DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
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June 29, 2016

CEPHEID
JIM KELLY, Ph.D.
EXECUTIVE DIRECTOR, REGULATORY AFFAIRS
904 CARIBBEAN DRIVE
SUNNYVALE CA 94089-1189

Re: K160901
Trade/Device Name: Xpert® Carba-R Assay
Regulation Number: 21 CFR 866.1640
Regulation Name: Antimicrobial susceptibility test powder
Regulatory Class: II
Product Code: POC, OOI
Dated: March 31, 2016
Received: April 1, 2016

Dear Dr. Kelly:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Ribhi Shawar -S

For Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

510(k) Number *(if known)*

K160901

Device Name

Xpert Carba-R

Indications for Use *(Describe)*

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 carbapenem-non-susceptible 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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

**CONTINUE ON A SEPARATE PAGE IF NEEDED.**

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**510(k) Summary**

As required by 21 CFR Section 807.92(c).

| | |
|---|---|
| Submitted by: | Cepheid<br>904 Caribbean Drive<br>Sunnyvale, CA 90489<br>Phone number: (847) 228-3299<br>Fax number: (847) 890-6589 |
| Contact: | Scott A. Campbell, PhD, MBA |
| Date of Preparation: | June 28, 2016 |
| Device: | |
| Trade name: | Xpert® Carba-R |
| Common name: | Xpert Carba-R Assay |
| Type of Test: | Qualitative nucleic acid amplification test of the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene sequences associated with carbapenem-non-susceptibility in gram-negative bacteria obtained from rectal swab specimens |
| Classification: | II |
| Regulation number: | 866.1640 |
| Classification name: | Antimicrobial susceptibility test powder |
| Product code: | POC, OOI |
| Classification Advisory Panel | Microbiology (83) |
| Prescription Use | Yes |
| Predicate Device Assay: | Cepheid Xpert ® Carba-R<br>[510(k) #K152614] |

Device Description:

The Xpert Carba-R Assay is an automated real-time polymerase chain reaction (PCR) *in vitro* diagnostic test for qualitative detection of the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene sequences from rectal swab specimens. The Xpert Carba-R Assay is intended as an aid for infection control for monitoring the spread of carbapenem-non-susceptible organisms in healthcare settings.

The Xpert Carba-R Assay is performed on the Cepheid GeneXpert® Instrument Systems (GeneXpert Dx, GeneXpert Infinity-48, GeneXpert Infinity-48s, and GeneXpert Infinity-80 systems). The GeneXpert Instrument System platform automates sample preparation, amplification and real-time detection.

The GeneXpert Instrument Systems require the use of single-use, disposable cartridges (the Xpert Carba-R cartridges) that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained and specimens never come into contact with working parts of the instrument modules, cross-contamination between samples is minimized.

The Xpert Carba-R Assay cartridges contain reagents for the detection of *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene sequences. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are controls utilized by the GeneXpert Instrument System platform. The SPC is present to control for adequate processing of the target bacteria and to monitor the presence of inhibitors in the real-time PCR reaction to reduce the possibility of false negative results. The PCC verifies reagent rehydration, real-time PCR tube filling in the cartridge, probe integrity, and dye stability.

The single-use, multi-chambered fluidic cartridges are designed to complete sample preparation and real-time PCR for the detection of the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene sequences from rectal swab specimens in approximately 50 minutes. The GeneXpert Instrument Systems, comprised of the GeneXpert Dx Systems and the GeneXpert Infinity Systems, have 1 to 80 randomly accessible modules, depending upon the instrument, that are each capable of performing separate sample processing and real-time PCR and RT- PCR tests. Each module contains a syringe drive for dispensing fluids (i.e., the syringe drive activates the plunger that works in concert with the rotary valve in the cartridge to move fluids between chambers), an ultrasonic horn for lysing cells or spores, and a proprietary I-CORE® thermocycler for performing real-time PCR and RT-PCR and detection.

Rectal swab specimens are placed into a sample reagent. The sample is transferred to the sample chamber of the disposable fluidic cartridge (the Xpert Carba-R cartridge). The user initiates a test from the system user interface and places the cartridge into the GeneXpert instrument platform, which performs hands-off real-time, multiplex PCR for detection of the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene sequences. The results are automatically generated at the end of the process in a report that can be viewed and printed.

Device 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 carbapenem-non-susceptible 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.

