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

A. 510(k) Number:

K160901

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 sequences from rectal swab specimens.

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:

   POC—System, nucleic acid amplification test, DNA, antimicrobial resistance marker, direct specimen

   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 designed for the detection and differentiation of the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene sequences associated with carbapenem-non-susceptibility. The test utilizes automated real-time polymerase chain reaction (PCR).

   The Xpert Carba-R Assay is intended as an aid to infection control in the detection of carbapenem-non-susceptible bacteria that colonize patients in healthcare settings. The Xpert Carba-R Assay is not intended to guide or monitor treatment for carbapenem-non-susceptible bacterial infections. A negative Xpert Carba-R Assay result does not preclude the presence of other resistance mechanisms.

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

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

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

2. Indication(s) for use:

   Same as the Intended Use.

3. Special conditions for use statement(s):

For prescription use only.

The Xpert Carba-R Assay detects *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene sequences from rectal swab specimens and is not for bacterial identification or to report susceptibility status.

The detection of assay targets by the Xpert Carba-R Assay does not indicate the presence of viable organisms containing the resistance marker.

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.

Detection of *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and/or *bla*$_{IMP}$ gene sequences does not indicate the presence of viable organisms with these markers in the specimen.

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 the qualitative detection of the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene sequences from rectal swab specimens. The Xpert Carba-R Assay is intended as an aid for infection control 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 that allow 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 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 Carba-R Assay

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

   K152614

3. Comparison with predicate:

Similarities		
Item	Device	Predicate Device
	Cepheid Xpert Carba-R Assay (K160901)	Cepheid Xpert Carba-R Assay (K152614)
Intended Use	The Xpert® Carba-R Assay, performed on the GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test designed for the detection and differentiation of the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene sequences associated with carbapenem-non-susceptibility. The test utilizes automated real-time polymerase chain reaction (PCR). The Xpert Carba-R Assay is intended as an aid to infection control in the detection of carbapenem-non-susceptible bacteria that colonize patients in healthcare settings. The Xpert Carba-R Assay is not intended to guide or monitor treatment for carbapenem-non-susceptible bacterial infections. A negative Xpert Carba-R Assay result does not preclude the presence of other resistance mechanisms. The Xpert Carba R-Assay is for use with the following sample types: <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.	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.

Similarities		
Item	Device	Predicate Device
	Cepheid Xpert Carba-R Assay (K160901)	Cepheid Xpert Carba-R Assay (K152614)
	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.	
Technological Principles	Same	Fully-automated nucleic acid amplification (DNA); real-time PCR
Test Cartridge	Same	Disposable single-use, multi-chambered fluidic cartridge
Detection Probes	Same	TaqMan® Probes
Controls	Same	Internal sample processing control (SPC); Probe check control (PCC); External controls available
Assay Targets	Same	*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene sequences
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

Differences		
Item	Device	Predicate
Sample Type	Rectal swab specimens	Bacterial isolates from culture
Organism(s)	N/A	Carbapenem non-susceptible *Enterobacteriaceae, Pseudomonas aeruginosa, Acinetobacter baumannii*

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 rectal swab specimens. Each swab is 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 the 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 can 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. An 11-member panel was tested that included: 1) two different organisms for each of the five resistance genes detected by the Xpert Carba-R Assay and 2) one negative sample for all five gene targets. Each organism was spiked into a pooled negative rectal matrix at low positive (~1x LoD) and moderate positive levels (2-3x LoD). To measure site-to-site reproducibility, the 11-member panel was tested in replicates of four each day at three (3) sites over a six day testing period with two operators per site. Three lots of Xpert Carba-R Assay cartridges were used at each testing site. A total of 1584 samples suspended in rectal matrix were evaluated, where 144 replicates for each of the 11 different panel members were tested. In rectal matrix, 99.4% (1574/1584) of samples were successful and produced the expected result on the first attempt. Seven (7) *ERROR* cases, one (1) *INVALID* result, and two (2) *NO RESULT* outcomes were reported. All ten samples yielded valid results upon repeat testing. The results of the reproducibility study are summarized in Table 2 below.

Table 2. Reproducibility of the 11-Member Sample Panel

Resistance Gene	Site 1[a]			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	91.7% (22/24)	87.5% (21/24)	89.5% (43/48)	83.3% (20/24)	87.5% (21/24)	85.4% (41/48)	87.5% (21/24)	79.2% (19/24)	83.3% (40/48)	86.1% (124/144)
VIM Mod Pos	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)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
NDM Mod Pos	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	91.7% (22/24)	95.8% (23/24)	93.8% (45/48)	95.8% (23/24)	95.8% (23/24)	95.8% (46/48)	100% (24/24)	91.7% (22/24)	95.8% (46/48)	95.1% (137/144)
KPC Mod Pos	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	95.8% (23/24)	100% (24/24)	97.9% (47/48)	100% (24/24)	91.7% (22/24)	95.8% (46/48)	95.8% (23/24)	95.8% (23/24)	95.8% (46/48)	96.5% (139/144)
OXA-48 Mod Pos	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	95.8% (23/24)	100% (24/24)	97.9% (47/48)	95.8% (23/24)	100% (24/24)	97.9% (47/48)	91.7% (22/24)	100% (24/24)	95.8% (46/48)	97.2% (140/144)

[a]*Each site tested one GeneXpert Instrument System—GeneXpert Dx, Infinity-80, or Infinity-48.*

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]	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	32.9	0.2	0.5	0.2	0.7	0.0	0.1	0.0	0	0.6	1.8	0.7	2.0
IMP Mod Pos	IMP	144	34.5	0.0	0.0	0.2	0.5	0	0.0	0.1	0.2	0.7	2.0	0.7	2.1
IMP Low Pos	IMP	140	36.4	0.0	0.0	0.0	0.0	0.2	0.5	0.0	0	1.2	3.3	1.2	3.4
VIM Mod Pos	VIM	144	31.0	0.0	0.0	0.3	0.9	0	0.0	0.2	0.5	0.5	1.6	0.6	1.9
VIM Low Pos	VIM	144	33.8	0.0	0.0	0.6	1.8	0.3	0.9	0.3	1.0	1.4	4.0	1.6	4.6
NDM Mod Pos	NDM	144	33.7	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.6	1.7	0.6	1.7
NDM Low Pos	NDM	143	36.2	0.2	0.7	0.0	0.0	0.3	0.7	0.0	0.0	0.8	2.3	0.9	2.5
KPC Mod Pos	KPC	144	34.2	0.0	0.0	0.3	0.8	0.2	0.6	0.0	0.0	0.4	1.2	0.6	1.6
KPC Low Pos	KPC	141	35.8	0.0	0.0	0.5	1.5	0.0	0.0	0.3	0.9	0.7	1.9	0.9	2.6
OXA-48 Mod Pos	OXA-48	144	34.3	0.0	0.0	0.2	0.5	0.2	0.5	0.1	0.3	0.5	1.6	0.6	1.7
OXA-48 Low Pos	OXA-48	143	36.1	0.0	0.0	0.0	0.0	0.2	0.6	0.0	0.0	0.8	2.3	0.9	2.4

