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Verigene® Gram-Positive Blood Culture Nucleic Acid Test (BC-GP)

REF **20-005-018 (Test Kit) ● 20-012-018 (Utility Kit)**

IVD

KEY-CODE **NAN098**

INTENDED 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 blood culture bottles identified as positive by a continuous monitoring blood culture system and which contain gram-positive bacteria.

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

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

BACKGROUND INFORMATION

Bloodstream Infection (BSI) occurs when a pathogenic microorganism, such as a gram-positive bacterium, enters the bloodstream. **BC-GP** is a multiplexed, automated nucleic acid test for the identification of the genus, species, and genetic antimicrobial resistance determinants for a broad panel of the most common gram-positive blood culture bacteria. While conventional microbiological methods may require 2-4 days to produce bacterial identification and resistance results, **BC-GP** provides results within 2.5 hours of blood culture positivity. The Verigene System's unique instrumentation allows for random access test processing, enabling on-demand testing directly from positive blood culture bottles without the need for batched testing. A brief description of the organisms detected by **BC-GP** ("**BC-GP** panel members") and their clinical relevance follows.

Staphylococcus spp. is a genus of gram-positive bacteria that includes at least forty species. Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. However, certain species cause a wide variety of infections in humans and animals through either toxin production or invasion.

Staphylococcus aureus (SA) is a species of gram-positive bacteria frequently responsible for hospital-acquired infections, and increasingly, for community-acquired infections. SA infections, which can cause cellulitis,

endocarditis, osteomyelitis, and bacteremia, are often complicated by antimicrobial resistance.[1] Methicillin-resistant SA (MRSA), conferred by the organism's acquisition of the *mecA* gene, has proven to be especially burdensome for medical facilities.[1] Up to 60% of infections occurring in intensive care units can be attributed to MRSA.[2] Difficulty in differentiation between resistant and susceptible strains in a time-efficient manner has led to antimicrobial use that is often ineffectual, wasteful, or bears risk of proliferating resistant strains. Despite implementation of surveillance programs to reduce MRSA infections, the mortality rates and costs associated with MRSA bacteremia represent a significant healthcare burden.[2]

Staphylococcus epidermidis (SE) is a commensal bacterium found on human skin, but has also been increasingly associated with infection, especially among patients with compromised immune systems. Like SA, SE can acquire the *mecA* gene, leading to methicillin-resistant SE (MRSE) strains.[1,3] Since they inhabit the skin flora on healthy humans, SE and MRSE are a frequent cause of contamination in blood culture bottles. Patients with contaminated blood culture bottles are often presumptively treated for bloodstream infections for several days until the organism can be identified as a contaminant using conventional biochemical methods. In conjunction with other clinical findings, rapid identification of SE in contaminated blood cultures could prevent unnecessary treatment, which is responsible for significant increases in patient care costs and overuse of antimicrobials.[4]

Staphylococcus lugdunensis is native to human skin and can cause contamination in blood culture bottles. However, *S. lugdunensis* may also cause acute infections, including BSI's, which are clinically similar to infections caused by *S. aureus*.[5] Patients with long-term central venous catheters are at particular risk for these types of infections. *S. lugdunensis* can also cause highly destructive cases of endocarditis, which may require surgical treatment and are associated with a 23% mortality rate.[6]

Enterococci comprise a genus of gram-positive bacteria that are found naturally in the flora of the human digestive system, but can be opportunistically pathogenic.[7,8] Pathogenic enterococci colonies can be responsible for a wide range of dangerous medical conditions, including endocarditis, meningitis, and bacteremia.[7] The vast majority of enterococci infections are caused by just two species: *Enterococcus faecalis* (EFL) and *Enterococcus faecium* (EFC).[7] High-level vancomycin resistance, conferred by the acquisition of highly transmissible *vanA* and *vanB* genes, has made infection control increasingly difficult in the hospital setting.[7] Difficulty in differentiation between resistant and susceptible strains has led to antimicrobial use that is often ineffectual, wasteful, or bears risk of proliferating resistant strains. Rapid identification of both organism and susceptibility is essential to implementing efficient and appropriate therapy.[9]

Vancomycin-resistant enterococci (VRE) are bacterial strains of the genus *Enterococcus* that are resistant to the antibiotic vancomycin. To become VRE, enterococci typically acquire plasmids or transposons that encode genes that confer vancomycin resistance. High-level vancomycin-resistant *E. faecalis* and *E. faecium* clinical isolates were first documented in Europe in the late 1980s.[10,11] Since then, VRE have been associated with outbreaks of hospital-acquired (nosocomial) infections around the world. In the United States, vancomycin-resistant *E. faecium* was associated with 4% of healthcare-associated infections reported to the Centers for Disease Control and Prevention (CDC) National Healthcare Safety Network from January 2006 to October 2007.[12] VRE can be carried by asymptomatic people who have come into contact with the bacteria. The most likely place where such contact can occur is in a hospital.

