510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

K152614

B. Purpose for Submission:

To obtain a substantial equivalence determination for the Xpert® Carba-R Assay on the Cepheid GeneXpert Instrument Systems (GeneXpert Dx, GeneXpert Infinity-48, GeneXpert Infinity-48s, and GeneXpert Infinity-80 systems) in the qualitative detection of the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene sequence from colonies.

C. Measurand:

Target DNA sequence of the following genes:

bla$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$

D. Type of Test:

Qualitative real-time polymerase chain reaction (PCR) assay

E. Applicant:

Cepheid

F. Proprietary and Established Names:

Proprietary Name: Xpert® Carba-R

Common Name: Xpert Carba-R Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.1640 (Antimicrobial susceptibility test powder)

2. Classification:

   Class II

3. Product code:

   PMY- System, nucleic acid amplification test, DNA, carbapenem non-susceptible gram negative organism, colony

   OOI-Real-time nucleic acid amplification system

4. Panel:

   83-Microbiology

H. Intended Use:

1. Intended use(s):
   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).

   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.

2. Indication(s) for use:

   Same as the Intended Use.

3. Special conditions for use statement(s):

   For prescription use only.

   Organisms should be identified and carbapenem non-susceptibility status should be determined prior to testing with the Xpert Carba-R Assay.

   The Xpert Carba-R Assay detects *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ from pure colonies and is not for bacterial identification.

   The performance of the Xpert Carba-R Assay with bacteria other than *Enterobacteriaceae, Pseudomonas aeruginosa* or *Acinetobacter baumannii* has not been evaluated.

The Xpert Carba-R Assay is not a sub-typing tool and does not report variants of the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ genes.

The detection of other OXA-carbapenemase genes, besides *bla*$_{OXA-48}$ and *bla*$_{OXA-181}$, has not been evaluated in the study.

4. Special instrument requirements:

   The Xpert Carba-R Assay uses PCR technology on the GeneXpert Instrument Systems, which extract, amplify, and detect the target DNA.

I. 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 pure colonies of carbapenem non-susceptible *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. The Xpert Carba-R Assay is intended as an aid for infection control to monitor the spread of carbapenem-non-susceptible organisms in healthcare settings. The 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 Systems utilize single-use, disposable cartridges (Xpert Carba-R cartridges) containing PCR reagents and allows for automated sample preparation, amplification, and real-time detection of gene targets in approximately 50 minutes. A Sample Processing Control (SPC) and a Probe Check Control (PCC) have been incorporated into the assay design to address key failure modes that could result in a false negative determination.

The GeneXpert Instrument Systems (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. 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.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Xpert ® *vanA* Assay

2. Predicate 510(k) number(s):

   K092953

3. Comparison with predicate:

Similarities		
Item	Device Xpert® Carba-R Assay (K152614)	Predicate Cepheid Xpert® *vanA* (K092953)
Intended Use	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). 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.	The Cepheid Xpert® vanA Assay performed in the GeneXpert® Dx System is a qualitative *in vitro* diagnostic test designed for rapid detection of the *vanA* gene sequence associated with vancomycin resistance in bacteria obtained from rectal swab specimens from patients at risk for intestinal colonization with vancomycin-resistant bacteria. The test utilizes automated real-time polymerase chain reaction (PCR) to detect the *vanA* gene that is frequently associated with vancomycin-resistant *enterococci* (VRE). The Xpert *vanA* Assay is intended to aid in the recognition, prevention, and control of vancomycin resistant organisms that colonize patients in healthcare settings. The Xpert *vanA* Assay is not intended to diagnose infections caused by vancomycin-resistant bacteria nor to guide or monitor treatment for vancomycin-resistant bacterial infections. Concomitant cultures are necessary to recover organisms for confirmatory identification of vancomycin-resistant bacteria, antimicrobial susceptibility testing, and for epidemiological typing.
Technological Principles	Fully-automated nucleic acid amplification (DNA); real-time PCR	Same
Test Cartridge	Disposable single-use, multi-chambered fluidic cartridge	Same
Detection Probes	TaqMan® Probes	Same
Controls	Internal sample processing control (SPC) and probe	Same

Similarities		
Item	Device Xpert® Carba-R Assay (K152614)	Predicate Cepheid Xpert® *vanA* (K092953)
	check control (PCC) External controls available	
Time to obtain test results	Approximately 50 minutes to results	Approximately 45 minutes to results
Interpretation of test results	Diagnostic software	Same

Differences		
Item	Device	Predicate
Sample Type	Bacterial isolates from culture	Rectal swabs
Assay Targets	Detects bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP} gene sequences	Detects gene sequences for the *vanA* encoded resistance to vancomycin/teicoplanin
Organism(s)	*Enterobacteriaceae, Pseudomonas aeruginosa, Acinetobacter baumannii*	*Vancomycin-resistant bacteria (Enterococcus)*
Instrument System	GeneXpert Instrument System (includes GeneXpert Dx, Infinity-48, Infinity-48s, and Infinity-80)	GeneXpert Dx

K. Standard/Guidance Document Referenced (if applicable):

1. ASTM D4169-09, *Standard Practice for Performance Testing of Shipping Containers and Systems*.
2. CLSI EP5-A2, *Evaluation of Precision Performance of Quantitative Measurement Methods*; *Approved Guideline*—Second Edition, 2004
3. CLSI EP15-A2, *User Verification of Performance for Precision and Trueness*; *Approved Guideline*–Second Edition, 2006
4. CLSI M02-A11. *Performance standards for Antimicrobial Disk Susceptibility Tests*; Eleventh Edition, 2012
5. CLSI M07-A9. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*; Approved standard—Ninth Edition, 2012
6. CLSI M07-A10. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*; Approved standard—Tenth Edition, 2015
7. CLSI M100-S24. *Performance Standard for Antimicrobial Susceptibility Testing*; Approved standard—Twenty-fourth Informational Supplement, 2014
8. CLSI MM3-A2, *Molecular Diagnostic Methods for Infectious Disease*; *Approved Guideline*–Second Edition, 2006
9. EN 13640, *Stability Testing of in vitro Diagnostic Reagents*, June 2002
10. *General Principles of Software Validation*; Final Guidance for Industry and FDA Staff, issued January 11, 2002
11. Guidance for Industry and FDA Staff–*Format for Traditional and Abbreviated 510(k)s*,

issued August 12, 2005

12. Guidance for Industry and FDA Staff-*Class II Special Controls Guidance Document: Instrumentation for Clinical Multiplex Test Systems*, issued on March 10, 2005
13. Guidance for Industry and FDA Staff–Content of Premarket Submissions for Management of Cybersecurity in Medical Devices, issued on October 2, 2014
14. Guidance for Industry-*Cybersecurity for Networked Medical Devices Containing Off-the-Shelf (OTS) Software*, issued January 14, 2005
15. Guidance for Industry and FDA Staff–*Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices*, issued May 11, 2005
16. Guidance for Industry, FDA Reviewers and Compliance on Guidance for *Off-the-Shelf Software Use in Medical Devices*; issued September 9, 1999
17. Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff-*Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable*, issued April 25, 2006

L. Test Principle:

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 from isolates of pure cultures of carbapenem-non-susceptible *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. The bacterial isolates from culture are tested using a loopful of organism (equivalent to a 0.5 McFarland suspension) resuspended in 5 ml of Sample Reagent. The sample is vortexed then transferred (1.7 ml) to the sample chamber of a disposable Xpert Carba-R Assay 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 target sequences.

