# Assay Training: Xpert® Carba-R

Cepheid Technical Support

CE-IVD

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# Training Agenda

- Xpert Carba-R Training
  - Reagents
  - Sample collection
  - Kit storage and handling
  - Precautions
  - Preparing cartridge
- Quality Control
- Results analysis
- Discussion and Q&A

# Xpert Carba-R Training Objectives

**At the end of the training, user will be able to:**

- Properly store and handle the Xpert Carba-R cartridge kits.
- Follow proper laboratory safety precautions.
- Collect appropriate specimen types and transport specimen.
- Perform the cartridge set up and run the assay.
- Report the various software-generated results.
- Understand assay control strategy.

# Introduction on disease state
## *What is Carbapenem resistance (Carba-R)?*

- **Carbapenems are a type of beta-lactam antibiotic, often an antibiotic of last resort.**
  - **Carbapenem resistance (Carba-R) is a result of chromosomal and highly mobile plasmid mediated resistance genes associated with gram-negative enteric organisms, Pseudomonas, and Acinetobacters.**
  - **Infection with carbapenem non-susceptible organisms are associated with high mortality (up to 40-50%).[1]**
  - **Screening for colonized patients can expedite infection control practices and reduce likelihood of nosocomial spread.**

[1]Guidance for control of carbapenem-resistant Enterobacteriaceae (CRE): 2012 CRE Toolkit. Division of Healthcare Quality Promotion. Centers for Disease Control and Prevention. Atlanta, Georgia. 2012.

# The Cepheid Solution


- **Simultaneous detection and differentiation**
  - Five Carba-R gene targets
  - Two internal controls for each individual sample
  - Sample Processing Control (SPC)
  - Probe Check Control (PCC)
- **High sensitivity and specificity**
- **Simple and easy to use**
  - Closed cartridge system
- **Results in 48 minutes**
- **On-demand results 24/7**
- **Random access**

# Intended Use

The Cepheid Xpert Carba-R Assay, performed on the GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test designed for rapid detection and differentiation of the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP-1}$ gene sequences associated with carbapenem-non-susceptibility in gram-negative bacteria obtained from rectal swab specimens from patients at risk for intestinal colonization with carbapenem-non-susceptible bacteria. The test utilizes automated real-time polymerase chain reaction (PCR). The Xpert Carba-R Assay is intended to aid in the detection of carbapenem-non susceptible bacteria that colonize patients in healthcare settings. The Xpert Carba-R Assay is not intended to guide or monitor treatment for carbapenem-non-susceptible bacterial infections.

Concomitant cultures are necessary to recover organisms for epidemiological typing, antimicrobial susceptibility testing, and for further confirmatory identification of carbapenem-non-susceptible bacteria.

# System and Reagent Requirements

## GeneXpert Systems

- 6 color modules
- GX DX Software v4.3 or higher

## Test kits (CE-IVD)

- GXCARBAR-CE-10

## Sample collection kits

- Cepheid catalog number 900-0370

# Xpert Carba-R Kit

| | Xpert Carba-R Assay |
|---|---|
| **Catalogue Number** | **GXCARBAR-CE-10** |
| **Tests per kit** | 10 |
| **Contents per test cartridge** | Dry & Liquid Reagents |
| **Transfer pipettes** | 10 |
| **Sample Reagent vials** | 10 |
| **Storage** | 2-28°C |

# Xpert Carba-R Sample Collection, Transport, and Storage

Cepheid Sample Collection Device (P/N 900-0370)

SCORE MARK

- **Sample Type:**
  - **Rectal Swab Collection**
- **Sample Collection:**
  - **Collect a paired rectal swab by carefully inserting both swab tips approximately 1 cm beyond the anal sphincter and rotate gently.**
- **Sample Transport and Storage:**
  - **Directly after collection, the swab sample can be held for up to 6 hours at 15– 28$^0$C. Thereafter, the swab can be stored at 2 – 28$^0$C for 7 days.**

# Sample Collection

GeneXpert®

## Rectal Specimen Collection Protocol

for use with Xpert® assays:

- Xpert Carba-R

1

A Copan Venturi Transystem double swab must be used to collect the specimen.
*Cepheid Collection Device #900-0370*

2

Carefully insert both swab tips approximately 1 cm beyond the anal sphincter and rotate gently.

3

Place the swabs back in the tube.

