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

A. 510(k) Number:

K163390

B. Purpose for Submission:

To obtain a Substantial Equivalence Determination for a new device, iC-GPC Assay on the iC-System.

C. Measurand:

Nucleic acid sequences of the following gram positive organisms and associated resistance markers: *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Enterococcus faecium, mecA, vanA* and *vanB.*

D. Type of Test:

Qualitative *in vitro* diagnostic test utilizing polymerase chain reaction (PCR) for the multiplex amplification of specific targets followed by detection of the amplified products with microarray hybridization.

E. Applicant

iCubate, Inc.

F. Proprietary and Established Names:

iC-GPC Assay
iC-System

G. Regulatory Information:

1. Regulation section:

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

2. Classification:

   Class II

3. Product codes:

   PAM: Gram positive bacteria and their resistance markers
   NSU: Instrumentation for clinical multiplex test systems

4. Panel:

   Microbiology

H. Intended Use:

1. Intended use(s):

   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.

2. Indication(s) for use:

   Same as the Intended Use

3. Special conditions for use statement(s):

   - For prescription use only
   - The iC-GPC Assay has only been validated for use with the following 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, BD BACTEC Lytic/10 Anaerobic/F, VersaTREK REDOX 1, VersaTREK REDOX 2, BacT/ALERT SA Standard Aerobic, BacT/ALERT FA Aerobic FAN, and BacT/ALERT FA Plus Aerobic.

4. Special instrument requirements:

   For use on the iC-System

I. Device Description:

The iC-GPC Assay detects gram positive bacteria and resistance markers from positive blood culture specimens that are determined to contain gram positive cocci by Gram stain.

The assay utilizes PCR for the multiplex amplification of specific gram positive bacterial and resistance marker targets and detects the amplified targets with microarray hybridization. The assay utilizes proprietary ARM-PCR (Amplicon Rescued Multiplex PCR) technology allowing multiple targets to be amplified in one reaction. Testing is performed using a self-contained disposable cassette that is processed by the iC-Processor. After processing, the cassette is read using the iC-Reader. Final results are generated by the iC-Reader aided by computer software for data acquisition, analysis and display in the iC-Report.

The iC-GPC Cassettes are provided in individually sealed plastic pouches which must be stored at 2-8°C.

The analytes detected and reported by the iC-GPC Assay include the following:

Organism	**iC-GPC Targets**
Staphylococcus epidermidis (SE)	*gse*A, *mecA*
Staphylococcus aureus (SA)	*nuc*, *mecA*
Streptococcus pneumoniae (SPN)	*lytA*
Enterococcus faecalis (EFLS)	*ddl*, *vanB*
Enterococcus faecium (EFCM)	*fcm*, *vanA*

Materials Provided:

- iC-GPC Cassettes (shipped and stored at 2-8°C)

Materials required but not provided (instruments and equipment):

- iC-Processor
- iC-Reader
- iC-Report Software
- 2-8°C Refrigerator
- ≤70°C Freezer
- Automated blood culture monitoring system
- Micro-pipettes & tips
- Blood culture bottles
- Gram staining reagents

Test report and interpretation:

After reading a cassette, data is transferred from the iC-Reader to the iMac computer. Data is analyzed using iC-Report software and a final result is generated. The user may view or print the final result.

iC-GPC provides a qualitative result for the presence ("Detected") or absence ("Not Detected") of iC-GPC target organisms. Detected organisms and resistance markers are reported separately. If a resistance marker is identified without an associated organism concurrently detected, the resistance marker will not be reported. Positive *mecA* resistance marker results will only be reported for specimens that are also positive for *Staphylococcus aureus* or *Staphylococcus epidermidis.* Positive *vanA* and *vanB* resistance marker results will only be reported for specimens that are also positive for *Enterococcus faecium* or *Enterococcus faecalis.*

If multiple organisms are detected, all organisms detected will be reported with the following disclaimer:

'The report is indeterminate; the sample may contain multiple organisms. Isolate the organisms and perform alternate tests for organism identification and potential resistance.'

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Verigene Gram Positive Blood Culture NucleicAcid Test (BC-GP)

2. Predicate 510(k) number:

   K122514

3. Comparison with predicate:

Similarities		
Item	Device	Predicate (K122514)
Intended Use	Qualitative multiplexed in vitro diagnostic device for the detection and identification of potentially pathogenic gram-positive bacteria and associated resistance markers from positive blood culture bottles.	Same
Organisms and resistance markers detected	*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Enterococcus faecium*, *mecA*, *vanA* and *vanB*	Same plus additional organisms (see differences table below)
Analyte	DNA	Same
Specimen Type	Positive Blood Culture specimens containing gram positive cocci	Positive Blood Culture specimens containing gram positive bacteria
Specimen Processing and Purification	Automated by instrument	Same
Controls	Device includes one internal positive control of heat-killed *Bacillus thuringiensis*. External controls are not provided.	Device includes two internal controls, one containing intact *Bacillus subtilis* and the other containing an assay-specific DNA target. External controls are not provided.

