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

I. Background Information:

A. 510(k) Number

K190341

B. Applicant

iCubate, Inc.

C. Proprietary and Established Names

iC-GN iC-Cassette for use on the iC-System
iC-GN Assay

D. Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PEN	Class II	21 CFR 866.3365 - Multiplex Nucleic Acid Assay For Identification Of Microorganisms And Resistance Markers From Positive Blood Cultures	MI - Microbiology

II. Submission/Device Overview:

A. Purpose for Submission:

The purpose of the submission is to obtain clearance for the iC-GN Assay for use on the iC-System.

B. Measurand:

Bacterial Species: *Acinetobacter baumannii* complex, *Enterobacter cloacae* complex, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus* species, and *Serratia marcescens*

Resistance Markers
KPC (bla_{KPC})- associated with resistance to carbapenems
NDM (bla_{NDM})- associated with resistance to carbapenems
CTX-M group 1($bla_{CTX\text{-}M}$ group 1)- associated with resistance to extended spectrum beta-lactams

C. Type of Test:
The iC-GN Assay utilizes polymerase chain reaction (PCR) for the multiplex amplification of specific targets and detects the amplified targets with microarray hybridization. Targets are detected directly from patient positive blood cultures confirmed by Gram stain to contain Gram-negative bacilli. The iC-GN Assay utilizes proprietary ARM-PCR (Amplicon Rescued Multiplex PCR) technology allowing for multiple targets to be amplified in one reaction. Testing is done in a self-contained, automated, disposable cassette using the iCubate processor (iC-Processor). After the reaction is complete, the cassette is read on the iCubate reader (iC-Reader). Results from the iC-Reader are interpreted by iC-Report software and a final report is displayed on the iMac computer. Results are qualitative.

II. Intended Use/Indications for Use:

A. Intended Use(s):
See Indications for Use below.

B. Indication(s) for Use:
The iCubate, Inc. iC-GN Assay for use on the iC-System is a qualitative, multiplexed, in vitro diagnostic test for the detection and identification of potentially pathogenic Gram-negative bacteria which may cause bloodstream infection (BSI). The iC-GN Assay is performed directly on positive blood cultures, confirmed by Gram stain to contain Gram-negative bacilli. Cultures demonstrating mixed Gram stain results should not be tested on the assay. The iC-GN Assay is validated for use with select BACTEC, BacT/ALERT and VersaTREK blood culture bottles. The iC-GN Assay is indicated for use in conjunction with other clinical and laboratory findings, such as culture, to aid in the diagnosis of bacterial bloodstream infections; however, it is not used to monitor bloodstream infections.

The iC-GN Assay detects target DNA and identifies the following:

Bacterial Genera and Species
Acinetobacter baumannii complex, *Enterobacter cloacae* complex, *Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus* species, *Serratia marcescens*

Resistance Markers
KPC (blaKPC)- associated with resistance to carbapenems, NDM (blaNDM)- associated with resistance to carbapenems, CTX-M group 1(blaCTX-M group 1)- associated with resistance to extended spectrum beta-lactams

In mixed growth, the iC-GN Assay does not specifically attribute detection of KPC, NDM, or CTX-M group 1 to a specific genera or species.

Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing, identification of organisms not detected by the iC-GN Assay, differentiation of mixed growth, association of antimicrobial resistance marker genes to a specific organism, or for epidemiological typing.

C. Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D. Special Instrument Requirements:
Requires the iC-System

III. Device/System Characteristics:

A. Device Description:
The iC-GN Assay utilizes polymerase chain reaction (PCR) for the multiplex amplification of specific targets and detects the amplified targets with microarray hybridization. Targets are detected directly from patient positive blood cultures confirmed by Gram stain to contain Gram-negative bacilli. The iC-GN Assay utilizes proprietary ARM-PCR (Amplicon Rescued Multiplex PCR) technology allowing for multiple targets to be amplified in one reaction. Testing is done in a self-contained, automated, disposable cassette using the iCubate processor (iC-Processor). After the reaction is complete, the cassette is read on the iCubate reader (iC-Reader). Results from the iC-Reader are interpreted by iC-Report software and a final report is displayed on the iMac computer.

To operate, the user opens the iC-Cassette cap and pipettes an aliquot of the diluted positive blood culture sample into the sample/PCR well in the bottom well plate of the cassette. Once inoculated, the cassette cap is closed, and all extraction, amplification and detection processes are completed in the cassette, a closed system. Extraction, amplification and detection sequences are defined by an assay script controlled by the iC–Processor.

The processing script is defined within a barcode label positioned on the top of each iC-Cassette which communicates with the iC-Processor. To access and pierce the foil-sealed reagent wells located in the bottom well plate of the cassette, the processor manipulates the cassette to move the cassette pipette horizontally and vertically. The script directs the transfer of reagents between the wells in the bottom well plate and finally to the array within the cassette. The iC-Processor is capable of processing four (4) iC-Cassettes with random access.

Once processing is complete, the cassette is manually transferred from the iC-Processor to the iC-Reader where the microarray within the cassette is read. The iC-Reader is capable of reading up to four (4) iC-Cassettes at one time. The results are interpreted via the iC-Report software and displayed for the user on the iMac. Raw data and result interpretations are stored within the iMac; raw data is accessible to iCubate service personnel only and not to the end user.

When finished with a loaded iC-GN Cassette, it should be disposed as biohazardous waste.

B. Principle of Operation:

Amplicon Rescued Multiplex PCR (ARM-PCR)

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:
   iC-System which was cleared under K163390.

IV. Substantial Equivalence Information:

A. Predicate Device Name(s):
Verigene Gram-Negative Blood Culture Nucleic Acid Test (bc-gn)

B. Predicate 510(k) Number(s):
K132843

C. Comparison with Predicate(s):

Device & Predicate Device(s):	K190341	K132843
Device Trade Name	**iC-GN Assay for use on the iC-System**	**Verigene Gram-Negative Blood Culture Nucleic Acid Test (CG-GN)**
General Device Characteristic Similarities		
Intended Use/ Indications for Use	The iCubate, Inc. iC-GN Assay for use on the iC-System is a qualitative, multiplexed, *in vitro* diagnostic test for the detection and identification of potentially pathogenic Gram-negative bacteria, which may cause bloodstream infection (BSI). The iC-GN Assay is performed directly on positive blood cultures, confirmed by Gram stain to contain Gram-negative bacilli. Cultures demonstrating mixed Gram stain results should not be tested on the assay. The iC-GN Assay is validated for use with select	The Verigene Gram-Negative Blood Culture Nucleic Acid Test (BC-GN), performed using the sample-to-results Verigene System, is a qualitative multiplexed in vitro diagnostic test for the simultaneous detection and identification of selected gram-negative bacteria and resistance markers. BC-GN is performed directly on blood culture media using blood culture bottles identified as positive by a continuous

	BACTEC, BacT/ALERT and *VersaTREK* blood culture bottles. The iC-GN Assay is indicated for use in conjunction with other clinical and laboratory findings, such as culture, to aid in the diagnosis of bacterial bloodstream infections; however, it is not used to monitor bloodstream infections. The iC-GN Assay detects target DNA and identifies the following: ***Bacterial Genera and Species*** *Acinetobacter baumannii complex, Enterobacter cloacae complex, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus species, Serratia marcescens* ***Resistance Markers*** *KPC (blaKPC)- associated with resistance to carbapenems, NDM (blaNDM)- associated with resistance to carbapenems, CTX-M group 1(blaCTX-M group 1)- associated with resistance to extended spectrum beta-lactams* In mixed growth, the iC-GN Assay does not specifically attribute KPC, NDM, or CTX-M to a specific genera or species. iC-GN is indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial bloodstream infections; however, is not to be used to monitor these infections. Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing, identification of organisms not detected by iC-GN, differentiation of mixed growth, association of antimicrobial resistance marker genes to a specific organism, or for epidemiological typing.	monitor blood culture system and which contain gram-negative bacteria as determined by Gram stain. BC-GN detects and identifies the following: *Acinetobacter* spp. *Citrobacter* spp. *Enterobacter* spp. *Proteus* spp. *Escherichia coli* *Klebsiella pneumoniae* *Klebsiella oxytoca* *Pseudomonas aeruginosa* BC-GN is indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial bloodstream infections; however, it is not to be used to monitor these infections. Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing, identification of organisms not detected by BC-GN, differentiation of mixed growth, association of antimicrobial resistance marker genes to a specific organism, of for epidemiological typing.
Sample Type	Positive Blood Culture	Positive Blood Culture
General Device Characteristic Differences		
Instrument Requirements	iC-System	Verigene System
Test Principle	ARM-PCR	Gold nanoparticle probe-based PCR
Compatible Blood Culture Bottles	BD BACTEC Standard/10 Aerobic/F BD BACTEC Standard/10 Anaerobic/F BD BACTEC Plus Aerobic/F BD BACTEC Plus Anaerobic/F	BACTEC™ Plus Aerobic/F BacT/ALERT FA FAN

	BD BACTEC Lytic/10 Anaerobic/F BacT/Alert SA Standard Aerobic BacT/Alert SN Standard Anaerobic BacT/Alert FA Aerobic FAN BacT/Alert FN Anaerobic FAN BacT/Alert FA Plus Aerobic BacT/Alert FN Plus Anaerobic VersaTREK REDOX 1 VersaTREK REDOX 2	
Throughput	Four (4) samples/iC-Processor	One (1) Sample/Processor

V. Standards/Guidance Documents Referenced:

1. *Guidance for the Content of Premarket Submissions for Software Contained in Medical* Devices
2. *Content of Premarket Submissions for Management of Cybersecurity in Medical Devices*
3. Validation of Analytical Procedures: Test and Methodology Q2(R1), ICH Harmonised Tripartite Guideline, Oct. 1994
4. Q2B Validation of Analytical Procedures: Methodology, Guidance for Industry, Nov. 1996

VI. Performance Characteristics (if/when applicable):

A. Analytical Performance:

1. Limit of Detection (LoD):
   A study was performed to determine the limit of detection for each iC-GN Assay target, defined as the lowest concentration (CFU/mL) of analyte that can be detected approximately 95% of the time. For the eleven targets detected by the iC-GN Assay, a panel of twenty-seven representative strains were evaluated, a minimum of three per target. For complex and genus level targets, at least two representative species were evaluated. LoD testing was conducted in two phases: the first phase was to narrow the range for LoD analysis. In phase II, the approximated 95% performance point determined in phase I was confirmed by testing a minimum of twenty replicates on each of three unique cassette lots. Plating and subsequent colony counts were used to determine organism concentrations. The final limit of detection for each target, is provided below.

