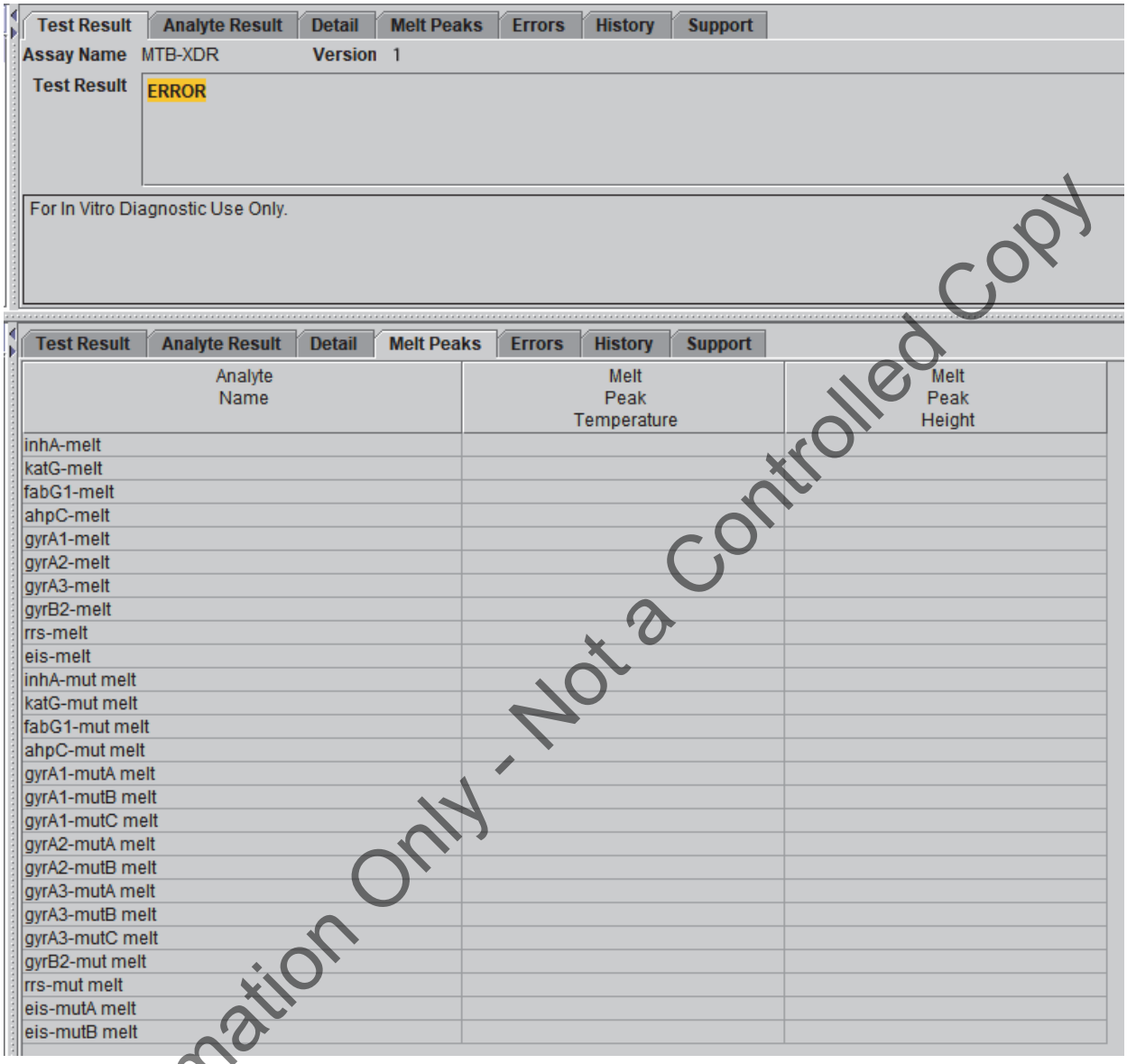


Figure 15. ERROR

16 Retests

16.1 Reasons to Repeat the Test

If any test results mentioned below occur, repeat the test according to the instructions in Section 16.2, Retest Procedure.

- An **INVALID** result indicates that the SPC failed. The sample was not properly processed, or PCR is inhibited or the sample was not properly collected.
- An **ERROR** result could be due to, but not limited to, Probe Check Control failed or the maximum pressure limits were exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress or a power failure occurred.
- An **INDETERMINATE** result indicates that resistance to a given drug could not definitively be concluded based on the assay algorithm (see Limitations for further explanations). Retesting with a different sample may or may not lead to a different result.

16.2 Retest Procedure

For retest, use a new cartridge (do not re-use a cartridge). If you have leftover sputum (should be ≥1.0 ml) or reconstituted sediment (should be ≥0.5 ml), always use new SR to decontaminate and liquefy the sputum before running the assay. Follow sample processing instructions according to Section 12.1, Procedure for Unprocessed Sputum or Section 12.2, Procedure for Decontaminated Concentrated Sputum Sediments.

If sufficient leftover SR-treated sample is available that has been stored for no longer than 2.5 hours up to 35 °C or has been stored no longer than 4 hours at 2–8 °C of the initial addition of SR to the sample, the leftover SR treated sample can be processed using a new cartridge. When retesting, always use a new cartridge and start the test within 30 minutes of adding processed sample to cartridge. See Section 12.3, Preparing the Cartridge.