Streptococcus spp. is a genus of gram-positive bacteria that can cause a wide variety of medical conditions. Streptococci can be responsible for diseases like bacterial pneumonia, meningitis, necrotizing fasciitis, erysipelas, and endocarditis.

Streptococci are classified into alpha-hemolytic and beta-hemolytic groups by their ability to lyse red blood cells.[13] Alpha-hemolytic streptococci form green halos of partially lysed blood cells in culture, and include *Streptococcus pneumoniae* and *Streptococcus anginosus* group spp..[13] *S. pneumoniae* is the most common *Streptococcus* isolated in culture.[14] The *S. anginosus* group includes *Streptococcus constellatus*, *Streptococcus intermedius,* and *Streptococcus anginosus*. Although *S. anginosus* group bacteria are less

commonly isolated in culture, infection is associated with high rates of mortality in the clinical setting.[15] Beta-hemolytic streptococci form clear halos of fully lysed blood cells in culture, and are categorized into several groups. The two most clinically-relevant species are *S. pyogenes* and *S. agalactiae*, also known as Group A *Streptococcus* (GAS) and Group B *Streptococcus* (GBS), respectively.[13] GAS is the cause of over 120,000 infections annually, including life-threatening conditions such as toxic shock syndrome and "flesh eating" necrotizing fasciitis.[16] GBS has become a major concern during pregnancy, when infection is commonly passed to the newborn, causing neonatal sepsis. The frequency of non-pregnancy GBS infections has also increased significantly in recent years.[17]

Listeria spp. is a virulent foodborne pathogen. It is the leading cause of foodborne pathogen-related death. The majority of deaths are individuals with compromised immune systems: pregnant women, newborns, the elderly, and the immunosupressed. The genus *Listeria* consists of gram-positive rod-shaped bacteria. Pathogenic *L. monocytogenes* has been known to cause severe infections and food poisoning, most commonly in immunocompromised hosts such as the elderly and newborns.[18]

PRINCIPLES AND PROCEDURES OF BC-GP AND THE VERIGENE SYSTEM

Verigene System

BC-GP is performed using the Verigene System, which is comprised of test consumables and shared instrumentation. The Verigene instrumentation is a bench-top sample-to-result molecular diagnostics workstation consisting of two modules: the Verigene Processor *SP* and the Verigene Reader. The Verigene Reader serves as the central control unit for the Verigene System as well as the user interface, storing and tracking information throughout the assay process and interpreting and generating test results once the assay is complete. The Verigene Processor *SP* automates (i) Sample Preparation – Cell lysis and magnetic bead-based bacterial DNA isolation from positive blood culture specimens obtained from patients, and (ii) Hybridization of bacteria-specific target DNA that employs a gold nanoparticle probe-based technology in a microarray format. The Verigene Processor *SP* utilizes single-use consumables to perform **BC-GP**, including an Extraction Tray, Utility Tray, and Verigene Test Cartridge.

All Verigene tests are formatted in self-contained test specific Verigene Test Cartridges which facilitate the analysis of a nucleic acid sample. Nucleic acids are prepared for testing directly from a positive blood culture media using magnetic glass particles in an extraction tray and input automatically into a Test Cartridge inside the Verigene Processor *SP.* A separate Tip Holder Assembly contains two pipette tips that are used to transfer and mix reagents during the assay. The user tests a sample by loading the single-use disposables into the Verigene Processor *SP* and pipetting the positive blood culture sample into the Extraction Tray. The user initiates the test protocol on the Verigene Reader by scanning or entering the barcode ID located on the Test Cartridge along with sample information. Following assay completion, the user collects data on the Verigene Reader by scanning the barcode ID on the Substrate Holder and inserting it into the Verigene Reader for analysis which constitutes detection and identification of hybridized bacterial DNA.

Test Procedure

Bacterial DNA is extracted from the organisms present in a positive blood culture media specimen, fragmented and denatured. This fragmented, single-stranded bacterial DNA hybridizes to complementary sequence-specific DNA oligonucleotides, known as capture oligonucleotides, arrayed on the surface of a substrate (glass slide). A second DNA oligonucleotide is then hybridized to the bacterial DNA that was captured initially. This oligonucleotide is known as a mediator oligonucleotide containing two sequence domains: one domain is complementary to the bacterial DNA target and a second domain is complementary to a common oligonucleotide attached to a signal-generating gold nanoparticle. After washing away any DNA not affixed to the captures, the gold nanoparticle is exposed to the captured mediator/target complex where it hybridizes to any captured mediator oligonucleotides. Presence of the silver-enhanced gold nanoparticle probes at a particular location on the substrate is assessed optically. The Verigene System with Processor *SP* has been previously cleared by the FDA for another similar application[19].

