GeneXpert®
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Xpert® Carba-R

REF **GXCARBAR-10**

Cepheid®

***In Vitro* Diagnostic Medical Device**

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Xpert® Carba-R

Rx only

For *In Vitro* Diagnostic Use Only

1 Proprietary Name

Xpert® Carba-R

2 Common or Usual Name

Xpert Carba-R Assay

3 Intended Use

The Xpert® Carba-R Assay, performed on the GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test designed for the detection and differentiation of the bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP} gene sequences associated with carbapenem-non-susceptibility. The test utilizes automated real-time polymerase chain reaction (PCR).

The Xpert Carba-R Assay is intended as an aid to infection control 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. A negative Xpert Carba-R Assay result does not preclude the presence of other resistance mechanisms.

The Xpert Carba-R Assay is for use with the following sample types:

Rectal Swab Specimens

The assay is performed on rectal swab specimens from patients at risk for intestinal colonization with carbapenem-non-susceptible bacteria. Concomitant cultures are necessary to recover organisms for epidemiological typing, antimicrobial susceptibility testing, and for further confirmatory bacterial identification.

Pure Colonies

The assay is performed on pure colonies of *Enterobacteriaceae*, *Acinetobacter baumannii*, or *Pseudomonas aeruginosa*, when grown on blood agar or MacConkey agar. For testing pure colonies, the Xpert Carba-R Assay should be used in conjunction with other laboratory tests including phenotypic antimicrobial susceptibility testing.

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] In addition, the Class D oxacillinase, OXA-48, which often mediates low-level carbapenem resistance, 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 swab samples on gram-negative 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 fast and accurate method of determining whether a rectal swab specimen or a carbapenem-non-susceptible bacterial isolate harbors one of these five common classes of carbapenem resistance genes would be a significant aid to infection control programs especially during outbreaks, since it has the potential to: 1) identify the specific resistance gene present in the organism, and 2) differentiate those organisms with the most common transmissible carbapenem resistance genes that encode carbapenemase enzymes from organisms that are resistant due to other beta-lactamases and/or changes in the organism's cell wall, which may not necessarily require placement of the patient in contact precautions.

5 Principle of the Procedure

The GeneXpert 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*$_{IMP}$ 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*$_{IMP}$ (IMP) gene sequences associated with carbapenem-non-susceptibility in gram-negative bacteria.

6 Reagents and Instruments

6.1 Materials Provided

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

Xpert Carba-R Assay Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
• Reagent 1	3 mL per cartridge
• Reagent 2 (Guanidinium chloride)	2.5 mL per cartridge
Xpert Carba-R Assay Sample Reagent Vials	**10**
• Sample Reagent	5.0 mL per vial
Disposable (1.7 mL) Transfer Pipettes	**10**
CD	**1**
• 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 at 2–28 °C.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use reagents or cartridges that have passed the expiration date.
- 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 System or GeneXpert Infinity Systems (catalog number varies by configuration): six-color GeneXpert Instrument, computer with proprietary GeneXpert Software Version 4.3 or higher, barcode scanner, and operator manual
- Specimen Collection Device: Cepheid Catalog Number 900-0370
- Blood Agar (e.g., Remel™ Blood Agar: Catalog Number R01200 or equivalent)
- MacConkey Agar (e.g., Remel™ MacConkey Agar: Catalog Number R01550 or equivalent)
- 10 µg Meropenem discs (e.g., BD BBL™ Sensi-Disc™ Antimicrobial Susceptibility Test Discs, Meropenem[4,5], catalog number 231704 or equivalent)
- Sterile forceps
- Disposable, sterile 10 µL inoculating loops (e.g., Copan: Catalog Number COPS-10, or Hardy Diagnostics: Catalog Number L2002A or equivalent)
- Vortex mixer
- Printer: If a printer is required, contact Cepheid Technical support to arrange for the purchase of a recommended printer.

6.4 Materials Available but Not Provided

- Multivalent External Control:
  Xpert Carba-R QC Panel M219, catalog number M219 from Maine Molecular Quality Controls, Inc. (MMQCI, Scarborough, ME), as external positive control (inactivated *Escherichia coli* carrying plasmid with KPC, NDM, VIM, IMP, OXA-48 gene sequences) and external negative control (inactivated *E. coli*).
- Individual External Controls:
  Carbapenemase-producing bacteria *K. pneumoniae* KPC-2 (catalog number ATCC BAA-1705); *K. pneumoniae* NDM-1 (catalog number ATCC BAA-2146); *K. pneumoniae* VIM-1 (catalog number NCTC 13439); *K. pneumoniae* OXA-48 (catalog number NCTC 13442); and *Escherichia coli* IMP-1 (catalog number NCTC 13476) as external positive controls.

7 Warnings and Precautions

- For *in vitro* diagnostic use.
- For prescription use only.
- 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[10, 11] and the Clinical and Laboratory Standards Institute.[12]
- Follow your institution’s safety procedures for working with chemicals and handling biological samples/agar plates with pure colonies.
- 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 samples, are recommended to avoid contamination of samples 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.
- 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 repeat the cleaning of the work area with 70% ethanol. Wipe work surfaces dry completely before proceeding.

8 Chemical Hazards[17, 18]

Reagent 2 contains Guanidinium chloride (Safety Data Sheet chemical warnings):

- Signal Word: Warning
- **CLP/GHS Hazard Statements**: H302: Harmful if swallowed, H315: Causes skin irritation, H319: Causes serious eye irritation.
- **Precautionary Statements**:
  - P264: Wash hands thoroughly after handling.
  - P280: Wear protective gloves/eye protection/face protection.
  - P273: Avoid release to the environment.
  - P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
  - P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  - P332 + P313: If skin irritation occurs: Get medical advice/attention.
  - P312: Call a POISON CENTER or physician if you feel unwell.
  - P501: Dispose of contents/container to location in accordance with local and regional/national/international regulations.

9 Sample Preparation and Storage

Rectal Swab Samples:

- Collection of a paired rectal swab: Carefully insert both swab tips approximately 1 cm beyond the anal sphincter and rotate gently. See “Materials Required but Not Provided” for the swabs to be used and Figure 1 and Figure 2 for examples of acceptable and unacceptable swabs for use with the Xpert Carba-R Assay.
- Place swab pair back into the original transport tube.
- Swabs in the transport tube can be stored at 15–28 °C for up to five days.

See Figure 1 for examples of acceptable swab specimens to be used with the Xpert Carba-R Assay. See Figure 2 for examples of highly soiled swab specimens that should not be used with the Xpert Carba-R Assay.

Figure 1. Examples of Acceptable Swab Specimens for Xpert Carba-R Testing

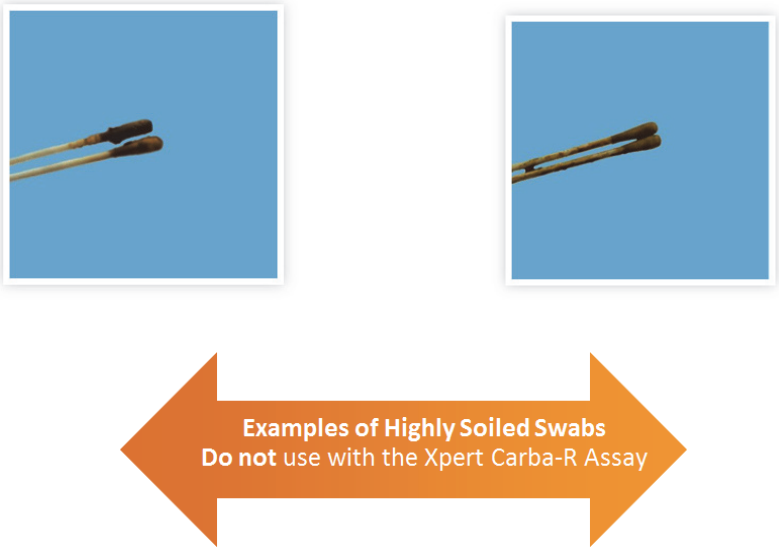

Figure 2. Examples of Unacceptable Swab Specimens for Xpert Carba-R Assay Testing

Bacterial Isolates:

1. Organisms should be identified and carbapenem non-susceptibility status should be determined in accordance with the current FDA approved drug package insert and the latest version of CLSI guideline M100[13] prior to testing on Xpert Carba-R Assay.
2. Inoculate the organism onto either a blood or MacConkey agar plate, streak for isolation, and place a 10 µg meropenem disk in the first streak quadrant as a means to ensure that the isolate retains its non-susceptibility to carbapenem.
3. Incubate the plate at 35 °C for 18–24 hours in ambient air.
4. Use the direct colony suspension method by touching isolated colonies with a swab or loop to prepare a 0.5 McFarland suspension of the bacterial isolate as outlined in the CLSI M07 Approved Standard[14]. The steps are also described below.
   A. Make a suspension of isolated colonies selected from an agar plate (e.g., a nonselective medium such as blood agar that has been incubated for 18-hours to 24-hours) directly in broth or saline.
   B. Adjust the suspension to achieve a turbidity equivalent to a 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 10^8 CFU/mL for *E. coli* ATCC (American Type Culture Collection) 25922.
   C. Use either a photometric device or, if performed visually, use adequate light to compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

10 Procedure

10.1 Preparing the Cartridge

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

1. Remove a Xpert Carba-R Assay cartridge, a Sample Reagent vial and a transfer pipette from the kit. Open the vial of the Sample Reagent.
2. To add the sample to the cartridge:

- For rectal swab samples, to add the swab sample to the cartridge:
  - From the paired swabs, place one swab into the Sample Reagent vial. Replace the unused swab into the transport tube and store.

Note Refer to Section 9 for storage conditions of the rectal swab samples. The leftover second swab may be used for repeat testing.

Note Refer to Section 14, Retest Procedure, to repeat the test for rectal swab samples.

  - 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.
- For bacterial isolates, to add the 0.5 McFarland suspension of the isolate to the cartridge:
  - Vortex the 0.5 McFarland suspension. Using a 10 µL loop, transfer 10 µL of the 0.5 McFarland suspension to a 5 mL vial of Sample Reagent. Swirl the loop a minimum of three times in the Sample Reagent. After the initial test, the leftover sample in the Sample Reagent vial can be retained at 2–28 °C for up to five days if a retest is required.

Note Refer to Section 14, Retest Procedure, for instructions on how to repeat the test for bacterial isolate samples.

Note Ensure that the 10 µL loop is filled with sample and the sample suspension in the loop does not burst when transferring the 0.5 McFarland suspension to the Sample Reagent.

3. Cap the Sample Reagent vial tightly and vortex at high speed for 10 seconds.
4. Open the cartridge lid. Open the Sample Reagent cap. Using the transfer pipette provided, aspirate the prepared sample (Sample Reagent containing the sample from Step 2) up to the mark on the pipette (which is approximately 1.7 mL; see Figure 3) and then transfer the material into the Sample Chamber large opening (see Figure 4) of the Xpert Carba-R Assay cartridge.
5. Close the cartridge lid and place the cartridge into the GeneXpert instrument within 30 minutes of adding the sample to the cartridge.

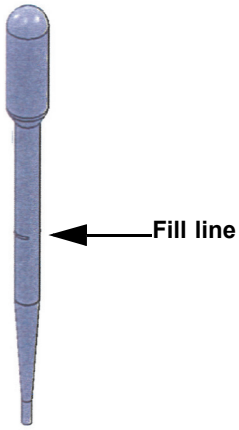

Figure 3. Transfer Pipette to Transfer Sample to Cartridge

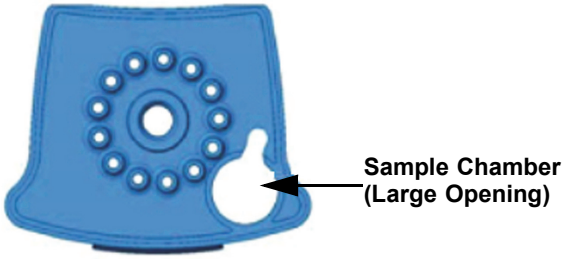

Figure 4. Xpert Carba-R Assay Cartridge (Top View)

10.2 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. The default workflow is described below.

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 14.

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.

10.3 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.

11 Quality Control

CONTROL

Built-in Quality Controls

Each test includes a Sample Processing Control and a Probe Check Control.

- **Sample Processing Control (SPC)**—Ensures the sample was processed correctly. 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 described in Section 6.4 are available but not provided and may be used in accordance with local, state, and federal accrediting organizations, as applicable. Always use external controls, according to the manufacturer’s instructions.

12 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
IMP DETECTED; **VIM NOT DETECTED;** **NDM NOT DETECTED;** **KPC NOT DETECTED;** **OXA48 NOT DETECTED** See Figure 5.	IMP target DNA sequence is detected; VIM, NDM, KPC, and OXA-48 target DNA sequences are not detected. • PCR amplification of the IMP 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 target DNA amplification may compete with this control. • PCC: PASS; all probe check results pass.
IMP NOT DETECTED; **VIM DETECTED;** **NDM NOT DETECTED;** **KPC NOT DETECTED;** **OXA48 NOT DETECTED** See Figure 6.	VIM target DNA sequence is detected; IMP, 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, 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.
IMP NOT DETECTED; **VIM DETECTED;** **NDM DETECTED;** **KPC NOT DETECTED;** **OXA48 NOT DETECTED** See Figure 7.	VIM and NDM target DNA sequences are detected; IMP, 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, 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.
IMP DETECTED; **VIM NOT DETECTED;** **NDM DETECTED;** **KPC NOT DETECTED;** **OXA48 NOT DETECTED** See Figure 8.	IMP and NDM target DNA sequences are detected; VIM, KPC, and OXA-48 target DNA sequences are not detected. • PCR amplification of the IMP 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 and NDM target DNA amplifications may compete with this control. • PCC: PASS; all probe check results pass.
IMP DETECTED; **VIM DETECTED;** **NDM NOT DETECTED;** **KPC NOT DETECTED;** **OXA48 DETECTED** See Figure 9.	IMP, VIM, and OXA-48 target DNA sequences are detected; NDM and KPC target DNA sequences are not detected. • PCR amplification of the IMP, 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, VIM, and OXA-48 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
IMP DETECTED; **VIM DETECTED;** **NDM DETECTED;** **KPC NOT DETECTED;** **OXA48 DETECTED** See Figure 10.	IMP, VIM, NDM, and OXA-48 target DNA sequences are detected; KPC target DNA sequence is not detected. • PCR amplification of the IMP, 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, VIM, NDM, and OXA-48 target DNA amplifications may compete with this control. • PCC: PASS; all probe check results pass.
IMP DETECTED; **VIM DETECTED;** **NDM DETECTED;** **KPC DETECTED;** **OXA48 DETECTED** See Figure 11.	IMP, VIM, NDM, KPC, and OXA-48 target DNA sequences are detected. • PCR amplification of the IMP, 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, VIM, NDM, KPC, and OXA-48 target DNA amplifications may compete with this control. • PCC: PASS; all probe check results pass.
IMP NOT DETECTED; **VIM NOT DETECTED;** **NDM NOT DETECTED;** **KPC NOT DETECTED;** **OXA48 NOT DETECTED** See Figure 12.	IMP, VIM, NDM, KPC, and OXA-48 target DNA sequences are not detected. • IMP, 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 13.	Presence or absence of IMP, VIM, NDM, KPC, and OXA-48 target DNA sequences cannot be determined. Use the instructions in Section 14, 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, VIM, NDM, KPC, and OXA-48 target DNA sequences cannot be determined. Use the instructions in Section 14, 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.
NO RESULT	Presence or absence of IMP, VIM, NDM, KPC, and OXA-48 target DNA sequences cannot be determined. Use the instructions in Section 14, 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