4

Specimens that can be tested within 6 hours can be stored at 15-28 ºC. Specimens stored at 2-28 ºC are stable for up to 7 days.

defining *on-demand* molecular diagnostics

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# Good Laboratory Practice

| | |
|---|---|
| **PCR laboratory setup** | • Cartridge/reagent preparation → Sample addition → Detection |
| **Specimen and reagent storage** | • Store specimens separately from reagents to prevent reagent contamination. |
| **Equipment** | • Use filtered pipette tips, when needed, for QC dilutions.<br>• Follow the manufacturer's recommendation for calibration and maintenance of the lab equipment. |

# Good Laboratory Practice, cont'd

| | |
|---|---|
| **Housekeeping** | • Clean work surfaces with a final concentration of 1:10 dilution of household bleach and then a 70% ethanol or 70% isopropanol solution. Wipe work surfaces dry.<br>• If contamination occurs, thoroughly clean the contaminated area with 1:10 dilution of household bleach, DNA AWAY, or 3% (w/v) hydrogen peroxide and rinse thoroughly with water. Wipe work surfaces dry. |
| **Personnel** | • Wear clean lab coats and gloves.<br>• Change gloves between processing samples. |
| **Lab bench area** | • Clean the lab bench area routinely.<br>• Keep the back of the instrument dust free. |

# Xpert Carba-R Kit Storage and Handling

- Store test kits at 2-28ºC. Do not use expired cartridges.
- Each single-use cartridge is used to process one test. Do not reuse processed cartridges.
- Do not open a cartridge until ready to add the sample eluted from the swab to the cartridge..
  - Cartridge should be placed onto the instrument within 30 minutes of adding the sample into the cartridge.
- Avoid cross contamination during sample handling steps.
  - Change gloves between processing each sample.
  - Change gloves before leaving work area and upon entry into work area.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results.
- Do not use a cartridge that has a damaged reaction tube.



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# Xpert Carba-R Cartridge Preparation

## Xpert® Cartridge Preparation Carba-R

Refer to the package insert for detailed instructions, precautions, and warnings.

For a copy of the SDS, visit www.cepheid.com or www.cepheidinternational.com

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1 Obtain one Xpert cartridge and one Sample Reagent vial for each sample.

2 Insert the swab into the Sample Reagent vial.

3 Break the swab at the score mark near the opening of the vial.

4 Recap the Sample Reagent vial and vortex for 10 seconds.

5 Open the Xpert cartridge lid.

6 Aspirate the Sample Reagent up to the line on the supplied pipette.

7 Empty the pipette into the sample chamber.

8 Close the Xpert cartridge lid.

9 Start the test within the timeframe specified in the package insert.

*Note: Do not hold the swab below the score mark. Use gauze or its equivalent to minimize the risk of contamination.*



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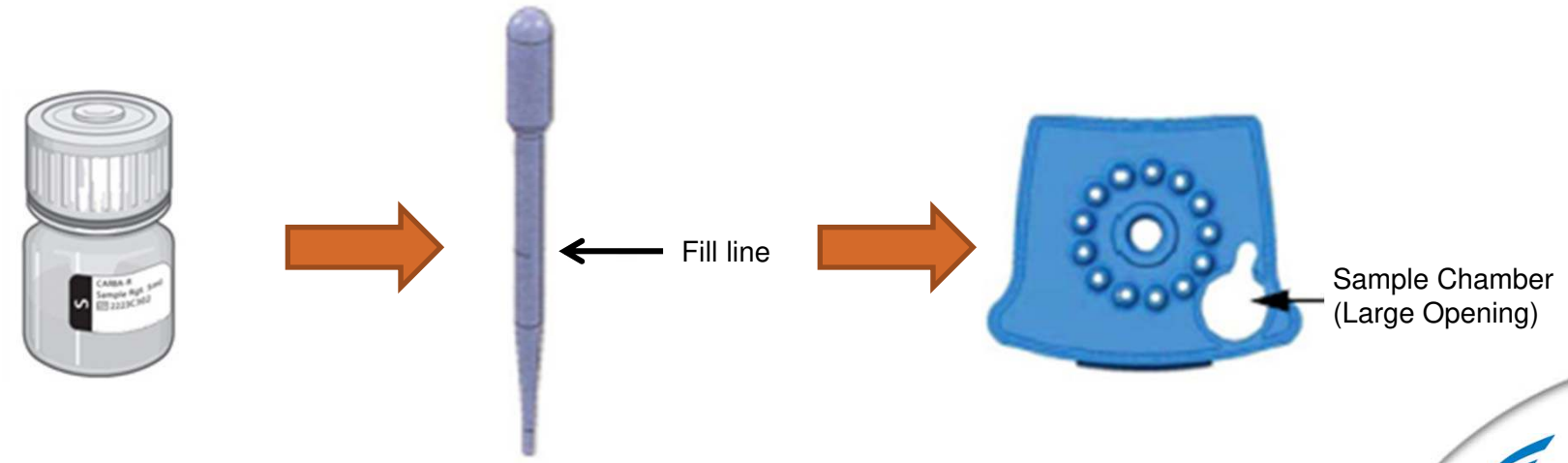
# Running the Test

- **Place a single swab into the Sample Reagent vial; break off at score-mark using sterile gauze to minimize risks of contamination.**
  - **Place the unused swab into the transport tube and store at 2-28ºC.**
- **Vortex the Sample Reagent vial for 10 seconds at high speed.**
- **Open the Sample Reagent vial.**
- **Fill the transfer pipette to the mark on the pipette.**
  - **Avoid air bubbles.**
  - **The remaining sample in the sample reagent vial can be retained at 2-28ºC for up to 4 days in case a retest is required.**



# Running the Test- new cartridge image

1. Put the eluted sample in the cartridge, then place the cartridge in the instrument.
2. The sample combines with the SPC.
3. A filter captures the sample and the SPC.
4. Ultrasonically lysed cells release nucleic acids.
5. Purified DNA mixes with dried-down bead reagents.
6. Simultaneous PCR amplification and detection occurs.
7. Results are ready to view and print in about 48 minutes!