Substantial Equivalence:

The Cepheid Xpert Carba-R Assay is substantially equivalent to the Xpert® Carba-R Assay, 510(k) #K152614. Both assays utilize the same GeneXpert cartridge and detect target gene sequences (*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$) using real-time PCR amplification and fluorogenic target-specific hybridization detection. The performance of the Xpert Carba-R Assay for an expanded indication was determined in a multi-site clinical study in which the performance of the Xpert Carba-R Assay was evaluated relative to culture and reference DNA sequence analysis. The results of the study demonstrated that the performance of the Xpert Carba-R Assay is substantially equivalent to the predicate device. Please refer to K152614 for information on the performance of the Xpert Carba-R Assay with pure colonies of carbapenem non-susceptible *Enterobacteriaceae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*.

Table 5-1 shows the similarities and differences between the Xpert Carba-R Assay and the predicate device.

**Table 5-1: Comparison of Similarities and Differences of the Xpert Carba-R Assay with the Predicate Device**

| Similarities | | |
|---|---|---|
| **Item** | **Device** | **Predicate Device** |
| | **Cepheid Xpert Carba-R Assay** | **Cepheid Xpert Carba-R Assay K152614** |
| General 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).**<br><br>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.**<br><br>The Xpert Carba-R Assay is for use with the following sample types:<br>Rectal Swab Specimens<br><br>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.<br><br>Pure Colonies<br><br>The assay is performed on carbapenem-non-susceptible 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 | The Xpert® Carba-R Assay, performed on the GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test 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-susceptible pure colonies of *Enterobacteriaceae, Acinetobacter baumannii,* or *Pseudomonas aeruginosa* grown on blood agar or MacConkey agar**. **The test utilizes automated real-time polymerase chain reaction (PCR).**<br><br>**A negative Xpert Carba-R Assay result does not preclude the presence of other resistance mechanisms.** The Xpert Carba-R Assay **should be used in conjunction with other laboratory tests including phenotypic antimicrobial susceptibility testing**. The Xpert Carba-R Assay is intended **as an aid for infection control** in detecting and differentiating genetic markers of resistance to monitor the spread of carbapenem-non-susceptible organisms **in healthcare settings**. **The Xpert Carba-R Assay is not intended to guide or monitor treatment for carbapenem-non-susceptible bacterial infections.** |

| Similarities | | |
|---|---|---|
| **Item** | **Device** | **Predicate Device** |
| | **Cepheid Xpert Carba-R Assay** | **Cepheid Xpert Carba-R Assay K152614** |
| | laboratory tests including phenotypic antimicrobial susceptibility testing. | |
| Type of test | Same | Qualitative |
| Technological Principles | Same | Fully-automated nucleic acid amplification (DNA); real-time PCR |
| Test Cartridge | Same | Disposable single-use, multi-chambered fluidic cartridge |
| Probes | Same | TaqMan® Probes |
| Controls | Same | Internal sample processing control (SPC) and probe check control (PCC)<br><br>External controls available |
| Instrument System | Same | GeneXpert Instrument System (includes GeneXpert Dx, Infinity-48, Infinity-48s, and Infinity-80) |
| Time to obtain test results | Same | Approximately 50 minutes to results |
| Interpretation of test results | Same | Diagnostic software of the GeneXpert Instrument System |
| Laboratory Users | Same | Operators in CLIA Moderate or High Complexity labs |
| **Differences** | | |
| | **New Device** | **Predicate Device** |
| **Item** | **Cepheid Xpert Carba-R Assay** | **Cepheid Xpert Carba-R Assay K152614** |
| Sample Types | Bacterial isolates from culture, **rectal swab specimens** | Bacterial isolates from culture |

The Xpert Carba-R Assay has the same general intended use as the predicate device and has the same technological characteristics as the predicate device. The differences between the Xpert Carba-R Assay and the predicate device do not raise questions of safety and effectiveness. The clinical study demonstrates that the Xpert Carba-R Assay is acceptable for its intended use with inexperienced laboratory users and is substantially equivalent to the predicate device described above.

Non-Clinical Studies:

**Analytical Sensitivity (Limit of Detection)**

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 5-2.

**Table 5-2. 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 |

**Analytical Reactivity (Inclusivity)**

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), 13 $bla_{NDM}$ (NDM), 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 5-3.

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 5-3). Xpert Carba-R Assay target DNA sequences were not detected in three bacterial strains as shown in Table 5-3. 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 Limitations in the package insert.