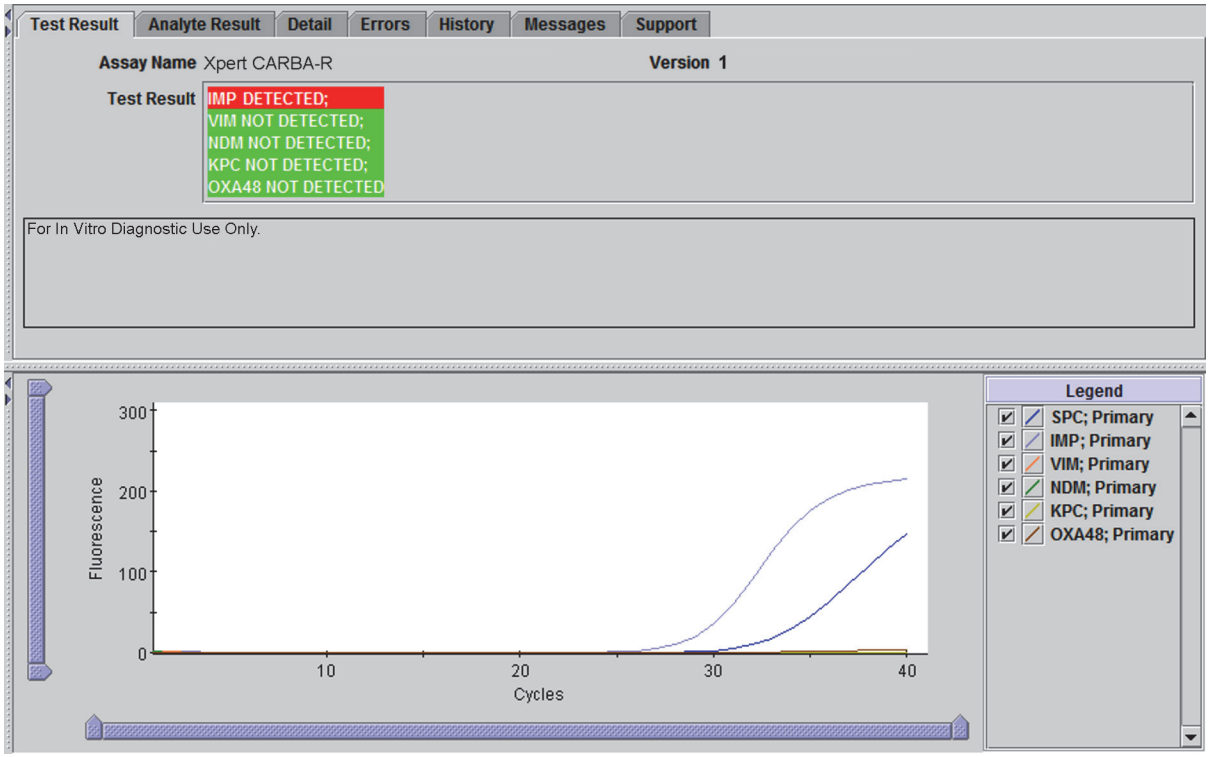

Figure 5. Carba-R Assay—IMP Detected

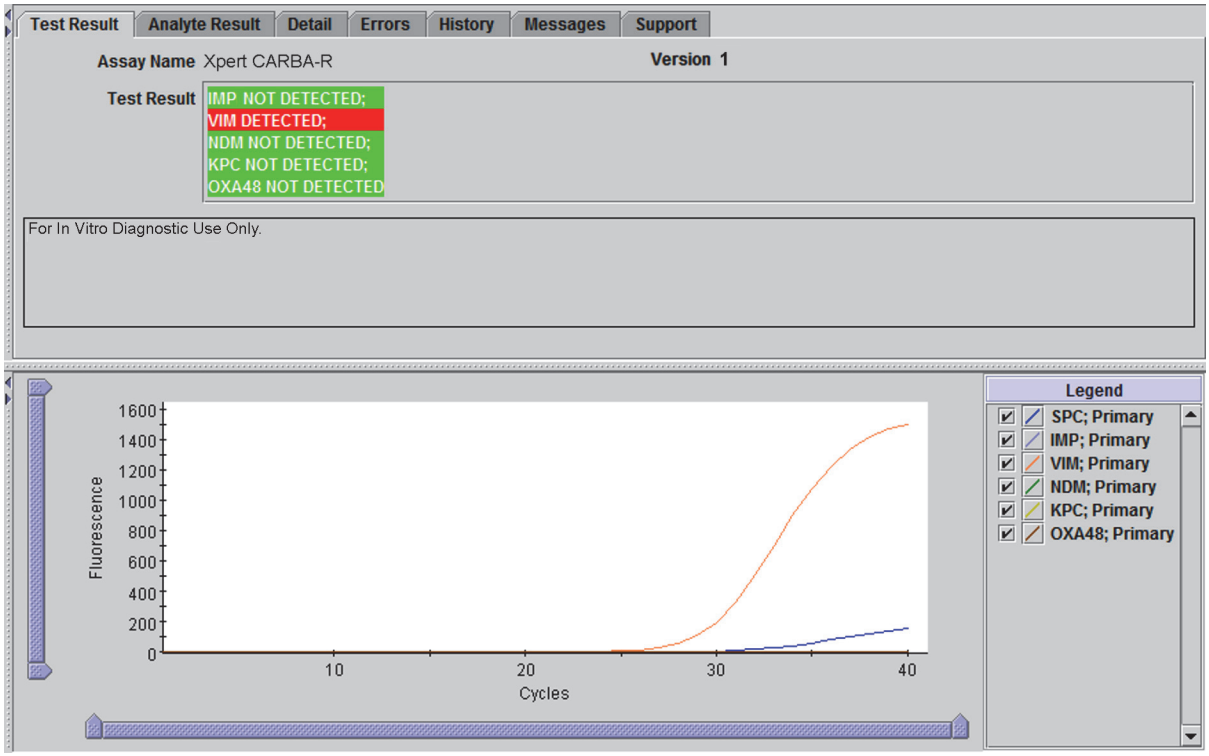

Figure 6. Carba-R Assay—VIM Detected

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

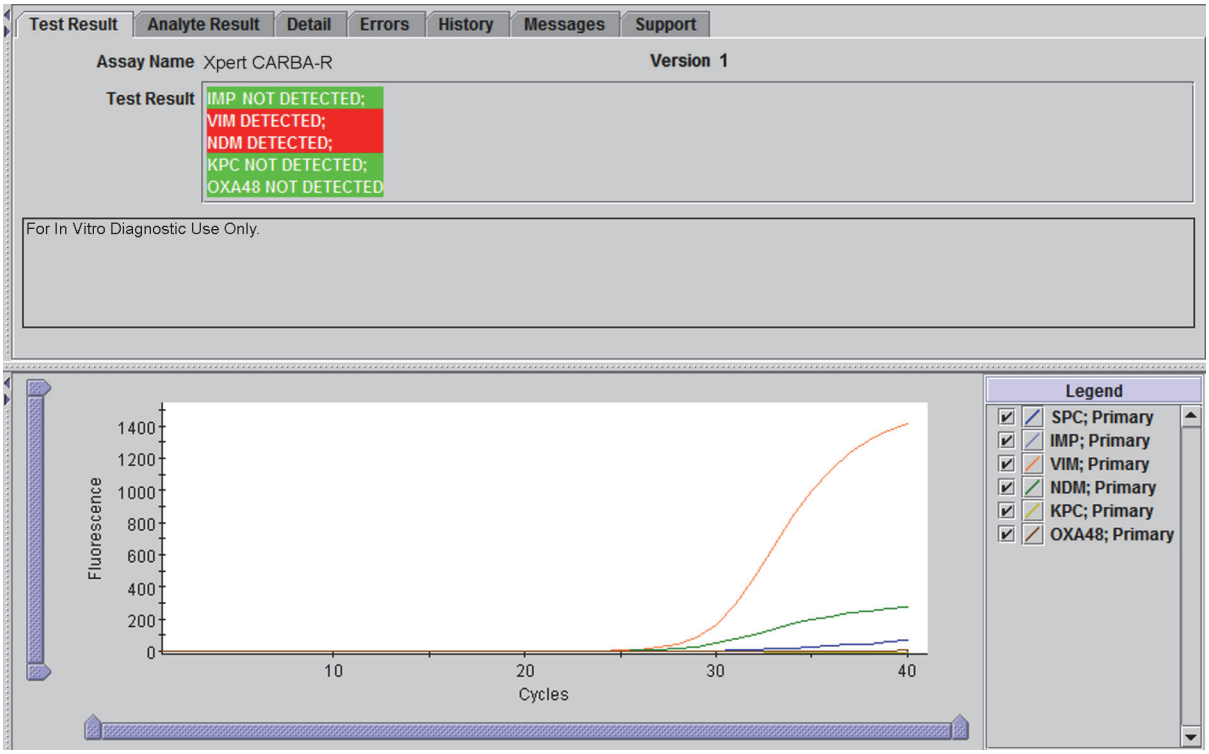

Figure 7. Carba-R Assay—VIM and NDM Detected

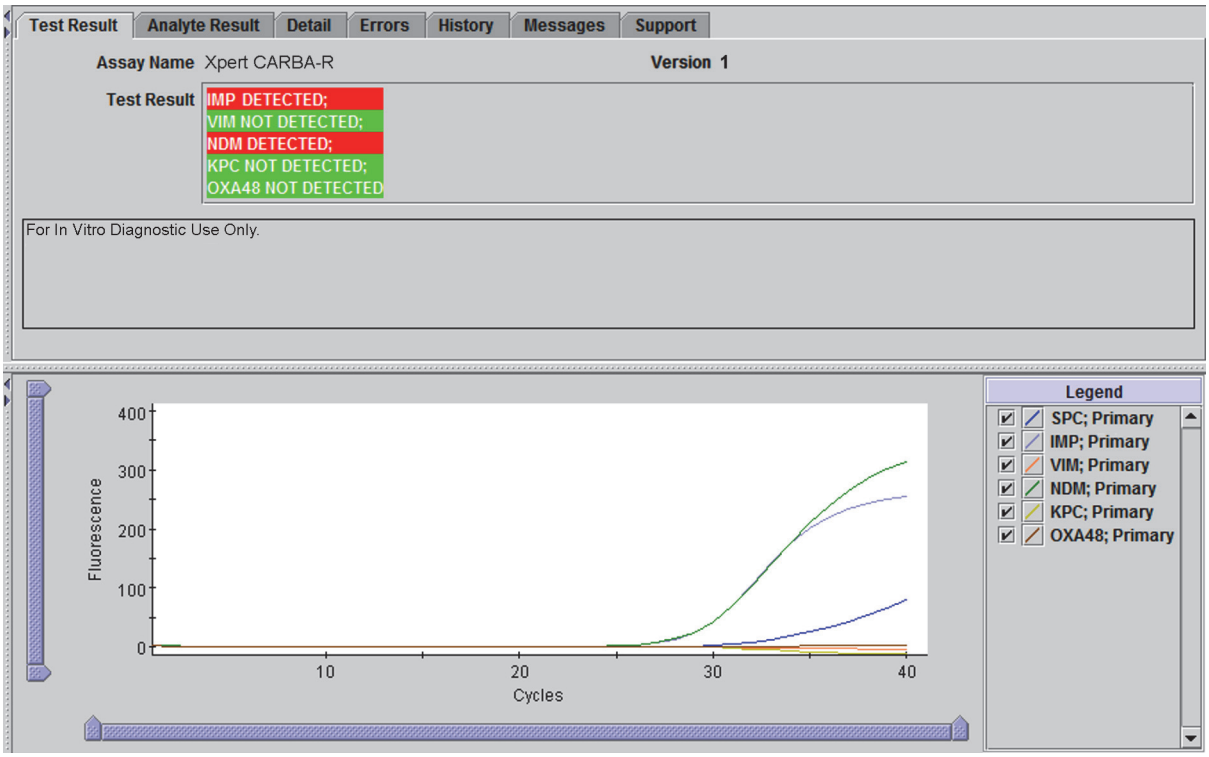

Figure 8. Carba-R Assay—IMP and NDM Detected

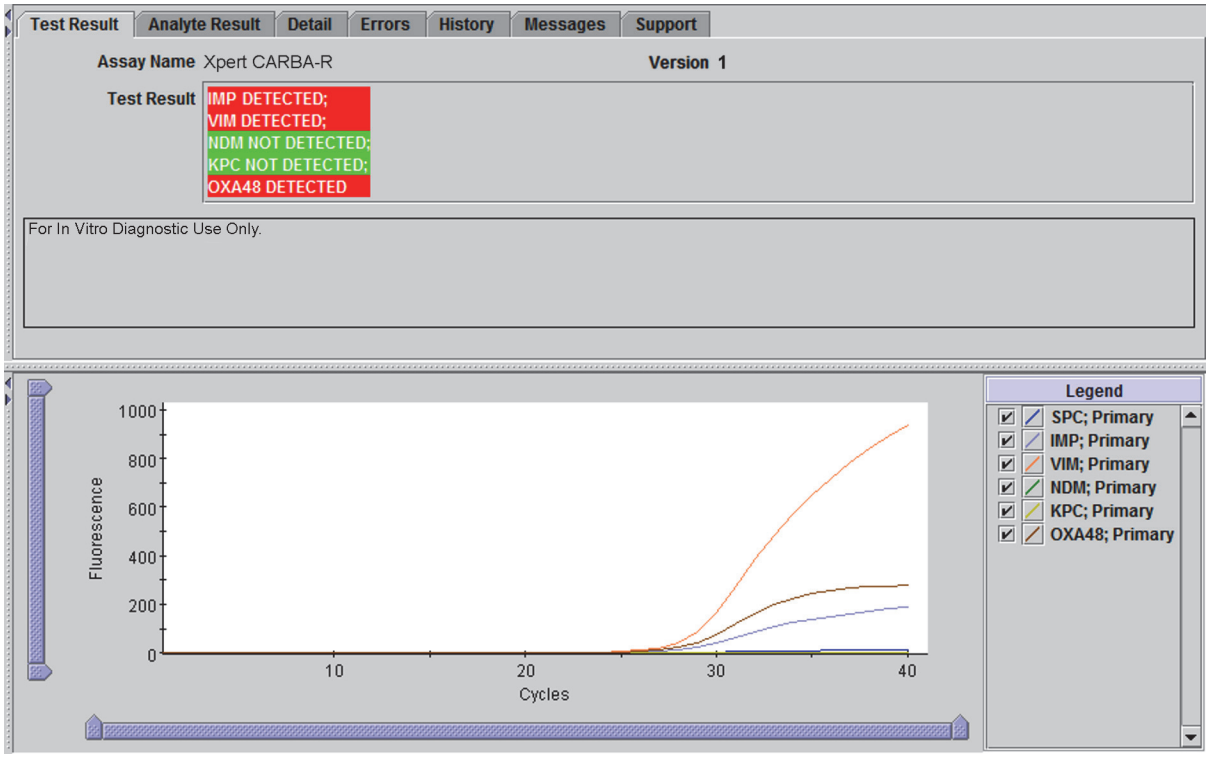

Figure 9. Carba-R Assay—IMP, VIM, and OXA-48 Detected

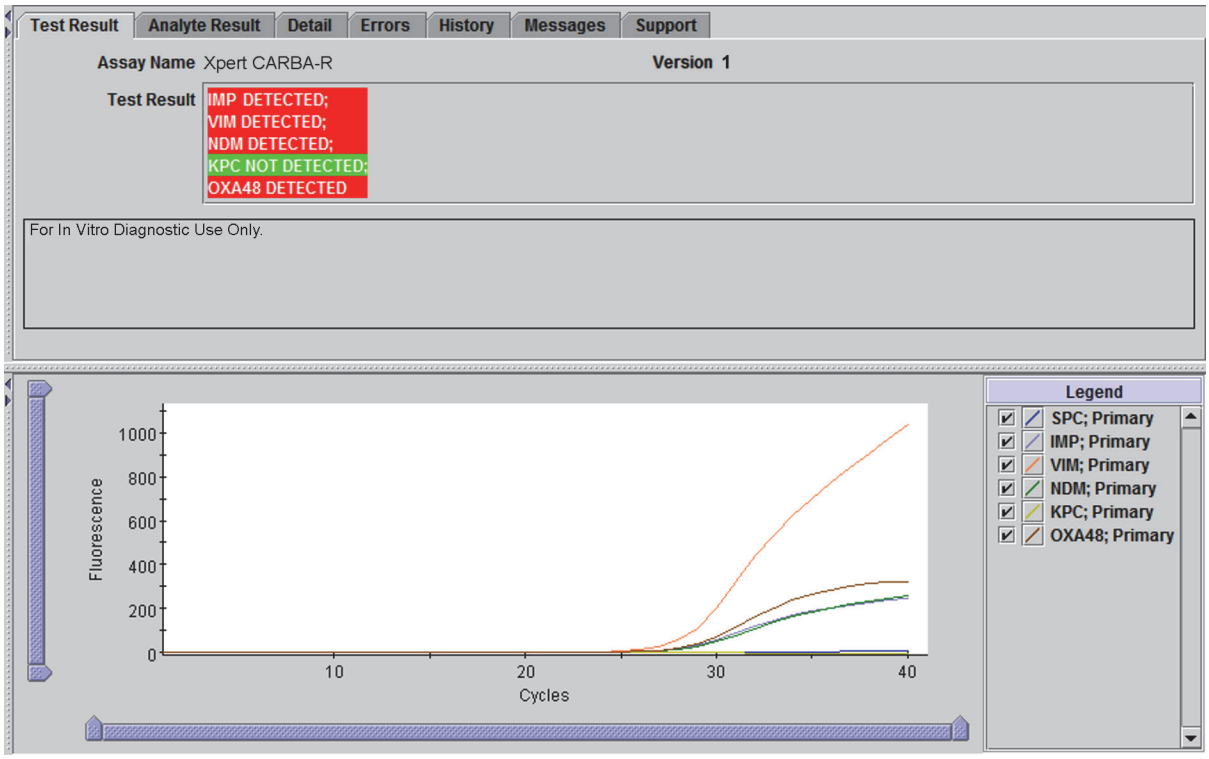

Figure 10. Carba-R Assay—IMP, VIM, NDM, and OXA-48 Detected

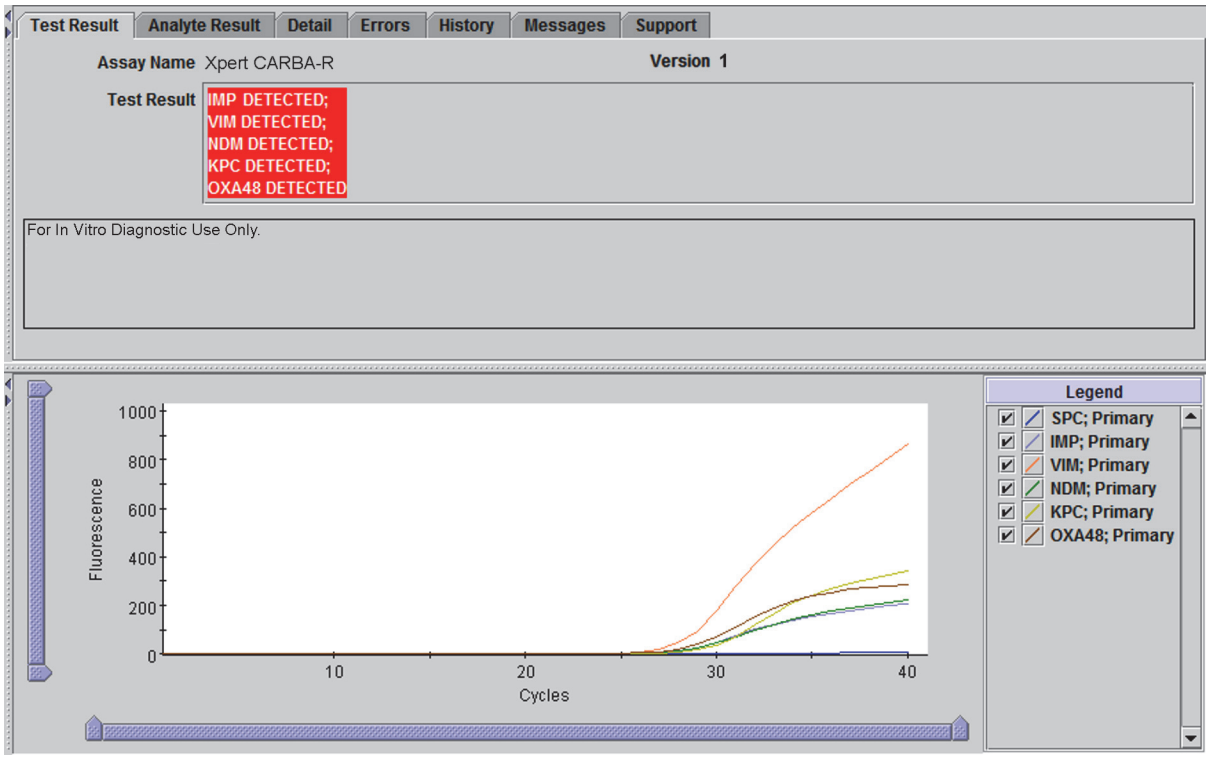

Figure 11. Carba-R Assay—IMP, VIM, NDM, KPC, and OXA-48 Detected

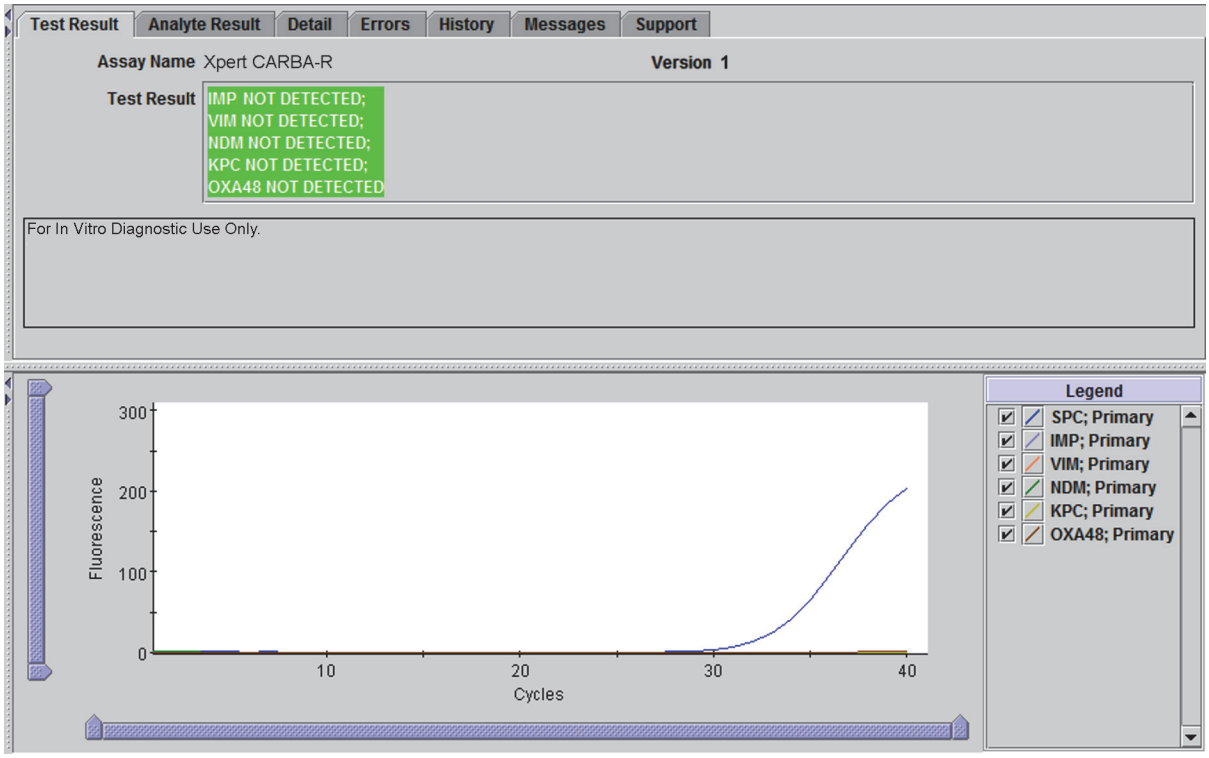

Figure 12. Carba-R Assay—IMP, VIM, NDM, KPC, and OXA-48 Not Detected

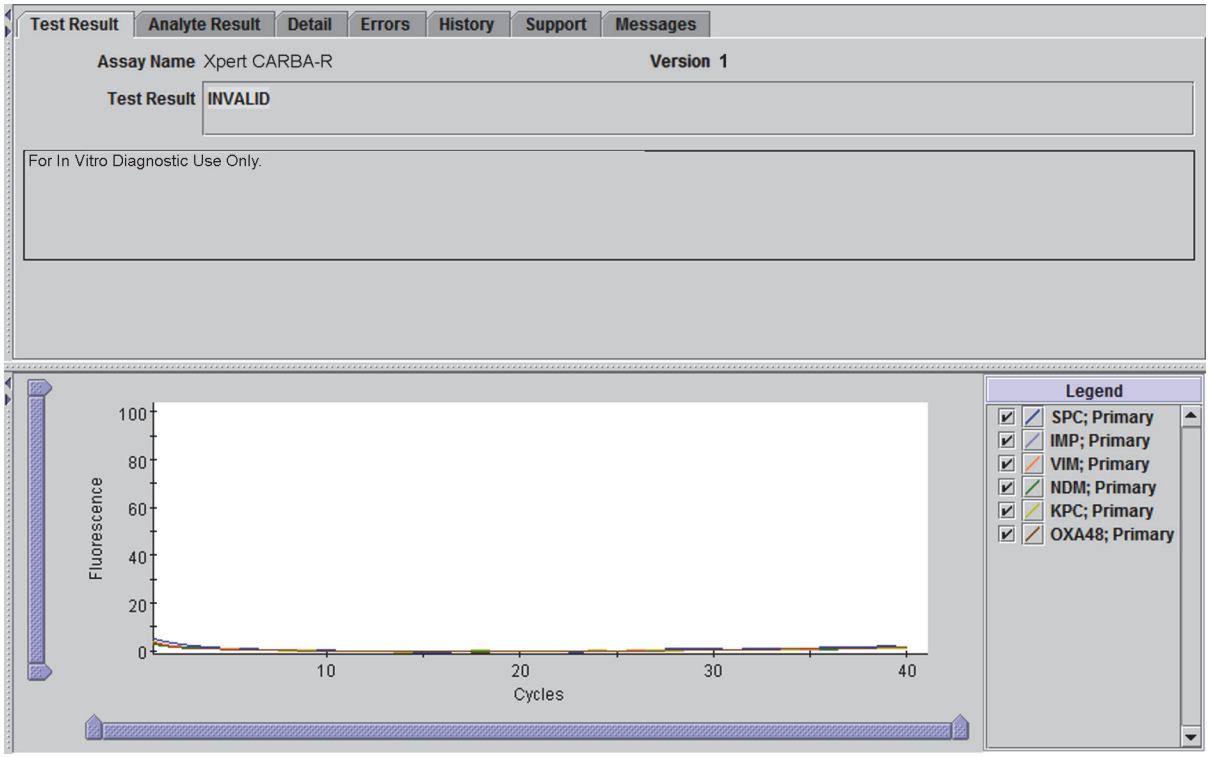

Figure 13. Carba-R Assay—Invalid

13 Reasons to Repeat the Test

Repeat the test using a new cartridge (do not re-use the cartridge) and new Sample Reagent vial. For the retest procedure, see Section 14, Retest Procedure.

- 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 Control fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.

14 Retest Procedure

14.1 Rectal Swab Sample Retest Procedure

1. Remove a new cartridge, a new Sample Reagent vial, and a new transfer pipette from the kit.
2. Remove the leftover swab from the transport container.