MATERIALS PROVIDED

A. Verigene **BC-GP** Kit (Catalog number **20-005-018**)

- 20 Verigene **BC-GP** Test Cartridges

  Each Test Cartridge comes preloaded with all required reaction solutions, including wash solutions, oligonucleotide probe solution and signal amplification solutions required to generate a test result. The Test Cartridges are labeled as: **BC-GP; 20-006-018**

- 20 Verigene **BC-GP** Extraction Trays (with Tip Holder Assemblies)

  Each Extraction Tray comes preloaded with all required solutions, including lysis/binding buffer, digestion enzymes, wash solutions, and buffer solutions necessary to extract nucleic acids and generate a test result. The Extraction Trays are contained within a carrier labeled as: **BC-GP; 20-009-018**

B. Verigene **BC-GP** Utility Kit; (Catalog number **20-012-018**)

20 Verigene **BC-GP** Utility Trays

Each Utility Tray comes preloaded with all required solutions, including digestion enzymes and the *B. subtilis* Internal Processing Control necessary to extract nucleic acids and generate a test result. The Utility Trays are contained within a carrier labeled as: **BC-GP; 20-011-018**

MATERIALS NEEDED BUT NOT PROVIDED

A. Instruments and Equipment

- Verigene Reader; Catalog number 10-0000-02
- Verigene Processor *SP*; Catalog number 10-0000-07
- 2 – 8°C refrigerator
- Automated blood culture monitoring system
- Micro-pipettors & tips
- Vortex mixer
- ≤ -70C freezer (optional)

B. Consumables and Reagents

- Blood culture bottles
- Gram staining reagents

REAGENT STORAGE, HANDLING, STABILITY

Component	***Storage Conditions***	***Comments***
Extraction Tray	2 – 28°C	Do not freeze.
Utility Tray	≤ 8°C	Shipped frozen. Upon receipt, may be stored frozen or refrigerated. Do not re-freeze after thawing
Test Cartridge	2 – 8°C	Do not freeze.
Tip Holder Assembly	2 – 30°C	Do not freeze.

METHODS

A. Specimen Collection & Storage

1. Draw blood using aseptic techniques into the blood culture bottle following manufacturer's instructions.
2. Incubate bottle in automated blood culture monitoring system until the bottle is flagged positive for microbial growth following manufacturer's instructions.
3. When the bottle is positive for microbial growth, perform a Gram stain.
4. For gram-positive bacteria test 350 µL of the blood culture media using **BC-GP**. Ensure the blood culture bottle is thoroughly mixed by inverting several times (>4) before retrieving test sample volume.
5. 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 the *mecA* gene to an organism and/or association of the *vanA/vanB* gene to an organism.
6. Positive blood culture media may be stored at room temperature (18-24ºC) for up to 12 hours or remain in the automated blood culture monitoring system at 35ºC for up to 8 hours prior to testing.
7. Inadequate or inappropriate specimen collection, storage, or transport may yield false negative results.
8. Training in specimen collection and handling is highly recommended because of the importance of specimen quality.

B. Test Procedure

Please refer to the Verigene System User's Manual for additional details on performing tests on the Verigene System as well as routine and daily maintenance.

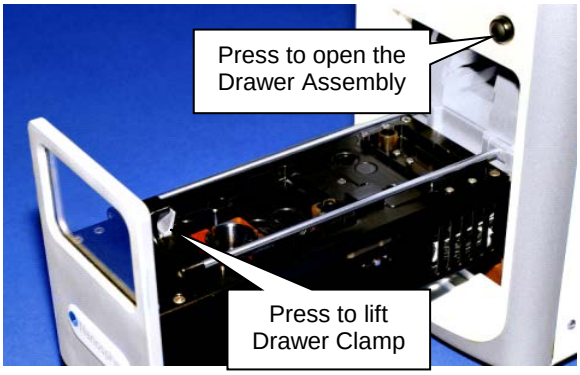
1. Preparing the work area for testing

   Sanitize vortex mixers, centrifuges, pipettes, countertops, and any other equipment used for sample processing with a lint-free decontaminating cloth before and after sample preparation.

2. Test set up

   a) Remove an Extraction Tray, Utility Tray, Tip Holder Assembly, and Test Cartridge from the refrigerator. If the Utility Tray was stored in the freezer, thaw at room temperature for 10 minutes. Begin test run within 30 minutes or store Utility Tray at < 8°C until ready to initiate testing.