Table 1: iC-GN Assay LoD Results			
Target	**Strain**	**Concentration (CFU/mL)**	**Defined Target LoD (CFU/mL)**
***A. baumannii* complex**	307-0294	5.3×10^5	$5.3 \times 10^5 – 5.2 \times 10^6$
	CDC-83	5.2×10^6	
	ATCC 23055	9.0×10^5	
***E. cloacae* complex**	Z101	5.0×10^6	$4.9 \times 10^5 – 5.5 \times 10^6$
	CDC-164	5.5×10^6	
	ATCC 700323	4.9×10^5	
E. coli	ATCC 43895	7.7×10^5	$7.7 \times 10^5 – 8.4 \times 10^5$
	ATCC BAA-2326	7.9×10^5	
	CDC-55	8.4×10^5	
K. oxytoca	Z115	6.2×10^5	$5.4 \times 10^5 – 1.1 \times 10^6$
	ATCC 13182	5.4×10^5	
	CDC-147	1.1×10^6	
K. pneumoniae	ATCC 35657	1.9×10^6	$6.0 \times 10^5 – 4.2 \times 10^6$
	CDC-40	3.6×10^6	
	CDC-42	1.9×10^6	
	KPC-2	4.2×10^6	
	LACNY 11	6.0×10^5	
***Proteus* species**	Z050	1.1×10^6	$6.9 \times 10^5 – 1.1 \times 10^6$
	CDC-59	9.9×10^5	
	Z028	7.6×10^5	
	Z129	6.9×10^5	
P. aeruginosa	Z139	1.2×10^6	$5.0 \times 10^5 – 1.2 \times 10^6$
	CDC-231	5.0×10^5	
	CDC-250	6.9×10^5	
S. marcescens	ATCC 43297	7.2×10^5	$6.4 \times 10^5 – 8.1 \times 10^5$
	ATCC 21212	8.1×10^5	
	CDC-91	6.4×10^5	
CTX-M group 1	ATCC BAA-2326 (CTX-M-15)	7.9×10^5	$7.9 \times 10^5 – 2.3 \times 10^6$
	CDC-40 (CTX-M-15)	2.3×10^6	
	CDC-42 (CTX-M-15)	1.9×10^6	
KPC	CDC-147 (KPC-3)	2.3×10^6	$1.5 \times 10^5 – 4.2 \times 10^6$
	KPC-2	4.2×10^6	
	CDC-231 (KPC-5)	1.5×10^5	
NDM	CDC-83 (NDM-1)	5.2×10^6	$3.3 \times 10^5 – 5.2 \times 10^6$
	CDC-55 (NDM-1)	4.0×10^6	
	CDC-250 (NDM-1)	3.3×10^5	

2. Bottle Ring:

A study was performed to establish the levels of each iC-GN target organism at two clinically relevant concentrations: initial bottle positivity (bottle "ring") and eight hours beyond initial positivity. Twenty-seven representative organisms were evaluated, a minimum of three per iC-GN target. Organisms were grown in BD BACTEC Plus Aerobic blood culture bottles with human blood added and placed on the BD BACTEC System. Within two hours of initial bottle positivity, the bottles were removed for plating and subsequent colony counts to determine organism concentrations. The bottles were then returned to the incubator and approximately eight hours after initial bottle positivity, the bottles were again removed for plating and subsequent colony counts to determine organism concentrations. Three bottles were grown for each strain. The average concentrations at initial bottle positivity and eight hours beyond initial bottle positivity are provided in below. The concentrations at initial bottle positivity, representative of the lowest levels that may be observed in a clinical setting, are above the limits of detection determined for each strain. The results of the study are as expected and establish that the established LoD is such that the assay has is able to detect clinically relevant levels of bacteria.

Table 2: iC-GN Target Organism Concentrations at Bottle "Ring"

Organism	Strain ID	Initial Bottle Positivity Average Concentration (CFU/mL)	Bottle Positivity + 8 Average Concentration (CFU/mL)
Acinetobacter baumannii	307-0294	4.24×10^8	8.27×10^8
Acinetobacter baumannii	CDC-83	3.39×10^8	7.23×10^8
Acinetobacter calcoaceticus	ATCC 23055	6.78×10^7	2.93×10^8
Enterobacter cloacae	Z101	2.17×10^8	1.97×10^9
Enterobacter cloacae	CDC-164	5.62×10^8	2.31×10^9
Enterobacter hormaechei	ATCC 700323	4.36×10^8	2.75×10^9
Escherichia coli	ATCC 43895	1.50×10^8	9.48×10^8
Escherichia coli	ATCC BAA-2326	6.23×10^8	1.52×10^9
Escherichia coli	CDC-55	4.93×10^8	1.51×10^9
Klebsiella oxytoca	Z115	5.32×10^8	2.07×10^9
Klebsiella oxytoca	ATCC 13182	4.16×10^8	4.52×10^9
Klebsiella oxytoca	CDC-147	9.67×10^8	1.31×10^9
Klebsiella pneumoniae	ATCC 35657	9.78×10^8	1.08×10^9
Klebsiella pneumoniae	CDC-40	2.16×10^8	1.36×10^9
Klebsiella pneumoniae	CDC-42	2.55×10^8	1.10×10^9
Klebsiella pneumoniae	KPC-2	7.70×10^8	1.66×10^9
Klebsiella pneumoniae	LACNY 11	5.43×10^7	1.67×10^9
Proteus mirabilis	Z050	1.71×10^8	7.40×10^8
Proteus mirabilis	CDC-59	7.37×10^7	8.10×10^8
Proteus penneri	Z028	8.88×10^7	4.33×10^8
Proteus vulgaris	Z129	4.37×10^7	5.00×10^8
Pseudomonas aeruginosa	Z139	9.18×10^7	1.37×10^{10}

Table 2: iC-GN Target Organism Concentrations at Bottle "Ring"			
Organism	**Strain ID**	**Initial Bottle Positivity Average Concentration (CFU/mL)**	**Bottle Positivity + 8 Average Concentration (CFU/mL)**
Pseudomonas aeruginosa	CDC-231	3.26×10^8	7.98×10^8
Pseudomonas aeruginosa	CDC-250	1.64×10^8	8.97×10^8
Serratia marcescens	ATCC 43297	8.55×10^8	2.03×10^9
Serratia marcescens	ATCC 21212	1.07×10^8	8.83×10^8
Serratia marcescens	CDC-91	7.28×10^8	1.67×10^9

3. Blood Culture Bottle Equivalency:

Commonly used blood culture bottle (BCB) media types were evaluated to demonstrate that variability in BCB media composition does not interfere with iC-GN Assay performance. Twenty-seven (27) representative iC-GN target organisms plus one non-target organism were tested in thirteen (13) BCB media types. Target organisms were tested near LoD concentrations (2-3×LoD). Each strain was tested in triplicate in each BCB media type. Target performance is based on all expected targets detected and no false positive targets detected. Non-target performance is based on all expected negative results. In the event of a false negative result, the strain was retested in replicates of ten. In the event of a false positive result or other failure, the strain was retested in triplicate. The results of iC-GN BCB equivalency testing are summarized in the table below. Performance in all bottle types met the acceptance criteria of ≥ 95% performance; all bottle types are validated for use with the iC GN Assay.

Table 3: iC-GN Assay BCB Equivalency Results					
BCB Media Type	**Overall Performance (%)**	**False Negatives (%)**	**False Positives (%)**	**PC Check Failures (%)**	**System Failures (%)**
BACTEC Standard Aerobic	93/94 (98.9%)	1/94 (1.1%)	0/94 (0.0%)	3/97 (3.1%)	0/97 (0.0%)
BACTEC Standard Anaerobic	85/86 (98.8%)	0/86 (0.0%)	1/86 (1.2%)	0/87 (0.0%)	1/87 (1.1%)
BACTEC Plus Aerobic	93/94 (98.9%)	1/94 (1.1%)	0/94 (0.0%)	2/97 (2.1%)	1/97 (1.1%)
BACTEC Plus Anaerobic	95/96 (98.6%)	1/96 (1.0%)	0/96 (0.0%)	2/100 (2.0%)	2/100 (2.0%)
BACTEC Lytic/10 Anaerobic	81/81 (100.0%)	0/81 (0.0%)	0/81 (0.0%)	0/81 (0.0%)	0/81 (0.0%)
BACT/ALERT SA Standard Aerobic	97/99 (98.0%)	1/99 (1.0%)	1/99 (1.0%)	4/103 (3.9%)	0/103 (0.0%)
BACT/ALERT SN Standard Anaerobic	87/88 (98.9%)	0/88 (0.0%)	1/88 (1.1%)	2/90 (2.2%)	0/90 (0.0%)

Table 3: iC-GN Assay BCB Equivalency Results					
BCB Media Type	**Overall Performance (%)**	**False Negatives (%)**	**False Positives (%)**	**PC Check Failures (%)**	**System Failures (%)**
BACT/ALERT FA Aerobic FAN	94/96 (97.9%)	0/96 (0.0%)	2/96 (2.1%)	1/97 (1.0%)	0/97 (0.0%)
BACT/ALERT FN Anaerobic FAN	92/94 (97.9%)	0/94 (0.0%)	2/94 (2.1%)	2/97 (2.1%)	1/97 (1.0%)
BACT/ALERT FA Plus Aerobic	94/95 (98.9%)	1/95 (1.1%)	0/95 (0.0%)	1/97 (1.1%)	1/97 (1.1%)
BACT/ALERT FN Plus Anaerobic	87/87 (100.0%)	0/87 (0.0%)	0/87 (0.0%)	2/90 (2.2%)	1/90 (1.1%)
VersaTREK REDOX 1	81/81 (100.0%)	0/81 (0.0%)	0/81 (0.0%)	0/81 (0.0%)	0/81 (0.0%)
VersaTREK REDOX 1	92/93 (98.9%)	1/93 (1.1%)	0/93 (0.0%)	1/94 (1.1%)	0/94 (0.0%)