Differences		
Item	Device	Predicate (K122514)
Instrument	iC-System	Verigene System
Organisms and Resistance Markers Detected	*Staphylococcus aureus* *Staphylococcus epidermidis* *Streptococcus pneumoniae* *Enterococcus faecalis* *Enterococcus faecium* *mecA* *vanA* *vanB*	Same plus the following additional organisms: *Staphylococcus* species *Staphylococcus lugdenensis* *Streptococcus spp.* *Streptococcus, pyogenes* *Streptococcus agalactiae* *Streptococcus anginosus group* *Enterococcus faecalis* *Enterococcus faecium* *Listeria* spp.
Test Principle	Heat lysis followed by two-step ARM-PCR, microarray hybridization and detection utilizing fluorescently labeled probes.	Cell lysis and magnetic bead-based bacterial DNA isolation followed by detection and identification of bacterial-specific DNA in a microarray format using gold nanoparticle probe-based hybridization technology.
Instrumentation	iC-Processor and iC-Reader	Verigene Reader and Processor SP
Time to Result	4.5 hours	2.5 hours

K. Standard/Guidance Document Referenced:

None cited

L. Test Principle:

The iC-GPC Assay is a multiplexed molecular assay that uses proprietary ARM-PCR (Amplicon Rescued Multiplex PCR) technology. The following chemical reactions take place in the iC-GPC Cassette:

1. DNA extraction – Nucleic acid extraction is performed using heat to lyse the gram positive bacteria and release DNA to be used in subsequent multiplex ARM-PCR amplification.

2. 1st stage multiple PCR – The ARM-PCR protocol includes two steps of amplification.

The first step of amplification utilizes a set of nested sequence-specific primers designed for each target. These primers are used to enrich targets as well as incorporate tags and priming sites for the 2nd stage PCR reaction.

3. 2nd stage PCR- Following the 1st stage PCR, a 2nd stage PCR is conducted using universal primers specific for tags incorporated into the amplicon during the 1st stage reaction. The 2nd stage PCR exponentially amplifies the target sequence and prepares it for microarray hybridization by enriching for single strand amplicons.

4. Microarray Hybridization- Upon completion of the 2nd stage PCR, amplified DNA is bound by gene specific fluorescently labeled probes and hybridizes to positions on the microarray specific for each target. Binding to a specific spot is measured by emission from the fluorescently labeled probes upon excitation with a laser in the iC-Reader and is interpreted by iC-Report to generate a data report.

5. The iC-Report software controls the operation of the iC-Processor and iC-Reader, analyzes data, and generates a test report after a run. The entire process takes approximately four and a half hours.

M. Performance Characteristics:

1. Analytical performance:

   a. Evaluation of organism concentration at bottle positivity (bottle ring)

   A study was performed to establish the concentration of each iC-GPC Assay target organism at initial bottle positivity. A total of nineteen organisms 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 after which 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 1 below. These concentrations, representative of the levels that may be observed in a clinical setting, are equivalent to or greater than the respective target LoDs (see below) and were used to determine target organism concentrations for analytical studies.

Table 1: Target Organism Concentrations at Bottle Positivity

Organism/Strain ID	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

Organism/Strain ID	Average Concentration (CFU/mL)
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

b. Reproducibility

A multi-site reproducibility study was conducted to assess the site-to-site, operator-to operator, and lot-to-lot reproducibility of the iC-GPC Assay. A representative panel of iC-GPC target organisms and one non-target organism were tested at two concentrations, the concentration present at bottle ring and the concentration present after eight hours incubation post bottle ring. Testing was performed by two independent operators at each of three sites, two external and one in-house. The panel was tested in replicates of three across five, non-consecutive days. Testing was evaluated across three cassette lots and four iC-Systems.

Five organisms, representing each of the iC-GPC Assay targets, plus one non-target organism were evaluated (see table 2 below). Blood culture bottles were inoculated with low organism concentrations and appropriate volumes of human blood and incubated on the automated blood culture instrument until signaled as positive by the instrument. Within one hour after initial positivity, an aliquot was removed from each bottle. Smaller aliquots were prepared and frozen at -20°C until testing was performed. The bottle was returned to the blood culture instrument for approximately eight additional hours of incubation. After this extended incubation, the bottle was removed, and aliquots were prepared and frozen at -20°C until testing.

Table 2: Reproducibility Test Panel

Organism	Strain ID	iC-GPC Targets
Staphylococcus epidermidis	ATCC 700566	*gseA, mecA*
Staphylococcus aureus	ATCC 700699	*nuc, mecA*
Streptococcus pneumoniae	ATCC 6301	*lytA*
Enterococcus faecalis	ATCC 51299	*ddl, vanB*
Enterococcus faecium	ATCC 700221	fcm, *vanA*
Corynebacterium striatum	MCW	---

Testing was performed by two operators at each of three sites; two external and one internal. Study specimens were randomized and blinded and tested over five non-consecutive days. Each panel member was tested in triplicate per site, per day and per operator. Three lots of iC-GPC Cassettes were included in the study.

Results from testing are shown in Table 3 below. Two false negative results were observed, one bottle negative for both *S. aureus* and *mecA*. Six false positive results were observed, five *E. faecalis* and one *Staphylococcus epidermidis*/*mecA*. All false positive results occurred in blood culture bottles containing the non-targeted organism *Corynebacterium striatum*, with three false positive results (two *E. faecalis* and one *S. epidermidis*/*mecA*) occurring at initial bottle ring and the three additional false positive results (all *E. faecalis*) occurring after eight hours incubation post bottle ring. A total of 15 tests (1.4%) generated invalid results and were not included in the data analysis.

Table 3: iC-GPC Reproducibility Study Results by Target Organism and 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%)

Organism/Gene Target/ Concentration	Overall Performance	Overall Performance % [95% CI]	False Negatives	False Positives	Positive Controls Check Failures	System Failures
***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%)

Results from the reproducibility study demonstrated that the iC-GPC Assay generates reproducible results for each targeted organism and resistance marker in positive blood culture specimens containing organism concentrations present at bottle ring and after eight hours additional incubation.

c. Linearity/assay reportable range:

Not applicable

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

Specimen stability:

A study was conducted to evaluate the stability of positive blood culture specimens stored under various conditions for use with the iC-GPC Assay. Samples replicates were prepared with six different organisms, representing each of the iC-GPC Assay targets and one non-target organism, *Corynebacterium striatum* (see Table 4 below). Samples were prepared by inoculating low concentrations of organisms into blood culture bottles with human blood added followed by incubation on an automated blood culture instrument until positivity.

