DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

August 8, 2017

ICUBATE, INC.
C/O FRAN WHITE
MDC ASSOCIATES, LLC
180 CABOT STREET
BEVERLY, MA 01915

Re: K163390
Trade/Device Name: iC-GPC Assay, iC-System
Regulation Number: 21 CFR 866.3365
Regulation Name: Multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures
Regulatory Class: Class II
Product Code: PAM, NSU
Dated: July 14, 2017
Received: July 17, 2017

Dear Ms. White:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of

medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely,

Steven R. Gitterman -S for

Uwe Scherf, M.Sc., Ph.D.
Director, Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K163390

Device Name
iC-GPC Assay for use on the iC-System

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

The iC-GPC Assay detects organism DNA and identifies the following bacterial species and resistance markers:

Bacterial Species
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus pneumoniae
Enterococcus faecalis
Enterococcus faecium

Resistance Markers
mecA- associated with methicillin resistance
vanA- associated with vancomycin resistance
vanB- associated with vancomycin resistance

The iC-GPC Assay detects the *mecA* resistance marker, inferring *mecA*-mediated methicillin resistance, and the *vanA* and *vanB* resistance markers, inferring *vanA*/*vanB*-mediated vancomycin resistance. In mixed growth, the iC-GPC Assay does not specifically attribute *van*-mediated vancomycin resistance to either *E. faecalis* or *E. faecium*, or *mecA*-mediated methicillin resistance to either *S. aureus* or *S. epidermidis*.

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

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) SUMMARY

Date of Summary: August 2, 2017

Product Name iC-GPC Assay™ for use on the iC-System™

Sponsor

iCubate®
601 Genome Way
Huntsville, AL 35806

Correspondent

MDC Associates, LLC
Fran White, President
180 Cabot Street
Beverly, MA 01915
Phone: (978) 705-5011
Fax: (800) 498-9121

Device Trade or Proprietary Name
iC-GPC Assay™ for use on the iC-System™

Common Name
Gram positive bacteria and their resistance markers

Regulation
21 CFR 866.3365, Multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures.

Product Codes
PAM, NSU

Classification
Class II

Substantial Equivalency

TABLE 1. Comparison of New Device with Predicate Device

<table>
<tr><th>Characteristic</th><th>iC-GPC Assay™ for use on the iC-System™
(New Device)</th><th>VERIGENE® Gram Positive Blood Culture Nucleic Acid Test (BC-GP) (K122514)
(Primary Predicate Device)</th></tr>
<tr><td colspan="3">Similarities</td></tr>
<tr><td>Intended Use</td><td>The iCubate iC-GPC Assay™ for use on the iC-System™ is a qualitative, multiplexed, in vitro diagnostic test for the detection and identification of potentially pathogenic gram positive bacteria, which may cause bloodstream infection (BSI). The iC-GPC Assay™ is performed directly on positive blood cultures, confirmed by Gram Stain to contain gram positive cocci. Cultures demonstrating mixed Gram stain results should not be tested on the assay. The iC-GPC Assay™ is validated for use with select BACTEC™, BacT/ALERT® and VersaTREK® blood culture bottles. The iC-GPC Assay™ is indicated for use in conjunction with other clinical and laboratory findings, such as blood culture isolate identification and antimicrobial susceptibility testing, to aid in the diagnosis of bacterial bloodstream infections; however, it is not used to monitor bloodstream infections.

The iC-GPC Assay™ detects organism DNA and identifies the following bacterial species and resistance markers:

<table><tr><th>Bacterial Species</th><th>Resistance Markers</th></tr><tr><td>Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus pneumoniae
Enterococcus faecalis
Enterococcus faecium</td><td>mecA- associated with methicillin resistance
vanA- associated with vancomycin resistance
vanB- associated with vancomycin resistance</td></tr></table>
The iC-GPC Assay™ detects the mecA resistance marker, inferring mecA-mediated methicillin resistance, and the vanA and vanB resistance markers, inferring vanA/vanB-mediated vancomycin resistance. In mixed growth, the iC-GPC Assay™ does not specifically attribute van-mediated vancomycin resistance to either E.</td><td>The Verigene® Gram Positive Blood Culture Nucleic Acid Test (BC-GP) performed using the sample-to-result Verigene System is a qualitative, multiplexed in vitro diagnostic test for the simultaneous detection and identification of potentially pathogenic gram-positive bacteria which may cause bloodstream infection (BSI). BC-GP is performed directly on positive blood culture using BACTEC™ Plus Aerobic/F and BacT/ALERT FA FAN® Aerobic blood culture bottles, which contain gram positive bacteria. BC-GP 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.

BC-GP detects and identifies the following bacterial genera and species:
Staphylococcus spp.
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus lugdunensis
Streptococcus spp.
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus agalactiae
Streptococcus anginosus group
Enterococcus faecalis
Enterococcus faecium
Listeria spp.

In addition, BC-GP detects the mecA resistance marker, inferring mecA-mediated methicillin resistance, and the vanA and vanB resistance markers, inferring vanA/vanB-mediated vancomycin resistance. In mixed growth, BC-GP does not specifically attribute van-mediated vancomycin resistance to either E. faecalis or E. faecium, or mecA-mediated methicillin resistance to either S. aureus or S. epidermidis.</td></tr>
</table>

Characteristic	iC-GPC Assay™ for use on the iC-System™ (New Device)	VERIGENE® Gram Positive Blood Culture Nucleic Acid Test (BC-GP) (K122514) (Primary Predicate Device)
	faecalis or *E. faecium*, or *mecA*-mediated methicillin resistance to either *S. aureus* or *S. epidermidis*. Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing, identification of organisms not detected by the iC-GPC Assay™, differentiation of mixed growth, association of antimicrobial resistance marker genes to a specific organism, or for epidemiological typing.	
Indication for Use	The iC-GPC Assay™ 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 the iC-GPC Assay™, differentiation of mixed growth, association of antimicrobial resistance marker genes to a specific organism, or for epidemiological typing.	BC-GP 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 BC-GP, differentiation of mixed growth, association of antimicrobial resistance marker genes to a specific organism, or for epidemiological typing.
Sample Type	Positive Blood Culture	Positive Blood Culture
Differences		
Instrument Requirements	iC-System™	VERIGENE® System
Test Principal	Arm-PCR	Gold nanoparticle probe-based PCR
Compatible Blood Culture Bottles	BACTEC™ Standard Aerobic/Anaerobic, BACTEC™ Plus Aerobic/Anaerobic, BACTEC™ Lytic/10 Anaerobic, BacT/ALERT Standard Aerobic, BacT/ALERT FA Aerobic FAN®, BacT/ALERT FA Plus Aerobic, VersaTREK® REDOX 1/2	BACTEC™ Standard Aerobic/Anaerobic, BACTEC™ Plus Aerobic/Anaerobic, BACTEC™ Lytic/10 Anaerobic, BACTEC™ Peds Plus, BacT/ALERT Standard Aerobic/Anaerobic, BacT/ALERT FA Aerobic FAN®, BacT/ALERT FN Anaerobic FAN®, BacT/ALERT PF Pediatric FAN, VersaTREK® REDOX 1/2
Throughput	Four (4) samples/iC-Processor™	One (1) sample/processor

Intended Use

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

The iC-GPC Assay™ detects organism DNA and identifies the following bacterial species and resistance markers:

Bacterial Species	Resistance Markers
Staphylococcus aureus *Staphylococcus epidermidis* *Streptococcus pneumoniae* *Enterococcus faecalis* *Enterococcus faecium*	*mecA*- associated with methicillin resistance *vanA*- associated with vancomycin resistance *vanB*- associated with vancomycin resistance

The iC-GPC Assay™ detects the *mecA* resistance marker, inferring *mecA*-mediated methicillin resistance, and the *vanA* and *vanB* resistance markers, inferring *vanA/vanB*-mediated vancomycin resistance. In mixed growth, the iC-GPC Assay™ does not specifically attribute *van*-mediated vancomycin resistance to either *E. faecalis* or *E. faecium*, or *mecA*-mediated methicillin resistance to either *S. aureus* or *S. epidermidis*.

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

Limitations

For prescription use only.

Please refer to the iC-GPC Assay™ labeling for a more complete list of warnings, precautions and contraindications.

Methodology

The iC-GPC Assay™ utilizes polymerase chain reaction (PCR) for the amplification of specific targets and detects the amplified target DNA with fluorescence-based microarray hybridization. The iC-GPC Assay™ uses proprietary ARM-PCR (Amplicon Rescued Multiplex PCR) technology allowing for multiple targets to be amplified in one reaction. Targets are detected directly from patient positive blood cultures, confirmed by Gram stain to contain gram positive cocci. Testing is performed within a closed, disposable cassette that contains all the reagents required to complete the iC-GPC Assay™, including a universal microarray.

To operate, the user opens the iC-GPC Cassette™ cap and pipettes an aliquot of the positive blood culture sample into the sample well of the cassette. The cassette is then inserted into the iC-Processor™, which performs the processes of DNA extraction, multiplex amplification, and microarray hybridization. After processing is complete, the cassette is inserted into the iC-Reader™ for fluorescence-based detection and data analysis. Final results are generated by iC-Report, computer software for data acquisition, analysis, and display.

Extraction, amplification, and hybridization 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-GPC 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 internal 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 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 4 iC-Cassettes™ at one time. The results are interpreted via the iC-Report™ software and displayed for the user on an iMac® computer. Raw data and result interpretations are stored within the iMac®; raw data is accessible to authorized personnel only and is not available to the end user.

Performance Data

For ease of reference, the following table defines iC-GPC target organisms, corresponding gene targets, and common acronyms used in the following study descriptions.

TABLE 1: iC-GPC Assay Targets		
Organism	**Target Gene**	**Acronym**
Staphylococcus epidermidis	*gseA*	SE
Staphylococcus aureus	*nuc*	SA
Streptococcus pneumoniae	*lytA*	SPN
Enterococcus faecalis	*ddl*	EFLS
Enterococcus faecium	fcm (ddl_{EFCM})	EFCM

Bottle Ring

A study was performed to establish the lowest level of each iC-GPC Assay target organism at initial bottle positivity (bottle "ring"). The nineteen organisms used to define target limits of detection were evaluated. Organisms were inoculated into BD BACTEC Plus Aerobic blood culture bottles with human blood added. Bottles were allowed to incubate on the blood culture system until initial bottle positivity. Bottles were removed from the incubator within two hours of bottle ring, and plating and subsequent colony counts were performed to confirm organism concentrations. A minimum of five bottles were evaluated for each strain. The average concentrations at bottle ring are presented in Table 2 below. These concentrations, representative of the lowest levels that may be observed in a clinical setting, are equivalent to or greater than the respective target limits of detection (see below).

TABLE 2: Target Organism Concentrations at Bottle "Ring"	
Organism	**Average Concentration (CFU/mL)**
SE 700566	2.68×10^7
SE 35984	2.04×10^7
SE 12228	2.59×10^7
SE 49134	1.01×10^7
SA 700699	5.00×10^8
SA BAA 1768	5.24×10^7
SA BAA 977	1.14×10^8
SA 25923	6.06×10^8
SPN 6301	5.40×10^6
SPN 700673	5.95×10^6
EFLS 51299	4.72×10^{10}
EFLS 700802	2.22×10^8
EFLS JMI 12536	2.27×10^8
EFLS 29212	5.53×10^{10}
EFLS BAA 2128	5.77×10^7
EFCM 700221	1.16×10^9
EFCM 51559	5.87×10^8
EFCM 35667	2.86×10^7
EFCM BAA 2127	2.70×10^8

Reproducibility

To confirm site-to-site, operator-to-operator, system-to-system, and lot-to-lot reproducibility of the iC-GPC Assay, a representative panel of target organisms and one non-target organism were tested at two concentrations: initial bottle positivity and eight hours beyond initial bottle positivity. Organisms were grown to the appropriate concentration in BD BACTEC Plus Aerobic/F 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 in-house. The six organism panel was tested in replicates of three across five, non-consecutive days. Testing was evaluated across three cassette lots and four iC-Systems. Table 3 below summarizes results by iC-GPC Assay target and concentration. Testing confirmed that performance of the iC-GPC Assay for use on the iC-System is reproducible across sites, operators, systems, and lots.