4. Inclusivity:

To demonstrate the inclusivity of the iC-GN Assay, eighty-two (82) representative strains were evaluated, a minimum of ten strains for each target analyte. Strains were tested at the lowest level of bottle positivity, considered within two hours of bottle "ring." Organisms were grown in BD BACTEC Plus Aerobic blood culture bottles with human blood added on the BD BACTEC System. Each strain was tested in triplicate. Performance calculations are based on all expected targets detected and no false positive targets detected. In the event of a false negative result, the strain was retested in replicates of ten. In the event of a false positive result or other failure, the strain was retested in triplicate. Two strains were not detected by the iC-GN Assay: *Acinetobacter calcoaceticus* ATCC 31926 was not detected as *A. baumannii* complex and *Enterobacter kobei* ATCC BAA-260 was not detected as E. cloacae complex. The results of iC-GN Inclusivity testing are summarized below. The results of the inclusivity testing support identification of the organisms and resistance markers claimed in the IFU, with the exceptions noted above. It was determined that the risks associated with failure to detect a small number of organisms included in the IFU could be mitigated via labeling. These exceptions are communicated to end users in the Limitations section of the device Package Insert.

Table 4: iC-GN Assay Inclusivity Results			
Organism	**Strain**	**Targets**	**Performance**
Enterobacter kobei	ATCC BAA-260	ECX	0/13[3]
Escherichia coli	ATCC 10536	EC	3/3
Escherichia coli	ATCC BAA-2469	EC, NDM-1	3/3
Escherichia coli	NCTC 9001	EC	3/3
Escherichia coli	NCTC 10538	EC	5/5
Escherichia coli	NCTC 13476	EC	3/3

Table 4: iC-GN Assay Inclusivity Results			
Organism	**Strain**	**Targets**	**Performance**
Escherichia coli	CDC-48	EC, CTX-M-15, NDM-1	3/3
Escherichia coli	CDC-61	EC, KPC-3	3/3
Escherichia coli	CDC-104	EC, KPC-4	7/8[4]
Escherichia coli	CDC-119	EC, CTX-M-15, NDM-1	3/3
Escherichia coli	CDC-162	EC, CTX-M-15, NDM-7	3/3
Klebsiella oxytoca	ATCC 8724	KO	3/3
Klebsiella oxytoca	ATCC 43086	KO	3/3
Klebsiella oxytoca	ATCC 43165	KO	3/3
Klebsiella oxytoca	ATCC 43863	KO	3/3
Klebsiella oxytoca	ATCC 49134	KO	3/3
Klebsiella oxytoca	ATCC 49334	KO	3/3
Klebsiella oxytoca	ATCC 51817	KO	3/3
Klebsiella oxytoca	ATCC 700324	KO	3/3
Klebsiella oxytoca	NCTC 11686	KO	3/3
Klebsiella oxytoca	CDC-71	KO	3/3
Klebsiella pneumoniae	ATCC-13882	KPN	3/3
Klebsiella pneumoniae	ATCC BAA-1705	KPN, KPC-2	3/3
Klebsiella pneumoniae	NCTC 9633	KPN	3/3
Klebsiella pneumoniae	NCTC 13438	KPN, KPC-3	3/3
Klebsiella pneumoniae	NCTC 13443	KPN, CTX-M-15, NDM-1	3/3
Klebsiella pneumoniae	CDC-44	KPN, CTX-M-15	3/3
Klebsiella pneumoniae	CDC-46	KPN, CTX-M-15	5/5
Klebsiella pneumoniae	CDC-49	KPN, CTX-M-15, NDM-1	3/3
Klebsiella pneumoniae	CDC-66	KPN, CTX-M-15	3/3
Klebsiella pneumoniae subsp. ozaenae	ATCC 11296	KPN	3/3
Proteus mirabilis	ATCC 7002	Proteus	3/3
Proteus mirabilis	ATCC 21100	Proteus	3/3
Proteus mirabilis	ATCC 43071	Proteus	3/3
Proteus mirabilis	NCIMB 13283	Proteus	3/3
Proteus mirabilis	CDC-155	Proteus, KPC-6	3/3
Proteus mirabilis	CDC-156	Proteus, KPC-2	3/3
Proteus mirabilis	CDC-159	Proteus, NDM-1	3/3
Proteus penneri	ATCC 33519	Proteus	3/3
Proteus vulgaris	ATCC 9484	Proteus	3/3
Proteus vulgaris	ATCC 29905	Proteus	3/3
Pseudomonas aeruginosa	ATCC 10145	PA	3/3
Pseudomonas aeruginosa	ATCC 19429	PA	3/3
Pseudomonas aeruginosa	ATCC BAA-1744	PA	3/3
Pseudomonas aeruginosa	CDC-54	PA	3/3

Table 4: iC-GN Assay Inclusivity Results			
Organism	**Strain**	**Targets**	**Performance**
Pseudomonas aeruginosa	CDC-64	PA	3/3
Pseudomonas aeruginosa	CDC-90	PA, KPC-5	3/3
Pseudomonas aeruginosa	CDC-94	PA	3/3
Pseudomonas aeruginosa	CDC-105	PA	3/3
Pseudomonas aeruginosa	CDC-108	PA	3/3
Pseudomonas aeruginosa	CDC-246	PA, NDM-1	5/5
Serratia marcescens	ATCC 8100	SM	3/3
Serratia marcescens	ATCC 13880	SM	3/3
Serratia marcescens	ATCC 14041	SM	3/3
Serratia marcescens	ATCC 14756	SM	3/3
Serratia marcescens	ATCC 29634	SM	3/3
Serratia marcescens	ATCC 29635	SM	3/3
Serratia marcescens	ATCC 43861	SM	3/3
Serratia marcescens	ATCC 43862	SM	3/3
Serratia marcescens	NCTC 9743	SM	3/3
Serratia marcescens	CDC-99	SM	3/3

1) 2/2 false negative ABX in initial testing. 7/9 false negative ABX in repeat testing. See limitation.
2) 1/3 false positive ABX in initial testing. 1/3 false positive ABX in repeat testing. Strain repeated in replicates of 10, 10/10 repeats passed.
3) 3/3 false negative ECX in initial testing. 10/10 false negative ECX in repeat testing. See limitation.
4) 1/3 processor error in initial testing. 1/3 false positive KPN in repeat testing. Strain repeated in triplicate, 3/3 repeats passed.

An *in silico* analysis was performed for each resistance marker. The predicted reactivity of each resistance marker detected by the iC-GN Assay is summarized in the tables below.

Table 5: Predicted (*in silico*) Reactivity for CTX-M group 1			
Associated Target Organism	***Variant Detected***	***Associated Target Organism***	***Variant Detected***
Acinetobacter baumannii complex	CTX-M-3	*Klebsiella oxytoca*	CTX-M-3
	CTX-M-15		CTX-M-15
Enterobacter cloacae complex	CTX-M-1		CTX-M-35
	CTX-M-3		CTX-M-36
	CTX-M-15		CTX-M-162
	CTX-M-22	*Klebsiella pneumoniae*	CTX-M-1
	CTX-M-37		CTX-M-3
	CTX-M-55		CTX-M-15
	CTX-M-167		CTX-M-22
	CTX-M-177		CTX-M-28
	CTX-M-187		CTX-M-32
	CTX-M-224		CTX-M-54
Escherichia coli	CTX-M-1		CTX-M-55
	CTX-M-2		CTX-M-71
	CTX-M-3		CTX-M-72
	CTX-M-4		CTX-M-118
	CTX-M-5		CTX-M-124
	CTX-M-6		CTX-M-129
	CTX-M-7		CTX-M-130
	CTX-M-8		CTX-M-133
	CTX-M-9		CTX-M-135
	CTX-M-10		CTX-M-138
	CTX-M-11		CTX-M-139
	CTX-M-12		CTX-M-173
	CTX-M-15		CTX-M-176
	CTX-M-28		CTX-M-183
	CTX-M-29		CTX-M-188
	CTX-M-32		CTX-M-197
	CTX-M-33		CTX-M-204
	CTX-M-36		CTX-M-208
	CTX-M-42		CTX-M-210
	CTX-M-55		CTX-M-220
	CTX-M-58	*Proteus* species	CTX-M-15
	CTX-M-69		CTX-M-66
	CTX-M-71		CTX-M-116
	CTX-M-79		CTX-M-136
	CTX-M-82		CTX-M-164
	CTX-M-90		CTX-M-167
	CTX-M-101		CTX-M-212
	CTX-M-102	*Pseudomonas aeruginosa*	CTX-M-1
	CTX-M-103		CTX-M-15
	CTX-M-109		CTX-M-32
	CTX-M-117		CTX-M-3
	CTX-M-120		CTX-M-15

	CTX-M-125		CTX-M-22
	CTX-M-127		CTX-M-55
	CTX-M-128	*Serratia marcescens*	CTX-M-221
	CTX-M-131		
	CTX-M-132		
	CTX-M-134		
	CTX-M-137		
	CTX-M-138		
	CTX-M-139		
	CTX-M-140		
	CTX-M-141		
	CTX-M-142		
	CTX-M-143		
	CTX-M-146		
	CTX-M-158		
	CTX-M-163		
	CTX-M-166		
	CTX-M-167		
	CTX-M-170		
	CTX-M-172		
	CTX-M-175		
	CTX-M-178		
	CTX-M-179		
	CTX-M-180		
	CTX-M-181		
	CTX-M-182		
	CTX-M-184		