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

At ~1x LoD, the expected target was not detected in some samples (See Table 2 above). The lowest % total agreement, 86.1% (124/144), was observed with the low positive IMP sample. All moderate positive samples (2-3x LoD) gave the expected result in rectal matrix. All negative samples were correctly identified as negative (144/144). A total of 76 control samples were run with only 1 *INVALID* reported; all remaining positive and negative controls gave the expected results. Agreement between sites, operators, and lots was evaluated using Fisher's Exact test. The data presented in Table 2 and Table 3 demonstrated an acceptable 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. For each day of the study, an external negative control and two types of experimental positive controls were tested. One external positive control consisted of *E. coli* cells containing a plasmid with an insert carrying amplicon sequences from all five Xpert Carba-R target analyte genes (Multivalent External Positive Control). The second external positive control consisted of individual carbapenemase-producing bacteria, each harboring only one of the Xpert Carba-R target carbapenemase genes. On each testing day, one negative control, the five-gene construct positive control, and two of five individual bacterial controls were tested. Of the 970 external control samples run, 99.2% (962/970) gave a valid result on the first attempt. Re-testing of the eight indeterminate controls gave the expected results.

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 *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.

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

A number of studies were conducted to establish the specimen stability of rectal swabs with the Xpert Carba-R Assay. These studies were performed to 1) establish the specimen stability for rectal swabs after specimen collection but prior to transfer to the Xpert Carba-R Sample Reagent and 2) establish the specimen stability of rectal swabs eluted into Xpert Carba-R Sample Reagent.

Stability of Rectal Swabs
In order to support a claim for the period of time that rectal swabs could be held before transfer to Xpert Carba-R Sample Reagent and further processing, a mixture of five carbapenemase-producing bacteria (at approximately 3x LoD) was spiked onto negative matrix swabs to create known positive samples. Both positive and negative matrix swab samples were tested with the Xpert Carba-R Assay after being stored for 1, 3, 5, and 7 days at 2°C, 8°C, 15°C and 28°C. Four (4) replicate negative matrix swab samples and 14 replicate positive matrix swab samples were tested per time point and temperature condition. At the time of testing, swabs were then added to Xpert Carba-R Sample Reagent. Eight replicate positive and negative matrix swab samples were tested at time t=0.

Under the conditions of this study, positive and negative specimens at all storage conditions and temperatures tested were correctly identified using the Xpert Carba-R Assay. The data supports that rectal swabs are stable from the time of collection prior to transfer to Xpert Carba-R Sample Reagent for up to 7 days when stored at 2°C –28°C before testing with the Xpert Carba-R Assay.

Stability after Rectal Swabs Resuspended in Xpert Carba-R Sample Reagent
To establish a claim for the stability of rectal swabs in the Xpert Carba-R Sample Reagent, pooled negative rectal swab matrix was spiked at approximately 3x LoD with a mixture of five carbapenemase-producing bacteria that harbored targets of the Xpert Carba-R Assay. Aliquots of the positive swab matrix (with bacteria) in Sample Reagent were prepared to create positive samples. Negative samples in the study consisted of aliquots of negative pooled swab matrix in Xpert Carba-R Sample Reagent. Positive and negative matrix samples were tested with the Xpert Carba-R Assay after being stored for 1, 3, 5 and 7 days at 2°C, 8°C, 15°C, and 28°C. Four (4) replicate negative matrix samples and 14 replicate positive matrix samples were tested per time point and temperature condition. Positive and negative matrix swab replicates were also tested at time t=0.

Under the conditions of this study, positive and negative samples at all storage conditions and temperatures tested were correctly reported using the Xpert Carba-R Assay. The data supports that sample material collected from rectal swabs are stable in Xpert Carba-R Sample Reagent for up to 7 days when stored at 2°C–28°C prior to testing with the Xpert Carba-R Assay.

The study supports the following product claim specimen storage conditions prior to testing with the Xpert Carba-R Assay: 1) up to 5 days at 15°C-28°C for rectal swab specimens in transport tubes before transfer to Xpert Carba-R Sample Reagent and 2) up to 5 days at 2°C-28°C for rectal swab specimens suspended in Xpert Carba-R Sample Reagent.

d. Detection limit:

A study was conducted to determine the Limit of Detection (LoD) of the Xpert Carba-R Assay for carbapenemase-producing organisms harboring the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ gene sequence targets seeded into rectal swab

background matrix. The LoD was defined as the lowest concentration (reported as cells/swab and cells/ml in Sample Reagent) of sample that can be reproducibly distinguished from negative samples with 95% confidence. Pre-screened negative rectal swab background matrix was used to confirm that the Limit of Blank (LoB) was zero. Two strains harboring each assay target were tested. Point estimates and two-sided 95% confidence intervals for the analytical LoD (reported as CFU/swab) were determined using probit regression analysis. The LoD estimate was verified by preparing two independent dilutions of each bacterial culture to the point estimate LoD value. Twenty measurements (10 from each dilution) were tested, and the study was completed using at least two unique lots of Xpert Carba-R Assay reagents.

Of the 2,774 runs in the LoD study to estimate the LoD of various targets in rectal matrix (excluding the external controls), 32 runs (1.2%) provided indeterminate GeneXpert results (2 *NO RESULT*, 9 *ERROR*, 21 *INVALID*). All 32 runs were repeated yielding valid Carba-R results as expected. A total of 195 of 198 external control runs provided valid GeneXpert results.

All the data was collected with the GeneXpert Dx software version 4.4a on the GeneXpert Dx GX-IV and GX-XVI instruments and with the Infinity Xpertise software version 6.1 on the Infinity-80 instrument. Table 4 shows the estimated LoD results by probit analysis for the test panel.