17 Limitations

- The performance of the Xpert MTB/XDR assay was validated using the procedures provided in this package insert. Modifications to XDR test procedure should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- The performance of the Xpert MTB/XDR assay 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 and assay training.
- A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- Because the detection of MTB complex DNA is dependent on the number of organisms present in the sample, reliable assay results are dependent on proper specimen collection, handling, and storage. Erroneous test results might occur from improper specimen collection, failure to follow the recommended sample collection procedure, handling or storage, technical error, sample mix-up, or an insufficient concentration of starting material. Careful compliance to the instructions in this insert is necessary to avoid erroneous results.
- Test results might be affected by previous or current antibiotic therapy. Therefore, therapeutic success or failure cannot be assessed using this test because DNA might persist following tuberculosis therapy.
- A positive test result does not necessarily indicate the presence of viable organisms. It is however, presumptive for the presence of MTB complex DNA including mutations associated with INH, FLQ, AMK, KAN, CAP and ETH resistance.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown XDR-MTB strains resulting in a drug-sensitive result.
- The Xpert MTB/XDR assay does not provide confirmation of susceptibility to INH, FLQ, AMK, KAN, CAP and ETH since mechanisms of resistance other than those detected by the assay may exist that may be associated with a lack of clinical response to treatment.
- Testing of blood, cerebral spinal fluid (CSF), gastric aspirate, stool, tissue, urine has not been evaluated for use in Xpert MTB/XDR assay.
- Although induced sputum specimens were not included in the clinical performance evaluation of the Xpert MTB/XDR assay, isotonic or hypertonic solutions, bronchodilators, and inhaled bronchodilators commonly used in the collection of induced sputum were tested and do not interfere with the assay. Saline induction may result in insufficient number the organisms recovered and could affect detection of *M. tuberculosis*.
- Concentrated sputum sediments used in the performance evaluation of the Xpert MTB/XDR assay were prepared following the NALC-NaOH method described in Kent and Kubica[11]. Use of other methods of sediment preparation may alter the performance of the test.
- A negative test does not exclude the possibility of isolating MTB complex DNA from the sputum sample. The Xpert MTB/XDR assay may be used in conjunction with mycobacterial culture to address the risk of false negative results and to recover the organism for further characterization and susceptibility testing.
- Specimens with "MTB Trace DETECTED" results when tested with the Xpert MTB/RIF Ultra assay are expected to be below the Limit of Detection of the MTB/XDR assay and are not recommended for testing with the Xpert MTB/XDR assay.
- The Xpert MTB/XDR assay by design does not differentiate between the species of the MTB-complex (i.e., *MTB, M. bovis, M. africanum, M. canettii, M. microti, M. caprae, M. pinnipedi, M. mungi, and M. orygis*). In addition, culture must also be performed to determine if an NTM strain is present in addition to MTB-complex.
- Lower sensitivity has been reported in the literature in pediatric patients due to the diffuse nature of MTB infection in the lungs of this patient group, and difficulties encountered in obtaining adequate specimens[16,17].

- Mixed infections with MTB and *M. marinum* may result in "INDETERMINATE" results for FLQ at >10^4 CFU/mL of *M. marinum* in presence of ≤408 CFU/mL of MTB.
- In rare instances, the *rrs* primers and probes may cross-react with environmental microbes or sputum microflora which may result in "INDETERMINATE" results for AMK, KAN and CAP.
- The Xpert MTB/XDR assay determines ETH resistance associated only with the mutations in the *inhA* promoter region. The absence of mutations in the inhA promoter region does not exclude ETH resistance. Mutations conferring ETH resistance are reported to be present in genomic regions not targeted by the Xpert MTB/XDR assay.[15]
- The association of mutations in the *oxyR-ahpC* and *gyrB* genes with INH and FLQ resistance respectively, has not yet been conclusively established; however published studies have reported these mutations are found in INH and FLQ resistance strains[18,19].
- Presence of deletions or rare mutations in any of the target genes could lead to "INDETERMINATE" results for a particular drug.
- In case of samples with a mixed population of both susceptible and resistant strains, there is a likelihood that the Xpert MTB/XDR assay may not detect the mutation, if the resistant population is present at undetectable levels for the assay.
- In samples with very low bacterial load or a mixture of both susceptible and resistant strains, the Xpert MTB/XDR assay may not reliably distinguish between low and high FLQ resistance.

18 Clinical Performance

Two clinical studies were performed. Xpert MTB/XDR assay clinical performance was estimated with frozen archived unprocessed sputum and concentrated sputum sediment specimens in Clinical Study 1 and with prospective sputum specimens and MGIT culture in Clinical Study 2.

18.1 Sputum Specimens

A blinded clinical study was conducted to evaluate the performance of the Xpert MTB/XDR assay relative to microbiological and molecular reference methods. i.e. phenotypic drug susceptibility (pDST) testing and sequencing respectively, for the detection of drug resistance to INH, ETH, FLQs and SLID (AMK, KAN and CAP). In addition, the clinical performance of the Xpert MTB/XDR assay was compared to the Xpert MTB/RIF or the Xpert MTB/RIF Ultra assay for the detection of MTB. Two sites with known high prevalence for MDR and XDR TB provided frozen archived unprocessed sputum or concentrated sputum sediment specimens known to be positive or negative by MTB culture

Table 5 shows the sensitivity and specificity of the Xpert MTB/XDR assay relative to pDST for drug resistance. The sensitivity was >90% for INH, FLQ and AMK, >85% for KAN and CAP, and >64% for ETH; the specificity was >98% for all drugs.

Table 5. Xpert MTB/XDR Assay vs. pDST for Drug Resistance

Drugs	N	TP	FN	TN	FP	Sensitivity (%)	95%CI	Specificity (%)	95% CI
INH	478	244	23	209	2	91.4	87.4 – 94.2	99.1	96.6 – 99.7
FLQ	417	148	11	254	4	93.1	88.0 - 96.1	98.5	96.1 – 99.4
AMK	405	79	7	317	2	91.9	84.1 – 96.0	99.4	97.7 – 99.8
KAN	343	58	8	276	1	87.9	77.9 – 93.7	99.6	98.0 – 99.9
CAP	167	21	4	142	0	84.0	65.3 – 93.6	100.0	97.4 – 100.0
ETH	230	75	41	112	2	64.7	55.6 – 72.8	98.3	93.8 – 99.5

Table 6 shows the sensitivity and specificity of the Xpert MTB/XDR assay relative to sequencing for drug resistance. The sensitivity was >93% for FLQ and greater than 96% for INH, AMK, KAN, CAP and ETH; the specificity was 100.0% for all drugs listed in the table except INH which was 98.7%.

Table 6. Xpert MTB/XDR Assay vs. Sequencing for Drug Resistance

Drug	N	TP	FN	TN	FP	Sensitivity (%)	95%CI	Specificity (%)	95%CI
INH	471	241	3	224	3	98.8	96.5 - 99.6	98.7	96.2 - 99.5
FLQ	469	152	11	306	0	93.3	88.3 - 96.2	100.0	98.8 – 100.0
AMK	463	81	3	379	0	96.4	90.0 - 98.8	100.0	99.0 – 100.0
KAN	463	88	3	372	0	96.7	90.8 - 98.9	100.0	99.0 – 100.0
CAP	463	78	3	382	0	96.3	89.7 - 98.7	100.0	99.0 – 100.0
ETH	473	104	3	366	0	97.2	92.1 – 99.0	100.0	99.0 – 100.0

Table 7 shows the positive percent agreement (PPA) and the negative percent agreement (NPA) of the Xpert MTB/XDR assay relative to the Xpert MTB/RIF assay for MTB detection to be 98.9% and 93.8%, respectively.

Table 7. Xpert MTB/XDR Assay vs. Xpert MTB/RIF Assay for MTB Detection

		Xpert MTB/RIF Assay		
		MTB Detected	**MTB Not Detected**	**Total**
Xpert MTB/XDR Assay	MTB Detected	273	2[a]	275
	MTB Not Detected	3[b]	30	33
	Total	276	32	308
PPA			98.9% (95%CI: 96.9-99.6)	
NPA			93.8% (95%CI: 79.9-98.3)	

Table 8 shows the PPA and NPA of the Xpert MTB/XDR assay relative to the Xpert MTB/RIF Ultra assay for MTB detection to be 99.5% and 100.0%, respectively.

