EVALUATION OF AUTOMATIC CLASS III DESIGNATION (DE NOVO) FOR VERIGENE® GRAM POSITIVE BLOOD CULTURE NUCLEIC ACID TEST (BC-GP)

REGULATORY INFORMATION

FDA identifies this generic type of device as: Multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures

A multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures is a qualitative in vitro device intended to simultaneously detect and identify microorganism nucleic acids from blood cultures that test positive by Gram stain or other microbiological stains. The device detects specific nucleic acid sequences for microorganism identification as well as for antimicrobial resistance. This device aids in the diagnosis of bloodstream infections when used in conjunction with other clinical and laboratory findings. However, the device does not replace traditional methods for culture and susceptibility testing.

NEW REGULATION NUMBER: 21 CFR 866.3365

CLASSIFICATION: II

PRODUCT CODE: PAM

DEVICE NAME: VERIGENE® GRAM POSITIVE BLOOD CULTURE NUCLEIC ACID TEST (BC-GP)

BACKGROUND :

DEVICE NAME: VERIGENE® GRAM POSITIVE BLOOD CULTURE NUCLEIC ACID TEST (BC-GP)

510(K): K113450

DATE OF 510(K) NSE DECISION: JUNE 12, 2012

DATE OF DE NOVO PETITION: JUNE 14, 2012

PETITIONER CONTACT: NANOSPHERE, INC. – MR. MARK DEL VECHIO

PETITIONER'S RECOMMENDED CLASSIFICATION: II

PETITIONER'S RECOMMENDED CONTROLS:

- Class II Special Controls Guidance Document: Multiplex Nucleic Acid Assay for Identification of Microorganism and Resistance Markers from Positive Blood Cultures
- General controls

INDICATIONS FOR USE

The Verigene® Gram Positive Blood Culture Nucleic Acid Test (BC-GP) performed using the sample-to-result Verigene System is a qualitative, multiplexed *in vitro* diagnostic test for the simultaneous detection and identification of potentially pathogenic gram-positive bacteria which may cause bloodstream infection (BSI). BC-GP is performed directly on positive blood culture using *BACTEC™ Plus Aerobic/F* and *BacT/ALERT FA FAN®* Aerobic blood culture bottles, which contain gram positive bacteria. BC-GP is indicated for use in conjunction with other clinical and laboratory findings, such as culture, to aid in the diagnosis of bacterial bloodstream infections; however, it is not used to monitor bloodstream infections.

BC-GP detects and identifies the following bacterial genera and species:

Staphylococcus spp.	*Streptococcus spp.*	*Enterococcus*
Staphylococcus aureus	*Streptococcus*	*faecalis*
Staphylococcus	*pneumoniae*	*Enterococcus*
epidermidis	*Streptococcus pyogenes*	*faecium*
Staphylococcus	*Streptococcus agalactiae*	
lugdunensis	*Streptococcus anginosus*	*Listeria spp.*
	group	

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

BC-GP is indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial bloodstream infections; however, is not to be used to monitor these infections. Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing, identification of organisms not detected by BC-GP, differentiation of mixed growth, association of antimicrobial resistance marker genes to a specific organism, or for epidemiological typing.

LIMITATIONS

For prescription use only

PLEASE REFER TO THE BC-GP LABELING FOR A MORE COMPLETE LIST OF WARNINGS, PRECAUTIONS AND CONTRAINDICATIONS.

DEVICE DESCRIPTION

The Verigene® Gram Positive Blood Culture Nucleic Acid Test (BC-GP) is a molecular assay which relies on detection of specific nucleic acid targets in a microarray format. For each of the

bacterial nucleic acid sequences detected by the BC-GP test, Capture and Mediator oligonucleotides are utilized for gold nanoparticle probe-based endpoint detection. The Capture oligonucleotides bind to a specific portion of the nucleic acid target and are themselves bound onto a substrate in the microarray. The Mediator oligonucleotides bind to a different portion of the same nucleic acid target and allow binding of a gold nanoparticle probe to a portion complementary to a gold nanoparticle probe. Specific silver enhancement of the bound gold nanoparticle probes at the capture sites results in gold-silver aggregates that scatter light with high efficiency.

The BC-GP test is performed on the Verigene® System, a 'sample-to-result', fully automated, bench-top molecular diagnostics workstation. The Verigene System consists of two components: the Verigene Reader and the Verigene Processor SP. The BC-GP test utilizes single-use disposable test consumables and a self-contained Verigene Test Cartridge for each sample tested. For the BC-GP test, the Verigene System allows automated nucleic acid extraction from Gram-positive bacteria-containing blood culture specimens and target detection of bacteria-specific DNA.

The Reader is the Verigene System's user interface, which serves as the central control unit for all aspects of test processing and results generation. The Reader's graphical user interface guides the user through test processing and test results using a barcode scanner. The user inserts the Test Cartridge into the Verigene Processor SP, which executes the test procedure, automating the steps of (1) Sample Preparation – Cell lysis and magnetic bead-based bacterial DNA isolation from blood culture samples and (2) Verigene Hybridization Test – Detection and identification of bacterial-specific DNA in a microarray format by using gold nanoparticle probe-based technology.

After test processing is complete, to obtain the test results the user removes the Test Cartridge from the Processor SP, removes the reagent pack from the substrate holder, and inserts the substrate holder into the Reader for analysis. Light scatter from the capture spots is imaged by the Reader and intensities from the microarray spots are used to make decisions regarding the presence (Detected) or absence (Not Detected) of a bacterial nucleic acid sequence/analyte.

SUMMARY OF NONCLINICAL/BENCH STUDIES

a. *Limit of Detection Studies*:

The limit of detection (LoD) of the BC-GP assay was assessed and confirmed by using bacterial strains with established titers. By definition, the LoD is the lowest target concentration that can be detected approximately 95% of the time. For each strain, the dilution series began with the sample obtained at 'bottle positivity'. The dilution series were prepared by using a diluent matrix that was comprised of blood culture broth containing charcoal, human blood, and a common commensal skin bacterium at a minimum concentration of ~10^7 CFU/mL. Each dilution was tested in replicates of four. The putative LoD was the lowest concentration level where all the replicates for the analyte were

'Detected'. Once the putative LOD was established, 20 replicate samples were tested to confirm the LoD.

Thirteen (13) strains of *S. aureus* (SA) and *S. epidermidis* (SE), both methicillin sensitive and resistant, ranged from 1.9 x 10^5 to 5.7 x 10^5 CFU/mL for *S. aureus* and 2.0 x 10^6 to 7.5 x 10^6 CFU/mL for *S. epidermidis*.

