Food and Drug Administration
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Silver Spring, MD 20993-0002
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510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K190275

B Applicant

GenePOC Inc.

C Proprietary and Established Names

GenePOC Carba

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PMY	Class II	21 CFR 866.1640 - Antimicrobial Susceptibility Test Powder	IM - Immunology & MI - Microbiology
OOI	Class II	21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the GenePOC Carba assay performed on the revogene instrument.

B Measurand:

Conserved DNA sequences of the following carbapenemase genes: bla_{KPC}, bla_{NDM}, bla_{VIM}, $bla_{OXA\text{-}48\text{-}like}$ and bla_{IMP}.

C Type of Test:

Qualitative real-time DNA amplification and detection assay.

III Intended Use/Indications for Use:

A Intended Use(s):

The GenePOC Carba assay, performed on the revogene instrument, is a qualitative in vitro diagnostic test designed for the detection and differentiation of the bla_{KPC}, bla_{NDM}, bla_{VIM}, $bla_{OXA\text{-}48\text{-}like}$, and bla_{IMP} gene sequences associated with carbapenem-non-susceptible pure colonies of *Enterobacteriaceae*, *Acinetobacter baumannii*, or *Pseudomonas aeruginosa*, when grown on blood agar or MacConkey agar. The test utilizes automated real-time Polymerase Chain Reaction (PCR).

The GenePOC Carba assay should be used in conjunction with other laboratory tests including phenotypic antimicrobial susceptibility testing. A negative GenePOC Carba assay result does not preclude the presence of other resistance mechanisms.

The GenePOC Carba assay is intended as an aid for infection control in the detection of carbapenem-non-susceptible bacteria that colonize patients in healthcare settings. The identification of a bla_{IMP}, bla_{NDM} or bla_{VIM} metallo-beta-lactamase gene (i.e., the genes that encode the IMP, NDM and VIM metallo-beta-lactamases, respectively) may be used as an aid to clinicians in determining appropriate therapeutic strategies for patients with known or suspected carbapenem non-susceptible infections.

B Indication(s) for Use:

Same as Intended Use

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

Organisms should be identified and carbapenem non-susceptibility status should be determined prior to testing with the GenePOC Carba assay.

The performance of the GenePOC Carba assay with bacteria other than *Enterobacteriaceae*, *Acinetobacter baumannii* or *Pseudomonas aeruginosa*, has not been evaluated.

In silico predictions of the inclusivity of the GenePOC Carba primers and probes were performed using sequence data available in GenBank in 2018. Analysis of new variant sequences of the targeted carbapenemase genes deposited in GenBank after 2018 has not been performed.

D Special Instrument Requirements:

GenePOC, Inc. revogene instrument

IV Device/System Characteristics:

A Device Description:

The GenePOC Carba assay is a qualitative *in vitro* diagnostic real-time PCR-based test for the detection and differentiation of five β-lactamase genes (*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA\text{-}48\text{-}like}$ and *bla*$_{IMP}$) that are associated with non-susceptibility of Gram negative bacteria to carbapenem antimicrobial agents. The assay is intended for use on isolated, pure colonies of *Enterobacteriaceae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* grown on solid culture media as an aid in detection of carbapenem non-susceptible organisms for the purposes of infection control and to aid in determining appropriate therapeutic strategies for patients with known or suspected infection with such organisms.

The GenePOC Carba assay kit comprises 24 individual pouches, each of which contains a single-use, disposable microfluidic cartridge (PIE), Sample Buffer Tube (SBT) and a Disposable Transfer Tool (DTT). Each PIE contains a slurry of glass beads for sample homogenization and bacterial lysis, a Process Control (PrC), a buffer for dilution of the lysate and primers and probes for detection of each of the targeted carbapenemase genes. The PrC is used to verify the sample processing and amplification steps, including the detection of potential assay inhibitors, microfluidic movement of liquid and reagent integrity.

Each PIE is an integrated closed device in which a sample is dispensed and processed through different microfluidic chambers and channels for nucleic acid extraction and subsequent real-time PCR amplification/detection. User intervention is required to prepare a standardized 0.5 McFarland bacterial suspension from previously identified, carbapenem non-susceptible colonies, add 15µL of this suspension to the SBT and subsequently transfer an aliquot from the SBT to the PIE, and load the PIE into the revogene carousel. Sample preparation and real-time PCR are completed in approximately 70 minutes. Upon completion of a run, the results for each target gene are interpreted automatically by the revogene instrument and reported as Positive, Negative, Indeterminate (instrument error) or Unresolved (PrC failure).

B Principle of Operation:

The GenePOC Carba assay is for the qualitative detection and differentiation of the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA\text{-}48\text{-}like}$ and bla$_{IMP}$ genes in isolated colonies of carbapenem non-susceptible *Enterobacteriaceae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* grown on sheep blood or MacConkey agar. The assay is performed on the revogene instrument (K170558) in single-use, disposable microfluidic cartridges (PIEs) that automate sample processing, microfluidic movement of liquid and real-time PCR amplification/detection. No operator intervention is necessary once the sample is loaded onto the revogene instrument.

To perform the test, a previously identified carbapenem non-susceptible bacterial strain is streaked on blood or MacConkey agar to obtain isolated colonies. A 10µg meropenem disc is placed in the first quadrant of the plate to monitor retention of non-susceptibility to carbapenems. After incubation for 18-24 hours at 35±2°C, isolated colonies are sampled and used to prepare a 0.5 McFarland suspension in saline. Fifteen microliters of the standardized suspension are then

added to a Sample Buffer Tube (SBT) and, after mixing, a Disposable Transfer Tool (DTT) provided in the assay kit is used to transfer an aliquot of the diluted suspension to a GenePOC Carba PIE which is then sealed and loaded into the revogene instrument.

The revogene can process between one and eight samples (PIEs) in a single run. Each PIE is an integrated, self-contained device that performs sample homogenization/bacterial lysis using glass beads, sample dilution and PCR amplification/detection. Fluid movement within each PIE is controlled by centrifugation through microfluidic channels. An internal Process Control (PrC) is included in the homogenization chamber to monitor reagent and process integrity.

The results are interpreted automatically by the system from measured fluorescent signals and may be viewed, printed, transferred, and/or stored by the user.

C Instrument Description Information:

Modes of Operation	Yes	No
Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?	☒	☐
Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?	☐	☒
Software		
FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types.	☒	☐

1. Instrument Name:

   GenePOC revogene instrument

2. Specimen Identification:

   The revogene instrument has two barcode readers to identify reagents and patient specimens. The Sample Buffer Tube (SBT) and microfluidic cartridge (PIE) are each pre-labeled with a unique barcode at the time of manufacture to identify both the specimen and assay type, providing traceability of each sample to the PIE, SBT and assay identifiers.

3. Specimen Sampling and Handling:

   User intervention is required to prepare a standardized bacterial suspension from isolated colonies of a previously identified, carbapenem non-susceptible bacterial isolate, transfer an aliquot of the suspension to the Sample Buffer Tube (SBT) and from there to the microfluidic cartridge (PIE). All further processing is automated once the PIE is loaded into the revogene instrument and the run is initiated. Results are interpreted automatically by the system.

4. Calibration:

The revogene instrument is factory calibrated at the time of manufacture. Calibration is verified annually by a trained technician.

5. Quality Control:

Each microfluidic cartridge (PIE) contains a Process Control (PrC) in the homogenization chamber that is designed to monitor process and reagent integrity. The PrC is mixed with the patient sample and undergoes simultaneous lysis, PCR amplification/detection.

Commercially available strains of bacteria may be used as External Controls for the GenePOC Carba assay in accordance with the requirements of local, state or federal regulations and accrediting agencies (refer to **Section VII A(5)**).