Table 8. Xpert MTB/XDR Assay vs. Xpert MTB/RIF Ultra Assay for MTB Detection

		Xpert MTB/RIF Ultra Assay		
		MTB Detected	**MTB Not Detected**	**Total**
Xpert MTB/XDR Assay	MTB Detected	207	0	207
	MTB Not Detected	1[a]	14	15
	Total	208	14	222
PPA			99.5% (95%CI: 97.3-99.9)	
NPA			100.0% (95%CI: 78.5-100.0)	

a. The Xpert MTB/RIF Ultra result was **MTB Trace DETECTED**.

Of the 531 the Xpert MTB/XDR assay runs performed in conjunction with this study, 15 gave non-determinate ("Error", "Invalid", or "No Result") results on the first attempt. Upon retest of these 15 specimens one result remained non-determinate. The non-determinate rate on initial test was 2.8% (15/531) and the non-determinate rateon final test was 0.2% (1/531).

A multi-center clinical study (Clinical Study 2) was conducted to evaluate the performance of the Xpert MTB/XDR assay relative to pDST and sequencing for the detection of resistance to INH, ETH, FLQ and SLID (AMK, KAN and CAP) in sputum specimens. Prospectively collected sputum specimens from four sites with known high prevalence of MDR TB were enrolled. Unprocessed sputum specimens and MGIT culture isolate specimens that were known to be positive by MTB culture were analyzed for drug resistance.

Table 9 shows the sensitivity and specificity of the Xpert MTB/XDR assay relative to pDST for all drug resistance in sputum specimens. The sensitivity was >90% for INH, FLQ and KAN, >85% AMK, >70% for CAP, and >50% for ETH. The specificity was ≥ 92% for all drugs.

Table 9. Xpert MTB/XDR Assay vs. pDST for Drug Resistance

Drugs	N	TP	FN	TN	FP	Sensitivity (%)	95% CI	Specificity (%)	95% CI
INH	587	452	24	106	5	95.0	92.6 - 96.6	95.5	89.9 - 98.1
FLQ	583	203	13	347	20	94.0	90.0 - 96.4	94.6	91.7 - 96.4
AMK	571	54	9	500	8	85.7	75.0 - 92.3	98.4	96.9 - 99.2
KAN	573	155	14	372	32	91.7	86.6 – 95.0	92.1	89.0 - 94.3
CAP	573	50	17	503	3	74.6	63.1 - 83.5	99.4	98.3 - 99.8
ETH	588	169	148	258	13	53.3	47.8 - 58.7	95.2	92.0 - 97.2

Table 10 shows the sensitivity and specificity of the Xpert MTB/XDR assay relative to sequencing for all drug resistance in sputum specimens. The sensitivity was >90% for INH, FLQ, and KAN (rounded up from 89.5%), >70% AMK, >65% for CAP, and >95% for ETH. The specificity was ≥ 98% for all drugs.

Table 10. Xpert MTB/XDR Assay vs. Sequencing for Drug Resistance

Drugs	N	TP	FN	TN	FP	Sensitivity (%)	95% CI	Specificity (%)	95% CI
INH	515	411	17	85	2	96.0	93.7 - 97.5	97.7	92 - 99.4
FLQ	513	201	6	303	3	97.1	93.8 - 98.7	99.0	97.2 - 99.7
AMK	501	50	18	430	3	73.5	62 - 82.5	99.3	98 - 99.8
KAN	503	170	20	308	5	89.5	84.3 - 93.1	98.4	96.3 - 99.3
CAP	504	45	23	435	1	66.2	54.3 - 76.3	99.8	98.7 - 100
ETH	517	160	6	347	4	96.4	92.3 - 98.3	98.9	97.1 - 99.6

18.2 MGIT Specimens

The multi-center clinical study (Clinical Study 2) was conducted to also evaluate the performance of the Xpert MTB/XDR assay relative to pDST and sequencing for the detection of resistance to INH, ETH, FLQ and SLID (AMK, KAN and CAP) in MTB positive specimens. Prospectively collected sputum specimens from four sites with known high prevalence of MDR TB were enrolled. Unprocessed sputum specimens and MGIT culture isolate specimens that were known to be positive by MTB culture were analyzed for drug resistance.

Table 11 shows the sensitivity and specificity of the Xpert MTB/XDR assay relative to pDST for all drug resistance. The sensitivity was >90% for INH, FLQ, and KAN, >85% AMK, >75% for CAP, and 55% for ETH. The specificity was ≥ 92% for all drugs.

Table 11. Xpert MTB/XDR Assay vs. pDST for Drug Resistance

Drugs	N	TP	FN	TN	FP	Sensitivity (%)	95% CI	Specificity (%)	95% CI
INH	596	459	23	109	5	95.2	92.9 - 96.8	95.6	90.1 - 98.1
FLQ	594	208	12	356	18	94.5	90.7 - 96.9	95.2	92.5 - 96.9
AMK	593	57	8	520	8	87.7	77.5 - 93.6	98.5	97.0 - 99.2
KAN	594	163	11	388	32	93.7	89.0 - 96.4	92.4	89.4 - 94.6
CAP	595	52	17	524	2	75.4	64.0 – 84.0	99.6	98.6 - 99.9
ETH	597	177	145	258	17	55.0	49.5 - 60.3	93.8	90.3 - 96.1

Table 12 shows the sensitivity and specificity of the Xpert MTB/XDR assay relative to sequencing for drug resistance. The sensitivity was >96% for INH, FLQ, and ETH, >85% for KAN, >70% for AMK, and >62% for CAP. The specificity was ≥ 97% for all drugs.

Table 12. Xpert MTB/XDR Assay vs. Sequencing for Drug Resistance

Drugs	N	TP	FN	TN	FP	Sensitivity (%)	95% CI	Specificity (%)	95% CI
INH	522	418	15	88	1	96.5	94.4 - 97.9	98.9	93.9 - 99.8
FLQ	521	205	5	309	2	97.6	94.5 – 99.0	99.4	97.7 - 99.8
AMK	520	52	20	446	2	72.2	61.0- 81.2	99.6	98.4 - 99.9
KAN	520	177	20	319	4	89.8	84.8 - 93.3	98.8	96.9 - 99.5
CAP	522	45	27	450	0	62.5	51.0 - 72.8	100.0	99.2 – 100.0
ETH	523	167	4	344	8	97.7	94.1 - 99.1	97.7	95.6 - 98.8

Of the 1211 Xpert MTB/XDR assay runs performed in this study (606 on sputum specimens, 605 on MGIT specimens), 35 gave non-determinate rate results on the initial test. Upon retest of these 35 specimens, two remained non-determinate. The non-determinate rate on initial test was 2.9% (35/1211) and the non-determinate rate on final test was 0.2% (2/1211).