Twelve (12) strains of *E. faecalis* and *E. faecium*, both vancomycin sensitive and resistant, ranged from 1.1 x 10^7 to 5.7 x 10^7 CFU/mL for *E. faecalis* and 2.4 x 10^6 to 3.7 x 10^7 CFU/mL for *E. faecium.*

Sixteen (16) strains of *Streptococcus* spp., *Staphylococcus* spp. (other than *S. aureus* and *S. epidermidis*), and *Listeria* spp. ranged from (CFU/mL):

Streptococcus spp.	1.8 x 10^6 to 1.2 x 10^8
S. agalactiae	1.2 x 10^7 to 2.2 x 10^7
S. pyogenes	9.5 x 10^6 to 6.3 x 10^7
S. anginosus group	1.4 x 10^7 to 1.2 x 10^8
S. lugdunensis	3.4 x 10^6 to 4.0 x 10^6
S. pneumoniae	1.8 x 10^6 to 9.9 x 10^6
Staphylococcus spp.	2.9 x 10^6 to 4.0 x 10^6
Listeria spp.	7.5 x 10^6 to 1.2 x 10^7

b. <u>*Inclusivity Studies*</u>:

In addition to the strains tested in the LoD studies, testing was performed on additional strains of each targeted organism/resistance marker. Bottles were spiked with each organism in blood culture bottles containing resins and whole blood. The inoculated blood culture bottles were placed in the automated blood culture system and incubated until positive by audible alarm (bottle ring). Positive specimens were plated to determine purity and quantitation (CFU/ml). Concentrations of tested positive blood culture specimens ranged from ~10^6 to ~10^9 CFU/mL at bottle ring. Testing was performed in duplicate with the BC-GP test. The following describes the organisms tested in the inclusivity studies:

<u>*SA and SE*</u> (both with and without the resistance marker *mecA*) - 98 methicillin-resistant *S. aureus* (MRSA) strains (including 65 representative NARSA strains), 18 methicillin-sensitive *Staphylococcus aureus* (SA), eight (8) borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) strains, six (6) methicillin-resistant *Staphylococcus epidermidis* (MRSE), and seven (7) methicillin-sensitive *Staphylococcus epidermidis* (SE).

<u>*E. faecium and E. faecalis*</u> (both with and without resistance markers *vanA* and *vanB)* - 79 strains including 14 vancomycin-resistant *Enterococcus faecalis* (11 of which were classified as *E. faecalis*, *vanA* and 3 were classified as *vanB*), 17 vancomycin-sensitive *Enterococcus faecalis*, 33 vancomycin-resistant *Enterococcus faecium* (26 of which were

classified as *vanA* and 7 were classified as *vanB*), and 15 vancomycin-sensitive *Enterococcus faecium*.

Streptococcus spp., *Staphylococcus spp*. and *Listeria spp*. - 233 bacterial strains, 184 of which belonged to the Streptococcus genus, including 34 *Streptococcus agalactiae*, 10 *Streptococcus anginosus*, 60 *Streptococcus pneumoniae*, and 16 *Streptococcus pyogenes*, 12 *Listeria sp*., and 45 Staphylococcus strains (of which four were *Staphylococcus lugdunensis*). One or more strains each of 13 different *Staphylococcu*s species (other than SA, SE, and *S. lugdenensis*), 18 different *Streptococcus* species (other than *S. pneumoniae*, *S. agalactiae*, and *S. pyogenes*, and 6 different *Listeria* species were tested in the studies. For the *Streptococcus anginosus* group, 10 total strains covering the three species *S. anginosus*, *S. intermedius*, and *S. constellatus* were tested. All strains tested gave positive results with the BC-GP assay, with the exception of *Listeria grayi* and BORSA strains. Two strains of *L. grayi* were tested and both were not detected. Subsequent in silico sequence analysis verified that this species will likely not be detected by the BC-GP assay. Therefore, the following limitation was placed in the package insert: "For *Listeria* species detection, *L. grayi* should not be detected by the test, as suggested by *in silico* analysis and confirmed with analytical testing." Tested BORSA strains were characterized as *mec*A negative and expectantly identified by the BC-GP test as *mec*A negative.

In order to support the genus level claims, a separate *in silico* analysis was performed by comparing the homology of the BC-GP assay probes against available GenBank entries for *Streptococcus spp, Staphylococcus sp*. and *Listeria sp*. The analysis, combined with wet-testing results, suggested that the BC-GP assay should detect 41 different species of *Staphylococcus*, 54 species of *Streptococcus,* and six species of *Listeria*.

c. Analytical Specificity (Exclusivity)

Analytical specificity was assessed using organisms phylogenetically related to panel organisms detected by the BC-GP test as well as those commonly present as contaminants in blood culture specimens. The exclusivity samples were divided into two distinct panels of organisms. The first panel consisted of 127 “non-BC-GP panel” organisms which would not have been expected to be detected by the BC-GP test.

- Fifty-two (79) gram-positive bacteria, including 15 *Micrococcus* strains, two each of seven *Enterococcus* strains and one of an eighth, a *Kytococcus,* a *Kocuria*, a *Peptostreptococcus*, two each of *Planococcus, Rothia, Leuconostoc, Granulicatella, Lactococcus,* and *Pediococcus* strains
- Forty (40) gram-negative bacteria
- One (1) acid-fast bacillus (attenuated *Mycobacterium tuberculosis)*
- One (1) mollicute (*Mycoplasma pneumonia*)
- Six (6) yeast strains

The second panel consisted of over 450 “BC-GP panel” organisms, which in total comprised the analytical inclusivity study samples. The size and characteristics of the second exclusivity sample set is as follows:

- *Staphylococcus* strains (178), including *MRSA* (98), *MSSA* (18), *BORSA* (8), *MRSE* (6), *MSSE* (7), *and 45 "non-SA/SE" Staphylococcus* spp
- *Enterococcus* strains (79), including *E. faecium VS* (15), *E. faecium VRE* (33), *E. faecalis VS* (17), and *E. faecalis VRE* (14)
- *Streptococcus* strains (184), including *S. agalactiae* (34), *S. pneumoniae* (60), *S. anginosus* (10), and *S. pyogenes* (16)
- *Listeria* strains (12)

All testing was performed in BACTEC plus bottles. Positive blood cultures were checked for purity and quantitated with final concentrations determined to be between 10^6 to 10^9 CFU/ml. Testing was performed in duplicate for each strain and depending on the organism, bottle preparation was performed in one of the following ways:

- Organisms were spiked into blood culture bottles containing resins and whole blood, and then incubated on the blood culture system until bottle ring, followed by testing with the BC-GP assay.
- High concentrations of organisms were spiked into blood culture bottles and immediately tested with the BC-GP assay.
- Previously-isolated genomic DNA was spiked into blood culture bottles for both *Yersinia enterocolitica* and *Cryptococcus neoformans* due to the risks associated with handling live cultures of these organisms.

The BC-GP test demonstrated acceptable specificity when challenged with these two panels, with no cross-reactivity observed for any of the organisms and/or strains tested with the exception of two strains of *Enterococcus avium,* which were identified as *E. faecium*, and *Lactococcus lactis*, which was identified as *Streptococcus* species. This information was presented in the package insert.

d. Interference

The potential inhibitory effects of substances that may be encountered in blood and associated with the blood culturing process were tested with the BC-GP test at the concentration levels listed in the table below.

Testing was performed using positive blood cultures containing representative strains of SA and SE (both with and without the resistance marker *mecA*), *E. faecalis* and *E. faecium* (with and without *vanA* and *vanB), Streptococcus* spp., *S. agalactiae, S. anginosus group, S. pneumoniae, S. pyogenes, Staphylococcus* spp., *S. lugdunensis* and *Listeria* spp. A negative blood culture sample was also tested with each interferent to assess the impact of the interferent on the processing of the BC-GP. For each organism and for the negative samples, the potential interferents (in table below) were added directly into individual positive blood cultures (or negative) and tested in triplicate. All the replicates for all the strains tested (and

the negative sample controls) gave the expected results indicating that these potential interferents do not interfere with the BC-GP test.

