Assay Technical Training
Xpert® MTB/RIF Ultra

For CE-IVD Use Only

CE IVD In Vitro Diagnostic Medical Device

301-7915 Rev. B July 2017

Training Agenda

- **Xpert® MTB/RIF Ultra**
  - Clinical utility
  - Reagents
  - Sample collection
  - Kit storage and handling
  - Preparing the cartridge
  - Quality Controls
  - Results analysis
- **Discussion**

Training Objectives

- **At the end of the training, user will be able to:**
  - Properly store and handle the Xpert® MTB/RIF Ultra cartridge kit
  - Follow proper laboratory safety precautions
  - Collect and transport appropriate specimen
  - Prepare a cartridge and run the assay
  - Report the various software generated results
  - Understand the assay control strategy

The Cepheid Solution

- Simultaneous detection
  - MTB Complex and Rifampicin resistance associated mutations
- On-board internal controls for each sample
  - Probe Check Control (PCC)
  - Specimen Processing Control (SPC)
- Results in approximately:
  - 65 minutes for a NEGATIVE
  - 77 minutes for a POSITIVE
- Closed cartridge system minimizes risk of contamination
- On-demand results
- Random access

Intended Use

The Xpert MTB/RIF Ultra Assay, 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 MTB positive specimens, it also detects rifampicin-resistance associated mutations of the *rpoB* gene.

The Xpert MTB/RIF Ultra Assay is intended for use with specimens from untreated patients for whom there is clinical suspicion of tuberculosis (TB) and who have received no anti-tuberculosis 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.

Targets and Probes

Targets

- IS1081 and IS6110 (multi-copy insertion element target present in most clinical TB strains)
- Rifampicin resistance determining region (RRDR) of *rpoB* gene (81 base pair)

Probes

- 1 probe for SPC (Sample Processing Control)
- 2 probes bind to *IS1081-IS6110*
- 4 probes bind to Rifampicin-R mutations in *rpoB*

A melt analysis with the 4 *rpoB* probes differentiates between the conserved wild-type sequence and mutations associated with RIF resistant.

Assay Requirements

GeneXpert Systems

- GeneXpert Dx Software **v4.7b** or higher
- Xpertise Software **v6.4b** or higher

Test Kits (CE-IVD)

- GXMTB/RIF-Ultra-10 and GXMTB/RIF-Ultra-50

Sample Collection

- Leak proof, sterile screw-capped collection containers

Other materials

- Personal Protective Equipment (PPE)
- 1:10 Bleach
- 70% ethanol or denatured ethanol

Optional

- Uninterruptible Power Supply /Surge Protector
- Printer
- Vortex

Good Laboratory Practice

Personnel Protective Equipment (PPE)	• Wear clean lab coats and gloves • Change gloves between processing samples
Lab Bench area	• Clean work surfaces routinely with: ✓ 1:10 dilution of household bleach ✓ 70% Ethanol Solution • After cleaning, ensure work surfaces are dry
Specimens, Samples, and Kits Storage	• Store specimens and sample away from kit to prevent contamination
Equipment(s)	• Use filtered tips when recommended • Follow the manufacturer's requirements for calibration and maintenance of equipment(s)

Kit Handling

Xpert MTB/RIF Ultra Kit Contents

Catalog Number	GXMTB/RIF-Ultra-10 GXMTB/RIF-Ultra-50
Cartridges Per Kit	10 or 50
Reagent Vials	10 or 50
Transfer Pipettes	12 or 60
Kit CD	Assay Definition File (ADF)
	Assay Import Instructions
	Package Insert (PDF)
Storage	2-28 ˚C

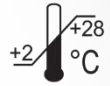

Kit of 10 cartridges


Kit of 50 cartridges

Cartridges contain chemically hazardous substances-please see Package Insert and Safety Data Sheet for more detailed information.

Xpert MTB/RIF Ultra Assay Kit Storage and Handling

- Store the Xpert MTB/RIF Ultra Assay cartridges and reagents at 2–28˚C



- Follow your institution's safety procedures for working with chemicals and handling biological samples
- Do not use Collection Reagent tubes that have not been validated by Cepheid
- Open the Assay cartridge lid only when adding the Sample, close the lid and proceed with processing

Warnings and Precautions

- Do not shake the cartridge
- Do not use a cartridge that… :
  - appears wet, has leaked or if the lid seal appears to have been broken
  - appears damaged
  - has been dropped after removing it from packaging
  - has been dropped or shaken after adding the sample to it
  - has a damaged reaction tube
  - has been used: each cartridge is single-use to process one test
  - is expired
  - Do not reuse spent disposable pipettes

Warnings and Precautions

- 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.

Xpert MTB/RIF Ultra Assay Limitations

- The Xpert MTB/RIF Ultra Assay has only been validated with Sputum and Sputum sediment using Cepheid's recommended procedure as per Package Insert.
- A positive result does not necessarily indicate the presence of viable organisms. It is, however, presumptive for the presence of MTB and Rifampicin resistance.
- Mutations or polymorphism in primer or probe binding regions may affect detection of new or unknown MDR MTB or Rifampicin resistant strains resulting in a false negative result.
- The Xpert MTB/RIF Ultra Assay has only been evaluated in Patients > 18 year old.

For detailed information, refer to the current Package Insert

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Specimen Collection, Storage and Transport

Sputum Sample Collection

- Subject must be seated or standing
- Rinse the patient's mouth twice with water
- Explain to the patient how to:
  - open and close the collection device
  - produce good sputum (collecting real sputum, not saliva)
  - inhale deeply 2/3 times, breath out hard each time, cough deeply from the chest
  - avoid contamination of the exterior of the container (carefully spitting and closing the container)
  - collect and safely deliver the sputum to the laboratory
  - keep the best sample

Sputum is usually thick and mucous.
It may be fluid and contain pieces of purulent material (pus). Color varies from opaque white to green. Bloody specimens will appear reddish or brown.
Clear saliva or nasal discharge is not suitable as a TB specimen.