19 Analytical Performance

19.1 Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the analytical limit of detection (LoD) of the Xpert MTB/XDR assay with two lots of reagents across three testing days. An MTB positive result is based on the detection of the single copy *inhA* target. The higher LoD observed per strain and per lot as determined by probit analysis was selected for verification. Verification of the estimated LoD claim was performed on one reagent lot across a minimum of three testing days. The LoD was established *using a representative MTBC member, Mycobacterium bovis BCG (Bacille Calmette-Guerin)* spiked into a MTB negative, unprocessed sputum and into a MTB negative, concentrated sputum sediment.

The LoD is the lowest concentration reported in CFU/mL that can be reproducibly distinguished from negative samples with ≥ 95% confidence. Replicates of 20 were evaluated at five to eight concentrations with two different reagent lots over the 3 days and the LoD was determined using Probit analysis.

The higher LoD 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 a minimum of three testing days with a claim based on a minimum of 19 of 20 positive replicates. The LoD point estimates in CFU/mL are provided in Table 13.

Table 13. Analytical Sensitivity (Limit of Detection)

Specimen Type	LoD Point Estimate, CFU/mL
Unprocessed Sputum	136
Sediment	86

19.2 Analytical Specificity (Exclusivity)

The analytical specificity of the Xpert MTB/XDR assay was evaluated by testing a panel of 57 organisms consisting of 21 bacteria, 1 fungus, 7 viruses and 28 Non- tuberculous mycobacteria (NTM) representing common respiratory pathogens or those potentially encountered in the respiratory tract and/or oropharyngeal flora. Three replicates of each bacterial and yeast strain were tested at concentrations of ≥ 1 x 10^6 CFU/mL. All viruses were tested at ≥1x10^5 (Tissue Culture Infectious Dose) $TCID_{50}$/ml. DNA or RNA were tested for 2 bacterial and 1 fungal strain at concentrations of ≥10^6 copies/ml, as whole organisms were not available or could not be accessed due to biosafety restrictions. Three replicates of each virus were tested at concentrations of ≥ 1 x 10^5 $TCID_{50}$/mL. The analytical specificity was 100%. The organisms tested are listed in Table 14, Table 15, and Table 16. None of the organisms tested resulted in cross-reactivity with the MTB detection probe generating “MTB NOT DETECTED” result for all the organisms and for all the replicates tested. The tables below list the organisms tested for the analytical specificity assay. *Aspergillus fumigatus* was analytically tested and showed no interference or cross reactivity. Cross reactivity with any other fungal species is not evident by *in silico* analysis.

Table 14. Analytical Specificity of the Xpert MTB/XDR Assay (Bacteria/Fungi)

Organism
Acinetobacter baumannii
Chlamydophila pneumoniae[a]
Citrobacter freundii
Corynebacterium xerosis
Enterobacter cloacae
Escherichia coli
Haemophilus influenzae
Klebsiella pneumoniae
Moraxella catarrhalis
Neisseria meningitidis[a]
Neisseria mucosa
Nocardia asteroids
Pseudomonas aeruginosa
Staphylococcus aureus
Staphylococcus epidermidis
Stenotrophomonas maltophilia

Table 14. Analytical Specificity of the Xpert MTB/XDR Assay (Bacteria/Fungi) (Continued)

Organism
Streptococcus agalactiae
Streptococcus mitis
Streptococcus mutans
Streptococcus pneumoniae
Streptococcus pyogenes
Aspergillus fumigatus[a]

a. Genomic DNA

Table 15. Analytical Specificity of the Xpert MTB/XDR Assay (Viruses)

Organism
Coronavirus 229E
Human metapneumovirus (hMPV) 16 Type A1
Parainfluenza Virus Type 1
Parainfluenza Virus Type 2
Parainfluenza Virus Type 3
Respiratory Syncytial Virus
Rhinovirus 1A

Table 16. Analytical Specificity of the Xpert MTB/XDR Assay (NTM)

Organism
Mycobacterium asiaticum
Mycobacterium avium NJH
Mycobacterium celatum
Mycobacterium chelonae
Mycobacterium flavescens
Mycobacterium fortuitum subsp. Fortuitum
Mycobacterium gastri
Mycobacterium gordonae
Mycobacterium gordonae
Mycobacterium gordonae
Mycobacterium genavense
Mycobacterium haemophilum
Mycobacterium malmoense
Mycobacterium marinum
Mycobacterium phlei
Mycobacterium scrofulaceum
Mycobacterium simiae
Mycobacterium szulgai
Mycobacterium terrae
Mycobacterium thermoresistibile
Mycobacterium triviale
Mycobacterium vaccae
Mycobacterium xenopi
Mycobacterium avium
Mycobacterium intracellulare
Mycobacterium abscessus
Mycobacterium marinum
Mycobacterium kansasii

19.3 Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the Xpert MTB/XDR assay was evaluated using a phylogenetically diverse panel consisting of susceptible and drug resistant MTB strains to evaluate the accuracy of the drug susceptibility results of the assay. The panel of twenty-two (22) MTB-complex (MTBC)-strains included eight (8) drug susceptible strains with wild-type target genes (Table 17) and fourteen (14) well characterized drug resistant strains (Table 18). All strains were tested in triplicate at concentrations at or near 3 X LoD of the *inhA* promotor target. The copy number tested for genomic DNA lysates was based on a fluorescent dye binding assay specific for double-stranded DNA (dsDNA).

The drug susceptible strains were tested and include five strains of MTB (AR2, GD139, AH1, HR36, H37Rv) and three MTB-complex mycobacterial species (*M. bovis, M. canetti* and *M. microti)*. The MTB strains were selected to broadly represent the range of genetic diversity and include one representative from each of the major phylogenetic lineages based on SNP-cluster groups (SCGs)[20].

The 14 drug resistant MTB strains were tested using genomic DNA lysates from well characterized specimens which contain 16 clinically significant canonical mutations with at least one of each of the eight regions targeted by the assay. These mutations are commonly present in multi-drug resistant or extensively drug resistant strains of MTB worldwide with the exception of a mutation in the *gyrB* gene.

Table 17 summarizes the results with drug susceptible strains showing number of correct results for each of the individual analytes in the assay. All panel members generated "MTB DETECTED; RESISTANCE NOT DETECTED" The Xpert MTB/XDR assay correctly identified all replicates of the strains tested near the limit of detection with wild type results for all probes except *oxyR-ahpC*. Since the *oxyR-ahpC* target has a higher LoD than the other targets in the assay, some replicates tested did not yield Tm results.