Results of the assay run are interpreted by the GeneXpert Instrument System software from measured fluorescent signals and embedded calculation algorithms. The results are automatically generated at the end of the process in a report that can be viewed and printed. Basic users see test results reported as *"red"* highlighted for *DETECTED* results and *"green"* highlighted for *NOT DETECTED* results. Additional results that can be reported include: *INVALID*, *ERROR*, and *NO RESULT*.

Interpretation of Results

The Xpert Carba-R Assay provides test results for the IMP, VIM, NDM, KPC, and OXA-48 target DNA sequences. A Sample Processing Control (SPC) and a Probe Check Control (PCC) have been designed for the assay as internal controls to enable the GeneXpert Instrument System to detect specific failure modes related to assay performance. The PCC is considered to PASS if the fluorescence generated meets the validated acceptance criteria. If the PCC fails for any of the IMP, VIM, NDM, KPC, and OXA-48 targets, or SPC target, a probe check error is reported and the test will not continue. The assay also reports if the test has an *INVALID*, *ERROR* or *NO RESULT*. Under these conditions, the test will need to be repeated using a new sample, a new cartridge, and/or new reagents. Retest procedures are described in the Xpert Carba-R package insert. An interpretation table for test results is shown in Table 1.

Table 1. Interpretation of Test Results for the Xpert Carba-R Assay

Result Report	Interpretation of Results
DETECTED	Target(s): For a valid *"DETECTED"* test result, PCR amplification of the target DNA sequence gives Ct value(s) within the valid range and a fluorescence endpoint above the threshold setting for IMP, VIM, NDM, KPC, and/or OXA-48; SPC: Not applicable (if at least one target detected); PCC: PASS; all probe check results pass.
NOT DETECTED	Target(s): For a valid *"NOT DETECTED"* test result, no valid Ct(s) are reported for the IMP, VIM, NDM, KPC, and/or OXA-48 target DNA sequences; SPC: PASS, PCR amplification of the SPC DNA sequence gives a Ct value within the valid range and a fluorescence endpoint above the threshold setting; PCC: PASS; all probe check results pass.
ERROR	Target(s): Presence or absence of IMP, VIM, NDM, KPC, and OXA-48 target DNA sequences cannot be determined; SPC: NO RESULT; PCC: FAIL*, one or more of the probe check results failed. *If the probe check passed, the error is caused by a system component failure.
INVALID	Target(s): Presence or absence of IMP, VIM, NDM, KPC, and OXA-48 target DNA sequences cannot be determined; SPC: FAIL, No PCR amplification of the SPC DNA sequence or the SPC Ct is not within valid range and the fluorescence endpoint is below threshold setting; PCC: PASS; all probe check results pass.
NO RESULT	Target(s): Presence or absence of IMP, VIM, NDM, KPC, and OXA-48 target DNA sequences cannot be determined; SPC: NO RESULT; PCC: Not applicable. A *"NO RESULT"* indicates that insufficient data were collected. For example, the operator stopped a test that was in progress or a power failure occurred.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   The reproducibility of the Xpert Carba-R Assay was established in a multi-center study. A panel of 13 bacterial samples included the following: 1) two different organisms for each of the five resistance genes detected by the Xpert Carba-R Assay, 2) two different organisms that harbored two gene targets, and 3) one organism negative for all five gene targets. A 0.5 McFarland equivalent of bacterial cell suspension was prepared for each sample. To measure site-to-site reproducibility, the (13)-member panel was tested in replicates of four each day at three (3) sites over a six day testing period. Three lots of Xpert Carba-R Assay cartridges were used at each testing site. A total of 1872 samples consisting of 144 replicates for the 13

different panel members were tested for the study using two operators at each site. Twenty-five (25) test runs from one instrument module were excluded, resulting in 1847 test runs included in the analyses. For the Xpert Carba-R Assay, 99.4% (1836/1847) of samples were successful and produced the expected result on the first attempt. Six (6) *ERROR* cases, two (2) *INVALID* results, and three (3) *NO RESULT* outcomes were reported. All eleven samples yielded valid results upon repeat testing. The results of the reproducibility study are summarized in Table 2 below.

Table 2. Reproducibility of the 13-Member Sample Panel

Resistance Gene* (sample number)	Site 1 (GeneXpert Dx)			Site 2 (Infinity-80)			Site 3 (Infinity-48)			% Total Agreement by Sample
	Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
KPC (S1)	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 (S2)	100% (23/23)	100% (22/22)	100% (45/45)	95.8% (23/24)	100% (24/24)	97.9% (47/48)	100% (24/24)	100% (24/24)	100% (48/48)	99.3% (140/141)
VIM (S1)	100% (22/22)	100% (23/23)	100% (45/45)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (141/141)
VIM (S2)	100% (22/22)	100% (24/24)	100% (46/46)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (142/142)
IMP (S1)	100% (23/23)	100% (24/24)	100% (47/47)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (143/143)
IMP (S2)	100% (23/23)	100% (23/23)	100% (46/46)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (142/142)
OXA-48 (S1)	100% (23/23)	100% (23/23)	100% (46/46)	100% (24/24)	91.7% (22/24)	95.8% (46/48)	100% (24/24)	100% (24/24)	100% (48/48)	98.6% (140/142)
OXA-48 (S2)	100% (23/23)	100% (22/22)	100% (45/45)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (141/141)
NDM (S1)	100% (22/22)	100% (21/21)	100% (43/43)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (139/139)
NDM (S2)	100% (23/23)	100% (23/23)	100% (46/46)	91.7% (22/24)	100% (24/24)	95.8% (46/48)	100% (24/24)	100% (24/24)	100% (48/48)	98.6% (140/142)
OXA-48/NDM (S1)	100% (24/24)	100% (23/23)	100% (47/47)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (143/143)
OXA-48/NDM (S2)	100% (23/23)	100% (24/24)	100% (47/47)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (143/143)
NEG	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)

**S1=Sample 1*
S2=Sample 2

The reproducibility of the Xpert Carba-R Assay was also evaluated by assessing the fluorescent signal (expressed in Ct values) for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between sites, days, and operators for each panel member are presented in Table 3 below.

