GeneXpert®
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Xpert® Carba-R

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GXCARBAR-CE-120

In Vitro Diagnostic Medical Device IVD CE

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Xpert® Carba-R

For *In Vitro* Diagnostic Use.

1 Proprietary Name

Xpert® Carba-R

2 Common or Usual Name

Xpert Carba-R Assay

3 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.

4 Summary and Explanation

The global spread of carbapenemase-producing Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter* species (i.e., carbapenem non-susceptible organisms, CNSOs) is a critical medical and public health issue.[1,2] These bacteria are often resistant to all beta-lactam agents and frequently are co-resistant to multiple classes of other antimicrobial agents, leaving very few treatment options.[3] Tracing the spread of CNSOs is complicated by the diversity of carbapenem-hydrolyzing enzymes that have emerged and the ability of the genes to spread among multiple bacterial species. Some of the resistance genes, such as the *Klebsiella pneumoniae* carbapenemase (KPC) determinants, are associated with successful clonal lineages of bacteria (e.g., *K. pneumoniae* ST258),[4] which have a selective advantage in hospital settings where antimicrobial use is high. Opportunities for transmission of organisms are often frequent, with further dissemination of the resistance genes via transmissible plasmids and integrons. *K. pneumoniae* strain ST258 has caused multiple epidemics globally, especially in the United States[1] and Israel.[5] Similarly, organisms containing the gene encoding New Delhi metallo-beta-lactamase (NDM) have been introduced into Europe by individuals who, in many cases, have visited India or Pakistan.[6] A third mechanism of carbapenem resistance, mediated by Verona integron-mediated metallo-beta-lactamase (VIM), has been a concern in Europe for several years. Additional metallo-beta-lactamases, such as those in the imipenemase (IMP) class, have been recognized in Japan and other Asian countries for many years, and are now spreading globally,[3] while the Class D oxacillinase, OXA-48, which often mediates low-level carbapenem resistance but not resistance to the extended-spectrum beta-lactamases, is now spreading rapidly in Europe.[7,8] Currently, the standard method for detecting patients who are colonized with carbapenem-non-susceptible organisms is to culture rectal or peri-rectal swab samples on non-selective agar plates, such as MacConkey agar, followed by antimicrobial susceptibility testing of lactose fermenting colonies, or by using selective screening agar media.[9] The former is laborious and can require several days to generate a final result, while the latter approach varies considerably in sensitivity and specificity based on the selective medium used. A rapid and accurate method for screening patients for colonization with CNSOs will facilitate the ability of infection control programs to interrupt the spread of CNSOs in hospitals and other healthcare venues. Screening patients in the United States for colonization with CNSOs is recommended by the Centers for Disease Control and Prevention (CDC) whenever a carbapenem-resistant strain of Enterobacteriaceae has been recognized in a hospital.[10] Many European countries, including the United Kingdom, France, and the Netherlands, also have national policies that advocate screening patients for CNSOs on admission to a hospital, especially if they previously have been hospitalized in a foreign country.[9]

5 Principle of the Procedure

The GeneXpert (GX) Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequence in simple or complex samples using real-time PCR assays. The systems consist of an instrument, personal computer, and preloaded software for performing tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the system, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

The Xpert Carba-R Assay includes reagents for the detection of bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP-1} gene sequences as well as a sample processing control (SPC) to control for adequate processing of the target bacteria and to indicate the presence of inhibitor(s) in the PCR reaction. The SPC also ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the PCR reagents are functional. An additional internal control, the Probe Check Control (PCC), verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The primers and probes in the Xpert Carba-R Assay detect proprietary sequences for the bla_{KPC} (KPC), bla_{NDM} (NDM), bla_{VIM} (VIM), bla_{OXA-48} (OXA-48), and bla_{IMP-1} (IMP-1) gene sequences associated with carbapenem-non-susceptibility in gram-negative bacteria.

6 Reagents and Instruments

6.1 Material Provided

The Xpert Carba-R Assay kit contains sufficient reagents to process 10 samples. The Xpert Carba-R Assay kit contains sufficient reagents to process 120 samples. The kits contain the following:

Xpert Carba-R Assay Cartridges with Integrated Reaction Tubes	**10 per kit**	**120 per kit**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge	1 of each per cartridge
• Reagent 1	3.0 mL per cartridge	3.0 mL per cartridge
• Reagent 2 (Guanidinium chloride)	2.5 mL per cartridge	2.5 mL per cartridge
Xpert Carba-R Assay Sample Reagent Vials	**10 per kit**	**120 per kit**
• Sample Reagent	5.0 mL per vial	5.0 mL per vial
Disposable (1.7 mL) Transfer Pipettes	**10 per kit**	**120 per kit**
CD	**1 per kit**	**1 per kit**
• Assay Definition Files (ADF)		
• Instructions to import ADF into software		
• Package Insert		

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 commingling of the material with other animal materials.

6.2 Storage and Handling

- Store the Xpert Carba-R Assay cartridges and reagents at 2 °C to 28 °C.
- Do not open a cartridge until you are ready to perform testing.
- Do not use reagents or cartridges that have passed the expiration date.
- The Sample Reagent is a clear, colorless liquid. Do not use the Sample Reagent if it has become cloudy or discolored.
- Use the cartridge within 30 minutes after opening the cartridge lid.
- Do not use a cartridge that has leaked.

6.3 Materials Required but Not Provided

- GeneXpert Dx Instrument or GeneXpert Infinity Systems (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, Operator Manual.
  - For GeneXpert Dx System: GeneXpert Dx software version 4.3 or higher
- Specimen Collection Device: Cepheid Catalog number 900-0370
- Printer: If a printer is required, contact Cepheid Technical support to arrange for the purchase of a recommended printer.
- Vortex mixer

7 Warnings and Precautions

- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[11] and the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards).[12]
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Consult your institution's environmental waste personnel on proper disposal of used cartridges and unused reagents. Check state and local regulations as they may differ from federal disposal regulations. This material may exhibit characteristics of hazardous waste requiring specific disposal requirements. Institutions should check their country hazardous waste disposal requirements.
- Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens or reagents.
- Do not substitute Xpert Carba-R Assay Sample Reagent with other reagents.
- Do not open the Xpert Carba-R Assay cartridge lid until you are ready to add the sample eluted from the swab.
- 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 place the sample ID label on the cartridge lid or on the bar code label.

- Each single-use Xpert Carba-R Assay cartridge is used to process one test. Do not reuse spent cartridges.
- Do not use a cartridge that has a damaged reaction tube.
- Wear clean lab coats and gloves. Change gloves between processing each sample.
- In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with a solution of 1:10 dilution of household chlorine bleach and then a 70% ethanol or 70% isopropanol solution. Wipe work surfaces dry completely before proceeding.

- Reagent 2 contains Guanidinium chloride (H302, harmful if swallowed; H315, causes skin irritation; and H319, causes serious eye irritation).

8 Specimen Collection, Transport, and Storage

1. Collect a paired rectal swab by carefully inserting both swab tips approximately 1 cm beyond the anal sphincter and rotate gently.
2. Place swab pair back into the original transport tube.

3. Swabs in the transport tube can be stored at 15 – 28 °C for up to six hours and thereafter at 2 – 28 °C for seven days.

4. Rectal swabs placed into sample reagent on the day of collection can be stored at 2 – 28 °C for up to four days.

9 Procedure

9.1 Preparing the Cartridge

Important **Place the cartridge into the GeneXpert instrument within 30 minutes of adding the sample into the cartridge.**

To add the swab sample to the cartridge:

1. Remove the cartridge and Sample Reagent vial from the kit.
2. Open one vial of the Sample Reagent provided and place one swab into the vial.
3. Replace the unused swab into the transport tube and store at 2 – 28 °C. See Section 8.

Note Wrap sterile gauze around both the stem of the swab and the mouth of the tube to minimize the risk of contamination.

4. Hold the swab by the stem near the rim of the vial, lift the swab a few millimeters from the bottom of the vial and bend the stem over the edge of the vial to break it off at the score mark, leaving the swab short enough to allow the swab to fit into the vial and to allow the cap to close tightly.
5. Close the Sample Reagent cap and vortex at high speed for 10 seconds.
6. Open the cartridge lid. Using the transfer pipette provided, aspirate the Sample Reagent up to the mark on the pipette (which is approximately 1.7 mL; see Figure 1) and then transfer the material into the sample chamber of the Xpert Carba-R cartridge. See Figure 2. The remaining sample in the sample reagent vial can be retained at 2 – 28 °C for up to four days from day of collection in case a retest is required.

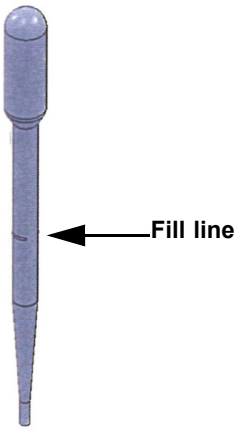

Figure 1. Transfer Pipette to Transfer Sample to Cartridge

7. Close the cartridge lid and place the cartridge into the GeneXpert instrument within 30 minutes.

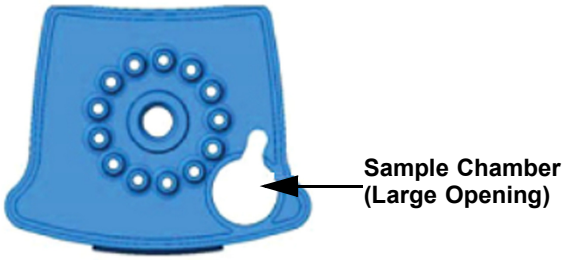

Figure 2. Xpert Carba-R Assay Cartridge (Top View)

9.2 Starting the Test

Important **Before starting the test, make sure the Xpert Carba-R 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* or the *GeneXpert Infinity System Operator Manual.***

Note The steps you follow can be different if the system administrator changed the default workflow of the system.