Table 5a: Predicted (*in silico*) Reactivity for KPC

Associated Target Organism	***Variant Detected***	***Associated Target Organism***	***Variant Detected***
Acinetobacter baumannii complex	KPC-2	*Klebsiella pneumoniae*	KPC-1
	KPC-3		KPC-2
	KPC-10		KPC-3
Enterobacter cloacae complex	KPC-1		KPC-4
	KPC-2		KPC-5
	KPC-3		KPC-6
	KPC-4		KPC-7
	KPC-13		KPC-8
	KPC-18		KPC-11
	KPC-47		KPC-14
Escherichia coli	KPC-2		KPC-15
	KPC-3		KPC-16
	KPC-12		KPC-17
	KPC-18		KPC-19
	KPC-20		KPC-22
	KPC-21		KPC-23
	KPC-28		KPC-25
Klebsiella oxytoca	KPC-2		KPC-26
	KPC-3		KPC-27

Proteus species	KPC-1		KPC-29
	KPC-2		KPC-30
Pseudomonas aeruginosa	KPC-2		KPC-31
	KPC-5		KPC-32
Serratia marcescens	KPC-2		KPC-33
			KPC-34
			KPC-35
			KPC-36
			KPC-37
			KPC-38
			KPC-39
			KPC-42
			KPC-43
			KPC-59

Table 5b: Predicted (*in silico*) Reactivity for NDM

Associated Target Organism	*Variant Detected*	*Associated Target Organism*	*Variant Detected*
Acinetobacter baumannii complex	NDM-1	*Klebsiella oxytoca*	NDM-1
	NDM-2		NDM-3
	NDM-3		NDM-4
	NDM-4	*Klebsiella pneumoniae*	NDM-1
	NDM-5		NDM-3
	NDM-7		NDM-4
	NDM-14		NDM-5
Enterobacter cloacae complex	NDM-1		NDM-6
	NDM-4		NDM-7
	NDM-5		NDM-9
	NDM-7		NDM-10
	NDM-22		NDM-16
Escherichia coli	NDM-1		NDM-23
	NDM-2		NDM-28
	NDM-3	*Proteus* species	NDM-1
	NDM-4	*Pseudomonas aeruginosa*	NDM-1
	NDM-5		NDM-5
	NDM-6	*Serratia marcescens*	NDM-1
	NDM-7		NDM-4
	NDM-9		NDM-12
	NDM-11		
	NDM-12		
	NDM-13		
	NDM-15		
	NDM-16		
	NDM-17		
	NDM-18		
	NDM-19		
	NDM-20		
	NDM-21		
	NDM-27		

5. Exclusivity:

To demonstrate the exclusivity of the iC-GN Assay, a comprehensive panel of non-target organisms that may be encountered in positive blood cultures was evaluated. A total of 114 strains were tested including organisms phylogenetically related to iC-GN target organisms as well as common blood culture contaminants. Potential cross-reactivity was

evaluated by testing exclusivity panel organisms at the highest possible concentrations, considered eight hours beyond initial bottle positivity or the equivalent. Organisms were grown in BD BACTEC Plus Aerobic blood culture bottles with human blood added on the BD BACTEC System. Each strain was tested in triplicate. Performance is based on the observation of all expected negative results. In the event of a false positive result or other failure, the organism was retested in replicates of three (3) or ten (10). Three (3) strains demonstrated reproducible cross-reactivity with iC-GN Assay targets: *Acinetobacter haemolyticus* cross-reacted with *Acinetobacter baumannii* complex, *Klebsiella variicola* cross-reacted with *Klebsiella pneumoniae,* and *Serratia odorifera* cross-reacted with *Serratia marcescens*. Exclusivity results are in the table below. Exclusivity testing confirms that the device detects organisms not listed in the IFU at low frequency. It was determined that the risks associated with the detection of organisms not listed in the IFU could be mitigated via labeling. The organisms not in the IFU that were detected are communicated to the end user in the limitations section of the Package Insert.

Table 6: iC-GN Assay Exclusivity Results

Organism	Strain	Concentration (CFU/mL)	Performance
Acinetobacter haemolyticus	ATCC 19002	7.20×10^8	0/3[1]
Acinetobacter lwoffi	Z141	2.45×10^8	3/3
Acinetobacter radioresistens	ATCC 43998	5.20×10^7	3/3
Acinetobacter schindleri	ATCC BAA618	3.50×10^8	3/3
Acinetobacter ursingii	ATCC BAA617	3.80×10^8	3/3
Aerococcus viridans	Z219	2.24×10^7	3/3
Aeromonas hydrophila	Z161	8.10×10^8	3/3
Alcaligenes faecalis	Z218	9.70×10^8	3/3
Aspergillus niger	Z105	1.62×10^8	3/3
Bacillus cereus	Z091	ND	3/3
Bacteroides fragilis	Z029	8.40×10^9	3/3
Brevundimonas vesicularis	ATCC 11426	3.80×10^8	5/5
Burkholderia cepacia	ATCC 25416	5.40×10^8	3/3
Campylobacter coli	Z293	3.90×10^8	3/3
Campylobacter jejuni	Z086	4.60×10^8	11/13[2]
Candida albicans	Z006	ND	3/3
Candida glabrata	Z007	3.20×10^7	3/3
Candida krusei	Z009	1.90×10^7	3/3
Candida parapsilosis	Z011	9.00×10^6	3/3
Candida tropicalis	Z012	3.50×10^7	4/4
Cedecea davisae	ATCC 33431	6.20×10^8	3/3
Citrobacter amalonaticus	Z051	8.4×10^8	3/3
Citrobacter braakii	ATCC 51113	4.90×10^8	3/3
Citrobacter freundii	Z064	2.25×10^8	3/3
Citrobacter koseri	Z039	1.14×10^9	3/3

Table 6: iC-GN Assay Exclusivity Results			
Organism	**Strain**	**Concentration (CFU/mL)**	**Performance**
Citrobacter sedlakii	ATCC 51115	9.80×10^8	2/2
Clostridium difficile (NAP-1 toxigenic)	NAP1	4.87×10^7	4/4
Clostridium difficile (non-toxigenic)	Z228	5.93×10^7	3/3
*Clostridium novyi**	Z179	1.14×10^7	5/5
Corynebacterium amycolatum	Z284	9.26×10^8	3/3
Corynebacterium genitalium	Z328	1.35×10^8	3/3
Corynebacterium jeikeium	Z232	8.50×10^8	4/4
Corynebacterium striatum	MCW000	2.07×10^9	5/6[3]
Cronobacter muytjensii	ATCC 51329	2.79×10^8	3/3
Cronobacter sakazakii	ATCC 29544	6.90×10^8	3/3
Cryptococcus neoformans	Serotype A	2.15×10^8	3/3
Edwardsiella tarda	Z183	8.70×10^7	4/5[4]
Enterobacter aerogenes	Z052	1.77×10^9	5/5
Enterobacter amnigenus	ATCC 51816	7.50×10^8	3/3
Enterococcus avium	Z171	2.58×10^8	5/6[5]
Enterococcus casseliflavus	Z002	2.44×10^9	4/4
Enterococcus cecorum	Z208	1.03×10^9	5/6[6]
Enterococcus faecalis	ATCC 51299	2.13×10^9	3/3
Enterococcus faecium	ATCC 700221	7.20×10^8	3/3
Enterococcus gallinarum	Z209	1.35×10^9	3/3
Enterococcus hirae	Z193	2.37×10^8	3/3
Enterococcus raffinosus	ATCC 49427	5.40×10^8	3/3
Escherichia fergusonii	ATCC 35469	8.70×10^8	3/3
Escherichia hermannii	Z184	1.01×10^9	5/5
Escherichia vulneris	ATCC 33821	7.50×10^8	3/3
Fusobacterium varium	Z361	2.49×10^9	3/3
Hafnia alvei	ATCC 51815	1.37×10^9	3/3
Haemophilus influenzae	ATCC 10211	3.09×10^9	3/3
Haemophilus parainfluenzae	ATCC 9796	1.33×10^8	3/3
Klebsiella variicola	ATCC 31488	4.40×10^8	0/3[7]
Kluyvera ascorbata (KPC+)	CDC-0144	1.40×10^9	3/3
Kocuria kristinae	Z250	7.20×10^7	3/3
Kytococcus schroeteri	ATCC BAA2410	1.50×10^7	3/3
Lactobacillus acidophilus	Z048	6.00×10^8	3/3
Lactobacillus plantarum	17-5	5.30×10^8	3/3
Lactobacillus reuteri	Z333	5.80×10^7	5/5
Lactococcus lactis	Z169	9.30×10^7	3/3
Leclercia adecarboxylata	ATCC 23216	1.01×10^9	3/3
Leminorella grimontii	Z364	4.00×10^9	3/3
Leuconostoc mesenteroides	Z197	4.00×10^7	5/5