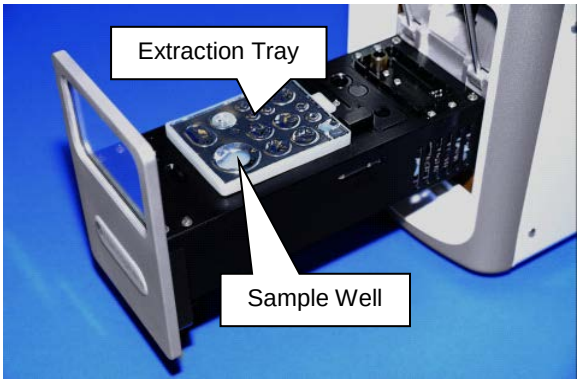
   b) The image below shows an empty Verigene Processor *SP*. Open the Drawer Assembly by pressing the black open/close button located on the front of the Verigene Processor *SP*. Open the Drawer Clamp by pressing in the silver latch and lifting the Clamp prior to loading the consumables.



3. Loading the Extraction Tray

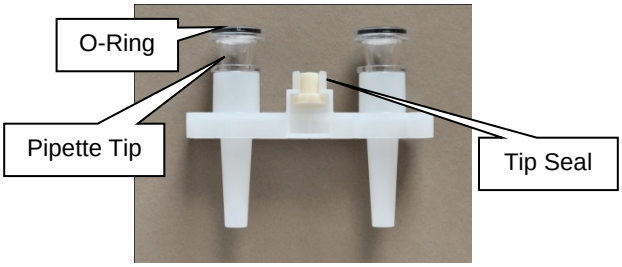
a) (*optional*) Prior to loading the Extraction Tray, thoroughly shake the Tray to resuspend the magnetic beads which have settled during storage. Check for complete resuspension by visually inspecting the well containing the beads. The well containing the magnetic beads is easily distinguished as the beads are black in color. Following adequate resuspension, gently tap the tray on the bench to ensure that the reagents settle to the bottom of each well.

b) The Extraction Tray can only be loaded in one direction in the Drawer Assembly. When loaded correctly, the Sample Well is located in the front right hand corner of the Drawer Assembly. Place the Extraction Tray in the Drawer Assembly and press down on the corners of the tray to ensure it is level. The image below shows a properly loaded Extraction Tray.



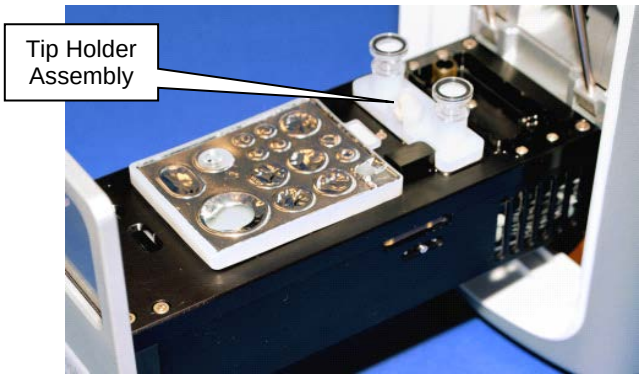
4. Loading the Tip Holder Assembly

a) The Tip Holder Assembly is a plastic holder that contains two Pipette Tips and a rubber Tip Seal. Each Pipette Tip contains an O-ring on top.



b) Before using the Tip Holder Assembly, check the top of each Pipette Tip for the O-ring and check for the rubber Tip Seal sitting straight and flush between the tips. If either is missing, replace with a new Tip Holder Assembly.

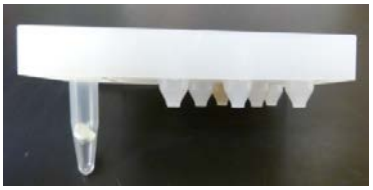
c) Insert the Tip Holder Assembly into the Drawer Assembly. The image below shows a properly loaded Tip Holder Assembly. The Tip Assembly can only be loaded in one direction in the Drawer Assembly. For orientation, there are two holes on the deck of the Drawer Assembly that fit each Pipette Tip and the opening to the Tip Seal should face away from Processor *SP*.