V Substantial Equivalence Information:

A Predicate Device Name(s):
Xpert Carba-R

B Predicate 510(k) Number(s):
K152614

C Comparison with Predicate(s):

Device & Predicate Device(s):	**K190275**	**K152614**
Device Trade Name	**GenePOC Carba**	**Xpert Carba-R Assay**
General Device Characteristic Similarities		
Intended Use/Indications For Use	The GenePOC Carba assay, performed on the revogene instrument, is a qualitative *in vitro* diagnostic test designed for the detection and differentiation of the bla_{KPC}, bla_{NDM}, bla_{VIM}, $bla_{OXA\text{-}48\text{-}like}$, and bla_{IMP} gene sequences associated with carbapenem-non-susceptible pure colonies of *Enterobacteriaceae*, *Acinetobacter baumannii*, or *Pseudomonas aeruginosa*, when grown on blood agar or	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}, $bla_{OXA\text{-}48}$, 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

Device & Predicate Device(s):	K190275	K152614
	MacConkey agar. The test utilizes automated real-time Polymerase Chain Reaction (PCR). The GenePOC Carba assay should be used in conjunction with other laboratory tests including phenotypic antimicrobial susceptibility testing. A negative GenePOC Carba assay result does not preclude the presence of other resistance mechanisms. The GenePOC Carba assay is intended as an aid for infection control in the detection of carbapenem-non-susceptible bacteria that colonize patients in healthcare settings. The identification of a bla_{IMP}, bla_{NDM} or bla_{VIM} metallo-β-lactamase gene (i.e., the genes that encode the IMP, NDM and VIM metallo-β-lactamases, respectively) may be used as an aid to clinicians in determining appropriate therapeutic strategies for patients with known or suspected carbapenem non-susceptible infections.	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.
Technological Principles	Automated DNA extraction, nucleic acid amplification and fluorescence-based real-time Polymerase Chain reaction (PCR) detection	Same
Test Cartridge	Disposable, single-use, multichambered fluidic cartridge	Same

Device & Predicate Device(s):		**K190275**	**K152614**
Targeted Carbapenemase Genes		*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$ and *bla*$_{IMP}$	Same
Bacterial Species		*Enterobacteriaceae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*	Same
Sample Type		Isolated colonies of carbapenem non-susceptible Gram negative bacteria on blood or MacConkey agar	Same
General Device Characteristic Differences			
Instrument System		revogene instrument	GeneXpert Instrument System (GeneXpert Dx, Infinity-48, Infinity-48s and Infinity-80)
Gene variants detected (based on analytical studies) [1]	*bla*$_{KPC}$	2, 3, 4	2, 3, 4
	bla$_{NDM}$	1, 4-7	1, 2, 4, 5
	bla$_{IMP}$	1, 4, 8, 9, 11	1, 2, 4, 6, 10, 11
	bla$_{OXA-48}$	48, 181, 204, 232	48, 181
	bla$_{VIM}$	1, 2, 10, 19	1, 2, 4, 10, 19
Additional gene variants whose detection is predicted based on *in silico* analysis [1]	*bla*$_{KPC}$	5-38	5-16
	bla$_{NDM}$	2, 3, 8-24	3, 6-9
	bla$_{IMP}$	2, 5, 6, 10, 17-20, 23-26, 28-30, 32, 33, 37, 38, 40, 42, 45, 47-49, 53-56, 59, 60, 62, 66, 69-72, 74-79	3, 8, 9, 13, 19 to 22, 24, 25, 27, 28, 30, 31, 33, 37, 40, 42
	bla$_{OXA-48}$	162, 199, 204, 244, 245, 252, 370, 484, 505, 514, 515, 519, 546, 547, 566	162, 163, 204, 232, 244, 245, 247
	bla$_{VIM}$	3-6, 8, 9, 11, 12, 14-18, 20, 23-46, 48-50, 52-55, 57, 59, 60	5-9, 11 to 18, 20, 23-38

[1] **Note:** The variants listed reflect the respective labeling of each device and the analytical studies and *in silico* analyses conducted at the time of 510(k) clearance.

VI Standards/Guidance Documents Referenced:

CLSI. *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline.* CLSI document EP25-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.

CLSI. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition.* CLSI document EP28-A3c. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The precision and reproducibility of the GenePOC Carba assay was evaluated in a study conducted at three sites using an 11-member panel of samples comprised of representative strains of bacteria that between them carried each of the targeted carbapenemase gene markers (**Table 1**). Each panel member was prepared at standardized concentration of 0.5 McFarland and frozen prior to distribution to the study sites for testing.

Table 1. Panel members used to evaluate the precision and reproducibility of the GenePOC Carba assay

Panel Member	Strain	Species	Carbapenemase Gene Variant
1	ATCC BAA-2146	*Klebsiella pneumoniae*	$bla_{\text{NDM-1}}$
2	CCRI 23064	*Escherichia coli*	$bla_{\text{NDM-5}}$
3	CCUG 59348	*Klebsiella pneumoniae*	$bla_{\text{KPC-2}}$
4	CCRI 21578	*Enterobacter cloacae*	$bla_{\text{KPC-4}}$
5	ATCC BAA-2523	*Escherichia coli*	$bla_{\text{OXA-48}}$
6	CCRI 22264	*Klebsiella pneumoniae*	$bla_{\text{OXA-181}}$
7	NCTC 13476	*Escherichia coli*	$bla_{\text{IMP-1}}$
8	CCRI 19583	*Klebsiella pneumoniae*	$bla_{\text{IMP-4}}$
9	NCTC 13440	*Klebsiella pneumoniae*	$bla_{\text{VIM-1}}$
10	CCRI 19585	*Klebsiella pneumoniae*	$bla_{\text{VIM-1}}$
11	CCRI 22760	*Enterobacter cloacae*	Negative

ATCC: American Type Culture Collection
NCTC: National Collection of Type Cultures
CCRI: Culture Collection of the Centre de Researche en Infectiologie
CCUG: Culture Collection, University of Gothenburg

A nested study design was used to characterize within-laboratory precision, between-laboratory reproducibility as well as lot-to-lot reproducibility, whereby a subset of samples

that was tested with one lot of reagents in the Lot-to-Lot Reproducibility Study also served as panel members in the Within Laboratory Precision and Between Laboratory Reproducibility Studies (**Table 2**). As a result, overall there was a total of 200 replicates of each positive panel member and 400 replicates of the negative panel member.

Table 2. Reproducibility and Precision Study design for the GenePOC Carba assay

Parameter	**Analysis**		
	Within-Laboratory Precision	**Lot-to-Lot Reproducibility**	**Between-Laboratory Reproducibility**
Sites	1	1	3
Operators/Site	2	2	2
Reagent Lots	1	3	1
Days of Testing/Reagent Lot	5	5	5
Replicates per Panel Member/Day/Operator	4 positive 8 negative [1]	4 positive 8 negative	4 positive 8 negative
Replicates/Panel Member/Lot	40 positive 80 negative	40 positive 80 negative	120 positive 240 negative
Total Replicates Per Panel Member	40 positive 80 negative	120 positive 240 negative	120 positive 240 negative
	200 positive [2] 400 negative		

[1] Because only one carbapenemase negative strain was used in the study, 8 replicates of **Panel Member #11** from **Table 1** were tested on each day by each operator

[2] Because of the nested study design, overall there was a total of 200 replicates of each positive panel member and 400 replicates of the negative panel member.

The qualitative test results from the GenePOC Carba assay Reproducibility and Precision Study are summarized in **Tables 3** and **4**. An analysis of Ct and endpoint fluorescence values obtained for each panel member at each study site is provided in **Table 5**.

Table 3. Summary of results from the Reproducibility and Precision Study for the GenePOC Carba assay

Analysis	Agreement by Panel Member	
	Positive [1]	**Negative** [2]
Within-Laboratory Precision	40/40 100% (91.2-100%) [3]	80/80 100% (95.4-100%)
Lot-to-Lot Reproducibility	120/120 100% (96.9-100%)	239/240 99.6% (97.7-99.9%)
Between-Laboratory Reproducibility	120/120 100% (96.9-100%)	237/240 98.8% (96.4-99.6%
Overall	200/200 100% (98.1-100%)	396/400 99.0% (98.1-100%)

[1] In each study, there was 100% positive agreement for Panel Members #1-10. A total of 2000 samples were tested across all 10 positive panel members (10 panel members x 200 replicates each).
[2] Panel Member #11
[3] Two-sided 95% score confidence interval

On initial testing, 34/2400 (1.4%) samples produced Indeterminate results and a further eight (8) (8/2400 = 0.3%) were reported as Unresolved. In each case, upon retesting of these samples, valid results were obtained. Eleven false positive results (11/2400 = 0.5%) were observed over the course of the study (bla_{IMP}: 8; bla_{NDM}: 3) (**Table 4**). The GenePOC Carba assay exhibited acceptable reproducibility and precision within and between sites/instruments/reagent lots and days of testing.