The results in Table 18 shows the assay also correctly identified expected resistance mutations in all 14 strains resistant to Isoniazid with mutations in *inhA* promoter, *katG* and *oxyR-ahpC* intergenic region; SLIDs resistance with mutations *rrs* and *eis* promoter region; and FLQ resistance with mutations in *gyrA*.

Table 17. Analytical Reactivity (Inclusivity) for Drug Susceptible Strains

Sample	Strain Lineage	inhA	katG	fabG1	oxyR-*ahpC*[a]	gyrA1	gyrA2	gyrA3	gyrB2	rrs	eis
(*M.bovis BCG)*	Not assigned	PASS	PASS	PASS	FAIL	PASS	PASS	PASS	PASS	PASS	PASS
M.bovis	Not assigned	PASS	PASS	PASS	FAIL	PASS	PASS	PASS	PASS	PASS	PASS
MTB (AR2)	2	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS
MTB (GD139)	3	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS
MTB (AH1)	4	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS
MTB (HR36)	5	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS
*MTB (*HR37Rv)	4	PASS	PASS	PASS	FAIL	PASS	PASS	PASS	PASS	PASS	PASS
M.canetti	Not assigned	PASS	PASS	PASS	FAIL	PASS	PASS	PASS	PASS	PASS	PASS
M.microti	Not assigned	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS

a. The LoD for oxyR-*ahpC* is higher than that of *inhA* used for determination of MTB positivity. "PASS" indicates all the replicates tested generated the expected wild type Tm; "FAIL" indicates at least one or more replicates generated no Tm values.

Table 18. Analytical Reactivity (Inclusivity) for Drug Resistant Strains (# positive results / total tested)

Strain ID	Gene	Expected Mutation	MTB Detected	Mutant Probe Tm Detected (# positive/tested)	Correct RESISTANCE DETECTED Calls (# positive/tested)
Clinical	*gyrA*	GAC 94 TAC	3 / 3	gyrA1-MutB (3/3); gyrA3-MutC (3/3)	FLQ [3/3]
	katG	AGC 315 ACC		katG Mut (3/3)	INH [3/3]
	fabG1	G609A		fabG1 Mut (3/3)	INH [3/3]

Table 18. Analytical Reactivity (Inclusivity) for Drug Resistant Strains (# positive results / total tested) (Continued)

Strain ID	Gene	Expected Mutation	MTB Detected	Mutant Probe Tm Detected (# positive/tested)	Correct RESISTANCE DETECTED Calls (# positive/tested)
Clinical	*gyrA*	GGC 88 GCC, GCG 90 GTG, TCG 91 CCG	3 / 3	gyrA1-MutB (2/3),[a] gyrA1-MutC (2/3), gyrA2-MutA (3/3), gyrA3-MutB (1/3)	FLQ [3/3]
	katG	AGC 315 ACC		katG Mut (3/3)	INH [3/3]
	rrs	A1401G		rrs-Mut (3/3)	AMK, CAP, KAN [3/3]
Clinical	*gyrA*	GAC 94 GGC	3 / 3	gyrA3-MutB (3/3)	FLQ [3/3]
	katG	AGC 315 ACC		katG Mut (3/3)	INH [3/3]
	rrs	A1401G		rrs-Mut (3/3)	AMK, CAP, KAN [3/3]
14-14194	*gyrA*	GAC 94 GCC	3 / 3	gyrA1-MutA, gyrA2-MutA	FLQ [3/3]
	katG	AGC 315 ACC		katG-Mut (3/3)	INH [3/3]
	inhA promoter	C -15 T		inhA-Mut (3/3)	INH, ETH [3/3]
15-14175	*katG*	AGC 315 ACC	3 / 3	katG-Mut (3/3)	INH [3/3]
	eis	-10G/A		eis-Mut (3/3)	KAN [3/3]
15-14191	*katG*	AGC 315 ACC	3 / 3	katG-Mut (3/3)	INH [3/3]
	eis	-10G/A		eis-Mut (3/3)	KAN [3/3]
16-05612	*katG*	AGC 315 ACC	3 / 3	katG-Mut (3/3)	INH [3/3]
	inhA promoter	C -15 T		inhA-Mut (3/3)	INH, ETH [3/3]
	eis	-12C/T		eis-Mut (3/3)	KAN [3/3]
16-05613	*katG*	AGC 315 ACC	3 / 3	katG-Mut (3/3)	INH [3/3]
	inhA promoter	C -15 T		inhA-Mut (3/3)	INH, ETH [3/3]
	eis	-12C/T		eis-Mut (3/3)	KAN [3/3]
14-13764	*katG*	AGC 315 ACC	3 / 3	katG-Mut (3/3)	INH [3/3]
	ahpC	-48G/A		ahpc-Mut (3/3)	INH [3/3]
14-13806	*katG*	AGC 315 ACC	3 / 3	katG-Mut (3/3)	INH [3/3]
	ahpC	-48G/A		ahpc-Mut (3/3)	INH [3/3]
Clinical	*gyrA*	GCG 90 GTG, GAC 94 GGC	3 / 3	gyrA3-MutB (3/3)	FLQ [3/3]
	inhA promoter	C -15 T		inhA-Mut (3/3)	INH [3/3]
	ahpC	G-6A		ahpC (2/3)[b]	INH [3/3]
Clinical	*katG*	AGC 315 ACC	3 / 3	katG Mut (3/3)	INH [3/3]
Clinical	*gyrB2*	ACC 539 AAC	3 / 3	gyrB2 WT[c]	*No resistance detected [0/3]
	rrs	A1410G		rrs-Mut (3/3)	AMK, CAP, KAN [3/3]
	gyrA	GCG 90 GTG		gyrA1 MuB (3/3), gyrA2 MutA (3/3), gyrA3 MutB (3/3)	FLQ [3/3]
	ahpC	g -6 a		ahpC Mut (3/3)	INH [3/3]
	inhA promoter	C -15 T		inhA Mut (3/3)	INH, ETH [3/3]
Clinical	*gyrA*	TCG 91 CCG	3 / 3	gyrA1-MutB (3/3), gyrA2-MutA (3/3), gyrA3-MutC (3/3)	FLQ [3/3]
	inhA promoter	C -15 T		inhA Mut (3/3)	INH, ETH [3/3]

a. This sample containing three different mutations in the *gyrA* gene did not generate mutant Tms for all the three *gyrA* probes all the time. However, for the correct resistance call to be made, at least one probe needs to generate a mutant Tm. The call was correctly made for all the replicates, since at least one *gyrA* probe always generated at least one mutant Tm when tested.
b. This sample is a *katG* / *ahp*C double mutant. The replicate with a missed *ahp*C mutant Tm was called INH-R due to the presence of the *katG* mutation, which was detected by the assay.
c. This specific mutation is not detected by the assay. However, there is limited clinical evidence that this mutation may actually contribute to FLQ resistance (Low confidence mutation for FLQ-Resistance).