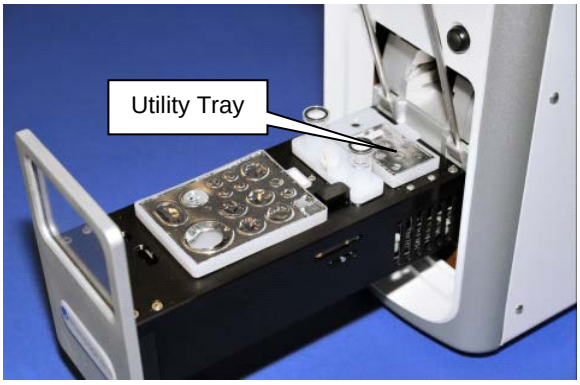
5. Loading the Utility Tray

a) (*optional*) Gently vortex the Utility Tray and gently tap the tray on the bench to settle the reagents.

b) Remove and save the cap from the *B. subtilis* Process Control (PC) Tube and fully insert the PC Tube into the Utility Tray. Visually inspect the tube to ensure the *B. subtilis* pellet is seated in the lower half of the PC tube as shown in the picture below.



c) Insert the Utility Tray into the Drawer Assembly. The image below shows a properly loaded Utility Tray. The Utility Tray can only be loaded in one direction in the Drawer Assembly. When loaded properly, the tray sits flat.



d) Lower and Latch the Drawer Clamp over the Trays while supporting the Drawer with the opposite hand. The image below shows a closed Drawer Clamp over properly loaded trays and Tip Holder Assembly. The Drawer Clamp will latch onto the Drawer Assembly when closed properly, and the user will be unable to lift the Drawer Clamp without pressing in the silver latch.

6. Ordering a Test

a) All Tests must be ordered through the Verigene Reader. No tests can be processed on the Verigene Processor *SP* without the user entering the Test Cartridge ID and Sample ID to the Verigene Reader.

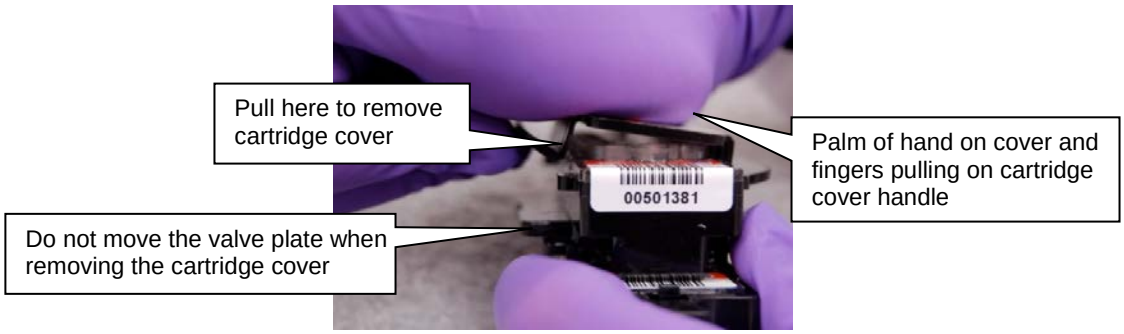
i. Login to the Verigene Reader as a 'user'.
ii. If the user would like to start a new Session, proceed to the next step (iii). If the user would like to order a test in a previously created session, they can select the desired Session from the drop down 'SESSION' menu then proceed to step (v). Up to 60 cartridges can be entered into a single session.
iii. From the Menu Bar, SESSION tab, select Start New Session where the Session Setup window will appear.
iv. Touch Session ID button and enter information by using the data entry keyboard. This can be any unique identifier in a format defined by the laboratory. The operator ID is automatically entered as the currently logged in 'user'.
v. Touch the Processing option on the Navigation Bar at the bottom of the screen.

b) Enter the Test Cartridge ID by scanning the barcode using the barcode scanner attached to the Reader. The user may manually enter in the Test Cartridge ID by selecting MENU and 'Enter Barcode' and then keying in the Test Cartridge ID number with the Reader's keyboard.
c) *(optional)* Scan the Test Cartridge Cover's 2D barcode using a barcode gun-style scanner to display the Test Cartridge's Reference Number, Expiration Date, and Lot Number on reports. Note: the wand style barcode scanner will not read 2D barcodes.

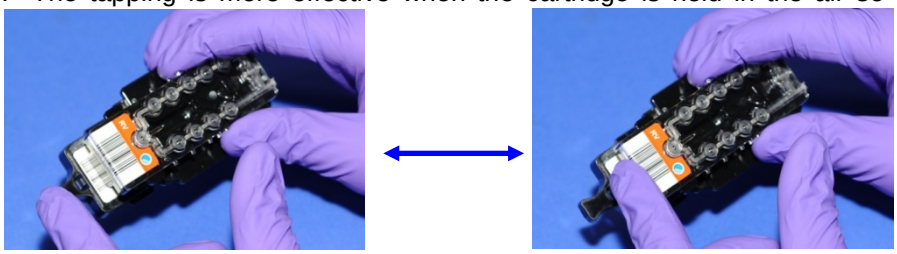
7. Loading a Test Cartridge

a) Hold the Test Cartridge by the handle with one hand, using the other hand apply pressure with the palm of the hand and remove the cartridge cover by bending the cover away and over the Reagent Pack edge. Ensure that the valve plate is not moved during cover removal (see illustration below).