TABLE 3: iC-GPC Reproducibility Performance by Target & Concentration

Organism/Gene Target/ Concentration	Overall Performance	Overall Performance % [95% CI]	False Negatives	False Positives	Positive Controls Check Failures	System Failures
S. epidermidis (gseA) Bottle Ring	89/89	100.0 [95.86-100.0]	0/89 (0.00%)	1/976 (0.10%)	0/90 (0.00%)	1/90 (1.11%)
S. epidermidis (gseA) Bottle Ring + 8 hours	90/90	100.0 [95.91-100.0]	0/90 (0.00%)	0/975 (0.00%)	0/90 (0.00%)	0/90 (0.00%)
S. aureus (nuc) Bottle Ring	89/89	100.0 [95.86-100.0]	0/89 (0.00%)	0/976 (0.00%)	1/90 (1.11%)	0/90 (0.00%)
S. aureus (nuc) Bottle Ring + 8 hours	89/90	98.9 [93.97-99.80]	1/90 (1.12%)	0/975 (0.00%)	0/90 (0.00%)	0/90 (0.00%)
S. pneumoniae (lytA) Bottle Ring	88/88	100.0 [95.82-100.0]	0/88 (0.00%)	0/977 (0.00%)	1/90 (1.11%)	1/90 (1.11%)
S. pneumoniae (lytA) Bottle Ring + 8 hours	89/89	100.0 [95.86-100.0]	0/89 (0.00%)	0/976 (0.00%)	0/90 (0.00%)	1/90 (1.11%)
E. faecalis (ddl) Bottle Ring	89/89	100.0 [95.86-100.0]	0/89 (0.00%)	2/976 (0.20%)	0/90 (0.00%)	1/90 (1.11%)
E. faecalis (ddl) Bottle Ring + 8 hours	89/89	100.0 [95.86, 100.0]	0/89 (0.00%)	3/976 (0.31%)	1/90 (1.11%)	0/90 (0.00%)
E. faecium (fcm) Bottle Ring	90/90	100.0 [95.91-100.0]	0/90 (0.00%)	0/975 (0.00%)	0/90 (0.00%)	0/90 (0.00%)
E. faecium (fcm) Bottle Ring + 8 hours	89/89	100.0 [95.86-100.0]	0/89 (0.00%)	0/976 (0.00%)	1/90 (1.11%)	0/90 (0.00%)
mecA Bottle Ring	177/178	99.4 [96.89-99.90]	1/178 (0.56%)	1/887 (0.11%)	1/180 (0.56%)	1/180 (0.56%)
mecA Bottle Ring + 8 hours	180/180	100.0 [97.91-100.0]	0/180 (0.00%)	0/885 (0.00%)	0/180 (0.00%)	0/180 (0.00%)
vanA Bottle Ring	90/90	100.0 [95.91-100.0]	0/90 (0.00%)	0/975 (0.00%)	0/90 (0.00%)	0/90 (0.00%)
vanA Bottle Ring + 8 hours	89/89	100.0 [95.86-100.0]	0/89 (0.00%)	0/976 (0.00%)	1/90 (1.11%)	0/90 (0.00%)
vanB Bottle Ring	89/89	100.0 [95.86-100.0]	0/89 (0.00%)	0/976 (0.00%)	0/90 (0.00%)	1/90 (1.11%)
vanB Bottle Ring + 8 hours	89/89	100.0 [95.86, 100.0]	0/89 (0.00%)	0/976 (0.00%)	1/90 (1.11%)	0/90 (0.00%)

Limit of Detection (LoD)

A study was conducted to determine the limit of detection, defined as the lowest concentration (CFU/mL) of analyte that can be detected approximately 95% of the time, for the eight targets detected by the iC-GPC Assay. A panel of nineteen organisms was evaluated, including a minimum of two bacterial strains per target analyte. Three concentrations were tested for each LoD panel member in replicates of twenty on three unique cassette lots. The LoD of each target is considered the lowest concentration with an approximately 95% detection rate, presented in Table 4 below.

TABLE 4: iC-GPC Assay Target LoD

iC-GPC Assay Target	Phenotype Identification	Defined LoD (CFU/mL)
gseA	*Staphylococcus epidermidis*	$1.6 \times 10^6 – 1.7 \times 10^7$
nuc	*Staphylococcus aureus*	$1.7 \times 10^6 – 4.4 \times 10^6$
mecA	Associated with methicillin resistance	$7.4 \times 10^5 – 9.5 \times 10^6$
lytA	*Streptococcus pneumoniae*	$1.3 \times 10^6 – 6.0 \times 10^6$
ddl	*Enterococcus faecalis*	$3.0 \times 10^5 – 5.8 \times 10^6$
fcm	*Enterococcus faecium*	$4.9 \times 10^6 – 7.9 \times 10^6$
vanA	Associated with vancomycin resistance	$7.2 \times 10^5 – 1.1 \times 10^7$
vanB	Associated with vancomycin resistance	$3.9 \times 10^6 – 5.8 \times 10^6$

Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) for each iC-GPC Assay target was evaluated using multiple well-characterized and clinically relevant strains chosen to represent temporal, geographic, and genetic diversity. Inclusivity panel organisms included the following:

- 5 *Staphylococcus epidermidis*, *mecA* negative strains
- 6 *Staphylococcus epidermidis, mecA* positive strains
- 5 *Staphylococcus aureus, mecA* negative strains
- 58 *Staphylococcus aureus, mecA* positive strains, representing various pulse-field gel electrophoresis types and including the following:
  - Vancomycin-intermediate SA (VISA)
  - Panton-Valentine Leukocidin (PVL)-producing SA
  - ATCC 43300 (hetero-resistant, *mecA* positive)
- 2 borderline oxacillin-resistant *Staphylococcus aureus* strains (BORSA)
- 10 *Streptococcus pneumoniae* strains
- 5 *Enterococcus faecalis, vanA/vanB* negative strains
- 5 *Enterococcus faecalis, vanA* or *vanB* positive strains
- 5 *Enterococcus faecium, vanA/vanB* negative strains
- 5 *Enterococcus faecium, vanA* or *vanB* positive strains

A total of 106 organisms were evaluated for iC-GPC Assay Inclusivity testing. Strains were tested near the target LoD (2-3x LoD) or at the lowest level of bottle positivity. Testing was performed in triplicate. In the event of a false negative result, the stain was retested near the target LoD in replicates of ten. All expected targets were detected by the iC-GPC Assay. Results of inclusivity testing are summarized in Table 5 below.

TABLE 5: iC-GPC Inclusivity Results			
Species	**Strain ID**	**Other IDs/Notes**	**Targets Detected/ Total Replicates**
S. epidermidis	Z318	N/A	3/3
S. epidermidis	Z0801689	HER 1292	3/3
S. epidermidis	ATCC 700583	N/A	3/3
S. epidermidis	Z291	ATCC 14990	3/3
S. epidermidis	Z049	N/A	3/3
S. epidermidis, mecA (+)	Z0801651	RP62A	3/3
S. epidermidis, mecA (+)	Z256	N/A	3/3
S. epidermidis, mecA (+)	Z257	N/A	3/3
S. epidermidis, mecA (+)	Z258	N/A	12/13
S. epidermidis, mecA (+)	Z259	N/A	3/3
S. epidermidis, mecA (+)	ATCC 29887	N/A	3/3
S. aureus	Z021	ATCC 6538P	3/3
S. aureus	ATCC 12600	N/A	3/3
S. aureus	Z0801675	N/A	3/3
S. aureus	Z057	N/A	3/3
S. aureus	Z153	N/A	3/3
S. aureus, mecA (+)	HM-466	131	3/3
S. aureus, mecA (+)	HM-467	177	3/3
S. aureus, mecA (+)	NR-10129	TCH60	3/3
S. aureus, mecA (+)	NR-10189	HFH-30364, USA400, **PVL+**	3/3
S. aureus, mecA (+)	NR-10192	HFH-30106, non USA100-1100	3/3
S. aureus, mecA (+)	NR-13524	H342087	3/3
S. aureus, mecA (+)	NR-28983	S0385	3/3
S. aureus, mecA (+)	NR-45872	HIP07930, USA600	3/3
S. aureus, mecA (+)	NR-45880	LIM1	3/3
S. aureus, mecA (+)	NR-45890	BR 5, **VISA**	3/3
S. aureus, mecA (+)	NR-46062	H2138 (isolate 10)	3/3
S. aureus, mecA (+)	NR-46063	P1V44, **VISA**	3/3
S. aureus, mecA (+)	NR-46072	1078, USA700	3/3
S. aureus, mecA (+)	NR-46080	AIS 2006061, USA1000, **PVL+**	3/3
S. aureus, mecA (+)	NR-46081	HIP12899, USA1100, **PVL+**	3/3
S. aureus, mecA (+)	NR-46218	GA-442, USA700	3/3
S. aureus, mecA (+)	NR-46070	USA300-0114	3/3
S. aureus, mecA (+)	NR-46171	CA-126, USA100	3/3
S. aureus, mecA (+)	NR-46172	CA-127, USA300, **PVL+**	3/3
S. aureus, mecA (+)	NR-46177	CA-347, USA600	3/3
S. aureus, mecA (+)	NR-46180	CA-409, USA200	3/3
S. aureus, mecA (+)	NR-46182	CA-513, USA800	3/3
S. aureus, mecA (+)	NR-46191	CO-34, USA300, **PVL+**	3/3
S. aureus, mecA (+)	NR-46197	CO-72, USA800	3/3

TABLE 5: iC-GPC Inclusivity Results			
Species	**Strain ID**	**Other IDs/Notes**	**Targets Detected/ Total Replicates**
S. aureus, mecA (+)	NR-46199	CT-110, USA100	3/3
S. aureus, mecA (+)	NR-46207	CT-58, USA500	3/3
S. aureus, mecA (+)	NR-46215	GA-356	3/3
S. aureus, mecA (+)	NR-46221	GA-656, USA800	3/3
S. aureus, mecA (+)	NR-46223	GA-92, USA300, **PVL+**	3/3
S. aureus, mecA (+)	NR-46224	NY-315, USA600	3/3
S. aureus, mecA (+)	NR-46250	OR-130, USA100	3/3
S. aureus, mecA (+)	NR-46251	OR-131, USA200	3/3
S. aureus, mecA (+)	NR-46261	TN-112, USA300	3/3
S. aureus, mecA (+)	NR-46269	TN-82, USA200	3/3
S. aureus, mecA (+)	NR-41875	M0001	3/3
S. aureus, mecA (+)	NR-41876	M0006	3/3
S. aureus, mecA (+)	NR-41877	M0055	3/3
S. aureus, mecA (+)	NR-41878	M0102	3/3
S. aureus, mecA (+)	NR-41879	M0108	3/3
S. aureus, mecA (+)	NR-41880	M0197	3/3
S. aureus, mecA (+)	NR-41881	M0200	3/3
S. aureus, mecA (+)	NR-41882	M0288	3/3
S. aureus, mecA (+)	NR-41883	M0334	3/3
S. aureus, mecA (+)	NR-41887	M0663	3/3
S. aureus, mecA (+)	NR-41889	M0934	3/3
S. aureus, mecA (+)	NR-41890	M0943	3/3
S. aureus, mecA (+)	NR-41895	M1510	12/13
S. aureus, mecA (+)	NR-41896	M1565	3/3
S. aureus, mecA (+)	NR-10187	HFH-29994, USA100	3/3
S. aureus, mecA (+)	NR-13525	F338081	3/3
S. aureus, mecA (+)	NR-13526	W342179	3/3
S. aureus, mecA (+)	NR-13533	S247312	3/3
S. aureus, mecA (+)	NR-13546	SU-1	3/3
S. aureus, mecA (+)	NR-30544	HI049, USA300, **PVL+**	3/3
S. aureus, mecA (+)	NR-45924	LinR #12	3/3
S. aureus, mecA (+)	ATCC 700698	N/A	3/3
S. aureus, mecA (+)	ATCC BAA44	N/A	3/3
S. aureus, mecA (+)	ATCC 43300	Hetero-resistant	3/3
BORSA	MCW 109	N/A	3/3
BORSA	MCW 141	N/A	3/3
S. pneumoniae	Z022	Clinical isolate, serotype 19F	3/3
S. pneumoniae	Z073	Clinical isolate, serotype 19F	3/3
S. pneumoniae	Z319	Clinical isolate, serotype 12F	3/3
S. pneumoniae	Z278	ATCC BAA-255, R6 (non-virulent)	3/3