# Quality Control

**Refer to the Package Insert for further details**

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# Cepheid Assay Control Strategy

- **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.**
    - **Instrument system control: Check status**
    - **Reagent control: Probe Check**
    - **Sample processing control: SPC and/or SAC**
    - **Amplification control: SPC and/or SAC and/or IC**

# Instrument System Control – Check Status

- **The Instrument 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.**

# Reagent Control - Probe Check Control

- **After sample preparation, bead reconstitution, and tube filling (prior to thermal cycling), multiple fluorescent readings are taken at different temperatures.**
- **The readings are compared to default settings established by Cepheid.**
- **The Probe Check controls for:**
  - **Missing Target Specific Reagent (TSR) and/or Enzyme Reagent beads, which contain all primers, probes, and internal control template**
  - **Incomplete reagent reconstitution**
  - **Incomplete reaction tube fill**
  - **Probe degradation**
- **If the Probe Check fails, an ERROR test result will be reported.**

# Sample Processing Control - SPC

- The Sample Processing Control (SPC) assesses the effectiveness of the sample preparation steps, including reaction tube filling.
- SPC is *Bacillus globigii* spores.
- The SPC controls for:
  - Missing primer/probe or enzyme beads
  - Incomplete reagent reconstitution
  - Incomplete reaction tube fill
  - Enzyme degradation
  - Sample lysis, nucleic acid extraction, and integrity of nucleic acid
  - Sample inhibition
- The SPC can be negative or positive in an analyte-positive sample.
- If the SPC fails in an analyte-negative sample, an INVALID test result will be reported.

# Commercially Available External Controls

| Organism Name | Beta-lactamases Present | Source |
|---|---|---|
| *Klebsiella pneumoniae* | KPC-3 | NCTC 13438 |
| *Klebsiella pneumoniae* | KPC | ATCC BAA-1705 |
| *Escherichia coli* | IMP | NCTC 13476 |
| *Pseudomonas aeruginosa* | VIM-10 | NCTC 13437 |
| *Klebsiella pneumoniae* | VIM-1 | NCTC 13439 |
| *Klebsiella pneumoniae* | VIM-1 | NCTC 13440 |
| *Klebsiella pneumoniae* | NDM-1 | NCTC 13443 |
| *Klebsiella pneumoniae* | NDM-1 | ATCC BAA-2146 |
| *Klebsiella pneumoniae* | OXA-48 | NCTC 13442 |

