January 9, 2018

Cepheid
Jim Kelly
Executive Director, Regulatory Affairs
904 Caribbean Drive
Sunnyvale, California 94089

Re: K173263
Trade/Device Name: Xpert Carba-R
Regulation Number: 21 CFR 866.1640
Regulation Name: Antimicrobial susceptibility test powder
Regulatory Class: Class II
Product Code: POC, PMY, OOI
Dated: October 10, 2017
Received: October 12, 2017

Dear Jim 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 Part 801 and Part 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.

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.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/) and CDRH Learn (http://www.fda.gov/Training/CDRHLearn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (http://www.fda.gov/DICE) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Ribhi Shawar -S

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K173263

Device Name
Xpert Carba-R Assay

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. 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:

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.

The identification of a *bla*$_{IMP}$, *bla*$_{NDM}$, or *bla*$_{VIM}$ metallo-beta-lactamase gene (i.e., the genes that encode the IMP, NDM, and VIM metallo-beta-lactamases, respectively) may be used as an aid to clinicians in determining appropriate therapeutic strategies for patients with known or suspected carbapenem-non- susceptible bacterial infections.

Rectal and Perirectal Swab Specimens

The assay is performed on rectal and perirectal 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.

The Xpert Carba-R Assay, when performed on rectal and perirectal swab specimens, is not intended to guide or monitor treatment for carbapenem-non-susceptible bacterial infections or to determine infection from carbapenem-non-susceptible bacteria.

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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8.0 510(k) Summary

As required by 21 CFR Section 807.92(c).

Submitted by:	Cepheid 904 Caribbean Drive Sunnyvale, CA 90489 Phone number: (408) 745-4183 Fax number: (408) 744-1479
Contact:	Jim Kelly, Ph.D.
Date of Preparation:	January 04, 2018
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, perirectal swab specimens, and bacterial isolates
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 [510(k) #K160901]

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 or perirectal swab specimens or isolates of pure cultures of carbapenem-non-susceptibility gram-negative bacteria. 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 or perirectal swab specimens or isolates of pure cultures of carbapenem-non- susceptibility gram-negative bacteria 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 or perirectal swab specimens or bacterial isolates from culture 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. 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:

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.

The identification of a *bla*$_{IMP}$, *bla*$_{NDM}$, or *bla*$_{VIM}$ metallo-beta-lactamase gene (i.e., the genes that encode the IMP, NDM, and VIM metallo-beta-lactamases, respectively) may be used as an aid to clinicians in determining appropriate therapeutic strategies for patients with known or suspected carbapenem-non- susceptible bacterial infections.

Rectal and Perirectal Swab Specimens

The assay is performed on rectal and perirectal 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.

The Xpert Carba-R Assay, when performed on rectal and perirectal swab specimens, is not intended to guide or monitor treatment for carbapenem-non-susceptible bacterial infections or to determine infection from carbapenem-non-susceptible bacteria.

Substantial Equivalence:

The Cepheid Xpert Carba-R Assay is substantially equivalent to the Xpert® Carba-R Assay, 510(k) #K160901. Both assays utilize the same GeneXpert cartridge and detect target gene sequences 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 and in a second separate clinical study using rectal and perirectal swab specimens to demonstrate the equivalency of both sample types. 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 K160901 for information on the

performance of the Xpert Carba-R Assay with rectal swab specimens.

Table 8-1 shows the similarities and differences between the Xpert Carba-R Assay and the predicate device.

Table 8-1: 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 K160901**
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). 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. A negative Xpert Carba-R Assay result does not preclude the presence of other resistance mechanisms.	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: <u>Pure Colonies</u> 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. The identification of a bla_{IMP}, bla_{NDM}, or bla_{VIM} metallo-beta-lactamase gene (i.e., the genes that encode the IMP, NDM, and VIM metallo-beta-lactamases, respectively) may be used	The Xpert Carba R-Assay is for use with the following sample types: <u>Pure Colonies</u> 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.