Blood Specimen Potential Interferents

Interferent	**Reference Level**	**Concentration Tested**
Hemoglobin	1-2 g/L	14 g/L
Triglyceride (Intralipid)	Normal <150 mg/dL High 300-500 mg/dL	3000 mg/dL
Conjugated	0.1-0.4 mg/dL	20 mg/dL
Unconjugated	0.1-0.8 mg/dL	20 mg/dL
γ-globulin	0.7-1.7 g/dL	6 g/dL
Sodium Polyanetholesulfonate (SPS)	0.02%-0.05% w/v (anticoagulant in blood culture bottles)	0.25% w/v

In a separate interference study, representative strains of SA and SE (both with and without the resistance marker *mecA*), *E. faecalis* and *E. faecium* (with and without *van*A and *van*B*)*, *Streptococcus* spp., *S. agalactiae, S. anginosus group, S. pneumoniae, S. pyogenes, Staphylococcus* spp., *S. lugdunensis* and *Listeria* spp were separately grown in BACTEC/F (Plus/Aerobic; Plus/Anaerobic; Standard/10 Aerobic; and PEDS Plus) and BacT/Alert (SA Aerobic, FA FAN Aerobic and PF Pediatric FAN) blood culture bottles. As expected, bottles containing targeted organisms gave positive results and negative blood culture bottles gave negative results, demonstrating that the bottle types tested did not have an impact on the performance of the BC-GP Test. Although this study demonstrated analytically that the additional bottle types do not interfere with the BC-GP Test, the majority of clinical study specimens were from BACTEC Plus Aerobic/F and BacT/ALERT FA FAN culture bottles. Therefore the Intended Use of the device will include only these two bottle types.

e. *Competitive Inhibition*

In order to assess competitive inhibition with the BC-GP test, representative bacterial strains targeted by the BC-GP assay were tested at their respective LoD levels when mixed with other targeted organisms at high concentration levels. The high concentration levels were obtained by incubating blood cultures for each strain at least eight hours beyond initial bottle positivity. The bottles were then spiked with the targeted organism at LoD. The mixed

specimens were tested in triplicate to assess inhibition. The organism combinations tested are listed in the tables below.

Organisms Tested for Competitive Inhibition Study (SA and SE)

Organisms	***Low Titer (CFU/mL)***	***High Titer (CFU/mL)***
S.aureus	4.6×10^6	
S. aureus (SA)	5.7×10^6	
S. epidermidis (MRSE)	7.5×10^6	
S. epidermidis (SE)	6.9×10^6	
S. pneumoniae		2.8×10^8
S. pyogenes		5.9×10^8
S. agalactiae		1.3×10^9
S. anginosus		7.9×10^8
E. faecium (*van*A)		1.1×10^9
E. faecium (*van*B)		8.3×10^8

Organisms Tested for Competitive Inhibition Study (*Enterococcus*)

Organisms	***Low Titer (CFU/mL)***	***High Titer (CFU/mL)***
E. faecalis	3.3×10^7	
E. faecalis, vanA	1.8×10^7	
E. faecalis, vanB	1.1×10^7	
E. faecium	7.4×10^6	
E. faecium, vanA	9.3×10^6	
E. faecium, vanB	6.8×10^6	
S. aureus / MRSA		8.9×10^7
S. epidermidis		2.6×10^8
S. agalactiae		1.3×10^8
S. anginosus		7.9×10^8
S. pneumoniae		2.8×10^8
L. monocytogenes		8.9×10^7

Organisms Tested for Competitive Inhibition Study (Other Targeted Bacteria)

Organisms	***Low Titer (CFU/mL)***	***High Titer (CFU/mL)***
S. anginosus	1.4×10^7	
S. agalactiae	1.2×10^7	
S. pneumoniae	1.8×10^8	
S. pyogenes	9.5×10^6	

S. lugdunensis	3.4×10^6	
L. monocytogenes	1.2×10^7	
S. aureus / MRSA		2.0×10^9
*S. epidermidis/*MRSE		5.5×10^8
S. aureus/MSSA		4.0×10^8
*S. epidermidis/*MSSE		7.4×10^8
E. faecalis (vanB)		1.3×10^8
E. faecium (vanA)		8.7×10^8
S. anginosus		1.2×10^9
S. agalactiae		8.6×10^8
S. pneumoniae		9.7×10^8
S. pyogenes		1.2×10^8
S. lugdenensis		2.0×10^9
L. monocytogenes		2.0×10^9

All replicates of the mixed samples gave expected results for both bacterial targets with the exception of one of three replicates of a high titer of *S. agalactiae* mixed with a low titer of *S. epidermidis.* For this one replicate, *S. epidermidis* was not detected. An additional 20 replicates of this organism mixture were tested, resulting in correct detection of both organisms in 20/20 replicates. The original result was likely related to the SE being at LoD levels. In conclusion, results of the competitive inhibition study demonstrated no evidence of competitive inhibition for organisms with concentrations near the LoD mixed with organisms at high concentrations.

f. *Assay Cutoff*

In order to determine the cut-off values for the GC-GP assay, a set of bacterial strains were tested to represent all the analytes detected by the BC-GP Test. Blood culture bottles were spiked with each individual organism and grown in automated blood culture instruments to "bottle ring." A minimum of four replicates for each positive blood culture specimen were tested with the BC-GP Test. The test decisions from each test were compiled to generate a data set that was used to initially determine BC-GP Assay Test cutoff values. A set of retrospective blood culture specimens were then tested with the BC-GP Test to verify the previously determined cut off values. Results were analyzed using logistic fit and ROC statistics. The results obtained from this the cutoff determination matched the results from culture-based biochemical results, thus verifying the final cutoff values.

g. *Carry-Over and Cross Contamination Studies*

A study was performed to assess the potential for carryover/cross-contamination with the BC-GP by alternately running ‘high positive’ samples followed by ‘true negative’ samples. All of the high positive samples yielded the expected ‘Detected’ results for the intended

bacteria and 'Not Detected' results for the other analytes. The true negative samples gave a 'Not Detected' call for all analytes. The studies confirmed that there was no evidence of carryover/cross-contamination from the high positive samples, or any other internal or external sources, in either of the steps of the BC-GP: Sample Extraction and Verigene Hybridization.

h. *Precision/Reproducibility:*

The precision and reproducibility of the BC-GP test was evaluated using a 40-member sample panel consisting of two concentrations of pure isolates of various strains of organisms grown in blood culture bottles a) until bottle positivity (Concentration 1 - approx. 10^7-10^8 CFU/mL) and b) bottle positivity plus an additional 8 hours of incubation on the blood culture instrument (Concentration 2 - approx. 10^8-10^9 CFU/mL). The sample panel was composed of a combination of 19 organisms and strains, including two strains of MRSA, and one strain each of MRSE, MSSA, MSSE, EFC, EFL, EFC/*vanA*, EFC/*vanB*, EFL/*vanA*, EFL/*vanB*, *S. agalactiae, S. anginosus, S. mitis, S. warneri, S. pneumoniae, S. pyogenes, S. lugdunensis* and *Listeria monocytogenes*. Two negative controls were also contained in the sample panel, one containing blood culture media only and one containing the organism *Corynebacterium urealyticum.*

Precision

Precision was established in-house at Nanosphere by testing the 40-member sample panel in duplicate twice daily by two operators for twelve (12) non-consecutive days, generating a total of forty-eight (48) replicates per sample. Precision was evaluated across multiple reagent lots, days, operators, runs, instruments and replicates. The percent agreement and two-sided 95%CI for the panel members combined was:

100% (576/576; 99.4%-100%) for *Staphylococcus aureus* and *S. epidermidis*
99.8% (575/576; 99.0% -100%) for *Enterococcus faecalis* and *E. faecium*
99.9% (767/768; 99.4%-100%) for *Streptococcus* spp., *S. agalactiae, S. anginosus group, S. pneumoniae, S. pyogenes, Staphylococcus* spp., *S. lugdunensis,* and *Listeria* spp.