Do not remove the Test Cartridge cover until immediately prior to inserting the Test Cartridge into the Processor *SP*.

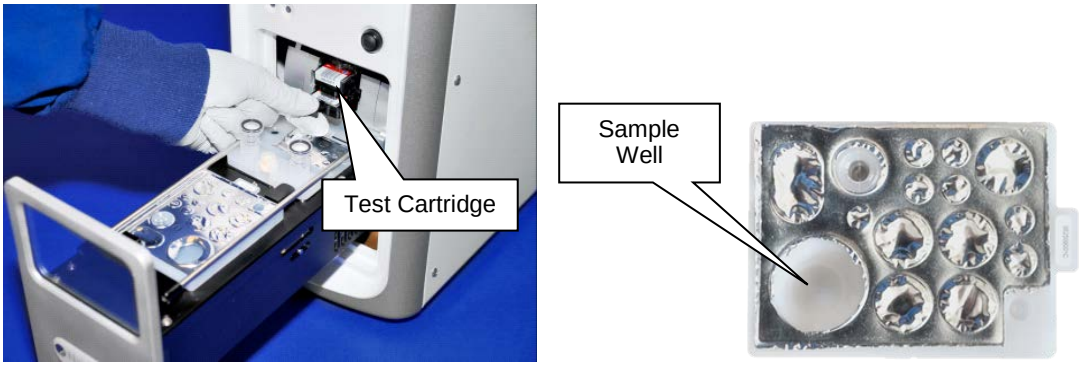


b) (*optional*) The user may settle the reagents in the cartridge before loading into the Verigene Processor *SP*. The optimal method for settling the reagents is to hold the Test Cartridge's reagent container on the side opposite the handle and tap the reagent container's barcode with your index finger. When tapping the cartridge, allow the force of the tapping to move the cartridge and your right hand. The tapping is more effective when the cartridge is held in the air so that it moves slightly.



c) Insert the Test Cartridge into the Hybridization Module of the Verigene Processor *SP* until it reaches a stopping point. The image below shows the user loading a Test Cartridge into the Verigene Processor *SP*.

d) Note: If the Test Cartridge is not inserted properly, the Processor SP will display a message on the information screen when the user attempts to close the Drawer Assembly.



8. Loading the Sample

   a. At the Reader enter the sample number/ID by scanning or using the Reader's touch-screen keyboard. Press Yes to confirm the sample ID (see image below). Ensure Hybridization and Extraction options are selected.

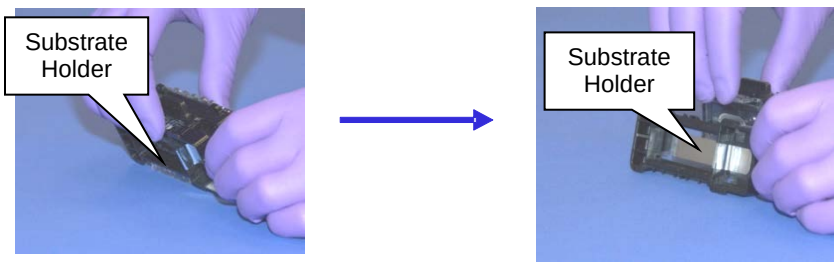
   b. In the subsequent dialogue box, select or de-select bacteria species or resistance markers from the list to activate or de-activate results reporting for those targets. Press Yes to confirm. The Verigene Reader will automatically default to the previously selected targets.

Note: *Once a test run is started, results for de-selected targets cannot be retrieved.*

   c) Gently vortex the gram-positive blood culture sample and pipette 350 µL of the gram-positive blood culture sample into the bottom of the Sample Well in the Extraction Tray (refer to image above for Sample Well location).
   d) Close the Drawer Assembly by pressing the OPEN/CLOSE button on the Processor *SP*. The Processor will automatically verify that each consumable is properly loaded and begin sample processing.
   e) Confirm countdown has started on the Processor *SP* display screen before leaving the area.
   f) In order to set up additional tests on other Processor *SP* instruments follow the same procedure. To avoid contamination and sample mix-ups, only set up one test at a time, change gloves after handling a sample, and decontaminate pipettes and sample tubes between tests.

9. Upon completion of a test run

   a) The Verigene Reader will ring to notify the user when the test is completed and the Processor *SP* will display a message indicating the test is finished. The Test Cartridge should be removed from the Processor *SP* upon completion of the test or within 12 hours of completion.
   b) Open the Drawer Assembly by pressing the OPEN/CLOSE button. Cap the PC tube for disposal.
   c) Remove the Test Cartridge and immediately orient to the side.
   d) While keeping the test cartridge on its side, separate the Reagent Pack.