TABLE 5: iC-GPC Inclusivity Results			
Species	**Strain ID**	**Other IDs/Notes**	**Targets Detected/ Total Replicates**
S. pneumoniae	Z279	PHE NCTC 11910, serotype 23F	3/3
S. pneumoniae	Z280	PHE NCTC 11897, serotype 9V	3/3
S. pneumoniae	Z282	ATCC 33400, NCTC 7465, serotype 1	3/3
S. pneumoniae	Z295	ATCC 49619, serotype 19F	3/3
S. pneumoniae	Z261	Clinical isolate	3/3
S. pneumoniae	Z262	Clinical isolate	3/3
E. faecalis	Z0801637	N/A	3/3
E. faecalis	ATCC 33186	N/A	3/3
E. faecalis	ATCC 49532	N/A	3/3
E. faecalis	Z289	ATCC 19433	3/3
E. faecalis	Z266	ATCC 6055	3/3
E. faecalis, *vanB* (+)	Z0801693	**vanB**	3/3
E. faecalis, *vanA* (+)	Z324	**vanA**	3/3
E. faecalis, *vanA* (+)	Z267	PHE NCTC 12201- **vanA**	3/3
E. faecalis, *vanA* (+)	Z269	PHE NCTC 12203- **vanA**	3/3
E. faecalis, *vanB* (+)	ATCC 51575	**vanB**	3/3
E. faecium	Z322	N/A	3/3
E. faecium	ATCC 8459	N/A	3/3
E. faecium	Z265	ATCC 9756	3/3
E. faecium	Z290	ATCC 19434	3/3
E. faecium	Z320	ATCC 19634	3/3
E. faecium, *vanA* (+)	Z0801892	**vanA**	3/3
E. faecium, *vanB* (+)	Z323	**vanB**	3/3
E. faecium, *vanA* (+)	Z270	PHE NCTC 12202- **vanA**	3/3
E. faecium, *vanA* (+)	Z271	PHE NCTC 12204- **vanA**	3/3
E. faecium, *vanA* (+)	Z260	**vanA**	3/3

Analytical Exclusivity

Analytical specificity of the iC-GPC Assay was evaluated by testing a comprehensive panel of non-target microorganisms that may be encountered in positive blood cultures. Exclusivity panel members included organisms phylogenetically related to iC-GPC target organisms as well as common blood culture contaminants. A total of ninety-four (94) exclusivity organisms were tested. Potential cross-reactivity was evaluated by testing the exclusivity panel organisms at high concentrations in blood culture bottle/blood media. Exclusivity results are presented in Table 6 below; performance is based on the observation of all expected negative results. In the event of a false positive result, the organism was retested in replicates of three (3) or ten (10). One organism, *Streptococcus bovis,* demonstrated reproducible cross-reactivity with the iC-GPC target *Enterococcus faecalis.*

TABLE 6: iC-GPC Exclusivity Results		
Exclusivity Organism	**Test Concentration (CFU/mL, or as designated, bottle ring or $TCID_{50}$/mL)**	**Exclusivity**
Abiotrophia defectiva	3.09×10^8	3/3
Acinetobacter baumannii	6.55×10^8	3/3
Acinetobacter lwoffi	5.70×10^8	3/3
Aerococcus viridans	5.05×10^7	3/3
Aeromonas hydrophila	3.50×10^8	3/3
Alcaligenes faecalis	1.01×10^{10}	3/3
Anaerococcus tetradius	3.55×10^8	3/3
Aspergillus niger	8.10×10^7	3/3
Bacillus cereus	7.30×10^6	3/3
Bacteroides fragilis	4.20×10^9	3/3
Campylobacter coli	2.55×10^8	3/3
Campylobacter jejuni	2.24×10^8	3/3
Candida albicans	8.00×10^7	3/3
Candida catenulata	1.14×10^9	3/3
Candida dubliniensis	7.05×10^6	3/3
Candida glabrata	1.15×10^8	3/3
Candida guilliermondii	1.03×10^7	3/3
Candida krusei	2.82×10^7	5/6[1]
Candida parapsilosis	3.15×10^7	3/3
Candida tropicalis	7.65×10^7	2/2
Citrobacter amalonaticus	2.35×10^9	3/3
Citrobacter freundii	6.40×10^8	3/3
Citrobacter koseri	3.29×10^9	3/3
Citrobacter sedlakii	1.70×10^9	3/3
Clostridium difficile (NAP-1 toxigenic)	2.44×10^7	3/3
Clostridium difficile (non-toxigenic)	2.97×10^7	3/3
Clostridium oedematiens (novyi)	5.70×10^6	3/3
Collinsella aerofaciens	5.95×10^7	3/3
Corynebacterium amycolatum	3.79×10^8	5/6[2]
Corynebacterium genitalium	2.50×10^8	3/3
Corynebacterium jeikeium	4.19×10^8	3/3
Coxsackie virus	$1 \times 10^{6.34}$ ($TCID_{50}$)	3/3
Cryptococcus neoformans	1.08×10^8	3/3
Cytomegalovirus	$1 \times 10^{5.07}$ ($TCID_{50}$)	3/3
Echovirus	$1 \times 10^{7.77}$ ($TCID_{50}$)	3/3
Edwardsiella tarda	5.05×10^9	3/3
Eggerthella lenta	1.42×10^8	3/3
Enterobacter aerogenes	6.50×10^9	3/3
Enterobacter cloacae	2.72×10^9	3/3

TABLE 6: iC-GPC Exclusivity Results		
Exclusivity Organism	**Test Concentration (CFU/mL, or as designated, bottle ring or $TCID_{50}$/mL)**	**Exclusivity**
Enterococcus avium	5.85×10^7	3/3
Enterococcus casseliflavus	9.60×10^6	3/3
Enterococcus cecorum	3.80×10^8	3/3
Enterococcus dispar	6.10×10^8	3/3
Enterococcus gallinarum	3.20×10^8	3/3
Enterococcus hirae	1.04×10^9	3/3
Enterococcus raffinosus	4.67×10^8	3/3
Enterovirus Type 71	$1 \times 10^{6.10}$ ($TCID_{50}$)	3/3
Escherichia coli	1.27×10^9	3/3
Escherichia hermannii	3.09×10^9	3/3
Fusobacterium varium	1.25×10^9	3/3
Klebsiella oxytoca	7.25×10^9	3/3
Klebsiella pneumoniae	2.27×10^9	3/3
Kocuria kristinae	3.45×10^8	3/3
Kytococcus schroeteri	1.85×10^8	3/3
Lactobacillus acidophilus	5.30×10^7	3/3
Lactobacillus plantarum subsp.plantarum	1.53×10^9	3/3
Lactobacillus reuteri	1.24×10^8	5/6[3]
Lactococcus lactis	2.57×10^9	3/3
Leminorella grimontii	1.22×10^9	3/3
Leuconostoc mesenteroides	3.17×10^7	3/3
Micrococcus luteus	1.13×10^7	3/3
Oerskovia enterophila	1.33×10^{10}	3/3
Pediococcus pentosaceus	2.82×10^9	3/3
Planococcus citreus	1.14×10^9	3/3
Propionibacterium acnes	9.75×10^7	3/3
Proteus mirabilis	9.25×10^8	3/3
Proteus penneri	1.11×10^9	3/3
Proteus vulgaris	1.13×10^9	3/3
Providencia alcalifaciens	1.62×10^9	10/11[4]
Providencia rettgeri	1.05×10^9	3/3
Providencia stuartii	1.48×10^9	3/3
Pseudomonas aeruginosa	2.14×10^8	3/3
Pseudomonas putida	4.95×10^8	3/3
Rothia mucilaginosus	1.60×10^8	3/3
Salmonella spp (typhimurium)	4.45×10^9	3/3
Staphylococcus capitis	4.05×10^7	3/3
Staphylococcus delphini	2.12×10^9	3/3
Staphylococcus haemolyticus	1.95×10^7	3/3

TABLE 6: iC-GPC Exclusivity Results		
Exclusivity Organism	**Test Concentration (CFU/mL, or as designated, bottle ring or $TCID_{50}$/mL)**	**Exclusivity**
Staphylococcus hominis	1.87×10^8	3/3
Staphylococcus intermedius	8.55×10^7	5/6[5]
Staphylococcus lugdunensis	3.05×10^9	3/3
Staphylococcus lutrae	6.00×10^9	3/3
Staphylococcus pettenkoferi	1.36×10^9	3/3
Staphylococcus schleiferi	2.67×10^9	3/3
Staphylococcus schleiferi subsp. coagulans	2.67×10^8	5/6[6]
Staphylococcus warneri	6.48×10^8	3/3
Streptococcus agalactiae	6.05×10^8	13/14[7]
Streptococcus anginosus	4.65×10^8	13/14[8]
Streptococcus bovis	5.50×10^8	3/14[9]
Streptococcus dysgalactiae	8.35×10^6	3/3
Streptococcus intermedius	Bottle ring + 8 hours	11/12[10]
Streptococcus mitis	Bottle ring + 8 hours	3/3
Streptococcus pseudopneumoniae	Bottle ring + 8 hours	3/3
Streptococcus pyogenes	8.20×10^8	3/3
Streptococcus salivarus	8.85×10^7	3/3
Streptococcus uberis	7.45×10^7	3/3

1) Candida *krusei:* 1/3 false positive *E. faecalis* in initial testing, 3/3 repeats negative.
2) *Corynebacterium amycolatum:* 1/3 false positive *S. epidermidis* in initial testing, 3/3 repeats negative.
3) *Lactobacillus reuteri:* 1/3 false positive *mecA* in initial testing, 3/3 repeats negative. Note that *mecA* would not be reported as the *S. aureus* and *S. epidermidis* targets were negative as expected.
4) *Providencia alcalifaciens*: 1/3 false positive *S. aureus* in initial testing, 8/8 repeats negative.
5) *Staphylococcus intermedius*: 1/3 false positive *mecA* in initial testing, 3/3 repeats negative. Note that *mecA* would not be reported as the *S. aureus* and *S. epidermidis* targets were negative as expected.
6) *Staphylococcus schleiferi* subsp. *coagulans*: 1/3 false positive *mecA* in initial testing, 3/3 repeats negative. Note that *mecA* would not be reported as the *S. aureus* and *S. epidermidis* targets were negative as expected.
7) *Streptococcus agalactiae:* 1/3 false positive *mecA* in initial testing, 11/11 repeats negative. Note that *mecA* would not be reported as the *S. aureus* and *S. epidermidis* targets were negative as expected.
8) *Streptococcus anginosus:* 1/2 false positive *E. faecalis* in initial testing, 12/12 repeats negative.
9) *Streptococcus bovis:* 1/2 false positive *E. faecalis* in initial testing, 10/12 false positive *E. faecalis* in repeat testing
10) *Streptococcus intermedius:* 1/3 false positive *E. faecalis* in initial testing, 9/9 repeats negative

Microbial Interference

Potential microbial interference was evaluated by testing high concentrations of 85 exclusivity organisms in combination with iC-GPC target organisms present near LoD concentrations. Microbial interference results are presented in Table 7 below; performance is based on all expected targets detected. In the event of a false negative result, the combination was repeated with the target organism near target LoD concentrations in replicates of 10. If additional false negative results were observed, testing was repeated with the target organism at bottle ring concentrations. Microbial interference was not observed for the majority of organism combinations. For those combinations with unexpected initial false negative results, additional replicate testing at the same concentration or at a higher "bottle ring" concentration generated 100% iC-GPC Assay target detection as expected.