3. Insert the swab into a new Sample Reagent vial. 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.
4. Cap the new Sample Reagent vial tightly and vortex at high speed for 10 seconds.
5. Open the cartridge lid. Using the provided transfer pipette, aspirate the Sample Reagent to the mark on the pipette, and then transfer the material into the Sample Chamber of the Xpert Carba-R Assay cartridge.
6. Close the cartridge lid and place the cartridge into the GeneXpert instrument within 30 minutes. Follow Section 10.2, Starting the Test.

14.2 Bacterial Isolates Retest Procedure

1. Remove a new cartridge, a new Sample Reagent vial, and a new transfer pipette from the kit.
2. Transfer the entire contents of the leftover sample in Sample Reagent vial to the new Sample Reagent vial.
3. Cap the new Sample Reagent vial tightly and vortex at high speed for 10 seconds.
4. Open the cartridge lid. Using the provided transfer pipette, aspirate the Sample Reagent to the mark on the pipette, and then transfer the material into the Sample Chamber of the Xpert Carba-R Assay cartridge.
5. Close the cartridge lid and place the cartridge into the GeneXpert instrument within 30 minutes. Follow Section 10.2, Starting the Test.

Note For bacterial isolates, do not perform the retest procedure more than once as repeated dilutions may give false negative results.