Table 4. LoD for Organisms in Rectal Matrix Harboring Carbapenemase Genes using the Xpert Carba-R Assay in 5 ml Sample Reagent

Organism	**Target Gene**	**Rectal Matrix**	
		LOD Claim CFU/swab[a]	**Estimated LoD In Sample Reagent CFU/ml[b]**
Acinetobacter baumannii	IMP-1	174	35
Klebsiella pneumoniae	IMP-1	306	61
Klebsiella pneumoniae	VIM-1	305	61
Escherichia coli	VIM-4	815	163
Klebsiella pneumoniae	NDM-1	251	50
Klebsiella pneumoniae	NDM	74	15
Klebsiella pneumoniae	KPC-3	373	75
Enterobacter cloacae	KPC	779	156
Enterobacter cloacae	OXA-48	154	31
Escherichia coli	OXA-48	104	21

a. Colony counts per swab confirmed by plating of each organism.
b. Estimated LoD for swab in 5 ml of Sample Reagent using 0.2 conversion factor: 0.2 x CFU/swab

Analytical Reactivity

An Inclusivity Study was conducted to test reactivity of the Xpert Carba-R Assay with 72 well-characterized bacterial isolates. The panel consisted of the following molecular resistance marker groups: (11) bla_{KPC} isolates, (13) bla_{NDM} isolates, (11) bla_{VIM} isolates, (8) bla_{OXA-48} isolates, (5) $bla_{NDM/OXA-181}$ isolates, (6) $bla_{OXA-181}$, (17) bla_{IMP} isolates, and (1) $bla_{KPC/VIM}$ isolate. Strains were seeded at 3x LoD and tested in pooled negative rectal swab matrix. 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	Concentration Tested (CFU/ml)	Rectal Matrix (Mean Ct)
KPC Isolates				
NCTC 13438	*Klebsiella pneumoniae*	KPC-3	153	34.4
31551	*Klebsiella pneumoniae*	KPC-4	50	34.5
ATCC BAA-1705	*Klebsiella pneumoniae*	KPC-2	130	35.9
CFVL	*Enterobacter cloacae*	KPC-2	160	34.4
KBM18	*Enterobacter aerogenes*	KPC-2	250	33.7
COL	*Escherichia coli*	KPC-2	147	33.1
BM9	*Klebsiella pneumoniae*	KPC-3	330	34.6
CGNC	*Serratia marcescens*	KPC-2	300	33.9
PA3	*Klebsiella pneumoniae*	KPC-2	100	34.1
PA-COL	*Pseudomonas aeruginosa*	KPC-2	250	34.4
164-3	*Klebsiella oxytoca*	KPC	70	33.7
NDM Isolates				
NCTC 13443	*Klebsiella pneumoniae*	NDM-1	80	33.5
ATCC BAA-2146	*Klebsiella pneumoniae*	NDM-1	80	33.3
34262	*Klebsiella pneumoniae*	NDM	80	33.6
GEN	*Acinetobacter baumannii*	NDM-1	130	33.4
3047	*Enterobacter cloacae*	NDM-1	70	36.2
7892	*Proteus mirabilis*	NDM-1	30	32.6
CAN	*Salmonella spp.*	NDM-1	70	33.7
EGY	*Acinetobacter baumannii*	NDM-2	40	35.0
I5	*Escherichia coli*	NDM-4	30	33.4
405	*Escherichia coli*	NDM-5	30	33.7
CF-ABE	*Citrobacter freundii*	NDM	30	33.6
73999	*Pseudomonas aeruginosa*	NDM	50	33.1
39365	*Providencia rettgeri*	NDM-1	70	33.3
VIM Isolates				
NCTC 13437	*Pseudomonas aeruginosa*	VIM-10	500	31.9

Strain ID	Organism	Confirmed Genetic Resistance Marker	Concentration Tested (CFU/ml)	Rectal Matrix (Mean Ct)
NCTC 13439	*Klebsiella pneumoniae*	VIM-1	130	32.6
NCTC 13440	*Klebsiella pneumoniae*	VIM-1	70	32.9
758	*Pseudomonas aeruginosa*	VIM	250	32.7
PA-87	*Klebsiella pneumoniae*	VIM	200	33.3
B92A	*Pseudomonas aeruginosa*	VIM	2000	31.4
Col1	*Pseudomonas aeruginosa*	VIM-2	500	32.5
BM19	*Serratia marcescens*	VIM-2	250	33.6
KOW7	*Escherichia coli*	VIM-4	250	33.0
DIH	*Klebsiella pneumoniae*	VIM-19	250	33.4
MSH2014-3	*Enterobacter cloacae*	VIM	500	31.8
OXA-48 and OXA-181 Isolates				
NCTC 13442	*Klebsiella pneumoniae*	OXA-48	40	33.2
OM11	*Klebsiella pneumoniae*	OXA-48	60	33.4
501	*Enterobacter cloacae*	OXA-48	80	34.2
DUW	*Klebsiella pneumoniae*	OXA-48	120	33.9
OM22	*Escherichia coli*	OXA-48	80	33.5
BOU	*Enterobacter cloacae*	OXA-48	80	33.6
TUR	*Enterobacter cloacae*	OXA-48	120	33.9
11670	*Escherichia coli*	OXA-48	100	33.3
MSH2014-64	*Klebsiella pneumoniae*	OXA-181	280	32.0
MSH2014-72	*Escherichia coli*	OXA-181	100	34.7
166643	*Klebsiella pneumoniae*	OXA-181	20	34.1
42194	*Klebsiella pneumoniae*	OXA-181	20	33.5
74	*Escherichia coli*	OXA-181	100	33.4
CDC0051	*Klebsiella ozaenae*	OXA-181	250	34.2
IMP Isolates				
NCTC 13476	*Escherichia coli*	IMP-1	250	34.2
695	*Acinetobacter baumannii*	IMP-1	1720	33.5
2340	*Enterobacter cloacae*	IMP-1	250	34.6
IMPBMI	*Klebsiella pneumoniae*	IMP-1	100	32.5
6852	*Klebsiella pneumoniae*	IMP-1	100	33.5
Yonsei_1	*Acinetobacter baumannii*	IMP-1	1000	33.9
Yonsei_2	*Acinetobacter baumannii*	IMP-1	500	34.4
70450-1	*Pseudomonas aeruginosa*	IMP-1	250	33.7
3994	*Pseudomonas* spp.	IMP-10	250	34.5
MKAM	*Pseudomonas aeruginosa*	IMP-1	500	33.4
5344	*Pseudomonas aeruginosa*	IMP-2	60	33.9
CDC0161	*Enterobacter aerogenes*	IMP-4	50,000	36.6
3985	*Pseudomonas aeruginosa*	IMP-11	2000	35.5
4032	*Pseudomonas aeruginosa*	IMP-6	80	32.7
3424	*Pseudomonas aeruginosa*	IMP-7	$1x10^6$	0
32443	*Klebsiella pneumoniae*	IMP-13	$1x10^6$	0