Table 3. Reproducibility of the Fluorescent Signal

Resistance Gene (sample number)	Assay Channel (Analyte)	N[a]	Between Site		Between Lot		Between Day		Between Operator		Within Assay		Total	
			SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
KPC (S1)	KPC	144	1.1	4.4	0	0	0	0	0.6	2.6	0.6	2.6	1.4	5.8
KPC (S2)	KPC	143	0.8	3.1	0.1	0.2	0.2	0.9	0.5	2.0	0.8	3.1	1.2	4.9
VIM (S1)	VIM	141	1.1	5.1	0	0	0	0	0.5	2.3	0.8	3.7	1.5	6.7
VIM (S2)	VIM	142	0.3	1.3	0.2	0.8	0	0	0.8	3.8	0.7	3.1	1.1	5.1
IMP (S1)	IMP	143	0.3	1.0	0	0	0.3	1.2	0.6	2.3	0.8	3.1	1.0	4.2
IMP (S2)	IMP	142	1.4	6.3	0.1	0.5	0	0	0.6	2.8	0.7	3.2	1.7	7.6
OXA-48 (S1)	OXA-48	140	0.6	2.6	0	0	0	0	0.7	2.8	0.8	3.5	1.2	5.2
OXA-48 (S2)	OXA-48	141	1.1	4.9	0.3	1.5	0	0	0.5	2.0	0.7	3.3	1.5	6.4
NDM (S1)	NDM	139	1.2	5.3	0	0	0	0	0.6	2.4	0.7	3.1	1.5	6.6
NDM (S2)	NDM	140	0.9	4.0	0.3	1.4	0	0	0.8	3.3	0.8	3.3	1.5	6.3
NDM/OXA-48 (S1)	NDM	143	1.3	5.4	0.2	0.8	0	0	0.6	2.5	0.7	3.1	1.6	6.8
	OXA-48	143	1.2	6.2	0.3	1.4	0	0	0.5	2.4	0.7	3.7	1.5	7.7
NDM/OXA-48 (S2)	NDM	143	1.2	5.3	0.2	1.1	0	0	0.5	2.4	0.8	3.5	1.6	6.9
	OXA-48	143	1.2	6.0	0.2	1.2	0	0	0.5	2.5	0.7	3.8	1.5	7.6
NEG	SPC	144	0.1	0.3	0.1	0.3	0	0	0.2	0.5	0.4	1.3	0.5	1.5

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

Two NDM samples and two OXA-48 samples were negative for all targets. One KPC sample was positive for both KPC and OXA-48 gene targets. The remaining panel samples were positive for the expected targets. All negative samples were correctly identified as negative (144/144). Agreement between sites, operators, and lots was evaluated using Fisher's Exact test. The data presented in Table 2 and Table 3 demonstrated good reproducibility for the Xpert Carba-R Assay on the GeneXpert Instrument Systems.

b. Linearity/assay reportable range:

Not applicable.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

External Controls

Commercially-available external controls can also be run in accordance with local, state, and federal accrediting organizations, as applicable. External controls include the following bacteria harboring target genes:

Multivalent External Positive Control—External positive control (inactivated *Escherichia coli* carrying plasmid with KPC, NDM, VIM, IMP, and OXA-48 gene sequences)

Individual Positive Controls

- *K. pneumoniae* KPC (ATCC BAA-1705)
- *K. pneumoniae* NDM (ATCC BAA-2146)
- *K. pneumoniae* VIM (NCTC 13439)
- *K. pneumoniae* OXA-48 (NCTC 13442)

- *Escherichia coli* IMP (NCTC 13476)

External Negative Control—Inactivated *E. coli* containing a plasmid with no resistance gene inserted.

Internal Control (IC) Reaction Analysis

Internal controls enable the system to detect specific failure modes that could potentially result in an incorrect test result. Each Xpert Carba-R Assay includes a Sample Processing Control (SPC) and Probe Check Control (PCC) pre-loaded in the cartridge and provided with the assay.

Sample Processing Control (SPC)

The SPC contains genomic DNA of *Bacillus globigii* that is included in each cartridge to verify adequate processing of the sample. The SPC verifies that lysis of bacteria has occurred if the organisms are present and verifies the effectiveness of each sample preparation step—reaction tube filling, reaction components are present and functioning, and monitoring for the presence of potential inhibitor(s) in the PCR assay. Test results are reported as *INVALID* if the SPC fails to meet the valid minimum or maximum Ct specification (range 3.0 to 40.0 Ct).

Probe Check control (PCC)

The PCC verifies reagent rehydration, real-time PCR tube filling in the cartridge, probe integrity, and dye stability. The PCC is considered to PASS if the fluorescence generated meets the validated acceptance criteria. If the PCC fails for any of the IMP, VIM, NDM, KPC, and OXA-48 targets or SPC target, a probe check error is reported and the test will not continue. If a probe check error is reported, the test may be repeated using a new sample, new cartridge, and new reagents.

Stability Studies

The objectives of the Xpert Carba-R Assay Stability and Shipping Study were to: 1) evaluate the shelf-life of the Xpert Carba-R Assay, 2) determine the open package stability to support claimed temperature/time limits for the assay, and 3) determine shipping stability for the assay.

Shelf-life and Expiration Dating Study

Three lots of the Xpert Carba-R Assay are being evaluated for stability at four temperatures and predefined time point intervals. Each Xpert Carba-R lot has been tested with samples containing high and low levels of target strains and with a negative sample type (an organism that is not detectable by the assay). Analysis of lot performance was based on the studies of the Cts for IMP, VIM, NDM, KPC, and OXA-48 targets from both low and high positive samples, as well as the SPC target from negative samples. Currently, six months of real-time data are available for the three stability lots. Real-time stability studies for determining shelf-life and expiration dating are ongoing. The storage temperature range for the Xpert Carba-R Assay is 2°C - 28°C.