Source: http://www.stoptb.org/wg/gli/assets/documents/TBLabDiagnosisSputum%20Microscopy_Handbook.pdf-(Images courtesy of A. Van Deun)

Specimen Collection, Transport and Storage

Sample type	Volume	Transportation	Storage Conditions
Unprocessed Sputum	1-4 mL		 up to 3 days  from day 4 to 10
Sputum sediment	0.5-2.5 mL		 up to 7 days

- Collect sputum or induced sputum following your institution's standard procedures.
- Do not accept specimens with obvious food particles or other solid particles

Cartridge Preparation

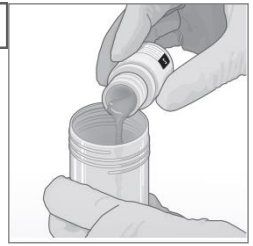
Cartridge Preparation – Unprocessed Sputum

1

Obtain one Xpert cartridge, sample reagent (SR), and sputum collection container for each sample.

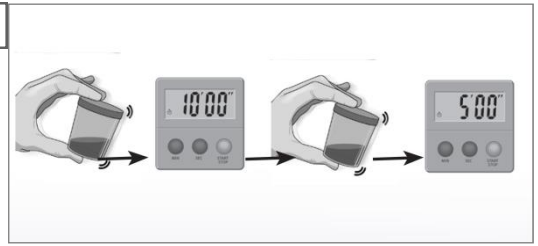
Note: Minimum Sputum volume for one test is 1 ml

2



Estimate volume of sputum. Add 2 volumes of SR to 1 volume of Sputum. Replace container lids.

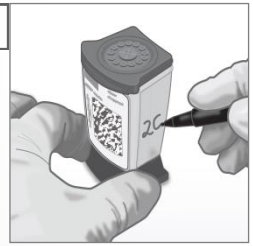
3



- Shake* vigorously 10 to 20 times or vortex for at least 10 seconds.
- Incubate at room temperature for 10 minutes.
- Shake or vortex again vigorously 10 to 20 times.
- Incubate for another 5 minutes **See Note below.

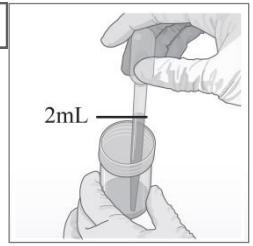
**One back and forth motion equals one shake*
*** Shake and incubate an additional 5 minutes if the sample is not completely liquefied*

4



Bring the cartridge to room temperature. Write on the side of the cartridge or affix an ID label. Open the cartridge lid.

5

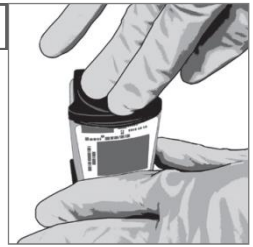


Aspirate the liquefied sample just above the line on the pipette.

6

Slowly empty the sample into the sample chamber of the cartridge.

7



Close the lid firmly.
Start the test within the time frame specified in the package insert.

Adapted from 301-7000

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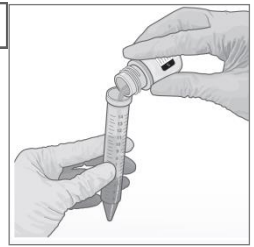
Cartridge Preparation – Sputum Sediment

Sputum sediment prepared according to the Kent and Kubica method is re-suspended in 67mM Phosphate/H2O buffer.*

1

Obtain one Xpert cartridge, sample reagent (SR), and sediment collection container for each sample.

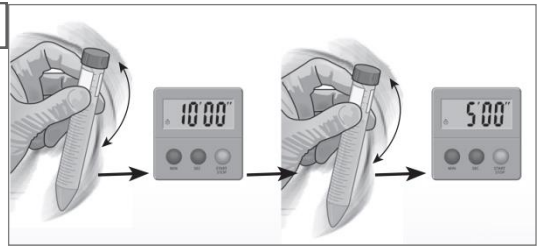
2



Collect at least 0.5mL of sediment*. For volumes ≥ 0.7mL add 2 volumes of reagent. Total volume must be at least 2 mL.

** For volume ~0.5-0.7mL, add 3 volumes of sample reagent to the sediment.*

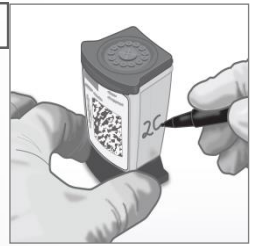
3



- Shake** vigorously 10 to 20 times or vortex for at least 10 seconds.
- Incubate at room temperature for 10 minutes.
- Shake or vortex again vigorously 10 to 20 times.
- Incubate for another 5 minutes **See Note below.

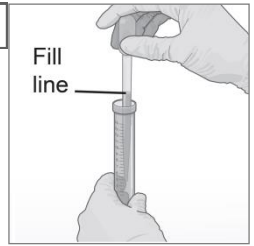
*** Shake and incubate an additional 5 minutes if the sample is not completely liquefied*

4



Bring the cartridge to room temperature. Write on the side of the cartridge or affix an ID label. Open the cartridge.

5

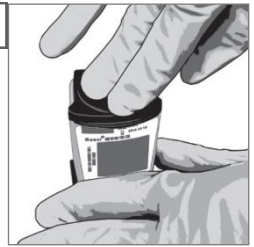


Aspirate the liquefied sample just above the line on the pipette.

6

Slowly empty the sample into the sample chamber of the cartridge.

7



Close the lid firmly.
Start the test within the time frame specified in the package insert.

Adapted from 301-7001

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Run a Test

1 Create Test

GeneXpert

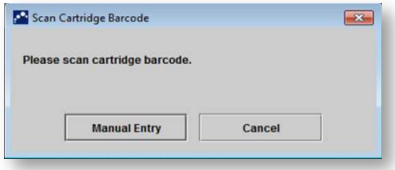
Start the test within 4 hours after adding the sample to the cartridge

GeneXpert Infinity

Place the cartridge on the conveyor within 30 minutes of adding the sample.

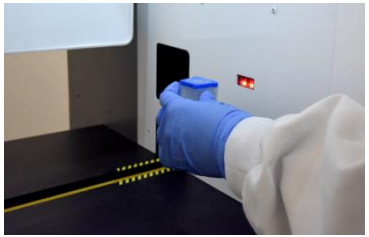
2 Scan barcode messages:

Cartridge/ Patient and/or Sample ID



By default, do not click on **Manual Entry** or **Cancel**

3 Scan the cartridge



"For complete details on how to run a test, refer to the Package Insert and the GeneXpert Dx or Xpertise Dx Operator Manuals.