Similarities		
Item	**Device**	**Predicate Device**
	Cepheid Xpert Carba-R Assay	**Cepheid Xpert Carba-R Assay K160901**
	as an aid to clinicians in determining appropriate therapeutic strategies for patients with known or suspected carbapenem-non- susceptible bacterial infections. <u>Rectal and Perirectal Swab Specimens</u> The assay is performed on rectal and perirectal 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. The Xpert Carba-R Assay, when performed on rectal and perirectal swab specimens, is not intended to guide or monitor treatment for carbapenem-non-susceptible bacterial infections or to determine infection from carbapenem-non-susceptible bacteria.	<u>Rectal Swab Specimens</u> 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.
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) 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

Similarities		
Item	**Device**	**Predicate Device**
	Cepheid Xpert Carba-R Assay	**Cepheid Xpert Carba-R Assay K160901**
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 K160901**
Sample Types	Bacterial isolates from culture, rectal swab **and perirectal swab specimens**	Bacterial isolates from culture and **rectal swab specimens**

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) –Perirectal 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 perirectal 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 perirectal 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 perirectal swab matrix are shown in **Table 8-2.**

Table 8-2: LoD Estimates and Verification for Organisms Harboring Carbapenemase Genes using the Xpert Carba-R Assay in Perirectal 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*	90	**118**	118	24	19/20
IMP-1 *Klebsiella pneumoniae*	269	**635**	635	127	20/20
VIM-1 *Klebsiella pneumoniae*	**901**	514	901	180	20/20
VIM-4 *Escherichia coli*	**446**	403	446	89	20/20
NDM-1 *Klebsiella pneumoniae* ATCC BAA-2146	**133**	113	133	27	20/20
NDM *Klebsiella pneumoniae*	**56**	54	56	11	20/20
KPC-3 *Klebsiella pneumoniae* NCTC 13438	**358**	292	358	72	20/20
KPC *Enterobacter cloacae*	1259	**1303**	1303	261	20/20
OXA-48 *Enterobacter cloacae*	**223**	166	223	45	20/20
OXA-48 *Escherichia coli*	126	**137**	137	27	20/20

Analytical Reactivity (Inclusivity)

The analytical reactivity of the Xpert Carba-R Assay with perirectal swab matrices 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 perirectal swab matrix and their test concentrations are presented in **Table 8-3**.

For testing in perirectal swab matrix, organisms were seeded into pooled negative perirectal swab matrix. All bacterial strains were tested in triplicate at approximately 3X LoD. 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 8-3). Xpert Carba-R Assay target DNA sequences were not detected in three bacterial strains as shown in Table 8-3. 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 8-4**.

Table 8-3: Analytical Reactivity of the Xpert Carba-R Assay in Perirectal Swab Matrix

Strain ID	Organism	Resistance Marker with Variant Information	Concentration Tested in Perirectal 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
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

Strain ID	Organism	Resistance Marker with Variant Information	Concentration Tested in Perirectal Swab Matrix (CFU/mL)
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
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 8-4: 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 organisms seeded into perirectal swab matrix. 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 8-5** and **Table 8-6**) and 24 commensal bacterial strains and other enteric microorganisms were also evaluated in the study (**Table 8-7**). Human cells were also tested in perirectal swab matrix (**Table 8-8**). Resistance mechanisms were determined by individual PCR assays, DNA sequence analysis, or Check-Points array version CT102.

For perirectal 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 perirectal 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 8-5, Table 8-6,** and **Table 8-7**). The analytical specificity of the assay was 100%.

Table 8-5: 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 8-6: 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
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

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

Organism	Strain ID	Confirmed Resistance Mechanisms	Carbapenem susceptibility (S/I/R)[a]		
			ETP[a]	IMP[a]	MEM[a]
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 8-7: 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
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

Strain ID	Organism	Concentration Tested (CFU/mL unless otherwise specified)
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 perirectal swab matrix only.

Table 8-8: 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 titer samples were formulated at concentrations of 5 x 10^6 CFU/swab and low titer targets were formulated at approximately 2X LoD for the respective strain in perirectal 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 8-9**). Samples were tested in replicates of eight.