Shipping Stability
The purpose of the shipping stability study was to validate the ability of the packaging systems to protect the Xpert Carba-R Assay test kits during transport. Kits were subjected to transport simulation conditions under pre-defined temperatures and humidity conditions. Packages were inspected and functional testing performed to establish shipped sample performance. The Xpert Carba-R Assay packaging was determined to be adequate in protecting the product from damage. Product performance testing was conducted on one assay lot.

Sample Stability
A study was conducted to establish the sample stability of carbapenem-non-susceptible bacterial isolates seeded into Xpert Carba-R Assay Sample Reagent. Table 4 shows the one negative and five positive bacterial strains tested. The five positive organisms comprised of carbapenemase-producing bacteria harboring each of the gene targets detected by the Xpert Carba-R Assay. The study was performed first by sub-culturing the bacteria onto blood agar plates and placing a meropenem disc in the first streak quadrant of each plate as a means to ensure that the bacterial isolate retained its non-susceptibility to carbapenem. The agar plates were incubated at 35°C for 18-24 hours. A bacterial suspension equivalent to a 0.5 McFarland suspension was prepared in tryptic soy broth for each plate. An aliquot of bacterial suspension was mixed with Sample Reagent and tested with the Xpert Carba-R Assay at t=0 day. One sample test volume in Sample Reagent was stored at 4°C for up to 8 days and tested on those days with the Xpert Carba-R Assay. A second test volume was stored at 30°C for up to 8 days and similarly tested. A negative control consisting of a 0.5 McFarland suspension of a carbapenem non-susceptible organism (that did not produce carbapenemase) was also prepared and tested.

Table 4. Bacterial Strains for Sample Stability Testing

Carba-R Target	Organism
bla$_{KPC}$	*Enterobacter cloacae*
bla$_{NDM}$	*Klebsiella pneumoniae*
bla$_{VIM}$	*Klebsiella pneumoniae*
bla$_{OXA-48}$	*Escherichia coli*
bla$_{IMP}$	*Acinetobacter baumannii*
Negative	*Enterobacter cloacae*

All positive and negative samples tested at each storage condition/temperature were correctly identified using the Xpert Carba-R Assay. The data supports that carbapenem non-susceptible bacterial isolate cells are stable in Xpert Carba-R Sample Reagent for up to 8 days when stored at 4°C – 30°C prior to testing with the Xpert Carba-R Assay. Therefore, the study supports storage claim of 2–28°C for up to 96 hours in Xpert Carba-R Sample Reagent for carbapenem non-susceptible bacterial isolates.

All external positive and negative controls gave the expected GeneXpert test result. Data for this study was collected using GeneXpert Dx software version

4.4a on the GeneXpert Dx GX-IV instrument.

Cartridge Hold Time Study
Samples prepared for testing with the Xpert Carba-R Assay and the GeneXpert Infinity System may not be available for testing for up to two hours after the cartridge is loaded onto the System (in a multiple-module system). In order to assess the stability of cartridge storage times, one lot of the Xpert Carba-R Assay was subjected to a sample hold time study where the sample was added to cartridges, held at three storage conditions (room temperature, 25°C/75% relative humidity, and 35°C), and processed at scheduled time intervals ranging from 0-5 hours. Positive and negative samples were tested under all storage conditions as a panel with 4 negative and 14 positive samples. Two types of external positive controls were employed in the study—multivalent external positive control and monovalent positive controls. Acceptable performance was observed for up to five hours of hold time between sample addition and cartridge processing for positive and negative samples under all conditions tested. The maximum hold time recommended from sample addition to the Xpert Carba-R Assay cartridges to processing on the GeneXpert Infinity System is 4.5 hours. All external positive and negative controls gave the expected GeneXpert test result.

d. Detection limit:

Not applicable

e. Analytical reactivity:

An inclusivity study was conducted to test reactivity of the Xpert Carba-R Assay with a 71-member panel (See Table 5) of well-characterized bacterial isolates that included the following molecular resistance marker groups and representative sub-groups: (11) *bla*$_{KPC}$ isolates, (13) *bla*$_{NDM}$ isolates, (11) *bla*$_{VIM}$ isolates, (8) *bla*$_{OXA-48}$ isolates, (5) *bla*$_{NDM/OXA-181}$ isolates, (5) *bla*$_{OXA-181}$, (17) *bla*$_{IMP}$ isolates, and (1) *bla*$_{KPC/VIM}$ isolate. The study was also used to demonstrate equivalency in growth on blood agar and MacConkey agar plates for the detection of target genes by the Xpert Carba-R Assay.

Bacterial isolates were grown on blood agar and MacConkey agar plates and incubated at 35°C for 18-24 hours. A meropenem disc was placed in the first streak quadrant of each plate as a means to ensure that the bacterial isolate retained its non-susceptibility to carbapenem. For each bacterial strain tested, a 0.5 McFarland cell suspension was prepared in tryptic soy broth for each plate. A 10 µl loop of the suspension was diluted in 5 ml Xpert Carba-R Sample Reagent, vortexed briefly, then duplicate 1.7 ml aliquots were tested with the Xpert Carba-R Assay. Each organism was tested in replicates of four (4)—two replicates per agar plate.

For each day of the study, an external negative control and two types of experimental positive controls were tested. The negative control consisted of *Escherichia coli* cells containing a plasmid with no resistance gene sequence. The two types of external positive controls employed in the study included:

- multivalent external positive control (containing all five gene targets)
- two monovalent positive controls (containing individual carbapenemase-producing bacteria, each harboring only one of the Xpert Carba-R target carbapenemase genes on a rotating basis).

On each testing day, one negative control, the five-gene construct positive control, and two of five individual bacterial controls (single gene target) were tested.

For a list of strains tested during the Analytical Reactivity Study, please refer to Table 5 below.