Only two samples (E. faecalis/*van*A and *S. lugdenensis*, both at a BP concentration of ~10^8 CFU/mL) yielded <100% agreement (97.9%, [47/48; 88.9-99.9]). Overall, there were 23 initial no-call results observed, all but one of which repeat tested successfully; the one final no-call result accounted for the discordant *S. lugdenensis* panel member result.

Reproducibility

Reproducibility was determined at three external sites by testing the same 40-member sample panel in duplicate twice daily by two (2) operators for five (5) non-consecutive days,

which generated a total of sixty (60) replicates per sample. The percent agreement for the panel members for all sites combined was:

100% (720/720; 99.5-100) for *Staphylococcus aureus* and *S. epidermidis,*
100% (720/720; 99.5-100) for *Enterococcus faecalis* and *E. faecium,*
100% (960/960; 99.6% -100%) for *Streptococcus* spp., *S. agalactiae, S. anginosus group, S. pneumoniae, S. pyogenes, Staphylococcus* spp., *S. lugdunensis,* and *Listeria* spp.

Overall, there were 73 initial no-call results observed, all of which repeat tested successfully.

i. Fresh versus Frozen Study

The stability of specimens subjected to up to two freeze/thaw cycles was evaluated using blood culture specimens which were tested before and after freezing (at or below -70 ºC to allow paired comparisons. Representative strains of SA and SE (both with and without the resistance marker *mecA*), *E. faecalis* and *E. faecium* (with and without *vanA* and *vanB), Streptococcus* spp., *S. agalactiae, S. anginosus group, S. pneumoniae, S. pyogenes, Staphylococcus* spp., *S. lugdunensis* and *Listeria* spp. were tested. Organisms were grown in blood culture bottles and incubated on a continuous monitoring blood culture system. Cultured samples were tested at baseline at the following two time points: (i) bottle positivity and (ii) bottle positivity plus eight (8) hours. Four replicates of each samples were then tested after two freeze/thaw cycles. A comparison of matched pairs of specimens demonstrated 100% agreement between the fresh and frozen samples for each analyte tested for all conditions evaluated. In summary, the results of the fresh versus frozen study demonstrated that the use of the use of frozen specimens used in the clinical study was acceptable for testing with the BC-GP test.

BIOCOMPATIBILITY/MATERIALS

N/A

SHELF LIFE/STERILITY

The BC-GP Assay, Verigene Reader and the Verigene Processor SP have no sterility requirements.
The BC-GP Test will be tested to achieve a minimum of 12 weeks stability.

ANIMAL STUDIES

N/A

ELECTROMAGNETIC COMPATIBILITY AND ELECTRICAL SAFETY

The Verigene Reader and the Verigene Processor SP meet all applicable international standards for electromagnetic compatibility and electrical safety.

MAGNETIC RESONANCE (MR) COMPATIBILITY

N/A

MECHANICAL SAFETY

N/A

SOFTWARE

Version: Verigene Reader Software Version 1.8.2b1 and Verigene Processor SP Software Version 3.3b2		
Level of Concern: Moderate		
	Y	**N**
Software description:	X	
Device Hazard Analysis:	X	
Software Requirements Specifications:	X	
Architecture Design Chart:	X	
Design Specifications:	X	
Traceability Analysis/Matrix:	X	
Development:	X	
Verification & Validation Testing:	X	
Revision level history:	X	
Unresolved anomalies:	X	

The information provided in the submission is adequate for each of the items listed above. Additionally, documentation provided for the final verification for the Verigene Reader software version 1.8.2b1 and Verigene Processor SP software version 3.3b2 is adequate.

SUMMARY OF CLINICAL INFORMATION

A method comparison study (n=1767) was conducted at five external, geographically-diverse clinical study sites to evaluate the comparative performance of BC-GP to applicable conventional biochemical, culture, and bidirectional sequencing reference methods. The total evaluable specimens tested in the studies included; 1251 fresh, 175 frozen, and 216 contrived blood culture specimens. Eligible study subjects included individuals receiving routine care requiring blood culture testing. Blood culture specimens were collected from the patients and incubated on either the BACTEC or the BacT Alert continuous monitoring blood culture systems. Bottles that were flagged positive by the instrument were gram stained and then bottles confirmed to contain gram positive organisms were then tested with the BC-GP Test.

Additionally, simulated specimens were utilized for very rare organisms such as *S. lugdunensis*, *S. agalactiae*, *S. anginosus* group, *S. pyogenes*, and *Listeria* species. These specimens were prepared by spiking blood culture bottles containing whole blood with bacterial suspensions of bacterial isolates. Prepared blood culture bottles were then grown to positivity on the continuous monitoring

blood culture system, until flagged positive. Gram stains were performed to verify the presence of gram positive bacteria, and then testing was performed with the BC-GP Test.

BC-GP Test results were compared with results from traditional laboratory reference methods (i.e. culture followed by testing blood culture isolates with conventional biochemicals, Vitek2, and cefoxitin disc testing. Cefoxitin discs were used as the reference method for confirming *mec*A mediated resistance in *S. aureus* and *S. epidermidis*. Vancomycin resistance and the presence of vanA or vanB in *E. faecium* and *E. faecalis* was performed using vancomycin E-tests followed by bidirectional sequencing on resistant organisms.

The initial No-call rate in the clinical study was 4.7% (77/1642) , and the final No-call rate was 1.1% (18/1642). The total rate of initial No-Calls and Pre-analysis (pre-AE) Errors was 6.2% (101/1642). The final No-Call and Pre-AE rate was 1.2% (20/1642).

The total specimens excluded from the BC-GP clinical study dataset (n=125) are listed by site and reason for exclusion in the following table:

Site	***No. Excluded***	***Reason for Exclusion***								
		Training Sample	*Culture/RM results Unavailable, Unidentified, or No Growth*	*Sample not tested on BC-GP or tested after time limit*	*Inadequate Sample Mixing*	*Incorrect Sample Type or Patient already tested*	*QC or protocol error*	*Gram stain not gram positive*	*Sample used in Analytical Studies*	*Dual organism grown from single stock (reference culture)*
MCW	32		1	6	1	6	2		10	6
OSU	30	5	4	1		9	3			8
LIJ	15	4	4	2		1		3		1
MN	19	3	1	4		7	1		1	2
NSU	28	2	4			13				9
Frozen	***1***			1						
Total	***125***									

Test performance is stratified by the manner in which the blood culture media specimen was obtained for clinical testing; i.e.; prospectively-collected fresh or stored frozen prior to testing, designated as the *"Specimen Type"* in the following tables.