- **Other options:**
  - **Known patient positive and negative samples**

# Results Analysis

## Refer to the Package Insert for further details

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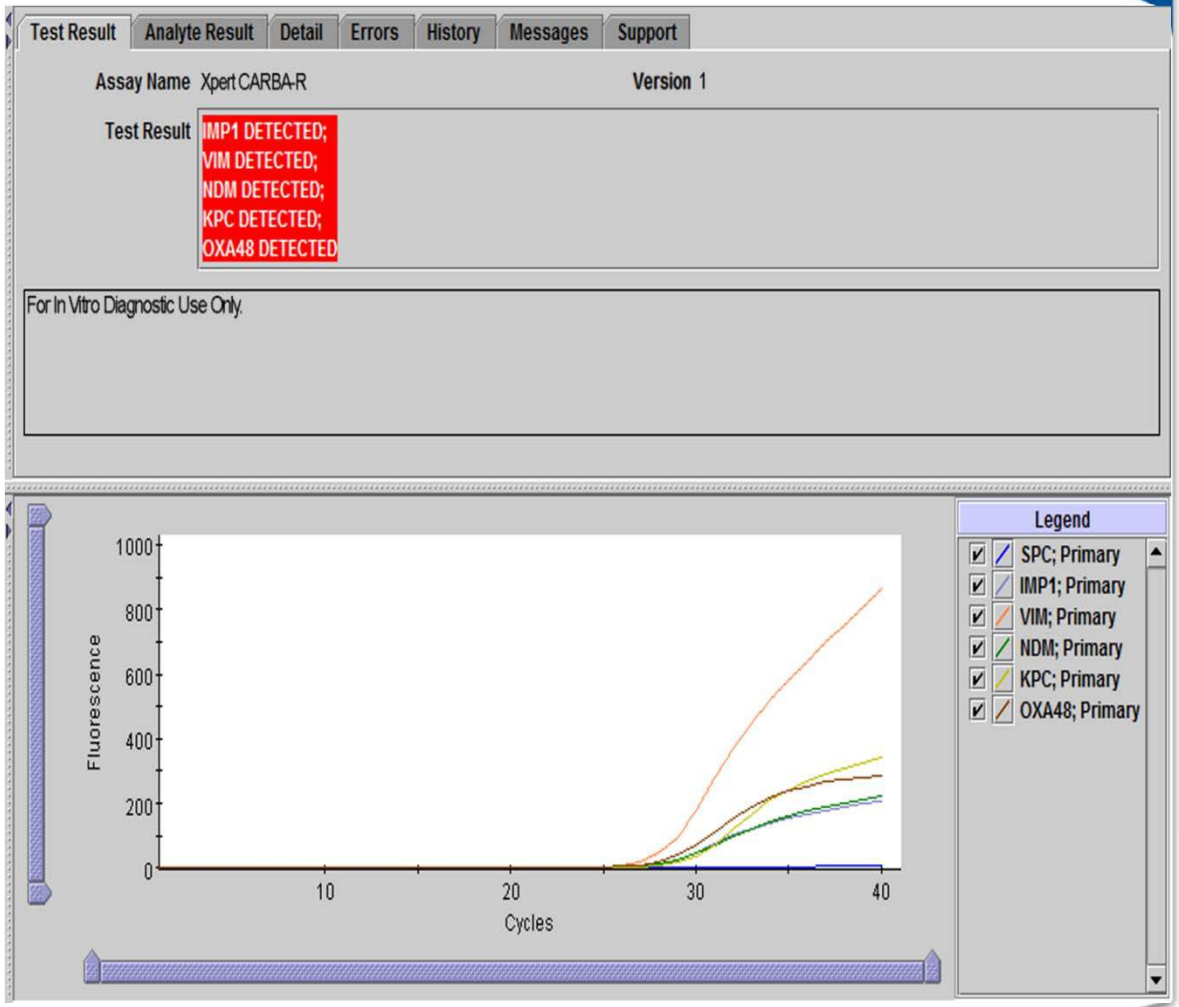
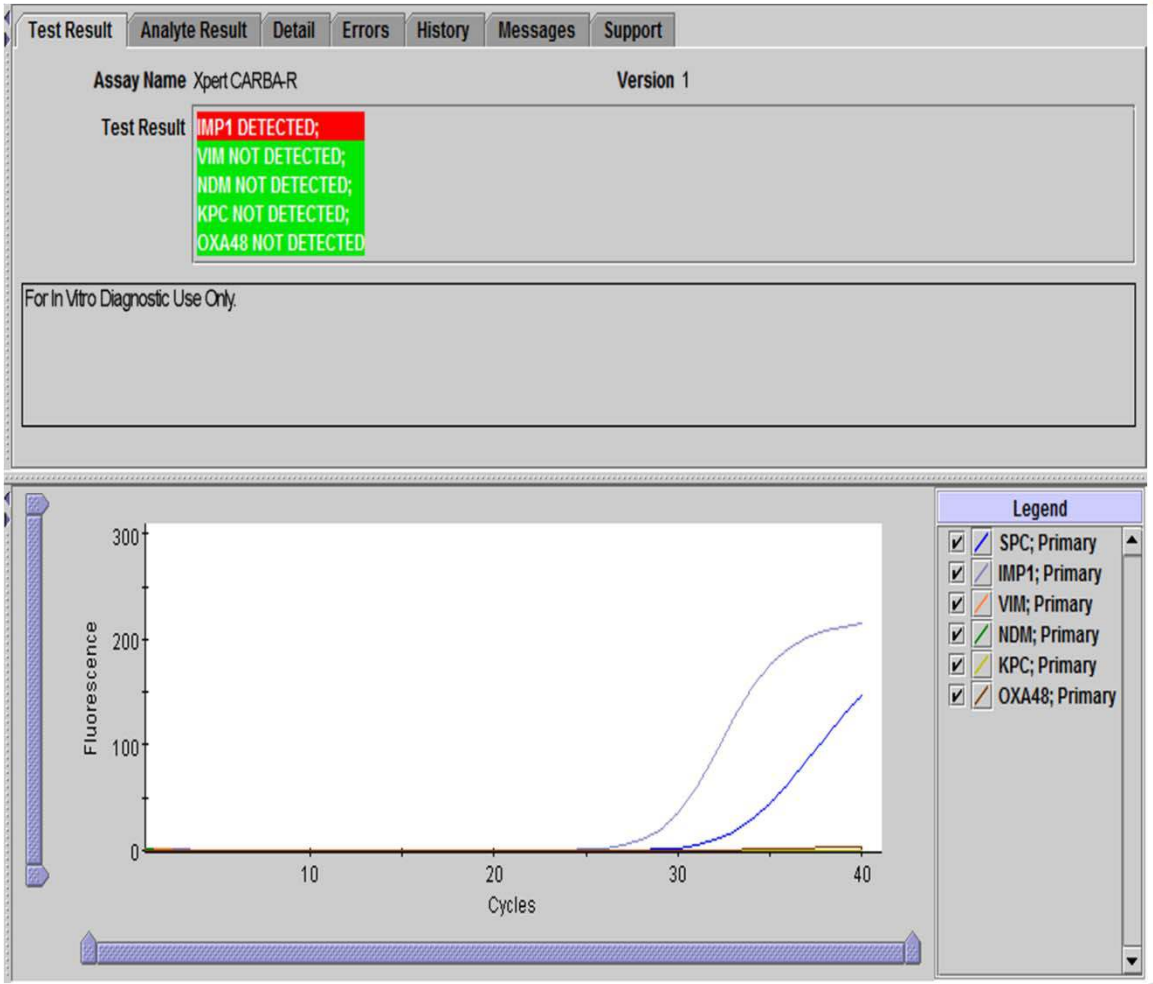
# Xpert Carba-R Results:
# IMP1 Pos/ VIM Pos/ NDM Pos/ KPC Pos/ OXA48 Pos