15 Limitations

15.1 General Limitations

- The Xpert Carba-R Assay detects *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ from rectal swab specimens or pure colonies, and is not for bacterial identification. Detection of these gene sequences does not indicate the presence of viable organisms.
- The Xpert Carba-R Assay is not a sub-typing tool and does not report variants of the *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{NDM}$, *bla*$_{KPC}$, or *bla*$_{OXA-48}$ genes.
- Certain bacterial species, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have been shown to exhibit resistance to carbapenems due to intrinsic resistance mechanisms.
- The detection of other OXA-carbapenemase genes, besides *bla*$_{OXA-48}$ and *bla*$_{OXA-181}$, has not been evaluated in the study.
- The *in silico* analyses used to predict variants detected by the assay were based on a comparison of target gene sequences available in GenBank to the Xpert Carba-R Assay primer/probe oligonucleotides and amplicon sequence for each gene target. BLAST searches for *in silico* analysis were performed in 2014-2015. *In silico* analysis of new variant gene sequences deposited into the database after 2015 for the five target genes have not been performed.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of current, new or unknown *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ variants, resulting in a false negative result.
- The Xpert Carba-R Assay will generate a negative IMP result when testing samples containing IMP-7, IMP-13, or IMP-14 gene sequences.
- Performance of the Xpert Carba-R Assay with non-target carbapenemase genes, other than *bla*$_{SPM}$, *bla*$_{SME}$, and *bla*$_{IMI}$, is unknown.
- As the detection of *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene sequences is dependent on the number of organisms present in the sample, reliable results are dependent on proper sample handling and storage.
- Testing with the Xpert Carba-R Assay should be used as an adjunct to other available methods.
- 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.

15.2 Rectal Specimen Limitations

- The performance of the Xpert Carba-R Assay has not been evaluated with rectal swab specimens from pediatric patients.
- Analytical studies using combinations of two bacterial populations on contrived swab specimens indicate that when one carbapenemase-producing bacterial species is inoculated near the LoD and another carbapenemase-producing bacterial species is present at concentrations equal or greater than 5 x 10^6 CFU/swab, the low concentration target may not be detected. Co-colonization with two or more carbapenemase-producing organisms has been reported with Xpert Carba-R Assay, but is rare. Lack of detection of a second target should have minimal impact on patient management since isolation procedures would be instituted for patients showing any positive result for a carbapenemase-producing organism.
- Interference with the Xpert Carba-R Assay may be observed with barium sulfate at > 0.1% w/v, and Pepto-Bismol at > 0.01% w/v in tests with rectal swab matrix samples.
- In rectal swab samples containing the VIM target, interference may occur if fecal fat is present at a concentration of 0.25% w/v, resulting in delayed cycle threshold values.

- In addition to *Pseudomonas aeruginosa* and *Acinetobacter baumannii* groups tested in the contrived study, other non-*Enterobacteriaceae* were also evaluated: *Pseudomonas stutzeri* (1)*, Pseudomonas oryzihabitans* (1)*, Pseudomonas putida* (2), and *Empedobacter brevis* (1). The performance of the Xpert Carba-R Assay with other non-*Enterobacteriaceae* besides these six species has not been evaluated and is therefore unknown.
- The Xpert Carba-R Assay showed reduced positive percent agreement (PPA of 55.6%) for detection of the bla_{VIM} gene sequence in *Pseudomonas aeruginosa.* Four (4) false negative results were observed with the assay in specimens in which *Pseudomonas aeruginosa* containing the bla_{VIM} sequence was recovered by the reference method.
- The Xpert Carba-R Assay showed reduced positive percent agreement (PPA of 85.7%) for the detection of the bla_{IMP} gene sequence in *Acinetobacter baumannii* during the Contrived Study. In addition, a low % total agreement (86.1%) across sites for the Reproducibility Study was observed with samples containing low concentrations of organism harboring the bla_{IMP} gene sequence.
- Carbapenem-resistant anaerobes potentially present in fecal specimens have not been evaluated by the Xpert Carba-R Assay.
- The detection of bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and/or bla_{IMP} from rectal specimens may be from organisms other than *Enterobacteriaceae, Pseudomonas aeruginosa* and *Acinetobacter baumannii.*
- The performance of the Xpert Carba-R Assay with susceptible isolates containing bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and/or bla_{IMP} gene sequences has not been fully evaluated.

15.3 Pure Colonies Limitations

- For pure colonies, the performance of the Xpert Carba-R Assay with bacteria other than Enterobacteriaceae, Pseudomonas aeruginosa, or Acinetobacter baumannii has not been evaluated. Organisms should be identified, and carbapenem non-susceptibility status should be determined prior to testing on Xpert Carba-R Assay.
- Erroneous test results might occur from improper culture techniques, failure to follow the recommended procedure to prepare the 0.5 McFarland suspension, 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.

16 Expected Values

In the Xpert Carba-R Assay clinical study, a total of 1187 rectal swab specimens and contrived specimens were evaluated across 5 study sites within and outside of the United States. Xpert Carba-R Assay results in comparison to culture and bidirectional DNA sequence analysis by gene target for each of the prospective combined and contrived specimens is presented in Table 2.

In a separate Xpert Carba-R Assay clinical study, a total of 467 bacterial isolates were evaluated across 4 study sites within and outside of the United States. Xpert Carba-R Assay results in comparison to bidirectional DNA sequence analysis by gene target for each of the two agar types are presented in Table 7, Table 8, Table 9, Table 10, and Table 11.

17 Performance Characteristics

17.1 Clinical Performance – Rectal Swab Specimens

Performance characteristics of the Xpert Carba-R Assay with rectal swab specimens were determined in a multi-site investigational study. The positive percent agreement (PPA) and negative percent agreement (NPA) of the Xpert Carba-R Assay was evaluated relative to a reference method of culture (MacConkey enrichment broth) and PCR/bi-directional DNA sequence analysis.

Five geographically diverse sites (three across the United States and two in Europe) prospectively collected paired rectal swab specimens from subjects who were hospitalized or in a long-term care facility. Highly soiled rectal swab specimens, according to the directions in Section 9 (Sample Preparation and Storage) were excluded from the study. Due to low prevalence of each of the Xpert Carba-R Assay target genes in the absence of an outbreak, contrived specimens were also included in the study.

One rectal swab of the pair was used for Xpert Carba-R Assay testing. The second rectal swab was inoculated into MacConkey enrichment broth and used for reference method testing. A reference culture laboratory determined the presence of carbapenem non-susceptible organisms by culturing the MacConkey enrichment broth from each of the specimens. The MacConkey enrichment broth was screened for the presence of carbapenem-non-susceptible organisms initially by plating the broth on MacConkey agar plates with a meropenem disk. For specimens that exhibited growth of gram-negative bacteria around the meropenem disk, confirmation of carbapenem non-susceptibility was determined on isolated colonies by using the disk diffusion method (per CLSI document M02) as well as CLSI document M100. DNA extracted from the carbapenem non-susceptible isolates was purified, quantified, and amplified using primers specific to all 5 target genes; amplified regions included more bases than the regions amplified by the Xpert Carba-R Assay. The production of the appropriate size amplification product was confirmed on Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA).

If bands shown on the Bioanalyzer corresponded to the expected size of the amplicon from any of the five target genes detected by the Xpert Carba-R Assay, the amplicon for the isolate was sent to an independent laboratory for reference bi-directional sequencing analysis, which was validated for detection of the five targets in the Xpert Carba-R Assay. If no bands were shown on the Bioanalyzer for any of the five target genes, the isolate was not sent for sequence analysis and the reference method result was considered negative for the five target genes.

Prospective Specimen Results Obtained with the Xpert Carba-R Assay in Comparison to the Reference Method

A total of 802 prospective rectal swab specimens were initially enrolled in this clinical study, of which 785 were eligible for inclusion. From the 785 eligible specimens, 755 specimens were included in the final dataset after exclusions based on protocol deviations (including 16 *Stenotrophomonas maltophilia* organisms that were excluded due to their intrinsic resistance to the carbapenems tested).

When tested with prospective rectal swab specimens, the Xpert Carba-R Assay demonstrated a PPA range from 60.0% to 100% for the four assay targets (bla_{KPC}, bla_{NDM}, bla_{VIM}, and bla_{OXA-48}) relative to the reference method (Table 2). The NPA for the bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP} gene sequences ranged from 98.6%-99.9%relative to the reference method (Table 2).

For specimens with discordant results (the Xpert Carba-R Assay was positive for a target gene but a carbapenem-non-susceptible organism was not isolated by reference culture), discordant analysis was performed using bi-directional sequencing on DNA extracted directly from the MacConkey enrichment broth. Discrepant testing results are footnoted in Table 2.

Table 2. Xpert Carba-R Performance vs. Reference Culture + Sequencing – Prospective Specimens

Specimen Type	Target	N	TP	FP	TN	FN	PPA% (95 CI)	NPA% (95 CI)
Prospective[g]	IMP	755	0	1[a]	754	0	N/A	99.9% (99.3-100.0)
	VIM	755	6	8[b]	737	4	60.0% (31.3-83.2)	98.9% (97.9-99.5)
	NDM	755	7	3[c]	745	0	100.0% (64.6-100.0)	99.6% (98.8-99.9)
	KPC	755	29	6[d,e]	720	0	100.0% (88.3-100.0)	99.2% (98.2-99.6)
	OXA-48	755	29	10[f]	715	1	96.7% (83.3-99.4)	98.6% (97.5-99.2)

N = Number, TP = True Positive, FP = False Positive, TN = True Negative, FN = False Negative

a. 1 discordant specimen was confirmed as FP after discordant analysis.

b. 2 of the 8 FPs were determined to be TPs after discordant analysis.

c. 1 of the 3 FPs was determined to be TP after discordant analysis.

d. 1 of the 6 FPs was determined to be TP after discordant analysis.

e. Site reported that subject was on ertapenem during time of specimen collection.

f. 3 of the 10 FPs were determined to be TPs after discordant analysis.

g. Of the 755 prospective rectal swab specimens evaluated in the study, 636 specimens did not yield a culture isolate. From the remaining 119 specimens, 112 carbapenem-non-susceptible organisms were recovered by the Reference Culture in addition to 7 carbapenem susceptible organisms [*Pseudomonas aeruginosa* (5); *Escherichia coli* (1), and *Enterobacter cloacae* (1)].

Performance of the Xpert Carba-R Assay on the prospective specimens is shown in Table 3 by species. Only organisms for which at least one positive specimen was collected are included in Table 3.

Table 3. Xpert Carba-R Performance vs. Reference Culture + Sequencing by Organism Type – Prospective Specimens

Species[a]	Target	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
Enterobacter cloacae	IMP	4	0	0	4	0	NA	100% (51.0-100.0)
	VIM	4	1	0	3	0	100% (20.7-100.0)	100% (43.9-100.0)
	NDM	4	0	0	4	0	NA	100% (51.0-100.0)
	KPC	4	0	0	4	0	NA	100% (51.0-100.0)
	OXA-48	4	1	0	3	0	100% (20.7-100.0)	100% (43.9-100.0)
E. coli	IMP	10	0	0	10	0	NA	100% (72.3-100.0)
	VIM	10	0	0	10	0	NA	100% (72.3-100.0)
	NDM	10	3	0	7	0	100% (43.9-100.0)	100% (64.6-100.0)
	KPC	10	2	0	8	0	100% (34.2-100.0)	100% (64.6-100.0)
	OXA-48	10	3	0	7	0	100% (43.9-100.0)	100% (64.6-100.0)
Klebsiella oxytoca	IMP	1	0	0	1	0	NA	100% (20.7-100.0)
	VIM	1	0	0	1	0	NA	100% (20.7-100.0)
	NDM	1	0	0	1	0	NA	100% (20.7-100.0)
	KPC	1	0	0	1	0	NA	100% (20.7-100.0)
	OXA-48	1	1	0	0	0	100% (20.7-100.0)	NA
Klebsiella pneumoniae	IMP	60	0	1	59	0	NA	98.3% (94.0-100.0)
	VIM	60	0	1	59	0	NA	98.3% (94.0-100.0)
	NDM	60	4	1	55	0	100% (51.0-100.0)	98.2% (90.6-99.7)
	KPC	60	27	1	32	0	100% (87.5-100.0)	97.0% (89.6-100.0)
	OXA-48	60	24	3	32	1	96.0% (80.5-99.3)	91.4% (77.6-97.0)

Table 3. Xpert Carba-R Performance vs. Reference Culture + Sequencing by Organism Type – Prospective Specimens (Continued)

Species[a]	Target	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
Pseudomonas aeruginosa	IMP	30	0	0	30	0	NA	100% (88.7-100.0)
	VIM	30	5	0	21	4	55.6% (26.7-81.1)	100% (84.5-100.0)
	NDM	30	0	1	29	0	NA	96.7% (83.3-99.4)
	KPC	30	0	1	29	0	NA	96.7% (83.3-99.4)
	OXA-48	30	0	0	30	0	NA	100% (88.7-100.0)

a. *Acinetobacter baumannii* (13) and *Enterobacter amnigensus* (1) were recovered but did not contain target sequences by the Reference Method.

Multiple targets were detected by the Xpert Carba-R Assay in eight prospective specimens. The details are provided in Table 4, along with the discrepant sequencing result.

Table 4. Prospective Specimens with Multiple Targets Detected

Specimen	Targets Detected by Xpert Carba-R Assay	Targets Detected by Reference Sequencing	Discrepant Testing Results - Targets Detected by Reference Sequencing
1	KPC, OXA-48	NEG	NEG
2	VIM, KPC	NEG[a]	NEG[a]
3	VIM, OXA-48	OXA-48	OXA-48
4	KPC, OXA-48	KPC	KPC, OXA-48
5	NDM, OXA-48	NDM	NDM, OXA-48
6	VIM, NDM	NEG[a]	NEG
7	NDM, KPC	KPC	NDM, KPC
8	VIM, KPC	VIM	VIM, KPC

a. An organism was not isolated from reference culture, therefore, reference sequencing was not performed.

Contrived Specimen Results Obtained with the Xpert Carba-R Assay in Comparison to the Reference Method

A total of 432 contrived specimens prepared in rectal swab matrix were also tested as part of the clinical study.

In addition to *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* groups tested in the contrived study, 5 other non-*Enterobacteriaceae* strains were also evaluated: *Pseudomonas stutzeri* (1), *Pseudomonas oryzihabitans* (1), *Pseudomonas putida* (2), and *Empedobacter brevis* (1).

When tested with contrived specimens, the Xpert Carba-R Assay demonstrated a range of PPA from 95% to 100% across the assay targets (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP}). The NPA for the bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP} gene sequences was 100% relative to the reference method (Table 5).

Table 5. Xpert Carba-R Performance vs. Reference Method – Contrived Specimens

Target	N	TP	FP	TN	FN	PPA%(95 CI)	NPA%(95 CI)
IMP	432	76	0	352	4	95.0% (87.8-98.0)	100.0% (98.9-100.0)
VIM	432	81	0	350	1	98.8% (93.4-99.8)	100.0% (98.9-100.0)
NDM	432	80	0	352	0	100.0% (95.4-100.0)	100.0% (98.9-100.0)
KPC	432	80	0	352	0	100.0% (95.4-100.0)	100.0% (98.9-100.0)
OXA-48	432	79	0	352	1	98.8% (93.3-99.8)	100.0% (98.9-100.0)

17.2 Clinical Performance – Bacterial Isolates

Performance characteristics of the Xpert Carba-R Assay with bacterial isolates were determined in a multi-site investigational study by comparing the Xpert Carba-R Assay to reference bi-directional sequencing of the amplified DNA target. Study samples included bacterial isolates grown from both blood agar and MacConkey agar.