1. Turn on the GeneXpert instrument system:
   - If using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. The GeneXpert software will launch automatically or may require double-clicking the GeneXpert Dx software shortcut icon on the Windows® desktop.

     or

   - If using the GeneXpert Infinity instrument, power up the instrument. The Xpertise software will launch automatically or may require double clicking the Xpertise software 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 System window, click **Create Test** (GeneXpert Dx) or click **Orders** and **Order Test** (Infinity).
4. Scan in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and is shown in the View Results window.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is associated with the test results and is shown in the View Results window.
6. Scan the barcode on the Xpert Carba-R Assay cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

Note If the barcode on the Xpert Carba-R cartridge does not scan, then set up a new test by following the retest procedure in Section 13.

7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity). Enter your password, if requested.
8. For the GeneXpert Infinity System, place the cartridge on the conveyor belt. The cartridge will be automatically loaded, the test will run, and the used cartridge will be placed into the waste container.

   or

   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. Then remove the cartridge.

   D. The used cartridges should be disposed in the appropriate specimen waste containers according to your institution's standard practices.

9.3 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* or the *GeneXpert Infinity System Operator Manual*.

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

10 Quality Control

CONTROL

Built-in Quality Controls

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

- **Sample Processing Control (SPC)**—Ensures the sample was correctly processed. The SPC contains spores of *Bacillus globigii* in the form of a dry bead that is included in each cartridge to verify adequate processing of the sample. The SPC verifies that lysis of bacteria has occurred if the organisms are present and 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 validated 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. Probe Check passes if it meets the assigned acceptance criteria.

External Controls

External controls may be used in accordance with local, state, and federal accrediting organizations, as applicable.

11 Interpretation of Results

The results are interpreted by the GeneXpert System from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window. Screenshots and interpretations for all possible combinations of results with the five target analytes in the Xpert Carba-R assay are not shown; however, the following examples are indicative of the type of results that can be expected.

Note The following table and figures show only representative examples of the types of results that can be expected with the Xpert Carba-R Assay. Not all possible combinations of results with the five target analytes are shown.

Table 1. Xpert Carba-R Assay Representative Results and Interpretation

Result	Interpretation
IMP1 DETECTED; VIM NOT DETECTED; NDM NOT DETECTED; KPC NOT DETECTED; OXA48 NOT DETECTED See Figure 3.	IMP-1 target DNA sequence is detected; VIM, NDM, KPC, and OXA-48 target DNA sequences are not detected. • PCR amplification of the IMP-1 target DNA gives a Ct value within the valid range and a fluorescence endpoint above the threshold setting; VIM, NDM, KPC, and OXA-48 target DNA sequences are absent or below the assay detection level. • SPC: Not applicable. The SPC is ignored because IMP-1 target DNA amplification may compete with this control. • PCC: PASS; all probe check results pass.
IMP1 NOT DETECTED; VIM DETECTED; NDM NOT DETECTED; KPC NOT DETECTED; OXA48 NOT DETECTED See Figure 4.	VIM target DNA sequence is detected; IMP-1, NDM, KPC, and OXA-48 target DNA sequences are not detected. • PCR amplification of the VIM target DNA gives a Ct value within the valid range and a fluorescence endpoint above the threshold setting; IMP-1, NDM, KPC, and OXA-48 target DNA sequences are absent or below the assay detection level. • SPC: Not applicable. The SPC is ignored because VIM target DNA amplification may compete with this control. • PCC: PASS; all probe check results pass.
IMP1 NOT DETECTED; VIM DETECTED; NDM DETECTED; KPC NOT DETECTED; OXA48 NOT DETECTED See Figure 5.	VIM and NDM target DNA sequences are detected; IMP-1, KPC, and OXA-48 target DNA sequences are not detected. • PCR amplification of the VIM and NDM target DNAs give Ct values within the valid ranges and fluorescence endpoints above the threshold settings; IMP-1, KPC, and OXA-48 target DNA sequences are absent or below the assay detection level. • SPC: Not applicable. The SPC is ignored because VIM and NDM target DNA amplifications may compete with this control. • PCC: PASS; all probe check results pass.

Table 1. Xpert Carba-R Assay Representative Results and Interpretation (Continued)

Result	Interpretation
IMP1 DETECTED; VIM NOT DETECTED; NDM DETECTED; KPC NOT DETECTED; OXA48 NOT DETECTED See Figure 6.	IMP-1 and NDM target DNA sequences are detected; VIM, KPC, and OXA-48 target DNA sequences are not detected. • PCR amplification of the IMP-1 and NDM target DNAs give Ct values within the valid ranges and fluorescence endpoints above the threshold settings; VIM, KPC, and OXA-48 target DNA sequences are absent or below the assay detection level. • SPC: Not applicable. The SPC is ignored because IMP-1 and NDM target DNA amplifications may compete with this control. • PCC: PASS; all probe check results pass.
IMP1 DETECTED; VIM DETECTED; NDM NOT DETECTED; KPC NOT DETECTED; OXA48 DETECTED See Figure 7.	IMP-1, VIM, and OXA-48 target DNA sequences are detected; NDM and KPC target DNA sequences are not detected. • PCR amplification of the IMP-1, VIM, and OXA-48 target DNAs give Ct values within the valid ranges and fluorescence endpoints above the threshold settings; KPC and NDM target DNA sequences are absent or below the assay detection level. • SPC: Not applicable. The SPC is ignored because IMP-1, VIM, and OXA-48 target DNA amplifications may compete with this control. • PCC: PASS; all probe check results pass.
IMP1 DETECTED; VIM DETECTED; NDM DETECTED; KPC NOT DETECTED; OXA48 DETECTED See Figure 8.	IMP-1, VIM, NDM, and OXA-48 target DNA sequences are detected; KPC target DNA sequence is not detected. • PCR amplification of the IMP-1, VIM, NDM, and OXA-48 target DNAs give Ct values within the valid ranges and fluorescence endpoints above the threshold settings; the KPC target DNA sequence is absent or below the assay detection level. • SPC: Not applicable. The SPC is ignored because IMP-1, VIM, NDM, and OXA-48 target DNA amplifications may compete with this control. • PCC: PASS; all probe check results pass.
IMP1 DETECTED; VIM DETECTED; NDM DETECTED; KPC DETECTED; OXA48 DETECTED See Figure 9.	IMP-1, VIM, NDM, KPC, and OXA-48 target DNA sequences are detected. • PCR amplification of the IMP-1, VIM, NDM, KPC, and OXA-48 target DNAs give Ct values within the valid ranges and fluorescence endpoints above the threshold settings. • SPC: Not applicable. The SPC is ignored because IMP-1, VIM, NDM, KPC, and OXA-48 target DNA amplifications may compete with this control. • PCC: PASS; all probe check results pass.
IMP1 NOT DETECTED; VIM NOT DETECTED; NDM NOT DETECTED; KPC NOT DETECTED; OXA48 NOT DETECTED See Figure 10.	IMP-1, VIM, NDM, KPC, and OXA-48 target DNA sequences are not detected. • IMP-1, VIM, NDM, KPC, and OXA-48 target DNA sequences are absent or below the assay detection level. • SPC: PASS; PCR amplification of the SPC DNA sequence gives a Ct value within the valid range and a fluorescence endpoint above the threshold setting. • PCC: PASS; all probe check results pass.
INVALID See Figure 11.	Presence or absence of IMP-1, VIM, NDM, KPC, and OXA-48 target DNA sequences cannot be determined. Use the instructions in Section 13, Retest Procedure, to repeat the test. • 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 threshold setting. • PCC: PASS; all probe check results pass.
ERROR	Presence or absence of IMP-1, VIM, NDM, KPC, and OXA-48 target DNA sequences cannot be determined. Use the instructions in Section 13, Retest Procedure, to repeat the test. • SPC: NO RESULT • PCC: FAIL*; one or more of the probe check results failed. 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.