Create a Test on GeneXpert Dx Software

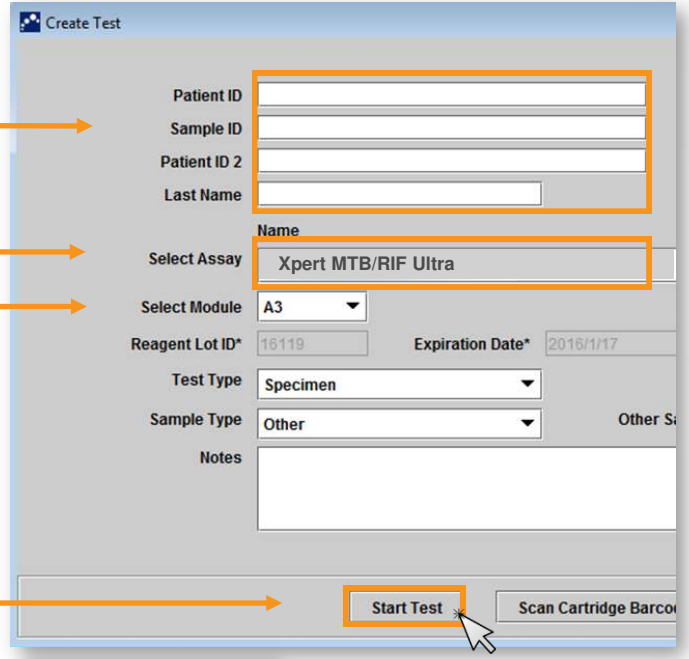
4 Complete the fields as required

5 The Assay Protocol is selected automatically

6 The module is selected automatically
DO NOT CHANGE IT!!!

7 Click on Start Test

8 A green light will flash on the module
Load the cartridge into module and close the door

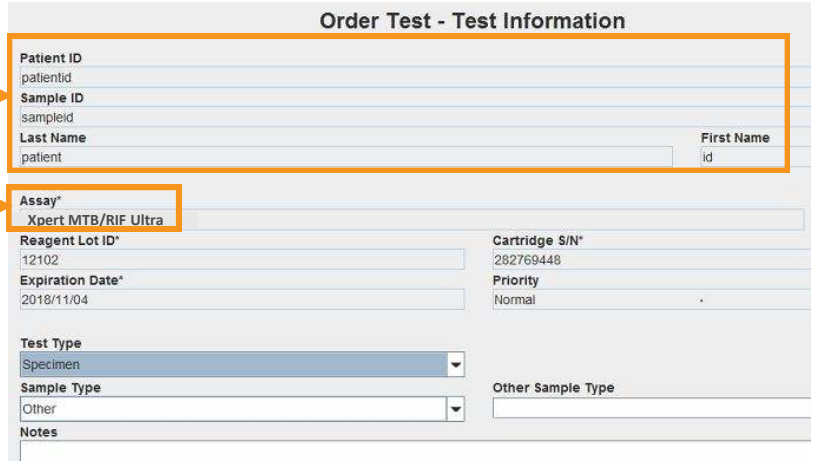


Create a Test on Xpertise Dx Software

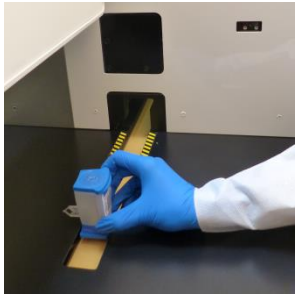
4 Complete the fields as required

5 The Assay Protocol is selected automatically

6 Click on SUBMIT



7 Place the cartridge into the conveyor belt



Automated Xpert Protocol

1 Sample is added to the cartridge

2 The cartridge is loaded into the System

3 Nucleic acids are purified

4 Purified nucleic acids mix with the PCR reagents

5 Simultaneous amplification and detection occurs

6 Results are ready to view

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Quality Controls

Cepheid Control Strategy

CONTROL

- **System System Control – Check Status**
  - System control checks the optics, temperature of the module and mechanical integrity of each cartridge.
  - If the system controls fail, an ERROR test result will be reported.

- **Assay Quality Controls**
  - Each Xpert cartridge is a self-contained test device
  - Cepheid designed specific molecular methods to include internal controls that enable the system to detect specific failure modes within each cartridge.
    - Specimen Processing Control (SPC)
    - Probe Check Controls (PCC)

Internal Quality Controls

- **Probe Check Controls (PCC)**
  - Before the PCR step, fluorescence signal is measured on all probes and compared with default factory settings to monitor
    - bead rehydration
    - reaction tube filling
    - probe integrity
    - dye stability
- **Sample Processing Controls (SPC)**
  - non-infectious spore in each cartridge
    - Verifies adequate sample processing
    - Verifies lysis, presence of the organism and detects PCR inhibition
    - Should be positive in a negative sample
    - Can be positive or negative in a positive sample