Strain ID	Organism	Confirmed Genetic Resistance Marker	Concentration Tested (CFU/ml)	Rectal Matrix (Mean Ct)
0092	*Pseudomonas aeruginosa*	IMP-14	$1x10^6$	0
Isolates with more than one genetic marker target				
GR-04/KP-69	*Klebsiella pneumoniae*	KPC-2/VIM	80	33.9
B108A	*Klebsiella pneumoniae*	OXA-181/NDM	10	32.6, 35.7
KP-OMA3	*Klebsiella pneumoniae*	OXA-181/NDM	60	32.6, 34.3
1300920	*Klebsiella pneumoniae*	OXA-181/NDM	15	32.8, 36.5
MSH2014-69	*Klebsiella pneumoniae*	OXA-181/NDM	20	33.8,34.2
C10192-DISCS	*Enterobacter aerogenes*	OXA-181/NDM	10	33.6, 35.8

Under the conditions of this study, 69 of 72 carbapenemase-producing bacterial strains were detected with the Xpert Carba-R Assay. Three carbapenemase-producing bacterial strains (IMP-7, IMP-13, and IMP-14) were not detected with the Xpert Carba-R Assay even at 10^6 CFU/ml. For a summary of these results, please refer to Table 6 below.

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	19	OXA-48, OXA-181 *(OXA-48 variant)*	-----	OXA-162, 163, 204, 232, 244, 245, 247
IMP	17	IMP-1 (9 strains), IMP-2, 4[a], 6, 10, 11	IMP-7[b], 13[c], 14[b]	IMP-3, 8, 9, 13[c], 19, 20, 21, 22, 24, 25, 27, 28, 30, 31, 33, 37, 40, 42

[a]IMP-4 gene (Enterobacter aerogenes) was detected at much higher CFU/ml (5 $x10^4$ CFU/ml) than other IMP variants detected by the assay.

[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 (Limitations in package insert).

[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 (Limitation in package insert).

e. Analytical specificity:

The analytical specificity of the Xpert Carba-R Assay was evaluated with 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. Panel members were re-suspended in negative rectal matrix. A set of 31 commensal/enteric microorganisms was also evaluated in the study, as well as human cells. Bacteria were seeded into negative matrix at $\geq 10^6$ CFU/ml in triplicate and tested with the Xpert Carba-R Assay. Viruses were tested at > $1x10^5$ $TCID_{50}$/ml or greater than 2.5×10^7 RNA copies/ml. Human cells were tested at $1x10^5$ cells/ml. For Analytical Specificity Study panel, please refer to Table 7 and Table 8 below.

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 Mechanism(s)[a]	Carbapenem Susceptibility (S/I/R)[b]		
			ETP[b]	IMP[b]	MEM[b]
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 flexneri	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

Organism	Strain ID	Confirmed Resistance Mechanism(s)[a]	Carbapenem Susceptibility (S/I/R)[b]		
			ETP[b]	IMP[b]	MEM[b]
Klebsiella pneumoniae	33560	CTX-M (15); SHV-11; TEM-1	S	S	S
Klebsiella pneumoniae	33603	SHV-2	R	I	R
Klebsiella pneumoniae	33617	SHV-27	S	S	S
Klebsiella pneumoniae	33643	SHV (-5, -55); TEM	S	S	S
Klebsiella pneumoniae	34430	SHV; TEM; CTX-M-15	S	S	S
Klebsiella pneumoniae	34680	TEM; CTX-M-2	R	S	R
Klebsiella pneumoniae	34732	CTX-M (15); SHV; TEM	R	S	S
Enterobacter cloacae	PA_174	GX-/Culture+; SHV; TEM	S	S	S
Enterobacter aerogenes	STU 645	SHV (WT+238S+240K)	R	S	R
Enterobacter aerogenes	STU 669	SHV (WT+238S+240K)	R	R	R
Escherichia coli	C3015	AmpC (CMY II); TEM	R	R	R
Enterobacter aerogenes	RI_100	AmpC (DHA); SHV	R	R	R
Klebsiella pneumoniae	B4A	SHV (WT + 238S +240K)	R	R	R
Klebsiella pneumoniae	B13A	SHV (WT + 238S +240K)	R	S	S
Enterobacter cloacae	RI_474	AmpC (ACT/MIR)	R	I	I
Enterobacter amnigenus	B71	AmpC (ACT/MIR)	R	R	R
Klebsiella pneumoniae	DD82A	SHV (WT + 238S + 240K)	R	S	R
Klebsiella pneumoniae	B100	CTX-M (-1, type-15 like); SHV (WT+238S); TEM	R	S	R
Enterobacter cloacae	135B	TEM	S	S	S
Klebsiella pneumoniae	B157	SHV; TEM	R	R	R
Escherichia coli	T2914280	CTX-M (-1, -15); TEM	R	S	R
Providencia stuartii	DD188	TEM (104K + 164S)	R	I	I
Enterobacter cloacae	DD189	AmpC (ACT/MIR)	R	S	S
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

Organism	Strain ID	Confirmed Resistance Mechanism(s)[a]	Carbapenem Susceptibility (S/I/R)[b]		
			ETP[b]	IMP[b]	MEM[b]
Enterobacter cloacae	S35766	AmpC(ACT/MIR)	S	S	S
Enterobacter cloacae	X1856910	AmpC (ACT/MIR); TEM	R	I	I
Klebsiella pneumoniae	W3758164	CTX-M (-1, -15 like); SHV; TEM.	R	I	R
Klebsiella pneumoniae	X2135758	CTX-M (-1, -15 like); SHV	R	S	S
Klebsiella pneumoniae	W3809535	CTX-M (-1, -15 like); SHV	R	R	R
Pseudomonas aeruginosa	CDC0064	SPM	R	R	R
Serratia marcescens	CDC0099	SME	R	R	R
Serratia marcescens	CDC0121	SME	R	R	R
Serratia marcescens	CDC0122	SME	R	R	R
Serratia marcescens	CDC0123	SME	R	R	R
Serratia marcescens	CDC0124	SME	R	R	R
Serratia marcescens	CDC0130	SME	R	R	R
Serratia marcescens	CDC0131	SME	R	R	R
Enterobacter cloacae group	CDC0132	IMI	R	R	R
Enterobacter cloacae complex	CDC0164	IMI	R	R	R

[a] *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 and Human Cells

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
Clostridium difficile	ATCC 9689
Enterobacter cloacae	ATCC 700621
Enterococcus faecium	ATCC 9756
Klebsiella oxytoca	ATCC 13182
Acinetobacter baumannii	ATCC BAA-747