To be included in the study, isolates must have been previously identified as *Enterobacteriaceae*, *Pseudomonas aeruginosa*, or *Acinetobacter baumannii*. For determination of sensitivity, isolates must have been either intermediate or resistant to meropenem, ertapenem and/or imipenem per CLSI M100-S24[15]. Isolates of *Pseudomonas aeruginosa* or *Acinetobacter baumanii* must have been intermediate or resistant to either imipenem or meropenem. These organisms are intrinsically resistant to ertapenem. For evaluation of specificity, isolates may have been susceptible or resistant to meropenem, ertapenem, and imipenem per CLSI M100-S24[15]. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates should have been susceptible to both imipenem and meropenem. Isolates were tested only once in the study.

A total of 489 bacterial isolates (431 clinical stock isolates and 58 fresh isolates) were initially enrolled in this clinical study, of which 485 were eligible for inclusion. The ineligible isolates included four isolates previously enrolled in the study.

From the 485 eligible isolates, 467 isolates (410 clinical stock isolates and 57 fresh isolates) were included in the final dataset used for the analyses presented in this report; two isolates were excluded because reference testing was not performed; and sixteen isolates were excluded because they were not identified as *Enterobacteriaceae*, *A. baumannii*, or *P. aeruginosa.*

For Xpert Carba-R Assay testing, well-isolated colonies that grew on each of the agar types were diluted to a 0.5 McFarland standard equivalent suspension using the direct colony suspension method per CLSI M07-A9.[16]

For reference sequencing, DNA from culture isolates was purified, quantified, and amplified using primers specific to all 5 target genes that were designed to amplify larger regions from the assay targets than the primers included in the Xpert Carba-R Assay. The production of the appropriate size of amplification product was confirmed on Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA).

If bands shown on the Bioanalyzer corresponded to the expected size of the amplicon from any of the five target genes detected by the Xpert Carba-R Assay, the amplicon for the isolate was sent to an independent laboratory for reference bi-directional sequencing analysis, which was validated for detection of the five targets in the Xpert Carba-R Assay. If no bands were shown on the Bioanalyzer for any of the five target genes, the isolate was not sent for sequence analysis and the reference method result was considered negative for the five target genes.

Multiple targets were detected by the Xpert Carba-R Assay in samples from ten isolates. The details are provided in Table 6, along with the reference sequencing result.

Table 6. Isolates with Multiple Targets Detected

Isolate	Agar Type[a]	Targets Detected by Xpert Carba-R Assay	Targets Detected by Reference Sequencing
1	BA, MC	NDM, OXA-48	NDM, OXA-48
2	BA	VIM, KPC	VIM
3	BA, MC	NDM, OXA-48	NDM, OXA-48
4	BA, MC	NDM, OXA-48	NDM, OXA-48
5	BA, MC	NDM, OXA-48	NDM, OXA-48
6	BA, MC	NDM, OXA-48	NDM, OXA-48
7	BA, MC	NDM, OXA-48	NDM, OXA-48
8	BA, MC	NDM, OXA-48	NDM, OXA-48
9	BA, MC	NDM, OXA-48	NDM, OXA-48
10	BA, MC	NDM, OXA-48	NDM, OXA-48

a. BA = blood agar; MC = MacConkey agar

When tested with isolates from blood agar, the Xpert Carba-R Assay demonstrated an overall sensitivity and specificity of 100.0% (95% CI: 99.0-100) and 98.1% (95% CI: 93.2-99.5), respectively, relative to reference sequencing performed from the blood agar isolates (Table 7). The combined result was defined as positive for the Xpert Carba-R Assay if any of the targets were positive, and negative for the Xpert Carba-R Assay if all of the targets were negative.

Table 7. Xpert Carba-R (blood agar) vs. Reference Sequencing (Isolate Grown on Blood Agar) — Combined

Target	N	TP	FP	TN	FN	Sensitivity % (95 CI)	Specificity % (95 CI)
Combined	467	364[a]	2[a]	101	0	100.0% (99.0-100)	98.1% (93.2-99.5)

a. Combined results represent results by isolate. Multiple target results were observed for some isolates.

When tested with isolates from blood agar, the Xpert Carba-R Assay demonstrated a sensitivity and specificity of >99% for each of the five assay targets, relative to reference sequencing performed from the blood agar isolates (Table 8).

For isolates with discordant results between the Xpert Carba-R Assay and reference sequencing, discrepant testing was performed using bi-directional sequencing on isolates from MacConkey agar plates. Discrepant testing results are footnoted in Table 8 and Table 10.

Table 8. Xpert Carba-R (blood agar) vs. Reference Sequencing (Isolate Grown on Blood Agar) — By Target

Target	N	TP	FP	TN	FN	Sensitivity % (95 CI)	Specificity % (95 CI)
IMP	467	40	1[a]	426	0	100% (91.2-100)	99.8% (98.7-100)
VIM	467	82	1[b]	384	0	100% (95.5-100)	99.7% (98.5-100)
NDM	467	78	0	389	0	100% (95.3-100)	100% (99.0-100)
KPC	467	84	1[c]	382	0	100% (95.6-100)	99.7% (98.5-100)
OXA-48	467	89	0	378	0	100% (95.9-100)	100% (99.0-100)

a. The bi-directional DNA sequencing result for this false positive IMP isolate exhibited 92.95% sequence homology which was slightly below the 95% cutoff criteria. Discrepant testing was not performed.
b. Discrepant testing results: 1 of 1 was VIM positive.
c. This false positive isolate is likely due to KPC cross-contamination at the level of sample preparation. Discrepant testing did not produce a sequence match with the KPC target. Discrepant testing produced a sequence match for the VIM target, therefore this isolate is classified as a TP in the "Combined" assessment presented in Table 7, above.

When tested with isolates from MacConkey agar, the Xpert Carba-R Assay demonstrated an overall sensitivity and specificity of 100% (95% CI: 99.0-100) and 97.1% (95% CI: 91.8-99.0), respectively, relative to reference sequencing performed from the blood agar isolates (Table 9). The combined result was defined as positive for the Xpert Carba-R Assay if any of the targets were positive, and negative for the Xpert Carba-R Assay if all of the targets were negative.

Table 9. Xpert Carba-R (MacConkey agar) vs. Reference Sequencing (Isolate Grown on Blood Agar— Combined

Target	N	TP	FP	TN	FN	Sensitivity % (95 CI)	Specificity % (95 CI)
Combined	467	364[a]	3	100	0	100% (99.0-100)	97.1% (91.8-99.0)

a. Combined results represent results by isolate. Multiple target results were observed for some isolates.

When tested with isolates from MacConkey agar, the Xpert Carba-R Assay demonstrated a sensitivity and specificity of >99% for each of the five assay targets, relative to reference sequencing performed from the blood agar isolates (Table 10).

Table 10. Xpert Carba-R (MacConkey agar) vs. Reference Sequencing (Isolate Grown on Blood Agar) — By Target

Target	N	TP	FP	TN	FN	Sensitivity, % (95 CI)	Specificity, % (95 CI)
IMP	467	40	1[a]	426	0	100% (91.2-100)	99.8% (98.7-100)
VIM	467	82	1[b]	384	0	100% (95.5-100)	99.7% (98.5-100)
NDM	467	78	1[c]	388	0	100% (95.3-100)	99.7% (98.6-100)
KPC	467	84	0	383	0	100% (95.6-100)	100% (99.0-100)
OXA-48	467	89	0	378	0	100% (95.9-100)	100% (99.0-100)

a. The bi-directional DNA sequencing result for this false positive IMP isolate exhibited 92.95% sequence homology which was slightly below the 95% cutoff criteria. Discrepant testing was not performed.
b. Discrepant testing results: 1 of 1 was VIM positive.
c. The clinical site reported that in-house characterization of this false positive isolate prior to study testing resulted in a positive NDM gene target. Discrepant testing did not produce a sequence match for any of the 5 gene targets.

The Xpert Carba-R Assay performance by specific organism group is shown in Table 11 for both blood agar and MacConkey Agar medium. The overall result was defined as positive for the Xpert Carba-R Assay if any of the targets were positive, and negative for the Xpert Carba-R Assay if all of the targets were negative.

Table 11. Xpert Carba-R vs. Reference Sequencing

Medium	Organisms	Target	N	TP	FP	TN	FN	Sensitivity % (95 CI)	Specificity % (95 CI)
Blood Agar	*Enterobacteriaceae*	IMP	343	4	0	339	0	100% (51.0-100)	100% (98.9-100)
		VIM	343	51	1	291	0	100% (93.0-100)	99.7% (98.1-99.9)
		NDM	343	73	0	270	0	100% (95.0-100)	100% (98.6-100)
		KPC	343	83	1	259	0	100% (95.6-100)	99.6% (97.9-99.9)
		OXA-48	343	89	0	254	0	100% (95.9-100)	100% (98.5-100)
		Overall	343	291[a]	1[a]	51	0	100% (98.7-100)	98.1% (89.9-99.7)
	Pseudomonas aeruginosa	IMP	80	16	1	63	0	100% (80.6-100)	98.4% (91.7-99.7)
		VIM	80	31	0	49	0	100% (89.0-100)	100% (92.7-100)
		NDM	80	0	0	80	0	NA	100% (95.4-100)
		KPC	80	1	0	79	0	100% (20.7-100)	100% (95.4-100)
		OXA-48	80	0	0	80	0	NA	100% (95.4-100)
		Overall	80	48	1	31	0	100% (92.6-100)	96.9% (84.3-99.5)
	Acinetobacter baumannii	IMP	44	20	0	24	0	100% (83.9-100)	100% (86.2-100)
		VIM	44	0	0	44	0	NA	100% (92.0-100)
		NDM	44	5	0	39	0	100% (56.6-100)	100% (91.0-100)
		KPC	44	0	0	44	0	NA	100% (92.0-100)
		OXA-48	44	0	0	44	0	NA	100% (92.0-100)
		Overall	44	25	0	19	0	100% (86.7-100)	100% (83.2-100)

Table 11. Xpert Carba-R vs. Reference Sequencing (Continued)

Medium	Organisms	Target	N	TP	FP	TN	FN	Sensitivity % (95 CI)	Specificity % (95 CI)
MacConkey Agar	*Enterobacteriaceae*	IMP	343	4	0	339	0	100% (51.0-100)	100% (98.9-100)
		VIM	343	51	1	291	0	100% (93.0-100)	99.7% (98.1-99.9)
		NDM	343	73	1	269	0	100% (95.0-100)	99.6% (97.9-99.9)
		KPC	343	83	0	260	0	100% (95.6-100)	100% (98.5-100)
		OXA-48	343	89	0	254	0	100% (95.9-100)	100% (98.5-100)
		Overall	343	291[a]	2	50	0	100% (98.7-100)	96.2% (87.0-98.9)
	Pseudomonas aeruginosa	IMP	80	16	1	63	0	100% (80.6-100)	98.4% (91.7-99.7)
		VIM	80	31	0	49	0	100% (89.0-100)	100% (92.7-100)
		NDM	80	0	0	80	0	NA	100% (95.4-100)
		KPC	80	1	0	79	0	100% (20.7-100)	100% (95.4-100)
		OXA-48	80	0	0	80	0	NA	100% (95.4-100)
		Overall	80	48	1	31	0	100% (92.6-100)	96.9% (84.3-99.5)
	Acinetobacter baumannii	IMP	44	20	0	24	0	100% (83.9-100)	100% (86.2-100)
		VIM	44	0	0	44	0	NA	100% (92.0-100)
		NDM	44	5	0	39	0	100% (56.6-100)	100% (91.0-100)
		KPC	44	0	0	44	0	NA	100% (92.0-100)
		OXA-48	44	0	0	44	0	NA	100% (92.0-100)
		Overall	44	25	0	19	0	100% (86.7-100)	100% (83.2-100)

a. Overall results represent results by isolate. Multiple target results were observed for some isolates.

Xpert Carba-R Assay results by phenotype are presented in Table 12 and Table 13 below. Phenotypic results were based on the organism identification and susceptibility results for each of the isolates. The combined result was defined as positive for the Xpert Carba-R Assay if any of the five assay targets were positive, and negative for the Xpert Carba-R Assay if all five of the assay targets were negative. A non-susceptible phenotype means the isolate was intermediate or resistant to at least one carbapenem. A susceptible phenotype means the isolate was susceptible to imipenem, meropenem, and ertapenem.

Table 12. Xpert Carba-R (blood agar) vs. Phenotype — Combined

		Phenotypic Results		
Xpert Carba-R		Non-susceptible	Susceptible	Total
	Gene Detected	356	10	366
	Gene Not Detected	95	6	101
	Total	451	16	467

Table 13. Xpert Carba-R (MacConkey agar) vs. Phenotype — Combined

		Phenotypic Results		
Xpert Carba-R		Non-susceptible	Susceptible	Total
	Gene Detected	357	10[a]	367
	Gene Not Detected	94[b]	6	100
	Total	451	16	467

a. The 10 isolates that are phenotypically carbapenem susceptible but positive by the Xpert Carba-R Assay may contain mutations that inactivate or down regulate expression of the carbapenem resistance gene detected by the Xpert Carba-R Assay.

b. The 94 isolates that are phenotypically carbapenem non-susceptible but negative by the Xpert Carba-R Assay may contain other mechanisms of carbapenem resistance, such as AmpC beta-lactamases or extended spectrum beta-lactamases in combination with porin mutations, or potentially other carbapenem resistance genes that are not detected by the Xpert Carba-R Assay.

Among the 934 tests performed (467 isolates x 2 agar types), one had an initial **NO RESULT** outcome (0.10%, 95% CI 0.00-0.58). The isolate yielded valid results upon repeat assay. The overall valid reporting rate of the assay was 100% (934/934).

18 Analytical Performance

18.1 Analytical Sensitivity (Limit of Detection) – Rectal Swabs

The analytical sensitivity or Limit of Detection (LoD) of the Xpert Carba-R Assay was assessed using carbapenemase-producing organisms seeded into pooled negative human 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. Bacteria were titered by plate counts and spiked onto clean swabs. Swabs were placed into pooled negative rectal swab matrix and replicates of 20 were evaluated at a minimum of five different concentrations over four days. The LoD for each of the ten carbapenemase-producing organisms was estimated by probit analysis. The LoD is defined as the lowest concentration of target cells (CFU/swab) 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.

The claimed LoD for each pair of carbapenemase-producing organism in rectal swab matrix are shown in Table 14.