Table 4: Specimen Stability Test Panel

Organism	Strain ID	iC-GPC Targets
Staphylococcus epidermidis	ATCC 700566	*gseA, mecA*
Staphylococcus aureus	ATCC 700699	*nuc, mecA*
Streptococcus pneumoniae	ATCC 6301	*lytA*
Enterococcus faecalis	ATCC 51299	*ddl, vanB*
Enterococcus faecium	ATCC 700221	fcm, *vanA*
Corynebacterium striatum	MCW	---

Samples were stored under three temperature conditions: incubated (35-37°C, on the automated blood culture instrument), ambient temperature (20-25°C), and refrigerated (2-8°C). Samples were tested in triplicate for each organism and storage condition at each of the following time points: 8, 24, and 48 hours beyond initial bottle positivity. For each time point and storage condition, results of the study demonstrated 100% detection for all bottle replicates containing targeted organisms and 100% negative results for bottles containing the non-targeted organism.

Results from the specimen stability study support the following stability claims for positive specimens stored under the following conditions: incubated (35- 37°C), room temperature (20-25°C), and refrigerated (2-8°C) for up to 48 hours beyond initial bottle positivity.

Fresh versus frozen and freeze/thaw studies:

A study was performed to evaluate the effect of freezing blood culture specimens on performance of iC-GPC Assay. The same panel of organisms presented in Table 4 above was evaluated in contrived blood culture specimens with human blood added. Samples were evaluated with organism concentrations both at bottle ring and eight hours post bottle ring concentrations. For each organism and organism concentration, three replicates were evaluated after both two and four weeks of frozen storage between -10°C and -30°C. Study results demonstrated 100% detection of samples containing targeted organisms and 100% negative results for bottles containing the non-targeted organism for each time point and organism concentration evaluated.

Additional testing was conducted with the same sample panel to evaluate the effect on positive blood culture specimens subjected to one or two freeze-thaw cycles. Again, positive blood culture samples with organism concentrations at bottle ring and eight hours post bottle ring concentrations were evaluated. Results of the study showed 100% detection as expected and demonstrated no effect on iC-GPC Assay performance for blood culture specimens subjected to two freeze/thaw cycles.

Results from the fresh versus frozen and the freeze/thaw studies demonstrated equivalent iC-GPC Assay performance between fresh and frozen specimens and for specimens subjected to one or two freeze thaw cycles. These results support the use of frozen clinical specimens in the clinical study as well as frozen contrived specimens in the reproducibility study.

Assay controls:

Internal DNA amplification and processing control: An internal positive control of heat-killed *Bacillus thuringiensis* is loaded into the test sample prior to DNA extraction. The positive control is lysed, amplified using primers specific for a *B. thuringiensis cry* gene, processed, and hybridized to the array at the same time as the sample. Reporting of the positive control in the iC-Report confirms

the assay was successful and ran to completion. In cases where targets are present at high concentrations, it is possible the target sequence will be preferentially amplified over the positive control. If the positive control is not detected on the array but a target is reported, the assay is still considered valid. If neither positive control nor target is reported, the assay is considered to have failed; an end report of "Positive controls check failed" will be generated.

Recommended External Controls: The iC-GPC Assay does not include external controls. The following recommendations for external control testing are included in the iC-GPC package insert:

Cultured organisms for each of the iC-GPC Assay targets should be tested occasionally in accordance with laboratory defined quality control requirements. It is recommended that external quality controls are performed before testing newly manufactured cassette lots and any time system performance is in question. A fresh culture of recommended quality control organisms should be isolated on TSA 5% sheep blood agar plates or equivalent media. From the growth plate, transfer the organisms into saline, and adjust organism concentration to 0.5 MacFarland or $1x10^8$ CFU/ml equivalent. Then, test in accordance to iC-GPC Assay instructions. If the external control fails, the test should be repeated. The strains listed in Table 5 can be used for external control testing.

Table 5: External Controls, Recommended Organisms

Organism	ATCC ID Number	Target
S. pneumoniae	6301	*S. pneumoniae*
E. faecalis	51299	*E. faecalis* and *vanB*
S. aureus	700699	*S. aureus* and *mecA*
E. faecium	700221	*E. faecium* and *vanA*
S. epidermidis	700566	*S. epidermidis* and *mecA*

External control testing, clinical study: A total of 551 quality control samples were evaluated in the clinical study and 24 quality control failures were observed.

Six quality control failures were due to incorrect organism calls with three samples generating false positive results and three samples generating false negative results. A total of 17 quality control samples generated internal control failures or instrument failures: twelve positive control check failures, two processor errors and two array registration errors. One additional sample was processed but not read by the site.

Quality control failures stratified by targeted organism are shown in Table 6 below.

Table 6: Quality Control Results, Clinical Study

QC Testing	*S. aureus* (*nuc*, *mecA*)		*S. epidermidis* (*gse*A, *mecA*)		*S. pneumoniae* (*lytA*)		*E. faecalis* (*ddl*1, *vanB*)		*E. faecium* (*fcm*, *vanA*)		Total Tests Performed	Percentage Pass/Fail
Pass	101	95.3%	102	98.1%	101	91.8%	109	97.3%	110	95.7%	527	95.6%
Fail	5	4.7%	2	1.9%	9	8.2%	3	2.7%	5	4.3%	24	4.4%

e. Detection limit:

A study was conducted to evaluate the Limit of Detection (LoD) of the iC-GPC Assay for each targeted analyte. A total of 19 bacterial strains were evaluated with a minimum of two strains per iC-GPC Assay target. The strains evaluated are shown in Table 7.