Organism	Strain ID
Citrobacter freundii	ATCC 33128
Morganella morganii	ATCC 49948
Stenotrophomonas maltophilia	ATCC 51331
Citrobacter koseri	ATCC 27028
Providencia stuartii	ATCC 49809
Peptostreptococcus anaerobius[a]	ATCC 49037
Streptococcus agalactiae	CCUG 29780 / ATCC 12401
Bifidobacterium adolescentis	ATCC 15703
Enterobacter aerogenes	ATCC 51697
Proteus mirabilis	ATCC 43071
Acinetobacter spp.	CCUG 34787
Citrobacter freundii	CCUG 418
Corynebacterium diphtheriae	CCUG 33629
Helicobacter pylori	CCUG 17874
Listeria monocytogenes	CCUG 33548
Providencia alcalifaciens	CCUG 6325
Campylobacter jejuni	CCUG 43594/ATCC 33560
Viruses	
Adenovirus B Type 7A/NY	MRVP/ZeptoMetrix
Enterovirus Type 71/NY	MRVP/ZeptoMetrix
Norovirus GII	Clinical Sample – Cepheid
Human Cells	
Bladder Cell Carcinoma (hgDNA)	ATCC HTB-4

[a]*Peptostreptococcus anaerobius was tested at 5 x 10^5CFU/ml.*

Of the 94 potentially cross-reactive organisms and nucleic acids 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. Of the 286 tests in rectal matrix, four (1.4%) runs were indeterminate (4 *INVALID*). All four indeterminate runs were successfully repeated and were reported as *NOT DETECTED* for all five targets (KPC, NDM, VIM, IMP and OXA-48) as expected. All external positive and negative controls were correctly reported as expected.

All the data were collected on the GeneXpert Dx (GX-IV) instrument using the GeneXpert Dx software version 4.4a.

f. Assay cut-off:

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. Assay cut-off values have not changed from those described in K152614.

g. Interfering substances:

A study was conducted to assess the inhibitory effects of substances potentially encountered in rectal swab specimens on the performance of the Xpert Carba-R Assay. Twenty-four substances were evaluated at *"worst case scenario"* concentrations. Eight replicate positive samples were tested per substance. Negative samples consisted of pooled negative rectal swab matrix with/without the interfering substance (not seeded with carbapenemase-producing organisms). Controls consisted of positive and negative samples with no interfering substances added. Positive samples were prepared from a mix of five carbapenemase-producing organisms harboring KPC, NDM, VIM, IMP and OXA-48 gene sequences seeded into pooled negative rectal swab matrix to give concentrations that were 3x analytical LoD (Table 9). The list of potentially interfering substances tested is shown in Table 10.

Table 9. Organisms used to Prepare the Mixed Positive Sample

Organism	Target Gene
Klebsiella pneumoniae	KPC
Klebsiella pneumoniae	NDM
Escherichia coli	VIM
Enterobacter cloacae	OXA-48
Acinetobacter baumannii	IMP-1

Table 10. Potentially Interfering Substances Tested

Substance ID	Substance/Class	Active Ingredient	Concentration Tested
Aleve (1)	Non-steroidal anti-inflammatory medication	Naproxen	0.25% w/v
Barium sulfate (2)	Imaging compound	N/A	0.25% and 0.1% w/v
Antimicrobial (3)	Antibiotic (oral)	Cephalexin	0.25% w/v
Antimicrobial (4)	Antibiotic (oral)	Ciprofloxacin	0.25% w/v
Condom (5)	Condom with spermicidal lubricant	Nonoxynol-9	1 condom[a]

Cortizone (6)	Creams/ointment/ suppositories	Hydrocortisone	0.25% w/v
ExLax (7)	Laxative	Sennosides	0.25% w/v
Fecal Fat (8)	Lipids	Stearic acid/Palmitic acid/Cholesterol	0.25% w/v
Imodium (9)	Anti-diarrheal medication	Loperamide hydrochloride/bismuth subsalicylate	0.25% w/v
Kaopectate (10)	Anti-diarrheal medication	Loperamide hydrochloride/bismuth subsalicylate	0.25% w/v
K-Y Jelly (11)	Topical cream	N/A	0.25% w/v
Milk of Magnesia (12)	Antacids	Calcium carbonate/aluminum hydroxide/magnesium hydroxide/simethicone	0.25% w/v
Mineral Oil-enema (13)	Enemas	N/A	0.25% w/v
Neosporin (14)	Antibiotic (topical)	Polymixin B/ Neomycin/ Bacitracin	0.25% w/v
Nystatin (15)	Anti-fungal/ anti-itch Vaginal	Nystatin	0.25% w/v
Pepcid (16)	Antacid	Famotidine	0.25% w/v
Pepto-Bismol (17)	Anti-diarrheal medication	Loperamide hydrochloride/bismuth subsalicylate	0.25%, 0.1%, 0.05%, 0.025%, 0.01% w/v
Petroleum jelly (18)	Topical cream	N/A	0.25% w/v
Preparation H (19)	Anti-hemorrhoid creams/ointments	Phenylephrine	0.25% w/v
Prilosec (20)	Acid reducer; antacid	Oemprazole	0.25% w/v
Saline-enema (21)	Enemas	N/A	0.25% w/v
Tagamet (22)	Antacid	Cimetidine	0.25% w/v
Vagisil (23)	Anti-fungal/ anti-itch Vaginal	Benzocaine, resorcinol	0.25% w/v
Wet Ones (24)	Moist Towelettes	Benzalkonium chloride, ethanol	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.*

Results showed that assay targets were detected in the presence of 22 of the 24 potentially interfering substances when tested at 0.25% w/v with the Xpert Carba-R Assay. It was noted that for fecal fat diluted into positive rectal matrix at 0.25% w/v, the VIM target showed a 2.475 Ct delay compared to the mean Ct of the control; however, the VIM target was still detected by the assay. In addition, a change in the Ct value was observed with the rectal swab matrix sample for the OXA target, which showed a 1.237 Ct delay compared to the mean Ct of the control in the presence of barium sulfate at 0.1% w/v. Interference with the Xpert Carba-R Assay may be observed with barium sulfate at > 0.1% w/v, Pepto-Bismol at > 0.01% w/v, and fecal fat at 0.25% w/v (for VIM) in tests with rectal swab matrix samples. Of the 404 tests (not including those test concentrations where barium sulfate and Pepto-Bismol by themselves showed high invalid rates), 2 tests provided indeterminate GeneXpert results (2 *ERROR*). Both indeterminate GeneXpert results were successfully repeated.

External controls gave the expected GeneXpert test result. All the data were collected using the GeneXpert Dx software version 4.4a on the GeneXpert Dx (GX-IV and GX-XVI) instruments.