Table 4. Summary of qualitative GenePOC Carba assay results from the Reproducibility and Precision Study, stratified by carbapenemase gene variant

Site [1]	Panel Member / *bla* variant [2]		Number Tested	GenePOC Positive					GenePOC Negative	Agreement with Expected Result	
				NDM	KPC	OXA-48 [3]	IMP	VIM		*N*	%
Site 1	1	NDM-1	120	120	0	0	1	0	0	119	99.2
	2	NDM-5	120	120	0	0	0	0	0	120	100
	3	KPC-2	120	0	120	0	0	0	0	120	100
	4	KPC-4	120	0	120	0	1	0	0	119	99.2
	5	OXA-48	120	0	0	120	0	0	0	120	100
	6	OXA-181	120	0	0	120	0	0	0	120	100
	7	IMP-1	120	0	0	0	120	0	0	120	100
	8	IMP-4	120	0	0	0	120	0	0	120	100
	9	VIM-1 [4]	120	0	0	0	0	120	0	120	100
	10	VIM-1 [5]	120	1	0	0	0	120	0	119	99.2
	11	Negative	240	0	0	0	1	0	239	239	99.6
Site 2	1	NDM-1	40	40	0	0	1	0	0	39	97.5
	2	NDM-5	40	40	0	0	0	0	0	40	100
	3	KPC-2	40	0	40	0	0	0	0	40	100
	4	KPC-4	40	0	40	0	1	0	0	39	97.5
	5	OXA-48	40	0	0	40	0	0	0	40	100
	6	OXA-181	40	0	0	40	0	0	0	40	100
	7	IMP-1	40	0	0	0	40	0	0	40	100
	8	IMP-4	40	0	0	0	40	0	0	40	100
	9	VIM-1 [4]	40	0	0	0	0	40	0	40	100
	10	VIM-1 [5]	40	0	0	0	0	40	0	40	100
	11	Negative	80	0	0	0	1	0	79	79	98.8
Site 3	1	NDM-1	40	40	0	0	1	0	0	39	97.5
	2	NDM-5	40	40	0	0	0	0	0	40	100
	3	KPC-2	40	0	40	0	0	0	0	40	100
	4	KPC-4	40	1	40	0	0	0	0	39	97.5
	5	OXA-48	40	0	0	40	0	0	0	40	100
	6	OXA-181	40	0	0	40	0	0	0	40	100
	7	IMP-1	40	0	0	0	40	0	0	40	100
	8	IMP-4	40	0	0	0	40	0	0	40	100
	9	VIM-1 [4]	40	0	0	0	0	40	0	40	100
	10	VIM-1 [4]	40	0	0	0	0	40	0	40	100
	11	Negative	80	1	0	0	1	0	78	78	97.5
All Sites	1	NDM-1	200	200	0	0	3	0	0	197	98.5
	2	NDM-5	200	200	0	0	0	0	0	200	100
	3	KPC-2	200	0	200	0	0	0	0	200	100
	4	KPC-4	200	1	200	0	2	0	0	197	98.5
	5	OXA-48	200	0	0	200	0	0	0	200	100
	6	OXA-181	200	0	0	200	0	0	0	200	100
	7	IMP-1	200	0	0	0	200	0	0	200	100
	8	IMP-4	200	0	0	0	200	0	0	200	100
	9	VIM-1 [4]	200	0	0	0	0	200	0	200	100
	10	VIM-1 [4]	200	1	0	0	0	200	0	199	99.5
	11	Negative	400	1	0	0	3	0	396	396	99.0

[1] Data from **Site 1** include results obtained with 3 different lots of GenePOC Carba reagents and 3 revogene instruments
[2] Refer to **Table 1** for a detailed description of the Panel Members
[3] Reported by the GenePOC Carba assay as positive for a $bla_{\text{OXA-48-like}}$ carbapenemase gene
[4] **Panel Members #9 and #10** were prepared from different isolates that carry $bla_{\text{VIM-1}}$ (**Table 1**)

Table 5. Summary of Ct and endpoint fluorescence values obtained with each panel member in the GenePOC Carba Reproducibility and Precision Study

Panel Member / *bla* variant [1]		Ct Value											
		Site 1 (n = 120) [2]			Site 2 (n = 40)			Site 3 (n = 40)			Overall		
		Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
1	**NDM-1**	29.5	1.3	4.5	30.0	1.0	3.3	30.2	0.7	2.2	29.8	1.2	4.0
2	**NDM-5**	28.9	1.3	4.6	29.5	1.3	4.5	31.0	1.5	4.8	29.5	1.6	5.4
3	**KPC-2**	30.8	2.2	7.1	29.5	1.7	5.7	29.4	1.4	4.7	30.2	2.0	6.8
4	**KPC-4**	29.6	2.0	6.7	28.9	1.7	5.7	31.0	1.8	5.8	29.8	2.0	6.7
5	**OXA-48**	28.6	1.4	5.0	29.4	1.1	3.7	32.1	1.4	4.5	29.4	1.9	6.6
6	**OXA-181**	28.2	1.3	4.7	27.7	1.2	4.5	28.2	0.8	2.9	28.1	1.2	4.4
7	**IMP-1**	29.0	1.0	3.5	28.4	1.3	4.4	30.9	1.0	3.1	29.3	1.4	4.6
8	**IMP-4**	29.2	1.3	4.4	28.8	1.2	4.2	30.0	0.8	2.8	29.3	1.2	4.2
9	**VIM-1** [3]	28.1	1.3	4.5	28.1	1.4	5.0	28.5	1.0	3.4	28.2	1.2	4.1
10	**VIM-1** [3]	28.1	0.93	3.3	27.4	0.98	3.6	27.3	0.7	2.5	27.6	1.2	4.4
11	**Negative** [4, 5]	33.0	2.5	7.6	33.6	1.3	3.9	35.2	1.1	3.2	33.6	2.3	6.7
Panel Member / *bla* variant [1]		**Endpoint Fluorescence**											
		Site 1 (n = 120) [2]			**Site 2 (n = 40)**			**Site 3 (n = 40)**			**Overall**		
		Mean	**SD**	**%CV**	**Mean**	**SD**	**%CV**	**Mean**	**SD**	**%CV**	**Mean**	**SD**	**%CV**
1	**NDM-1**	793	104	13.1	750	78	10.4	760	104	13.7	778	101	13.0
2	**NDM-5**	789	85	10.8	715	80	11.1	727	88	12.1	762	91	11.9
3	**KPC-2**	1287	164	12.7	1231	169	13.7	1712	162	9.5	1361	241	17.7
4	**KPC-4**	1383	154	11.2	1313	128	9.7	1466	200	13.7	1386	166	12.0
5	**OXA-48**	807	96	11.9	716	92	12.8	672	131	19.5	762	118	15.4
6	**OXA-181**	688	109	15.8	633	112	17.7	713	125	17.5	682	115	16.9
7	**IMP-1**	1631	258	15.8	1558	222	14.3	1384	279	20.2	1567	272	17.3
8	**IMP-4**	1354	257	19.0	1243	233	18.8	1326	242	18.2	1326	252	19.0
9	**VIM-1** [3]	1833	183	10.0	1415	162	11.5	1646	171	10.4	1712	241	14.1
10	**VIM-1** [3]	1387	171	12.3	1256	178	14.1	1693	186	11.0	1422	227	16.0
11	**Negative** [4, 5]	794	198	25.0	716	135	18.9	673	170	25.2	755	188	25.0

SD: Standard Deviation; %CV: Percent Coefficient of Variation

[1] Refer to **Table 1** for a detailed description of the Panel Members

[2] Data from **Site 1** include results obtained with 3 different lots of GenePOC Carba reagents and 3 revogene instruments

[3] **Panel Members #9** and **#10** were prepared from different isolates that carry *bla*$_{VIM-1}$ (**Table 1**)

[4] Ct and endpoint values shown are for the Process Control

[5] For **Panel Member #11**: n = 240 at **Site 1** and n = 80 at **Sites 2** and **3**

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:

Analytical Specificity

The analytical specificity of the GenePOC Carba assay was evaluated by testing a panel of 50 carbapenem susceptible and non-susceptible isolates of *Enterobacteriaceae*, *A. baumannii* and *P. aeruginosa*. The carbapenem susceptibility phenotype of each isolate (susceptible, intermediate or resistant) was determined by disc diffusion and antimicrobial resistance gene content was characterize by whole Genome Sequencing and/or targeted PCR (**Table 6**). Most of the isolates carried β-lactamase gene(s) that do not confer non-susceptibility to carbapenems. Those that were non-susceptible (i.e., intermediate or resistant) to carbapenems either harbored carbapenemase markers that are not targeted by the GenPOC Carba assay or non-susceptibility was conferred by a non-carbapenemase-mediated mechanism. Each isolate was tested with the GenePOC Carba assay in triplicate using a starting suspension equivalent to ~4 McFarland (i.e., ~5-10 times higher concentration than the standard workflow for the GenePOC Carba assay which uses a starting suspension density of 0.5 McFarland). No false

positive results were observed other than a single replicate of *K. pneumoniae* isolate CCRI-59359 that yielded a positive result for *bla*$_{\text{OXA-48-like}}$. Three additional replicates of this strain were tested and all yielded the expected negative results for each of the five *bla* gene targets.