- **IMP1, VIM, NDM, KPC and OXA48 target DNA sequences are detected.**
- **SPC: Not applicable (N/A). The SPC is ignored because IMP1, VIM, NDM, KPC and OXA48 target DNA amplifications can compete with this control.**
- **Probe Check: PASS. All probe check results pass.**



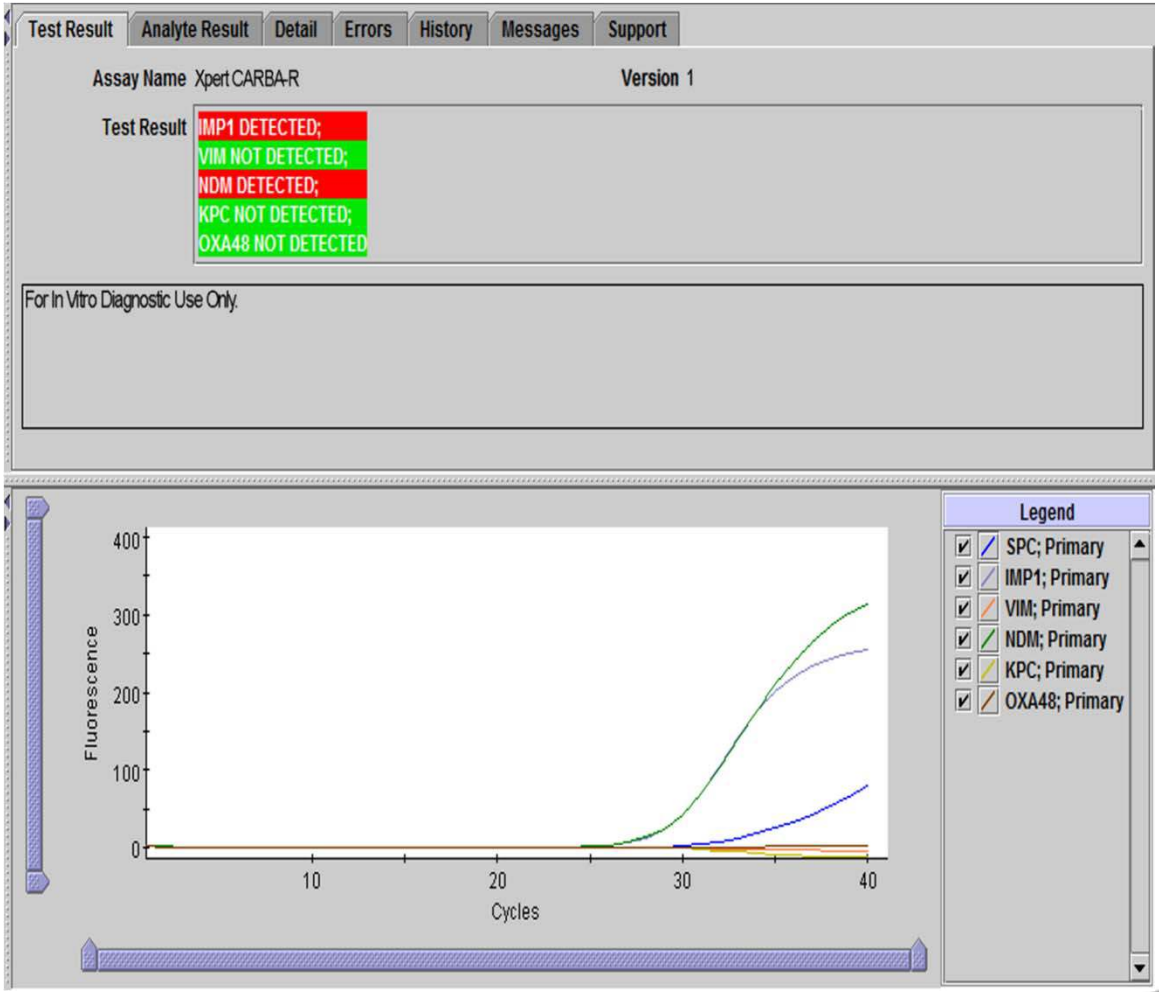
# Xpert Carba-R Results:
# IMP1 Pos/ VIM Neg/ NDM Neg/ KPC Neg/ OXA48 Neg

- **IMP1 target DNA sequence is detected; VIM, NDM, KPC and OXA48 target DNA sequences are not detected.**
- **SPC: Not applicable (NA). SPC is ignored because IMP1 target DNA amplification can compete with this control.**
- **Probe Check: PASS. All probe check results pass.**