Table 5. Strains Tested in Analytical Reactivity

Strain ID	Organism	Confirmed Genetic Resistance Marker
KPC Isolates		
NCTC 13438	*Klebsiella pneumoniae*	KPC-3
31551	*Klebsiella pneumoniae*	KPC-4
ATCC BAA-1705	*Klebsiella pneumoniae*	KPC-2
CFVL	*Enterobacter cloacae*	KPC-2
KBM18	*Enterobacter aerogenes*	KPC-2
COL	*Escherichia coli*	KPC-2
BM9	*Klebsiella pneumoniae*	KPC-3
CGNC	*Serratia marcescens*	KPC-2
PA3	*Pseudomonas aeruginosa*	KPC-2
COL	*Pseudomonas aeruginosa*	KPC-2
164-3	*Klebsiella oxytoca*	KPC
NDM Isolates		
NCTC 13443	*Klebsiella pneumoniae*	NDM-1
ATCC BAA-2146	*Klebsiella pneumoniae*	NDM-1
34262	*Klebsiella pneumoniae*	NDM
GEN	*Acinetobacter baumannii*	NDM-1
3047	*Enterobacter cloacae*	NDM-1
7892	*Proteus mirabilis*	NDM-1
CAN	*Salmonella spp.*	NDM-1
EGY	*Acinetobacter baumannii*	NDM-2
I5	*Escherichia coli*	NDM-4
405	*Escherichia coli*	NDM-5
CF-ABE	*Citrobacter freundii*	NDM
73999	*Pseudomonas aeruginosa*	NDM
39365	*Providencia rettgeri*	NDM-1
VIM Isolates		
NCTC 13437	*Pseudomonas aeruginosa*	VIM-10
NCTC 13439	*Klebsiella pneumoniae*	VIM-1
NCTC 13440	*Klebsiella pneumoniae*	VIM-1
758	*Pseudomonas aeruginosa*	VIM
N/A	*Klebsiella pneumoniae*	VIM
N/A	*Pseudomonas aeruginosa*	VIM
Col1	*Pseudomonas aeruginosa*	VIM-2

Strain ID	Organism	Confirmed Genetic Resistance Marker
BM19	*Serratia marcescens*	VIM-2
KOW7	*Escherichia coli*	VIM-4
DIH	*Klebsiella pneumoniae*	VIM-19
MSH2014-3	*Enterobacter cloacae*	VIM
OXA Isolates		
NCTC 13442	*Klebsiella pneumoniae*	OXA-48
OM11	*Klebsiella pneumoniae*	OXA-48
501	*Enterobacter cloacae*	OXA-48
DUW	*Klebsiella pneumoniae*	OXA-48
OM22	*Escherichia coli*	OXA-48
BOU	*Enterobacter cloacae*	OXA-48
TUR	*Enterobacter cloacae*	OXA-48
11670	*Escherichia coli*	OXA-48
MSH2014-64	*Klebsiella pneumoniae*	OXA-181
MSH2014-72	*Escherichia coli*	OXA-181
166643	*Klebsiella pneumoniae*	OXA-181
42194	*Klebsiella pneumoniae*	OXA-181
74	*Escherichia coli*	OXA-181
IMP Isolates		
NCTC 13476	*Escherichia coli*	IMP-1
695	*Acinetobacter baumannii*	IMP-1
2340	*Enterobacter cloacae*	IMP-1
IMPBMI	*Klebsiella pneumoniae*	IMP-1
6852	*Klebsiella pneumoniae*	IMP-1
Yonsei_1	*Acinetobacter baumannii*	IMP-1
Yonsei_2	*Acinetobacter baumannii*	IMP-1
70450-1	*Pseudomonas aeruginosa*	IMP-1
3994	*Pseudomonas* spp.	IMP-10
MKAM	*Pseudomonas aeruginosa*	IMP-1
5344	*Pseudomonas aeruginosa*	IMP-2
G029	*Salmonella spp*	IMP-4
3985	*Pseudomonas aeruginosa*	IMP-11
4032	*Pseudomonas aeruginosa*	IMP-6
3424	*Pseudomonas aeruginosa*	IMP-7
32443	*Klebsiella pneumoniae*	IMP-13
92	*Pseudomonas aeruginosa*	IMP-14
Isolates with more than one genetic marker target		
GR-04/KP-69	*Klebsiella pneumoniae*	KPC-2/VIM
B108A	*Klebsiella pneumoniae*	NDM/OXA-181
KP-OMA3	*Klebsiella pneumoniae*	NDM/OXA-181
1300920	*Klebsiella pneumoniae*	NDM/OXA-181
MSH2014-69	*Klebsiella pneumoniae*	NDM/OXA-181
C10192-DISCS	*Enterobacter aerogenes*	NDM/OXA-181

All organisms tested exhibited adequate growth on both blood agar and MacConkey agar plates. Xpert Carba-R Assay target genes were detected in 68 of the 71 bacterial strains with the Xpert Carba-R Assay. Target sequences were not detected in three strains (Table 6). One replicate sample for one of the (12) KPC-containing strains reported an indeterminate result (*INVALID*). The sample was successfully repeated and was correctly reported as *"KPC DETECTED."*

Table 6. 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 with Target	Type(s) Detected	Type(s) not Detected	
KPC	12	KPC-2, 3, 4	-----	KPC-5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16
NDM	18	NDM-1, 2, 4, 5	-----	NDM-3, 6, 7, 8, 9
VIM	12	VIM-1, 2, 4, 10, 19	-----	VIM-5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38
OXA-48	18	OXA-48, OXA-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 (Limitation in package insert).*

[b]*IMP-13 gene (Klebsiella pneumoniae): although predicted to be detected by in silico analysis, the IMP-13 gene was not detected by the assay (Limitation in package insert).*

f. Analytical specificity:

The Xpert Carba-R Assay was examined for analytical specificity by testing a panel of 62 well-characterized carbapenem-susceptible bacteria or bacteria with carbapenem non-susceptibility due to genes or mechanisms other than the Xpert Carba-R target genes. Twenty-four commensal/enteric microorganisms were also evaluated in the study. All organisms were grown aerobically on blood agar and MacConkey agar plates. Bacterial suspensions tested were equivalent to a 0.5 McFarland cell suspension. A 100 µl aliquot of each suspension was diluted into 5 ml Sample Reagent to a final concentration of $>1 \times 10^6$ CFU/ml. Each organism was tested a total of four times (from each type of plate). Table 7 and Table 8 list the organisms tested during the Analytical Specificity Study.