Table 7: LoD Test Panel

Organism	Strain ID	iC-GPC Targets
Staphylococcus epidermidis	ATCC 700566	*gseA, mecA*
Staphylococcus epidermidis	ATCC 35984	*gseA, mecA*
Staphylococcus epidermidis	ATCC 12228	*gseA*
Staphylococcus epidermidis	ATCC 49134	*gseA*
Staphylococcus aureus	ATCC 700699	*nuc, mecA*
Staphylococcus aureus	ATCC BAA1768	*nuc, mecA*
Staphylococcus aureus	ATCC BAA977	*nuc*
Staphylococcus aureus	ATCC 25923	*nuc*
Streptococcus pneumoniae	ATCC 6301	*lytA*
Streptococcus pneumoniae	ATCC 700673	*lytA*
Enterococcus faecalis	ATCC 51299	*ddl, vanB*
Enterococcus faecalis	ATCC 700802	*ddl, vanB*
Enterococcus faecalis	JMI 12536	*ddl, vanA*
Enterococcus faecalis	ATCC 29212	*ddl*
Enterococcus faecalis	ATCC BAA2128	*ddl*
Enterococcus faecium	ATCC 700221	fcm, *vanA*
Enterococcus faecium	ATCC 51559	fcm, *vanA*
Enterococcus faecium	ATCC 35667	fcm
Enterococcus faecium	ATCC BAA2127	fcm

The LoD study included preliminary estimations of target LoDs based on testing of 10 sample replicates of each strain at several organism concentrations. Additional testing was performed with three organism concentrations spanning the estimated LoD, with replicates of 20 tested on three different lots for each dilution. All samples were prepared in a blood culture media/blood matrix.

The final LoD for each strain was calculated as the lowest organism concentration (CFU/mL) demonstrating a detection rate of approximately 95%. Final LoD organism concentrations were determined by plating and subsequent colony counts. A total of 91 runs with positive controls check failures (2.11%) and 21 system failures (0.49%) were observed in the study and were excluded from the data analysis. Results from LoD testing are shown in Table 8.

Table 8: Final LoDs for Targeted Analytes

iC-GPC Organism target	Defined LoD (CFU/mL)
Staphylococcus epidermidis	$1.6 \times 10^6 – 1.7 \times 10^7$
Staphylococcus aureus	$1.7 \times 10^6 – 4.4 \times 10^6$
mecA	$7.4 \times 10^5 – 9.5 \times 10^6$
Streptococcus pneumoniae	$1.3 \times 10^6 – 6.0 \times 10^6$
Enterococcus faecalis	$3.0 \times 10^5 – 5.8 \times 10^6$
Enterococcus faecium	$4.9 \times 10^6 – 7.9 \times 10^6$
vanA	$7.2 \times 10^5 – 1.1 \times 10^7$
vanB	$3.9 \times 10^6 – 5.8 \times 10^6$

f. 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 with testing performed in triplicate. Strains were tested near the target LoD (2-3x LoD) or at the lowest level of bottle positivity. Colony counts were used to confirm final concentration tested. In the event of a false negative result, the strain was retested near the target LoD in replicates of ten.

Results from the study showed that all expected targets were detected by the iC-GPC Assay with the exception of two false negative results: one false negative for *S. epidermidis* and one false negative *S. aureus*/*mecA*. Repeat testing of the two strains generated 10/10 replicates with the expected positive results. In this study there were four runs with positive controls check failures (1.13%) and one system error (0.28%). Results of inclusivity testing are summarized in Table 9 below.

Table 9: Inclusivity Study Results

Species	Strain ID	Other IDs/Notes	Replicates 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

Species	Strain ID	Other IDs/Notes	Replicates Detected/ Total Replicates
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
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

Species	Strain ID	Other IDs/Notes	Replicates Detected/ Total Replicates
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
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- van*A*	3/3
E. faecalis, vanA (+)	Z269	PHE NCTC 12203- van*A*	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

.

Note: The iC-GPC software logic only allows reporting of *vanA*/*B* targets with *Enterococcus faecium* and *Enterococcus faecalis*. Therefore the *vanA* target will not be reported for positive specimens containing *S. aureus* strains that carry this marker (i.e., VISA/VRSA strains).

g. Analytical specificity (Exclusivity)

The analytical specificity of the iC-GPC Assay was evaluated by testing a comprehensive panel of non-targeted microorganisms that may be encountered in positive blood cultures. Exclusivity panel members included organisms phylogenetically related to iC-GPC target organisms, organisms commonly recovered from positive blood cultures as well as common blood culture contaminants. A total of 94 exclusivity organisms were tested. Potential cross-reactivity was evaluated by testing panel organisms at high concentrations in blood culture bottle media with the appropriate volume of human blood added according to bottle instructions. In the event of a false positive result, additional replicates were evaluated. Samples excluded from the sample set for data analyses were 17 samples with positive controls check failures (4.4%) and 3 samples with system errors (0.8%).

Due to false positive *E. faecalis* results observed with samples containing *Streptococcus bovis* (strain Z167), a second strain of *Streptococcus bovis* (ATCC 9145) was also tested. In a total of 14 sample replicates containing *Streptococcus bovis*, 11 samples generated false positive results for the *E. faecalis* target indicating cross reactivity of this organisms with the iC- GPC Assay target. The package insert includes a limitation indicating cross-reactivity of *Streptococcus bovis* with the *Enterococcus faecalis* target of the iC-GPC Assay.