Table 6. Isolates used to characterize the analytical specificity of the GenePOC Carba assay

Species	Isolate	β-Lactamase Gene(s) Identified (100% Coverage)	Resistance Phenotype (S/I/R)			
			ETP	MEM	IMP	DOR
Acinetobacter baumannii	CCRI-1016	TEM-90, Mbl, BlaA2, OXA-653, Zn-dependent hydrolase	N/A	S	S	S
Acinetobacter baumannii	CCRI-1017	TEM-206, SCO-1, Mbl, BlaA2, OXA-673, Zn-dependent hydrolase	N/A	S	S	S
Enterobacter aerogenes	CCRI-3853	AmpC	S	S	S	S
Enterobacter aerogenes	CCRI-3879	AmpC	S	S	S	S
Enterobacter aerogenes [1]	CCRI-19495	SHV-5, AmpC	R	R	R	R
Enterobacter amnigenus [2]	CCRI-22353	ACT-15	R	R	R	R
Enterobacter cloacae	CCRI-3852	ACT-7	I	S	S	S
Enterobacter cloacae	CCRI-3854	ACT-42	I	S	I	S
Enterobacter cloacae	CCRI-23473	No β-Lactamase Gene Identified	R	R	R	R
Enterobacter cloacae	CCRI-21536	ACT-5	R	R	R	R
Enterobacter cloacae	CCRI-21540	ACT-7	R	S	I	S
Enterobacter cloacae	CCRI-21603	ACT-7	R	R	R	R
Enterobacter cloacae	CCRI-21692	ACT-14	R	S	R	I
Enterobacter cloacae	CCRI-22075	ACT-7	R	R	R	R
Enterobacter cloacae	CCRI-22097	ACT-16	R	R	R	R
Enterobacter cloacae	CCRI-23318	CTX-M-15, TEM-206, CMH-1	R	R	R	R
Escherichia coli	CCRI-21970	AmpC1, AmpC2, MrdA, AmpH, CMY-44	R	I	R	I
Escherichia coli	CCUG 58541	CTX-M-14, TEM-104, MrdA, AmpC2, AmpH	R	S	S	S
Escherichia coli	NCTC 13441	CTX-M-15, TEM-198, MrdA, OXA-13, AmpC2	S	S	S	S
Escherichia coli	CCRI- 21710	AmpC2, MrdA, CTX-M-15, AmpH, OXA-13	S	S	S	S
Escherichia coli	CCRI-778	AmpC2, MrdA	S	S	S	S
Escherichia coli	CCRI-779	TEM-206, AmpC1, MrdA, AmpC2, AmpH	S	S	S	S
Escherichia coli	CCRI-785	TEM-206, AmpC1, MrdA, AmpC2, AmpH	S	S	S	S
Escherichia coli	CCRI-878	AmpC2, MrdA, AmpH	S	S	S	S
Escherichia coli	CCUG 55970	CTX-M-9, AmpC2, TEM-206, MrdA, AmpC1, AmpH	S	S	S	S
Escherichia coli	CCUG 55971	CTX-M-15, TEM-143, AmpC2	S	S	S	S

Species	Isolate	β-Lactamase Gene(s) Identified (100% Coverage)	Resistance Phenotype (S/I/R)			
			ETP	MEM	IMP	DOR
Escherichia coli	CCUG 55972	CTX-M-2, AmpC1, AmpC2, AmpH	S	S	S	S
Escherichia coli	CCUG 58540	CTX-M-15, AmpC2, TEM-206, MrdA, AMPH, OXA-13	S	S	S	S
Escherichia coli	CCUG 58542	CTX-M-15, MrdA, OXA-13, AmpC2	S	S	S	S
Klebsiella pneumoniae	CCUG 58546	SHV-44, AmpH	R	S	S	S
Klebsiella pneumoniae	NCTC 13465	SHV-85, TEM-206, AmpH	S	S	S	S
Klebsiella pneumoniae	CCUG 54718	CTX-M-15, TEM-33, OXA-13, AmpH	S	S	S	S
Klebsiella pneumoniae	CCUG 59358	SHV-14, OXA-13, LAP-2	S	S	S	S
Klebsiella pneumoniae	CCUG 59349	CTX-M-15, AmpH, OXA-13, TEM- 105, SHV-11	S	S	S	S
Klebsiella pneumoniae	CCUG 59359 [3]	TEM-15, SHV-70, AmpH	S	S	S	S
Klebsiella pneumoniae	CCUG 59360	SHV-12, TEM-168, AmpH, OXA-93	S	S	S	S
Klebsiella pneumoniae	CCRI-784	SHV-27, AmpH	S	S	S	S
Klebsiella pneumoniae	CCRI-1015	TEM-171, SCO-1, PER-2, OXA-93, SHV-39, AmpH	S	S	S	S
Klebsiella pneumoniae [4]	CCRI-806	OKP-B-11	S	S	S	S
Proteus mirabilis	CCRI-21789	No β-Lactamase Gene Identified	S	S	I	S
Proteus mirabilis	CCRI-825	TEM-33, CTX-M-2, OXA-23	S	S	S	S
Proteus mirabilis	CCRI-826	TEM-215	S	S	S	S
Proteus mirabilis	CCRI-831	TEM-206, CTX-M-2, OXA-23	S	S	S	S
Pseudomonas aeruginosa	CCRI-873	OXA-503	N/A	S	S	S
Pseudomonas aeruginosa	CCRI-1228	OXA-503	N/A	S	S	S
Pseudomonas aeruginosa	C72	SPM	N/A	R	R	R
Salmonella sp.	CCRI- 8892	CTX-M-5, TEM-166, OXA-13	S	S	S	S
Salmonella sp.	CCRI- 8893	CTX-M-5, TEM-95, OXA-13	S	S	S	S
Serratia marcescens	CCRI-21537	SRT-1	S	S	I	S
Serratia marcescens	CCRI-23334	SME-4, SRT-1	R	R	R	R

ETP; ertapenem; MEM; meropenem; IMP: imipenem; DOR: doripenem; S: Susceptible; I: Intermediate; R: Resistant; N/A: Not applicable: *A. baumannii* and *P. aeruginosa* are intrinsically resistant to ertapenem; NCTC: National Collection of Type Cultures; CCRI: Culture Collection of the Centre de Researche en Infectiologie; CCUG: Culture Collection, University of Gothenburg

[1] *Klebsiella aerogenes* by DNA sequencing

[2] *Enterobacter cloacae* by DNA sequencing

[3] On initial testing, 1/3 replicates produced a false positive result for bla_{NDM}; 3/3 additional replicates produced the expected negative result for all five target genes.

[4] *Klebsiella quasipneumoniae* by DNA sequencing

In Silico Analysis
In addition to laboratory testing, the specificity of the GenePOC Carba assay primers and probes was evaluated *in silico* by using the Basic Local Alignment Search Tool (BLAST) to interrogate the GenBank nucleotide collection (nr/nt) database. All possible primer combinations within each PCR master mix (bla_{KPC}/bla_{NDM}/PrC for Master Mix 1 and bla_{IMP}/$bla_{OXA\text{-}48\text{-}like}$/$bla_{VIM}$ for Master Mix 2) were evaluated for the potential to amplify non-specific products. The only potential amplification products identified were those for the targeted carbapenemase resistance genes. These results are acceptable.

Interfering Substances Study
The potential for the culture medium used for growth of bacterial colonies to interfere with the GenePOC Carba assay was evaluated by testing isolated colonies grown on blood and MacConkey agar plates from three different manufacturers. Testing was performed with representative strains of bacteria that harbored each of the carbapenemase resistance markers targeted by the GenePOC Carba assay, as well as a negative control strain. Each isolate was grown on each culture medium and suspended to a standardized concentration of 0.5 McFarland using saline from two different suppliers, resulting in a total of 12 test conditions for each organism. Three replicates of each strain were tested under each condition ((3 types of blood agar + 3 types of MacConkey agar) x 2 preparations of saline x 6 strains x 3 replicates = 216 samples). The expected results were obtained for 214/216 (99.1%) samples. The two (2) discordant results occurred with a strain of *K. pneumoniae* that was reported, as expected, as positive for bla_{NDM} but which also produced positive results for bla_{IMP} (1 replicate) and bla_{VIM} (1 replicate) under different test conditions. Retesting of freshly prepared bacterial suspensions with the same combinations of culture media and saline produced the expected results (three (3) replicates per condition). The results of the Interfering Substances Study were determined to be acceptable.