Table 1. Xpert Carba-R Assay Representative Results and Interpretation (Continued)

Result	Interpretation
NO RESULT	Presence or absence of IMP-1, VIM, NDM, KPC, and OXA-48 target DNA sequences cannot be determined. Use the instructions in Section 13, Retest Procedure, to repeat the test. Insufficient data were collected to produce a test result (for example, the operator stopped a test that was in progress or a power failure occurred). • SPC: NO RESULT • PCC: Not applicable

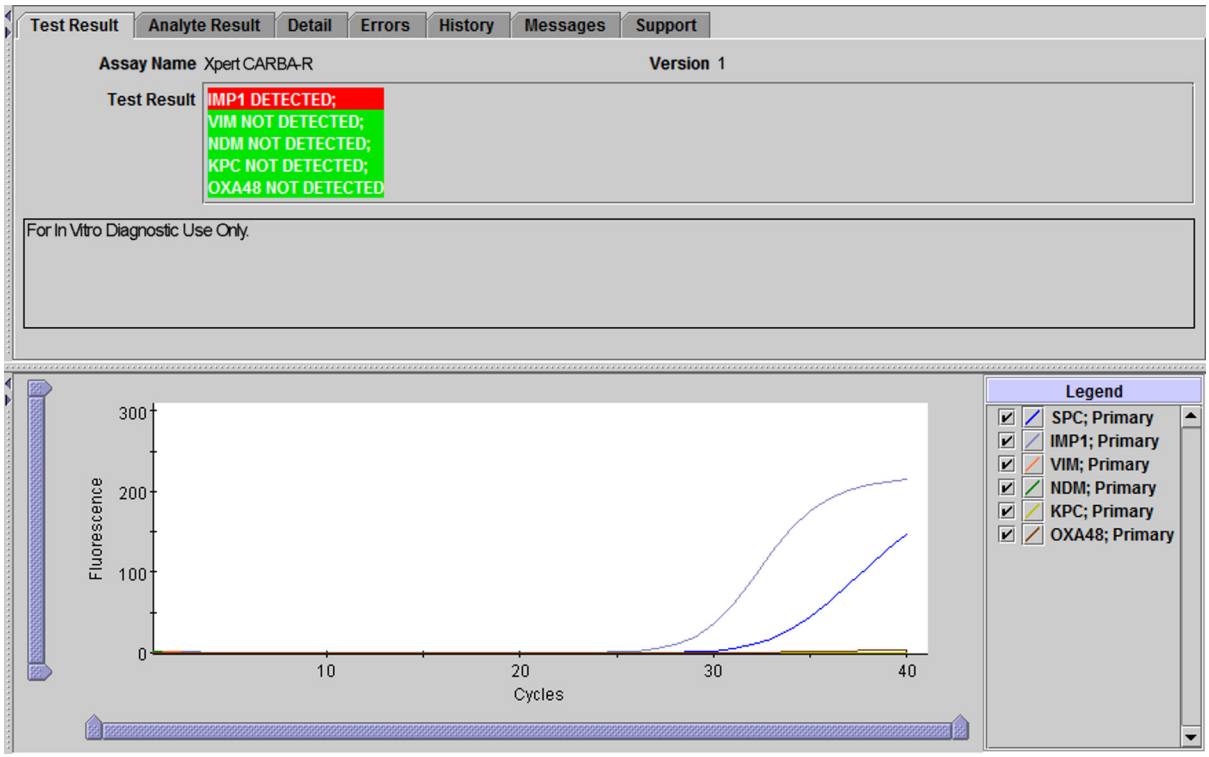

Figure 3. Carba-R Assay—IMP-1 Detected

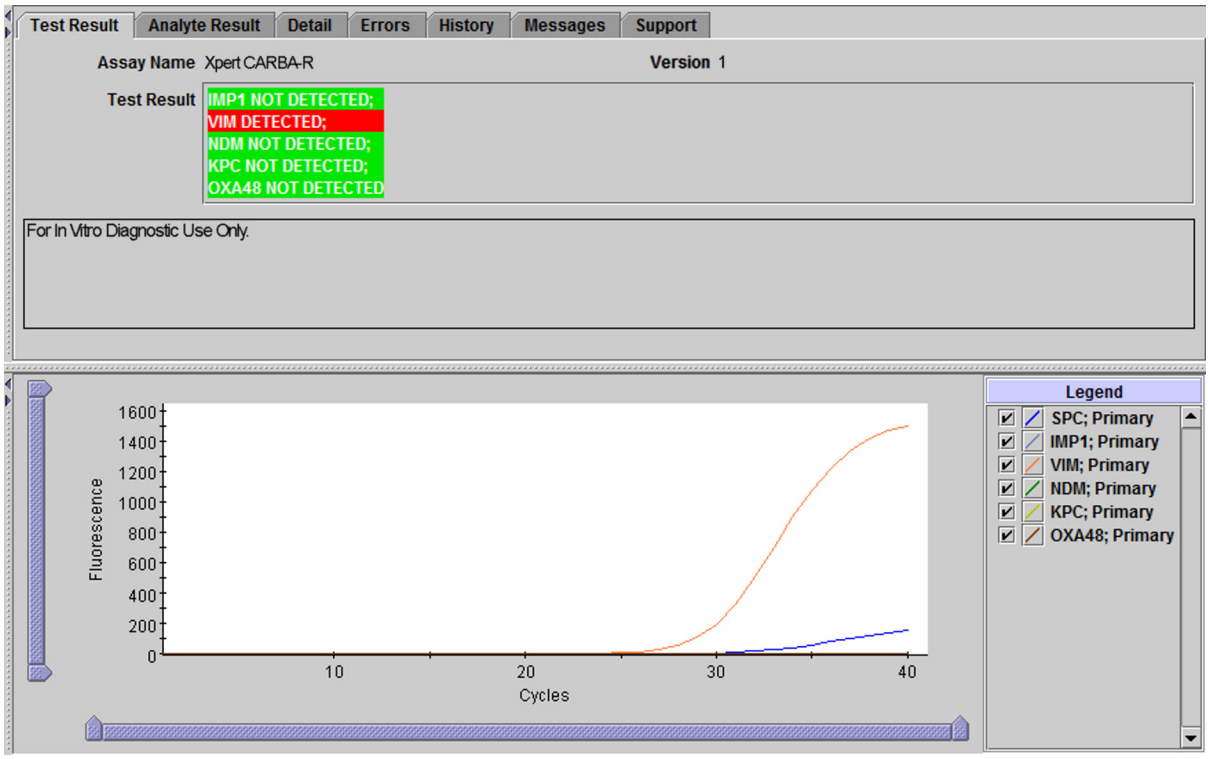

Figure 4. Carba-R Assay—VIM Detected

Note Examples of NDM positive, KPC positive, and OXA positive samples are not shown.