Table 6: iC-GN Assay Exclusivity Results			
Organism	**Strain**	**Concentration (CFU/mL)**	**Performance**
Listeria monocytogenes	ATCC 19115	2.03×10^9	3/3
Micrococcus luteus	Z100	1.80×10^8	3/3
Moraxella catarrhalis	ATCC 25238	1.27×10^9	3/3
Morganella morganii	ATCC 25830	1.23×10^9	3/3
Neisseria gonorrhoeae	ATCC 19424	ND	3/3
Neisseria lactamica	ATCC 23970	2.90×10^8	3/3
Neisseria meningitidis	Serotype A	2.55×10^8	5/5
Neisseria mucosa	ATCC 49233	5.80×10^8	4/4
Neisseria sicca	ATCC 9913	1.43×10^8	3/3
Pantaea agglomerans	ATCC 27155	2.00×10^6	3/3
Pasturella multocida	ATCC 12945	2.84×10^9	2/2
Pediococcus pentosaceus	Z226	1.91×10^8	3/3
Planococcus citreus	ATCC 14404	1.95×10^8	3/3
Pluralibacter gergoviae	ATCC 33028	1.27×10^9	3/3
Propionibacterium acnes	Z144	7.90×10^8	5/5
Providencia alcalifaciens	Z292	3.10×10^9	3/3
Providencia rettgeri	Z370	2.20×10^9	3/3
Providencia stuartii	Z213	1.70×10^9	3/3
Pseudomonas fluorescens	ATCC 13525	2.43×10^8	3/3
Pseudomonas luteola	ATCC 43273	1.09×10^8	3/3
Pseudomonas mendocina	ATCC 25411	1.23×10^9	3/3
Pseudomonas nitroreducens	ATCC 33634	5.30×10^8	3/3
Pseudomonas oryzihabitans	ATCC 43272	1.70×10^7	4/5[8]
Pseudomonas putida	Z030	3.30×10^8	3/3
Pseudomonas stutzeri	ATCC 17588	6.20×10^8	3/3
Raoultella planitcola	ATCC 33558	1.25×10^9	3/3
Rothia mucilaginosus	Z033	5.50×10^7	3/3
Salmonella enterica	ATCC BAA1715	2.23×10^9	3/3
Serratia fonticola	ATCC 29844	1.18×10^9	3/3
Serratia liquefaciens	ATCC 27592	1.24×10^9	11/12[9]
Serratia odorifera	ATCC 33077	2.19×10^9	11/13[10]
Serratia rubidaea	ATCC 19278	1.54×10^8	5/5
Staphylococcus aureus	ATCC 700699	4.30×10^7	3/3
Staphylococcus capitis	Z192	2.13×10^8	3/3
Staphylococcus epidermidis	ATCC 700566	5.90×10^7	3/3
Staphylococcus haemolyticus	Z067	2.70×10^7	5/5
Staphylococcus hominis	Z031	9.90×10^7	3/3
Staphylococcus intermedius	Z112	3.30×10^7	3/3
Staphylococcus lugdunensis	Z097	2.43×10^8	3/3
Staphylococcus schleiferi	Z294	2.52×10^9	3/3
Stenotrophomonas maltophilia	ATCC BAA84	1.74×10^9	5/5

Table 6: iC-GN Assay Exclusivity Results			
Organism	**Strain**	**Concentration (CFU/mL)**	**Performance**
Streptococcus agalactiae	Z019	5.80×10^8	3/3
Streptococcus anginosus	Z179	9.50×10^8	3/3
Streptococcus bovis	Z167	8.00×10^8	3/3
Streptococcus dysgalactiae	Z068	2.65×10^8	3/3
Streptococcus intermedius	Z126	1.40×10^7	5/6[11]
Streptococcus pneumoniae	ATCC 6301	3.80×10^8	5/6[12]
Streptococcus pyogenes	Z018	4.80×10^7	3/3
Veillonella parvula	Z379	6.70×10^9	5/6[13]

1) 3/3 false positive *A. baumannii* complex in initial testing. See limitation.
2) 2/3 false positive *E. coli* in initial testing. 10/10 repeats negative.
3) 1/3 false positive *S. marcescens* in initial testing. 3/3 repeats negative.
4) 1/3 positive control check failure in initial testing. 1/3 false positive *S. marcescens* in repeat testing.
5) 1/3 false positive *S. marcescens* in initial testing. 3/3 repeats negative.
6) 1/3 false positive *E. coli* in initial testing. 3/3 repeats negative.
7) 3/3 false positive *K. pneumoniae* in initial testing. See limitation.
8) 1/3 false positive *S. marcescens* in initial testing. 2/2 repeats negative.
9) 1/3 false positive *S. marcescens* in initial testing. 9/9 repeats negative.
10) 1/3 false positive *S. marcescens* in initial testing. 1/10 false positive *S. marcescens* in repeat testing. See limitation.
11) 1/3 false positive *S. marcescens* in initial testing. 3/3 repeats negative.
12) 1/3 false positive *S. marcescens* in initial testing. 3/3 repeats negative.
13) 1/3 false positive *E. coli* in initial testing. 3/3 repeats negative.

6. Interfering Substances:

iC-GN Assay performance was evaluated in the presence of potentially inhibiting substances that may be encountered in blood and blood culture media. Eight representative target organisms plus one non-target organism were evaluated. Organisms were tested at the lowest levels of bottle positivity, considered within two hours of bottle "ring." Potential interferents were tested at concentrations exceeding the highest concentrations that may be encountered in blood and blood culture media (for concentrations tested see 510(k) Summary for K190341, Table 13 (p.24-25)). Target performance is based on all expected targets detected and no false positive targets detected. Non-target performance is based on all negative results. In the event of a false negative result, the organism/interferent combination was retested in replicates of ten (10). In the event of a false positive result or other failure, the organism/interferent combination was retested in triplicate. If the discordant result was observed in repeat testing, the combination was retested at a decreased inhibitor concentration. below. Interference testing was performed in BD BACTEC Plus Aerobic blood culture bottle media, which has a sodium polyanetholesulfonate (SPS) concentration of 0.05% w/v. Additional SPS at a concentration greater than 0.05% w/v was found to interfere with the performance of some iC-GN Assay targets, resulting in increased false negative results and positive control check failures. Interference results are included in the table below. It was determined that the risks associated with the interfering substance, SPS, could be mitigated via labeling. The information regarding interference associated with SPS is provided for the end user in the Package Insert.

Table 7: Interfering Substances Test Panel		
Interference Compound	**linically Relevant Concentration**	**Test Concentration**
Hemoglobin	1-2 g/L	10 g/L
Conjugated Bilirubin	0.1-0.4 mg/dL	10 mg/dL
Unconjugated Bilirubin	0.1-0.8 mg/dL	10 mg/dL
Protein (γ-globulin + albumin)	0.7-1.7 g/dL	4 g/dL
Triglyceride	300-500 mg/dL	1500 mg/dL
Human Genomic DNA	NA	1×10^6 cells/mL
Sodium Polyanetholesulfonate (SPS)	0.02-0.05% w/v	0.1% w/v
Cefepime	16 µg/mL	80 µg/mL
Ceftriaxone	16 µg/mL	80 µg/mL
Fluconazole	25 µg/mL	100 µg/mL
Gentamicin	20 µg/mL	80 µg/mL
Meropenem	16 µg/mL	80 µg/mL
Piperacillin	32 µg/mL	160 µg/mL
Vancomycin	20 µg/mL	100 µg/mL

Table 7a: iC-GN Assay Interfering Substances Performance												
	Target Performance											
Interference Compound	**ABX**	**ECX**	**EC**	**KO**	**KPN**	**PM**	**PA**	**SM**	**SE**	**KPC -2**	**NDM-1**	**CTX-M -15**
Hemoglobin	3/3	3/3	3/3	3/3	3/3	3/3	14/14	3/3	5/5	3/3	14/14	3/3
Conjugated Bilirubin	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Unconjugated Bilirubin	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Protein (γ-globulin + albumin)	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Triglyceride	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Human Genomic DNA	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
SPS (0.1%)	3/12[1]	3/3	3/3	3/3	3/3	3/3	2/12[2]	3/3	4/4	3/3	8/12[3]	3/3

Table 7a: iC-GN Assay Interfering Substances Performance												
	Target Performance											
Interference Compound	**ABX**	**ECX**	**EC**	**KO**	**KPN**	**PM**	**PA**	**SM**	**SE**	**KPC -2**	**NDM-1**	**CTX-M -15**
SPS (0.5%)	3/3	--	--	--	--	--	3/3	--	--	--	3/3	--
Cefepime	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Ceftriaxone	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Fluconazole	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Gentamicin	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Meropenem	3/3	3/3	5/5	3/3	3/3	5/6[4]	3/3	3/3	3/3	3/3	3/3	5/5
Piperacillin	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3

7. Microbial Interference:

Potential microbial interference was evaluated by testing high concentrations of Gram-negative exclusivity organisms in combination with low concentrations of iC-GN target organisms. A total of sixty (60) Gram-negative exclusivity strains were tested at the highest possible concentrations, considered eight hours beyond initial bottle positivity or the equivalent. Eight (8) representative iC-GN target organisms were tested at concentrations below the lowest levels of bottle positivity. Each organism combination was tested in triplicate. Performance was based on all expected targets detected and no false positive targets detected. In the event of a false negative result, the combination was retested in replicates of ten (10). In the event of a false positive result or other failure, the combination was retested in replicates of three (3) or ten (10). Microbial interference results are presented in the table below. A small number of organisms initially were observed to cause false negative results, however additional testing showed that the initial results were not reproducible. It was determined that the risks associated with the microbial interference could be mitigated via labeling. The organisms that showed an increased risk of microbial interference are listed in the Limitations section of the Package Insert.

Table 8: iC-GN Assay Microbial Interference Results											
Organism	**ABX**	**ECX**	**EC**	**KO**	**KPN**	**PM**	**PA**	**SM**	**CTX-M-15**	**KPC-2**	**NDM-1**
A. lwoffi	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
A. radioresistens	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
A. schindleri	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
A. ursingii	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
A. hydrophila	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
A. faecalis	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
B. fragilis	3/3	3/3	3/3	3/3	3/3	4/5[1]	3/3	3/3	3/3	3/3	3/3
B. vesicularis	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
B. cepacia	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
C. coli	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3