Table 7. Analytical Specificity Panel with Organisms Having a Resistance Mechanism other than Targets of the Xpert Carba-R Assay

Organism	Strain ID	Confirmed Resistance Mechanisms[a]	Carbapenem susceptibility (S/I/R)[b]		
			ETP[b]	IMP[b]	MEM[b]
Escherichia coli	NCTC 13441	CTX-M (15)	S	S	S
Klebsiella pneumoniae	NCTC 13465	CTX-M (25)	S	S	S
Enterobacter cloacae	Clinical isolate	OmpC/OmpF deficient	R	R	R
Citrobacter freundii	Clinical isolate	TEM (WT+164S)	S	S	S
Enterobacter cloacae	Clinical isolate	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	Clinical isolate	CTX-M (2); TEM	R	S	S
Enterobacter cloacae	Clinical isolate	CTX-M (2); TEM	R	R	R
Serratia marcescens	Clinical isolate	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.	Clinical isolate	CTX-M (2); TEM	S	S	S
Shigella flexnerii	Clinical isolate	CTX-M (2); TEM	S	S	S
Enterobacter cloacae	PA_3	AmpC; CTX-M-15; TEM	S	S	S
Klebsiella pneumoniae	Clinical isolate	SHV	S	S	S
Klebsiella pneumoniae	Clinical isolate	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	Clinical isolate	SHV-27	S	S	S
Klebsiella pneumoniae	Clinical isolate	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	Clinical isolate	SHV (WT+238S+240K)	R	S	R

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

Organism	Strain ID	Confirmed Resistance Mechanisms[a]	Carbapenem susceptibility (S/I/R)[b]		
			ETP[b]	IMP[b]	MEM[b]
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] *Presence of these markers of resistance were determined by individual PCR assays, DNA sequence analysis, or by other research-based methods.*

[b] *S/I/R = Susceptible/Intermediate/Resistant; ETP = Ertapenem, IMP = Imipenem, MEM = Meropenem*

Table 8. Panel of Commensal and Other Enteric Microorganisms Tested in this Study

Organism	Strain ID
Escherichia coli	ATCC 25922
Enterococcus faecalis	ATCC 29212
Klebsiella pneumoniae	ATCC 700603
Escherichia coli	ATCC 35218
Staphylococcus aureus	ATCC 25923
Pseudomonas aeruginosa	ATCC 27853
Enterobacter cloacae	ATCC 700621
Enterococcus faecium	ATCC 9756
Klebsiella oxytoca	ATCC 13182
Acinetobacter baumannii	ATCC BAA-747
Citrobacter freundii	ATCC 33128
Morganella morganii	ATCC 49948
Stenotrophomonas maltophilia	ATCC 51331
Citrobacter koseri	ATCC 27028
Providencia stuartii	ATCC 49809
Streptococcus agalactiae	CCUG 29780 / ATCC 12401
Enterobacter aerogenes	ATCC 51697
Proteus mirabilis	ATCC 43071
Acinetobacter spp.	CCUG 34787
Citrobacter freundii	CCUG 418
Corynebacterium diphtheriae	CCUG 33629
Helicobacter pylori	CCUG 17874

Organism	Strain ID
Listeria monocytogenes	CCUG 33548
Providencia alcalifaciens	CCUG 6325

Of the 86 potentially cross-reactive organisms tested, including organisms exhibiting antibiotic resistance mechanisms other than production of KPC, NDM, VIM, IMP and OXA-48, none were detected with the Xpert Carba-R Assay. Four runs were indeterminate [(1) *NO RESULT* and (3) *INVALID*)]. All four indeterminate runs were successfully repeated and were reported as *NOT DETECTED* for all five targets. All data were collected on the GeneXpert Dx (GX-IV) instrument using GeneXpert Dx software version 4.4a.

For each day of the study, an external negative control and two types of experimental positive controls were tested as described in the Analytical Reactivity Study above.

g. Interfering substances:

Not Applicable

h. Carry-over:

The purpose of the Carry-over Study was to determine the carry-over rate of contamination in negative samples due to the nucleic acid extraction and amplification of high positive samples in the GeneXpert cartridge. In this study, a negative sample was tested in a GeneXpert module on a GeneXpert Dx GX-IV instrument immediately following the testing of a high titer positive sample (1×10^6 CFU/ml) in the same GeneXpert module. Negative samples consisted of inactivated *E. coli* cells without target sequences diluted in Sample Reagent to 1×10^5 CFU/ml. The positive sample was composed of inactivated *E. coli* cells (with plasmid containing all five Xpert Carba-R target analyte genes—KPC, NDM, VIM, IMP and OXA-48 targets) diluted in Sample Reagent. After an initial negative sample test, sample testing proceeded by alternating negative and high positive sample runs a total of 50 times for two GeneXpert modules. For each day of the study, an external negative control and two types of experimental positive controls were tested as described in the Analytical Reactivity Study. There were 102 total runs where all 50 high positive samples were correctly reported as *DETECTED* for all five Xpert Carba-R Assay targets. All 52 negative samples reported *NOT DETECTED* results for all five Xpert Carba-R Assay targets as expected. There were no errors or invalid results reported. Study results indicated no evidence of sample or amplicon carry-over contamination in the GeneXpert Dx GX-IV modules.

i. Assay cut-off:

Optimized lot specific parameters (LSP) and assay settings for the Xpert Carba-R Assay were determined using pre-clinical data. The pre-clinical study was conducted using patient rectal swabs to determine the performance of the Xpert Carba-R Assay

relative to reference DNA sequence analysis of organisms growing on agar media that were non-susceptible to at least one carbapenem. Contrived samples, prepared by seeding carbapenemase-producing bacteria into stool matrix, were also tested with the Xpert Carba-R Assay. Pre-clinical study sensitivity and specificity values for different Ct cut-offs were evaluated for both prospective and contrived samples.

For IMP, VIM, NDM, KPC, and OXA-48 gene targets, the valid cycle threshold (Ct) range was 3.0 to 38.0. For the SPC, the valid Ct range was set from 3.0 to 40.0. A Ct value outside the valid range is reported as *NOT DETECTED.* The Ct cut-offs are included as automatic calculations in the assay definition file (ADF) provided with the Xpert Carba-R Assay. The lot specific parameters and assay settings described were confirmed in the clinical study, and clinical study results indicated that the cut-off values selected for detection of the five carbapenemase gene targets yielded acceptable performance.

2. Comparison studies:

   a. Method comparison with predicate device:

   Not applicable

   b. Matrix comparison:

   Not applicable

3. Clinical studies:

   a. Clinical Sensitivity:

   In a multi-center study, performance of the Xpert Carba-R Assay was evaluated with bacterial isolates grown from both blood agar and MacConkey agar relative to reference DNA amplification of the target DNA followed by bi-directional sequencing. Testing was performed from growth of the isolate on blood agar. To include isolates in the study, organism identification must have been determined prior to testing with the Xpert Carba-R Assay in order to confirm that isolates belonged to the *Enterobacteriaceae* family or were identified as *Pseudomonas aeruginosa* or *Acinetobacter baumannii*. Organism susceptibility status (susceptible, intermediate or resistant) to meropenem, ertapenem and/or imipenem was determined using CLSI standard test methods (M07-A9) and the interpretive criteria found in the FDA drug label and CLSI M100-S24.