19.4 Interfering Substances Study

Performance of the Xpert MTB/XDR assay was evaluated in the presence of 35 potentially interfering substances that may be present in the sputum. Potentially interfering substance classes include endogenous substances that are may be present in the specimen and exogenous substances that might be introduced into the specimen. Isotonic or hypertonic solutions, bronchodilators, and inhaled bronchodilators commonly used in the collection of induced sputum were tested and do not interfere with the assay. Saline induction may result in insufficient number the organisms recovered and could affect detection of M. tuberculosis.

The substances tested are listed in Table 19 with active ingredients and concentrations tested shown. Negative samples (n = 8) were tested per each substance to determine the effect on the performance of the sample processing control (SPC). Positive samples (n = 8) *Mycobacterium bovis, Bacille Calmette-Guerin (BCG)* spiked at 3x the analytical Limit of Detection for TB positivity were tested per substance. All substances were tested in MTB-negative pooled human sputum background included in this study. All positive and negative replicates were identified correctly using the Xpert MTB/XDR assay, except for Zicam gel (50% w/v; resulted in "MTB NOT DETECTED" in 11.1% of the replicates tested).

Table 19. Potentially Interfering Substances in the Xpert MTB/XDR Assay

Substance/Class	Description / Active Ingredient	Concentration Tested
Blood (human)	Blood 5% (v/v)	5% (v/v)
Human DNA/Cells	HELA 229 cell line	10^6 cells/mL
White Blood Cells (human)	WBC/Pus matrix (30% buffy coat; 30% plasma; 40% PBS)	100% (v/v)
Antimycotic; Antibiotic	Nystatin 500KU (100%)	20% (v/v)
Germicidal Mouthwash	Chlorhexidine gluconate (0.12%) oral rinse, USP	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 12.5mM Citrate
Gastric Acid	pH 3 to 4 solution in water, neutralized with sodium bicarbonate	100% (v/v)
Anesthetics (endotracheal intubation)	Lidocaine HCl 4%	4% (v/v)
Nebulizing solutions	NaCl 5% (w/v)	5% (w/v)
Mucin	Mucin 5% (w/v)	5% (w/v)
Antibacterial, systemic	Levofloxacin 25 mg/mL	5 mg/mL
Nasal corticosteroids	Fluticasone 500 mcg/spray	5 µg/mL;
Inhaled bronchodilators	Albuterol Sulfate (2 mg/5mL)	100 µg/mL
Oral anesthetics	Orajel (20% Benzocaine)	5% (w/v)
Anti-viral drugs	Acyclovir	50 µg/mL
Antibiotic, nasal ointment	Neosporin (400U Bactracin, 3.5mg Neomycin, 5000U Polymyxin B)	5% (w/v)
Tobacco	Nicogel 40% tobacco extract	0.5%
Anti-tuberculosis drugs	Streptomycin 1mg/mL	25µg/mL
Anti-tuberculosis drugs	Ethambutol 1mg/mL	50 µg/mL
Anti-tuberculosis drugs	Isoniazid 50mg/5ml	50 µg/mL

Table 19. Potentially Interfering Substances in the Xpert MTB/XDR Assay (Continued)

Substance/Class	Description / Active Ingredient	Concentration Tested
Oral expectorants	Guaifenesin (400mg/tablet)	5 mg/mL
Anti-tuberculosis drugs	Pyrazinamide (500mg/tablet)	100 µg/mL
Nasal gel (Homeopathic)	Zicam gel	50% (w/v)
		20% (w/v)
Nasal spray	Phenylephrine 1%	0.5% (v/v)
Anti-tuberculosis drugs	Rifampicin (300mg/tablet)	25 µg/mL
Allergy relief medicine (Homeopathic)	100% Pure Tea tree oil (<5% Cineole, >35% Terpinen-4-01)	0.5% (v/v)
Nebulizing solutions	Pentamidine isethionate	300ng/ml
Anti-tuberculosis drugs	Amoxicillin	25 µg/mL
Bronchodilator	Epinephrine	1mg/mL
Anti-tuberculosis drugs	Amikacin	70ug/ml
Anti-tuberculosis drugs	Capreomycin	50ug/ml
Anti-tuberculosis drugs	Kanamycin	50ug/ml
Anti-tuberculosis drugs	Ethionamide	50ug/ml
Flu Mist Qual Nasal	Influenza Virus Vaccine Live-nasal	5%

19.5 Carry-over Contamination Study

A study was conducted to demonstrate that carry-over, cross contamination does not occur when using the single-use, self-contained Xpert MTB/XDR cartridges. The study consisted of processing a negative sample immediately following processing a high concentration of *Mycobacterium bovis-Bacille Calmette-Guerin* (BCG) at 1 x 10^{+6} CFU/mL in human sputum the same Gene Xpert module. This testing scheme was repeated at least 20 times in two GeneXpert modules producing a total of 41 runs resulting in 20 positives and 21 negatives per module.

All 20 positive samples were correctly reported as **MTB DETECTED; INH Resistance NOT DETECTED; FLQ Resistance NOT DETECTED; AMK Resistance NOT DETECTED; KAN Resistance NOT DETECTED; CAP Resistance NOT DETECTED; ETH Resistance NOT DETECTED**. All 21 negative samples were correctly reported as **MTB NOT DETECTED**. Under the conditions of this study, there was no evidence of any carry–over contamination when testing with very high positive *BCG* sample at the concentration of 1.0 x 10^{+6} CFU/mL.

19.6 Competitive Interference Study

Competitive interference of the assay caused by the presence of high concentrations of high concentrations of non-tuberculous Mycobacteria (NTM) on the detection of low levels of MTB in the Xpert MTB/XDR assay was evaluated by testing the representative member of the MTBC. BCG at ~ 3 x LoD (411 CFU/mL) in the presence of different NTM strains at 1 x 10E+06 CFU/mL concentration in a background of negative control buffer. MTB positivity is based on detection of *inhA* promoter valid melt peak height and melt peak temperature. Resistance detection is based on valid mut melt peak height and mut melt peak temperature for individual analytes (*inhA, katG, gyrA1, gyrA2, gyrA3, gyrB2* and *eis*). *oxyR-ahpC and fabG1* analytes were excluded due to lower sensitivity and *rrs* was excluded due to known interference with microflora. All samples containing BCG should have results as **MTB DETECTED; INH Resistance NOT DETECTED; FLQ Resistance NOT DETECTED; AMK Resistance NOT DETECTED; KAN Resistance NOT DETECTED; CAP Resistance NOT DETECTED; ETH Resistance NOT DETECTED**.