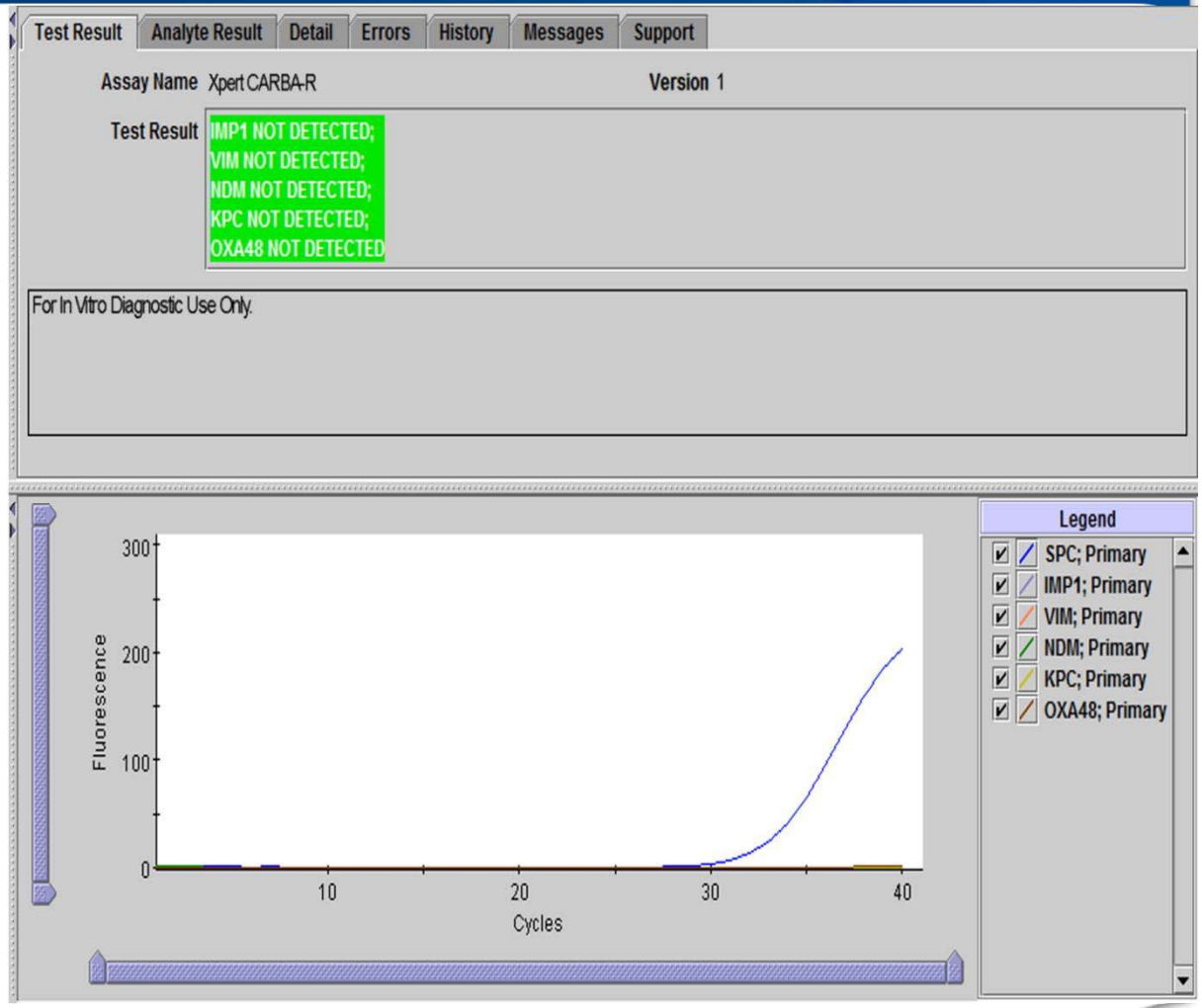
# Xpert Carba-R Results:
# IMP1 Pos/ VIM Neg/ NDM Pos/ KPC Neg/ OXA48 Neg

- **IMP1 and NDM target DNA sequences are detected; VIM, KPC and OXA48 target DNA sequences are not detected.**
- **SPC: Not applicable (NA). SPC is ignored because IMP1 and NDM target DNA amplification can compete with this control.**
- **Probe Check: PASS. All probe check results pass.**