Commercially Available External Controls

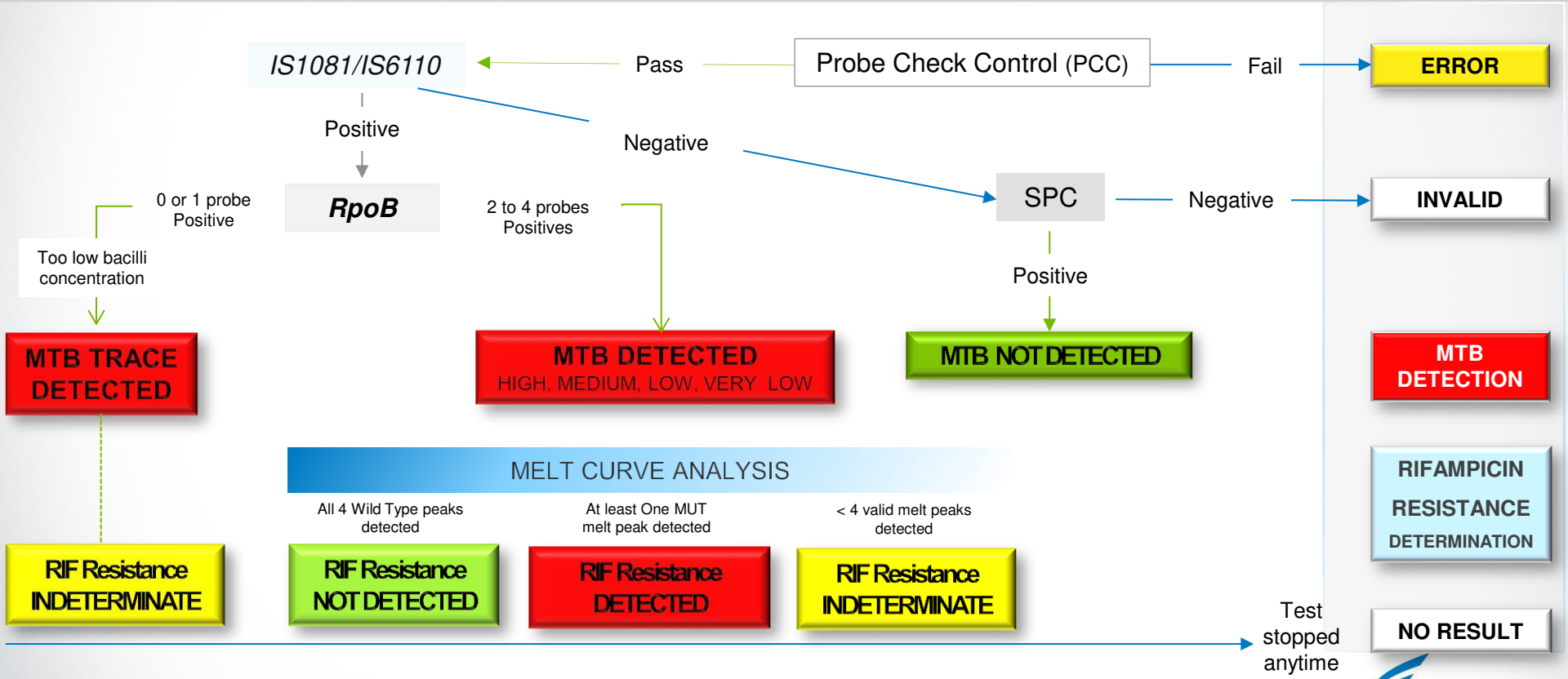
Catalog Number	Description	Configuration	Storage
	MTB/RIF Ultra External Control manufactured by SmartSpots		
DCS-5	SmartSpots Positive Control (HBDC specific)	4 dried spots	Room Temperature
	MTB/RIF Ultra Control Bundle External Control manufactured by MMQCI for Cepheid http://www.mmqci.com/qc-m114.php		
CTRL-M114-5 (MMQCI Cat. number: M114-5)	TBWT-04 (No mutation/ WT H37v)	5 x 1 mL	2-8˚C
	TBMDR1-04 (mutations in: *rpoB, inhA, katG*)	5 x 1 mL	2-8˚C
	TB NEG (Negative control: No TB DNA, no cells)	5 x 1 mL	2-8˚C

For DCS-5 Controls, follow the instruction from manufacturer SmartSpot : http://www.tbgxmonitor.com/
For CRTL-M114-5 Controls, follow the instructions from manufacturer MMQCI : http://www.mmqci.com/qc-m114.php

- Other vendors for quality control material are also available than the one outlined above.
- External controls should be used in accordance with local, state accrediting organizations, as applicable

Result Interpretation

Result Interpretation Algorithm



Xpert MTB/RIF Ultra – All Possible Results

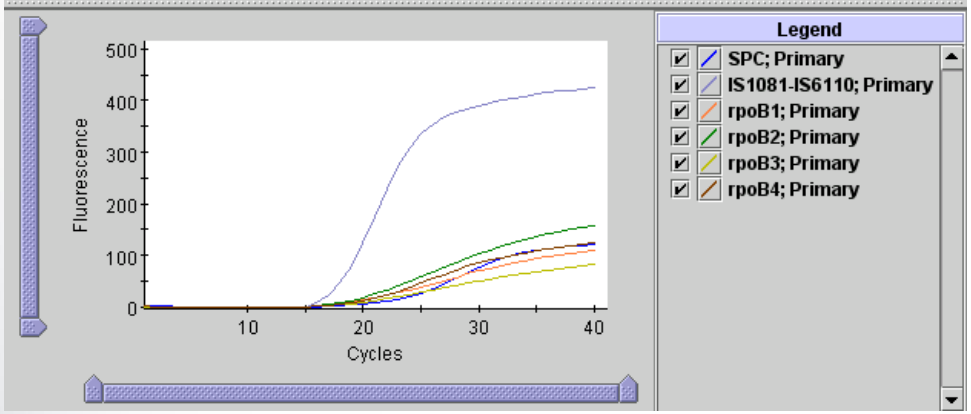
IS1081 / IS6110	rpoB 1/2/3/4	Melt Analysis performed	TB Results	RIF Results
+	2 and more +	Yes	MTB DETECTED HIGH	RIF Resistance DETECTED
+	All +	Yes	MTB DETECTED HIGH	RIF Resistance NOT DETECTED
+	2 and more +	Yes	MTB DETECTED HIGH	RIF Resistance INDETERMINATE
+	2 and more +	Yes	MTB DETECTED MEDIUM	RIF Resistance DETECTED
+	All +	Yes	MTB DETECTED MEDIUM	RIF Resistance NOT DETECTED
+	2 and more +	Yes	MTB DETECTED MEDIUM	RIF Resistance INDERMINATE
+	2 and more +	Yes	MTB DETECTED LOW	RIF Resistance DETECTED
+	All +	Yes	MTB DETECTED LOW	RIF Resistance NOT DETECTED
+	2 and more +	Yes	MTB DETECTED LOW	RIF Resistance INDERTERMINATE
+	2 and more +	Yes	MTB DETECTED VERY LOW	RIF Resistance DETECTED
+	All +	Yes	MTB DETECTED VERY LOW	RIF Resistance NOT DETECTED
+	2 and more +	Yes	MTB DETECTED VERY LOW	RIF Resistance INDETERMINATE
+	0 or 1 +	No	MTB TRACE DETECTED	RIF Resistance INDETERMINATE
FAIL	0,1 or 2 +	No	MTB NOT DETECTED	
FAIL	FAIL	No	INVALID	
FAIL	FAIL	No	INVALID	