Summary of Clinical Test Performance versus Reference Method(s) – *Staphylococcus* and *Listeria*

Specimen Type		*n=*	*% Agreement (95% CI)*		*Reference Method*
			Positive	*Negative*	
Staphylococcus aureus (SA)					Culture and Conventional Biochemical and Vitek2
Prospective	Fresh	1251	99.1% 322/325 (97.3-99.8)	100% 926/926 (99.6-100)	
	Frozen	175	100% 10/10 (69.2-100)	100% 165/165 (97.8-100)	
	Total	1426	99.1% 332/335 (97.4-99.8)	100% 1091/1091 (99.7-100)	
Simulated		216	-	100% 216/216 (98.3-100)	
Staphylococcus epidermidis (SE)					
Prospective	Fresh	1251	93.0% 294/316 (89.6-95.6)	98.7% 923/935 (97.8-99.3)	
	Frozen	175	100% 2/2 (15.8-100)	100% 173/173 (97.9-100)	
	Total	1426	93.1% 296/318 (89.7-95.6)	98.9% 1096/1108 (98.1-99.4)	
Simulated		216	100% 2/2 (15.8-100)	100% 214/214 (98.3-100)	
mecA					Culture and Cefoxitin Disk Diffusion
Prospective	Fresh	1251	94.1% 366/389 (91.2-96.2)	97.8% 843/862 (96.6-98.7)	
	Frozen	175	100% 9/9 (66.4-100)	100% 166/166 (97.8-100)	
	Total	1426	94.2% 375/398 (91.5-96.3)	98.2% 1009/1028 (97.1-98.9)	
Simulated		216	-	100% 216/216 (98.3-100)	

Specimen Type		*n=*	*% Agreement (95% CI)*		*Reference Method*
			Positive	*Negative*	
Staphylococcus species					Culture and Conventional Biochemical and Vitek2
Prospective	Fresh	1251	97.9% 895/914 (96.8-98.7)	99.4% 335/337 (97.9-99.9)	
	Frozen	175	100% 30/30 (88.4-100)	99.3% 144/145 (96.2-99.9)	
	Total	1426	98.0% 925/944 (96.9-98.8)	99.4% 479/482 (98.2-99.9)	
Simulated		216	100% 25/25 (86.3-100)	100% 191/191 (98.1-100)	
Staphylococcus lugdunensis					
Prospective	Fresh	1251	87.5% 7/8 (47.4-99.7)	100% 1243/1243 (99.7-100)	
	Frozen	175	100% 12/12 (73.5-100)	100% 163/163 (97.8-100)	
	Total	1426	95.0% 19/20 (75.1-99.9)	100% 1406/1406 (99.7-99.9)	
Simulated		216	100% 20/20 (83.2-100)	99.5% 195/196 (97.2-99.9)	
Listeria species					
Prospective	Fresh	1251	100% 3/3 (29.2-100)	100% 1248/1248 (99.7-100)	
	Frozen	175	-	100% 175/175 (97.9-100)	
	Total	1426	100% 3/3 (29.2-100)	100% 1423/1423 (99.7-100)	
Simulated		216	100% 34/34 (89.7-100)	100% 182/182 (98.0-100)	

Summary of Clinical Test Performance versus Reference Method(s) – *Enterococcus*

Specimen Type		n=	% Agreement (95% CI) Positive	% Agreement (95% CI) Negative	Reference Method
Enterococcus faecalis (EFL)					Culture and Conventional Biochemical and Vitek2
Prospective	Fresh	1251	96.0% 72/75 (88.8-99.2)	99.9% 1175/1176 (99.5-99.9)	
	Frozen	175	100% 21/21 (83.9-100)	100% 154/154 (97.6-100)	
	Total	1426	96.9% 93/96 (91.1-99.4)	99.9% 1329/1330 (99.6-99.9)	
Simulated		216	92.3% 12/13 (64.0-99.8)	100% 203/203 (98.2-100)	
Enterococcus faecium (EFC)					
Prospective	Fresh	1251	94.4% 34/36 (81.3-99.3)	100% 1215/1215 (99.7-100)	
	Frozen	175	100% 32/32 (89.1-100)	100% 143/143 (97.5-100)	
	Total	1426	97.1% 66/68 (89.8-99.6)	100% 1358/1358 (99.7-100)	
Simulated		216	100% 46/46 (92.3-100)	99.4% 169/170 (96.8-99.9)	

Specimen Type		n=	% Agreement (95% CI) Positive	% Agreement (95% CI) Negative	Reference Method
vanA					Culture and Bidirectional Sequencing
Prospective	Fresh	1251	91.9% 34/37 (78.1-98.3)	100% 1214/1214 (99.7-100)	
	Frozen	175	96.9% 31/32 (83.8-99.9)	97.9% 140/143 (94.0-99.6)	
	Total	1426	94.2% 65/69 (85.8-98.4)	99.8% 1354/1357 (99.4-99.9)	
Simulated		216	100% 15/15 (78.2-100)	100% 201/201 (98.2-100)	
vanB					
Prospective	Fresh	1251	0/0	100% 1251/1251 (99.7-100)	
	Frozen	175	100% 3/3 (29.2-100)	100% 172/172 (97.9-100)	
	Total	1426	100% 3/3 (29.2-100)	100% 1423/1423 (99.7-100)	
Simulated		216	97.3% 36/37 (85.8-99.9)	100% 179/179 (98.0-100)	

Summary of Clinical Test Performance versus Reference Method(s) – *Streptococcus*

Specimen Type		n=	% Agreement (95% CI) Positive	% Agreement (95% CI) Negative	Reference Method
Streptococcus spp.					Culture and Conventional Biochemical and Vitek2
Prospective	Fresh	1251	91.7% 143/156 (86.2-95.5)	99.5% 1090/1095 (98.9-99.9)	
	Frozen	175	98.4% 63/64 (91.6-99.9)	100% 111/111 (96.7-100)	
	Total	1426	93.6% 206/220 (89.6-96.5)	99.6% 1201/1206 (99.0-99.9)	
Simulated		216	100% 92/92 (96.1-100)	99.2% 123/124 (95.6-99.9)	
S. agalactiae					
Prospective	Fresh	1251	97.5% 39/40 (86.8-99.9	100% 1211/1211 (99.7-100)	
	Frozen	175	100% 31/31 (88.8-100)	100% 144/144 (97.5-100)	
	Total	1426	98.6% 70/71 (92.4-99.9)	100% 1355/1355 (99.7-100)	
Simulated		216	100% 6/6 (54.1-100)	100% 210/210 (98.3-100)	

Specimen Type		n=	% Agreement (95% CI) Positive	% Agreement (95% CI) Negative	Reference Method
S. pneumoniae					Culture and Conventional Biochemical and Vitek2
Prospective	Fresh	1251	100% 25/25 (86.3-100)	99.6% 1221/1226 (99.1-99.9)	
	Frozen	175	100% 13/13 (75.3-100)	100% 162/162 (97.8-100)	
	Total	1426	100% 38/38 (90.8-100)	99.6% 1383/1388 (99.2-99.9)	
Simulated		216	100% 8/8 (63.1-100)	100% 208/208 (98.2-100)	
S. pyogenes					
Prospective	Fresh	1251	100% 10/10 (69.2-100)	100% 1241/1241 (99.7-100)	
	Frozen	175	92.9% 13/14 (66.1-99.8)	100% 161/161 (97.7-100)	
	Total	1426	95.8% 23/24 (78.9-99.9)	100% 1402/1402 (99.7-100)	
Simulated		216	98.2% 53/54 (90.1-99.9)	100% 162/162 (97.8-100)	