Table 8: iC-GN Assay Microbial Interference Results											
Organism	**ABX**	**ECX**	**EC**	**KO**	**KPN**	**PM**	**PA**	**SM**	**CTX-M-15**	**KPC-2**	**NDM-1**
C. jejuni	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
C. davisae	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
C. amalonaticus	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
C. braakii	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
C. freundii	3/3	3/3	3/3	3/3	3/3	2/2	3/3	3/3	3/3	3/3	3/3
C. koseri	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
C. sedlakii	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
C. muytjensii	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
C. sakazakii	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
E. tarda	3/3	3/3	3/3	3/3	3/3	3/3	5/5	3/3	3/3	3/3	5/5
E. aerogenes	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
E. amnigenus	3/3	3/3	5/5	3/3	3/3	3/3	3/3	3/3	5/5	3/3	3/3
E. fergusonii	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
E. hermannii	3/3	3/3	5/5	3/3	13/13	3/3	3/3	3/3	5/5	12/13[2]	3/3
E. vulneris	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
F. varium	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
H. alvei	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
H. influenzae	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
H. parainfluenzae	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
K. ascorbata	10/13[3]	3/3	11/12[4]	3/3	3/3	3/3	3/3	3/3	10/12[4]	3/3	3/3
L. adecarboxylata	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
L. grimontii	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
M. catarrhalis	12/13[5]	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
M. morganii	3/3	3/3	3/3	3/3	3/3	3/3	5/5	3/3	3/3	3/3	5/5
N. gonorrhoeae	5/5	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
N. lactamica	3/3	3/3	3/3	3/3	3/3	3/3	5/5	3/3	3/3	3/3	5/5
N. meningitidis	3/3	3/3	5/5	3/3	3/3	3/3	3/3	3/3	5/5	3/3	3/3
N. mucosa	5/5	3/3	5/5	3/3	3/3	3/3	3/3	3/3	5/5	3/3	3/3
N. sicca	5/6[6]	3/3	3/3	5/5	3/3	3/3	3/3	3/3	3/3	3/3	3/3
P. agglomerans	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
P. multocida	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
P. gergoviae	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
P. alcalifaciens	5/5	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
P. rettgeri	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
P. stuartii	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
P. fluorescens	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
P. luteola	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
P. mendocina	3/3	3/3	3/3	3/3	3/3	3/3	3/3	5/5	3/3	3/3	3/3
P. nitroreducens	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
P. oryzihabitans	3/3	3/3	3/3	3/3	5/5	3/3	3/3	3/3	3/3	5/5	3/3
P. putida	3/3	3/3	3/3	3/3	3/3	3/3	5/5	3/3	3/3	3/3	5/5

Table 8: iC-GN Assay Microbial Interference Results											
Organism	**ABX**	**ECX**	**EC**	**KO**	**KPN**	**PM**	**PA**	**SM**	**CTX-M-15**	**KPC-2**	**NDM-1**
P. stutzeri	3/3	5/5	3/3	3/3	3/3	3/3	5/5	3/3	3/3	3/3	5/5
R. planitcola	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
S. enterica	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
S. fonticola	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
S. liquefaciens	3/3	5/5	3/3	3/3	12/13[7]	3/3	3/3	3/3	3/3	13/13	3/3
S. odorifera	3/3	3/3	3/3	12/13[8]	3/3	3/3	3/3	3/3	3/3	3/3	3/3
S. rubidaea	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
S. maltophilia	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
V. parvula	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3

1) 1/3 array registration error in initial testing. 1/3 false positive *E. cloacae* complex in repeat testing.
2) 1/3 false negative KPC in initial testing. 10/10 repeats passed.
3) 3/3 false negative *A. baumannii* complex in initial testing; concentration was determined to be below the target limit of detection. 10/10 repeats passed.
4) 1/2 false negative CTX-M in initial testing. 1/10 false positive *K. pneumoniae* in repeat testing.
5) 1/3 false negative *A. baumannii* complex in initial testing. 10/10 repeats passed.
6) 1/3 false positive *E. coli* in initial testing. 3/3 repeats passed.
7) 1/3 false negative *K. pneumoniae* in initial testing. 10/10 repeats passed.
8) 1/3 false positive *S. marcescens* in initial testing. 10/10 repeats passed.

8. Competitive Inhibition:

iC-GN Assay performance was evaluated with combinations of target analytes that may be found in mixed positive blood cultures. One target organism was prepared at the lowest level of bottle positivity, considered within two hours of bottle "ring", while the second target organism was prepared at the highest possible concentration, considered eight hours after initial bottle positivity. All organisms were grown in BD BACTEC Plus Aerobic blood cultures bottles with human blood added on the BD BACTEC System. The organisms were combined at a ratio of one part "low" to four parts "high". Each low concentration organism was tested in combination with each high concentration organism in triplicate. Performance was based on all expected targets detected. In the event of a false negative result, the organism combination was retested in replicates of ten (10) at the same "low" and "high" organism ratio. In the event of a reproducible false negative result, the organism combination was retested in replicates of ten (10) at a ratio of one part "low" to one part "high." All high concentration iC-GN targets were detected. Due to competitive inhibition, low concentration targets were not detected in 1.7% of tests (3/178). When iC-GN target organisms were present at similar concentrations, all targets were detected. Competitive inhibition results are included in the table below. Based on the results of the competitive inhibition study, the following limitation was included in the Package Insert, "Due to competitive inhibition, target organisms present at low concentrations may not be detected by the iC-GN Assay when a second target organism is present at higher concentrations in a mixed culture".

Table 9: iC-GN Assay Competitive Inhibition Performance	
	Target Performance

Low Organism	High Organism	Low Organism	Low Marker	High Organism	High Marker
A. baumannii	ECX	3/3	NA	3/3	NA
	EC (CTX-M-15+)	3/3	NA	3/3	3/3
	KO	3/3	NA	3/3	NA
	KPN (KPC-2+)	3/3	NA	3/3	3/3
	PM	3/3	NA	3/3	NA
	PA (NDM-1+)	3/3	NA	3/3	3/3
	SM	3/3	NA	3/3	NA
E. cloacae	ABX	3/3	NA	3/3	1 FP KPC
	EC (CTX-M-15+)	3/3	NA	3/3	3/3
	KO	3/3	NA	3/3	NA
	KPN (KPC-2+)	3/3	NA	3/3	3/3
	PM	3/3	NA	3/3	NA
	PA (NDM-1+)	3/3	NA	3/3	3/3
	SM	3/3	NA	3/3	NA
E. coli (CTX-M-15+)	ABX	3/3	3/3	3/3	NA
	ECX	3/3	3/3	3/3	NA
	KO	3/3	3/3	3/3	NA
	KPN (KPC-2+)	3/3	3/3	3/3	3/3
	PM	3/3	3/3	3/3	NA
	PA (NDM-1+)	3/3	3/3	3/3	3/3
	SM	3/3	3/3	3/3	NA
K. oxytoca	ABX	3/3	NA	3/3	NA
	ECX	3/3	NA	3/3	NA
	EC (CTX-M-15+)	3/3	NA	3/3	3/3
	KPN (KPC-2+)	3/3	NA	3/3	3/3
	PM	3/3	NA	3/3	NA
	PA (NDM-1+)	3/3	NA	3/3	3/3
	SM	3/3	3/3	3/3	NA
K. pneumoniae (KPC-2+)	ABX	3/3	3/3	3/3	NA
	ECX	3/3	3/3	3/3	NA
	EC (CTX-M-15+)	3/3	3/3	3/3	3/3
	KO	3/3	3/3	3/3	NA
	PM	3/3	3/3	3/3	NA
	PA (NDM-1+)	3/3	3/3	3/3	3/3
	SM	3/3	3/3	3/3	NA
P. mirabilis	ABX	3/3	NA	3/3	NA
	ECX	3/3	NA	3/3	NA
	EC (CTX-M-15+)	3/3	NA	3/3	3/3
	KO	5/5	NA	5/5	NA
	KPN (KPC-2+)	3/3	NA	3/3	3/3
	PA (NDM-1+)	3/3	NA	3/3	3/3
	SM	3/3	NA	3/3	NA
	ABX	3/3	3/3	3/3	NA

P. aeruginosa (NDM-1+)	ECX	3/3	3/3	3/3	NA
	EC (CTX-M-15+)	12/13	11/13	13/13	13/13
	EC (CTX-M-15+) **1:1**	10/10	10/10	10/10	10/10
	KO	3/3	3/3	3/3	NA
	KPN (KPC-2+)	3/3	3/3	3/3	3/3
	PM	3/3	3/3	3/3	NA
	SM	3/3	3/3	3/3	NA
S. marcescens	KPN (KPC-2+)	3/3	NA	3/3	3/3
	PM	3/3	NA	3/3	NA
	PA (NDM-1+)	3/3	NA	3/3	3/3
	ABX	3/3	NA	3/3	NA
	ECX	3/3	NA	3/3	NA
	EC (CTX-M-15+)	3/3	NA	3/3	3/3
	KO	3/3	NA	3/3	NA

9. Reproducibility:

To confirm the site-to-site, operator-to-operator, system-to-system, and lot-to-lot reproducibility of the iC-GN Assay, a representative panel of target organisms and one non-target organism were evaluated at two clinically relevant concentrations: initial bottle positivity and eight hours beyond initial bottle positivity. Organisms were grown to the appropriate concentrations in BD BACTEC Plus Aerobic blood culture bottles with human blood added on the BD BACTEC System. Testing was performed by two independent operators at each of three sites, two external and one internal. Each operator tested the eighteen-organism panel in triplicate across five, non-consecutive days. Testing was performed on six iC-GN Cassette lots and multiple iC-Systems. Performance is based on all expected targets detected and no false positive targets detected. The table below summarizes the reproducibility results stratified by iC-GN target and concentration. Overall Reproducibility performance was 99.3%, confirming that iC-GN Assay performance is reproducible across sites, operators, systems and lots.