4. Assay Reportable Range:

Not applicable.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Process Control
Each GenePOC Carba PIE includes an integrated Process Control (PrC) that is designed to monitor sample homogenization, dilution, amplification and detection. The PrC is amplified and detected using a specific pair of PCR primers and a detector probe that are located in one of the two amplification chambers of the GenePOC Carba PIE.
External Controls
External Positive and Negative Controls should be tested in accordance with good laboratory practices and applicable regulations and the requirements accrediting agencies. The manufacturer does not provide External Controls for use with the GenePOC Carba assay but recommends use of standardized 0.5 McFarland suspensions of the strains listed in **Table 7**

as Positive Controls. For a Negative Control, a carbapenem non-susceptible isolate that does not carry any of the resistance genes targeted by the GenePOC carba assay is recommended.

Table 7. Strains recommended for use as external Positive Controls for the GenePOC Carba assay and summary of validation data

Species	Strain	*bla* Gene Target	Expected Result (%)
K. pneumoniae	ATCC BAA-2146	NDM-1	36/36 (100)
K. pneumoniae	CCUG 59348	KPC-2	36/36 (100)
E. coli	ATCC BAA-2523	OXA-48	36/36 (100)
E. coli	NCTC 13476	IMP-1	36/36 (100) [1]
K. pneumoniae	NCTC 13440	VIM-1	36/36 (100)

ATCC: American Type Culture Collection
NCTC: National Collection of Type Cultures
CCUG: Culture Collection, University of Gothenburg
[1] 1/36 replicates produced an Indeterminate result on initial testing but gave the expected result upon repeat from the same Sample Buffer Tube

Use of the strains shown in **Table 7** was validated by testing 36 replicates of each with three lots of GenePOC Carba reagents (12 replicates/lot). All controls produced the expected results.

External Positive and Negative Controls were also tested on each day of the Method Comparison Study described in **Section VII B(1)**. The strain used for the Positive Control was selected daily on a rotating basis so that a control for each gene target was tested at least twice at each study site. A total of 52 pairs of Positive and Negative External Controls was evaluated over the course of the study. Of these, 49 pairs (94.2%) produced the expected results for each of the targeted carbapenemase genes on initial testing and in each case, the controls that failed produced the expected results upon repeat.

Sample Stability
Studies were performed to evaluate the stability of bacterial suspensions in the Sample Buffer Tube (SBT) and of the GenePOC Carba PIE after removal from the dessicant pouch and addition of sample. Bacterial suspensions in the SBT were shown to be stable for 4 days at 25°C and 7 days at 2-8°C. GenePOC Carba PIEs were found to be stable for up to 1 hour at 25°C after addition of sample.

Reagent Stability
The shelf-life of the GenePOC Carba assay reagents will be established through a Real-time Stability Study conducted at 2-8°C and 25±3°C/60% relative humidity.

6. Detection Limit:

Analytical Sensitivity
The analytical sensitivity of the GenePOC Carba assay was not evaluated because bacterial suspensions for testing must be standardized to a density of 0.5 McFarland, which is substantially above the limit of detection (LoD) for assays based on nucleic acid amplification.

Inclusivity

The analytical reactivity (inclusivity) of the GenePOC Carba assay was evaluated using a panel of 58 carbapenem non-susceptible isolates that included multiple variants of each targeted gene marker. The non-susceptibility of each isolate was confirmed by disc diffusion with doripenem, ertapenem, imipenem and meropenem according to standard methods (CLSI, M02-13 and M100-S28). Genotypic characterization of carbapenemase resistance genes was performed by Whole Genome Sequencing. Each isolate was tested with the GenePOC Carba assay in triplicate. Samples that produced indeterminate or unexpected (i.e., false positive or false negative) results were retested. All the final results obtained with each of the 58 strains were as expected. A listing of isolates and gene variants used in the GenePOC Carba Inclusivity Study is shown **Tables 8** and **9**. The inclusivity of the GenePOC Carba assay for detection of carbapenemase resistance markers in *Enterobacteriaceae, A. baumannii* and *P. aeruginosa* was determined to be acceptable.

Table 8. Isolates used to evaluate the inclusivity of the GenePOC Carba assay, stratified by carbapenemase gene

Species	Isolate	Origin	Year	Carbapenemase Gene Variant
Isolates Harboring Multiple Genes (2)				
Klebsiella pneumoniae	CCRI-23061	Switzerland	2015	OXA-232, NDM-1
Pseudomonas aeruginosa	ATCC BAA-2793	Chile	2014	KPC-2, VIM-2
Isolates Harboring *bla*$_{KPC}$ (11)				
Enterobacter cloacae	CCRI-21578 [1]	Canada	2011	KPC-4
Escherichia coli	ATCC BAA-2340	USA	N/A	KPC
Klebsiella oxytoca	CCRI-21581	Canada	2011	KPC-3
Klebsiella pneumoniae	NCTC 13438	N/A1	N/A1	KPC-3
	ATCC BAA-1705	USA	2007	KPC-2
	CCRI-19587	Canada	2009	KPC-3
	CCRI-19570	USA	2003	KPC-2
	CCUG 59413	Sweden	2010	KPC-3
	CCUG 59348	Norway	2010	KPC-2
	CCUG 56233	Sweden	2088	KPC-2
Pseudomonas aeruginosa	CCRI-21587	Canada	2011	KPC-2
Isolates Harboring *bla*$_{NDM}$ (14)				
Enterobacter cloacae	ATCC BAA-2468	USA	N/A1	NDM-1
Escherichia coli	CCRI-22255	France	2013	NDM-1
	CCRI-23064	Switzerland	2015	NDM-5
	CCRI-23464	Canada	2016	NDM-5
	CCRI-23065	Switzerland	2015	NDM-6
	CCRI-23066	Switzerland	2015	NDM-7
Klebsiella pneumoniae	NCTC 13443	N/A	N/A	NDM-1
	ATCC BAA-2146	USA	N/A	NDM-1
	CCRI-21711	Canada	2011	NDM-1
	CCRI-22199	Canada	2012	NDM-1
	CCRI-22254	France	2013	NDM-4
	CCUG 60138	Sweden	2010	NDM-1
Providencia rettgeri	CCRI-22257	France	2013	NDM-1
Providencia stuartii	CCRI-22256	France	2013	NDM-1
Isolates Harboring *bla*$_{OXA\text{-}48\text{-}like}$ (11)				
Citrobacter freundii	CCRI-23374	Canada	2016	OXA-204
Enterobacter cloacae	CCRI-22266	France	2013	OXA-48
Escherichia coli	CCRI-22265	France	2013	OXA-48
	ATCC BAA-2523	N/A	N/A	OXA-48
Klebsiella pneumoniae	NCTC 13442	N/A	N/A	OXA-48
	CCRI-22263	France	2013	OXA-48
	CCRI-22264	France	2013	OXA-181
	CCRI-23060	Switzerland	2015	OXA-204

Species	Isolate	Origin	Year	Carbapenemase Gene Variant
	ATCC BAA-2524	N/A	N/A	OXA-48
	CCUG 64452	Sweden	2013	OXA-48
Providencia rettgeri	CCRI-22267	France	2013	OXA-181
Isolates Harboring *bla*$_{IMP}$ (11)				
Acinetobacter baumannii	CCRI-19488	Canada	2003	IMP-1
Citrobacter youngae	CCRI-21591	Canada	2011	IMP-4
Escherichia coli	NCTC 13476	N/A	N/A	IMP-1
Klebsiella pneumoniae	CCRI-19569	Japan	2003	IMP-1
	CCRI-19582	Turkey	2009	IMP-1
	CCRI-19583	Taiwan	2009	IMP-4
	CCRI-19588	Taiwan	2009	IMP-4
	CCRI-19584	Taiwan	2009	IMP-8
Pseudomonas aeruginosa	CCRI-21589	Canada	2011	IMP-1
	CCRI-21590	China	2000	IMP-9
Serratia marcescens	CCRI-22262	France	2013	IMP-11
Isolates Harboring *bla*$_{VIM}$ (9)				
Klebsiella pneumoniae	NCTC 13439	N/A	N/A	VIM-1
	NCTC 13440	N/A	N/A	VIM-1
	CCRI-19585	France	2009	VIM-1
	CCRI-22258	France	2013	VIM-1
	CCRI-22259	France	2013	VIM-19
Pseudomonas aeruginosa	NCTC 13437	N/A	N/A	VIM-10
	CCRI-21588	Canada	2011	VIM-2
	CCRI-22720	Argentina	2014	VIM-2
Serratia marcescens	CCRI-22261	France	2013	VIM-2

ATCC: American Type Culture Collection
CCRI: Culture Collection of the Centre de Researche en Infectiologie
NCTC: National Collection of Type Cultures
CCUG: Culture Collection, University of Gothenburg

Table 9. Summary of results from the GenePOC Carba Inclusivity Study stratified by carbapenemase gene variant and bacterial species