Table 14. LoD Estimates and Verification for Organisms Harboring Carbapenemase Genes using the Xpert Carba-R Assay in Rectal Swab Matrix

Target Gene and Organism	LoD Estimates (Probit) CFU/Swab		LoD Claim CFU/ Swab	Estimated LoD in Sample Reagent (CFU/mL)	Verification (Positives/20)
	Lot 1	Lot 2			
IMP-1 *Acinetobacter baumannii*	174	141	174	35	20/20
IMP-1 *Klebsiella pneumoniae*	303	306	306	61	20/20
VIM-1 *Klebsiella pneumoniae*	247	305	305	61	20/20
VIM-4 *Escherichia coli*	815	468	815	163	20/20
NDM-1 *Klebsiella pneumoniae* ATCC BAA-2146	117	251	251	50	20/20
NDM *Klebsiella pneumoniae*	74	57	74	15	19/20
KPC-3 *Klebsiella pneumoniae* NCTC 13438	373	292	373	75	20/20
KPC *Enterobacter cloacae*	779	537	779	156	20/20
OXA-48 *Enterobacter cloacae*	154	109	154	31	20/20
OXA-48 *Escherichia coli*	104	99	104	21	20/20

18.2 Analytical Reactivity (Inclusivity)

18.2.1 Rectal Swab Matrix Study

The analytical reactivity of the Xpert Carba-R Assay with rectal swab matrix was evaluated by testing a panel of 72 samples. This panel consisted of 11 bla_{KPC} (KPC), 11 bla_{VIM} (VIM), 8 bla_{OXA-48} (OXA-48), 5 bla_{NDM}/$bla_{OXA-181}$ (NDM/OXA-181), 6 $bla_{OXA-181}$ (OXA-181), 17 bla_{IMP} (IMP), and one bla_{KPC}/bla_{VIM} (KPC/VIM) well-characterized bacterial strains. The strains tested in rectal swab matrix and their test concentrations are presented in Table 15.

For testing in rectal swab matrix, organisms were seeded into pooled negative rectal swab matrix. All bacterial strains were tested in triplicate at approximately 3x LoD for each specimen type. Xpert Carba-R Assay target genes were detected in 69 of 72 carbapenemase-producing bacterial strains although IMP-4 was detected only using a higher concentration (Table 15). Xpert Carba-R Assay target DNA sequences were not detected in three bacterial strains as shown in Table 15. In one of the three bacterial strains, the IMP-13 gene was not detected by the assay, although it was predicted to be detected by *in silico* analysis. In two of the other three bacterial strains, the IMP-7 and IMP-14 genes were not predicted to be detected by *in silico* analysis and were not detected by the assay. See Section 15, Limitations in the package insert.

Table 15. Analytical Reactivity of the Xpert Carba-R Assay in Rectal Swab Matrix

Strain ID	Organism	Resistance Marker with Variant Information	Concentration Tested in Rectal Swab Matrix (CFU/mL)
NCTC 13438	*Klebsiella pneumoniae*	KPC-3	153
31551	*Klebsiella pneumoniae*	KPC-4	50
ATCC BAA-1705	*Klebsiella pneumoniae*	KPC-2	130
PA-Col	*Pseudomonas aeruginosa*	KPC-2	250
KBM18	*Enterobacter aerogenes*	KPC-2	250
BM9	*Klebsiella pneumoniae*	KPC-3	330
PA3	*Klebsiella pneumoniae*	KPC-2	100

Table 15. Analytical Reactivity of the Xpert Carba-R Assay in Rectal Swab Matrix (Continued)

Strain ID	Organism	Resistance Marker with Variant Information	Concentration Tested in Rectal Swab Matrix (CFU/mL)
CGNC	*Serratia marcescens*	KPC-2	300
CFVL	*Enterobacter cloacae*	KPC-2	160
COL	*Escherichia coli*	KPC-2	147
GR-04/KP-69	*Klebsiella pneumoniae*	KPC-2, VIM	80
164-3	*Klebsiella oxytoca*	KPC	70
NCTC 13437	*Pseudomonas aeruginosa*	VIM-10	500
NCTC 13439	*Klebsiella pneumoniae*	VIM-1	130
NCTC 13440	*Klebsiella pneumoniae*	VIM-1	70
758	*Pseudomonas aeruginosa*	VIM	250
PA-87	*Klebsiella pneumoniae*	VIM	200
B92A	*Pseudomonas aeruginosa*	VIM	2000
Col1	*Pseudomonas aeruginosa*	VIM-2	500
BM19	*Serratia marcescens*	VIM-2	250
KOW7	*Escherichia coli*	VIM-4	250
DIH	*Klebsiella pneumoniae*	VIM-19	250
MSH2014-3	*Enterobacter cloacae*	VIM	500
NCTC 13443	*Klebsiella pneumoniae*	NDM-1	80
ATCC BAA-2146	*Klebsiella pneumoniae*	NDM-1	80
34262	*Klebsiella pneumoniae*	NDM	80
GEN	*Acinetobacter baumannii*	NDM-1	130
3047	*Enterobacter cloacae*	NDM-1	70
7892	*Proteus mirabilis*	NDM-1	30
CAN	*Salmonella spp.*	NDM-1	70
EGY	*Acinetobacter baumannii*	NDM-2	40
I5	*Escherichia coli*	NDM-4	30
405	*Escherichia coli*	NDM-5	30
CF-ABE	*Citrobacter freundii*	NDM	30
73999	*Pseudomonas aeruginosa*	NDM	50
39365	*Providencia rettgeri*	NDM-1	70
NCTC 13442	*Klebsiella pneumoniae*	OXA-48	40
OM11	*Klebsiella pneumoniae*	OXA-48	60
501	*Enterobacter cloacae*	OXA-48	80
DUW	*Klebsiella pneumoniae*	OXA-48	120

Table 15. Analytical Reactivity of the Xpert Carba-R Assay in Rectal Swab Matrix (Continued)

Strain ID	Organism	Resistance Marker with Variant Information	Concentration Tested in Rectal Swab Matrix (CFU/mL)
OM22	*Escherichia coli*	OXA-48	80
BOU	*Enterobacter cloacae*	OXA-48	80
TUR	*Enterobacter cloacae*	OXA-48	120
11670	*Escherichia coli*	OXA-48	100
166643	*Klebsiella pneumoniae*	OXA-181	20
42194	*Klebsiella pneumoniae*	OXA-181	20
MSH2014-64	*Klebsiella pneumoniae*	OXA-181	280
MSH2014-72	*Escherichia coli*	OXA-181	100
74	*Escherichia coli*	OXA-181	100
CDC0051	*Klebsiella ozaenae* [a]	OXA-181	250
B108A	*Klebsiella pneumoniae*	NDM, OXA-181	10
C10192-DISCS	*Enterobacter aerogenes*	NDM, OXA-181	10
KP-OMA3	*Klebsiella pneumoniae*	NDM, OXA-181	60
1300920	*Klebsiella pneumoniae*	NDM, OXA-181	15
MSH2014-69	*Klebsiella pneumoniae*	NDM, OXA-181	20
NCTC 13476	*Escherichia coli*	IMP-1	250
695	*Acinetobacter baumannii*	IMP-1	1720
2340	*Enterobacter cloacae*	IMP-1	250
IMPBMI	*Klebsiella pneumoniae*	IMP-1	100
Yonsei_1	*Acinetobacter baumannii*	IMP-1	1000
Yonsei_2	*Acinetobacter baumannii*	IMP-1	500
6852	*Klebsiella pneumoniae*	IMP-1	100
MKAM	*Pseudomonas aeruginosa*	IMP-1	500
70450-1	*Pseudomonas aeruginosa*	IMP-1	250
3994	*Pseudomonas spp.*	IMP-10	250
CDC0161	*Enterobacter aerogenes*[a]	IMP-4	5.00E+04
5344	*Pseudomonas aeruginosa*	IMP-2	60
3985	*Pseudomonas aeruginosa*	IMP-11	2000
4032	*Pseudomonas aeruginosa*	IMP-6	80
3424	*Pseudomonas aeruginosa*	IMP-7 [b,c]	1.00E+06

Table 15. Analytical Reactivity of the Xpert Carba-R Assay in Rectal Swab Matrix (Continued)

Strain ID	Organism	Resistance Marker with Variant Information	Concentration Tested in Rectal Swab Matrix (CFU/mL)
32443	*Klebsiella pneumoniae*	IMP-13[c]	1.00E+06
92	*Pseudomonas aeruginosa*	IMP-14 [b,c]	1.00E+06

a. These organisms were not tested as bacterial isolates.
b. IMP-7 and IMP-14 genes (*Pseudomonas aeruginosa*) were not detected by the assay and were not predicted to be detected by *in silico* analysis (see Section 15, Limitations).
c. IMP-13 gene (*Klebsiella pneumoniae*): although predicted to be detected by *in silico* analysis, the IMP-13 gene was not detected by the assay (see Section 15, Limitations).

18.2.2 Bacterial Isolate Study

The analytical sensitivity of the Xpert Carba-R Assay with bacterial isolates was also evaluated by testing a panel of 71 samples consisting of 11 bla_{KPC} (KPC), 13 bla_{NDM} (NDM), 11 bla_{VIM} (VIM), 8 bla_{OXA-48} (OXA-48), 5 bla_{NDM}/$bla_{OXA-181}$ (NDM/OXA-181), 5 $bla_{OXA-181}$ (OXA-181), 17 bla_{IMP} (IMP), and one bla_{KPC}/bla_{VIM} (KPC/VIM) well-characterized bacterial strains. The strains tested as bacterial isolates are presented in Table 16.

For bacterial isolate testing, organisms were tested in replicates of four that were prepared by diluting 10 µL of 0.5 McFarland cell suspension for each bacterial strain in 5 mL of Sample Reagent. Testing was performed using both blood agar and MacConkey plates. Xpert Carba-R Assay target genes were detected in 68 of 71 bacterial strains from both plates (Table 16). Xpert Carba-R Assay target DNA sequences were not detected in three bacterial strains as shown in Table 16. In one of the three bacterial strains, the IMP-13 gene was not detected by the assay, although it was predicted to be detected by *in silico* analysis. In two of the three bacterial strains, the IMP-7 and IMP-14 genes that were not detected by the assay were also not predicted to be detected by *in silico* analysis. See the Limitations section in the package insert.

Table 16. Analytical Reactivity of the Xpert Carba-R Assay – Bacterial Isolates

Strain ID	Organism	Resistance Marker with Variant Information
NCTC 13438	*Klebsiella pneumoniae*	KPC-3
31551	*Klebsiella pneumoniae*	KPC-4
ATCC BAA-1705	*Klebsiella pneumoniae*	KPC-2
PA-Col	*Pseudomonas aeruginosa*	KPC-2
KBM18	*Enterobacter aerogenes*	KPC-2
BM9	*Klebsiella pneumoniae*	KPC-3
PA3	*Klebsiella pneumoniae*	KPC-2
CGNC	*Serratia marcescens*	KPC-2
CFVL	*Enterobacter cloacae*	KPC-2
COL	*Escherichia coli*	KPC-2
GR-04/KP-69	*Klebsiella pneumoniae*	KPC-2, VIM
164-3	*Klebsiella oxytoca*	KPC
NCTC 13437	*Pseudomonas aeruginosa*	VIM-10
NCTC 13439	*Klebsiella pneumoniae*	VIM-1
NCTC 13440	*Klebsiella pneumoniae*	VIM-1
758	*Pseudomonas aeruginosa*	VIM
PA-87	*Klebsiella pneumoniae*	VIM

Table 16. Analytical Reactivity of the Xpert Carba-R Assay – Bacterial Isolates (Continued)

Strain ID	Organism	Resistance Marker with Variant Information
B92A	*Pseudomonas aeruginosa*	VIM
Col1	*Pseudomonas aeruginosa*	VIM-2
BM19	*Serratia marcescens*	VIM-2
KOW7	*Escherichia coli*	VIM-4
DIH	*Klebsiella pneumoniae*	VIM-19
MSH2014-3	*Enterobacter cloacae*	VIM
NCTC 13443	*Klebsiella pneumoniae*	NDM-1
ATCC BAA-2146	*Klebsiella pneumoniae*	NDM-1
34262	*Klebsiella pneumoniae*	NDM
GEN	*Acinetobacter baumannii*	NDM-1
3047	*Enterobacter cloacae*	NDM-1
7892	*Proteus mirabilis*	NDM-1
CAN	*Salmonella spp.*	NDM-1
EGY	*Acinetobacter baumannii*	NDM-2
I5	*Escherichia coli*	NDM-4
405	*Escherichia coli*	NDM-5
CF-ABE	*Citrobacter freundii*	NDM
73999	*Pseudomonas aeruginosa*	NDM
39365	*Providencia rettgeri*	NDM-1
NCTC 13442	*Klebsiella pneumoniae*	OXA-48
OM11	*Klebsiella pneumoniae*	OXA-48
501	*Enterobacter cloacae*	OXA-48
DUW	*Klebsiella pneumoniae*	OXA-48
OM22	*Escherichia coli*	OXA-48
BOU	*Enterobacter cloacae*	OXA-48
TUR	*Enterobacter cloacae*	OXA-48
11670	*Escherichia coli*	OXA-48
MSH2014-64	*Klebsiella pneumoniae*	OXA-181
MSH2014-72	*Escherichia coli*	OXA-181
B108A	*Klebsiella pneumoniae*	NDM, OXA-181
C10192-DISCS	*Enterobacter aerogenes*	NDM, OXA-181
KP-OMA3	*Klebsiella pneumoniae*	NDM-1, OXA-181
166643	*Klebsiella pneumoniae*	OXA-181
42194	*Klebsiella pneumoniae*	OXA-181
1300920	*Klebsiella pneumoniae*	NDM, OXA-181
MSH2014-69	*Klebsiella pneumoniae*	NDM, OXA-181
74	*Escherichia coli*	OXA-181

Table 16. Analytical Reactivity of the Xpert Carba-R Assay – Bacterial Isolates (Continued)

Strain ID	Organism	Resistance Marker with Variant Information
NCTC 13476	*Escherichia coli*	IMP-1
695	*Acinetobacter baumannii*	IMP-1
2340	*Enterobacter cloacae*	IMP-1
IMPBMI	*Klebsiella pneumoniae*	IMP-1
6852	*Klebsiella pneumoniae*	IMP-1
Yonsei_1	*Acinetobacter baumannii*	IMP-1
Yonsei_2	*Acinetobacter baumannii*	IMP-1
70450-1	*Pseudomonas aeruginosa*	IMP-1
3994	*Pseudomonas spp.*	IMP-10
MKAM	*Pseudomonas aeruginosa*	IMP-1
5344	*Pseudomonas aeruginosa*	IMP-2
G029	*Salmonella spp*	IMP-4
3985	*Pseudomonas aeruginosa*	IMP-11
4032	*Pseudomonas aeruginosa*	IMP-6
3424	*Pseudomonas aeruginosa*	IMP-7[a,b]
32443	*Klebsiella pneumoniae*	IMP-13[a]
92	*Pseudomonas aeruginosa*	IMP-14[a,b]

a. Not detected by Xpert Carba-R (see Section 15, Limitations).
b. IMP-7 and IMP-14 genes were not detected by the assay and were not predicted to be detected by *in silico* analysis (see Section 15, Limitations).

The variants detected, and predictions for detecting other subtypes of each resistance gene based on *in silico* analysis, are presented in Table 17 (representing results from both the rectal swab matrix and bacterial isolate study).