MTB DETECTED MEDIUM; RIF Resistance DETECTED

MTB DETECTED MEDIUM;
RIF Resistance DETECTED

Test Result | Analyte Result | Detail | Melt Peaks | Errors | History | Support

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
SPC	25.5	120	NA	PASS
IS1081-IS6110	17.7	425	NA	PASS
rpoB1	25.1	111	POS	PASS
rpoB2	21.6	158	POS	PASS
rpoB3	25.4	83	POS	PASS
rpoB4	23.0	124	POS	PASS



The MTB target is present within the sample:

- At least one *rpoB* mutation has been detected.
- SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control.
- Probe Check: PASS

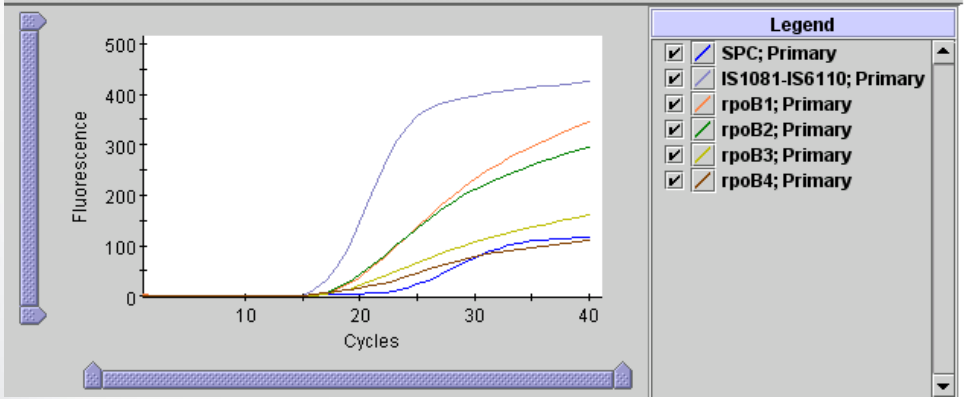
Analyte Name	Melt Peak Temperature	Melt Peak Height
rpoB1 melt		
rpoB2 melt		
rpoB3 melt		
rpoB4 melt	67.3	84.5
rpoB1 Mut melt	63.4	67.8
rpoB2 Mut melt	69.5	103.3
rpoB3 Mut melt	72.6	65.1
rpoB4 Mut melt A		
rpoB4 Mut melt B		

MTB DETECTED MEDIUM; RIF Resistance NOT DETECTED

MTB DETECTED MEDIUM;
RIF Resistance NOT DETECTED

Test Result | Analyte Result | Detail | Melt Peaks | Errors | History | Support

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
SPC	25.9	117	NA	PASS
IS1081-IS6110	17.4	426	NA	PASS
rpoB1	20.1	344	POS	PASS
rpoB2	19.2	295	POS	PASS
rpoB3	21.1	160	POS	PASS
rpoB4	22.7	111	POS	PASS



The MTB target is present within the sample:

- No mutation in the *rpoB* gene target sequence has been detected.
- SPC: NA (not applicable) An SPC is not required because MTB amplification can compete with this control
- Probe Check: PASS

Analyte Name	Melt Peak Temperature	Melt Peak Height
rpoB1 melt	69.2	67.3
rpoB2 melt	73.1	103.3
rpoB3 melt	75.8	87.9
rpoB4 melt	67.4	77.9
rpoB1 Mut melt		
rpoB2 Mut melt		
rpoB3 Mut melt		
rpoB4 Mut melt A		
rpoB4 Mut melt B		

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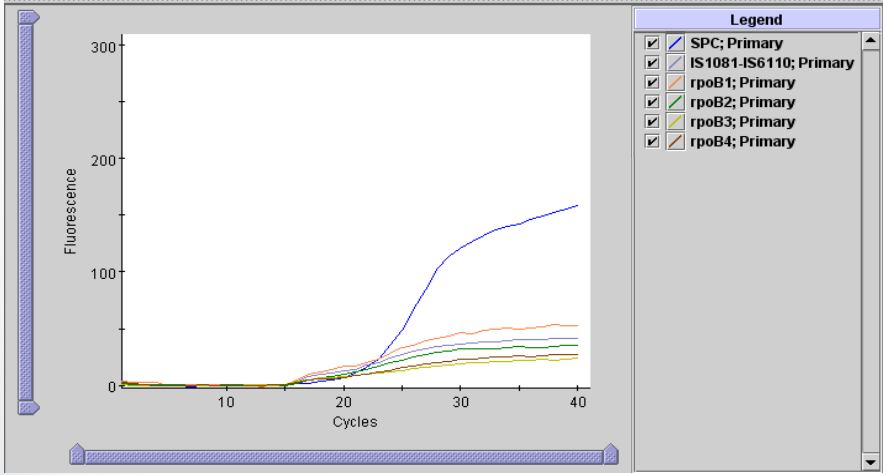
MTB DETECTED LOW; RIF Resistance INDETERMINATE

MTB DETECTED LOW;
RIF Resistance INDETERMINATE

Test Result | Analyte Result | Detail | Melt Peaks | Errors | History | Support

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
SPC	23.6	159	NA	PASS
IS1081-IS6110	35.7	41	NA	PASS
rpoB1	27.2	53	POS	PASS
rpoB2	29.1	35	POS	PASS
rpoB3	0.0	24	NEG	PASS
rpoB4	0.0	27	NEG	PASS



The MTB target is present within the sample:

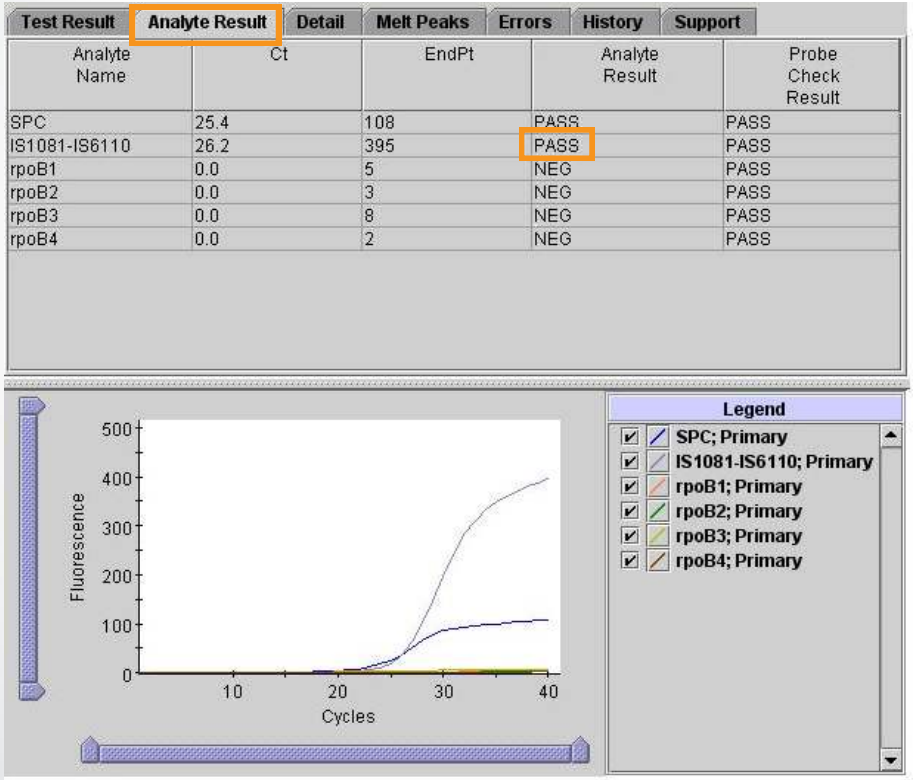
- *IS1081/IS6110* signal is detected
- RIF resistance could not be determined due to insufficient signal detection
- SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control.
- Probe Check: PASS

Analyte Name	Melt Peak Temperature	Melt Peak Height
rpoB1 melt		
rpoB2 melt		
rpoB3 melt		
rpoB4 melt		
rpoB1 Mut melt		
rpoB2 Mut melt		
rpoB3 Mut melt		
rpoB4 Mut melt A		
rpoB4 Mut melt B		

Cepheid

MTB Trace DETECTED

MTB Trace DETECTED;
RIF Resistance INDETERMINATE



The MTB target is present within the sample:

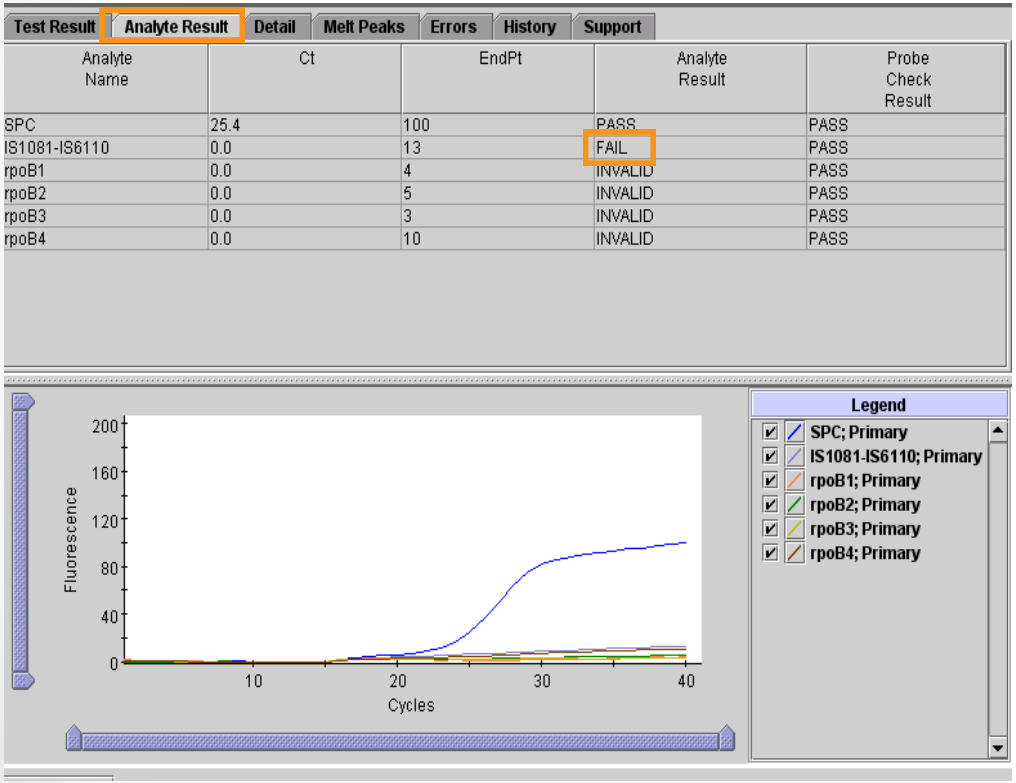
- *IS1081-IS6110* signal is detected.
- RIF resistance cannot be determined due to insufficient signal detection.
- SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control.
- Probe Check: PASS

A TRACE result call means that low levels of MTB are detected but no RIF resistant result is detected.

A TRACE result is always RIF Resistance INDETERMINATE

MTB NOT DETECTED

MTB NOT DETECTED



The MTB target is NOT detected within the sample:

- RIF resistance cannot be determined due to insufficient signal detection
- SPC: PASS. The SPC met the acceptance criteria.
- Probe Check: PASS