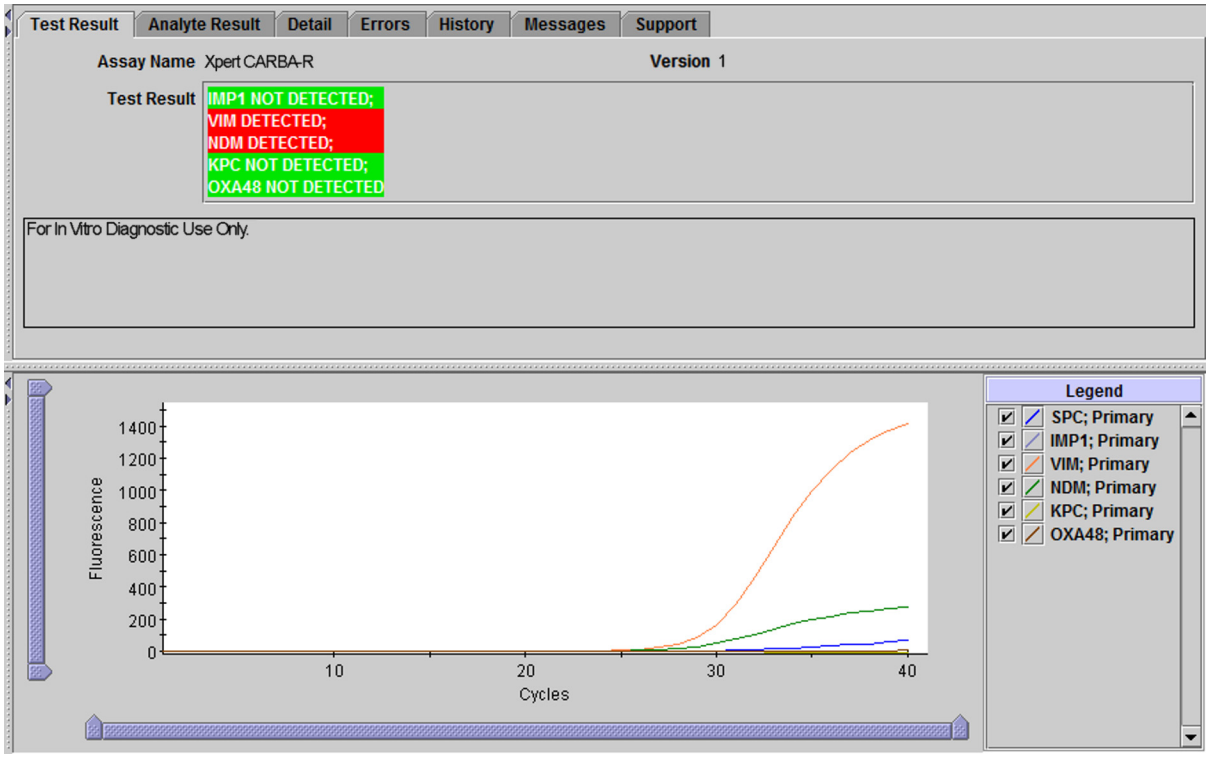

Figure 5. Carba-R Assay—VIM and NDM Detected

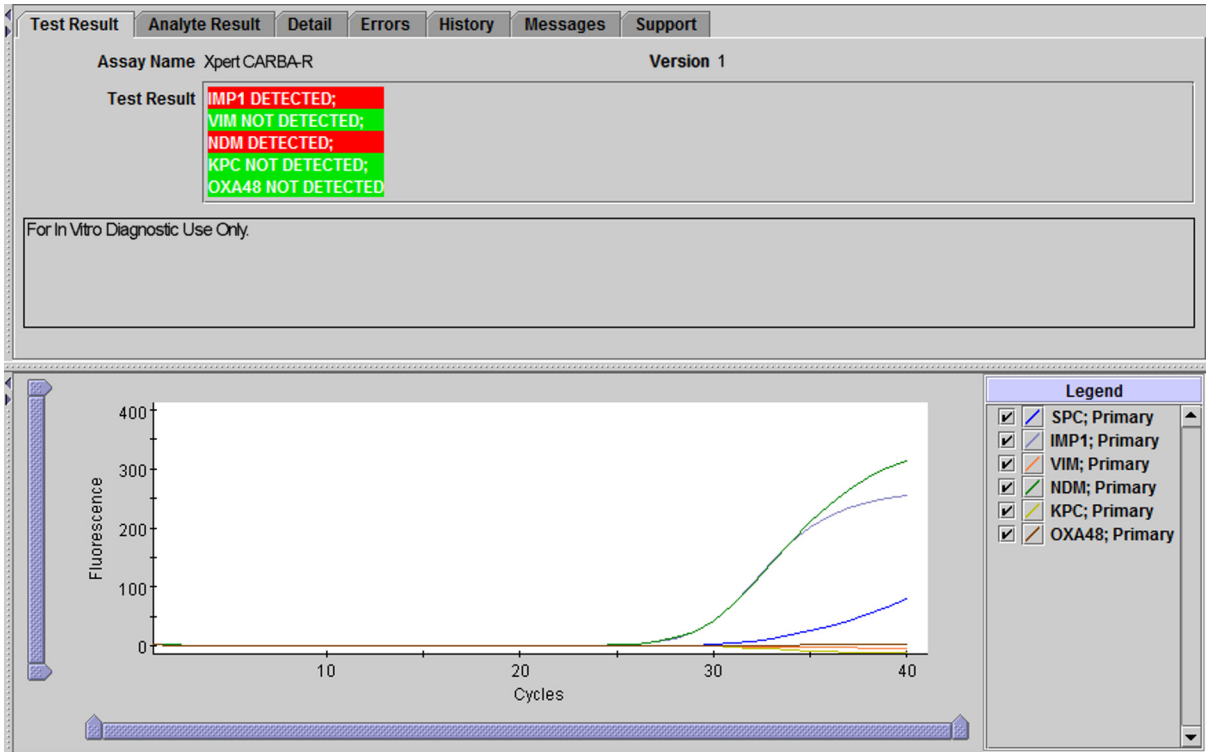

Figure 6. Carba-R Assay—IMP-1 and NDM Detected

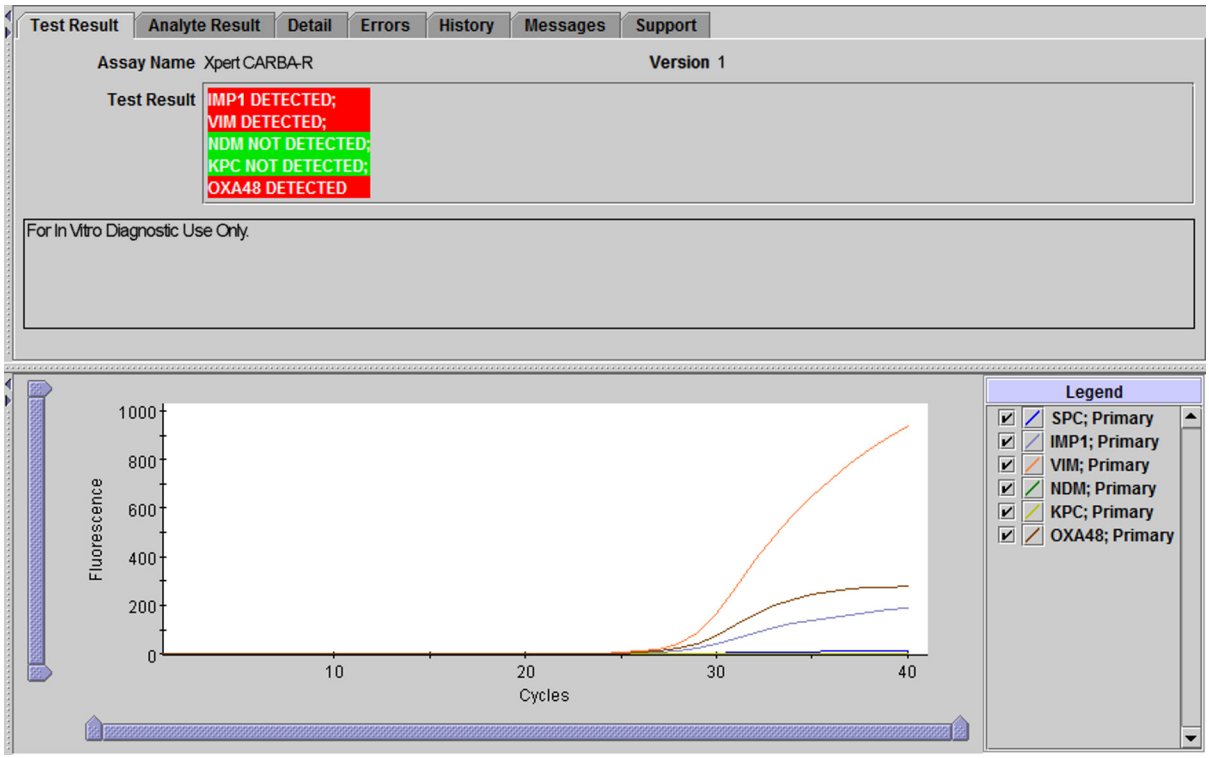

Figure 7. Carba-R Assay—IMP-1, VIM, and OXA-48 Detected

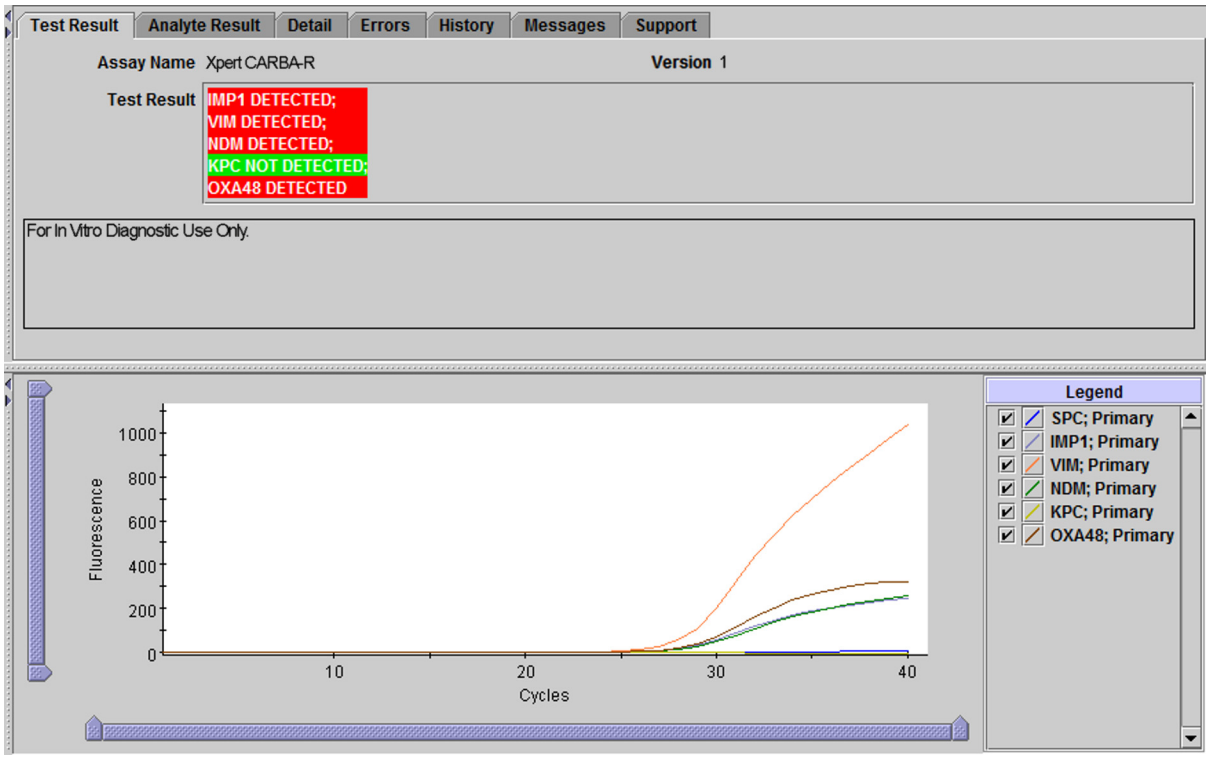

Figure 8. Carba-R Assay—IMP-1, VIM, NDM, and OXA-48 Detected

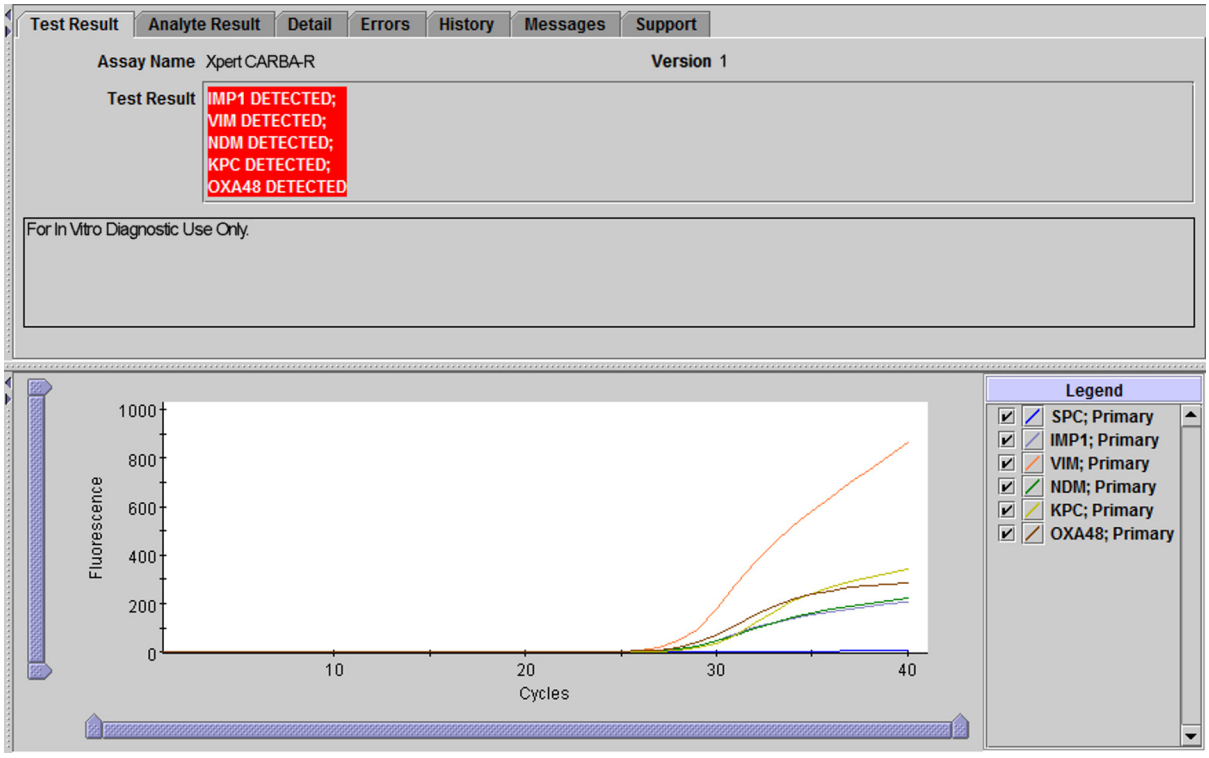

Figure 9. Carba-R Assay—IMP-1, VIM, NDM, KPC, and OXA-48 Detected

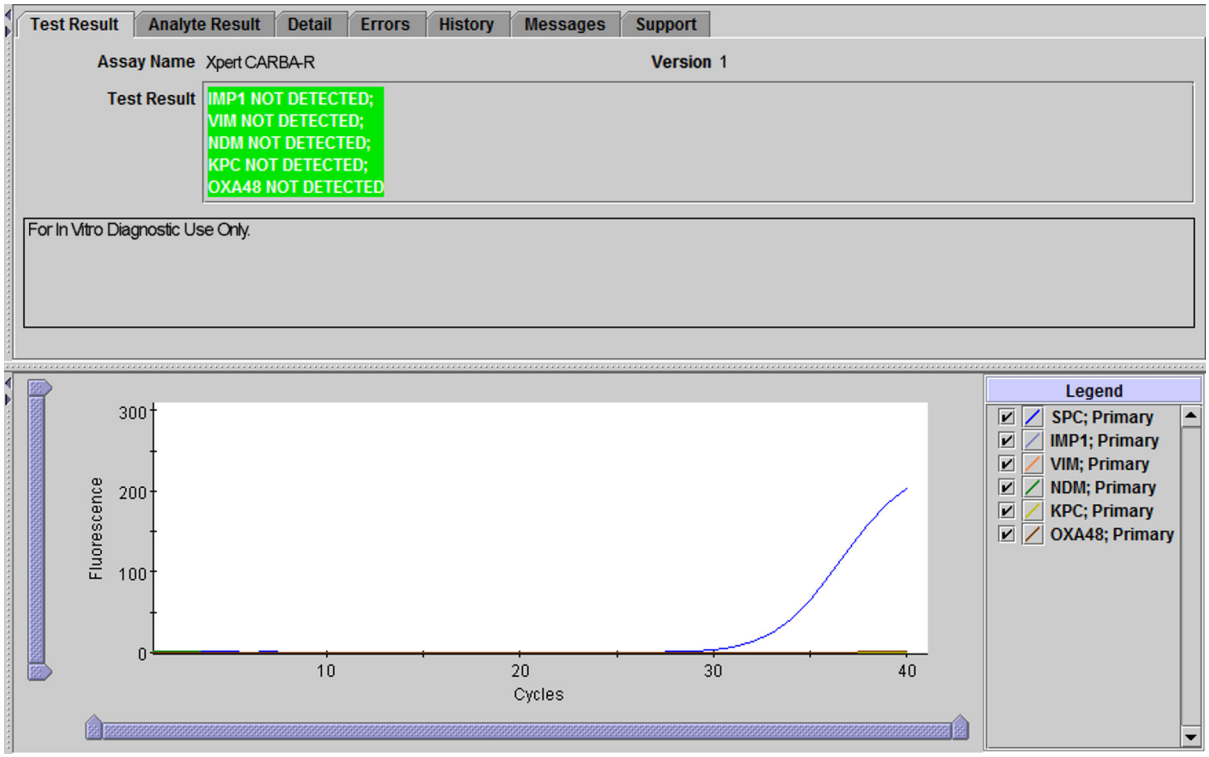

Figure 10. Carba-R Assay—IMP-1, VIM, NDM, KPC, and OXA-48 Not Detected

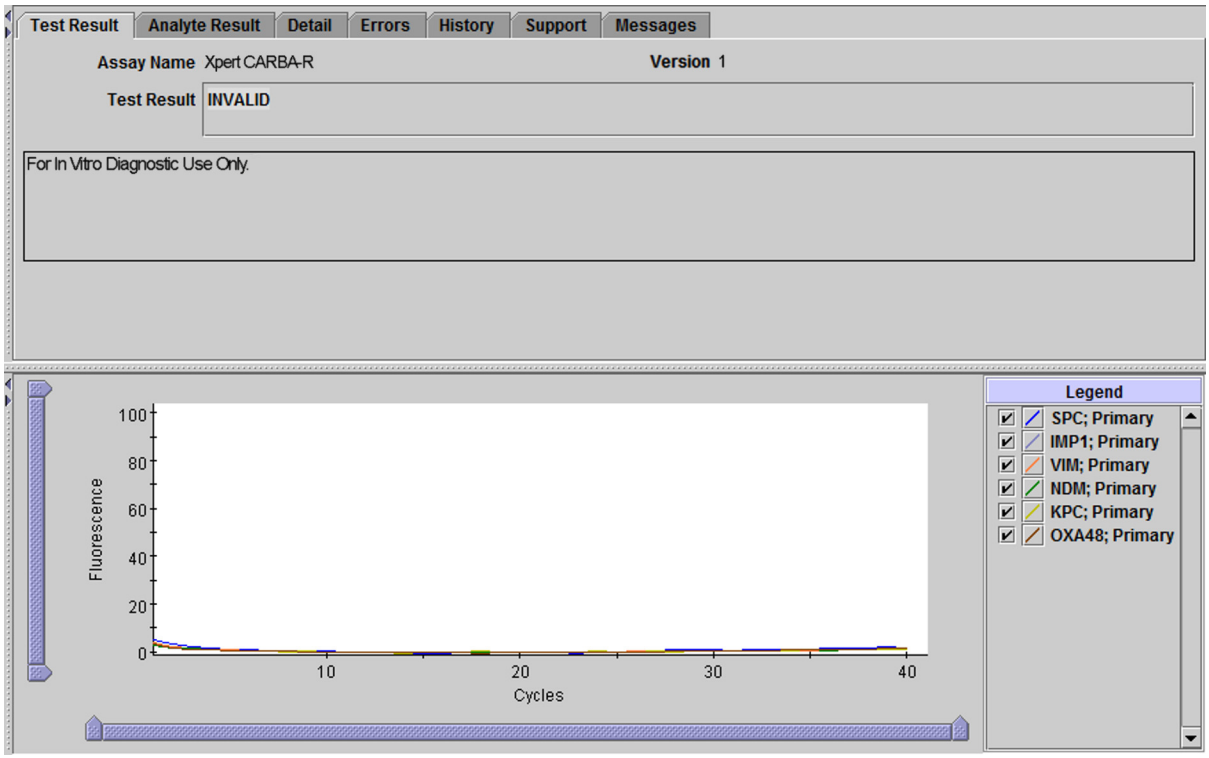

Figure 11. Carba-R Assay—Invalid

12 Reasons to Repeat the Test

Repeat the test using a new cartridge (do not re-use the cartridge) and new Sample Reagent vial for dilution.

- An **INVALID** result indicates that the control SPC failed. The sample was not properly processed or PCR is inhibited, or the volume of sample added was inadequate.
- 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, because the maximum pressure limits were exceeded, or a valve positioning error was detected.
- 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.
- If an External QC fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.

13 Retest Procedure

1. Remove a new cartridge and new Sample Reagent vial from the kit.
2. Transfer the remaining liquid from the original Sample Reagent vial containing the vortexed rectal swab sample (that had been stored at 2 – 28 °C; see Section 9.1) to the new Sample Reagent vial.
3. Close the Sample Reagent vial cap and vortex at high speed for 10 seconds.
4. Continue with subsequent testing steps starting at Step 6 of Section 9.1, Preparing the Cartridge.