S. anginosus group					
Prospective	Fresh	1251	100% 9/9 (66.4-100)	99.8% 1239/1242 (99.3-99.9)	
	Frozen	175	100% 3/3 (29.2-100)	100% 172/172 (97.9-100)	
	Total	1426	100% 12/12 (73.5-100)	99.8% 1411/1414 (99.4-99.9)	
Simulated		216	100% 23/23 (85.2-100)	99.5% 192/193 (97.2-99.9)	

The following tables provide the clinical performance of BC-GP for *mecA* detection with *S. aureus* and *S. epidermidis* (as determined by the reference method) and *vanA/vanB* detection with *E. faecalis* and *E. faecium* (as determined by the reference method). In the study protocol, *Staphylococcus* species (other than *S. aureus* and *S. epidermidis*) were not tested for methicillin resistance by cefoxitin disk analysis. However, 12 *Staphylococcus* species (as determined by the reference method) were positive for *S. epidermidis* and *mecA* by the BC-GP test and have been counted as false positives for *mecA*. These 12 specimens are accounted for in the total negative agreement calculation for the *mec*A target.

Detection of *mecA* with *S. aureus* and *S. epidermidis*, as Determined by Reference Method

Organism	*Resistance Marker*	*n=*	*% Agreement (95% CI) with Reference Method*	
			Positive [Cefoxitin (+)]	*Negative* [Cefoxitin (-) or no cefoxitin testing, as organism not isolated on culture]
S. aureus	*mecA* 94.2% 375/398 (91.5-96.3)	335	97.5% 157/161 (93.8-99.3)	98.8% 172/174 (95.9-99.9)
S. epidermidis (including 12 specimens identified as SE by BC-GP only)		330	92.0% 219/238 (87.8-95.1)	81.5% 75/92 (72.1-88.9)

Detection of *vanA/vanB* with *E. faecalis* and *E. faecium*, as Determined by Reference Method

Organism	*Resistance Marker*	*n=*	*% Agreement (95% CI) with Reference Method*	
			Positive (Bi-directional Sequencing (+))	*Negative* (Bi-directional Sequencing (-))
E. faecalis	*vanA* 95.2% 80/84 (88.3-98.7)	109	85.7% 12/14 (57.2-98.2)	100% 95/95 (96.2-100)
E. faecium		114	97.2% 69/71 (90.2-99.7)	93.0% 40/43 (80.9-98.5)
E. faecalis	*vanB* 97.5% 39/40 (86.8-99.9)	109	100% 7/7 (59.0-100)	100% 102/102 (96.5-100)
E. faecium		114	97.0% 32/33 (84.2-99.9)	100% 81/81 (95.6-100)

The following table contains additional genus/group-level specific BC-GP clinical and analytical performance data stratified by individual species within each genus (i.e.; *Staphylococcus* sp. (other than *SA, SE, S. lugdunensis), Streptococcus* sp. (other than *S. agalactiae, S. pyogenes, S. pneumoniae*) *Listeria* sp. and *Streptococcus anginosus* group.

Summary of Genus/Group-level Test Performance versus Reference Method(s) – Stratified by Species

Staphylococcus Genus		
Organism	*% (+) Agreement (95% CI)*	
	Clinical	*Analytical*
Combined *Staphylococcus* spp.	98.0% 950/969 (97.0-98.8)	100% 37/37 (90.5-100)
Staphylococcus arlettae	100% 1/1 (2.5-100)	NT*
Staphylococcus auricularis	100% 16/16 (79.4-100)	100% 2/2 (15.8-100)
Staphylococcus capitis	96.4% 54/56 (87.7-99.6)	100% 2/2 (15.8-100)
Staphylococcus caprae	100% 5/5 (47.8-100)	100% 2/2 (15.8-100)
Staphylococcus cohnii	NT	100% 2/2 (15.8-100)
Staphylococcus chromogenes	100% 2/2 (15.8-100)	NT
Staphylococcus haemolyticus	91.4% 32/35 (76.9-98.2)	100% 2/2 (15.8-100)
Staphylococcus hominis	98.5% 133/135 (94.8-99.8)	100% 3/3 (29.2-100)
Staphylococcus intermedius	100% 3/3 (29.2-100)	100% 1/1 (2.5-100)
Staphylococcus muscae	NT	100% 1/1 (2.5-100)
Staphylococcus pasteuri	NT	100% 2/2 (15.8-100)
Staphylococcus saccharolyticus	NT	100% 4/4 (39.8-100)
Staphylococcus saprophyticus	100% 4/4 (39.8-100)	100% 2/2 (15.8-100)
Staphylococcus schleiferi	100% 1/1 (2.5-100)	100% 2/2 (15.8-100)
Staphylococcus sciuri	NT	100% 2/2 (15.8-100)
Staphylococcus simulans	100% 5/5 (47.8-100)	100% 2/2 (15.8-100)

Streptococcus Genus		
Organism	*% (+) Agreement (95% CI)*	
	Clinical	*Analytical*
Combined *Streptococcus* spp.	95.5% 298/312 (92.6-97.5)	100% 183/183 (98.0-100)
Streptococcus bovis	100% 1/1 (2.5-100)	100% 4/4 (39.8-100)
Streptococcus dysgalactiae	100% 5/5 (47.8-100)	100% 2/2 (15.8-100)
Streptococcus dysgalactiae equisimilis	100% 2/2 (15.8-100)	100% 3/3 (29.2-100)
Streptococcus equi	NT	100% 2/2 (15.8-100)
Streptococcus equinus	NT	100% 2/2 (15.8-100)
Streptococcus gallolyricus	NT	100% 3/3 (29.2-100)
Streptococcus gallolyticus pasteurianus	NT	100% 1/1 (2.5-100)
Streptococcus gordonii	100% 1/1 (2.5-100)	100% 2/2 (15.8-100)
Streptococcus infantarius infantarius	NT	100% 1/1 (2.5-100)
Streptococcus infantarius subsp. coli	NT	100% 1/1 (2.5-100)
Streptococcus infantis	NT	100% 2/2 (15.8-100)
S. mitis, S. oralis, or *S. mitis/oralis*	95.2% 20/22 (70.8-98.9)	100% 19/19 (82.4-100)
Streptococcus mutans	50% 1/2 (1.3-98.7)	100% 2/2 (15.8-100)
Streptococcus parasanguinis	100% 4/4 (39.8-100)	100% 4/4 (39.8-100)
Streptococcus peroris	NT	100% 1/1 (2.5-100)
Streptococcus salivarius	50% 4/8 (15.7-84.3)	100% 5/5 (47.8-100)

Streptococcus anginosus Group		
Organism	*% (+) Agreement (95% CI)*	
	Clinical	*Analytical*
Streptococcus. anginosus group	100% 35/35 (90.0-100)	100% 10/10 (69.2-100)
Streptococcus anginosus	100% 7/7 (59.0-100)	100% 5/5 (47.8-100)
Streptococcus constellatus	100% 9/9 (66.4-100)	100% 3/3 (29.2-100)
Streptococcus intermedius	100% 8/8 (63.1-100)	100% 2/2 (15.8-100)
Streptococcus. anginosus group (not further Identified)	100% 11/11 (71.5-100)	NT