TABLE 7: iC-GPC Microbial Interference Results						
Exclusivity Organism	**Test Concentration (CFU/mL)**	**SE**	**SA**	**SPN**	**EFLS**	**EFCM**
		Test Concentration for initial three replicates (CFU/mL)				
		1.4×10^7	**1.4×10^7**	**Bottle Ring**	**1.2×10^7**	**1.2×10^7**
Abiotrophia defective	1.54×10^8	3/3	3/3	3/3	3/3	3/3
Acinetobacter baumannii	3.28×10^8	3/3	3/3	3/3	3/3	3/3
Acinetobacter lwoffi	2.85×10^8	3/3	3/3	3/3	3/3	3/3
Aerococcus viridans	2.53×10^7	3/3	3/3	3/3	12/13[1]	3/3
Aeromonas hydrophila	1.75×10^8	3/3	3/3	3/3	3/3	3/3
Anaerococcus tetradius	1.78×10^8	3/3	3/3	3/3	3/3	3/3
Bacillus cereus	3.65×10^6	3/3	3/3	3/3	3/3	3/3
Bacteroides fragilis	2.10×10^9	3/3	3/3	2/2	3/3	3/3
Campylobacter coli	1.28×10^8	3/3	18/22[2]	3/3	3/3	3/3
Campylobacter jejuni	1.12×10^8	3/3	3/3	3/3	3/3	3/3
Candida albicans	4.00×10^7	3/3	3/3	3/3	3/3	3/3
Candida catenulate	5.68×10^8	3/3	3/3	3/3	3/3	3/3
Candida dubliniensis	3.53×10^6	3/3	3/3	3/3	3/3	3/3
Candida glabrata	5.75×10^7	3/3	3/3	3/3	3/3	3/3
Candida guilliermondii	5.15×10^6	3/3	3/3	3/3	3/3	3/3
Candida krusei	1.41×10^7	3/3	3/3	3/3	20/22[3]	3/3
Candida parapsilosis	1.58×10^7	3/3	3/3	3/3	3/3	3/3
Candida tropicalis	3.83×10^7	3/3	3/3	3/3	3/3	3/3
Citrobacter amalonaticus	1.18×10^8	3/3	3/3	3/3	3/3	5/6[4]
Citrobacter freundii	3.20×10^8	3/3	3/3	3/3	3/3	3/3
Citrobacter koseri	1.64×10^9	3/3	3/3	3/3	3/3	3/3
Citrobacter sedlakii	8.50×10^8	3/3	3/3	3/3	3/3	3/3
Clostridium difficile (NAP-1 toxigenic)	1.22×10^7	3/3	5/6[5]	3/3	3/3	3/3
Clostridium difficile (non-toxigenic)	1.48×10^7	5/6[6]	3/3	3/3	3/3	3/3
Clostridium oedematiens (novyi)	2.85×10^6	3/3	3/3	3/3	3/3	3/3
Collinsella aerofaciens	2.98×10^7	3/3	3/3	3/3	3/3	3/3
Corynebacterium amycolatum	1.89×10^8	3/3	3/3	3/3	3/3	3/3
Corynebacterium genitalium	1.25×10^8	3/3	3/3	3/3	3/3	3/3
Corynebacterium jeikeium	2.09×10^8	3/3	3/3	3/3	2/2	3/3
Edwardsiella tarda	2.53×10^9	3/3	3/3	3/3	3/3	3/3
Enterobacter aerogenes	3.25×10^9	3/3	3/3	3/3	3/3	3/3
Enterobacter cloacae	1.36×10^9	3/3	3/3	3/3	3/3	3/3
Enterococcus avium	2.93×10^7	3/3	3/3	3/3	3/3	3/3
Enterococcus casseliflavus	4.80×10^6	3/3	3/3	3/3	3/3	3/3
Enterococcus cecorum	1.90×10^8	3/3	3/3	3/3	3/3	3/3
Enterococcus dispar	3.05×10^8	3/3	3/3	3/3	3/3	3/3

TABLE 7: iC-GPC Microbial Interference Results						
Exclusivity Organism	**Test Concentration (CFU/mL)**	**SE**	**SA**	**SPN**	**EFLS**	**EFCM**
		Test Concentration for initial three replicates (CFU/mL)				
		1.4×10^7	**1.4×10^7**	**Bottle Ring**	**1.2×10^7**	**1.2×10^7**
Enterococcus gallinarum	1.60×10^8	3/3	3/3	3/3	3/3	3/3
Enterococcus hirae	5.18×10^8	3/3	3/3	3/3	3/3	3/3
Enterococcus raffinosus	2.33×10^8	3/3	3/3	3/3	3/3	3/3
Escherichia coli	6.35×10^8	3/3	3/3	3/3	3/3	3/3
Escherichia hermannii	1.54×10^9	3/3	3/3	3/3	11/12[7]	3/3
Fusobacterium varium	6.23×10^8	3/3	3/3	3/3	3/3	3/3
Klebsiella oxytoca	3.63×10^9	3/3	3/3	3/3	3/3	3/3
Klebsiella pneumoniae	1.13×10^9	3/3	3/3	3/3	3/3	3/3
Kocuria kristinae	1.73×10^8	3/3	12/13[8]	3/3	3/3	3/3
Kytococcus schroeteri	9.25×10^7	3/3	3/3	3/3	3/3	3/3
Lactobacillus acidophilus	2.65×10^7	3/3	3/3	3/3	3/3	3/3
Lactobacillus plantarum subsp.plantarum	7.63×10^8	3/3	3/3	3/3	3/3	3/3
Lactobacillus reuteri	6.18×10^7	3/3	3/3	3/3	3/3	3/3
Lactococcus lactis	1.28×10^9	3/3	3/3	3/3	3/3	3/3
Leminorella grimontii	6.08×10^8	3/3	3/3	3/3	3/3	3/3
Leuconostoc mesenteroides	1.58×10^7	3/3	3/3	3/3	3/3	3/3
Micrococcus luteus	5.63×10^6	3/3	11/12[9]	3/3	3/3	3/3
Oerskovia enterophila	6.63×10^9	3/3	3/3	3/3	3/3	3/3
Pediococcus pentosaceus	1.41×10^9	3/3	3/3	3/3	3/3	3/3
Propionibacterium acnes	4.88×10^7	3/3	3/3	3/3	3/3	3/3
Proteus mirabilis	4.63×10^8	3/3	12/13[10]	3/3	11/12[11]	3/3
Proteus penneri	5.55×10^8	3/3	11/13[12]	3/3	3/3	3/3
Proteus vulgaris	5.63×10^8	3/3	3/3	3/3	3/3	3/3
Providencia alcalifaciens	8.08×10^8	3/3	3/3	3/3	3/3	3/3
Providencia rettgeri	5.25×10^8	3/3	3/3	3/3	3/3	3/3
Providencia stuartii	7.40×10^8	3/3	5/6[13]	3/3	3/3	3/3
Pseudomonas aeruginosa	1.07×10^8	3/3	3/3	3/3	3/3	3/3
Pseudomonas putida	2.48×10^8	3/3	3/3	3/3	3/3	3/3
Rothia mucilaginosus	8.00×10^7	3/3	3/3	3/3	3/3	3/3
Salmonella spp (typhimurium)	2.23×10^9	3/3	3/3	3/3	3/3	3/3
Staphylococcus capitis	2.03×10^7	3/3	21/23[14]	3/3	3/3	3/3
Staphylococcus delphini	1.06×10^9	3/3	12/13[15]	3/3	3/3	3/3
Staphylococcus haemolyticus	9.75×10^6	3/3	3/3	11/12[16]	3/3	3/3
Staphylococcus hominis	9.33×10^7	3/3	3/3	3/3	3/3	3/3
Staphylococcus intermedius	4.28×10^7	3/3	3/3	3/3	3/3	3/3
Staphylococcus lugdunensis	1.53×10^9	3/3	3/3	3/3	3/3	3/3
Staphylococcus lutrae	3.00×10^9	3/3	3/3	3/3	3/3	3/3

TABLE 7: iC-GPC Microbial Interference Results						
Exclusivity Organism	**Test Concentration (CFU/mL)**	**SE**	**SA**	**SPN**	**EFLS**	**EFCM**
		Test Concentration for initial three replicates (CFU/mL)				
		1.4×10^7	**1.4×10^7**	**Bottle Ring**	**1.2×10^7**	**1.2×10^7**
Staphylococcus pettenkoferi	6.78×10^8	12/13[17]	3/3	3/3	3/3	3/3
Staphylococcus schleiferi	1.33×10^9	3/3	3/3	3/3	3/3	3/3
Staphylococcus schleiferi subsp. coagulans	1.22×10^9	3/3	3/3	3/3	3/3	3/3
Staphylococcus warneri	3.20×10^8	3/3	3/3	3/3	3/3	3/3
Streptococcus agalactiae	3.03×10^8	3/3	3/3	3/3	3/3	3/3
Streptococcus anginosus	2.33×10^8	3/3	3/3	3/3	3/3	12/13[18]
Streptococcus bovis	2.75×10^8	5/6[19]	3/3	3/3	3/3	3/3
Streptococcus dysgalactiae	4.18×10^7	3/3	3/3	3/3	3/3	3/3
Streptococcus intermedius	8.25×10^7	3/3	3/3	3/3	3/3	3/3
Streptococcus mitis	Bottle ring + 8 hours	3/3	3/3	3/3	3/3	3/3
Streptococcus pseudopneumoniae	Bottle ring + 8 hours	3/3	3/3	3/3	3/3	3/3
Streptococcus pyogenes	4.10×10^8	3/3	3/3	3/3	3/3	3/3
Streptococcus salivarus	4.43×10^7	3/3	12/13[20]	3/3	3/3	2/2
Streptococcus uberis	3.73×10^7	3/3	3/3	3/3	3/3	3/3

1) 1 false negative *vanB* in initial testing (2/3), 10/10 repeats passed
2) 2 false negative *S. aureus* and 1 false negative *mecA* in initial testing (1/3), 2 false negative *S. aureus* and 2 false negative *mecA* in repeat testing near LoD (8/10). No false negatives in repeat testing at bottle ring concentration (9/9).
3) 1 false negative *vanB* in initial testing (2/3), 1 false negative *vanB* in repeat testing near LoD (9/10). No false negatives in repeat testing at bottle ring concentration (9/9).
4) 1 false positive *mecA* in initial testing (2/3), 3/3 repeats passed. Note that *mecA* would not be reported as the *S aureus* and *S. epidermidis* targets were negative as expected.
5) 1 false positive *S. epidermidis* in initial testing (2/3), 3/3 repeats passed
6) 1 false positive *S. aureus* in initial testing (2/3), 3/3 repeats passed
7) 1 false negative *vanB* in initial testing (2/3), 9/9 repeats passed
8) 1 false negative *S. aureus* and 1 false negative *mecA* in initial testing (2/3), 10/10 repeats passed
9) 1 false negative *S. aureus* and 1 false negative *mecA* in initial testing (2/3), 9/9 repeats passed
10) 1 false negative *S. aureus* in initial testing (2/3), 10/10 repeats passed
11) 1 false negative *vanB* in initial testing (2/3), 9/9 repeats passed
12) 2 false negative *mecA* in initial testing (1/3), 10/10 repeats passed
13) 1 false positive *S. epidermidis* in initial testing (2/3), 3/3 repeats passed
14) 1 false negative *mecA* in initial testing (2/3), 1 false negative *S. aureus* and 1 false negative *mecA* in repeat testing near LoD (9/10). No false negatives in repeat testing at bottle ring concentration (9/9).
15) 1 false negative *S. aureus* and 1 false negative *mecA* in initial testing (2/3), 10/10 repeats passed
16) 1 false negative *S. pneumoniae* in initial testing (1/2), 10/10 repeats passed at bottle ring concentration.
17) 1 false negative *mecA* in initial testing (2/3), 10/10 repeats passed
18) 1 false *negative E. faecium* in initial testing (2/3), 10/10 repeats passed
19) 1 false positive *E. faecalis* in initial testing (2/3), 3/3 repeats passed
20) 1 false negative *S. aureus* in initial testing (2/3), 10/10 repeats passed

Competitive Inhibition

iC-GPC Assay performance was evaluated with combinations of target analytes that may be found in mixed positive blood cultures. One target organism was tested at a concentration near LoD ("low organism") in combination with a second target organism at a concentration of bottle ring + 8 hours ("high organism"). Combinations of five organisms representing each of the iC-GPC Assay targets were tested, for a total of 20 combinations. Competitive inhibition results are summarized in Table 8 below. Results represent the number of detected targets out of the total number of replicates tested. All high concentration iC-GPC targeted organisms were detected in all organism combinations. Due to competitive inhibition, low concentration organism targets were only detected in 3/20 (15%) of organism combinations. Target organisms present near LoD concentrations may not be detected by the iC-GPC Assay when a second target organism is present in the blood culture specimen.