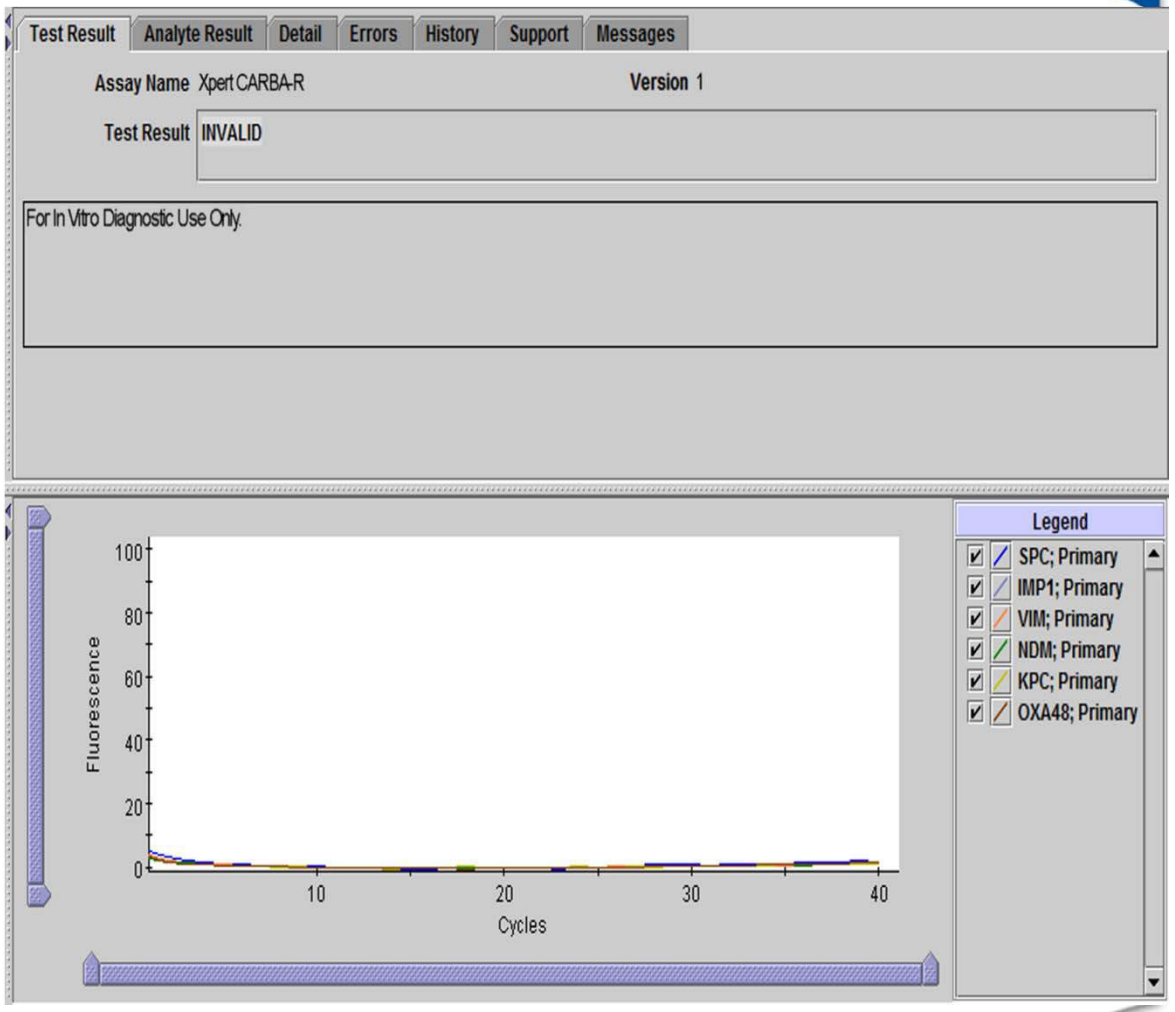
# Xpert Carba-R Results:
# IMP1 Neg/ VIM Neg/ NDM Neg/ KPC Neg/ OXA48 Neg

- **IMP1, VIM, NDM, KPC and OXA48 target DNA sequences are not detected.**
- **SPC: PASS; PCR amplification of the SPC DNA sequence gives a Ct value within the valid range and a fluorescence endpoint above the minimum setting.**
- **Probe Check: PASS. All probe check results pass.**



# Xpert Carba-R Results: INVALID

- **Presence or absence of IMP1, VIM, NDM, KPC and OXA48 target DNA sequences cannot be determined.**
- **SPC: FAIL; No PCR amplification of the SPC DNA sequence or the SPC Ct is not within valid range and the fluorescence endpoint is below minimum setting.**
- **PCC: PASS; all probe check results pass.**