Four replicates of each NTM/ BCG competitive mixture test condition along with a positive control condition with only BCG at ~3 x LoD were tested. None of the NTM strains tested interfered with the detection of 411 CFU/mL of BCG and generated the correct result as mentioned above. However, under the conditions of this study, competitive inhibitory effects were observed in the presence of only one of the two strains of *M.marinum* (ATCC 0927) tested. Interference with gyrA2 probes was observed only at challenge concentrations >10^4 CFU/mL resulting in FLQ resistance INDETERMINATE calls at these high challenge concentrations. Refer to Section 17, Limitations for further information.

Table 20. Competitive Interference by NTM on MTB detection and drug susceptibility detection

Test Condition / NTM Strain ID	NTM CFU/ mL	MTB Detected	INH	FLQ	AMK	KAN	CAP	ETH
MTB + M. avium / (NJH)	10E+06	PASS	PASS	PASS	PASS	PASS	PASS	PASS
MTB + M.gastir / (ATCC 15754)	10E+06	PASS	PASS	PASS	PASS	PASS	PASS	PASS
MTB + M.gordonae / (NJH)	10E+06	PASS	PASS	PASS	PASS	PASS	PASS	PASS
MTB + M.gordonae / (ATCC 14470)	10E+06	PASS	PASS	PASS	PASS	PASS	PASS	PASS
MTB + M.gordonae / (ATCC 35760)	10E+06	PASS	PASS	PASS	PASS	PASS	PASS	PASS
MTB + M.marinum / (NJH)	10E+06	PASS	PASS	PASS	PASS	PASS	PASS	PASS
MTB + M.marinum / (ATCC 0927)	10E+06	PASS	PASS	FAIL	PASS	PASS	PASS	PASS
	10E+05	PASS	PASS	FAIL	PASS	PASS	PASS	PASS
	10E+04	PASS	PASS	PASS	PASS	PASS	PASS	PASS
	10E+03	PASS	PASS	PASS	PASS	PASS	PASS	PASS
MTB + M.xenopi / (ATCC 700084)	10E+06	PASS	PASS	PASS	PASS	PASS	PASS	PASS
MTB + M.avian / (ATCC 15769)	10E+06	PASS	PASS	PASS	PASS	PASS	PASS	PASS
MTB + M.intracellulare / (ATCC 35771)	10E+06	PASS	PASS	PASS	PASS	PASS	PASS	PASS
MTB + M.abscessus / (ATCC 19977)	10E+06	PASS	PASS	PASS	PASS	PASS	PASS	PASS
MTB + M.kansasii / (ATCC 12478)	10E+06	PASS	PASS	PASS	PASS	PASS	PASS	PASS

"PASS" indicates all the replicates tested generated the expected "RESISTANCE NOT DETECTED" result for the relevant drugs;
"FAIL" indicates at least one or more replicates generated "RESISTANCE INDETERMINATE" result for the particular drug.

19.7 Fresh and Frozen Sputum Equivalency

Fresh and frozen sputum equivalency with the Xpert MTB/XDR assay was evaluated by testing *M.bovis* – Bacillus Calmette-Guerin (BCG) cells in a background of a pooled MTB negative unprocessed sputum at two concentrations representing 3X LoD (400 CFU/ml) and 1000XLoD (1.3×10^5 CFU/mL). Replicate samples at each concentration were frozen and stored at -80°C and at least 8 replicates thawed and tested after storage at 1 week, 2 weeks, 1 month, 3 months, 6 months and 9 months. The results were compared to unprocessed sputum spiked with the same concentrations tested at time point zero prior to freezing.

Assay performance was not affected, and correct results were obtained for all replicates tested at 3X LoD after -80°C storage at 2 weeks, 3 months and 6 months. A single replicate at the week 1 timepoint returned an "INH-Resistance Indeterminate" result due to the *katG* probe dropout and single replicate at 1 month resulted in an *ahpC* drop out, but correct results were observed for all replicates at 3 and 6 months. Correct results were obtained at the 9-month time point at 3X LoD in 8 out of 9 replicates (89%). No effect on the assay performance was observed when the sputum with 1000X LoD was stored at -80°C at all time points tested though 9 months. The results from this study support frozen storage at 80°C of unprocessed sputum for up to 6 months.

19.8 Inactivation of Mycobacteria in Sputum Samples

The disinfection capability of the Xpert MTB Sample Reagent was determined using a standardized quantitative tuberculocidal culture method.[21] 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.

20 Precision and Reproducibility

The precision and reproducibility of the Xpert MTB/XDR assay was established in a multicenter (three sites), blinded study utilizing a multi-factor nested design. The study consisted of a five-member sample panel and each panel member was prepared by spiking an MTB wild type (WT) strain and an MTB mutant (MUT) strain into artificial sputum matrix. The WT and MUT strains were made from plasmids carrying either MTB XDR wild type or mutant sequences for the genes targeted by the assay, encapsulated in killed, chemically fixed E. coli.

The panel members were prepared at ~1xLoD and ~3xLoD using the melt temperatures (Tm) of the *inhA* promoter target in the Xpert MTB/XDR assay, which generates the **MTB DETECTED/NOT DETECTED** result depending on the presence or absence of the wildtype or mutant *inhA* promoter specific Tm. Testing was conducted for six days with three lots of Xpert MTB/XDR cartridges. Each site had two operators (OP1 and OP2) who performed two runs each with two replicates/run each day. A replicate was a single cartridge test. The percent agreement for each panel member is presented in Table 21.

Table 21. Percent Agreement of Xpert MTB/XDR Assay for MTB and *inhA* Detection

Sample	Site 1			Site 2			Site 3			Total Agreement by Sample
	OP 1	OP 2	Subtotal	OP 1	OP 2	Subtotal	OP 1	OP 2	Subtotal	
MTB MUT 1xLoD	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	95.8% (23/24)	97.9% (47/48)	91.7% (22/24)	91.7% (22/24)	91.7% (44/48)	96.5% (139/144)
MTB MUT 3xLoD	95.8% (23/24)	100% (24/24)	97.92% (47/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	99.3% (143/144)
MTB WT 1xLoD	100% (24/24)	91.67% (22/24)	95.8% (46/48)	91.7% (22/24)	91.7% (22/24)	91.7% (44/48)	91.7% (22/24)	100% (24/24)	95.8% (46/48)	94.4% (136/144)
MTB WT 3xLoD	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
NEG	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	95.8% (23/24)	97.9% (47/48)	99.3% (143/144)

The performance of the Xpert MTB/XDR assay in MTB WT and MUT strains at low (~1x) and moderate (~3x) LoD panel samples for each gene target where MTB was detected is presented in Table 22.