Listeria Genus		
Combined *Listeria* spp.	100% 37/37 (90.5-100)	100% 12/12 (73.5-100)
Listeria grayi	NT	0% 0/2 (15.8-100)
Listeria innocua	100% 8/8 (63.1-100)	100% 1/1 (2.5-100)
Listeria ivanovii	100% 2/2 (15.8-100)	100% 1/1 (2.5-100)
Listeria monocytogenes	100% 24/24 (85.8-100)	100% 6/6 (54.1-100)
Listeria seeligeri	100% 1/1 (2.5-100)	100% 1/1 (2.5-100)
Listeria welshimeri	100% 1/1 (2.5-100)	100% 1/1 (2.5-100)
Listeria spp. unidentified	100% 1/1 (2.5-100)	NT

Staphylococcus warneri	90.0% 18/20 (68.3-98.8)	100% 2/2 (15.8-100)	*Streptococcus sanguinis*	75% 3/4 (19.4-99.4)	100% 3/3 (29.2-100)
Staphylococcus xylosus	100% 2/2 (15.8-100)	100% 2/2 (15.8-100)	*Streptococcus thoraltensis*	NT	100% 1/1 (2.5-100)
Staphylococcus species (CONS)	100% 29/29 (88.1-100)	NT	*Streptococcus viridans*	83.3% 20/24 (62.6-95.3)	NT
			Streptococcus uberis	100% 1/1 (2.5-100)	NT

*NT = "Not Tested

Mixed Culture Results: In total, there were 98 mixed specimens that were detected either by BC-GP, the reference culture methods or both. The two tables below list the distinct mixed specimen combinations detected by BC-GP in the clinical study and the additional distinct mixed specimen combinations detected by the reference/comparator methods, but not detected by BC-GP. In the clinical study, there were a total of six discrepant mixed specimens for which the BC-GP test detected organisms that were not detected by the reference culture (false positives). Additionally, there were 25 discrepant mixed specimens for which the reference culture detected organisms that were not detected by the BC-GP Test (false negatives). The majority (22/25) of the organisms not detected by the BC-GP Test in the false negative mixed specimens were organisms that can sometimes be considered as potential blood culture contaminants (*Staphylococcus sp*. (not SA), *S. viridans*, and *S. salivarius*.) For any mixed blood culture, there may be different rates of growth for each organism present, and therefore the organism present in lower concentrations (below "bottle ring") may not be detected. The following limitation was placed in the package insert regarding detection of organism by the BC-GP Test in mixed blood cultures:

- In mixed cultures containing Gram positive bacteria and other organisms, BC-GP may not identify all the detectable organisms in the specimen, depending upon the concentration of each target present.

Clinical Mixed Specimen Combinations Detected by BC-GP, but not by Reference Methods

Analyte 1	*Analyte 2*	*Observed Frequency*	*No. of Specimens with Discrepant Co-analytes*	*Discrepant Analyte(s)*[1]
E. faecalis and *vanA*	*E. faecium*	1	0	
E. faecalis	*Staphylococcus species*	3	0	
E. faecalis	*Streptococcus species*	1	1	*Streptococcus species*
E. faecium and *vanA*	*Staphylococcus species*	1	0	
Listeria	*S. anginosus group*	1	1	*S. anginosus group*
S. lugdunensis	*Listeria species*	1	0	
Staphylococcus species	*E. faecalis*	2	1	*Staphylococcus species*
Staphylococcus species and *S. epidermidis* and *mecA+*	*E. faecalis*	1	0	
Staphylococcus species and *S. aureus*	*Staphylococcus species* and *S. epidermidis* and *mecA+*	1	0	
Streptococcus species and *S. agalactiae*	*Staphylococcus species* and *S. aureus*	1	0	
Streptococcus species and *S. pneumoniae*	*Streptococcus species* and *S. anginosus group*	1	1	*S. anginosus group*
Streptococcus species and *S. pyogenes*	*E. faecalis*	1	1	*E. faecalis*
Streptococcus species	*Staphylococcus species*	2	1	*Staphylococcus species*
	TOTAL	17	6	

[1] Defined as an analyte that was detected by BC-GP, but not detected by the reference methods.

Additional Clinical Mixed Specimens Detected by Reference Methods, but not Detected by BC-GP

Analyte 1	*Analyte 2*	*Analyte 3*	*Observed Frequency*	*No. Specimens w/ Discrepant Co-analytes*	*Discrepant Analyte(s)*[1]
E. faecalis (vanS)	*S. haemolyticus*		2	2	*S. haemolyticus*
E. faecalis (vanS)	*S. aureus (methR)*		1	1	*S. aureus (methR)*
E. faecalis (vanS)	*GNR*		2	0	-
E. faecalis (vanS)	*S. epidermidis (methR)*		1	1	*S. epidermidis (methR)*
E. faecalis (vanS)	*yeast*	*S. warneri*	1	1	*S. warneri*
E. faecalis (vanS)	*Bacillus species (not anthracis)*		1	0	-
E. faecalis (vanR)	*S. epidermidis (methR)*		2	2	*S. epidermidis (methR)*
E. faecalis (vanR)	*S. agalactiae*		1	1	*(vanR)*
E. faecium (vanS)	*S. aureus (methR)*		1	1	*S. aureus (methR)*
E. faecium	*GNR*		1	0	-
E. faecium (vanR)	*S. warneri*		1	1	*S. warneri*
E. faecium (vanR)	*S. capitis*		1	1	*S. capitis*
E. faecium (vanR)	*S. epidermidis (methR)*		1	1	*S. epidermidis (methR)*
E. faecium (vanR)	*GNR*		1	0	-
E. faecium (vanR)	*Corynebacterium*		1	0	-
M. lylae	*M. luteus*		1	0	-
Streptococcus viridans group	*GNR*		1	1	*Strep viridans group*
K. rosea	*K. kristinae*		1	0	-
E. casseliflavus	*GNR*		1	0	-
Leuconostoc mesenteroides	*S. salivarius*		1	1	*S. salivarius*
S. hominis	*S. warneri*		1	0	-
S. capitis	*S. epidermidis (methS)*		2	1	*S. epidermidis (methS)*