   For reference DNA amplification followed by sequencing, DNA from culture isolates was purified, quantified, and amplified using primers specific to all 5 target that amplified larger regions than the assay targets. Amplicons of the appropriate size were confirmed on the Agilent 2100 Bioanalyzer and sent for reference bi-directional sequencing analysis.

For Xpert Carba-R Assay testing, well-isolated colonies that grew on each of the agar types were diluted to a 0.5 McFarland standard equivalent suspension using the direct colony suspension method. If discordant results between the Xpert Carba-R Assay and reference (DNA amplification and sequencing) were observed, discrepant testing was performed using bi-directional sequencing on isolates from MacConkey agar plates. All sites performing Xpert Carba-R Assay testing used GeneXpert Dx instruments with GeneXpert Dx System software version 4.3.

Calculations of sensitivity and specificity took into account the ability of the Xpert Carba-R Assay to report the presence or absence of target genes in the pool of test isolates. Because carbapenem non-susceptibilty can be due to mechanisms other than the presence of Xpert Carba-R target DNA sequences, results from all non-susceptible isolates may not coincide with the presence of target genes by the Xpert Carba-R Assay and may be included in the specificity calculation. In general, *Enterobacteriaceae* isolates included in sensitivity calculations were determined to be either intermediate or resistant to meropenem, ertapenem and/or imipenem per FDA drug label and CLSI M100-S24. Isolates of *Pseudomonas aeruginosa* or *Acinetobacter baumannii* must have been determined to be intermediate or resistant to either imipenem or meropenem (these organisms are known to be resistant to ertapenem). In specificity calculations, *Enterobacteriaceae* isolates may have been determined to be susceptible or intermediate/resistant to meropenem, ertapenem, and imipenem per FDA drug label and CLSI M100-S24. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates should have been susceptible to both imipenem and meropenem.

A total of 489 bacterial isolates (431 clinical stock isolates and 58 fresh isolates) were enrolled in the clinical study across four clinical sites. Four isolates that had been previously enrolled were excluded from the study, and two isolates were excluded because reference method testing was not performed. An additional sixteen samples were excluded because organisms were not identified as *Enterobacteriaceae, Pseudomonas aeruginosa, or Acinetobacter baumannii*. Thus, 467 compliant samples remained in the final analysis.

Isolates grown on both blood agar and MacConkey agar were evaluated with the Xpert Carba-R Assay for a total of 934 runs (467 each from blood agar and McConkey agar). Performance of the Xpert Carba-R Assay was assessed separately for each type of agar, resistance marker target, and overall (all 5 targets combined) relative to reference sequencing for each of the two agar types used to grow isolates. The study showed that 99.9% (933/934) of isolates yielded results on the first run. One isolate resulted in a *NO RESULT* outcome upon initial testing, but yielded a result upon repeat testing. The final invalid rate was reported as 0%.

When tested with isolates from blood agar, the Xpert Carba-R Assay demonstrated an overall sensitivity and specificity of 100% (95% CI: 99.0-100) and 98.1% (95% CI: 93.2-99.5), respectively, relative to the reference method performed from isolates grown on blood agar plates. Similar results were reported for isolates grown on

MacConkey agar, where the Xpert Carba-R Assay demonstrated an overall sensitivity and specificity of 100% (95% CI: 99.0-100) and 97.1% (95% CI: 91.8-99.0), respectively. The combined performance was evaluated based on defining a result as positive for the Xpert Carba-R Assay if any of the targets were positive and negative for the assay if all the targets were negative.

Combined performance, performance by target, and performance by organism group for the Xpert Carba-R Assay with isolates grown on blood agar and MacConkey agar are shown in Table 9 (Combined Performance), Table 10 (Individual Targets), and Table 11 (Organism Group) below.

Table 9. Xpert Carba-R Assay (Blood agar and MacConkey agar) vs Reference Method—Combined

Plate	Total Isolates	TP	FP	TN	FN	Sensitivity (95% CI)	Specificity (95% CI)
Blood agar	467	364	2	101	0	100% (99.0-100)	98.1% (93.2-99.5)
MacConkey agar	467	364	3	100	0	100% (99.0-100)	97.1% (91.8-99.0)

Table 10. Xpert Carba-R Assay (Blood agar and MacConkey agar) vs Reference Method—by Target

Plate	Target	Total	TP	FP	TN	FN	Sensitivity (95% CI)	Specificity (95% CI)
Blood agar	IMP	467	40	1[a]	426	0	100% (91.2-100)	99.8% (98.7-100)
	VIM	467	82	1[b]	384	0	100% (95.5-100)	99.7% (98.5-100)
	NDM	467	78	0	389	0	100% (95.3-100)	100% (99.0-100)
	KPC	467	84	1[c]	382	0	100% (95.6-100)	99.7% (98.5-100)
	OXA-48	467	89	0	378	0	100% (95.9-100)	100% (99.0-100)
MacConkey agar	IMP	467	40	1[a]	426	0	100% (91.2-100)	99.8% (98.7-100)
	VIM	467	82	1[b]	384	0	100% (95.5-100)	99.7% (98.5-100)
	NDM	467	78	1[d]	388	0	100% (95.3-100)	99.7% (98.6-100)
	KPC	467	84	0	383	0	100% (95.6-100)	100% (99.0-100)
	OXA-48	467	89	0	378	0	100% (95.9-100)	100% (99.0-100)

[a] *bi-directional DNA sequencing result for this false positive IMP isolate exhibited 92.95% sequence homology which was slightly below the 95% cut-off criteria. Discrepant testing was not performed.*

[b] *Discrepant testing results: 1 of 1 was VIM positive.*

[c] *This false positive sample was investigated and may be due to KPC cross-contamination at the level of sample preparation. Discrepant testing did not produce a sequence match with the KPC target. Discrepant testing produced a sequence match for the VIM target. This isolate was classified as a TP in the overall assessment presented in Table 9 above.*

[d] *The clinical site reported that in-house characterization of this false positive isolate prior to study testing resulted in a positive NDM gene target. Discrepant testing did not produce a sequence match for any of the 5 gene targets.*

Table 11. Xpert Carba-R Assay Performance (Blood agar and MacConkey agar) vs Reference Method—by Organism Group