Species	*bla*$_{NDM}$ Variants Detected					Total
	1	**4**	**5**	**6**	**7**	
Enterobacter cloacae	1	0	0	0	0	**1**
Escherichia coli	1	0	2	1	1	**5**
Klebsiella pneumoniae	5	1	0	0	0	**6**
Providencia rettgeri	1	0	0	0	0	**1**
Providencia stuartii	1	0	0	0	0	**1**
Total	**9**	**1**	**2**	**1**	**1**	**14**

Species	*bla*$_{KPC}$ Variant Detected				Total
	Not Known	**2**	**3**	**4**	
Enterobacter cloacae	0	0	0	1 [1]	**1**
Escherichia coli	1	0	0	0	**1**
Klebsiella oxytoca	0	0	1	0	**1**
Klebsiella pneumoniae	0	4	3	0	**7**
Pseudomonas aeruginosa	0	1	0	0	**1**
Total	**1**	**5**	**4**	**1**	**11**

Species	*bla*$_{OXA-48}$ Variants Detected			Total
	48	**181**	**204**	
Citrobacter freundii	0	0	1	**1**
Enterobacter cloacae	1	0	0	**1**
Escherichia coli	2	0	0	**2**
Klebsiella pneumoniae	4	1	1	**6**
Providencia rettgeri	0	1	0	**1**
Total	**7**	**2**	**2**	**11**

Species	*bla*$_{IMP}$ Variants Detected					Total
	1	**4**	**8**	**9**	**11**	
Acinetobacter baumannii	1	0	0	0	0	**1**
Citrobacter youngae	0	1	0	0	0	**1**
Escherichia coli	1	0	0	0	0	**1**
Klebsiella pneumoniae	2	2	1	0	0	**5**
Pseudomonas aeruginosa	1	0	0	1	0	**2**
Serratia marcescens	0	0	0	0	1	**1**
Total	**5**	**3**	**1**	**1**	**1**	**11**

Species	*bla*$_{VIM}$ Variants Detected				Total
	1	**2**	**10**	**19**	
Klebsiella pneumoniae	4	0	0	1	**5**
Pseudomonas aeruginosa	0	2	1	0	**4**
Serratia marcescens	0	1	0	0	**1**
Total	**4**	**3**	**1**	**1**	**9**

Species [2]	*bla* Variants Detected				Total
	bla$_{KPC}$	*bla*$_{VIM}$	*bla*$_{OXA-48}$	*bla*$_{NDM}$	
	2	**2**	**232**	**1**	
Klebsiella pneumoniae	0		1		**1**
Pseudomonas aeruginosa	1		0		**1**
Total	**1**		**1**		**2**

[1] On initial testing, 3/3 replicates were positive for *bla*$_{KPC}$ and 1/3 replicates also produced a false positive result for *bla*$_{NDM}$; upon repeat testing, 3/3 replicates were positive for *bla*$_{KPC}$ and none was positive for any other carbapenemase gene

[2] Isolates with multiple carbapenemase genes

In Silico Analysis

In addition to laboratory testing, the inclusivity of the GenePOC Carba assay was demonstrated through *in silico* analysis of the targeted carbapenemase genes using the Basic Local Alignment Search Tool (BLAST). Predictions of the likelihood of detecting different gene variants were made based on the sequence data available in GenBank in November, 2018. A summary of the results is presented in **Table 10**. The analysis showed that the GenePOC Carba assay was likely to detect most of the known clinically relevant subtypes of the targeted carbapenemase genes. A Limitation in the device labeling indicates that analysis of new variant sequences deposited in GenBank after 2018 has not been performed.

Table 10. Summary of *in silico* analysis of the inclusivity of the GenePOC Carba assay

Gene	Variant Sequences Available	Prediction based on *In Silico* Analysis [1]			
		Detectable [2]	Likely Detectable [3]	Potentially Detectable [4]	Not Detectable [5]
bla$_{NDM}$	24	24 (1-24)	--	--	--
bla$_{KPC}$	37	37 (2-38)	--	--	--
bla$_{OXA}$	30	12 (48, 162, 181, 199, 204, 244, 245, 252[6], 370, 484, 505, 566)	6 (232, 514 [6], 515 [6], 519, 546 [6], 547 [6])	--	12 (54 [6], 163 [7], 247 [7], 405 [7], 416 [6], 436, 438 [7], 439 [7], 517, 535 [6], 538 [6], 567)
bla$_{IMP}$	73	2 (16, 74)	49 (1, 2, 4-6, 8-10, 13-15, 17-20, 23-26, 28-30, 32, 33, 37, 38, 40, 42, 45, 47-49, 53-56, 59, 60, 62, 66, 69-72, 75-79)	18 (3, 7, 11, 21, 22, 27, 34, 41, 43, 44, 51, 52, 58, 61, 64, 67, 68, 73)	4 (12, 31, 35, 63) [8]
bla$_{VIM}$	58	52 (1-6, 8-12, 14-20, 23-46, 48-50, 52-55, 57, 59, 60)	--	3 (51, 56, 58)	3 (7 [9], 13, 47)

[1] Based on the percentage of homology of the primers and probe(s) to the target sequence and the number and location of mismatches. The number of variant sequences in each category is shown, followed by the applicable variant types in parentheses.
[2] 100% homology of each primer/detector probe with the target sequence
[3] 95-100% homology of each primer/detector probe with the target sequence
[4] <95% homology of one or more primers and/or the detector probe with the target sequence but ≤2 nucleotide mismatches over their entire length
[5] Failure to fulfill the parameters to be considered "Detectable," "Likely Detectable" or "Potentially Detectable"
[6] Associated with *Shewanella*, a rare human pathogen
[7] Associated with a mutation that results in the absence of carbapenemase activity (Dortet L. *et al*. Antimicrob. Agents Chemother 2015 59 (7): 3823-3828)
[8] Reported in *Pseudomonas aeruginosa* (types 31, 35 and 63) and *Pseudomonas putida* (type 12)
[9] Atypical *bla*$_{VIM}$ variant (Toleman M.A., *et al*. Antimicrob Agents Chemother 2004 48 (1): 329-332)

7. Assay Cut-Off:

Cut-off parameters for each of the five target genes and the Process Control were established by Receiver Operating Characteristic (ROC) curve analysis of endpoint fluorescence values

obtained from testing known positive and negative samples, in conjunction with analysis of the associated cycle threshold (Ct) values.

8. Accuracy (Instrument):

Not applicable.

9. Carry-Over:

Studies were performed to evaluate the likelihood of obtaining false positive results with the GenePOC Carba assay due to carry-over (run-to-run) or cross- (sample-to-sample) contamination. The potential for carry-over contamination was evaluated by performing 10 alternating runs of all positive and all negative samples. Similarly, the potential for cross-contamination was evaluated by testing alternating positive and negative samples within a revogene instrument carousel over a total of 10 runs. In each case, a single revogene instrument was used for all 10 runs. The positive samples comprised an isolate of bla_{KPC}-positive *K. pneumoniae* at a starting concentration of ~4 McFarland (i.e., ~5-10 times higher concentration than the prescribed workflow for the GenePOC Carba assay which uses a starting suspension density of 0.5 McFarland). The negative samples comprised a carbapenem non-susceptible isolate that did not possess any of the targeted carbapenemase genes. A summary of the results is presented in **Table 11**. Across the two studies, a total of three (3) false positive results was observed, none of which were for bla_{KPC}. The false positive results for bla_{IMP} (2) and bla_{VIM} (1) were attributed to contamination in the laboratory environment.

The device labeling contains instructions regarding the preparation and testing of External Negative controls to monitor performance and reduce the potential for reporting of erroneous results due to laboratory contamination. Additional precautions for handling previously used PIEs and for decontaminating the revogene instrument are also provided. This is acceptable.

Table 11. Summary of results from the GenePOC Carba Contamination Study

Gene Target	Positive Results/Total Tested			
	Carry-over		Cross	
	High Positive [1]	Negative	High Positive [1]	Negative
bla_{NDM}	0/40	0/40	0/40	0/40
bla_{KPC}	40/40	0/40	40/40	0/40
$bla_{OXA\text{-}48\text{-}like}$	0/40	0/40	0/40	0/40
bla_{IMP}	1/40	0/40	0/40	1/40
bla_{VIM}	1/40	0/40	0/40	0/40

[1] bla_{KPC}-positive containing $\geq 10^7$ CFU/mL in the Sample Buffer Tube

B Comparison Studies:

1. Method Comparison with Predicate Device:

The performance of the GenePOC Carba assay was evaluated in a study that was conducted at three sites (two in the U.S. and one ex-U.S.) with a mixture of prospectively collected and stock isolates of carbapenemase producing organisms. Prior to inclusion in the study, each isolate was confirmed by MALDI-TOF analysis to be either *Acinetobacter baumannii*,

Pseudomonas aeruginosa or a species that is a member of the *Enterobacteriaceae,* and to be non-susceptible to one or more carbapenems as determined by disc diffusion according to standard procedures (CLSI, M02-13 and M100-S28). The results obtained with the GenePOC Carba assay were compared to those produced by another FDA-cleared device for the detection of the targeted carbapenemase resistance markers from isolated colonies, used according to the manufacturer's instructions. Testing with the GenePOC assay was performed with organisms grown on both blood and MacConkey agar. The comparator method was performed independently using organisms grown on blood agar.