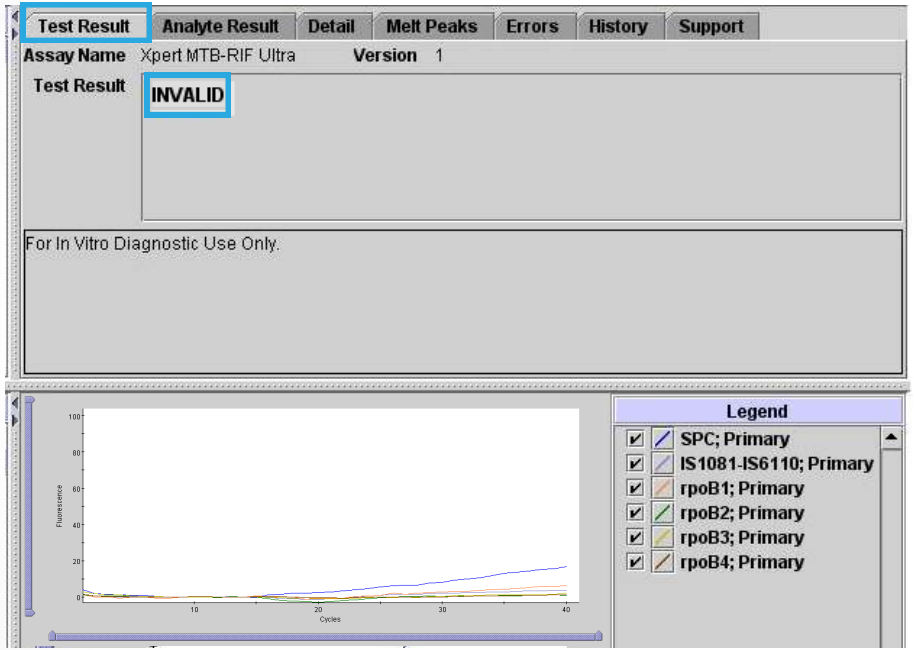
Troubleshooting

Factors That Negatively Affect Results

- Improper specimen collection
  - The bacterial load in the specimen is below the detection limit of the test
  - Performance with extra-pulmonary specimen types has not been assessed
- Improper transport or storage of collected specimen
  - Storage and transport conditions are specimen specific
  - Refer to the Package Insert for the appropriate handling instructions
- Improper testing procedure
  - Modification to the testing procedures may alter the performance of the test
  - Careful compliance with the package insert is necessary to avoid erroneous results

INVALID Result

INVALID

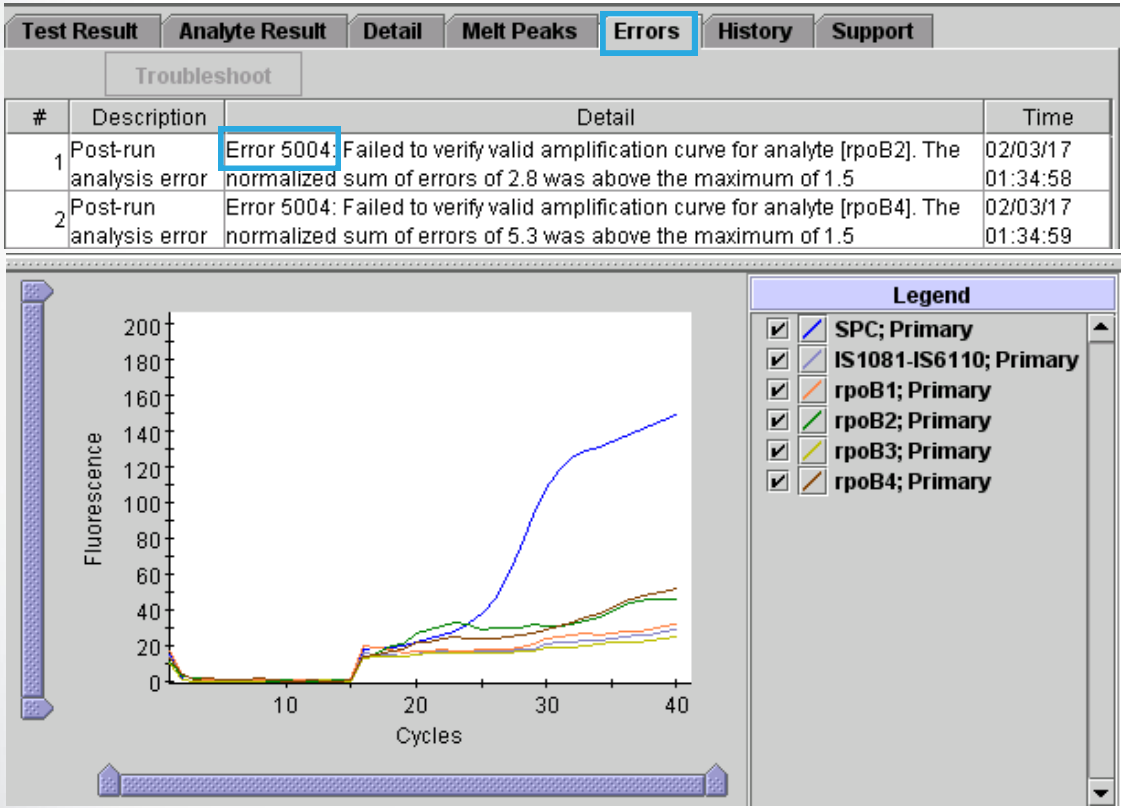


The presence or absence of MTB target cannot be determined

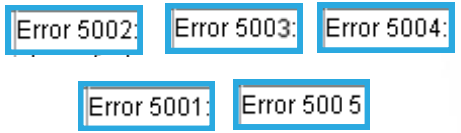
- SPC: FAIL. The SPC does not meet the acceptance criteria.
- Probe Check: PASS
- Possible Cause
  - Improper sample collection
  - Incorrect sample preparation
  - Improper storage of the cartridges
  - Inefficient sample processing in cartridge
  - Missing primer/probe or enzyme beads
  - Presence of interfering substances in the sample
- Solution
  - Repeat the test with a new cartridge and new sample