Plate	Organism Group	Target	N	TP	FP	TN	FN	Sensitivity (95 CI)	Specificity (95 CI)
Blood agar	*Enterobacteriaceae*	IMP	343	4	0	339	0	100% (51.0-100)	100% (98.9-100)
		VIM	343	51	1	291	0	100% (93.0-100)	99.7% (98.1-99.9)
		NDM	343	73	0	270	0	100% (95.0-100)	100% (98.6-100)
		KPC	343	83	1	259	0	100% (95.6-100)	99.6% (97.9-99.9)
		OXA-48	343	89	0	254	0	100% (95.9-100)	100% (98.5-100)
		Overall	343	291[a]	1[a]	51	0	100% (98.7-100)	98.1% (89.9-99.7)
	Pseudomonas aeruginosa	IMP	80	16	1	63	0	100% (80.6-100)	98.4% (91.7-99.7)
		VIM	80	31	0	49	0	100% (89.0-100)	100% (92.7-100)
		NDM	80	0	0	80	0	NA	100% (95.4-100)
		KPC	80	1	0	79	0	100% (20.7-100)	100% (95.4-100)
		OXA-48	80	0	0	80	0	NA	100% (95.4-100)
		Overall	80	48	1	31	0	100% (92.6-100)	96.9% (84.3-99.5)
	Acinetobacter baumannii	IMP	44	20	0	24	0	100% (83.9-100)	100% (86.2-100)
		VIM	44	0	0	44	0	NA	100% (92.0-100)
		NDM	44	5	0	39	0	100% (56.6-100)	100% (91.0-100)
		KPC	44	0	0	44	0	NA	100% (92.0-100)
		OXA-48	44	0	0	44	0	NA	100% (92.0-100)
		Overall	44	25	0	19	0	100% (86.7-100)	100% (83.2-100)
MacConkey agar	*Enterobacteriaceae*	IMP	343	4	0	339	0	100% (51.0-100)	100% (98.9-100)
		VIM	343	51	1	291	0	100% (93.0-100)	99.7% (98.1-99.9)
		NDM	343	73	1	269	0	100% (95.0-100)	99.6% (97.9-99.9)
		KPC	343	83	0	260	0	100% (95.6-100)	100% (98.5-100)
		OXA-48	343	89	0	254	0	100% (95.9-100)	100% (98.5-100)
		Overall	343	291[a]	2	50	0	100% (98.7-100)	96.2% (87.0-98.9)
	Pseudomonas aeruginosa	IMP	80	16	1	63	0	100% (80.6-100)	98.4% (91.7-99.7)

Plate	Organism Group	Target	N	TP	FP	TN	FN	Sensitivity (95 CI)	Specificity (95 CI)
		VIM	80	31	0	49	0	100% (89.0-100)	100% (92.7-100)
		NDM	80	0	0	80	0	NA	100% (95.4-100)
		KPC	80	1	0	79	0	100% (20.7-100)	100% (95.4-100)
		OXA-48	80	0	0	80	0	NA	100% (95.4-100)
		Overall	80	48	1	31	0	100% (92.6-100)	96.9% (84.3-99.5)
	Acinetobacter baumannii	IMP	44	20	0	24	0	100% (83.9-100)	100% (86.2-100)
		VIM	44	0	0	44	0	NA	100% (92.0-100)
		NDM	44	5	0	39	0	100% (56.6-100)	100% (91.0-100)
		KPC	44	0	0	44	0	NA	100% (92.0-100)
		OXA-48	44	0	0	44	0	NA	100% (92.0-100)
		Overall	44	25	0	19	0	100% (86.7-100)	100% (83.2-100)

[a]*Multiple target results were observed for some isolates.*

Ten isolates were identified where at least two Xpert Carba-R Assay targets were detected. These results are shown in Table 12 below.

Table 12. Isolates with Multiple Targets Detected

# of Samples with Multiple Targets	Agar Type[a]	Targets Detected by Xpert Carba-R Assay	Targets Detected by Reference Sequencing
9	BA, MC	NDM, OXA-48	NDM, OXA-48
1	BA	VIM, KPC	VIM

[a]*BA=Blood Agar, MC=MacConkey Agar*

External controls for the Xpert Carba-R Assay consisted of one negative sample, one sample positive for all (5) targets of the assay, and five different positive controls each containing a single target of the assay. The negative control and five-target positive controls were run on each day that study samples were tested, along with two of the single-target positive controls (on a rotating basis). Study samples were not run until correct results were obtained for each of the four controls. External control data was compiled across all sites and overall QC results were acceptable.

*b. Clinical specificity**:***

See comments in 3a above.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

Of the total 451 isolates grown on blood agar plates and reported to be carbapenem non-susceptible based on conventional phenotypic (AST) results, 356 isolates were determined to have one or more of the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene targets by the Xpert Carba-R Assay. Results from isolate growth on MacConkey agar plates showed similar results with 357 *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and/or *bla*$_{IMP}$ gene targets detected in 451 carbapenem non-susceptible isolates. Table 13 and Table14 show the results of the Xpert Carba-R Assay compared to susceptibility test results for the 467 isolates included in the clinical study.

Table 13. Xpert Carba-R Assay (Blood agar) vs Susceptibility Test Report

Xpert Carba-R Assay	Phenotype[a]		
	NS (non-susceptible)	**S (susceptible)**	**Total**
Gene Detected	356	10	366
Gene Not Detected	95	6	101
Total	451	16	467

Table 14. Xpert Carba-R Assay (MacConkey agar) vs Susceptibility Test Report

Xpert Carba-R Assay	Phenotype[a]		
	NS (non-susceptible)	**S (susceptible)**	**Total**
Gene Detected	357	10	367
Gene Not Detected	94	6	100
Total	451	16	467

[a] *A non-susceptible phenotype means the isolate was intermediate or resistant to at least one carbapenem. A susceptible phenotype means the isolate was susceptible to imipenem, meropenem, and ertapenem.*

N. Instrument Name:

GeneXpert Instrument Systems

O. System Descriptions:

1. Modes of Operation:

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes ___X_____ or No _____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _____ or No ____X____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X____ or No _____

3. Specimen Identification:

   Similar to previously cleared system.

4. Specimen Sampling and Handling:

   A bacterial suspension equivalent to a 0.5 McFarland suspension is prepared and a 10 µl loop of suspension is transferred to 5 ml of Xpert Carba-R Sample Reagent. An aliquot of sample (1.7 ml) is then transferred to the sample chamber of the disposable, single-use fluidic cartridge (Xpert Carba-R cartridge). The user initiates a test from the system user interface and places the cartridge into the GeneXpert Instrument System. Additional sample preparation, amplification, and real-time detection are all fully-automated and completed by the instrument system.

5. Calibration:

   No calibration is required by the user.

6. Quality Control:

   Quality control is addressed for each separately cleared assay to be run on the instrument. See section M1(c) for information on internal and external controls.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.