TABLE 8: iC-GPC Competitive Inhibition Performance

		Target Performance			
LoD Strain	**High Strain**	**Low Organism (~2-3x LoD)**	**Low Resistance Marker (~2-3x LoD)**	**High Organism (Bottle ring + 8 hours)**	**High Resistance Marker (Bottle ring + 8 hours)**
S. epidermidis (SE/*mecA*)	SA/*mecA*	0/3	3/3*	3/3	3/3*
	SPN	3/3	3/3	3/3	NA
	EFLS/*vanB*	0/3	0/3	3/3	3/3
	EFCM/*vanA*	0/3	0/3	3/3	3/3
S. aureus (SA/*mecA*)	SE/*mecA*	0/3	3/3*	3/3	3/3*
	SPN	3/3	3/3	3/3	NA
	EFLS/*vanB*	0/3	0/3	3/3	3/3
	EFCM/*vanA*	0/3	0/3	3/3	3/3
S. pneumoniae (SPN)	SE/*mecA*	0/3	NA	3/3	3/3
	SA/*mecA*	0/3	NA	3/3	3/3
	EFLS/*vanB*	0/3	NA	3/3	3/3
	EFCM/*vanA*	0/3	NA	3/3	3/3
E. faecalis (EFLS/*vanB*)	SE/*mecA*	0/3	0/3	3/3	3/3
	SA/*mecA*	1/3	0/3	3/3	3/3
	SPN	3/3	2/3	3/3	NA
	EFCM/*vanA*	0/3	0/3	3/3	3/3
E. faecium (EFCM/*vanA*)	SE/*mecA*	0/3	3/3**	3/3	3/3
	SA/*mecA*	0/3	3/3**	3/3	3/3
	SPN	3/3	3/3	3/3	NA
	EFLS/*vanB*	0/3	2/3	3/3	3/3
Total		**13/60 (21.7%)**	**25/48 (52.1%)**	**60/60 (100%)**	**48/48 (100%)**

*The source of the resistance marker cannot be distinguished between high concentration organism and low concentration organism.

**Results include all positive resistance marker detections, regardless of associated organism detection. In the absence of a positive *Enterococcus* detection, *vanA* would not be reported.

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-GPC Assay performance. Nineteen representative iC-GPC target

organisms plus one non-target organism were tested in 10 BCB media types (see Table 9 below). Target organisms were tested near LoD concentrations (2-3x LoD). Target performance is based on all expected targets identified and no false positive targets detected. Non-target performance is based on expected negative results. Performance in all BACTEC™ and VersaTREK® bottle types and BacT/ALERT® SA Standard Aerobic media met acceptance criteria of ≥ 95%; these bottles are validated for use with the iC-GPC Assay.

False positive *Enterococcus faecium* results were observed in 15/59 (25.4%) of BacT/ALERT® FA Aerobic FAN and 13/60 (21.7%) of BacT/ALERT® FA Plus Aerobic media. Testing in these bottle types was repeated at higher concentrations, approximately 5-10x LoD, with false positive *E. faecium* results observed in 8/59 (13.6%) and 5/60 (8.3%) of BacT/ALERT FA Aerobic FAN and BacT/ALERT FA Plus Aerobic media, respectively. Overall performance results are presented in the table below.

TABLE 9: iC-GPC Assay BCB Equivalency Results

Blood Culture Bottle Type	Overall Performance (%)	Target Performance (%)	False Negatives (%)	Non-Target Performance (%)	False Positives (%)
BACTEC™ Plus Aerobic	69/70 (98.6%)	66/67 (98.5%)	1/67 (1.5%)[1]	3/3 (100.0%)	0/70 (0.0%)
BACTEC™ Plus Anaerobic	70/72 (97.2%)	67/69 (97.1%)	1/69 (1.4%)[2]	3/3 (100.0%)	1/72 (1.4%)[3]
BACTEC™ Standard Aerobic	62/63 (98.4%)	59/60 (98.3%)	1/60 (1.7%)[4]	3/3 (100.0%)	0/63 (0.0%)
BACTEC™ Standard Anaerobic	77/80 (96.3%)	74/77 (96.1%)	2/77 (2.6%)[5]	3/3 (100.0%)	1/80 (1.3%)[6]
BACTEC™ Lytic/10 Anaerobic	78/81 (96.3%)	75/78 (96.2%)	2/78 (2.6%)[7]	3/3 (100.0%)	1/81 (1.2%)[8]
VersaTREK® REDOX 1	60/60 (100.0%)	57/57 (100.0%)	0/57 (0.0%)	3/3 (100.0%)	0/60 (0.0%)
VersaTREK® REDOX 2	59/59 (100.0%)	56/56 (100.0%)	0/56 (0.0%)	3/3 (100.0%)	0/59 (0.0%)
BacT/ALERT® SA Standard Aerobic	60/60 (100.0%)	57/57 (100.0%)	0/57 (0.0%)	3/3 (100.0%)	0/60 (0.0%)
BacT/ALERT® FA Aerobic FAN (2-3x LoD)	44/59 (74.6%)	44/56 (78.6%)	0/56 (0.0%)	0/3 (0.0%)	15/59 (25.4%)[9]
BacT/ALERT® FA Aerobic FAN (5-10x LoD)	51/59 (86.4%)	51/57 (89.5%)	0/57 (0.0%)	0/2 (0.0%)	8/59 (13.6%)[10]
BacT/ALERT® FA Plus Aerobic (2-3x LoD)	47/60 (78.3%)	46/57 (80.7%)	0/57 (0.0%)	1/3 (33.3%)	13/60 (21.7%)[11]
BacT/ALERT® FA Plus Aerobic (5-10x LoD)	55/60 (91.7%)	54/57 (94.7%)	0/57 (0.0%)	1/3 (33.3%)	5/60 (8.3%)[12]

1) 1 false negative *mecA*
2) 1 false negative *S. epidermidis*
3) 1 false positive *E. faecalis*
4) 1 false negative *S. epidermidis*
5) 1 false negative *S. epidermidis,* 1 false negative *vanA*
6) 1 false positive *S. epidermidis*
7) 1 false negative *S. epidermidis*, 1 false negative *S. pneumoniae*
8) 1 false positive *E. faecalis/vanB*
9) 15 false positive *E. faecium*
10) 8 false positive *E. faecium*
11) 12 false positive *E. faecium,* 1 false positive *S. aureus*
12) 5 false positive *E. faecium*

Further investigation suggests that the increased percentage of false positive *E. faecium* results observed in BacT/Alert® FA Aerobic FAN and BacT/Alert® FA Plus Aerobic media may be due to the presence of remnant nucleic acids and/or non-viable organisms in the culture media. FA Aerobic FAN media was further evaluated in clinical method comparison studies. In 96 clinical FA Aerobic FAN blood culture specimens tested, no false positive *E. faecium* results were observed, suggesting the risk of false positive results for *E. faecium* may be minimized when other organisms are present at bottle positivity concentrations in this bottle type.

Positive *E. faecium* results should be confirmed using alternative methods when using BacT/ALERT® FA Aerobic FAN or FA Plus Aerobic blood culture bottles.

The iC-GPC Assay is not recommended for use with BacT/ALERT® SN Standard Anaerobic, FN Anaerobic FAN, or FN Plus Anaerobic blood culture bottles.

Interfering Substances

iC-GPC Assay performance was evaluated in the presence of potentially inhibiting substances encountered in blood and blood culture media. Five representative target organisms plus one gram positive, non-target organism (Group B *Streptococcus*) were evaluated. Target organisms were tested near LoD concentrations in combination with potential interferents at "worst-case" concentrations. Target performance is based on all expected targets detected, while non-target performance is based on all negative results. In the event of a false negative result, the organism/interferent was retested in replicates of ten. In the event of a false positive result or other system failure, the organism/interferent was retested in replicates of three. If the discordant result was not observed upon repeat testing, the compound was not considered an iC-GPC Assay interferent (Table 10). If the discordant result was observed upon repeat testing, the combination was retested at a decreased inhibitor concentration until interference was no longer observed (Table 11). Interference testing was performed in BD BACTEC Plus Aerobic blood culture bottle media with a sodium polyanetholesulfonate (SPS) concentration of 0.05% w/v. Additional SPS at concentrations greater than 0.03% w/v was found to interfere with iC-GPC Assay performance. Total protein (gamma-globulin and albumin) in concentrations greater than 3g/dL was also found to interfere with iC-GPC Assay performance. Both interferents may result in false negative results or positive controls check failures.

TABLE 10: Non-Interfering Substances Performance

		Target Performance					
Potential Interferent	**Test Concentration**	**SE**	**SA**	**SPN**	**EFLS**	**EFCM**	**Group B Strep**
Hemoglobin	14 g/L	3/3	3/3	3/3	3/3	3/3	5/6[1]
Triglyceride	1000 mg/dL	3/3	3/3	3/3	3/3	3/3	3/3
Conjugated bilirubin	20 mg/dL	3/3	3/3	12/13[2]	12/13[3]	3/3	3/3
Unconjugated bilirubin	20 mg/dL	3/3	3/3	3/3	3/3	3/3	3/3
Human genomic DNA	1 x 10^6 cells/mL	3/3	3/3	3/3	12/13[4]	3/3	3/3
Vancomycin	100 mg/mL	3/3	3/3	12/13[5]	3/3	3/3	3/3
Piperacillin	160 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3
Meropenem	80 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3
Cefepime	80 mg/mL	3/3	12/13[6]	11/12[7]	3/3	3/3	3/3
Ceftriaxone	80 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3
Fluconazole	125 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3
Gentamicin	100 mg/mL	3/3	3/3	11/12[8]	12/14[9]	3/3	3/3

1) 1 false positive *E. faecalis,* 3/3 repeats passed

2) 1 false negative *S. pneumoniae*, 10/10 repeats passed
3) 1 false negative *vanB*, 10/10 repeats passed
4) 1 false negative *vanB*, 10/10 repeats passed
5) 1 false negative *S. pneumoniae*, 10/10 repeats passed
6) 1 false negative *mecA*, 10/10 repeats passed
7) 1 false negative *S. pneumoniae*, 9/9 repeats passed
8) 1 false negative *S. pneumoniae,* 9/9 repeats passed
9) 1 false negative *vanB,* 1 false positive *S. epidermidis,* 11/11 repeats passed

TABLE 11: Interfering Substances Performance

		Target Performance					
Interferent	**Test Concentration**	**SE**	**SA**	**SPN**	**EFLS**	**EFCM**	**Group B Strep**
Total Protein (γ-globulin + albumin)	3 g/dL	2/2	3/3	3/3	2/3[1]	3/3	3/3
Total Protein (γ-globulin + albumin)	6g/dL*	0/3	0/3	2/3	0/3	0/3	2/3
SPS	0.03% w/v	3/3	12/13[2]	3/3	3/3	3/3	5/6[3]
SPS	0.04% w/v*	2/3	2/3	2/2	1/3	3/3	3/3

*Concentration at which interference observed; concentration decreased for subsequent testing.

1) 1 false negative *vanB,* test not repeated
2) 1 false negative *S. aureus/mecA*, 10/10 repeats passed
3) 1 false positive *mecA*, 3/3 repeats passed

Carryover and Cross-Contamination

The iC-System is a closed system, designed to eliminate the potential for carryover or cross-contamination between samples. Testing was performed to confirm that no false positive results from carryover or cross-contamination will occur from a high positive sample into a negative sample. Two representative iC-GPC target organisms, *Staphylococcus aureus* ATCC BAA977 (*nuc* positive, *mecA* negative) and *Enterococcus faecium* ATCC 700221 (fcm positive, *vanA* positive), were tested as representative positive samples. A gram positive non-target organism, *Corynebacterium striatum,* was used as the negative sample. All organisms were tested at high concentrations, eight hours beyond initial bottle positivity. Positive, target organism samples were tested in an alternating pattern with negative, non-target organism samples across eight blades of an iC-Processor. Performance was based on accurate iC-GPC Assay target detection. No false positives indicative of sample carryover or cross-contamination were observed.

Method Comparison

A method comparison study was performed at four geographically dispersed clinical sites to evaluate the performance of the iC-GPC Assay™ for use on the iC-System™. Sites tested 966 leftover, de-identified specimens from aerobic and anaerobic 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: BD BACTEC™, bioMérieux BacT/ALERT®, and Thermo Fisher VersaTREK®. Positive blood cultures were confirmed by Gram stain to contain gram positive cocci prior to being entered into the study. Specimens demonstrating mixed Gram stain results were excluded from the study.