Exclusivity results are presented in Table 10 below; performance is based on the observation of expected negative results.

Table 10: Exclusivity Results

Exclusivity Organism	Test Concentration (CFU/mL, or as designated, bottle ring or $TCID_{50}$/mL)	Exclusivity (observed negative results)
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

Exclusivity Organism	Test Concentration (CFU/mL, or as designated, bottle ring or $TCID_{50}$/mL)	Exclusivity (observed negative results)
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
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

Exclusivity Organism	Test Concentration (CFU/mL, or as designated, bottle ring or $TCID_{50}$/mL)	Exclusivity (observed negative results)
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
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

Exclusivity Organism	Test Concentration (CFU/mL, or as designated, bottle ring or $TCID_{50}$/mL)	Exclusivity (observed negative results)
Streptococcus salivarius	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

h. Microbial interference

A study was conducted to evaluate the performance of the iC-GPC Assay for samples containing mixtures of targeted and non-targeted organisms. Samples were prepared in a blood culture media/blood matrix with target organisms near the assay LoD mixed with non-targeted organisms at high concentrations ranging from 10^6-10^9 CFU/mL or at concentrations present at eight hours post bottle ring. A total of 85 different non-targeted microorganisms were evaluated in combination with each of five targeted organism strains. Testing was performed in triplicate.

For those organism combinations generating initial false negative results, additional samples were tested in replicates of 10 with the target organism near target LoD. If additional false negative results were observed, testing was repeated with the target organism at bottle ring concentrations. 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% positive results with the iC-GPC Assay. Samples excluded from the sample set for data analysis included 22 samples with positive controls check failures (1.4%) and 10 samples with system errors (0.7%). Microbial interference study results are presented in Table 11 below; performance is based on all expected targets detected.

Table 11: Microbial Interference Study 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 defectiva	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

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
Staphylococcus pettenkoferi	6.78×10^8	12/13[17]	3/3	3/3	3/3	3/3

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 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 salivarius	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

i. Competitive inhibition

Performance of the iC-GPC Assay 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 12 below. Results represent the number of detected targeted organisms out of the total number of replicates tested. No positive control failures or other system errors were observed in the study.

Due to competitive inhibition, low concentration organism targets were detected in only 3/20 (15%) of organism combinations. All high concentration iC-GPC targeted organisms were detected in all organism combinations. A limitation was placed in the package insert indicating the following:

- 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.

Table 12: Competitive Inhibition Study Results

		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.

j. Blood culture bottle equivalency

Commonly used blood culture bottle media types were evaluated to demonstrate that variability in media composition does not interfere with iC-GPC Assay performance. A total of 19 representative strains of iC-GPC targeted organisms plus one non-target organism were tested in 10 bottled blood culture media types. Target organisms were tested near LoD concentrations (2-3x LoD). Expected performance for targeted organisms was based on all expected targets identified and no false positive targets detected. Expected performance for non-target organisms was based on expected negative results. A total of 10 runs with positive controls check failures (1.23%) and five system errors (0.62%) were observed in the study and excluded from the data analysis.

Performance of the iC-GPC Assay for all BACTEC and VersaTREK bottle types and for BacT/ALERT SA Standard Aerobic bottles met the acceptance criteria of ≥ 95% accurate results, demonstrating acceptable analytical performance for these bottle types with the iC-GPC Assay (see table 13 below).

False positive results for *Enterococcus faecium* were observed in 15/59 (25.4%) of BacT/ALERT FA Aerobic FAN and 13/60 (21.7%) of BacT/ALERT FA Plus Aerobic bottles for specimens prepared with target organisms present at 2-3x LoD. Additional testing for both bottle types was performed with higher organism concentrations of 5-10x LoD for each targeted organism. This additional testing resulted in false positive *E. faecium* results for 8/59 (13.6%) and 5/60 (8.3%) of BacT/ALERT FA Aerobic FAN and BacT/ALERT FA Plus Aerobic bottles respectively.

The potential cause of false positive *E. faecium* results was further investigated using multiple lots of uninoculated BacT/Alert FA Aerobic FAN and FA Plus Aerobic bottles. Results from this testing showed that false positive results continued to occur for these two bottle types with the iC-GPC Assay. Further testing of inoculated BacT/Alert FA Aerobic FAN and FA Plus Aerobic blood culture bottles was performed using a PCR assay that detects a different *E. faecium* DNAs sequence. This testing generated positive PCR results that were subsequently confirmed by sequencing and therefore confirming that the uninoculated bottle media contained *E. faecium* nucleic acids. Results from the investigation indicate that the iC-GPC Assay false positive *E. faecium* results observed in BacT/Alert FA Aerobic FAN and BacT/Alert FA Plus Aerobic media in the analytical bottle equivalency study are not likely due to a limitation of the iC-GPC Assay but rather are due to the presence of remnant nucleic acids and/or non-viable *E. faecium* organisms in the culture media.

Although analytical testing showed significant numbers of false positive *E. faecium* results for samples prepared in BacT/Alert FA Aerobic FAN and BacT/Alert FA Plus blood culture media, there were no false positive *E. faecium* results observed in clinical positive blood culture specimens for these two bottle types (i.e., no false positive *E. faecium* results observed in 96 BacT/Alert FA Aerobic FAN and for 18 BacT/Alert FA Plus blood culture bottles).

Because of the observed false positive results observed in analytical testing, the result interpretation section as well as the limitations section of the package insert indicate that all positive *E. faecium* results should be confirmed using alternative methods when testing BacT/ALERT FA Aerobic FAN or FA Plus Aerobic blood culture bottles.