Table 10 : iC-GN Assay Reproducibility Performance by Target						
Target/Concentration	**Overall Performance**	**Overall Performance % [95% CI]**	**False Negatives**	**False Positives**	**PC Check Failures**	**System Failures**
***A. baumannii* complex** Bottle Ring	90/90	100.0 [95.91-100.0]	0/90 (0.00%)	0/90 (0.00%)	0/90 (0.00%)	0/90 (0.00%)
***A. baumannii* complex** Bottle Ring + 8 hours	87/90	96.7 [90.65-98.86]	0/90 (0.00%)	3/90 (3.33%)	0/90 (0.00%)	0/90 (0.00%)
***E. cloacae* complex** Bottle Ring	86/88	97.7 [92.09-99.37]	1/88 (1.14%)	1/88 (1.14%)	2/90 (2.22%)	0/90 (0.00%)
***E. cloacae* complex** Bottle Ring + 8 hours	90/90	100.0 [95.91-100.0]	0/90 (0.00%)	0/90 (0.00%)	0/90 (0.00%)	0/90 (0.00%)
E. coli Bottle Ring	90/90	100.0 [95.91-100.0]	0/90 (0.00%)	0/90 (0.00%)	0/90 (0.00%)	0/90 (0.00%)
E. coli Bottle Ring + 8 hours	89/89	100.0 [95.86-100.0]	0/89 (0.00%)	0/89 (0.00%)	0/90 (0.00%)	1/90 (1.11%)
K. oxytoca Bottle Ring	89/90	98.9 [93.97-99.80]	0/90 (0.00%)	1/90 (1.11%)	0/90 (0.00%)	0/90 (0.00%)
K. oxytoca Bottle Ring + 8 hours	89/89	100.0 [95.86-100.0]	0/89 (0.00%)	0/89 (0.00%)	1/90 (1.11%)	0/90 (0.00%)
K. pneumoniae Bottle Ring	90/90	100.0 [95.91-100.0]	0/90 (0.00%)	0/90 (0.00%)	0/90 (0.00%)	0/90 (0.00%)
K. pneumoniae Bottle Ring + 8 hours	89/89	100.0 [95.86-100.0]	0/89 (0.00%)	0/89 (0.00%)	1/90 (1.11%)	0/90 (0.00%)
***Proteus* species** Bottle Ring	89/89	100.0 [95.86-100.0]	0/89 (0.00%)	0/89 (0.00%)	1/90 (1.11%)	0/90 (0.00%)
***Proteus* species** Bottle Ring + 8 hours	88/88	100.0 [95.92-100.0]	0/88 (0.00%)	0/88 (0.00%)	0/90 (0.00%)	2/90 (2.22%)
P. aeruginosa Bottle Ring	88/89	98.9 [93.91-99.80]	1/89 (1.12%)	0/89 (0.00%)	1/90 (1.11%)	0/90 (0.00%)
P. aeruginosa Bottle Ring + 8 hours	89/90	98.9 [93.97-99.80]	1/90 (1.11%)	0/90 (0.00%)	0/90 (0.00%)	0/90 (0.00%)
S. marcescens Bottle Ring	87/89	97.8 [92.17-99.38]	0/89 (0.00%)	2/89 (2.25%)	1/90 (1.11%)	0/90 (0.00%)
S. marcescens Bottle Ring + 8 hours	87/89	97.8 [92.17-99.38]	0/89 (0.00%)	2/89 (2.25%)	1/90 (1.11%)	0/90 (0.00%)

Table 10 : iC-GN Assay Reproducibility Performance by Target						
Target/Concentration	**Overall Performance**	**Overall Performance % [95% CI]**	**False Negatives**	**False Positives**	**PC Check Failures**	**System Failures**
CTX-M group 1 Bottle Ring	90/90	100.0 [95.91-100.0]	0/90 (0.00%)	0/90 (0.00%)	0/90 (0.00%)	0/90 (0.00%)
CTX-M group 1 Bottle Ring + 8 hours	89/89	100.0 [95.86-100.0]	0/89 (0.00%)	0/89 (0.00%)	0/90 (0.00%)	1/90 (1.11%)
KPC Bottle Ring	90/90	100.0 [95.91-100.0]	0/90 (0.00%)	0/90 (0.00%)	0/90 (0.00%)	0/90 (0.00%)
KPC Bottle Ring + 8 hours	89/89	100.0 [95.86-100.0]	0/89 (0.00%)	0/89 (0.00%)	1/90 (1.11%)	0/90 (0.00%)
NDM Bottle Ring	89/89	100.0 [95.86-100.0]	0/89 (0.00%)	0/89 (0.00%)	1/90 (1.11%)	0/90 (0.00%)
NDM Bottle Ring + 8 hours	89/90	98.9 [93.97-99.80]	1/90 (1.11%)	0/90 (0.00%)	0/90 (0.00%)	0/90 (0.00%)

B. Comparison Studies:

1. Method Comparison with the Predicate Device:
   A method comparison study was performed at five (5) geographically dispersed clinical sites. Sites tested 1002 leftover de-identified specimens from anaerobic and aerobic blood culture bottles flagged as positive by their respective continuous monitoring blood culture system. Three of the commonly used blood culture systems were included in the study: Thermo Fisher VersaTREK, BD BACTEC and BioMerieux BacT/ALERT.

   Patient positive blood cultures confirmed by Gram stain to be positive for Gram-negative bacilli were enrolled in the study. Any positive blood cultures showing an initial mixed Gram stain were not enrolled or were subsequently withdrawn from the study dataset.

   Final performance of the iC-GN Assay organism targets was compared to reference culture followed by MALDI identification per the study protocol. Final performance of the iC-GN Assay resistance marker targets was compared to PCR amplification followed by confirmatory bi-directional sequencing. Phenotypic antimicrobial susceptibility testing (AST) was also performed on all specimens to identify additional samples which required sequencing. Discordant samples were also sequenced.

   To supplement performance of observed lower prevalence organisms, 170 contrived samples were prepared using verified strains. Contrived samples were prepared at iCubate using BD BACTEC Plus Aerobic Blood Culture Bottles with 10 mL of human blood added (in accordance with BACTEC instructions). Organisms were spiked into bottles at concentrations of 5-30 CFU/bottle and incubated until bottles were flagged as positive. Aliquots of samples were frozen and provided to the sites (frozen) for testing.

   Of the 1107 positive blood culture specimens enrolled in the study, a total of 105 specimens were excluded/withdrawn from the study and excluded from all subsequent performance analyses. Of the 1002 specimens remaining, 976 were fresh prospective specimens and 26 (2.6%) were frozen prospective specimens. Nineteen (19) samples

were excluded from the performance analysis for *Proteus mirabilis* due to confirmed contamination of BD BACTEC Bottles with non-viable *Proteus* organisms or nucleic acid, leaving a total of 983 evaluable specimens for *Proteus mirabilis*.

The total specimens excluded from the iC-GN Assay Method Comparison Study (n=105) are listed by site and the reasons for exclusion are noted in the 510(k) Summary for K190341, Table 12 (p.25). The most common reasons for exclusion included incomplete reference testing and repeat iC-GN errors.

Throughout the course of the study, an initial error rate of 2.9 % (34/1181) was observed. Reasons for error included the following: *Positive controls check failure (27), Array registration error (6), and Processor/System error (1).* When an error was observed, repeat testing was performed with the iC-GN Assay per the protocol. Upon repeat testing, the error rate was reduced to 0.8% (9/1181). The 510(k) Summary for K190341, Table 13 (p.26) includes a table of the no-call results.

When performance of the iC-GN Assay was compared to reference culture followed by MALDI identification or PCR/bi-directional sequencing, there was no significant difference in performance noted between the five study sites or between the three blood culture systems. Performance for all positive bottle types/systems combined is presented in the tables below for detection of the iC-GN Assay targets as compared to culture and MALDI or PCR/bi-directional sequencing. Results are stratified by prospectively tested fresh specimens, prospectively collected/retrospectively tested frozen specimens and contrived specimens. These data are provided in the tables below.

Table 11a: iC-GN Assay Performance: *Acinetobacter baumannii* complex *(ppa)*					
Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	976	100% 7/7 (64.6-100)	99.9% 968-969** (99.4-100)	Culture & MALDI
	Frozen	26	- 0/0 -	100% 26/26 (87.1-100)	
	TOTAL	1002	100% 7/7 (64.6-100)	99.9% 994/995 (99.4-100)	
Contrived		170	100% 45/45 (92.1-100)	100% 125/125 (97.0-100)	

**1/1 false positive observed was negative for *A. baumannii* complex by PCR/bi-directional sequencing

Table 11b: iC-GN Assay Performance: *Enterobacter cloacae* complex *(ramA)*					
Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	976	94.5% 52/55* (85.1-98.1)	100% 921/921 (99.6-100)	Culture & MALDI
	Frozen	26	100% 5/5 (56.6-100)	100% 21/21 (84.5-100)	
	TOTAL	1002	95.0% 57/60 (86.3-98.3)	100% 942/942 (99.6-100)	
Contrived		170	100% 17/17 (81.6-100)	100% 153/153 (97.6-100)	

*1/3 false negatives observed was negative for *E. cloacae* complex by PCR/bi-directional sequencing; 2/3 were positive for *E. cloacae* complex by PCR/bi-directional sequencing

Table 11c: iC-GN Assay Performance: *Escherichia coli (uidA)*					
Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	976	98.4% 480/488* (96.8-99.2)	100% 488/488 (99.2-100)	Culture & MALDI
	Frozen	26	100% 6/6 (61.0-100)	100% 20/20 (83.9-100)	
	TOTAL	1002	98.4% 486/494 (96.8-99.2)	100% 508/508 (99.2-100)	
Contrived		170	100% 15/15 (79.6-100)	100% 155/155 (97.6-100)	

*4/8 false negatives observed were negative for E. coli by PCR/bi-directional sequencing; 3/8 were positive for E. coli by PCR/bi-directional sequencing; 1/8 was not available for sequencing

Table 11d: iC-GN Assay Performance: *Klebsiella oxytoca (pehX)*					
Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	976	95.8% 23/24* (79.8-99.3)	99.7% 949/952** (99.1-99.9)	Culture & MALDI
	Frozen	26	- 0/0 -	100% 26/26 (87.1-100)	
	TOTAL	1002	95.8% 23/24 (79.8-99.3)	99.7% 975/978 (99.1-99.9)	
Contrived		170	100% 30/30 (88.6-100)	100% 140/140 (97.3-100)	

*1/1 false negative observed was negative for *K. oxytoca* by PCR/bi-directional sequencing
**3/3 false positives observed were negative for *K. oxytoca* by PCR/bi-directional sequencing

Table 11e: iC-GN Assay Performance: *Klebsiella pneumoniae (parC)*					
Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	976	96.8% 150/155* (92.7-98.6)	99.3% 815/821** (98.4-99.7)	Culture & MALDI
	Frozen	26	100% 3/3 (43.9-100)	100% 23/23 (85.7-100)	
	TOTAL	1002	96.8% 153/158 (92.8-98.6)	99.3% 838/844 (98.4-99.7)	
Contrived		170	100% 21/21 (84.5-100)	99.3% 148/149 (96.3-99.9)	

*3/5 false negatives observed were negative for *K. pneumoniae* by PCR/bi-directional sequencing; 2/3 were positive for *K. pneumoniae* by PCR/bi-directional sequencing

**6/6 false positives observed were negative for *K. pneumoniae* by PCR/bi-directional sequencing

Table 11f: iC-GN Assay Performance: *Proteus mirabilis (rpoB)*					
Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	957	97.4% 37/38* (86.5-99.5)	99.5% 914/919** (98.7-99.8)	Culture & MALDI
	Frozen	26	100% 9/9 (70.1-100)	100% 17/17 (81.6-100)	
	TOTAL	983***	97.9% 46/47 (88.9-99.6)	99.5% 931/936 (98.8-99.8)	
Contrived		170	100% 12/12 (75.8-100)	100% 158/158 (97.6-100)	

*1/1 false negative observed was positive for *P. mirabilis* by PCR/bi-directional sequencing

**3/5 false positives observed were negative for *P. mirabilis* by PCR/bi-directional sequencing; 2/5 were not available for sequencing

*** 19 samples were excluded from *Proteus mirabilis* performance analysis due to confirmed *Proteus* contamination within the BD BACTEC Bottles, leaving a total of 983 evaluable specimens.