Competitive Interference

To evaluate the potential competitive inhibitory effect of multiple carbapenemase-producing organisms on the performance of the Xpert Carba-R Assay in rectal swab specimens, a study was performed by testing various combinations of carbapenemase-producing organisms seeded at high and low concentrations into natural matrix. High concentrations of organisms corresponded to ~$1x10^6$ CFU/ml, and low concentrations corresponded to 2x LoD. An inhibitory effect was observed for three out of five targets (IMP, VIM, and OXA-48) when a low concentration of each target was present in combination with a high concentration of another assay target. Table 11 reports the number of target replicates that were detected in the competitive interference study with rectal swab matrix.

Table 11. Number of Correct Results for Combinations in the Competitive Interference Study with the Xpert Carba-R Assay using a Rectal Swab Matrix

Sample number	Combination	Number of DETECTED results/ Number of replicates					Inhibited[a] (Yes or No)
		IMP	VIM	NDM	KPC	OXA	
1	High KPC/High NDM/Low VIM		7/8	8/8	8/8		No
2	High KPC/High NDM/Low OXA			8/8	8/8	8/8	No
3	High KPC/High NDM/Low IMP	5/8		8/8	8/8		Yes/IMP
4	High VIM/High OXA/Low KPC		8/8		8/8	8/8	No
5	High VIM/High OXA/Low NDM		8/8	7/8		8/8	No
6	High VIM/High OXA/Low IMP	6/8	8/8			8/8	Yes/IMP
7	High IMP/Low KPC	8/8			8/8		No
8	High IMP/Low NDM	8/8		8/8			No
9	High IMP/Low VIM	8/8				8/8	No
10	High IMP/Low OXA	8/8				6/8	Yes/OXA

11	High OXA/Low VIM		3/8			8/8	Yes/VIM
12	High VIM/Low OXA		8/8			7/8	No
13	High KPC/Low NDM			8/8	8/8		No
14	Negative	0/8	0/8	0/8	0/8	0/8	N/A

[a]*Target was considered inhibited if 6 or fewer replicates were detected per sample.*

For those targets not detected at 2x LoD in ≥ 7/8 replicates (IMP, VIM, OXA-48), an additional study was performed where the low target concentration was increased to 4x LoD to evaluate the competitive inhibitory effect. No inhibitory effect was observed for the three targets (IMP, VIM and OXA-48) at 4x LoD in the presence of high concentrations of other targets for the Xpert Carba-R Assay (Table 12).

Table 12. Number of Correct Results for Combinations in the Competitive Interference Study with the Xpert Carba-R Assay using a Low Target Concentration of 4x LoD.

Sample Number	Combination	Number of DETECTED results/ Number of replicates					Inhibited (Yes or No)
		IMP	VIM	NDM	KPC	OXA	
3	High KPC/High NDM/Low IMP	8/8		8/8	8/8		No
6	High VIM/High OXA/Low IMP	7/8	8/8			8/8	No
10	High IMP/Low OXA	8/8				8/8	No
11	High OXA/Low VIM		8/8			8/8	No
14	Negative	0/8	0/8	0/8	0/8	0/8	N/A

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 immediately following the testing of a high titer positive sample in the same GeneXpert module. The high positive sample was composed of inactivated *E. coli* cells containing a plasmid with all five Xpert Carba-R target analyte genes (KPC, NDM, VIM, IMP and OXA-48 gene targets) diluted in Sample Reagent with rectal swab matrix to a concentration of 1 x 10^6 CFU/ml. The testing pattern was repeated 25 times on two GeneXpert modules for a total of 102 tests (25 high positive samples and 26 negative samples per module) for the positive cells in rectal swab matrix. For each day of the study, an external negative control and two types of external positive controls were tested as described previously. All 52 negative samples reported *NOT DETECTED* results for all five Xpert Carba-R Assay targets as expected. All positive samples correctly reported all Xpert Carba-R targets as *DETECTED*. There was one invalid result reported, which gave a valid result upon re-testing. Study results indicated no evidence of sample or amplicon carry-over contamination in the GeneXpert modules.

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 characteristics of the Xpert Carba-R Assay were evaluated with rectal swab specimens. The positive percent agreement (PPA) and negative percent agreement (NPA) of the Xpert Carba-R Assay were evaluated relative to a Composite Reference Method (consisting of Reference Culture + PCR and Sequencing of the amplification product). Five geographically diverse sites were selected (three across the United States and two in Europe), and prospectively paired rectal swab specimens were collected from subjects who were hospitalized or in a long-term care facility. In the study, one swab of a pair was used for Xpert Carba-R Assay testing. Highly soiled rectal swabs were excluded from the study. Contrived specimens were included in the study due to the expected low prevalence for some Xpert Carba-R Assay target genes.

For the Reference Culture, the second swab specimen was inoculated into 11 ml MacConkey enrichment broth containing a 10 µg meropenem disk and incubated overnight at 35°C. An aliquot of MacConkey broth culture was spread onto a MacConkey agar plate, and a 10 µg meropenem disk was placed on the plate. After an overnight incubation at 35°C, the zone of clearing was measured. If growth was observed within a 28 mm zone (including the meropenem disk), species identification of the organisms was performed after subculture of colonies to sheep blood agar. Organism susceptibility status (susceptible, intermediate or resistant) to meropenem, ertapenem and imipenem was determined using CLSI standard test methods (M07-A9) and the interpretive criteria found in the FDA drug label and CLSI M100-S24. Carbapenem non-susceptible organisms were then subcultured to sheep blood agar (with a meropenem disk placed between the 1st and 2nd streak) and incubated overnight at 35°C. Three to five well-isolated colonies of the same morphotype, as described in CLSI M07-A9, were collected and sent for sequencing.

DNA from the carbapenem non-susceptible isolates was purified, quantified, and amplified using primers specific to all 5 target genes that were validated and amplify a larger region than the Xpert Carba-R primers. The production of the appropriate sized amplification products was confirmed on Agilent 2100 Bioanalyzer. 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. PCR and sequencing was not performed for specimens

where there was no growth in the 28 mm zone, if the results of antimicrobial susceptibility testing identified a susceptible isolate, or organisms had intrinsic resistance to all the carbapenems tested (e.g. *Stenotrophomonas maltophilia*).

A total of 802 prospective rectal swab specimens were initially enrolled in the clinical study, of which 785 were eligible for inclusion. The ineligible specimens included:

- (14) specimens from subjects with incomplete specimen information
- (1) specimen was too soiled
- (1) specimen was previously enrolled
- (1) specimen from subject not hospitalized or in long-term care facility

An additional 30 specimens were excluded due to:

- (10) shipping delay/testing delay
- (4) invalid GX control or GX indeterminate/invalid
- (16) organisms where there were no zone interpretations listed corresponding to intermediate or resistant to determine non-susceptibility

Thus, 755 rectal swab specimens remained compliant and were included in the final analysis for the prospective study. Performance of the Xpert Carba-R Assay was assessed separately for each type of resistance marker target and compared to Reference Culture + Sequencing result. With the rectal swab specimens, the study showed that 98.9% (747/755) of the specimens produced a valid result on the first run. Four (4) *INVALID* and Four (4) *ERRORS* occurred, which upon re-testing yielded a valid result.