The following limitations are included in the package insert regarding testing of BacT/Alert blood culture bottles with the iC-GPC Assay:

- There is an increased risk of *E. faecium* false positives when the iC-GPC Assay is used with bioMérieux BacT/ALERT FA Aerobic FAN and BacT/ALERT FA Plus Aerobic blood culture bottles due to remnant nucleic acids or non-viable organisms that have been reported to be present in the culture media. When using these bottle types, positive *E. faecium* test results should be confirmed using alternative methods.

- The iC-GPC Assay has not been validated for use with bioMérieux BacT/ALERT SN Standard Anaerobic, FN Anaerobic FAN, and FN Plus Anaerobic blood culture bottles due to an increased risk of *E. faecium* false positives from nucleic acids or non-viable organisms present in the culture media. Anaerobic BacT/ALERT bottles including SN Standard Anaerobic, FN Anaerobic FAN, and FN Plus Anaerobic should not be used with the iC-GPC Assay.

Study results from the blood culture equivalency study are presented in Table 13.

Table 13: iC-GPC Assay Blood Culture Equivalency Study 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	51/59 (86.4%)	51/57 (89.5%)	0/57 (0.0%)	0/2 (0.0%)	8/59 (13.6%)[10]

Blood Culture Bottle Type	Overall Performance (%)	Target Performance (%)	False Negatives (%)	Non-Target Performance (%)	False Positives (%)
FAN (5-10x LoD)					
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*

k. Interfering substances

iC-GPC Assay performance was evaluated in the presence of potentially inhibiting substances that may be present in blood and blood culture media. Samples containing five representative target organisms plus one gram positive, non-target organism (Group B *Streptococcus*) were evaluated. Samples were prepared with targeted organisms at near LoD concentrations with each potential interferent at "worst-case" concentrations that exceed the potentially highest concentration encountered in blood culture bottles inoculated with indicated blood volumes. Antibiotics were tested at five times the concentration expected in whole blood.

Target organism performance was based on all expected targets detected, while non-target organism performance was based on expected 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. For any discordant results observed both initially and with additional replicates, additional samples were evaluated with decreasing inhibitor concentrations until interference was no longer observed.

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. The presence of these substances in positive blood culture specimens may result in false negative results or positive controls check failures.

A total of 10 positive controls check failures (2.69%) and three system errors (0.81%) were observed during the study and results from these samples were excluded from

the data analysis. Table 14 includes results for those substances that did not demonstrate interference initially or after testing of additional replicates. Table 15 includes results for those substances that demonstrated interference.

Table 14: Interference Study Results – Non-interfering Substances

		Target Performance					
Potential Interferent	**Test**	**SE**	**SA**	**SPN**	**EFLS**	**EFCM**	**Group B**
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 15: Interfering Substance Study – Interfering Substances

		Target Performance					
Interferent	**Test**	**SE**	**SA**	**SPN**	**EFLS**	**EFCM**	**Group B**
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

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

l. Carryover/cross-contamination

The iC-System is a closed system, designed to reduce the potential for carryover or cross-contamination between samples. A study was conducted to evaluate the potential for false positive results from carryover or cross-contamination when high positive samples are tested consecutively with negative samples. 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 high 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 positive results were observed.

m. Assay cut-off:

The iC-GPC Assay identifies and reports detected analytes based on the relative intensity levels of fluorescent markers (amplified DNA bound by gene specific fluorescently labeled probes) hybridized to a glass microarray within the iC-Cassette. The assay software analyzes data captured from image processing and compares fluorescent signals from analyte-specific sensors in the sensor array to background fluorescence.

2. Clinical studies:

a. Method comparison with FDA-cleared multiplex assay:

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 blood culture 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. 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.

Blood culture specimens flagged as positive by the instrument were confirmed using Gram stain to contain gram positive cocci prior to being entered into the study. Specimens demonstrating mixed Gram stain results were excluded as indicated by the assay instructions for use.

Performance of the iC-GPC Assay was evaluated as 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 an FDA-cleared multiplex assay. Results from this additional testing were used only for discordant analysis with results included as footnotes under the performance tables.

Additionally, performance of the iC-GPC Assay was compared to conventional reference methods including subculture, identification of isolates using biochemical methods or MALDI-TOF identification and phenotypic antimicrobial susceptibility testing (AST).

An additional 168 contrived specimens were tested for low prevalence organisms and resistance markers. Contrived specimens were prepared using a variety of clinical strains and type strains of *S. pneumoniae*, *E. faecalis*, and *E. faecium*. Confirmation of strain identities was confirmed by sequencing for all clinical isolates as well as isolates containing *vanA*/*vanB* resistance markers. Contrived specimens were prepared in BD BACTEC Plus Aerobic blood culture bottles with the appropriate volume of blood added in accordance with bottle instructions. Organisms were spiked into bottles at low concentrations and incubated until flagged as positive by the instrument. Aliquots of specimens were frozen and provided to the three clinical sites for testing.

Of the 966 positive blood culture specimens evaluated in the study, a total of 53 specimens were excluded from the study results. Of the remaining 913 specimens remaining, 879 were fresh prospective specimens and 34 were frozen prospective specimens.

The total specimens excluded from the iC-GPC Assay Method Comparison Study (n=53) are listed by site and reason for exclusion in Table 16. The most common reasons for exclusion included repeat iC-GPC errors, repeat comparator assay errors, or exclusion of specimens with mixed Gram stain results.