Performance of the iC-GPC Assay was compared to an FDA-cleared multiplex assay that detects gram positive organisms and associated resistance markers directly from positive blood culture specimens. PCR and bi-directional sequencing were also performed for all specimens with positive results for resistance markers by either the iC-GPC Assay or the FDA-cleared multiplex assay. These PCR and bi-directional sequencing results were used to analyze discordant results between the iC-GPC Assay and the FDA-cleared multiplex assay.

Additionally, performance of the iC-GPC Assay was compared to conventional reference methods including subculture, identification of isolates using biochemical methods or MALDI-TOF, and phenotypic antimicrobial susceptibility testing (AST). PCR and bi-directional sequencing were not used for discordant analysis between iC-GPC and conventional reference method results.

A total of 966 positive blood culture specimens, confirmed by Gram stain to contain gram positive cocci, were enrolled in the iC-GPC Assay Method Comparison Study. A total of 53 samples were withdrawn from the study (see below). Of the 913 samples included in the study after identified samples were withdrawn, 879 were fresh prospective samples and 34 were frozen prospective samples.

An additional 168 contrived samples were evaluated for low prevalence organisms and resistance markers. Contrived specimens were prepared by inoculating low concentrations of organisms into BD BACTEC Plus Aerobic blood culture bottles with human blood added and incubating the bottles on the blood culture system until bottle positivity. Multiple strains of *S. pneumoniae*, *E. faecalis*, and *E. faecium* were used to prepare contrived specimens.

Performance vs. FDA-Cleared Multiplex Assay:

When performance of the iC-GPC Assay was compared to the FDA-cleared multiplex assay, there was no statistical difference in performance noted between the four study sites or between the three blood culture systems. The following blood culture bottle types were evaluated: BD BACTEC Standard Aerobic/Anaerobic, VersaTREK REDOX 1/2, BacT/ALERT SA Standard Aerobic, and BacT/ALERT FA Aerobic FAN.

Performance of the iC-GPC Assay in comparison to the FDA-cleared multiplex assay is provided in the tables below. Performance is stratified by prospectively tested fresh specimens, prospectively collected and retrospectively tested frozen specimens, and contrived specimens.

iC-GPC Assay Performance: *Staphylococcus aureus (nuc)*

Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	879	96.9% 249/257 (94.0-98.4)	99.7% 620/622 (98.8-99.9)	FDA-Cleared Multiplex Assay
	Frozen	34	100% 9/9 (70.1-100)	100% 25/25 (86.7-100)	
	TOTAL	913	97.0% 258/266* (94.2-98.5)	99.7% 645/647** (98.9-99.9)	
Contrived		168	- 0/0 -	100% 168/168 (97.8-100)	

*Of the 8 observed false negative *S. aureus* results by the iC-GPC Assay, 7/8 were positive for *S. aureus* by PCR/bi-directional sequencing.
**Of the 2 observed false positive *S. aureus* results by the iC-GPC Assay, 1/2 was positive for *S. aureus* by PCR/bi-directional sequencing. One sample was not available for discordant analysis.

iC-GPC Assay Performance: *Staphylococcus epidermidis (gseA)*

Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	879	98.3% 227/231 (95.6-99.3)	98.0% 635/648 (96.6-98.8)	FDA-Cleared Multiplex Assay
	Frozen	34	100% 4/4 (51.0-100)	96.7% 29/30 (83.3-99.4)	
	TOTAL	913	98.3% 231/235* (95.7-99.3)	97.9% 664/678** (96.6-98.8)	
Contrived		168	- 0/0 -	100% 168/168 (97.8-100)	

*Of the 4 observed false negative *S. epidermidis* results by the iC-GPC Assay, 3/4 were positive for *S. epidermidis* by PCR/bi-directional sequencing.
**Of the 14 observed false positive *S. epidermidis* results by the iC-GPC Assay, 9/14 were positive for *S. epidermidis* by PCR/bi-directional sequencing.

iC-GPC Assay Performance: *Streptococcus pneumoniae (lytA)*

Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	879	82.6%* 19/23 (62.9-93.0)	99.9% 855/856 (99.3-100)	FDA-Cleared Multiplex Assay
	Frozen	34	100% 4/4 (51.0-100)	100% 30/30 (88.6-100)	
	TOTAL	913	85.2% 23/27* (67.5-94.1)	99.9% 885/886** (99.4-100)	
Contrived		168	100% 36/36 (90.4-100)	100% 132/132 (97.2-100)	

*Of the 4 observed false negative *S. pneumoniae* results by the iC-GPC Assay, 0/4 were positive for *S. pneumoniae* by PCR/bi-directional sequencing and 0/4 culture isolates obtained from these specimens were positive for *S. pneumoniae* by MALDI ID.
**Of the 1 observed false positive *S. pneumoniae* result by the iC-GPC Assay, 1/1 was positive for *S. pneumoniae* by PCR/bi-directional sequencing.

iC-GPC Assay Performance: *Enterococcus faecalis (ddl)*

Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	879	96.6% 56/58 (88.3-99.0)	99.9% 820/821 (99.3-100)	FDA-Cleared Multiplex Assay
	Frozen	34	100% 3/3 (43.8-100)	100% 31/31 (89.0-100)	
	TOTAL	913	96.7% 59/61* (88.8-99.1)	99.9% 851/852** (99.3-100)	
Contrived		168	100% 90/90 (95.9-100)	100% 78/78 (95.3-100)	

*Of the 2 observed false negative *E. faecalis* results by the iC-GPC Assay, 1/2 was positive for *E. faecalis* by PCR/bi-directional sequencing.
**Of the 1 observed false positive *E. faecalis* result by the iC-GPC Assay, 0/1 was positive for *E. faecalis* by PCR/bi-directional sequencing.

iC-GPC Assay Performance: *Enterococcus faecium* (fcm)

Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	879	96.4% 27/28 (82.3-99.4)	99.8% 849/851 (99.1-99.9)	FDA-Cleared Multiplex Assay
	Frozen	34	100% 1/1 (20.7-100)	100% 33/33 (89.6-100)	
	TOTAL	913	96.6% 28/29* (82.8-99.4)	99.8% 882/884** (99.2-99.9)	
Contrived		168	100% 42/42 (91.6-100)	100% 126/126 (97.0-100)	

*Of the 1 observed false negative *E. faecium* result by the iC-GPC Assay, 1/1 was positive for *E. faecium* by PCR/bi-directional sequencing.
**Of the 2 observed false positive *E. faecium* results by the iC-GPC Assay, 1/2 were positive for *E. faecium* by PCR/bi-directional sequencing.

iC-GPC Assay Performance: *mecA* (methicillin resistance)

Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	879	96.1% 269/280 (93.1-97.8)	98.2% 588/599 (96.7-99.0)	FDA-Cleared Multiplex Assay
	Frozen	34	100% 5/5 (56.6-100)	100% 29/29 (88.3-100)	
	TOTAL	913	96.1% 274/285†* (93.2-97.8)	98.2% 617/628** (96.9-99.0)	
Contrived		168	- 0/0 -	100% 168/168 (97.8-100)	

†Of the 274 observed true positive *mecA* results by the iC-GPC Assay, 269/274 were positive for *mecA* by PCR/bi-directional sequencing.
*Of the 11 observed false negative *mecA* results by the iC-GPC Assay, 10/11 were positive for *mecA* by PCR/bi-directional sequencing.

**Of the 11 observed false positive *mecA* results by the iC-GPC Assay, 8/11 were positive for *mecA* by PCR/bi-directional sequencing.

iC-GPC Assay Performance: *vanA* (vancomycin resistance)					
Specimen Type		**N=**	**Percent Agreement**		**Comparator Method**
			Positive (95% CI)	**Negative (95% CI)**	
Prospective	Fresh	879	94.7% 18/19 (75.4-99.1)	99.4% 855/860 (98.6-99.8)	**FDA-Cleared Multiplex Assay**
	Frozen	34	100% 1/1 (20.7-100)	100% 33/33 (89.6-100)	
	TOTAL	913	95.0% 19/20†* (76.4-99.1)	99.4% 888/893** (98.7-99.8)	
Contrived		168	100% 40/40 (91.2-100)	100% 128/128 (97.1-100)	

†Of the 19 observed true positive *vanA* results by the iC-GPC Assay, 19/19 were positive for *vanA* by PCR/bi-directional sequencing.
*Of the 1 observed false negative *vanA* result by the iC-GPC Assay, 1/1 was positive for *vanA* by PCR/bi-directional sequencing.
**Of the 5 observed false positive *vanA* results by the iC-GPC Assay, 2/5 were positive for *vanA* by PCR/bi-directional sequencing.

iC-GPC Assay Performance: *vanB* (vancomycin resistance)					
Specimen Type		**N=**	**Percent Agreement**		**Comparator Method**
			Positive (95% CI)	**Negative (95% CI)**	
Prospective	Fresh	879	100% 2/2 (34.2-100)	99.9% 876/877 (99.4-100)	**FDA-Cleared Multiplex Assay**
	Frozen	34	- 0/0 -	100% 34/34 (89.8-100)	
	TOTAL	913	100% 2/2† (34.2-100)	99.9% 910/911* (99.4-100)	
Contrived		168	100% 56/56 (93.6-100)	100% 112/112 (96.7-100)	

†Of the 2 observed true positive *vanB* results by the iC-GPC Assay, 2/2 were positive for *vanB* by PCR/bi-directional sequencing.
*Of the 1 observed false positive *vanB* result by the iC-GPC Assay, 0/1 was positive for *vanB* by PCR/bi-directional sequencing.

The following tables provide the performance of prospective clinical samples in comparison to the FDA-cleared multiplex assay for *mecA* detection with *S. aureus/S. epidermidis, vanA* detection with *E. faecalis/E. faecium,* and *vanB* detection with *E. faecalis/E. faecium.*

Detection of *mecA* with *S. aureus*: Prospective					
Staphylococcus aureus/mecA		**FDA-Cleared Comparator Assay**			
		SA+/*mecA*+	SA+/*mecA*-	SA-	TOTAL
iCubate iC-GPC™	SA+/*mecA*+	111	3	0	114
	SA+/*mecA*-	0	144	2	146
	SA-	4	4	645	653
	TOTAL	115	151	647	913
Staphylococcus aureus/mecA				95% C.I.	
% Agreement (SA+/*mecA*+)		96.5% (111/115)		91.4%	98.6%
% Agreement (SA+/*mecA*-)		95.4% (144/151)		90.7%	97.7%
% Agreement (SA-)		99.7% (645/647)		98.9%	99.2%

Detection of *mecA* with *S. epidermidis*: Prospective					
Staphylococcus epidermidis/mecA		**FDA-Cleared Comparator Assay**			
		SE+/*mecA*+	SE+/*mecA*-	SE-	TOTAL
iCubate iC-GPC™	SE+/*mecA*+	163	2	6	171
	SE+/*mecA*-	4	62	8	74
	SE-	3	1	664	668
	TOTAL	170	65	678	913
Staphylococcus epidermidis/mecA				95% C.I.	
% Agreement (SE+/*mecA*+)		95.9% (163/170)		91.8%	98.0%
% Agreement (SE+/*mecA*-)		95.4% (62/65)		87.3%	98.4%
% Agreement (SE-)		97.9% (664/678)		96.6%	98.8%

Detection of *vanA* with *E. faecalis*: Prospective					
Enterococcus faecalis/vanA		**FDA-Cleared Comparator Assay**			
		Efs+/*vanA*+	Efs+/*vanA*-	Efs-	TOTAL
iCubate iC-GPC™	Efs+/*vanA*+	0	2	1	3
	Efs+/*vanA*-	0	57	0	57
	Efs-	0	2	851	853
	TOTAL	0	61	852	913
Enterococcus faecalis/vanA				95% C.I.	
% Agreement (Efs+/*vanA*+)		-		-	-
% Agreement (Efs+/*vanA*-)		93.4% (57/61)		84.3%	97.4%
% Agreement (Efs-)		99.9% (851/852)		99.3%	99.9%