In addition to the prospective study, well-characterized isolates carrying each assay target were tested in a contrived study. Strains were re-suspended in rectal matrix before testing with the Xpert Carba-R Assay at 1x, 3x, and 10x LoD concentrations. The following numbers of unique isolates were evaluated in the study spanning multiple gram negative species (Table 13 and Table 14):

Table 13. Number of Unique Bacterial Strains Tested by Target and Concentration Level

Target	1x LoD	3x LoD[a]	10x LoD[a]
IMP	30	25	25
KPC	30	25	25
OXA-48	29	25	25
VIM	30	26	26
NDM	30	25	25
Negatives[b]	15		

[a]*Strains tested at 3x and 10x LoD were chosen from the unique strains tested at 1x LoD.*
[b]*Thirty negative samples were included in the study(15 x2).*

Table. 14. Various Species Tested in the Contrived Study by Target

Target	Various Organisms included in the Study
VIM	*Acinetobacter baumannii*
	Enterobacter cloacae
	Enterobacter asburiae
	Escherichia coli
	Klebsiella pneumoniae
	Pseudomonas aeruginosa
	Pseudomonas putida
	Serratia marcescens
IMP	*Acinetobacter baumannii*
	Enterobacter cloacae
	Klebsiella pneumoniae
	Pseudomonas aeruginosa
	Pseudomonas stutzeri
NDM	*Acinetobacter baumannii*
	Citrobacter spp.
	Empedobacter brevis
	Enterobacter cloacae
	Escherichia coli
	Klebsiella pneumoniae
	Morganella morganii
	Proteus mirabilis
	Pseudomonas oryzihabitans
	Salmonella spp.
KPC	*Citrobacter koseri*
	Enterobacter cloacae
	Enterobacter aerogenes
	Escherichia coli
	Klebsiella pneumoniae
	Serratia marcescens
OXA-48	*Enterobacter cloacae*
	Enterobacter aerogenes
	Escherichia coli
	Klebsiella pneumoniae
No target	*Acinetobacter baumannii*
	Enterobacter cloacae
	Enterobacter aerogenes
	Klebsiella pneumoniae
	Morganella morganii

Target	Various Organisms included in the Study
	Pseudomonas aeruginosa
	Serratia marcescens
	Shigella flexneri

Discordant samples were tested with an alternate PCR method when the Xpert Carba-R Assay result was positive and the Reference Method result was negative. An aliquot of the MacConkey broth was used to extract DNA and amplify any potential targets by PCR before sending for DNA sequencing. These results were not used to change the original performance data (See results in the footnotes to Table 15).

Study results of the Xpert Carba-R Assay compared to the Reference Method are shown in Table 15 stratified by individual target for prospective and contrived studies.

Table 15. Clinical Performance Data for the Xpert Carba-R Assay vs. Reference Culture + Sequencing

Study	Target	TP	FP	TN	FN	PPA% (95 CI)	NPA% (95 CI)
Prospective (n=755)	IMP	0	1[a]	754	0	N/A	99.9% (99.3-100.0)
	VIM	6	8[b]	737	4	60.0% (31.3-83.2)	98.9% (97.9-99.5)
	NDM	7	3[c]	745	0	100.0% (64.6-100.0)	99.6% (98.8-99.9)
	KPC	29	6[d,e]	720	0	100.0% (88.3-100.0)	99.2% (98.2-99.6)
	OXA-48	29	10[f]	715	1	96.7% (83.3-99.4)	98.6% (97.5-99.2)
Contrived (n=432)	IMP	76	0	352	4[g]	95.0% (87.8-98.0)	100.0% (98.9-100.0)
	VIM	81	0	350	1[h]	98.8% (93.4-99.8)	100.0% (98.9-100.0)
	NDM	80	0	352	0	100.0% (95.4-100.0)	100.0% (98.9-100.0)
	KPC	80	0	352	0	100.0% (95.4-100.0)	100.0% (98.9-100.0)
	OXA-48	79	0	352	1[i]	98.8% (93.3-99.8)	100.0% (98.9-100.0)

[a] *0 of the 1FPs was determined to be TP after discordant analysis.*
[b] *2 of the 8 FPs were determined to be TPs after discordant analysis.*
[c] *1 of the 3 FPs was determined to be TP after discordant analysis.*
[d] *1of the 6 FPs was determined to be TP after discordant analysis.*
[e] *Site reported that subject was on ertapenem during time of specimen collection.*
[f] *3 of the 10 FPs were determined to be TPs after discordant analysis.*
[g] *4FNs were observed with the following organisms: Acinetobacter baumannii [(2) at 1X LoD and (1) at 3X LoD] and Pseudomonas aeruginosa [(1) at 1X LoD]*
[h] *1FN was observed with Enterobacter asburiae.*
[i] *1FN was observed with Klebsiella pneumoniae.*

The Xpert Carba-R Assay performance by specific organism group is shown in Table 16 for the prospective study.

Table 16. Xpert Carba-R Results (by Organism and Target) vs Reference Culture + Sequencing (Prospective Study)

PROSPECTIVE STUDY							
Organism[a, b]	Target	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
A. baumannii (n=13)	IMP	0	0	13	0	NA	100% (77.2-100)
	VIM	0	0	13	0	NA	100% (77.2-100)
	NDM	0	0	13	0	NA	100% (77.2-100)
	KPC	0	0	13	0	NA	100% (77.2-100)
	OXA-48	0	0	13	0	NA	100% (77.2-100)
E. amnigenus 2 (n=1)	IMP	0	0	1	0	NA	100% (20.7-100)
	VIM	0	0	1	0	NA	100% (20.7-100)
	NDM	0	0	1	0	NA	100% (20.7-100)
	KPC	0	0	1	0	NA	100% (20.7-100)
	OXA-48	0	0	1	0	NA	100% (20.7-100)
E. cloacae (n=4)	IMP	0	0	4	0	NA	100% (51.0-100)
	VIM	1	0	3	0	100% (20.7-100)	100% (43.9-100)
	NDM	0	0	4	0	NA	100% (51.0-100)
	KPC	0	0	4	0	NA	100% (51.0-100)
	OXA-48	1	0	3	0	100% (20.7-100)	100% (43.9-100)
E. coli (n=10)	IMP	0	0	10	0	NA	100% (72.3-100)
	VIM	0	0	10	0	NA	100% (72.3-100)
	NDM	3	0	7	0	100% (43.9-100)	100% (67.6-100)
	KPC	2	0	8	0	100% (34.2-100)	100% (64.6-100)
	OXA-48	3	0	7	0	100% (43.9-100)	100% (64.6-100)
K. oxytoca (n=1)	IMP	0	0	1	0	NA	100% (20.7-100)
	VIM	0	0	1	0	NA	100%