Table 17. Summary of Variants Detected by Wet Testing or Predicted to be Detected Based on *In Silico* Analysis

Marker (or Traditional Subgroup)	Wet Testing			Not Tested but Predicted to be Detected Based on *in silico* Analysis
	No. of Samples	Type(s) Detected	Type(s) not Detected	
KPC	12	KPC-2,3,4	--	KPC-5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16
NDM	18	NDM-1,2,4,5	--	NDM-3, 6, 7, 8, 9
VIM	12	VIM-1,2,4,10,19	--	VIM-5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38
OXA-48	18	OXA-48, 181 (OXA-48 variant)	--	OXA-162, 163, 204, 232, 244, 245, 247

Table 17. Summary of Variants Detected by Wet Testing or Predicted to be Detected Based on *In Silico* Analysis (Continued)

Marker (or Traditional Subgroup)	Wet Testing			Not Tested but Predicted to be Detected Based on *in silico* Analysis
	No. of Samples	Type(s) Detected	Type(s) not Detected	
IMP	17	IMP-1 (9 strains), IMP-2, 4, 6, 10, 11	IMP-7[a], 13[b], 14[a]	IMP-3, 8, 9, 13[b], 19, 20, 21, 22, 24, 25, 27, 28, 30, 31, 33, 37, 40, 42

a. IMP-7 and IMP-14 genes (*Pseudomonas aeruginosa*) were not detected by the assay and were not predicted to be detected by *in silico analysis* (see Section 15, Limitations).

b. IMP-13 gene (*Klebsiella pneumoniae*) was tested: although predicted to be detected by *in silico analysis*, the IMP-13 gene was not detected by the assay (see Section 15, Limitations).

18.3 Analytical Specificity (Cross-Reactivity)

The analytical specificity of the Xpert Carba-R Assay was evaluated for bacterial isolates and organisms seeded into rectal swab matrix. For both specimen types, a panel of 62 well-characterized bacterial strains of carbapenem-susceptible bacteria or bacteria with carbapenem non-susceptibility due to genes or mechanisms other than the Xpert Carba-R target genes (Table 18 and Table 19) and 24 commensal bacterial strains and other enteric microorganisms were also evaluated in the study (Table 20). Human cells were also tested in rectal swab matrix (Table 19). Resistance mechanisms were determined by individual PCR assays, DNA sequence analysis, or Check-Points array version CT102.

For rectal swab matrix samples, 62 strains were tested at concentrations >1 x 10^6 CFU/mL with the exception of *Peptostreptococcus anaerobius* that was tested at 5 x10^5 CFU/mL. Viruses were tested at >1 x 10^5 $TCID_{50}$/mL or greater than 2.5 x10^7 RNA copies/mL. A 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 94 potentially cross-reactive organisms and nucleic acids tested was detected with the Xpert Carba-R Assay.

For bacterial isolates, organisms were grown aerobically on blood agar and MacConkey agar plates. Two cell suspensions equivalent to a 0.5 McFarland cell suspension were prepared from isolated colonies on each type of agar plate. Each organism was tested a total of four times (two replicates from each of two 0.5 McFarland cell suspensions per organism) from each plate.

The Xpert Carba-R Assay did not cross react with any of the organisms tested (Table 18, Table 19, Table 20, and Table 21). The analytical specificity of the assay was 100%.

Table 18. Number of Carbapenem-Susceptible and Non-Susceptible Organisms for each Antibiotic

	Ertapenem	Imipenem	Meropenem
Susceptible	19	30	24
Intermediate	0	8	4
Resistant	43	24	34

Table 19. Cross-Reactivity Panel

Organism	Strain ID	Confirmed Resistance Mechanisms	Carbapenem Susceptibility (S/I/R)[a]		
			ETP[a]	IMP[a]	MEM[a]
Escherichia coli	NCTC 13441	CTX-M (-1, -type 15 like); TEM	S	S	S
Klebsiella pneumoniae	NCTC 13465	CTX-M (25)	S	S	S
Enterobacter aerogenes	810	OmpC/OmpF deficient; TEM	R	R	R
Citrobacter freundii	1698	TEM (WT+164S)	S	S	S
Enterobacter cloacae	5557	AmpC (ACT/MIR)	R	R	R
Klebsiella pneumoniae	kpn5	CTX-M-2	R	S	R
Klebsiella pneumoniae	kpn12	TEM; SHV; CTX-M	R	R	R
Escherichia coli	eco1	TEM; CTX-M-2	R	R	R
Escherichia coli	eco2	CTX-M (2); TEM	R	S	S
Enterobacter cloacae	cor1	CTX-M (2); TEM	R	R	R
Serratia marcescens	hpp21	CTX-M (2); TEM	S	S	S
Morganella morganii	fer29	CTX-M (2); TEM	S	R	S
Proteus mirabilis	gut25	CTX-M (2); TEM	S	R	S
Salmonella spp.	3209	CTX-M (2); TEM	S	S	S
Shigella flexnerii	3331	CTX-M (2); TEM	S	S	S
Enterobacter cloacae	PA_3	AmpC; CTX-M-15; TEM	S	S	S
Klebsiella pneumoniae	32189	SHV	S	S	S
Klebsiella pneumoniae	32443	CTX-M (1, -type 15 like); SHV	S	S	S
Klebsiella pneumoniae	32598	CTX-M (-1, -type 15 like); SHV; TEM	R	I	R
Klebsiella pneumoniae	33560	CTX-M (15); SHV-11; TEM-1	S	S	S
Klebsiella pneumoniae	33603	SHV-2	R	I	R
Klebsiella pneumoniae	33617	SHV-27	S	S	S
Klebsiella pneumoniae	33643	SHV (-5, -55); TEM	S	S	S
Klebsiella pneumoniae	34430	SHV; TEM; CTX-M-15	S	S	S
Klebsiella pneumoniae	34680	TEM; CTX-M-2	R	S	R
Klebsiella pneumoniae	34732	CTX-M (15); SHV; TEM	R	S	S
Enterobacter cloacae	PA_174	GX-/Culture+; SHV; TEM	S	S	S
Enterobacter aerogenes	STU 645	SHV (WT+238S+240K)	R	S	R
Enterobacter aerogenes	STU 669	SHV (WT+238S+240K)	R	R	R
Escherichia coli	C3015	AmpC (CMY II); TEM	R	R	R
Enterobacter aerogenes	RI_100	AmpC (DHA); SHV	R	R	R
Klebsiella pneumoniae	B4A	SHV (WT + 238S +240K)	R	R	R
Klebsiella pneumoniae	B13A	SHV (WT + 238S +240K)	R	S	S
Enterobacter cloacae	RI_474	AmpC (ACT/MIR)	R	I	I

Table 19. Cross-Reactivity Panel (Continued)

Organism	Strain ID	Confirmed Resistance Mechanisms	Carbapenem Susceptibility (S/I/R)[a]		
			ETP[a]	IMP[a]	MEM[a]
Enterobacter amnigenus	B71	AmpC (ACT/MIR)	R	R	R
Klebsiella pneumoniae	DD82A	SHV (WT + 238S + 240K)	R	S	R
Klebsiella pneumoniae	B100	CTX-M (-1, type-15 like); SHV (WT+238S); TEM	R	S	R
Enterobacter cloacae	135B	TEM	S	S	S
Klebsiella pneumoniae	B157	SHV; TEM	R	R	R
Escherichia coli	T2914280	CTX-M (-1, -15); TEM	R	S	R
Providencia stuartii	DD188	TEM (104K + 164S)	R	I	I
Enterobacter cloacae	DD189	AmpC (ACT/MIR)	R	S	S
Escherichia coli	B198B	CTX-M (-1, type -15 like); TEM	R	S	R
Klebsiella pneumoniae	T3019989-1	CTX-M (-1, type-15 like); SHV	R	I	R
Klebsiella pneumoniae	T3019989-2	CTX-M (-1, type-15 like); SHV	R	S	R
Enterobacter cloacae	ENC-THAI14	VEB-1, TEM	S	S	S
Escherichia coli	CB154006	CTX-M (9); TEM	R	I	I
Enterobacter cloacae	S35766	AmpC (ACT/MIR)	S	S	S
Enterobacter cloacae	X1856910	AmpC (ACT/MIR); TEM	R	I	I
Klebsiella pneumoniae	W3758164	CTX-M (-1, -15 like); SHV; TEM	R	I	R
Klebsiella pneumoniae	X2135758	CTX-M (-1, -15 like); SHV	R	S	S
Klebsiella pneumoniae	W3809535	CTX-M (-1, -15 like); SHV	R	R	R
Pseudomonas aeruginosa	CDC0064	SPM	R	R	R
Serratia marcescens	CDC0099	SME	R	R	R
Serratia marcescens	CDC0121	SME	R	R	R
Serratia marcescens	CDC0122	SME	R	R	R
Serratia marcescens	CDC0123	SME	R	R	R
Serratia marcescens	CDC0124	SME	R	R	R
Serratia marcescens	CDC0130	SME	R	R	R
Serratia marcescens	CDC0131	SME	R	R	R
Enterobacter cloacae group	CDC0132	IMI	R	R	R
Enterobacter cloacae complex	CDC0164	IMI	R	R	R

a. S/I/R = Susceptible/Intermediate/Resistant, ETP = Ertapenem, IMP = Imipenem, MEM = Meropenem

Table 20. Cross-reactivity Panel (Commensal and Other Enteric Microorganisms)

Strain ID	Organism	Concentration Tested (CFU/mL Unless Otherwise Specified)
ATCC 25922	*Escherichia coli*	2.67E+06
ATCC 29212	*Enterococcus faecalis*	3.15E+06
ATCC 700603	*Klebsiella pneumoniae*	5.20E+06
ATCC 35218	*Escherichia coli*	2.47E+06
ATCC 25923	*Staphylococcus aureus*	4.53E+06
ATCC 27853	*Pseudomonas aeruginosa*	3.17E+06
ATCC 9689	*Clostridium difficile*[a]	1.80E+07
ATCC 700621	*Enterobacter cloacae*	8.95E+06
ATCC 9756	*Enterococcus faecium*	6.54E+06
ATCC 13182	*Klebsiella oxytoca*	4.76E+06
ATCC BAA-747	*Acinetobacter baumannii*	2.27E+06
ATCC 33128	*Citrobacter freundii*	2.01E+06
ATCC 49948	*Morganella morganii*	8.19E+06
ATCC 51331	*Stenotrophomonas maltophilia*	3.15E+06
ATCC 27028	*Citrobacter koseri*	5.05E+06
ATCC 49809	*Providencia stuartii*	3.01E+06
ATCC 49037	*Peptostreptococcus anaerobius*[a]	5.00E+05
CCUG 29780 / ATCC 12401	*Streptococcus agalactiae*	5.21E+06
ATCC 15703	*Bifidobacterium adolescentis*[a]	1.10E+08
ATCC 51697	*Enterobacter aerogenes*	3.19E+06
ATCC 43071	*Proteus mirabilis*	1.78E+06
CCUG 34787	*Acinetobacter spp.*	2.40E+06
CCUG 418	*Citrobacter freundii*	2.95E+06
CCUG 33629	*Corynebacterium diphtheriae*	4.48E+06
CCUG 17874	*Helicobacter pylori*	1.61E+06
CCUG 33548	*Listeria monocytogenes*	4.77E+06
CCUG 6325	*Providencia alcalifaciens*	4.91E+06
CCUG 43594 / ATCC 33560	*Campylobacter jejuni*[a]	3.27E+06
MRVP/ZeptoMetrix	Adenovirus B Type 7A/NY[a]	1.40E+05 $TCID_{50}$/mL
MRVP/ZeptoMetrix	Enterovirus Type 71/NY[a]	4.40E+05 $TCID_{50}$/mL
Clinical Sample – Cepheid	Norovirus GII[a]	2.5 x 10^7 RNA copies/mL

a. These organisms were tested in rectal swab matrix only.

Table 21. Cell Line Representing Human Genomic DNA

Organism Name	Source
Bladder Cell Carcinoma (hgDNA)	ATCC HTB-4

18.4 Competitive Interference

A competitive interference study was performed to test whether a high titer of one or more carbapenemase-producing organisms would interfere with the detection of a second target carbapenemase-producing organism that was present at a low titer. High titered samples were formulated at concentrations of 5 x 10^6 CFU/swab and low titered targets were formulated at approximately 2x LoD for the respective strain in rectal swab matrix. One carbapenemase-producing bacterial strain for each gene analyte, i.e., the genes encoding KPC, NDM, VIM, OXA-48, and IMP, was used in this study. Each carbapenemase-producing bacterial strain type was tested at low titers in conjunction with a high titer of each of the other one or two carbapenemase-producing bacterial strain types (Table 22). Samples were tested in replicates of eight. An inhibitory effect was observed for three of the five targets (IMP, VIM, and OXA-48) when a low concentration of each target was present in combination with a high concentration of one or two other targets for samples tested in rectal swab matrix. The three targets (IMP, VIM, and OXA-48) were tested at a higher concentration (4x LoD) in combination with a high concentration of one of two other targets for samples in rectal swab matrix. No inhibitory effect was observed for the three targets (IMP, VIM and OXA-48) at 4x LoD in the presence of clinically relevant co-infections for the Xpert Carba-R Assay. The competitive inhibitory effect on the Carba-R targets (IMP, VIM and OXA-48) is addressed in Section 15, Limitations in the package insert.

Table 22. Combinations of Carbapenemase-producing Bacteria Tested with the Xpert Carba-R Assay

Combination
High KPC/High NDM/Low VIM
High KPC/High NDM/Low OXA
High KPC/High NDM/Low IMP
High VIM/High OXA/Low KPC
High VIM/High OXA/Low NDM
High VIM/High OXA/Low IMP
High IMP/Low KPC
High IMP/Low NDM
High IMP/Low VIM
High IMP/Low OXA
High OXA/Low VIM
High VIM/Low OXA
High KPC/Low NDM
Negative

18.5 Potentially Interfering Substances

The performance of the Xpert Carba-R Assay was evaluated with 24 potentially interfering substances that may be present in rectal swab specimens. Potentially interfering substances (IS) solutions were prepared and tested at concentrations specified in Table 23. Positive and negative samples were included in this study. Positive samples consisted of a mix of five carbapenemase-producing organisms harboring KPC, NDM, VIM, IMP-1 and OXA-48 gene sequences seeded into pooled negative rectal swab matrix at approximately 3x LoD. Eight replicate positive samples were tested per substance. Negative samples consisted of pooled negative rectal swab matrix not seeded with carbapenemase-producing organisms. Eight replicate negative samples were tested per substance to determine the effect on the performance of the sample processing control (SPC). Controls consisted of positive and negative samples with no interfering substances added. 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 controls lacking the substance. The positive and negative replicate samples for 22 potentially interfering substances were correctly identified using the Xpert Carba-R Assay. Interference with the Xpert Carba-R Assay may be observed with barium sulfate at > 0.1% w/v and Pepto-Bismol at > 0.01% w/v in tests with rectal swab

matrix samples. See Section 15, Limitations in the package insert. Rectal swab matrix samples, positive for a mix of five carbapenemase-producing organisms harboring KPC, NDM, VIM, IMP-1 and OXA-48 gene sequences that were tested with fecal fat at 0.25% w/v, did not yield any false negative results, however, delayed cycle threshold values were observed for the VIM target. This potential interference from the presence of 0.25% w/v fecal fat is provided in the Limitations section of the package insert.