Detection of *vanB* with *E. faecalis*: Prospective					
Enterococcus faecalis/vanB		**FDA-Cleared Comparator Assay**			
		Efs+/*vanB*+	Efs+/*vanB*-	Efs-	TOTAL
iCubate iC-GPC™	Efs+/*vanB*+	2	0	1	3
	Efs+/*vanB*-	0	57	0	57
	Efs-	0	2	851	853
	TOTAL	2	59	852	913
Enterococcus faecalis/vanB				95% C.I.	
% Agreement (Efs+/*vanB*+)		100.0% (2/2)		34.2%	100.0%
% Agreement (Efs+/*vanB*-)		96.6% (57/59)		88.5%	99.1%
% Agreement (Efs-)		99.9% (851/852)		99.3%	99.9%

Detection of *vanA* with *E. faecium*: Prospective					
Enterococcus faecium/vanA		**FDA-Cleared Comparator Assay**			
		Efm+/*vanA*+	Efm+/*vanA*-	Efm-	TOTAL
iCubate iC-GPC™	Efm+/*vanA*+	19	2	2	23
	Efm+/*vanA*-	0	7	0	7
	Efm-	1	0	882	883
	TOTAL	20	9	884	913
Enterococcus faecium/vanA				95% C.I.	
% Agreement (Efm+/*vanA*+)			95.0% (19/20)	76.4%	99.1%
% Agreement (Efm+/*vanA*-)			77.8% (7/9)	45.3%	93.7%
% Agreement (Efm-)			99.8% (882/884)	99.2%	99.9%

Detection of *vanB* with *E. faecium*: Prospective					
Enterococcus faecium/vanB		**FDA-Cleared Comparator Assay**			
		Efm+/*vanB*+	Efm+/*vanB*-	Efm-	TOTAL
iCubate iC-GPC™	Efm+/*vanB*+	0	0	1	1
	Efm+/*vanB*-	0	28	1	29
	Efm-	0	1	882	883
	TOTAL	0	29	884	913
Enterococcus faecium/vanB				95% C.I.	
% Agreement (Efm+/*vanB*+)			-	-	-
% Agreement (Efm+/*vanB*-)			96.6% (28/29)	82.8%	99.4%
% Agreement (Efm-)			99.8% (882/884)	99.2%	99.9%

The following tables provide the performance of contrived samples for *vanA/vanB* detection with *E. faecalis* and *E. faecium.*

Detection of *vanA* with *E. faecalis*: Contrived				
vanA/E. faecalis		**Expected Result**		
		POS	NEG	TOTAL
iCubate iC-GPC™	POS	16	0	16
	NEG	0	152	152
	TOTAL	16	152	168
vanA/E. faecalis			95% C.I.	
Positive % Agreement		100.0%	80.6%	100.0%
Negative % Agreement		100.0%	97.5%	100.0%

Detection of *vanA* with *E. faecium*: Contrived				
vanA/E. faecium		**Expected Result**		
		POS	NEG	TOTAL
iCubate iC-GPC™	POS	24	0	24
	NEG	0	144	144
	TOTAL	24	144	168
vanA/E. faecium			95% C.I.	
Positive % Agreement		100.0%	86.2%	100.0%
Negative % Agreement		100.0%	97.4%	100.0%

Detection of *vanB* with *E. faecalis*: Contrived				
vanB/E. faecalis		**Expected Result**		
		POS	NEG	TOTAL
iCubate iC-GPC™	POS	56	0	56
	NEG	0	112	112
	TOTAL	56	112	168
vanB/E. faecalis			95% C.I.	
Positive % Agreement		100.0%	93.6%	100.0%
Negative % Agreement		100.0%	96.7%	100.0%

Detection of *vanB* with *E. faecium*: Contrived				
vanB/E. faecium		**Expected Result**		
		POS	NEG	TOTAL
iCubate iC-GPC™	POS	0	0	0
	NEG	0	168	168
	TOTAL	0	168	168
vanB/E. faecium			95% C.I.	
Positive % Agreement		-	-	-
Negative % Agreement		100.0%	97.8%	100.0%

Performance vs. Traditional Culture and Susceptibility:
Performance of the iC-GPC Assay was compared to traditional laboratory reference methods: culture followed by testing blood culture isolates with conventional biochemicals/MALDI-TOF and AST testing (cefoxitin disc for *mecA* and vancomycin Etest for *vanA/vanB*).

Performance of the iC-GPC Assay in comparison to traditional laboratory reference methods is provided in the tables below. Performance is stratified by prospectively tested fresh specimens and prospectively collected, retrospectively tested frozen specimens.

iC-GPC Assay Performance: *Staphylococcus aureus (nuc)*					
Specimen Type		**N=**	**Percent Agreement**		**Reference Method**
			Positive (95% CI)	**Negative (95% CI)**	
Prospective	Fresh	849	95.8% 250/261 (92.6-97.6)	99.8% 587/588 (99.0-100)	**Culture & Biochemicals**
	Frozen	32	100% 9/9 (70.1-100)	100% 23/23 (85.7-100)	
	TOTAL	881	95.9% 259/270 (92.9-97.7)	99.8% 610/611 (99.1-100)	

iC-GPC Assay Performance: *Staphylococcus epidermidis (gseA)*					
Specimen Type		**N=**	**Percent Agreement**		**Reference Method**
			Positive (95% CI)	**Negative (95% CI)**	
Prospective	Fresh	849	95.9% 213/222 (92.5-97.9)	96.5% 605/627 (94.7-97.7)	**Culture & Biochemicals**
	Frozen	32	100% 3/3 (43.8-100)	96.6% 28/29 (82.8-99.4)	
	TOTAL	881	96.0% 216/225 (92.6-97.9)	96.5% 633/656 (94.8-97.7)	

iC-GPC Assay Performance: *Streptococcus pneumoniae (lytA)*

Specimen Type		N=	Percent Agreement		Reference Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	856	100% 18/18 (82.4-100)	99.9% 837/838 (99.3-100)	Culture & Biochemicals
	Frozen	33	100% 3/3 (43.8-100)	96.7% 29/30 (83.3-99.4)	
	TOTAL	889	100% 21/21 (84.5-100)	99.8% 866/868 (99.2-99.9)	

iC-GPC Assay Performance: *Enterococcus faecalis (ddl)*

Specimen Type		N=	Percent Agreement		Reference Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	858	96.6% 57/59 (88.5-99.1)	99.9% 798/799 (99.3-100)	Culture & Biochemicals
	Frozen	33	100% 3/3 (43.8-100)	100% 30/30 (88.6-100)	
	TOTAL	891	96.8% 60/62 (89.0-99.1)	99.9% 828/829 (99.3-100)	

iC-GPC Assay Performance: *Enterococcus faecium* (fcm)

Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	858	96.4% 27/28 (82.3-99.4)	99.6% 827/830 (98.9-99.9)	Culture & Biochemicals
	Frozen	33	100% 1/1 (20.7-100)	100% 32/32 (89.3-100)	
	TOTAL	891	96.6% 28/29 (82.8-99.4)	99.7% 859/862 (99.0-99.9)	

iC-GPC Assay Performance: *mecA* (methicillin resistance)

Specimen Type		N=	Percent Agreement		Comparator Method
			Positive (95% CI)	Negative (95% CI)	
Prospective	Fresh	848	90.0% 252/280 (85.9-93.0)	97.0% 551/568 (95.3-98.1)	Culture, Biochemicals & Cefoxitin Disc
	Frozen	32	100% 5/5 (56.6-100)	100% 27/27 (87.5-100)	
	TOTAL	880	90.2% 257/285 (86.2-93.1)	97.1% 578/595 (95.5-98.2)	

* Of the 28 observed false negative *mecA* results by the iC-GPC Assay, 14/28 were positive for *mecA* by multiple FDA-cleared multiplex assays. Repeat AST testing also confirmed samples to be cefoxitin sensitive. See note below.

iC-GPC Assay Performance: *vanA/B* (vancomycin resistance)					
Specimen Type		**N=**	**Percent Agreement**		**Comparator Method**
			Positive (95% CI)	**Negative (95% CI)**	
Prospective	Fresh	855	87.5% 21/24 (69.0-95.7)	99.8% 829/831 (99.1-99.9)	**Culture, Biochemicals & Vancomycin Etest**
	Frozen	33	100% 1/1 (20.7-100)	100% 32/32 (89.3-100)	
	TOTAL	888	88.0%* 22/25 (70.0-95.8)	99.8% 861/863 (99.2-99.9)	

*Of the 3 observed false negative results by the iC-GPC Assay, 1/3 was positive for *vanA/vanB* by the FDA-cleared multiplex assay.

The following tables provide the performance of prospective clinical samples in comparison to traditional laboratory reference methods for *mecA* detection with *S. aureus* and *S. epidermidis* and *vanA/vanB* detection with *E. faecalis* and *E. faecium.*

NOTE: Of the 21 observed false negative *mecA* results by the iC-GPC Assay for specimens identified to contain methicillin-resistant *S. aureus* by conventional identification methods and cefoxitin disc testing, 16 were also *mecA* negative by the FDA-cleared multiplex assay used for method comparison testing. To further investigate, isolates from 14 of the specimens identified at a single site were tested with an alternate real-time PCR assay that detects *mec*A and *mec*C. This assay confirmed the isolates to be negative for both *mec*A and *mec*C. Cefoxitin disc testing was also repeated on the 14 sample isolates. All isolates were found to be susceptible to cefoxitin with a zone size of 22mm. These tests confirm the iC-GPC result of *mecA* negative for 14 of the 21 discrepant results.

Detection of *mecA* with *S. aureus*: Prospective					
Staphylococcus aureus/mecA		**Culture, Biochemicals & Cefoxitin Disc Testing**			
		SA+/*mecA*+	SA+/*mecA*-	SA-	TOTAL
iCubate iC-GPC™	SA+/*mecA*+	113	1	0	114
	SA+/*mecA*-	16	128	1	145
	SA-	5	6	610	621
	TOTAL	134	135	611	880
Staphylococcus aureus/mecA				95% C.I.	
% Agreement (SA+/*mecA*+)		84.3%* (113/134)		77.2%	89.5%
% Agreement (SA+/*mecA*-)		94.8% (128/135)		89.7%	97.5%
% Agreement (SA-)		99.8% (610/611)		99.1%	100%

*See note above

Detection of *mecA* with *S. epidermidis*: Prospective					
Staphylococcus epidermidis/mecA		**Culture, Biochemicals & Cefoxitin Disc Testing**			
		SE+/*mecA*+	SE+/*mecA*-	SE-	TOTAL
iCubate iC-GPC™	SE+/*mecA*+	144	10	15	169
	SE+/*mecA*-	2	60	8	70
	SE-	5	4	632	641
	TOTAL	151	74	655	880
Staphylococcus epidermidis/mecA				95% C.I.	
% Agreement (SE+/*mecA*+)		95.4% (144/151)		90.7%	97.7%
% Agreement (SE+/*mecA*-)		81.2% (60/74)		70.7%	88.4%
% Agreement (SE-)		96.5% (632/655)		94.8%	97.7%

Detection of *vanA/vanB* with *E. faecalis*: Prospective					
E. faecalis/vanA/vanB		**Culture, Biochemicals & Vancomycin AST**			
		Efs+/*van*+	Efs+/*van*-	Efs-	TOTAL
iCubate iC-GPC™	Efs+/*van*+	1	1	1	3
	Efs+/*van*-	2	53	0	55
	Efs-	0	2	828	830
	TOTAL	3	56	829	888
E. faecalis/vanA/vanB				95% C.I.	
% Agreement (Efs+/*van*+)		33.3% (1/3)		6.2%	79.2%
% Agreement (Efs+/*van*-)		94.6% (53/56)		85.4%	98.2%
% Agreement (Efs-)		99.9% (828/829)		99.3%	100%

Detection of *vanA/vanB* with *E. faecium*: Prospective					
E. faecalis/vanA/vanB		**Culture, Biochemicals & Vancomycin AST**			
		Efm+/*van*+	Efm+/*van*-	Efm-	TOTAL
iCubate iC-GPC™	Efm+/*van*+	20	1	3	24
	Efm+/*van*-	0	7	0	7
	Efm-	1	0	856	857
	TOTAL	21	8	859	888
E. faecium/vanA/vanB				95% C.I.	
% Agreement (Efm+/*van*+)		95.2% (20/21)		77.3%	99.2%
% Agreement (Efm+/*van*-)		87.5% (7/8)		52.9%	97.8%
% Agreement (Efm-)		99.7% (856/859)		99.0%	99.9%

Mixed Culture Results:

In total, there were seven (7) mixed culture specimens that were detected by the iC-GPC Assay, the FDA-cleared multiplex assay, or both. The tables below lists the mixed target combinations detected by iC-GPC and the comparator assay in the clinical study. There was one (1) discrepant mixed sample for which iC-GPC detected a target that was not detected by the comparator assay. There was one (1) discrepant mixed sample for which the comparator assay detected targets that were not detected by iC-GPC. Due to competitive inhibition, target organisms present near LoD concentrations may not be detected by the iC-GPC Assay when a second target organism is present at higher concentrations.