14 Limitations

- For *In Vitro* Diagnostic Use.
- Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens or reagents.
- Erroneous test results might occur from improper specimen collection, failure to follow the recommended sample collection, handling and storage procedures, technical error, sample mix-up, or because the number of organisms in the specimen is too low to be detected by the test. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- Because the detection of bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP-1} gene sequences is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
- A positive test result does not necessarily indicate the presence of viable organisms.
- Testing with the Xpert Carba-R Assay should be used as an adjunct to other available methods.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP-1} variants resulting in a false negative result.
- In a mixed culture containing organisms having more than one of the five targeted gene sequences, the assay LoD may vary, especially when an extremely high concentration of one or more of the five gene sequences is present.
- As with all PCR-based *in vitro* diagnostic tests, extremely low levels of target below the LoD of the assay may be detected, but results may not be reproducible.
- Xpert Carba-R Assay results may sometimes be **INVALID** due to a failed SPC control, or result in an **ERROR** or **NO RESULT**, and require retesting that can lead to a delay in obtaining final results.

15 Performance Characteristics

Performance characteristics of the Xpert Carba-R Assay were evaluated in a multi-site prospective study at two institutions in the U.S. and two institutions in Europe (EU). Due to the low prevalence of organisms containing carbapenem-resistant genes in the absence of an outbreak, and the difficulty in obtaining fresh specimens containing carbapenem-non-susceptible organisms, the prospective specimens collected for this study were supplemented with contrived specimens (well characterized isolates spiked into negative rectal swab matrix).

Subjects included individuals whose routine care included collection of rectal swab specimens for screening of carbapenem resistant organisms, or individuals who provided informed consent. A double swab set was used to collect rectal specimens from eligible subjects. One swab from the set was used for reference culture and susceptibility testing; the other swab was used for testing with the Xpert Carba-R Assay. DNA from all carbapenem-non-susceptible isolates was extracted and sent to an independent laboratory for DNA sequence identification. Patient management continued at the site per standard practice.

Susceptibility testing was performed in accordance with the CLSI documents M2-A11, M7-A9, and M100-S23.[13,14,15] Meropenem discs were used in disk diffusion testing for detecting carbapenem resistance.

The Xpert Carba-R Assay results were compared to reference culture and to sequencing for culture-confirmed carbapenem-non-susceptible isolates.

A total of 633 specimens were tested by the Xpert Carba-R Assay for the target carbapenem-resistant gene sequences (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP-1}) and by the reference method. Relative to the reference method, the Xpert Carba-R Assay demonstrated overall sensitivity and specificity of 96.6% (95% CI: 92.2–98.9) and 98.6% (95% CI: 97.1–99.4) respectively (Table 2) on the combined set of contrived and prospective specimens. The Xpert Carba-R Assay results were defined as positive if one or more of the five target sequences was detected, and negative if none of the targets were detected.

Table 2. Overall Xpert Carba-R Performance vs. Reference Culture + Sequencing

		Culture + Sequencing		
		Pos	**Neg**	**Total**
Xpert Carba-R	**Pos**	142	7	149
	Neg	5	479	484
	Total	147	486	633
	Sensitivity: 96.6% (95% CI: 92.2–98.9) Specificity: 98.6% (95% CI: 97.1–99.4)			

Table 3 shows the positive predictive value (PPV), negative predictive value (NPV) and accuracy estimates of the Xpert Carba-R Assay as a function of prevalence.

Table 3. Overall PPV, NPV, and Accuracy Estimates of the Xpert Carba-R Assay as Function of Prevalence

Prevalence	PPV	NPV	Accuracy
0.00%	0.00%	100.00%	98.56%
10.00%	88.17%	99.62%	98.36%
20.00%	94.37%	99.14%	98.17%
30.00%	96.64%	98.54%	97.97%
40.00%	97.81%	97.75%	97.78%
50.00%	98.53%	96.66%	97.58%
60.00%	99.02%	95.08%	97.38%
70.00%	99.37%	92.55%	97.19%
80.00%	99.63%	87.87%	96.99%
90.00%	99.83%	76.30%	96.79%
100.00%	100.00%	0.00%	96.60%

Table 4 shows a tabulation of the Xpert Carba-R Assay results by individual target for all specimens. There were a total of 633 specimens, each with results for five individual targets for a total of 3165 results.

Table 4. Xpert Carba-R Assay Table of All Results by Individual Target

		Culture + Sequencing						
		IMP-1+	VIM+	NDM+	KPC+	OXA-48+	NEG	Total
Xpert Carba-R	IMP-1+	26	0	0	0	0	0	26
	VIM+	0	29	0	0	0	1	30
	NDM+	0	0	26	0	0	1	27
	KPC+	0	0	0	29	0	4	33
	OXA-48+	0	0	0	0	38	1	39
	NEG	1	2	0	1	2	3004[a]	3010
	Total	27	31	26	30	40	3011	3165

a. Negative pairs (3004 total) were broken down as follows: 606 both tests IMP-1 and NEG; 601 both tests VIM and NEG; 606 both tests NDM and NEG; 599 both tests KPC and NEG; 592 both tests OXA-48 and NEG.

Relative to the reference method, the Xpert Carba-R Assay demonstrated a sensitivity and specificity for the IMP-1 target of 96.3% and 100%, respectively. See Table 5.

Table 5. Xpert Carba-R Assay Performance—IMP-1

		Culture + Sequencing		
		Pos	Neg	Total
Xpert Carba-R	Pos	26	0	26
	Neg	1	606	607
	Total	27	606	633
	Sensitivity: 96.3% (95% CI: 81.0–99.9) Specificity: 100% (95% CI: 99.4–100)			

Relative to the reference method, the Xpert Carba-R Assay demonstrated a sensitivity and specificity for the VIM target of 93.5% and 99.8%, respectively. See Table 6.

Table 6. Xpert Carba-R Assay Performance—VIM

		Culture + Sequencing		
		Pos	Neg	Total
Xpert Carba-R	Pos	29	1	30
	Neg	2	601	603
	Total	31	602	633
	Sensitivity: 93.5% (95% CI: 78.6–99.2) Specificity: 99.8% (95% CI: 99.1–100)			

Relative to the reference method, the Xpert Carba-R Assay demonstrated a sensitivity and specificity for the NDM target of 100% and 99.8%, respectively. See Table 7.

Table 7. Xpert Carba-R Assay Performance—NDM

	Culture + Sequencing			
Xpert Carba-R		**Pos**	**Neg**	**Total**
	Pos	26	1	27
	Neg	0	606	606
	Total	26	607	633
	Sensitivity: 100% (95% CI: 86.8–100) Specificity: 99.8% (95% CI: 99.1–100)			

Relative to the reference method, the Xpert Carba-R Assay demonstrated a sensitivity and specificity for the KPC target of 96.7% and 99.3%, respectively. See Table 8.

Table 8. Xpert Carba-R Assay Performance—KPC

	Culture + Sequencing			
Xpert Carba-R		**Pos**	**Neg**	**Total**
	Pos	29	4	33
	Neg	1	599	600
	Total	30	603	633
	Sensitivity: 96.7% (95% CI: 82.8–99.9) Specificity: 99.3% (95% CI: 98.3–99.8)			

Relative to the reference method, the Xpert Carba-R Assay demonstrated a sensitivity and specificity for the OXA-48 target of 95.0% and 99.8%, respectively. See Table 9.

Table 9. Xpert Carba-R Assay Performance—OXA-48

	Culture + Sequencing			
Xpert Carba-R		**Pos**	**Neg**	**Total**
	Pos	38	1	39
	Neg	2	592	594
	Total	40	593	633
	Sensitivity: 95.0% (95% CI: 83.1–99.4) Specificity: 99.8% (95% CI: 99.1–100)			

16 Analytical Performance

16.1 Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the analytical limit of detection (LoD) of the Xpert Carba-R Assay with carbapenemase-producing organisms seeded into negative natural human pooled rectal swab matrix. The LoD was determined for two carbapenemase-producing bacteria for each gene analyte, i.e., the genes encoding KPC, NDM, VIM, OXA-48, and IMP-1. Bacteria were titered by plate counts and diluted into negative pooled rectal swab matrix. Replicates of 20 were evaluated at a minimum of six different concentrations and LoDs were estimated by probit analysis. For this study, the estimated LoD is defined as the lowest concentration of target cells that can be reproducibly distinguished from negative samples with 95% confidence. The study was performed with two different lots of Xpert Carba-R reagents and the claimed LoD is the higher of the two determinations. The estimated LoDs were verified by preparing and testing 10 replicates from two independent dilutions of each bacterium at each estimated LoD.

In all cases, the upper one-sided 95% CI of the proportion that was positive was greater than 95%, i.e., ≥ 19/20.

The LoD claim for each pair of carbapenemase-producing organisms is shown in Table 10.