INVALID Result

INVALID



- The presence or absence of MTB cannot be determined

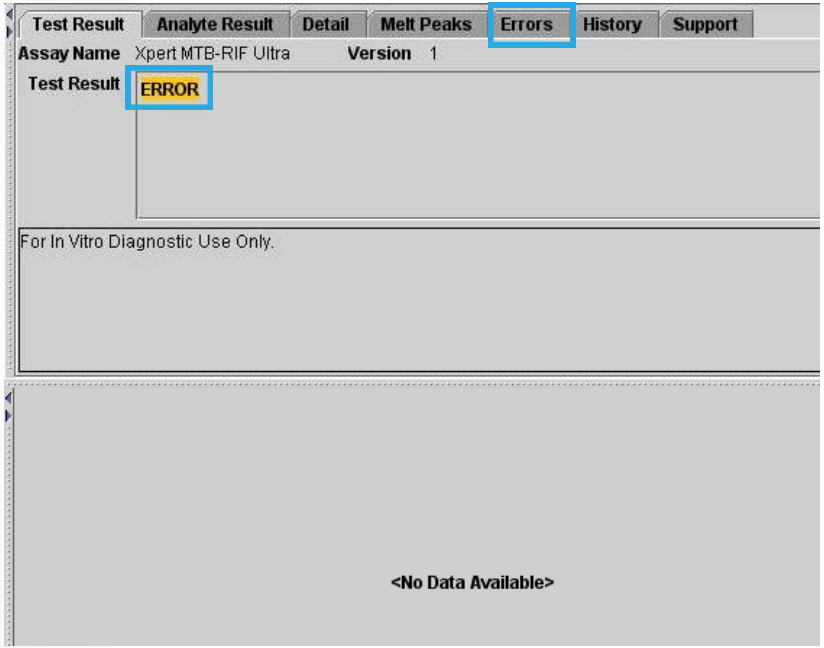


- **Cause**
  - Defect in reaction tube
  - Poor thermal contact of the reaction tube with the instrument thermal plates
- **Solution**
  - Repeat the test with a new cartridge

Cepheid

ERROR

ERROR



- The presence or absence of MTB cannot be determined
- MTB: NO RESULT.
- SPC: NO RESULT.
- If Probe Check: FAIL

Possible Cause

- Improper Sample collection
- Incorrect Sample volume added to the cartridge

- If Probe Check: PASS

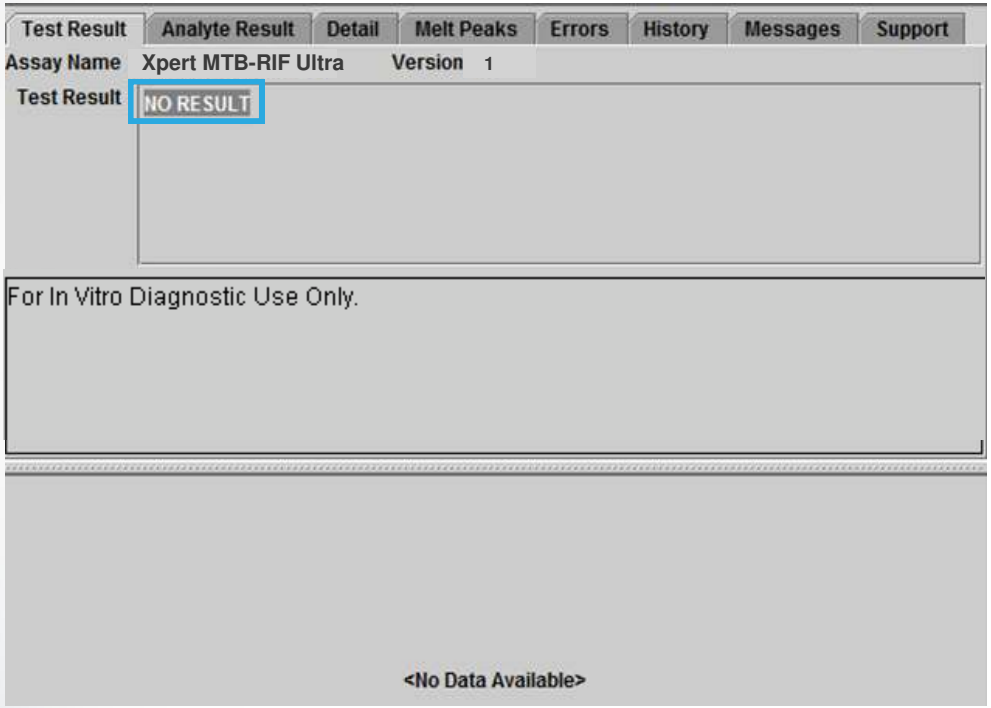
Cause

- Check the GeneXpert System module

- **Solution**
  - Repeat the test with a new cartridge

NO RESULT

NO RESULT



- The presence or absence of MTB cannot be determined.
- MTB: NO RESULT
- SPC: NO RESULT
- Probe Check: NA (not applicable)
- **Possible Cause**
  - A NO RESULT indicates that insufficient data were collected.
  - Test was stopped with stop test button
  - Electrical failure
- **Solution**
  - Secure the power
  - Repeat the test with a new cartridge

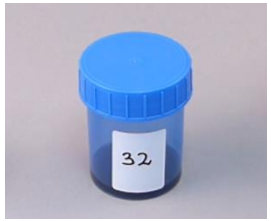
Re-test Procedure

1

Discard used cartridge

Follow your institution's safety guidelines for disposal of cartridges

2



Obtain the residual sample, mix according to Package Insert

If the leftover sample volume is insufficient, or the retest continues to return an INVALID, ERROR, or NO RESULT, collect a new sample

3

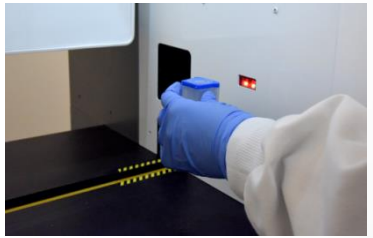
Obtain a new cartridge

Label appropriately as retest on the new cartridge

Process the sample per the package insert

4

Run the test on the System



Technical Assistance

- Before contacting Cepheid Technical Support, collect the following information:
  - Product name
  - Lot number
  - Serial number of the System
  - Error messages (if any)
  - Software version and, if applicable, Computer Service Tag number

- Log your complaint online using the following link ***http://www.cepheid.com/us/support*** **:** *Create a Support Case*

Region	Telephone	Technical Support Email
US	+ 1 888 838 3222	techsupport@cepheid.com
Australia and New Zealand	+ 1800 107 884 (AU) + 0800 001 028 (NZ)	techsupportANZ@cepheid.com
Brazil and Latin America	+ 55 11 3524 8373	latamsupport@cepheid.com
China	+ 86 021 5406 5387	techsupportchina@cepheid.com
France	+ 33 563 825 319	support@cepheideurope.com
Germany	+ 49 69 710 480 480	support@cepheideurope.com
India, Bangladesh, Bhutan, Nepal, and Sri Lanka	+ 91 11 48353010	techsupportindia@cepheid.com
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United Kingdom	+ 44 3303 332 533	support@cepheideurope.com
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Thank You.
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