Multiple Detections by iC-GPC						Total Targets Detected	Discrepant Targets	Discrepant Results (Targets Not Detected by FDA-Cleared Multiplex Assay)
Site	**ID**	**Target 1**	**Target 2**	**Target 3**	**Target 4**			
LAC	1081	Staphylococcus aureus	Enterococcus faecalis			2	0	NA
LAC	1149	Staphylococcus epidermidis	mecA	Enterococcus faecalis		3	0	NA
LAC	1150	Enterococcus faecalis	Enterococcus faecium	vanA		3	1	vanA
LAC	1289	Staphylococcus aureus	mecA	Enterococcus faecalis	vanB	4	0	NA
LAC	1403	Staphylococcus epidermidis	mecA	Enterococcus faecalis		3	0	NA
TGH	4029	Staphylococcus aureus	Enterococcus faecalis			2	0	NA

Multiple Detections by FDA-Cleared Multiplex Assay						Total Targets Detected	Discrepant Targets	Discrepant Results (Targets Not Detected by iC-GPC)
Site	**ID**	**Target 1**	**Target 2**	**Target 3**	**Target 4**			
LAC	1081	Staphylococcus aureus	Enterococcus faecalis			2	0	NA
LAC	1149	Staphylococcus epidermidis	mecA	Enterococcus faecalis		3	0	NA
LAC	1150	Enterococcus faecalis	Enterococcus faecium			2	0	NA
LAC	1289	Staphylococcus aureus	mecA	Enterococcus faecalis	vanB	4	0	NA
LAC	1403	Staphylococcus epidermidis	mecA	Enterococcus faecalis		3	0	NA
MCW	2134	Staphylococcus aureus	mecA	Enterococcus faecium	vanA	4	2	Staphylococcus aureus, mecA
TGH	4029	Staphylococcus aureus	Enterococcus faecalis			2	0	NA

Of the six (6) mixed culture specimens detected by the iC-GPC Assay, there were two (2) discrepant samples for which iC-GPC detected a target that was not detected by culture, biochemicals, and AST testing.

Multiple Detections by iC-GPC						Total Targets Detected	Discrepant Targets	Discrepant Results (Targets Not Detected by Culture/AST)
Site	**ID**	**Target 1**	**Target 2**	**Target 3**	**Target 4**			
LAC	1081	Staphylococcus aureus	Enterococcus faecalis			2	0	NA
LAC	1149	Staphylococcus epidermidis	mecA	Enterococcus faecalis		3	0	NA
LAC	1150	Enterococcus faecalis	Enterococcus faecium	vanA		3	3	Enterococcus faecium, vanA
LAC	1289	Staphylococcus aureus	mecA	Enterococcus faecalis	vanB	4	0	vanB (E-Test Intermediate)
LAC	1403	Staphylococcus epidermidis	mecA	Enterococcus faecalis		3	0	NA
TGH	4029	Staphylococcus aureus	Enterococcus faecalis			2	0	NA

There were fifty-six (56) mixed culture specimens detected by culture and biochemicals, including organisms not detected by the iC-GPC Assay. The table below lists the mixed target combinations detected by traditional reference methods. There were twelve (12) discrepant samples for which the reference methods detected targets that were not detected by iC-GPC (false negatives). There were nine (9) discrepant samples for which iC-GPC detected targets were not detected by the reference

methods (false positives). Of these, two (2) were positive *mecA* results associated with an iC-GPC target organism where reference testing associated the *mecA* with a non-target organism.

Multiple Detections by Culture, Biochemicals & AST					
Target 1	**Target 2**	**Target 3**	**Frequency**	**No. w/ Discrepant Targets**	**Discrepant Target**
Staphylococcus aureus	Enterococcus faecalis		3	1	Staphylococcus aureus
Staphylococcus aureus, mecA	Enterococcus faecalis		1	0	
Staphylococcus aureus, mecA	Enterococcus faecalis	Streptococcus agalactiae	1	1	Staphylococcus aureus, mecA, Enterococcus faecalis, Staphylococcus epidermidis*
Staphylococcus aureus, mecA	Enterococcus faecium, vanA	Candida glabrata	1	1	Staphylococcus aureus, mecA
	Stenotrophomonas rhizophila	Pseudomonas fulva			
Staphylococcus epidermidis, mecA	Enterococcus faecalis		4	2	Staphylococcus epidermidis (2), mecA (2)
Staphylococcus epidermidis, mecA	Staphylococcus epidermidis		1	0	NA
Staphylococcus epidermidis, mecA	Staphylococcus hominis		4	0	NA
Staphylococcus epidermidis	Staphylococcus hominis		2	1	Staphylococcus epidermidis
Staphylococcus epidermidis	Staphylococcus hominis	Staphylococcus capitis	1	1	Staphylococcus epidermidis
Staphylococcus epidermidis	Staphylococcus hominis, mecA		1	1	mecA*
Staphylococcus epidermidis, mecA	Staphylococcus haemolyticus		1	0	NA
Staphylococcus epidermidis	Staphylococcus haemolyticus		1	0	NA
Staphylococcus epidermidis, mecA	Staphylococcus haemolyticus, mecA		1	0	NA
Staphylococcus epidermidis	Staphylococcus haemolyticus, mecA		1	1	mecA*
Staphylococcus epidermidis, mecA	Staphylococcus capitus		1	0	Staphylococcus epidermidis, mecA
Staphylococcus epidermidis	Staphylococcus capitus		1	1	NA
Staphylococcus epidermidis, mecA	Staphylococcus capitus, mecA		1	1	Staphylococcus epidermidis, mecA
Staphylococcus epidermidis, mecA	Staphylococcus lugdunensis		1	0	NA
Staphylococcus epidermidis, mecA	Staphylococcus warneri, mecA		1	0	NA
Staphylococcus epidermidis, mecA	Staphylococcus species, mecA		1	0	NA
Staphylococcus epidermidis, mecA	Aerococcus viridans		1	0	NA
Staphylococcus epidermidis	Bacillus circulans		1	0	NA
Staphylococcus epidermidis	Streptococcus agalactiae		1	0	NA
Staphylococcus epidermidis	Streptococcus oralis		1	0	NA
Staphylococcus epidermidis	Low Disc: Strep. mitis/oralis		1	1	Staphylococcus epidermidis
Staphylococcus aureus, mecA	Streptococcus mitis		1	0	NA
Staphylococcus aureus, mecA	Unidentified Organism		1	1	Staphylococcus aureus, mecA
Enterococcus faecalis	Staphylococcus simulans	Aerococcus viridans	1	1	Enterococcus faecalis
Enterococcus faecalis	Staphylococcus haemolyticus, mecA		1	0	NA
Enterococcus faecalis	Klebsiella pneumoniae		1	0	NA
Staphylococcus hominis, mecA	Staphylococcus hominis		4	3	Staphylococcus epidermidis (2)**, Enterococcus faecalis*
Staphylococcus hominis, mecA	Staphylococcus capitis		2	1	Staphylococcus epidermidis*
Staphylococcus hominis	Staphylococcus lentus		1	0	NA
Staphylococcus hominis	Staphylococcus species		2	0	NA
Staphylococcus hominis	Aerococcus viridans		2	0	NA

Multiple Detections by Culture, Biochemicals & AST					
Target 1	**Target 2**	**Target 3**	**Frequency**	**No. w/ Discrepant Targets**	**Discrepant Target**
Staphylococcus haemolyticus	Staphylococcus capitis		1	1	Staphylococcus epidermidis*
Staphylococcus capitis	Low Disc: Streptococcus mitis/oralis		1	0	NA
Staphylococcus capitis	Staphylococcus warneri		1	1	Staphylococcus epidermidis*
Staphylococcus lentus, mecA	Low Disc: Strep. mitis/oralis		1	0	NA
Staphylococcus cohnii ssp urealyticus, mecA	Staphylococcus cohnii ssp cohnii, mecA		1	0	NA
Streptococcus sanguinis	Streptococcus gordonii		1	0	NA

*false positive result

Expected Values:

913 prospectively collected fresh and frozen blood culture specimens were obtained from four geographically dispersed clinical sites. The number and percentage of positive cases (positivity rate) determined by the iC-GPC Assay stratified by U.S. state for each of the organisms and resistance markers detected by the assay are presented below. Overall, the iC-GPC Assay detected at least one organism in 66.9% (611/913) prospectively collected specimens and at least one resistance marker in 33.4% (305/913) prospectively collected specimens.

Organism	**U.S. State**	NY	WI	NM	FL	TOTAL
	TOTAL n	**300**	**269**	**248**	**96**	**913**
S. aureus	POSITIVE n	86	57	92	31	266
	% Positivity	28.7%	21.2%	37.1%	32.3%	29.1%
S. epidermidis	POSITIVE n	63	85	66	21	235
	% Positivity	21.0%	31.6%	26.6%	21.9%	25.7%
S. pneumoniae	POSITIVE n	12	7	7	1	27
	% Positivity	4.0%	2.6%	2.8%	1.0%	3.0%
E. faecalis	POSITIVE n	22	26	6	7	61
	% Positivity	7.3%	9.7%	2.4%	7.3%	6.7%
E. faecium	POSITIVE n	5	22	1	1	29
	% Positivity	1.7%	8.2%	0.4%	1.0%	3.2%
Resistance Marker	**TOTAL n**	**300**	**269**	**248**	**96**	**913**
mecA	POSITIVE n	77	91	81	36	285
	% Positivity	25.7%	33.8%	32.7%	37.5%	31.2%
vanA	POSITIVE n	4	15	1	0	20
	% Positivity	1.3%	5.6%	0.4%	0.0%	2.2%
vanB	POSITIVE n	1	1	0	0	2
	% Positivity	0.3%	0.4%	0.0%	0.0%	0.2%

Error Rates and Sample Withdrawals:

Throughout the course of the study, an initial error rate of 7.6% (83/1098) was observed. Errors included positive controls check failures, array registration errors, and processor or other system errors. When a failed control or system error was observed, the sample was retested on the iC-GPC Assay. Upon repeat testing, the error rate was reduced to 1.6% (17/1098). Samples generating reproducible iC-GPC errors were withdrawn from the study.

No-Calls					
Internal Positive Control Failure		**Instrument Errors**		**Total Non-Reportable Rate**	
Initial #fail/#total Percent (95% CI)	**Final** #fail/#total Percent (95% CI)	**Initial** #fail/#total Percent (95% CI)	**Final** #fail/#total Percent (95% CI)	**Initial** #fail/#total Percent (95% CI)	**Final** #fail/#total Percent (95% CI)
5.9%	1.5%	1.6%	0.1%	7.6%	1.5%
65/1098	16/1098	18/1098	1/1098	83/1098	17/1098
[4.7-7.5%]	[0.9-2.4%]	[1.0-2.6%]	[0.0-0.5%]	[6.1-9.3%]	[1.0-2.5%]

The total specimens excluded from the iC-GPC Assay Method Comparison Study (n=53) are listed by site and reason for exclusion in the following table. The most common reasons for exclusion included repeat iC-GPC error, repeat comparator assay error, or specimens demonstrating mixed Gram stain results.

Withdrawn Samples					
	Reason for Withdrawal				
Site	**Mixed Gram Stain**	**Repeat iC-GPC Error**	**Repeat Comparator Assay Error**	**No Comparator Assay ID/ Other**	**TOTAL**
1	6	4	2	0	12
2	13	7	2	0	22
3	3	8	1	4	16
4	1	0	0	2	3
TOTAL	23	19	5	6	53

Additional specimens were excluded when iC-GPC Assay performance was compared to conventional reference methods for the following reasons: no culture identification, indeterminate culture identification, or non-specific AST results (i.e., intermediate susceptibility).