The variants detected, and predictions for detecting other subtypes of each resistance gene based on *in silico* analysis, are presented in Table 5-4.

**Table 5-3. 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 |
| 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 |

| Strain ID | Organism | Resistance Marker with variant information | Concentration Tested in Rectal Swab Matrix (CFU/mL) |
|---|---|---|---|
| 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 |
| 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 |

| Strain ID | Organism | Resistance Marker with variant information | Concentration Tested in Rectal Swab Matrix (CFU/mL) |
|---|---|---|---|
| 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 |
| 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 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 Limitations).

**Table 5-4. 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 | 19 | OXA-48, 181(OXA-48 variant) | -- | OXA-162, 163, 204, 232, 244, 245, 247 |
| 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 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 Limitations).

### Analytical Specificity (Cross-reactivity)

The analytical specificity of the Xpert Carba-R Assay was evaluated for bacterial isolates, organisms seeded into rectal swab matrix. For all three 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 5-5 and Table 5-6) and 24 commensal bacterial strains and other enteric

microorganisms were also evaluated in the study (Table 5-7). Human cells were also tested in rectal swab matrix (Table 5-8). 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 x$10^5$ CFU/mL. Viruses were tested at >1 x $10^5$ $TCID_{50}$/mL or greater than 2.5 x$10^7$ 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.

The Xpert Carba-R Assay did not cross react with any of the organisms tested (Table 5-6, Table 5-7, and Table 5-8). The analytical specificity of the assay was 100%.

**Table 5-5. 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 5-6. 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 |

| Organism | Strain ID | Confirmed Resistance Mechanisms | Carbapenem susceptibility (S/I/R)[a] | | |
|---|---|---|---|---|---|
| | | | ETP[a] | IMP[a] | MEM[a] |
| *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 |
| *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 |

| Organism | Strain ID | Confirmed Resistance Mechanisms | Carbapenem susceptibility (S/I/R)[a] | | |
|---|---|---|---|---|---|
| | | | ETP[a] | IMP[a] | MEM[a] |
| *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 5-7. 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 |

| Strain ID | Organism | Concentration Tested (CFU/mL unless otherwise specified) |
|---|---|---|
| ATCC 9689 | *Clostridium difficile* | 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* | 5.00E+05 |
| CCUG 29780 / ATCC 12401 | *Streptococcus agalactiae* | 5.21E+06 |
| ATCC 15703 | *Bifidobacterium adolescentis* | 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* | 3.27E+06 |
| MRVP/ZeptoMetrix | Adenovirus B Type 7A/NY | 1.40E+05 $TCID_{50}$/mL |
| MRVP/ZeptoMetrix | Enterovirus Type 71/NY | 4.40E+05 $TCID_{50}$/mL |
| Clinical Sample – Cepheid | Norovirus GII | 2.5 x $10^7$ RNA copies/mL |

**Table 5-8. Cell Line Representing Human Genomic DNA**

| Organism Name | Source |
|---|---|
| Bladder Cell Carcinoma (hgDNA) | ATCC HTB-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 5-9). 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 5-9. 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 |

**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 5-10. 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 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 5-10. 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 |

| Substance/Class | Active Ingredient | Concentration Tested |
|---|---|---|
| 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 |
| 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.

## Carry-Over Contamination

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 $1 \times 10^6$ 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**.

Clinical Studies

## Clinical Performance

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, 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 5-11). 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 5-11).

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 5-11.

**Table 5-11. 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. 1of 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 by Reference Culture. From the remaining 119 specimens, 112 carbapenem-non-susceptible organisms were recovered by 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 5-12 by species. Only organisms for which at least one positive specimen was collected are included in Table 5-12.

**Table 5-12. 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) |

| Species[a] | Target | N | TP | FP | TN | FN | PPA (95% CI) | NPA (95% CI) |
|---|---|---|---|---|---|---|---|---|
| *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) |
| *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 5-13, along with the discrepant sequencing result.

**Table 5-13. 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 PPA range 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 5-14).

**Table 5-14. 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) |

**Reproducibility 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 5-15.

**Table 5-15. 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) |
| **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 5-16.

**Table 5-16. 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.

## Conclusions

The results of the nonclinical analytical and clinical performance studies summarized above demonstrate that the Xpert Carba-R Assay is safe and effective for its intended use and is substantially equivalent to the predicate device.