Note: The performance of the FDA-cleared device used as the comparator for the GenePOC Carba assay was previously evaluated in comparison to PCR/bidirectional sequencing and exhibited 100% sensitivity and close to 100% specificity for detection of the targeted carbapenemase resistance markers from isolated colonies of carbapenem non-susceptible bacteria. Because the isolates included in the study were identified to the species level and their carbapenem non-susceptible status was determined by reference methods, use of this device as a reference comparator method to evaluate the sensitivity and specificity of the GenePOC Carba assay was determined to be acceptable.

Five hundred and thirty-two (532) bacterial isolates (475 stock and 57 prospective) were initially enrolled in the study. Of these, 16 were excluded from the analysis of performance for the following reasons: eleven (11) did not meet the inclusion criteria for species identification (either they were not among the targeted organisms or organism identification was inconclusive), three (3) were unavailable for analysis due to laboratory error and two (2) were found to be susceptible to all four carbapenems tested. Four (4) additional isolates were also excluded because they were associated with a Negative External Control failure on initial testing and produced Indeterminate results upon repeat. The performance of the GenePOC Carba assay was therefore evaluated using a total of 512 carbapenem non-susceptible isolates of *Enterobacteriaceae* (306), *Acinetobacter baumannii* (99) and *Pseudomonas aeruginosa* (107) (**Tables 12** and **13**). Performance stratified by each organism group is shown in **Tables 14** and **15** for colonies grown on blood and MacConkey agar, respectively.

Table 12. Performance of the GenePOC Carba assay for the detection of carbapenemase resistance markers in carbapenem non-susceptible isolates of *Enterobacteriaceae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* grown on blood agar

GenePOC Carba Performance with Colonies Grown on Blood Agar				
***bla*$_{NDM}$**		**Reference Comparator Method**		
		Positive	**Negative**	**Total**
GenePOC Carba	**Positive**	186	2 [1]	**188**
	Negative	2 [2]	322	**324**
	Total	**188**	**324**	**512**
Sensitivity		98.9% (186/188); 96.2-99.7%		
Specificity		99.4% (322/324); 97.8-99.8%		
***bla*$_{KPC}$**		**Reference Comparator Method**		
		Positive	**Negative**	**Total**
GenePOC Carba	**Positive**	113	3 [3]	**117**
	Negative	1 [4]	395	**396**
	Total	**114**	**398**	**512**
Sensitivity		99.1% (113/114); 95.2-99.8%		
Specificity		99.2% (395/398); 97.8-99.7%		
***bla*$_{OXA-48}$**		**Reference Comparator Method**		
		Positive	**Negative**	**Total**
GenePOC Carba	**Positive**	65	3 [5]	**68**
	Negative	0	444	**444**
	Total	**65**	**447**	**512**
Sensitivity		100% (65/65); 94.4-100%		
Specificity		99.3% (444/447); 98.0-99.8%		
***bla*$_{IMP}$**		**Reference Comparator Method**		
		Positive	**Negative**	**Total**
GenePOC Carba	**Positive**	27	19 [6]	**46**
	Negative	0	466	**466**
	Total	**27**	**485**	**512**
Sensitivity		100% (27/27); 87.5-100%		
Specificity		96.1% (466/485); 94.0-97.5%		
***bla*$_{VIM}$**		**Reference Comparator Method**		
		Positive	**Negative**	**Total**
GenePOC Carba	**Positive**	52	1 [7]	**53**
	Negative	0	459	**459**
	Total	**52**	**460**	**512**
Sensitivity		100% (52/52); 93.1-100%		
Specificity		99.8% (459/460); 98.8-100%		

Sensitivity and specificity are reported with two-sided 95% score confidence intervals

Note: Isolates with discordant results between the GenePOC Carba assay and the reference method were investigated using alternative PCR assays for each of the 5 target carbapenemase genes, followed by bidirectional sequencing. The results of this discordant analysis are summarized in the footnotes below:

[1] 1/2 positive for *bla*$_{NDM-1}$

[2] 1/2 positive for *bla*$_{NDM-1}$; 1/2 positive for *bla*$_{OXA-48}$

[3] 2/3 positive for *bla*$_{KPC-3/KPC-38}$

[4] 1/1 positive for *bla*$_{KPC-3/KPC-38}$

[5] 1/3 positive for *bla*$_{OXA-48}$

[6] 17/19 positive for *bla*$_{IMP}$ (11 *bla*$_{IMP-13/IMP-37}$; 2 *bla*$_{IMP-62}$; 1 each of *bla*$_{IMP-4}$, *bla*$_{IMP-15}$, *bla*$_{IMP-16}$ and *bla*$_{IMP-27/IMP-64}$)

[7] 1/1 Negative for *bla*$_{VIM}$

Table 13. Performance of the GenePOC Carba assay for the detection of carbapenemase resistance markers in carbapenem non-susceptible isolates of *Enterobacteriaceae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* grown on MacConkey agar

GenePOC Carba Performance with Colonies Grown on MacConkey Agar				
***bla*$_{NDM}$**		**Reference Comparator Method**		
		Positive	**Negative**	**Total**
GenePOC Carba	**Positive**	186	2 [1]	**188**
	Negative	2 [2]	322	**324**
	Total	**188**	**324**	**512**
Sensitivity		98.9% (186/188); 96.2-99.7%		
Specificity		99.4% (322/324); 97.8-99.8%		
***bla*$_{KPC}$**		**Reference Comparator Method**		
		Positive	**Negative**	**Total**
GenePOC Carba	**Positive**	114	3 [3]	**117**
	Negative	0	395	**395**
	Total	**114**	**398**	**512**
Sensitivity		100% (114/114); 96.7-100%		
Specificity		99.2% (395/398); 97.8-99.7%		
***bla*$_{OXA-48}$**		**Reference Comparator Method**		
		Positive	**Negative**	**Total**
GenePOC Carba	**Positive**	65	2 [4]	**67**
	Negative	0	445	**445**
	Total	**65**	**447**	**512**
Sensitivity		100% (65/65); 94.4-100%		
Specificity		99.6% (445/447); 98.4-99.9%		
***bla*$_{IMP}$**		**Reference Comparator Method**		
		Positive	**Negative**	**Total**
GenePOC Carba	**Positive**	27	21 [5]	**48**
	Negative	0	464	**464**
	Total	**27**	**485**	**512**
Sensitivity		100% (27/27); 87.5-100%		
Specificity		95.7% (464/485); 93.5-97.2%		
***bla*$_{VIM}$**		**Reference Comparator Method**		
		Positive	**Negative**	**Total**
GenePOC Carba	**Positive**	52	1 [6]	**53**
	Negative	0	459	**459**
	Total	**52**	**460**	**512**
Sensitivity		100% (52/52); 93.1-100%		
Specificity		99.8% (459/460); 98.8-100%		

Sensitivity and specificity are reported with two-sided 95% score confidence intervals

Note: Isolates with discordant results between the GenePOC Carba assay and the reference method were investigated using alternative PCR assays for each of the 5 target carbapenemase genes, followed by bidirectional sequencing. The results of this discordant analysis are summarized in the footnotes below:

[1] 1/2 positive for *bla*$_{NDM-1}$

[2] 1/2 positive for *bla*$_{NDM-1}$; 1/2 positive for *bla*$_{OXA-48}$

[3] 2/3 positive for *bla*$_{KPC-3/KPC-38}$

[4] 1/2 positive for *bla*$_{OXA-48}$

[5] 17/21 positive for *bla*$_{IMP}$ (11 *bla*$_{IMP-13/IMP-37}$, 2 *bla*$_{IMP-62}$; 1 each of *bla*$_{IMP-4}$, *bla*$_{IMP-15}$, *bla*$_{IMP-16}$ and *bla*$_{IMP-27/IMP-64}$

[6] 0/1 positive for *bla*$_{VIM}$

Table 14. Performance of the GenePOC Carba assay for the detection of carbapenemase resistance markers in carbapenem non-susceptible isolates grown on blood agar, stratified by organism group