							(20.7-100)
	NDM	0	0	1	0	NA	100% (20.7-100)
	KPC	0	0	1	0	NA	100% (20.7-100)
	OXA-48	1	0	0	0	100% (20.7-100)	NA
K. pneumoniae (n=60)	IMP	0	1	59	0	NA	98.3% (91.2-99.7)
	VIM	0	1	59	0	NA	98.3% (91.2-99.7)
	NDM	4	1	55	0	100% (51.0-100)	98.2% (90.6-99.7)
	KPC	27	1	32	0	100% (87.5-100)	97% (84.7-99.5)
	OXA-48	24	3	32	1	96.0% (80.5-99.3)	91.4% (77.6-97.0)
P. aeruginosa (n=30)	IMP	0	0	30	0	NA	100% (88.7-100)
	VIM	5	0	21	4	55.6% (26.7-81.1)	100% (84.5-100)
	NDM	0	1	29	0	NA	96.7% (83.3-99.4)
	KPC	0	1	29	0	NA	96.7% (83.3-99.4)
	OXA-48	0	0	30	0	NA	100% (88.7-100)

[a] *N refers to the total numbers of specimens where an organism was identified. This number contains samples that PCR positive and PCR negative for the presence of assay targets.*

[b] *16 Stenotrophomonas maltophilia isolates recovered by the Reference Method. Specimens where these isolates were identified were not included in the analysis because of the intrinsic resistance to multiple carbapenems.*

For Table 17, the results of the contrived study are stratified by the spiking concentration of the carbapenemase-producing organism tested with the Xpert Carba-R Assay.

Table 17. Performance of the Xpert Carba-R Assay in the Contrived Study (by Concentration)

Contrived Study[a,b]								
Concentration Tested	Target	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
1x LoD (n=150)	IMP	30	27	0	120	3	90.0% (74.4-96.5)	100% (96.9-100)
	VIM	30	29	0	120	1	96.7% (83.3-99.4)	100% (96.9-100)
	NDM	30	30	0	120	0	100% (88.7-100)	100% (96.9-100)
	KPC	30	30	0	120	0	100% (88.7-100)	100% (96.9-100)
	OXA-48	30[c]	29	0	120	1	96.7%	100%

							(83.3-99.4)	(96.9-100)
3x LoD (n=126)	IMP	25	24	0	101	1	96.0% (80.5-99.3)	100% (96.3-100)
	VIM	26	26	0	100	0	100% (87.1-100)	100% (96.3-100)
	NDM	25	25	0	101	0	100% (86.7-100)	100% (96.3-100)
	KPC	25	25	0	101	0	100% (86.7-100)	100% (96.3-100)
	OXA-48	25	25	0	101	0	100% (86.7-100)	100% (96.3-100)
10x LoD (n=126)	IMP	25	25	0	101	0	100% (86.7-100)	100% (96.3-100)
	VIM	26	26	0	100	0	100% (87.1-100)	100% (96.3-100)
	NDM	25	25	0	101	0	100% (86.7-100)	100% (96.3-100)
	KPC	25	25	0	101	0	100% (86.7-100)	100% (96.3-100)
	OXA-48	25	25	0	101	0	100% (86.7-100)	100% (96.3-100)

[a]*See Table 15 above for additional information on the FNs observed in the Contrived Study*
[b]*Based on the study design and definitions for positive and negatives, one stain of Empedobacter brevis (VIM) target was not included in the analysis. This strain was determined to be susceptible.*
[c]*1 isolate* was tested twice at different sites.

Eight isolates were identified in the prospective study where at least two Xpert Carba-R Assay targets were detected. These results are shown in Table 18 below.

Table 18. Prospective Specimens with Multiple Targets Detected

Targets Detected by Xpert Carba-R Assay	Targets Detected by PCR and Reference Sequencing	Targets Detected by Alternate PCR and Reference Sequencing
KPC, OXA-48	NEG[a]	NEG[a]
VIM, KPC	NEG[b]	NEG[a]
VIM, OXA-48	OXA-48	OXA-48
KPC, OXA-48	KPC	KPC, OXA-48
NDM, OXA-48	NDM	NDM, OXA-48
VIM, NDM	NEG[b]	NEG[a]
NDM, KPC	KPC	NDM, KPC
VIM, KPC	VIM	VIM, KPC

[a]*PCR yielded no bands for sequencing.*
[b]*An organism was not isolated from reference culture, therefore reference sequencing was not performed.*

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

were 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 755 rectal specimens in the study included for analysis, 10.1% of the specimens (76/755) contained a carbapenem non-susceptible organism with at least one of the assay gene targets (IMP, VIM, NDM, KPC, OXA-48) that was recovered by the Reference Method (Table 19). From 755 rectal specimens, a total of 112 carbapenem non-susceptible organisms (I or R to at least one carbapenem) were recovered by the Reference Culture.

Table 19. Detection of Non-susceptible Organisms with the Gene Targets By the Reference Culture + Sequencing Method

Site Information		**Rectal Specimens**	
Collection Site	Location	Total	# Positive by Reference Culture + Sequencing *(NS organism with at least one assay target)*
Site 1	US (Midwest)	16	0
Site 2	US (Midwest)	130	25
Site 3	US (Southeastern)	14	0
Site 4	Europe (Spain)	456	39
Site 5	Europe (Italy)	139	12
Totals		755	76

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:

   Specific instructions should be followed for the collection of rectal swab specimens. The user refers to a Reference Diagram supplied by Cepheid to determine the acceptability of swab specimens. Any specimens that are highly soiled per the Reference Diagram must be excluded from the study. Swabs are then placed in the appropriate collection device. One swab is added to Sample Reagent for Xpert Carba-R Assay testing. 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:

   The Xpert Check kit is used by the customer or by Cepheid personnel to perform the calibration check of the instrument. The Xpert Check is not provided with the instrument since the instrument is originally calibrated by Cepheid. A calibration check is recommended on an annual basis. In the GeneXpert Operator's Manual (Calibration Section), the user is instructed to contact Cepheid Technical Support for information about calibration.

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.