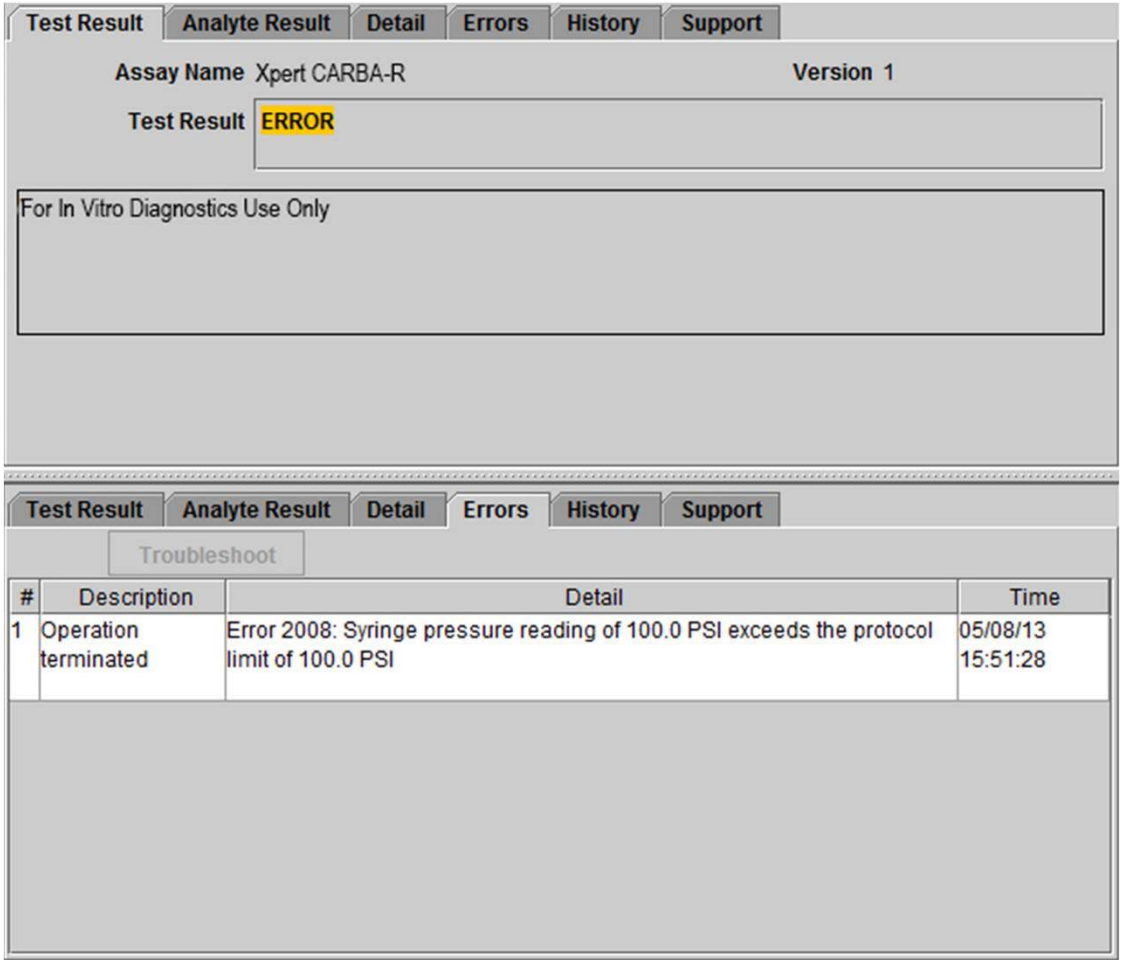


# Xpert Carba-R Result: ERROR

- **Presence or absence of IMP1, VIM, NDM, KPC and OXA48 target DNA sequences cannot be determined.**

- **SPC: NO RESULT**
- **PCC: FAIL*; all or one of the probe check results fail. The PCC probably failed because the reaction tube was filled improperly or a probe integrity problem was detected.**

* **If the probe check passed, the error is caused by a system component failure.**



# Xpert Carba-R Result: NO RESULT

- **Presence or absence of Carba-R target DNA cannot be determined. Use the instructions in Section 13, Retest Procedure in the Package Insert to repeat the test. Insufficient data were collected to produce a test result (for example, the operator stopped a test that was in progress).**

- **SPC: NO RESULT**
- **PCC: Not applicable**

# Reasons to Repeat the Assay

- An INVALID result indicates that the SPC control failed. The sample was not properly processed or PCR was inhibited.
- An ERROR result indicates that the Probe Check control failed and the assay was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, or because 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.

# Factors That Negatively Affect Results

- Improper specimen collection
  - Performance with other collection devices and specimen types has not been assessed.
  - For assays that contain the SAC control, a specimen that does not contain human cells will result in an invalid test result.
- 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.
  - Technical error or sample mix-up can impact test results.
  - Careful compliance with the Package Insert is necessary to avoid erroneous results.
- Interfering substance
  - False negative test results or invalid results may be observed in the presence of interfering substance.
- The number of organisms in the specimen is below the detection limit of the test
- Refer to Package Insert for non-determinate rate

# Interfering Substances

- **Of the 23 potentially inhibitory substances tested, Pepto-Bismol 0.25%w/v had a statistically significant inhibitory effect on the detection of IMP-1 in the Xpert Carba-R Assay. No other statistically significant inhibitory effects were observed.**

- **Please refer to the Xpert Carba-R Package Insert for additional data on potentially interfering substances.**

# Carba-R Retest Procedure

| | | |
|---|---|---|
| 1 | Discard used cartridge. |  |
| 2 | From the test kit, remove new: Xpert Carba-R cartridge, Sample Reagent vial, and transfer pipette. |  |
| 3 | Transfer the remaining liquid from the original Sample Reagent vial (stored ≤ 4 days at 2-8°C) to the new Sample Reagent vial. |  |
| 4 | Repeat the test with a new cartridge. |  |
| 5 | Follow the Package Insert on how to run a test. |  |

# Discussion and Q&A

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# Technical Support

- **Cepheid provides technical support in the field, on the phone, by fax, and by email.**
- **North America:**
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# Discussion and Q&A