An inhibitory effect was observed for two of the five targets (NDM and IMP) 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 perirectal swab matrix. The two targets (NDM and IMP) were tested at a higher concentration (4X LoD) in combination with a high concentration of one or two other targets for samples in perirectal swab matrix. No inhibitory effect was observed for the two targets (NDM and IMP) 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 (NDM, IMP, VIM and OXA-48) is addressed in the Limitations section in the package insert.

Table 8-9: 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 perirectal swab specimens. Potentially interfering substances (IS) solutions were prepared and tested at concentrations specified in

Table 8-10. 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 perirectal swab matrix at approximately 3X LoD. Eight replicate positive samples were tested per substance. Negative samples consisted of pooled negative perirectal 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 > 0.025% w/v in tests with perirectal swab matrix samples. See Limitations in the package insert.

Table 8-10: 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
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 perirectal swab matrix to a concentration of 1 x 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 perirectal 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 –Perirectal Swab Specimens

Performance characteristics of the Xpert Carba-R Assay perirectal 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 prospectively collected paired perirectal swab specimens from subjects who were hospitalized or in a long-term care facility. Highly soiled perirectal swab specimens, according to the directions in Section 9 of package insert (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 swab of the pair was used for Xpert Carba-R Assay testing. The second 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 five 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 963 prospective perirectal swab specimens were initially enrolled in this clinical study, of which 947 were eligible for inclusion. From the 947 eligible specimens, 924 specimens were included in the final dataset after exclusions based on protocol deviations (including 10 *Stenotrophomonas maltophilia*, one *Pseudomonas putida* and one *Pseudomonas stutzeri* organisms that were excluded due to their intrinsic resistance to the carbapenems tested).

When tested with prospective perirectal swab specimens, the Xpert Carba-R Assay demonstrated a PPA of 100% for the three assay targets (bla_{NDM}, bla_{KPC} and bla_{OXA-48}) relative to the reference method. The NPA for the bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP} gene sequences ranged from 99.6% to 100% relative to the reference method (**Table 8-11**).

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 8-11**.

Table 8-11: Xpert Carba-R Performance vs. Reference Culture + Sequencing – Prospective Perirectal Specimens

Specimen Type	Target	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
Perirectal[c]	IMP	924	0	0	924	0	N/A	100% (99.6-100)
	VIM	924	0	0	924	0	N/A	100% (99.6-100)
	NDM	924	1	0	923	0	100% (20.7-100)	100% (99.6-100)
	KPC	924	2	4[a]	918	0	100% (34.2-100)	99.6% (98.9-99.8)
	OXA-48	924	1	1[b]	922	0	100% (20.7-100)	99.9% (99.4-100)

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

a. Testing results by sequencing: 4 of 4 were KPC negative.

b. Testing results by sequencing: 1 of 1 was OXA-48 negative.

c. Of the 924 prospective perirectal swab specimens evaluated in the study, 891 specimens did not yield a culture isolate. From the remaining 33 specimens, 31 carbapenem-non-susceptible organisms were recovered by the Reference Culture in addition to two carbapenem susceptible organisms (*Pseudomonas aeruginosa*).

Performance of the Xpert Carba-R Assay on the prospective perirectal specimens is shown in Table 8-12 by species. Only organisms for which at least one positive specimen was collected are included in **Table 8-12**.

Table 8-12. Xpert Carba-R Performance vs. Reference Culture + Sequencing by Organism type – Prospective Perirectal Specimens

Species[a]	Target	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
Enterobacter aerogenes	IMP	1	0	0	1	0	NA	100% (20.7-100)
	VIM	1	0	0	1	0	NA	100% (20.7-100)
	NDM	1	0	0	1	0	NA	100% (20.7-100)
	KPC	1	1	0	0	0	100% (20.7-100)	NA
	OXA-48	1	0	0	1	0	NA	100% (20.7-100)
Klebsiella pneumoniae	IMP	3	0	0	3	0	NA	100% (43.9-100)
	VIM	3	0	0	3	0	NA	100% (43.9-100)
	NDM	3	1	0	2	0	100% (20.7-100)	100% (34.2-100)
	KPC	3	1	0	2	0	100% (20.7-100)	100% (34.2-100)
	OXA-48	3	1	0	2	1	96.2% (20.7-100)	100% (34.2-100)

a. *Acinetobacter baumannii* (1), and *Pseudomonas aeruginosa* (28) were recovered but did not contain target sequences by the Reference Method.