Table 22. Percent Agreement of Xpert MTB/XDR Assay in MTB MUT and WT Types Specimens

Drug	Percent Concordance			
	MTB MUT 1x LoD (95% CI) [n agree/total n]	MTB MUT 3xLoD (95% CI) [n agree/total n]	MTB WT 1x LoD (95% CI) [n agree/total n]	MTB WT 3x LoD (95% CI) [n agree/total n]
INH	100.00% (97.3-100) [139/139]	100.00% (97.4-100.0) [143/143]	89.1% (82.6-93.4) [115/129]	99.3% (96.2-99.9) [143/144]
FLQ	87.80% (81.3-92.2) [122/139]	100.00% (97.4-100.0) [143/143]	81.4% (73.8-87.2) [105/129]	95.8% (91.2-98.1) [138/144]
ETH	100.00% (97.3-100) [139/139]	100.00% (97.4-100.0) [143/143]	99.2% (95.7-99.9) [128/129]	100.0% (97.4-100.0) [144/144]
AMK	100.00% (97.3-100) [139/139]	100.00% (97.4-100.0) [143/143]	91.5% (85.4-95.2) [118/129]	98.6% (95.1-99.6) [142/144]

Table 22. Percent Agreement of Xpert MTB/XDR Assay in MTB MUT and WT Types Specimens (Continued)

Drug	Percent Concordance			
	MTB MUT 1x LoD (95% CI) [n agree/total n]	**MTB MUT 3xLoD (95% CI) [n agree/total n]**	**MTB WT 1x LoD (95% CI) [n agree/total n]**	**MTB WT 3x LoD (95% CI) [n agree/total n]**
CAP	99.30% (96.3-99.0) [138/139]	100.00% (97.4-100.0) [143/143]	98.4% (94.5-99.6) [127/129]	99.3% (96.2-99.9) [143/144]
KAN	100.00% (97.3-100) [139/139]	100.00% (97.4-100.0) [143/143]	91.5% (85.4-95.2) [118/129]	98.6% (95.1-99.6) [142/144]

21 References

1. WHO. Global Tuberculosis Report 2019. www.who.int/tb/publications/global_report
2. WHO. 2018, Rapid Communication: Key changes to treatment of multidrug- and rifampicin-resistant tuberculosis.
3. WHO, 2019, Rapid Communication: Key changes to the treatment of drug-resistant tuberculosis
4. Sulis G, Pai M 2020) Isoniazid-resistant tuberculosis: A problem we can no longer ignore. PLoS Med 17(1): e1003023
5. Soumitesh Chakravorty, Sandy S. Roh, et al Department of Medicine, New Jersey Medical School, Newark, New Jersey, USA Detection of Isoniazid-, Fluoroquinolone-, Amikacin-, and Kanamycin-Resistant Tuberculosis in an Automated, Multiplexed 10-Color Assay Suitable for Point-of-Care Use J Clin Microbiol Jan 2017 Volume 55 Issue 1.
6. Centers for Disease Control and Prevention. Biosafety in microbiological and biomedical laboratories. Chosewood, LC and Wilson, DE (eds) (2009). HHS Publication number (CDC) 21-1112.
7. Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards). Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (refer to latest edition).
8. Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards). Laboratory Detection and Identification of Mycobacteria; Document M48A(refer to latest edition).
9. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).
10. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z.
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. Camus JC, et al. 2002. Re-annotation of the genome sequence of Mycobacterium tuberculosis H37Rv. Microbiology 148:2967–73.)
13. Zhang et al. 1996. Molecular basis for the exquisite sensitivity of Mycobacterium tuberculosis to isoniazid. Proc Natl Acad Sci U S A. 1996;93(23):13212–13216
14. Maruri et al. 2012. A systematic review of gyrase mutations associated with fluoroquinolone-resistant Mycobacterium tuberculosis and a proposed gyrase numbering system. J Antimicrob Chemother.
15. Vadwai V, Ajbani K, Jose M, Vineeth VP, Nikam C, Deshmukh M, Shetty A, Soman R, Rodrigues C. Can inhA mutation predict ethionamide resistance? Int J Tuberc Lung Dis. 2013 Jan;17(1):129-30. doi: 10.5588/ijtld.12.0511. Epub 2012 Nov 8. PubMed PMID: 23146620
16. Dunn JJ, Starke JR, Revell PA. 2016. Laboratory diagnosis of Mycobacterium tuberculosis infection and disease in children. J Clin Microbiol 54:1434 –1441. doi:10.1128/JCM.03043
17. Diagnostic Standards and Classification of Tuberculosis in Adults and Children, Am J Respir Crit Care Med Vol 161. pp 1376–1395, 2000.
18. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC (2015) Genetic Mutations Associated with Isoniazid Resistance in Mycobacterium tuberculosis: A Systematic Review. PLoS ONE 10(3): e0119628. doi:10.1371/journal.pone.0119628
19. Farhat MR, Jacobson KR, Franke MF, Kaur D, Sloutsky A, Mitnick CD, Murray M. 2016. Gyrase mutations are associated with variable levels of fluoroquinolone resistance in Mycobacterium tuberculosis. J Clin Microbiol 54:727–733. doi:10.1128/JCM.02775-15.
20. Gagneux S, Small PM. Global phylogeography of Mycobacterium tuberculosis and implications for tuberculosis product development. Lancet Infect Dis. 2007 May;7(5):328-37.
21. 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.

22 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

23 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

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

Contact Information

United States

Telephone: + 1 888 838 3222

Email: techsupport@cepheid.com

France

Telephone: + 33 563 825 319

Email: support@cepheideurope.com

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

24 Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	CE marking – European Conformity
	Do not reuse
	Batch code
	Consult instructions for use
	Manufacturer
	Contains sufficient for <n> tests
	Control
	Expiration date
	Temperature limitation
	Biological risks
	Caution
	Flammable Liquids
	Skin Corrosion
	Severe Health Hazards
	Country of manufacture

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna,
Sweden

25 Revision History

Description of Changes: From 302-3514 Rev. B to 302-3514 Rev. C.

Purpose: To align with the requirements of Regulation (EU) 2017/746.

Revision	Description of Change
Section 3	Added Intended Purpose, Intended User/Environment per IVDR requirements.
Throughout	Instances of "Assay" used as a brand name changed to "assay"
Section 11	Added sections about sputum and sputum sediment transport, as well as a section describing MGIT
Section 17	Added new third bullet to Limitations
Section 18	Added Tables 9-12 with clinical performance data
Section 19	Added 19.7 regarding Fresh and Frozen Sputum Equivalency
Section 25	Added Revision History section.