10. Analyzing results

    a) Remove the protective tape from the back of the Substrate Holder.
    b) Use the Reader's barcode scanner to read the barcode on the Substrate and immediately insert the Substrate Holder into the Reader.
    c) When the barcode is accepted, a prompt to load the Substrate Holder will display.
    d) Scanning the barcode ensures that the test result is associated with the correct sample. When the load substrate prompt occurs, it will only display for 20 seconds. The analysis will only start if the substrate is loaded during the animated prompt.
    e) To properly insert the substrate into the Reader hold the substrate by the handle with the barcode facing away from you. Next, insert the Substrate Holder into the substrate compartment. The compartment is designed to place the holder in the correct position. Do not force the holder in, but do insert it into the compartment as far as it will go comfortably. Close the door of the substrate compartment.
    f) The analysis will automatically begin. A small camera icon will appear on the Reader letting the user know analysis has begun.
    g) The analysis is completed by the Reader when the camera icon is replaced with an upward facing arrow and the Reader rings.
    h) Confirm that a result other than 'No Call – No GRID' has been generated by touching the substrate icon for the test. A Substrate producing a 'No Call – No GRID' result should be rescanned and reanalyzed. Use the 'Interpretation of Results' section to analyze results.
    i) Once the scan is complete, dispose of used Test Substrate.

11. Printing results

    a) Touch the substrate icon in the Session's Processing screen. A window displaying the results will open; touch the 'Print' option on this screen to print a Detail Report.
    b) A Summary Report is available by moving to the Results screen of the Session on the bottom Navigation Bar; go to MENU then select 'Print Summary'. The Summary Report will provide the results for all Tests processed within the current Session.
    c) In the Results screen the Detail Reports can also be viewed and printed. First select the desired Test from the list, go to MENU and then touch 'Print Detail'.

C. Interpretation of Results

BC-GP provides a qualitative result for the presence ("Detected") or absence ("Not Detected") for all bacterial analytes in the test panel. The panel analytes are represented by target-specific spots on the Test Substrate (microarray). The image analysis of the Test Substrate provides image intensities for each panel analyte as well as imaging controls (IC) and negative controls (NC). Intensities at the panel analytes are required to be above an empirically determined 'Noise Threshold' after which they are normalized to generate 'Ratio-to-IC' and 'Ratio-to-NC' values. Cut-offs for both the normalized ratios were determined by ROC curve analysis.

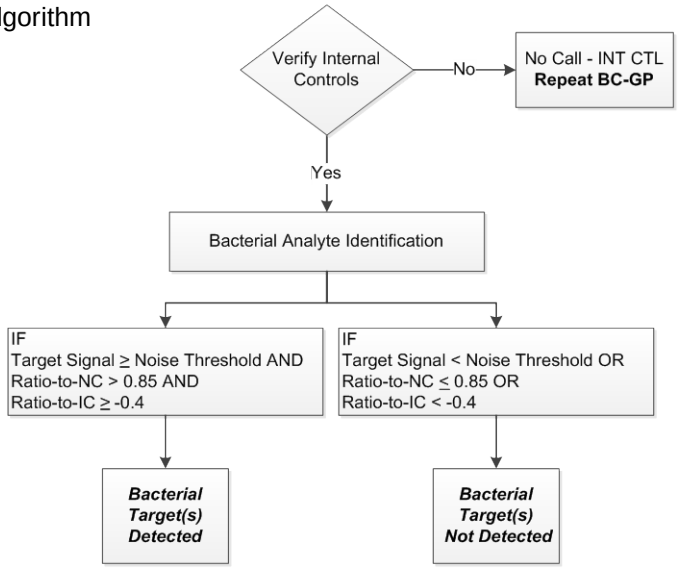
For a 'Detected' and 'Not Detected' result to be generated by **BC-GP**, three conditions (or "filters") must be met. These conditions serve as a single-set of clinical 'cut-off' or detection criteria.

- *Filter 1:* Signal ≥ Noise Threshold
- *Filter 2:* Normalized 'Ratio to Negative Control (NC)' > 0.85
- *Filter 3*: Normalized 'Ratio to Imaging Control (IC)' ≥ - 0.4

Two Internal Controls, INT CTL 1 (extraction control) and INT CTL 2 (hybridization control), guide decisions regarding the validity of the test process. Both INT CTL 1 and INT CTL 2 are treated as unique targets (or panel members), and their presence verified in order for a valid result to be generated. If the internal controls fail, a No Call – INT CTL 1 (for INT CTL 1 failure), a No Call – INT CTL 2 (for INT CTL 2 failure), or a No Call – INT CTL (for failure of both INT CTL 1 and INT CTL 2) are provided. If the internal controls are verified, the presence or absence of individual bacteria is reported based on the cut-off criteria.