Table 23. 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% and 0.1% w/v
Antibiotic (oral)	Cephalexin	0.25% w/v
Antibiotic (oral)	Ciprofloxacin	0.25% w/v
Condom with spermicidal lubricant	Nonoxynol-9	1 condom[a]
Creams/ointment/suppositories	Hydrocortisone	0.25% w/v
Laxative	Sennosides	0.25% w/v
Lipids	Stearic acid/Palmitic acid/Cholesterol (fecal fat)	0.25% w/v
Anti-diarrheal medication	Loperamide hydrochloride/bismuth subsalicylate (Imodium)	0.25% w/v
Anti-diarrheal medication	Loperamide hydrochloride/bismuth subsalicylate (Kaopectate)	0.25% w/v
Topical cream	K-Y Jelly	0.25% w/v
Antacids	Calcium carbonate/aluminum hydroxide/ magnesium hydroxide/simethicone (Milk of Magnesia)	0.25% w/v
Enemas	Mineral oil	0.25% w/v
Antibiotic (topical)	Polymixin B/ Neomycin/ Bacitracin (Neosporin)	0.25% w/v
Anti-fungal/ anti-itch Vaginal	Nystatin	0.25% w/v
Antacid	Famotidine (Pepcid)	0.25% w/v
Anti-diarrheal medication	Loperamide hydrochloride/bismuth subsalicylate (Pepto-Bismol)	0.25%, 0.1%, 0.05%, 0.025%, 0.01% w/v
Topical cream	Petroleum jelly	0.25% w/v

Table 23. Potentially Interfering Substances Tested (Continued)

Substance/Class	Active Ingredient	Concentration Tested
Anti-hemorrhoid creams/ointments	Phenylephrine (Preparation H)	0.25% w/v
Acid reducer; antacid	Oemprazole (Prilosec)	0.25% w/v
Enemas	Saline-enema	0.25% w/v
Antacid	Cimetidine (Tagamet)	0.25% w/v
Anti-fungal/anti-itch Vaginal	Benzocaine, resorcinol (Vagisil)	0.25% w/v
Moist towelettes	Benzalkonium chloride, ethanol (Wet Ones)	1 piece[b]

a. One condom added to 40 mL swab matrix.
b. One piece (5 inch x 7-1/2 inch) added to 40 mL swab matrix.

18.6 Carry-over Contamination Study

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination in negative samples run following very high positive 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 (KPC, NDM, VIM, IMP and OXA-48 targets). Positive cells were diluted in pooled negative rectal swab matrix to a concentration of 1 x 106 CFU/mL. The testing scheme was repeated 25 times on two GeneXpert modules for a total of 102 tests (25 high positive samples per module and 26 negative samples per module) for the rectal swab matrix. All 50 positive samples correctly reported all Xpert Carba-R targets as **DETECTED**. All 52 negative samples correctly reported all Xpert Carba-R targets as **NOT DETECTED**.

19 Reproducibility

19.1 Rectal Swab Matrix Study

Reproducibility of the Xpert Carba-R Assay was evaluated using two panels of 11 samples, prepared in pooled negative rectal swab matrix. Two operators at each of the three study sites tested one panel of 11 samples in replicates of four per day over six testing days (11 samples x 2 replicates x 2 times/day x 6 days x 2 operators x 3 sites). Three lots of Xpert Carba-R Assay cartridges were used at each of the 3 testing sites. The Xpert Carba-R Assay was performed according to the Xpert Carba-R Assay procedure. Results are summarized in Table 24.

Table 24. Summary of Reproducibility Results - % Agreement, Rectal Swab Matrix

Sample	Matrix[a]	Site 1			Site 2			Site 3			% Total Agreement by Sample
		Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
Neg	R	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
IMP Mod Pos	R	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
IMP Low Pos	R	91.7% (22/24)	87.5% (21/24)	89.5% (43/48)	83.3% (20/24)	87.5% (21/24)	85.4% (41/48)	87.5% (21/24)	79.2% (19/24)	83.3% (40/48)	86.1% (124/144)
VIM Mod Pos	R	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
VIM Low Pos	R	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
NDM Mod Pos	R	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
NDM Low Pos	R	91.7% (22/24)	95.8% (23/24)	93.8% (45/48)	95.8% (23/24)	95.8% (23/24)	95.8% (46/48)	100% (24/24)	91.7% (22/24)	95.8% (46/48)	95.1% (137/144)
KPC Mod Pos	R	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)

Table 24. Summary of Reproducibility Results - % Agreement, Rectal Swab Matrix (Continued)

Sample	Matrix[a]	Site 1			Site 2			Site 3			% Total Agreement by Sample
		Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
KPC Low Pos	R	95.8% (23/24)	100% (24/24)	97.9% (47/48)	100% (24/24)	91.7% (22/24)	95.8% (46/48)	95.8% (23/24)	95.8% (23/24)	95.8% (46/48)	96.5% (139/144)
OXA-48 Mod Pos	R	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
OXA-48 Low Pos	R	95.8% (23/24)	100% (24/24)	97.9% (47/48)	95.8% (23/24)	100% (24/24)	97.9% (47/48)	91.7% (22/24)	100% (24/24)	95.8% (46/48)	97.2% (140/144)

a. R=rectal

The reproducibility of the Xpert Carba-R Assay was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-sites, between-lots, between- days, between-operators, and within-assays for each panel member are presented in Table 25.

Table 25. Summary of Reproducibility Data, Rectal Swab Matrix

Sample	Matrix[a]	Assay Channel (Analyte)	N[b]	Mean Ct	Between-Site		Between-Lot		Between-Day		Between-Operator		Within-Assay		Total	
					SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Neg	R	SPC	144	32.9	0.2	0.5	0.2	0.7	0.0	0.1	0.0	0	0.6	1.8	0.7	2.0
IMP Mod Pos	R	IMP	144	34.5	0.0	0.0	0.2	0.5	0	0.0	0.1	0.2	0.7	2.0	0.7	2.1
IMP Low Pos	R	IMP	140	36.4	0.0	0.0	0.0	0.0	0.2	0.5	0.0	0	1.2	3.3	1.2	3.4
VIM Mod Pos	R	VIM	144	31.0	0.0	0.0	0.3	0.9	0	0.0	0.2	0.5	0.5	1.6	0.6	1.9
VIM Low Pos	R	VIM	144	33.8	0.0	0.0	0.6	1.8	0.3	0.9	0.3	1.0	1.4	4.0	1.6	4.6
NDM Mod Pos	R	NDM	144	33.7	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.6	1.7	0.6	1.7
NDM Low Pos	R	NDM	143	36.2	0.2	0.7	0.0	0.0	0.3	0.7	0.0	0.0	0.8	2.3	0.9	2.5
KPC Mod Pos	R	KPC	144	34.2	0.0	0.0	0.3	0.8	0.2	0.6	0.0	0.0	0.4	1.2	0.6	1.6
KPC Low Pos	R	KPC	141	35.8	0.0	0.0	0.5	1.5	0.0	0.0	0.3	0.9	0.7	1.9	0.9	2.6
OXA-48 Mod Pos	R	OXA-48	144	34.3	0.0	0.0	0.2	0.5	0.2	0.5	0.1	0.3	0.5	1.6	0.6	1.7
OXA-48 Low Pos	R	OXA-48	143	36.1	0.0	0.0	0.0	0.0	0.2	0.6	0.0	0.0	0.8	2.3	0.9	2.4

a. R=rectal
b. Results with non-zero Ct values out of 144.

19.2 Bacterial Isolate Study

Reproducibility of the Xpert Carba-R Assay was evaluated using a panel of 13 bacterial samples that included: two different organisms per each of the five resistance gene targets detected by the Xpert Carba-R Assay; two stock samples that included two gene targets; and one stock sample negative for all five gene targets. Two operators at each of the three study sites tested one panel of 13 samples in replicates of four per day. Each sample was used to make two 0.5 McFarland equivalent suspensions from which two replicates were tested over six testing days (13 samples x 2 replicates x 2 times/day x 6 days x 2 operators x 3 sites). Three lots of Xpert Carba-R Assay cartridges were used at each of the 3 testing sites. The Xpert Carba-R Assay was performed according to the Xpert Carba-R Assay procedure. Upon completion of the testing, 25 tests run on one instrument module were excluded resulting in a total of 1847 samples included in the analyses. Results are summarized in Table 26.

Table 26. Summary of Reproducibility Results – % Agreement, Bacterial Isolates

Resistance Gene (Sample #)	Site 1			Site 2			Site 3			% Total Agreement by Sample
	Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
KPC (1)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
KPC (2)	100% (23/23)	100% (22/22)	100% (45/45)	95.8% (23/24)	100% (24/24)	97.9% (47/48)	100% (24/24)	100% (24/24)	100% (48/48)	99.3% (140/141)
VIM (1)	100% (22/22)	100% (23/23)	100% (45/45)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (141/141)
VIM (2)	100% (22/22)	100% (24/24)	100% (46/46)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (142/142)
IMP (1)	100% (23/23)	100% (24/24)	100% (47/47)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (143/143)
IMP (2)	100% (23/23)	100% (23/23)	100% (46/46)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (142/142)
OXA (1)	100% (23/23)	100% (23/23)	100% (46/46)	100% (24/24)	91.7% (22/24)	95.8% (46/48)	100% (24/24)	100% (24/24)	100% (48/48)	98.6% (140/142)
OXA (2)	100% (23/23)	100% (22/22)	100% (45/45)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (141/141)
NDM (1)	100% (22/22)	100% (21/21)	100% (43/43)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (139/139)
NDM (2)	100% (23/23)	100% (23/23)	100% (46/46)	91.7% (22/24)	100% (24/24)	95.8% (46/48)	100% (24/24)	100% (24/24)	100% (48/48)	98.6% (140/142)
OXA,NDM (1)	100% (24/24)	100% (23/23)	100% (47/47)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (143/143)
OXA,NDM (2)	100% (23/23)	100% (24/24)	100% (47/47)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (143/143)
NEG	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)

The reproducibility of the Xpert Carba-R Assay was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-sites, between-lots, between-days, between-operators, and within-assays for each panel member are presented in Table 27.

Table 27. Summary of Reproducibility Data – Bacterial Isolates

Resistance Gene (Sample #)	Assay Channel (Analyte)	N[a]	Between-Site		Between-Lot		Between-Day		Between-Operator		Within-Assay		Total	
			SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
KPC (1)	KPC	144	1.1	4.4	0	0	0	0	0.6	2.6	0.6	2.6	1.4	5.8
KPC (2)	KPC	143	0.8	3.1	0.1	0.2	0.2	0.9	0.5	2.0	0.8	3.1	1.2	4.9
VIM (1)	VIM	141	1.1	5.1	0	0	0	0	0.5	2.3	0.8	3.7	1.5	6.7
VIM (2)	VIM	142	0.3	1.3	0.2	0.8	0	0	0.8	3.8	0.7	3.1	1.1	5.1
IMP (1)	IMP	143	0.3	1.0	0	0	0.3	1.2	0.6	2.3	0.8	3.1	1.0	4.2
IMP (2)	IMP	142	1.4	6.3	0.1	0.5	0	0	0.6	2.8	0.7	3.2	1.7	7.6
OXA (1)	OXA48	140	0.6	2.6	0	0	0	0	0.7	2.8	0.8	3.5	1.2	5.2
OXA (2)	OXA48	141	1.1	4.9	0.3	1.5	0	0	0.5	2.0	0.7	3.3	1.5	6.4
NDM (1)	NDM	139	1.2	5.3	0	0	0	0	0.6	2.4	0.7	3.1	1.5	6.6
NDM (2)	NDM	140	0.9	4.0	0.3	1.4	0	0	0.8	3.3	0.8	3.3	1.5	6.3
NDM/OXA (1)	NDM	143	1.3	5.4	0.2	0.8	0	0	0.6	2.5	0.7	3.1	1.6	6.8
	OXA48	143	1.2	6.2	0.3	1.4	0	0	0.5	2.4	0.7	3.7	1.5	7.7
NDM/OXA (2)	NDM	143	1.2	5.3	0.2	1.1	0	0	0.5	2.4	0.8	3.5	1.6	6.9
	OXA48	143	1.2	6.0	0.2	1.2	0	0	0.5	2.5	0.7	3.8	1.5	7.6
NEG	SPC	144	0.1	0.3	0.1	0.3	0	0	0.2	0.5	0.4	1.3	0.5	1.5

a. Results with non-zero Ct values out of 144.

20 References

1. Kallen AJ, et al. 2010. Current epidemiology of multidrug-resistant gram-negative bacilli in the United States. Infect Control Hosp Epidemiol. 31 Suppl 1: S51–54.
2. Nordmann P, et al. 2012. Carbapenemase-producing *Enterobacteriaceae*: a call for action! Clin Microbiol Infect. 18: 411–412.
3. Cornaglia G, et al. 2011. Metallo-beta-lactamases: a last frontier for beta-lactams? Lancet Infect Dis. 11: 381–393.
4. Kitchel B, et al. 2009. Molecular epidemiology of KPC-producing Klebsiella pneumoniae in the United States: Clonal expansion of MLST sequence type 258. Antimicrob Agents Chemother. 53:3365–3370.
5. Schwaber MJ, et al. 2011. Containment of a country-wide outbreak of carbapenem-resistant Klebsiella pneumoniae in Israeli hospitals via a nationally implemented intervention. Clin Infect Dis. 52: 848–855.
6. Kumarasamy KK, et al. 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 10: 597–602.
7. Cuzon G, et al. 2008. Plasmid-encoded carbapenem-hydrolyzing beta-lactamase OXA-48 in an imipenem-susceptible Klebsiella pneumoniae strain from Belgium. Antimicrob Agents Chemother. 52: 3463–3464.
8. Nordmann P, et al. 2011. Global spread of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis. 17: 1791–1798.
9. Grundmann H, et al. 2010. Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. Euro Surveill. 15:1-13.
10. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories (refer to latest edition). http://www.cdc.gov/biosafety/publications/
11. Centers for Disease Control and Prevention. Accessed January 20, 2016. Healthcare-associated Infections (HAIs). http://www.cdc.gov/hai/organisms/cre/cre-facilities.html
12. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline. Document M29 (refer to latest edition).
13. CLSI M100. Performance Standards for Antimicrobial Susceptibility Testing, CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA (refer to latest edition).
14. CLSI M07-A10. 2015. Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically; Tenth Edition, CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA.
15. CLSI M100-S24. 2014. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Edition, CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA.
16. CLSI M07-A9. 2015. Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically; Ninth Edition, CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA
17. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).
18. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

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23 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	In vitro diagnostic medical device
	Do not re-use
LOT	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Contains sufficient for <n> tests
CONTROL	Control
	Expiration date
	Temperature limitation
	Biological risks
	Warning
Rx only	For prescription use only

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IVD