Table 11g: iC-GN Assay Performance: *Pseudomonas aeruginosa (algD)*					
Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	976	95.1% 78/82* (88.1-98.1)	99.8% 892/894** (99.2-99.9)	Culture & MALDI
	Frozen	26	100% 1/1 (20.7-100)	100% 25/25 (86.7-100)	
	TOTAL	1002	95.2% 79/83 (88.3-98.1)	99.8% 917/919 (99.2-99.9)	
Contrived		170	100% 10/10 (72.2-100)	100% 160/160 (97.7-100)	

*4/4 false negatives observed were positive for *P. aeruginosa* by PCR/bi-directional sequencing
**2/2 false positives observed were negative for *P. aeruginosa* by PCR/bi-directional sequencing

Table 11h: iC-GN Assay Performance: *Serratia marcescens (gyrB)*					
Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	976	100% 29/29 (88.3-100)	99.6% 943/947** (98.9-99.8)	Culture & MALDI
	Frozen	26	0/0-	100% 26/26 (87.1-100)	
	TOTAL	1002	100% 29/29 (88.3-100)	99.6% 969/973 (98.9-99.8)	
Contrived		170	100% 20/20 (83.9-100)	99.3% 149/150 (96.3-99.9)	

**1/4 false positives observed was positive for *S. marcescens* by PCR/bi-directional sequencing; 3/4 were negative for *S. marcescens* by PCR/bi-directional sequencing

Table 11I: iC-GN Assay Performance: CTX-M					
Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	976	97.0% 64/66 (89.6-99.2)	99.9% 909/910 (99.4-100)	PCR/Bi-directional sequencing
	Frozen	26	100% 1/1 (20.7-100)	100% 25/25 (86.7-100)	
	TOTAL	1002	97.0% 65/67 (89.8-99.2)	99.9% 934/935 (99.4-100)	
Contrived		170	100% 15/15 (79.6-100)	100% 155/155 (97.6-100)	

Table 11J: iC-GN Assay Performance: KPC					
Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	976	100% 1/1 (20.7-100)	99.9% 974/975 (99.4-100)	PCR/Bi-directional sequencing
	Frozen	26	- 0/0 -	100% 26/26 (87.1-100)	
	TOTAL	1002	100% 1/1 (20.7-100)	99.9% 1000/1001 (99.4-100)	
Contrived		170	100% 50/50 (92.9-100)	99.2% 119/120 (95.4-99.9)	

Table 11K: iC-GN Assay Performance: NDM					
Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	976	- 0/0 -	100% 976/976 (99.6-100)	PCR/Bi-directional sequencing
	Frozen	26	- 0/0 -	100% 26/26 (87.1-100)	
	TOTAL	1002	- 0/0 -	100% 1002/1002 (99.6-100)	
Contrived		170	100% 50/50 (92.9-100)	100% 120/120 (96.9-100)	

2. Analysis of Mixed Culture Results:
   In the method comparison study, there were thirty (30) mixed culture specimens that were detected by the iC-GN Assay, culture and MALDI, or both. There were twelve (12) discrepant mixed samples for which iC-GN detected a target that was not detected by the comparator assay. There were four (4) discrepant mixed samples for which the comparator assay detected targets that were not detected by iC-GN. Due to competitive inhibition, target organisms present at low concentrations may not be detected by the iC-GN Assay when a second target organism is present at higher concentrations. See the 510(k) Summary, Tables 29 and 30 (p.31-32) which includes tables that list the mixed target combinations detected by iC-GN and the comparator method in the clinical study.

Table 12: Multiple Organism Detections by iC-GN as Compared to Culture/MALDI							
Multiple Detections by iC-GN					Total Targets Detected by iC-GN	No of Discrepant Targets	Discrepant Results (Targets Not Detected by culture/MALDI)
Site	ID	Target 1	Target 2	Target 3			
LAC	1102	*E. coli*	*K. pneumoniae*		2	0	
LAC	1118	*E. coli*	*K. pneumoniae*		2	0	
LAC	1141	*E. coli*	*K. pneumoniae*		2	0	
LAC	1220	*E. cloacae* complex	*E. coli*		2	0	
LAC	1236	*E. coli*	*K. oxytoca*	*K. pneumoniae*	3	1	*K. oxytoca*
LAC	1285	*K. pneumoniae*	*S. marcescens*		2	1	*S. marcescens*
LAC	1307	*E. coli*	*K. oxytoca*		2	0	
LAC	1378	*E. coli*	*K. pneumoniae*		2	1	*K. pneumoniae*
LAC	1382	*K. oxytoca*	*K. pneumoniae*		2	1	*K. oxytoca*
MCW	2023	*E. coli*	*K. pneumoniae*		2	0	

Table 12: Multiple Organism Detections by iC-GN as Compared to Culture/MALDI							
Multiple Detections by iC-GN					Total Targets Detected by iC-GN	No of Discrepant Targets	Discrepant Results (Targets Not Detected by culture/MALDI)
Site	ID	Target 1	Target 2	Target 3			
MCW	2032	*E. cloacae* complex	*K. oxytoca*		2	0	
MCW	2038	*K. oxytoca*	*K. pneumoniae*		2	0	
MCW	2041	*E. coli*	*P. mirabilis*		2	0	
MCW	2104	*E. coli*	*S. marcescens*		2	1	*S. marcescens*
MCW	2193	*K. pneumoniae*	*S. marcescens*		2	2	*K. pneumoniae, S. marcescens*
TC	3015	*K. oxytoca*	*P. aeruginosa*		2	2	*K. oxytoca, P. aeruginosa*
TC	3096	*E. coli*	*K. pneumoniae*		2	1	*K. pneumoniae*
TC	3131	*E. cloacae* complex	*K. pneumoniae*		2	0	
TC	3183	*K. pneumoniae*	*S. marcescens*		2	1	*S. marcescens*
TGH	4031	*E. coli*	*P. mirabilis*		2	1	*P. mirabilis*
TGH	4037	*E. coli*	*P. aeruginosa*		2	0	
TGH	4124	*E. cloacae* complex	*P. aeruginosa*		2	1	*P. aeruginosa*
TGH	4132	*E. cloacae* complex	*K. pneumoniae*		2	0	
IU	5025	*A. baumannii* complex	*K. pneumoniae*		2	1	*A. baumannii* complex
IU	5031	*E. coli*	*K. pneumoniae*		2	0	
IU	5042	*E. cloacae* complex	*K. pneumoniae*		2	0	
LAC	1102	*K. pneumoniae*	*E. coli*		2	0	
LAC	1118	*K. pneumoniae*	*E. coli*		2	0	
LAC	1141	*E. coli*	*K. pneumoniae*		2	0	
LAC	1220	*E. cloacae* complex	*E. coli*		2	0	
LAC	1236	*K. pneumoniae*	*E. coli*		2	0	
LAC	1268	*P. aeruginosa*	*P. mirabilis*		2	1	*P. aeruginosa*
LAC	1307	*E. coli*	*K. oxytoca*		2	0	
LAC	1338	*E. coli*	*K. pneumoniae*		2	1	*K. pneumoniae*
MCW	2023	*K. pneumoniae*	*E. coli*		2	0	
MCW	2032	*K. oxytoca*	*E. cloacae* complex		2	0	
MCW	2038	*K. pneumoniae*	*K. oxytoca*		2	0	
MCW	2041	*P. mirabilis*	*E. coli*		2	0	
TC	3006	*E. coli*	*K. pneumoniae*		2	1	*K. pneumoniae*
TC	3131	*E. cloacae* complex	*K. pneumoniae*		2	0	

Table 12: Multiple Organism Detections by iC-GN as Compared to Culture/MALDI							
Multiple Detections by iC-GN					Total Targets Detected by iC-GN	No of Discrepant Targets	Discrepant Results (Targets Not Detected by culture/MALDI)
Site	ID	Target 1	Target 2	Target 3			
TGH	4007	*E. coli*	*P. aeruginosa*		2	1	*P. aeruginosa*
TGH	4037	*E. coli*	*P. aeruginosa*		2	0	
TGH	4132	*K. pneumoniae*	*E. cloacae* complex		2	0	
IU	5031	*E. coli*	*K. pneumoniae*		2	0	
IU	5042	*K. pneumoniae*	*E. cloacae* complex		2	0	

C. Expected Values:

A total of 1002 prospectively collected fresh and frozen blood culture specimens were obtained from five geographically dispersed clinical sites. The number and percentage of positive cases (positivity rate) determined by the iC-GN Assay stratified by U.S. state for each of the organisms and resistance markers detected by the assay are presented below. Overall, the iC-GN Assay detected at least one organism in 89% (901/1002) prospectively collected specimens and at least one resistance marker in 6.8% (68/1002) prospectively collected specimens. Expected values are presented in the 510(k) Summary, Table 30 (p.32-33).

VII. Proposed Labeling:

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

VIII. Conclusion:

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