Multiple targets were detected by the Xpert Carba-R Assay in one prospective specimen. The details are provided in **Table 8-13**, along with the discrepant sequencing result.

Table 8-13. Prospective Perirectal 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
9	NDM, OXA-48	NDM, OXA-48	NA

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

A total of 432 contrived specimens prepared in perirectal 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 and NPA of 100% for the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene sequences was 100% relative to the reference method (**Table 8-14**).

Table 8-14. Xpert Carba-R Performance vs. Reference Method – Contrived Specimens

Matrix	Target	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
Perirectal	IMP	432	80	0	352	0	100% (95.4-100)	100% (98.9-100)
	VIM	432	82	0	350	0	100% (95.5-100)	100% (98.9-100)
	NDM	432	80	0	352	0	100% (95.4-100)	100% (98.9-100)
	KPC	432	80	0	352	0	100% (95.4-100)	100% (98.9-100)
	OXA-48	432	80	0	352	0	100% (95.4-100)	100% (98.9-100)

Perirectal Swab and Rectal Swab Equivalence Study

To demonstrate equivalence of perirectal swab specimens and rectal swab specimens, a study was conducted at one site enrolling fresh prospectively collected rectal and perirectal swab specimens from consented subjects who were hospitalized in-patients.

Paired swab sets provided in the Cepheid Specimen Collection Device were used to collect specimens from each subject. One paired swab set was used to collect the perirectal swab specimen and a second paired swab set was used to collect the rectal swab specimen. The perirectal swab specimen was collected first followed by the rectal swab specimen from the same subject. One swab from each paired swab set was used for Xpert Carba-R Assay testing. The second swab from each paired swab set was used for culture and susceptibility testing when either or both the perirectal or rectal swab specimen(s) were positive for one or more target(s) by the Xpert Carba-R Assay. No culture was performed if perirectal and rectal swab specimens were both negative by the Xpert assay.

Bi-directional DNA sequencing was performed on DNA extracted from isolated colonies that manifested carbapenem-non-susceptibility by the CLSI disk diffusion method or from MacConkey broth with meropenem disk if the culture result was negative and the Xpert Carba-R Assay result was positive.

A total of 207 specimens were initially enrolled in this clinical study, all of which were eligible for inclusion. Of the 207 eligible specimens, 201 specimens were included in the final dataset used for the analyses. Six swab specimens (4 perirectal swab specimens and 2 rectal swab specimens) were excluded due to indeterminate results from the Xpert Carba-R Assay.

Of the 201 specimens included in the data analyses, 92 (45.8%) were collected from female subjects and 109 (54.2%) from male subjects. Overall 45.8% (92/201) specimens were collected from subjects between 21 and 65 years of age and 54.2%

(109/201) were from subjects >65 years of age.

The performance (PPA and NPA) of the Xpert Carba-R Assay using perirectal swab specimens was determined relative to the results of the Xpert Carba-R Assay using rectal swab specimens from the same subject. The PPA and NPA estimates are shown in

Table 8-15. Relative to the Xpert Carba-R Assay rectal swab specimen result, the perirectal swab specimens demonstrated an overall PPA and NPA of 94.7% (95%CI: 75.4-99.1) and 97.8% (95%CI: 94.5-99.1), respectively.

Table 8-15. Xpert Carba-R Assay – Perirectal Swab Specimens vs Rectal Swab Specimens

		Xpert Carba-R Assay – Rectal Swab Specimens		
		Positive	**Negative**	**Total**
Xpert Carba-R Assay – Perirectal Swab Specimens	**Pos**	18[a]	4[b]	22
	Neg	1[c]	178	179
	Total	19	182	201
		PPA	94.7% (95%CI: 75.4-99.1)	
		NPA	97.8% (95%CI: 94.5-99.1)	

[a] For one specimen, Xpert testing on the rectal swab was positive for KPC and OXA-48 and on the perirectal swab was positive for OXA-48 only. The specimen was culture negative for both rectal and perirectal swabs. Sequence results from the MacConkey broths were negative for the perirectal swab and OXA-48 positive for the rectal swab.