Table 10. LoD for Carbapenemase-Producing Organisms

Organism	Strain ID	LoD (CFU/swab)
KPC *Klebsiella pneumoniae*	NCTC 13438	348
KPC *Enterobacter cloacae*	C8823	750
NDM *Klebsiella pneumoniae*	ATCC BAA-2146	246
NDM *Klebsiella pneumoniae*	C8658	306
OXA-48 *Escherichia coli*	OM22	213
OXA-48 *Enterobacter cloacae*	501	451
IMP-1 *Acinetobacter baumannii*	695	1165
IMP-1 *Klebsiella pneumoniae*	IMPBMI	258
VIM *Klebsiella pneumoniae*	C8667	274
VIM *Pseudomonas aeruginosa*	C10107	118

16.2 Analytical Reactivity (Inclusivity)

The analytical sensitivity of the Xpert Carba-R Assay was evaluated by testing a panel of 60 samples consisting of 20 well characterized bacterial strains for the $bla_{\text{OXA-48}}$ target (which includes $bla_{\text{OXA-181/232}}$ variants) and 10 well-characterized bacterial strains for each of the four other Carba-R targets. See Table 11. Organisms were tested in triplicate in pooled negative rectal swab matrix. All organisms were tested near the analytical limit of detection (LoD) and concentrations were confirmed by plating on non-selective media in triplicate and determining viable counts. Under the conditions of this study, all 60 bacterial strains were detected with the Xpert Carba-R Assay. Inclusivity was 100%.

Table 11. List of Carbapenemase-Producing Organisms and Concentrations (CFU/mL) Tested Using the Xpert Carba-R Assay

Organism	Strain ID	Confirmed Characteristic	Test Concentration (CFU/mL)
Klebsiella pneumoniae	NCTC 13438	KPC-3	100
Klebsiella pneumoniae	31551	KPC-4	100
Klebsiella pneumoniae	ATCC BAA-1705	KPC	100
Pseudomonas aeruginosa	COL	KPC-2	100
Enterobacter aerogenes	KBM18	KPC-2	100
Klebsiella pneumoniae	BM9	KPC-3	100

Table 11. List of Carbapenemase-Producing Organisms and Concentrations (CFU/mL) Tested Using the Xpert Carba-R Assay (Continued)

Organism	Strain ID	Confirmed Characteristic	Test Concentration (CFU/mL)
Pseudomonas aeruginosa	PA3	KPC-2	100
Serratia marcescens	CGNC	KPC-2	100
Enterobacter cloacae	CFVL	KPC-2	100
Escherichia coli	COL	KPC-2	100
Escherichia coli	NCTC 13476	IMP	100
Acinetobacter baumannii	695	IMP-1	450
Enterobacter cloacae	2340	IMP-1	100
Klebsiella pneumoniae	IMPBMI	IMP	100
Acinetobacter baumannii	Yonsei_1	IMP	500
Acinetobacter baumannii	Yonsei_2	IMP	500
Klebsiella pneumoniae	6852	IMP-1	100
Pseudomonas aeruginosa	MKAM	IMP-1	200
Pseudomonas aeruginosa	70450-1	IMP	100
Pseudomonas aeruginosa	3994	IMP-10	200
Pseudomonas aeruginosa	NCTC 13437	VIM-10	400
Klebsiella pneumoniae	NCTC 13439	VIM-1	100
Klebsiella pneumoniae	NCTC 13440	VIM-1	100
Pseudomonas aeruginosa	758	VIM	400
Klebsiella pneumoniae	PA_87	VIM	100
Pseudomonas aeruginosa	B92A	VIM	100
Pseudomonas aeruginosa	Col1	VIM-2	400
Serratia marcescens	BM19	VIM-2	100
Escherichia coli	KOW7	VIM-4	100
Klebsiella pneumoniae	DIH	VIM-19	200
Klebsiella pneumoniae	NCTC 13443	NDM-1	100
Klebsiella pneumoniae	ATCC BAA-2146	NDM-1	100
Klebsiella pneumoniae	34262	NDM	100
Acinetobacter baumannii	AB-GEN	NDM-1	100
Enterobacter cloacae	3047	NDM-1	100
Proteus mirabilis	7892	NDM-1	100
Salmonella spp.	CAN	NDM-1	100
Acinetobacter baumannii	EGY	NDM-2	100
Escherichia coli	I5	NDM-4	100
Escherichia coli	405	NDM-5	100
Klebsiella pneumoniae	NCTC 13442	OXA-48	100
Klebsiella pneumoniae	OM11	OXA-48	100
Enterobacter cloacae	501	OXA-48	100
Klebsiella pneumoniae	DUW	OXA-48	100

Table 11. List of Carbapenemase-Producing Organisms and Concentrations (CFU/mL) Tested Using the Xpert Carba-R Assay (Continued)

Organism	Strain ID	Confirmed Characteristic	Test Concentration (CFU/mL)
Escherichia coli	OM22	OXA-48	100
Enterobacter cloacae	BOU	OXA-48	100
Enterobacter cloacae	TUR	OXA-48	100
Escherichia coli	11670	OXA-48	100
Escherichia coli	AME	OXA-48	100
Klebsiella pneumoniae	11978	OXA-48	100
Klebsiella pneumoniae	166643	OXA-181	50
Klebsiella pneumoniae	42194	OXA-181	50
Klebsiella pneumoniae	MSH2014-6	OXA-181	150
Klebsiella pneumoniae	MSH2014-44	OXA-181	200
Klebsiella pneumoniae	MSH2014-64	OXA-181	150
Escherichia coli	MSH2014-72	OXA-181	100
Escherichia coli	MSH2014-73	OXA-181	100
Klebsiella pneumoniae	MSH2014-18	OXA-232	50
Klebsiella pneumoniae	MSH2014-51	OXA-232	50
Klebsiella pneumoniae	MSH2014-75	OXA-232	50

16.3 Analytical Cross-Reactivity (Exclusivity)

The analytical specificity of the Xpert Carba-R Assay was evaluated by testing a panel of 54 samples consisting of 22 well characterized bacterial strains of related resistance profiles (see Table 12), 28 well characterized bacterial strains representing common pathogens or non-pathogens potentially encountered in the gastrointestinal tract (see Table 13), three viral organisms representing viruses potentially present in the gastrointestinal tract (see Table 13), and one bladder carcinoma cell line to represent human genomic DNA (see Table 14).

All bacterial strains were grown and titered. Strains were tested at concentrations $\geq 10^5$ CFU/mL. Adenovirus and enterovirus were tested at concentrations $\geq 10^5$ $TCID_{50}$/mL; norovirus was tested as a norovirus positive clinical specimen at a concentration of 2.5 x 10^7 RNA copies/mL. The bladder cell-line (human genomic DNA) was tested at 1 x 10^5 cells/mL. Organisms were diluted into pooled negative rectal swab matrix and tested in triplicate. None of the 54 potentially cross-reactive organisms and nucleic acids tested was detected with the Xpert Carba-R Assay. Positive and negative controls were included in the study. The analytical specificity was 100%.

Table 12. List of Organisms of Related Resistance

Organism Name	Beta-lactamases Present	Test Concentration (CFU/mL)
Escherichia coli	CTX-M (15)	5.0 x 10^7
Klebsiella pneumoniae	CTX-M (25)	7.5 x 10^7
Enterobacter cloacae	OmpC/OmpF deficient	9.9 x 10^7
Citrobacter freundii	TEM (WT+164S)	7.3 x 10^7
Enterobacter cloacae	AmpC (ACT/MIR)	4.9 x 10^7
Escherichia coli	CTX-M (2); TEM; OXA-2	1.3 x 10^7
Enterobacter cloacae	CTX-M (2); TEM	9.5 x 10^7
Serratia marcescens	CTX-M (2); TEM	2.2 x 10^7

Table 12. List of Organisms of Related Resistance (Continued)

Organism Name	Beta-lactamases Present	Test Concentration (CFU/mL)
Morganella morganii	CTX-M (2); TEM	9.3×10^7
Proteus mirabilis	CTX-M (2); TEM	8.2×10^7
Salmonella spp.	CTX-M (U)	7.8×10^7
Shigella flexnerii	CTX-M (2); TEM	3.8×10^7
Klebsiella pneumoniae	SHV	4.1×10^7
Klebsiella pneumoniae	IMP-13; CTX-M; SHV-1;	8.8×10^7
Klebsiella pneumoniae	CTX-M (15); SHV-11; TEM-1	3.8×10^7
Klebsiella pneumoniae	CTX-M (15); SHV	5.3×10^7
Klebsiella pneumoniae	SHV-27	8.3×10^7
Klebsiella pneumoniae	SHV (-5, -55); TEM	5.8×10^7
Klebsiella pneumoniae	CTX-M (15); SHV; TEM	6.4×10^7
Enterobacter aerogenes	SHV (WT+238S+240K)	6.5×10^7
Enterobacter aerogenes	SHV (WT+238S+240K)	9.0×10^7
Escherichia coli	AmpC (CMY II); TEM	8.0×10^8