Table 16: Withdrawn Specimens

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

When performance of the iC-GPC Assay was compared to the FDA-cleared multiplex assay, there was no apparent difference in performance noted between the four study sites or between the three blood culture systems. Performance for all positive bottle types/systems combined is presented in Tables 17-24 respectively for detection of the *S. aureus*, *S. epidermidis*, *S. pneumoniae*, *E. faecalis*, *E. faecium*, *mecA*, *vanA* and *vanB* iC-GPC Assay targets as compared to the comparator assay. Results are

stratified by prospectively tested fresh specimens, prospectively collected/retrospectively tested frozen specimens and contrived specimens. Table 22 includes performance for detection of *mecA* with results linked to detection of *S. aureus* and *S. epidermidis* combined. Tables 23 and 24 include performance for detection of *vanA* and *vanB* respectively with results linked to detection of *E. faecalis* and *E. faecium* combined.

Table 17: 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.

Table 18: C-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.

Table 19: 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.

Table 20: 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

Table 21: 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.

Table 22: iC-GPC Assay Performance: *mecA*

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 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.

Table 23: iC-GPC Assay Performance: *vanA*

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

Table 24: iC-GPC Assay Performance: *vanB*

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 1 observed false positive *vanB* result by the iC-GPC Assay, 0/1 was positive for *vanB* by PCR/bi-directional sequencing

Tables 25-30 include performance of the iC-GPC Assay as compared to an FDA-cleared multiplex assay for each targeted resistant marker linked to detection of specific targeted organisms. Tables 25 and 26 include stratified performance for detection of *mec*A with *S. aureus* and *S. epidermidis* respectively. Tables 27-30 include stratified performance for *vanA* and *vanB* for detection with *E. faecalis* and *E. faecium*. Performance tables include all prospectively collected specimens, both fresh and frozen combined.

Table 25: Detection of *mecA* with *S. aureus*, Prospective Specimens

Staphylococcus aureus/mecA		FDA-Cleared Comparator Assay			
		SA+/*mecA*+	SA+/*mecA*-	SA-	TOTAL
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%

Table 26: Detection of *mecA* with *S. epidermidis*, Prospective Specimens					
Staphylococcus epidermidis/mecA		**FDA-Cleared Comparator Assay**			
		SE+/*mecA*+	SE+/*mecA*-	SE-	TOTAL
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%

Table 27: Detection of *vanA* with *E. faecalis*, Prospective Specimens					
Enterococcus faecalis/vanA		**FDA-Cleared Comparator Assay**			
		Efs+/*vanA*+	Efs+/*vanA*-	Efs-	TOTAL
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*+)			(0/0)	-	-
% Agreement (Efs+/*vanA*-)			93.4% (57/61)	84.3%	97.4%
% Agreement (Efs-)			99.9% (851/852)	99.3%	99.9%

Table 28: Detection of *vanB* with *E. faecalis*, Prospective Specimens					
Enterococcus faecalis/vanB		**FDA-Cleared Comparator Assay**			
		Efs+/*vanB*+	Efs+/*vanB*-	Efs-	TOTAL
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%

Table 29: Detection of *vanA* with *E. faecium*, Prospective Specimens					
Enterococcus faecium/vanA		**FDA-Cleared Comparator Assay**			
		Efm+/*vanA*+	Efm+/*vanA*-	Efm-	TOTAL
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%

Table 30: Detection of *vanB* with *E. faecium*: Prospective Specimens					
Enterococcus faecium/vanB		**FDA-Cleared Comparator Assay**			
		Efm+/*vanB*+	Efm+/*vanB*-	Efm-	TOTAL
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*+)			(0/0)	-	-
% Agreement (Efm+/*vanB*-)			96.6% (28/29)	82.8%	99.4%
% Agreement (Efm-)			99.8% (882/884)	99.2%	99.9%

Tables 31-33 include performance of the iC-GPC Assay for detection of *vanA* and *vanB* in contrived specimens prepared with *E. faecalis* or *E. faecium* strains that carry either *vanA* or *vanB* resistance markers

Table 31: Detection of *vanA* with *E. faecalis*: Contrived Specimens

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%

Table 32: Detection of *vanA* with *E. faecium*: Contrived Specimens

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%

Table 33: Detection of *vanB* with *E. faecalis*: Contrived Specimens

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%

b. Performance of iC-GPC compared to traditional culture and susceptibility testing

Performance of the iC-GPC Assay was also evaluated compared to results from traditional laboratory reference methods that included subculture of positive bottles to agar media, identification of culture isolates using conventional biochemical or MALDI-TOF identification and antimicrobial susceptibility testing (AST). AST testing was comprised of cefoxitin disc testing for all *Staphylococcus* isolates (phenotypic reference method for *mecA*) and vancomycin Etests for all *Enterococcus faecalis* and *Enterococcus faecium* (phenotypic reference method for *vanA*/*vanB*). Performance of the iC-GPC Assay in comparison to culture and AST testing is presented in Tables 34-40 for each targeted analyte (*S. aureus*, *S. epidermidis*, S. *pneumoniae*, *E. faecalis*, *E. faecium*, *mecA*, *vanA* and *vanB*). Performance is stratified by prospectively tested fresh specimens and prospectively collected, retrospectively tested frozen specimens.

Table 34: 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)	

Table 35: 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)	

Table 36: 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)	

Table 37: 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)	

Table 38: 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)	

Table 39: 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.-95.7)	99.8% 829/831 (99.1-99.9)	Culture, Biochemicals & 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)	

Table 40: iC-GPC Assay Performance: *mecA*/methicillin resistance

Specimen Type			Percent Agreement		Comparator Method
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 14 false negative samples to be cefoxitin sensitive. See note below.