[b] 2 of 4 were culture positive for both rectal and perirectal swabs, sequence results from isolates were both OXA-48 positive, 1 of 4 was culture negative for both rectal and perirectal swabs, sequence result the rectal sequence result was not available due to isolate not saved, the perirectal isolate was interpreted as carbapenem susceptible and per protocol sequencing was not required.

[c] Culture negative for both rectal and perirectal swabs, sequence results from MacConkey broths were both OXA-48 Positive.

Reproducibility Study

Reproducibility of the Xpert Carba-R Assay was evaluated using of a panel of 11 samples, prepared in pooled negative perirectal 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 8-16**.

Table 8-16: Summary of Reproducibility Results - % Agreement, Perirectal Swab Matrix

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	
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)
IMP Mod Pos	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	95.8% (23/24)	91.7% (22/24)	93.8% (45/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	91.7% (22/24)	95.8% (46/48)	96.5% (139/144)
VIM Mod Pos	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	100% (24/24)	91.7% (22/24)	95.8% (46/48)	91.7% (22/24)	91.7% (22/24)	91.7% (44/48)	95.8% (23/24)	83.3% (20/24)	89.6% (43/48)	92.4% (133/144)
NDM Mod Pos	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	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	87.5% (21/24)	100% (24/24)	93.8% (45/48)	97.9% (141/144)
KPC Mod Pos	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	91.7% (22/24)	91.7% (22/24)	91.7% (44/48)	91.7% (22/24)	95.8% (23/24)	93.8% (45/48)	100% (24/24)	91.7% (22/24)	95.8% (46/48)	93.8% (135/144)
OXA-48 Mod Pos	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	87.5% (21/24)	87.5% (21/24)	87.5% (42/48)	100% (24/24)	95.8% (23/24)	97.9% (47/48)	95.8% (23/24)	95.8% (23/24)	95.8% (46/48)	93.8% (135/144)

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 8-17.

Table 8-17: Summary of Reproducibility Data for Perirectal Swab Matrix

Sample	Assay Channel (Analyte)	N[a]	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	SPC	144	32.7	0.0	0.0	0.2	0.6	0.0	0.0	0.2	0.5	0.4	1.2	0.5	1.4
IMP Mod Pos	IMP	144	33.7	0.0	0.0	0.1	0.2	0.0	0.0	0.2	0.5	0.5	1.5	0.5	1.6
IMP Low Pos	IMP	142	36.0	0.2	0.5	0.0	0.0	0.1	0.3	0.2	0.5	0.8	2.1	0.8	2.3

Sample	Assay Channel (Analyte)	N[a]	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 (%)
VIM Mod Pos	VIM	144	31.2	0.1	0.2	0.1	0.3	0.0	0.1	0.2	0.5	0.4	1.3	0.5	1.5
VIM Low Pos	VIM	142	35.0	0.0	0.0	0.6	1.6	0.0	0.0	0.6	1.7	1.4	4.1	1.6	4.7
NDM Mod Pos	NDM	144	33.2	0.0	0.0	0.0	0.0	0.2	0.5	0.2	0.5	0.4	1.2	0.5	1.4
NDM Low Pos	NDM	143	35.7	0.2	0.5	0.0	0.0	0.2	0.6	0.0	0.0	0.9	2.4	0.9	2.5
KPC Mod Pos	KPC	144	34.6	0.0	0.0	0.3	1.0	0.0	0.0	0.2	0.5	0.4	1.3	0.6	1.7
KPC Low Pos	KPC	143	36.4	0.0	0.0	0.5	1.3	0.1	0.4	0.0	0.0	0.7	2.0	0.9	2.4
OXA-48 Mod Pos	OXA-48	144	34.4	0.1	0.2	0.2	0.6	0.0	0.0	0.2	0.5	0.5	1.5	0.6	1.7
OXA-48 Low Pos	OXA-48	144	36.4	0.0	0.0	0.0	0.0	0.4	1.2	0.0	0.0	1.0	2.7	1.1	2.9

a. 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.