Table 13. List of Commensal and Other Enteric Microorganisms

Organism Name	Source	Test Concentration (CFU/mL)
Escherichia coli	ATCC 25922	6.1×10^7
Enterococcus faecalis	ATCC 29212	2.0×10^7
Klebsiella pneumoniae	ATCC 700603	6.0×10^7
Escherichia coli	ATCC 35218	9.8×10^7
Staphylococcus aureus	ATCC 25923	1.3×10^8
Pseudomonas aeruginosa	ATCC 27853	2.9×10^7
Enterobacter cloacae	ATCC 700621	5.2×10^7
Enterococcus faecium	ATCC 9756	6.8×10^7
Klebsiella oxytoca	ATCC 13182	8.0×10^7
Acinetobacter baumannii	ATCC BAA-747	2.2×10^7
Citrobacter freundii	ATCC 33128	9.4×10^7
Morganella morganii	ATCC 49948	1.2×10^7
Stenotrophomonas maltophilia	ATCC 51331	4.9×10^7
Citrobacter koseri	ATCC 27028	$> 1.5 \times 10^8$
Providencia stuartii	ATCC 49809	5.3×10^7
Streptococcus agalactiae	CCUG 29780/ATCC 12401	3.1×10^7
Enterobacter aerogenes	ATCC 51697	7.8×10^7
Proteus mirabilis	ATCC 43071	3.4×10^7
Acinetobacter spp.	CCUG 34787	1.6×10^7
Bifidobacterium adolescent	CCUG 24604	2.3×10^7
Campylobacter jejuni	CCUG 43594/ATCC 33560	1.5×10^6

Table 13. List of Commensal and Other Enteric Microorganisms (Continued)

Organism Name	Source	Test Concentration (CFU/mL)
Citrobacter freundii	CCUG 418	> 1.5×10^8
Clostridium difficile (non-toxigenic)	ATCC 700057	4.5×10^7
Corynebacterium diphtheriae	CCUG 33629	4.0×10^7
Helicobacter pylori	CCUG 17874	1.3×10^7
Listeria monocytogenes	CCUG 33548	> 1.5×10^8
Peptostreptococcus anaerobius	CCUG 7835	5.0×10^5
Providencia alcalifaciens	CCUG 6325	7.8×10^7
Adenovirus B Type 7A/NY	MRVP/Zeptometrix	1.4×10^5 $TCID_{50}$/mL
Enterovirus Type 71/NY	MRVP/Zeptometrix	4.4×10^5 $TCID_{50}$/mL
Norovirus GII	Clinical Sample—Cepheid Solna	2.5×10^7 RNA copies/mL

Table 14. Cell Line Representing Human Genomic DNA

Organism Name	Source	Test Concentration (cells/mL)
Bladder Cell Carcinoma (hgDNA)	ATCC HTB-4	1.0×10^5

16.4 Potentially Interfering Substances

In a non-clinical study, 23 potentially interfering substances that may be present in rectal swab specimens were evaluated with the Xpert Carba-R Assay. Solutions of potentially interfering substances were prepared and tested at concentrations specified in Table 15. Eight replicate negative samples were tested per substance to determine the effect on the performance of the sample processing control (SPC).

To determine whether the presence of the potentially interfering substances caused false negative results, eight replicate positive samples were tested per substance. Positives consisted of a mix of five carbapenemase-producing organisms at concentrations of 2–4x analytical LoD previously determined for each organism. The substances and organisms were diluted into Sample Reagent for testing.

The effect of each potentially interfering substance on positive and negative replicates was evaluated by comparing target cycle threshold (Ct) values generated in the presence of the substance to Ct values from Sample Reagent controls lacking the substance.

In the presence of the 23 potentially interfering substances, no invalid results caused by inhibition of the SPC in negative samples was observed. Of the 23 potentially inhibitory substances tested, Pepto-Bismol (Bismuth subsalicylate) 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.

Table 15. Potentially Interfering Substances Tested

Substance/Class	Active Ingredient	Concentration Tested
Non-steroidal anti-inflammatory medication	Naproxen	0.25% w/v
Imaging compound	Barium sulfate	0.25% w/v
Antibiotic (oral)	Cephalexin	0.25% w/v
	Ciprofloxin	0.25% w/v
Antibiotic (topical)	Polymixin B/Neomycin/Bacitracin	0.25% w/v
Creams/ointment/suppositories	Hydrocortisone	0.25% w/v
Laxative	Sennosides	0.25% w/v
Enemas	Mineral oil	0.25% w/v
Anti-diarrheal medication	Loperamide hydrochloride	0.25% w/v
	Bismuth subsalicylate (2)	0.25% w/v
Topical cream	Chlorhexidine Gluconate and Methyl Hydroxybenzoate	0.25% w/v
	Petroleum jelly	0.25% w/v
Antacids	Calcium carbonate/aluminum hydroxide/magnesium hydroxide/ simethicone	0.25% w/v
	Cimetidine	0.25% w/v
	Famotidine	0.25% w/v
Acid reducer; antacid	Omeprazole	0.25% w/v
Anti-fungal/anti-itch Vaginal	Nystatin	0.25% w/v
	Benzocaine, resorcinol	0.25% w/v
Anti-hemorrhoid creams/ointments	Phenylephrine	0.25% w/v
Enemas	Saline	0.25% w/v
Condom with spermicidal lubricant	Nonoxynol-9	1 condom[a]
Moist towelettes	Benzalkonium chloride ethanol	1 piece[b]

a. One condom added to 40 mL Sample Reagent.
b. One piece (5 inch x 7½ inch) added to 40 mL Sample Reagent.

16.5 Carry-over Contamination Study

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination in negative samples. The study consisted of a negative sample processed in the same GeneXpert module immediately following a very high positive sample. The high positive sample is composed of inactivated E. coli cells containing a plasmid with an insert consisting of a synthetic oligonucleotide of the amplicon sequences from the five Xpert Carba-R target analyte genes. Positive cells were diluted into pooled negative rectal swab matrix to a concentration of 1 x 10^6 CFU/mL. The testing scheme was repeated 20 times on two GeneXpert modules for a total of 102 tests (25 high positive samples per module and 26 negative samples per module). All 50 positive samples correctly reported all Xpert Carba-R targets **DETECTED**. All 52 negative samples correctly reported all Xpert Carba-R targets **NOT DETECTED**.

16.6 Assay Reproducibility

Reproducibility of the Xpert Carba-R Assay was assessed in a five-day, multicenter study in which two operators at each of three sites blindly tested a 11-member precision panel. Each panel member was tested in replicates of three for a total of 90 replicates per panel member. This panel was composed of well characterized isolates spiked into negative rectal swab matrix. Data are summarized by assay target. See Table 16 and Table 17.

Table 16. Summary of Reproducibility Results—% Agreement by Study Site/Operator

Sample	Site 1		Site 2		Site 3		% Total Agreement by Sample
	Op 1	Op 2	Op 1	Op 2	Op 1	Op 2	
KPC low pos	80.00% (12/15)	86.70% (13/15)	80.00% (12/15)	93.30% (14/15)	86.70% (13/15)	93.30% (14/15)	86.7% (78/90)
KPC mod pos	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (90/90)
VIM low pos	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	93.30% (14/15)	86.70% (13/15)	96.7% (87/90)
VIM mod pos	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (90/90)
NDM low pos	100% (15/15)	100% (15/15)	73.30% (11/15)	86.70% (13/15)	100% (15/15)	100% (15/15)	93.3% (84/90)
NDM mod pos	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (90/90)
OXA-48 low pos	100% (15/15)	86.70% (13/15)	80.00% (12/15)	86.70% (13/15)	93.30% (14/15)	86.70% (13/15)	88.9% (80/90)
OXA-48 mod pos	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (90/90)
IMP-1 low pos	100% (15/15)	100% (15/15)	100% (15/15)	86.70% (13/15)	86.70% (13/15)	100% (15/15)	95.6% (86/90)
IMP-1 mod pos	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (90/90)
Neg	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)	100% (90/90)

Table 17. Summary of Reproducibility Data[a]

Sample	Assay Channel (Analyte)	N[b]	Mean Ct	Between-Site		Between-Day		Between-Operator		Within-Assay		Total	
				SD[c]	CV[d] (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
KPC low pos	KPC	84	36.1	0.13	0.4	0	0	0.08	0.2	1.14	3.2	1.15	3.2
KPC mod pos	KPC	90	34.0	0	0	0.21	0.6	0.15	0.4	0.53	1.6	0.59	1.7
VIM low pos	VIM	89	35.0	0.35	1	0	0	0.28	0.8	1.08	3.1	1.17	3.4
VIM mod pos	VIM	90	31.6	0.15	0.5	0	0	0.18	0.6	0.34	1.1	0.41	1.3
NDM low pos	NDM	87	35.8	0.16	0.4	0.07	0.2	0.17	0.5	0.86	2.4	0.89	2.5
NDM mod pos	NDM	90	33.2	0	0	0.13	0.4	0	0	0.58	1.8	0.60	1.8
OXA-48 low pos	OXA-48	87	36.6	0	0	0	0	0	0	0.99	2.7	0.99	2.7
OXA-48 mod pos	OXA-48	90	32.4	0.09	0.3	0	0	0	0	0.37	1.1	0.38	1.2
IMP-1 low pos	IMP-1	89	36.1	0	0	0.13	0.4	0.29	0.8	0.89	2.5	0.95	2.6
IMP-1 mod pos	IMP-1	90	33.7	0.04	0.1	0.09	0.3	0.15	0.4	0.49	1.5	0.52	1.5
Neg	SPC	90	33	0	0	0	0	0.27	0.8	0.63	1.9	0.69	2.1

a. Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, the variability as measured with SD and CV is set to 0.
b. Results with non-zero Ct values out of 90.
c. SD = standard deviation.
d. CV = coefficient of variation.

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18 Cepheid Headquarters Locations

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19 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

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

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20 Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	Do not reuse
	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Contains sufficient for <n> tests
	Control
	Expiration date
	CE marking – European Conformity
	Authorized Representative in the European Community
	Authorized Representative in Switzerland
	Importer
	Temperature limitation
	Biological risks
	Warning

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