GenePOC Carba Performance with Colonies Grown on Blood Agar [1]			
Organism Group	***bla* Gene Target**	**Sensitivity**	**Specificity**
Enterobacteriaceae	NDM	98.8% (85/86) 93.7-99.8% [1]	99.5% (219/220) 97.5-99.9%
	KPC	99.1% (112/113) 95.2-99.8%	98.4% (190/193) 95.5-99.5%
	OXA-48	100% (64/64) 94.3-100%	98.8% (239/242) 96.4-99.6%
	IMP	100% (14/14) 78.5-100%	98.3% (287/292) 96.1-99.3%
	VIM	100% (12/12) 75.8-100%	100% (294/294) 98.7-100%
Acinetobacter baumannii	NDM	98.7% (75/76) 92.9-99.8%	100% (23/23) 85.7-100%
	KPC	100% (1/1) 20.7-100%	100% (98/98) 96.2-100%
	OXA-48	Not Applicable	100% (99/99) 96.3-100%
	IMP	100% (8/8) 67.6-100%	100% (91/91) 96.0-100%
	VIM	100% (1/1) 20.7-100%	99.0% (97/98) 94.4-99.8%
Pseudomonas aeruginosa	NDM	100% (26/26) 87.1-100%	98.8% (80/81) 93.3-99.8%
	KPC	Not Applicable	100% (107/107) 96.5-100%
	OXA-48	100% (1/1) 20.7-100%	100% (106/106) 96.5-100%
	IMP	100% (5/5) 56.6-100%	86.3% (88/102) 78.3-91.6%
	VIM	100% (39/39) 91.0-100%	100% (68/68) 94.7-100%

[1] Sensitivity and specificity are reported with two-sided 95% score confidence intervals

Table 15. Performance of the GenePOC Carba assay for the detection of carbapenemase resistance markers in carbapenem non-susceptible isolates grown on MacConkey agar, stratified by organism group

GenePOC Carba Performance with Colonies Grown on MacConkey Agar [1]			
Organism Group	***bla* Gene Target**	**Sensitivity**	**Specificity**
Enterobacteriaceae	NDM	98.8% (85/86) 93.7-99.8%	99.5% (219/220) 97.5-99.9%
	KPC	100% (113/113) 96.7-100%	98.4% (190/193) 95.5-99.5%
	OXA-48	100% (64/64) 94.3-100%	99.2% (240/242) 97.0-99.8%
	IMP	100% (14/14) 78.5-100%	98.3% (287/292) 96.1-99.3%
	VIM	100% (12/12) 75.8-100%	99.7% (293/294) 98.1-99.9%
Acinetobacter baumannii	NDM	98.7% (75/76) 92.9-99.8%	100% (23/23) 85.7-100%
	KPC	100% (1/1) 20.7-100%	100% (98/98) 96.2-100%
	OXA-48	Not Applicable	100% (99/99) 96.3-100%
	IMP	100% (8/8) 67.6-100%	97.8% (89/91) 92.3-99.4%
	VIM	100% (1/1) 20.7-100%	100% (98/98) 96.2-100%
Pseudomonas aeruginosa	NDM	100% (26/26) 87.1-100%	98.8% (80/81) 93.3-99.8%
	KPC	Not Applicable	100% (107/107) 96.5-100%
	OXA-48	100% (1/1) 20.7-100%	100% (106/106) 96.5-100%
	IMP	100% (5/5) 56.6-100%	86.3% (88/102) 78.3-91.6%
	VIM	100% (39/39) 91.0-100%	100% (68/68) 94.7-100%

[1] Sensitivity and specificity are reported with two-sided 95% score confidence intervals

There were 22 isolates on blood agar and 24 on MacConkey agar that produce positive results for two or more carbapenemase genes (**Table 16**). All 21 isolates that were positive for both bla_{NDM} and bla_{OXA} by the reference method produced the expected results with the GenePOC Carba assay from both types of culture media.

Table 16. Summary of isolates that produced positive results for multiple carbapenemase genes with the GenePOC Carba assay

Culture Medium [1]	Number	Carbapenemase Gene(s) Reported	
		Reference Comparator	GenePOC Carba
Blood Agar	21	*bla*$_{NDM}$, *bla*$_{OXA-48}$	*bla*$_{NDM}$, *bla*$_{OXA-48-like}$ [1]
	1	*bla*$_{NDM}$	*bla*$_{NDM}$, *bla*$_{VIM}$ [2]
MacConkey Agar	21	*bla*$_{NDM}$, *bla*$_{OXA-48}$	*bla*$_{NDM}$, *bla*$_{OXA-48-like}$ [1]
	2	*bla*$_{NDM}$	*bla*$_{NDM}$, *bla*$_{IMP}$ [2]
	1	*bla*$_{KPC}$	*bla*$_{KPC}$, *bla*$_{VIM}$

[1] All 21 isolates that were positive for *bla*$_{NDM}$ and *bla*$_{OXA}$ by the reference comparator method were also positive by the GenePOC Carba assay from both culture media

[2] One (1) isolate that was positive for *bla*$_{NDM}$ only by the reference comparator method was positive for *bla*$_{NDM/VIM}$ from blood agar and *bla*$_{NDM/VIM}$ from MacConkey agar

The number and percentage of Unresolved and Indeterminate results after initial and repeat testing of colonies grown on blood and MacConkey agar is shown in **Table 17**.

Table 17. Summary of Unresolved and Indeterminate results observed with the GenePOC Carba assay

Culture Medium	Number (%) [n = 516] [1]			
	Unresolved		Indeterminate	
	Initial	Final	Initial	Final
Blood agar	1 (0.2) [2]	0 (0)	8 (1.6)	4 (0.8)
MacConkey agar	1 (0.2) [3]	0 (0)	6 (1.2)	4 (0.8)

[1] Denominator includes 4 isolates that were associated with a Negative Control failure on initial testing and which produced Indeterminate results upon repeat

[2] Sample reported Unresolved for *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{OXA-48-like}$ and *bla*$_{IMP}$ but Positive for *bla*$_{VIM}$

[3] Sample reported Unresolved for all five gene targets

2. Matrix Comparison:

Fresh vs Frozen Study

A study was performed to evaluate the effect on GenePOC Carba assay performance of freezing standardized suspensions of bacteria prior to testing. Representative carbapenemase non-susceptible isolates were grown under selective conditions and isolated colonies were used to prepare 0.5 McFarland suspensions that were tested "fresh" after storage at 2-8°C or after freezing at -20°C for ≥24 hours. All results were as expected. The results from this study supported use of frozen panel members in the Reproducibility and Precision Study described in **Section VII A1**.

C Clinical Studies:

1. Clinical Sensitivity:

Refer to **Section VII B(1)**, above.

2. Clinical Specificity:

Refer to **Section VII B(1)**, above.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The performance of the GenePOC Carba assay was evaluated using a collection of prospectively collected and archived isolates of carbapenem non-susceptible bacteria. Five hundred and twelve (512) isolates of *Enterobacteriaceae*, *A. baumannii* and *P. aeruginosa* were included in the study, of which 427 were found to be positive for one or more carbapenemase resistance genes using an FDA-cleared reference molecular assay. In comparison, the GenePOC Carba assay gave a positive result for one or more carbapenemase genes with 449 isolates. A summary of the carbapenemase resistance genes identified by the reference method and by the GenePOC Carba assay is shown in **Table 18**.

Table 18. Summary of carbapenemase resistance genes identified in the Clinical Study for the GenePOC Carba assay

Carbapenemase Resistance Gene	Number of Carbapenem Non-susceptible Isolates (%) [n = 512] [1]		
	Reference Comparator	GenePOC Carba	
		Blood Agar	MacConkey Agar
NDM	188 (36.7)	188 (37.5)	188 (37.5)
KPC	114 (22.3	116 (22.7)	117 (22.9)
OXA	65 (12.7)	68 (13.3)	67 (13.1)
IMP	27 (5.3)	46 (9.0)	48 (9.4)
VIM	52 (10.2)	53 (10.4)	53 (10.4)
Multiple	21 (4.1) [2]	22 (4.3) [3]	24 (4.7) [4]
Negative	87 (17.0)	63 (12.3)	63 (12.3)

[1] Percentages do not add up to 100 because some isolates were positive for two or more carbapenemase resistance genes
[2] 21/21 positive for bla_{NDM} and bla_{OXA}
[3] 21/22 positive for bla_{NDM} and bla_{OXA}; 1/22 positive for bla_{NDM} and bla_{VIM}
[4] 21/24 positive for bla_{NDM} and bla_{OXA}; 2/24 positive for bla_{NDM} and bla_{IMP}; 1/24 positive for bla_{KPC} and bla_{VIM}

F Other Supportive Instrument Performance Characteristics Data:

Not applicable.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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