Analyte 1	*Analyte 2*	*Analyte 3*	*Observed Frequency*	*No. Specimens w/ Discrepant Co-analytes*	*Discrepant Analyte(s)*[1]
S. haemolyticus	*K. kristinae*		1	0	-
S. hominis	*S. salivarius*		1	1	*Streptococcus spp.*
S. simulans	*S. hominis*		1	0	-
S. epidermidis (methR)	*S. hominis*	*S. auricularis*	1	1	*S. epidermidis (methR)*
S. simulans	*S. warneri*		1	0	-
S. epidermidis (methS)	*S. hominis*		3	2	*S. epidermidis (methS)*
S. hominis	*Staphylococcus species (CoNS)*		2	0	-
S. hominis	*S. schleiferi*		1	0	-
S. haemolyticus	*S. capitis*		1	0	-
S. haemolyticus	*S. hominis*		1	0	-
S. auricularis	*GNR*		1	0	-
S. hominis	*S. epidermidis (methR)*		6	2	*S. epidermidis (methR)*
S. hominis	*S. capitis*		2	0	
S. viridans group	*S. hominis*		1	1	*Streptococcus species*
S. epidermidis (methS)	*S. warneri*		1	0	
S. epidermidis	*Staphylococcus species (CoNS)*		1	0	
Corynebacterium	*S. epidermidis (methR)*		1	0	
S. capitis	*S. epidermidis (methR)*		4	0	
S. epidermidis (methR)	*S. hominis*		5	0	
S. hominis	*S. capitis*		1	0	
S. lugdunensis	*S. warneri*		1	1	*S. lugdunensis*
Staphylococcus species (CoNS)	*S. epidermidis (methS)*		1	0	
S. aureus (methR)	*S. hominis*		2	0	
S. hominis	*S. aureus (methS)*		1	0	
Staphylococcus spp (CoNS)	*S.epidermidis (methR)*		1	0	
S. capitis	*Globicatella sp.*		1	0	
S. salivarius	*R. mucilaginosa*		1	0	
Viridans Streptococcus	*S. mitis*		1	0	
S. agalactiae	*Proteus species*		1	0	
S. epidermidis (methR)	*S. agalactiae*		1	1	*S. epidermidis (methR)*
S. constellatus	*GNR*		1	0	
S. epidermidis (methR)	*S. pyogenes*		1	1	*S. epidermidis (methR)*
S. salivarius	*Viridans strep*	*Neisseria spp.*	1	0	
S. salivarius	*K. kristinae*		1	0	
Neisseria spp.	*S. mitis*	*S. sanguinis*	1	0	
S. mitis	*Granulicatella*		1	0	
L. pseudomesenteroides	*S. mitis*		1	0	
S. parasanguinis	*R. muciliginosa*		1	0	
		TOTAL	*81*	*25*	

[1] Defined as an analyte that was detected by the reference methods, but not detected by BC-GP.

LABELING

Labeling has been provided which is adequate and satisfies 21 CFR Parts 801 and 809 as well as the special control requirements.

RISKS TO HEALTH

FDA has identified the following risks to health associated with use of the Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures: false positive results, false negative results, and errors in interpretation. Failure of the device to detect and identify a targeted microorganism when such microorganism is present in the specimen (false negative result) may lead to a delay in finding the true cause of the bloodstream infection/bacteremia and to inappropriate antibiotic use. An incorrect positive test result (false positive result) also may lead to unnecessary or ineffective antibiotic therapy and delay in determining the true cause of the patient's illness. Failure of the device to detect a targeted gene associated with resistance when such gene is present in the detected microorganism (e.g., false negative results for *mec*A, *van*A, *van*B) may lead to treatment with ineffective antibiotics and lapses in infection control measures. An incorrect positive result for the presence of a targeted gene associated with resistance when such gene is present in the detected microorganism (false positive result) may also lead to inappropriate antibiotic therapy (frequently overly broad) to cover resistant microorganisms that are not present. The more potent antibiotics may have more side effects (e.g., renal toxicity, etc), and may lead to unnecessary and often costly implementation of infection control measures. Failure to correctly interpret test results in the context of other clinical and laboratory findings may lead to inappropriate or delayed treatment. For example, positive assay results do not rule out viral or other bacterial co-infections.

All risks to health have been identified for the subject device.
Risks to health have been addressed for this device in the special controls guideline document entitled "Class II Special Controls Guideline: Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures."

Identified Risks and Required Mitigations

Identified Risks	Required Mitigations
False negative result	The FDA document entitled "Class II Special Controls Guideline: Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures," which addresses this risk through: Device Description Containing the Information Specified in the Special Control Guideline, Performance Characteristics, and Labeling
False positive result	The FDA document entitled "Class II Special Controls Guideline: Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures," which addresses this risk through: Device Description Containing the Information Specified in the Special Control Guideline, Performance Characteristics, and

Identified Risks	Required Mitigations
	Labeling
Errors in Interpretation	The FDA document entitled "Class II Special Controls Guideline: Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures," which addresses this risk through: Device Description Containing the Information Specified in the Special Control Guideline, Performance Characteristics, and Labeling

BENEFITS RISKS ANALYSIS

We considered the following factors in our analysis of benefit: the accuracy of the device, availability of alternative treatments or diagnostics, the magnitude of the benefits, and the probability that patients would experience more than one benefit.

We considered the following factors in our analysis of risk; the probability and the consequences of false-positive or false-negative test results; the quality of the study design and robustness of the data; the risk mitigation by use of product labeling; and an analysis of available postmarket data for other similar multiplex assays.

The direct benefit of the device, established through pre-clinical and clinical studies, rests on accurate detection of gram-positive bacteria, including the test's specified drug resistance markers, in blood cultures. Analytical performance characteristics (limit of detection, inclusivity, exclusivity, interference, competitive inhibition, carry-over, precision/reproducibility and fresh/frozen matrix studies) were adequately validated for each of the BC-GP assay's analytes. The clinical study with over 1600 evaluable samples showed high concordance between results from the BC-GP assay and results from conventional methods (culture and identification) for the analytes.

Rapid availability of results from the BC-GP assay, compared to conventional methods for the BC-GP assay's analytes, presents a plausible additional benefit arising from earlier treatment prompted by the detection analytes. Ordinarily, the most readily available information to the physician (within ~12-24 hours) is the Gram stain performed after blood culture bottle positivity. The antibiotic may be changed based on the Gram stain results (to cover for either Gram-positive or Gram-negative organisms or both). Definitive organism targeted therapy is delayed an additional 1-2 days until the process for final identification of the organism and antimicrobial susceptibility is completed by traditional culture methods. The BC-GP test allows for simultaneous identification of these twelve types of bacteria and their resistance genes within about 2 1/2 hours after bacterial growth is first detected in the sample and after a Gram stain is performed and shows Gram-positive organisms.

The risks for this device are associated with inaccurate test results, which may lead to error in diagnosis and treatment. The probability of such events is small (from the analytical and clinical data presented), as has been observed for already similar marketed devices. The small risk for the BC-GP assay is mitigated through labeling specifying that the intended use of the BC-GP assay is “for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial bloodstream infections; Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility

testing, identification of organisms not detected by **BC-GP**, differentiation of mixed growth, association of antimicrobial resistance marker genes to a specific organism, or for epidemiological typing." Additionally, a limitation has been provided in the product labeling stating that a trained health care professional should interpret assay results together with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.

The benefits of the BC-GP assay outweigh the risks. Analytical and clinical performance characteristics are comparable to those from already marketed devices. Use of this device would allow faster identification of causative pathogens and their resistance markers in cases of bacteremia, which may lead to earlier and more definitive targeted therapy for patients with bloodstream infections/bacteremia likely resulting in improved clinical outcomes. Studies have shown that delayed administration of antibiotics is associated with a 7.6% decrease in survival rate for each hour that therapy is delayed.[1] The identified risks posed by the device have a low probability and are adequately mitigated.

1. Kumar A., Roberts D., Wood KE. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med.* 2006;34(6): 1589-1596.

SPECIAL CONTROLS:

In combination with the general controls of the FD&C Act, the Verigene® Gram Positive Blood Culture Nucleic Acid Test is subject to the following special controls:

The special controls for the BC-GP Assay are contained in the guideline document entitled "Class II Special Controls Guideline: Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures."

CONCLUSION

The De Novo petition for the Verigene® Gram Positive Blood Culture BC-GP Nucleic Acid Test is granted and the device is classified under the following:

Product Code: PAM, Gram-positive bacteria and their resistance markers
Device Type: Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures
Class: II
Regulation: 21 CFR 866.3365