Tables 41-44 include performance of the iC-GPC Assay for detection of *mecA* and *vanA*/*vanB* in comparison to traditional culture and AST testing for specific organism targets. Results include all fresh and frozen prospective clinical specimens with performance stratified for detection of *mecA* with *S. aureus*, detection of *mecA* with *S. epidermidis*, detection of *vanA*/*vanB* with *E. faecalis* and detection of *vanA*/*vanB* with *E. faecium*.

NOTE: There were 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 (See Table 41 below). Of these 21 specimens, five were false negative due to a missed detection for the *S. aureus* target. Of the remaining 16 false negative results for *mecA*, all were also *mecA* negative by the FDA-cleared multiplex assay used in the method comparison study. Further investigation into these false negative results revealed that 14 of the specimens were from a single clinical site. Additional testing of isolates from the 14 false negative specimens was performed. Isolates were tested with an alternate real-time PCR assay that detects *mecA* and *mecC*. This assay confirmed the isolates to be negative for both *mecA* and *mec*C. Cefoxitin disc testing was also repeated on the 14 specimen isolates. Upon repeat testing, all isolates were found to be susceptible to cefoxitin with a zone size of 22mm which is very close to the cutoff of 21 mm for susceptibility/resistance. Results from the additional testing confirm that the iC-GPC result of *mecA* negative was correct for 14 of the 21 false negative *mecA* results.

Table 41: Detection of *mecA* with *S. aureus*: Prospective Specimens

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

Table 42: Detection of *mecA* with *S. epidermidis*: Prospective Specimens

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%

Table 43: Detection of *vanA/vanB* with *E. faecalis*: Prospective Specimens					
E. faecalis/vanA/vanB		**Culture, Biochemicals & Vancomycin AST**			
		Efs+/*van*R	Efs+/*van*S	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%

Table 44: Detection of *vanA/vanB* with *E. faecium*: Prospective Specimens					
E. faecalis/vanA/vanB		**Culture, Biochemicals & Vancomycin AST**			
		Efm+/*van*R	Efm+/*van*S	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%

b. Analysis of Mixed Culture Results:

In the method comparison study, there were seven mixed culture specimens that were detected by the iC-GPC Assay, the FDA-cleared multiplex comparator assay, or with both assays. Tables 45 and 46 list the mixed target combinations detected by iC-GPC Assay and/or the comparator assay. There was one discrepant specimen for which the iC-GPC Assay detected a target that was not detected by the comparator assay. There was one discrepant specimen for which the comparator assay detected targets that were not detected by iC-GPC. It is noted that the assay labeling includes a limitation that 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.

Table 45: Multiple Organism Detections by iC-GPC as Compared to FDA-cleared multiplex assay

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

Table 46: Multiple Organism Detections by FDA-cleared multiplex assay as compared to iC-GPC

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

Table 47 includes a listing of specimens generating iC-GPC positive results for multiple organism targets as compared to results from reference culture methods. Of the six mixed culture specimens detected by the iC-GPC Assay, there were two discrepant specimens in which the iC-GPC Assay detected a target that was not detected by culture, biochemicals, and AST testing.

Table 47: Multiple Organism Detections by iC-GPC as Compared to Culture

Multiple Detections by FDA-cleared FDA Assay						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

Table 48 includes a listing of specimens for which multiple organisms were recovered by traditional reference culture methods. There were 56 mixed culture specimens identified by culture and standard of care isolate identification, including organisms not detected by the iC-GPC Assay. There were a total of 12 discrepant specimens for which the reference methods identified isolates that were not detected by iC-GPC (false negatives). There were nine discrepant specimens for which iC-GPC detected targets that were not detected by the reference methods (false positives).

Table 48: Multiple Organisms recovered by Culture as Compared to iC-GPC Assay

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*

Multiple Detections by Culture, Biochemicals & AST					
Target 1	**Target 2**	**Target 3**	**Frequency**	**No. w/ Discrepant Targets**	**Discrepant Target**
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

Multiple Detections by Culture, Biochemicals & AST					
Target 1	**Target 2**	**Target 3**	**Frequency**	**No. w/ Discrepant Targets**	**Discrepant Target**
Staphylococcus hominis	*Aerococcus viridans*		2	0	NA
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

3. Clinical cut-off:

   Not applicable

4. Expected values:

   A total of 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. Expected values are presented in Table 49.

Table 49: Positivity by the iC-GPC Assay as Observed in the Clinical Study

Organism	Sites	1	2	3	4	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%

N. Instrument Name:

iC-System

O. System Descriptions:

1. Modes of Operation:

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

   Yes _____ or No ____X_____

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

   Yes _____ or No ___X_____

2. Software:

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

   Yes __X_____ or No _____

3. Specimen Identification: Patient information is manually entered into the iMac computer for each iC-GPC test. For each patient specimen, the user then selects "Link Cassette" in the iC-System software and then scans the Cassette barcode using a barcode scanner.

4. Specimen Sampling and Handling:

   Positive blood culture specimens are extracted from the blood culture bottle using a syringe and placed into a sterile tube. A gram stain is then performed to determine the presence of gram positive cocci. If gram positive cocci are present and is not mixed, the user then pipets the well-mixed specimen into the bottom of the sample well in the iC-GPC Cassette. The user closes the cassette cap and locks the sliding lid into place. The loaded cassette is then placed onto the iC-Processor. When processing is complete, the Cassette automatically ejects and then the user inserts the iC-Cassette into the iC-Reader where the iC-Cassette is read. Results are automatically transferred to the iC-Software and subsequently reported.

5. Calibration:

   The user is not required to calibrate the instrument.

6. Quality Control:

   See Section M(1)(d) above for information on internal and external controls.

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

Not applicable

Q. Proposed Labeling:

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

R. Conclusion:

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