In summary, to generate a test result, a decision algorithm is followed (see **BC-GP** Decision Algorithm below), verifying the presence of the process control (INT CTL 1) and hybridization control (INT CTL 2). Both INT CTL 1 and INT CTL 2 signal intensities must meet the detection criteria for a "valid call" to be generated, and this call is made only after both INT CTL 1 and INT CTL 2 are verified during analysis of each test. This signifies that the extraction and hybridization processes were performed correctly. Finally, a 'Detected' call is provided at every **BC-GP** panel analyte that meets the detection criteria.

BC-GP Decision Algorithm



1. Calls for Valid Results

The table below lists the possible test results generated by **BC-GP**, representing identification of bacterial nucleic acid sequences/targets, when the Internal Controls INT CTL 1 and INT CTL 2 are verified as "Detected". An initial "Not Detected" test result may be repeated once, at the discretion of the user, in order to confirm the initial result. Should a "Detected" result be obtained upon repeat testing, it is appropriate to consider this latter test result reportable.

Calls for Valid Results –Internal Controls Verified

Organism/Gene	***Target Genes***	***Test Result Reported as "Detected"***		
		Genus	*Species*	*Resistance Marker*
Staphylococcus spp.(1)	*tuf*	*Staphylococcus*(7)	-	-
Staphylococcus aureus	*gyrB*	*Staphylococcus*	*S. aureus*	-
Staphylococcus epidermidis	*hsp60*	*Staphylococcus*	*S. epidermidis*	-
Staphylococcus aureus, mecA(2)	*gyrB* and *mecA*	*Staphylococcus*	*S. aureus*	*mecA*
Staphylococcus epidermidis, mecA(2)	*hsp60* and *mecA*	*Staphylococcus*	*S. epidermidis*	*mecA*
Staphylococcus lugdunensis	*sodA*	*Staphylococcus*	*S. lugdunensis*	-
Enterococcus faecalis	*hsp60*	-	*E. faecalis*	-
Enterococcus faecalis, vanA(3)	*hsp60 and vanA*	-	*E. faecalis*	*vanA*
Enterococcus faecalis, vanB(3)	*hsp60 and vanB*	-	*E. faecalis*	*vanB*
Enterococcus faecium	*hsp60*	-	*E. faecium*	-
Enterococcus faecium, vanA(3)	*hsp60 and vanA*	-	*E. faecium*	*vanA*
Enterococcus faecium, vanB(3)	*hsp60 and vanB*	-	*E. faecium*	*vanB*
Streptococcus spp.(4)	*tuf*	*Streptococcus*(8)	-	-
Streptococcus agalactiae	*hsp60*	*Streptococcus*	*S. agalactiae*	-
Streptococcus anginosus group(5)	*gyrB*	*Streptococcus*	*S. anginosus group*	-
Streptococcus pneumoniae	*gyrB*	*Streptococcus*	*S. pneumoniae*	-
Streptococcus pyogenes	*hsp60*	*Streptococcus*	*S. pyogenes*	-
Listeria spp.(6)	*tuf*	*Listeria*	-	-
All Analytes "Not Detected"	N/A	-	-	-

(1) Evaluated *Staphylococcus* spp. shown to be detected by **BC-GP** include: *S. arlettae, S. aureus, S. auricularis, S. capitis, S. caprae, S. chromogenes, S. cohnii, S. epidermidis, S. haemolyticus, S. hominis, S. intermedius, S. lugdunensis, S. muscae, S. pasteuri, S. saccharolyticus, S. saprophyticus, S. schleiferi, S. sciuri, S. simulans, S. warneri,* and *S. xylosus.*

(2) This information is provided only to convey that detection of both the sequence for the organism and mecA must occur in order for **BC-GP** to yield a valid "mecA" call. In mixed growth, **BC-GP** does not specifically attribute *mecA*-mediated methicillin resistance to either *Staphylococcus aureus* or *Staphylococcus epidermidis.*

(3) This information is provided only to convey that detection of both the sequence for the organism and *vanA* or *vanB* must occur in order for **BC-GP** to yield a valid "vanA" or "vanB" call. In mixed growth, **BC-GP** does not specifically attribute *vanA*- or *vanB*-mediated vancomycin resistance to either *Enterococcus faecalis* or *Enterococcus faecium.*

(4) Evaluated *Streptococcus* spp. shown to be detected by **BC-GP** include: *S. agalactiae, S. anginosus, S. bovis, S. constellatus, S. dysgalactiae, S. dysgalactiae* subsp *equisimilis, S. equi, S. equinus, S. gallolyricus, S. gallolyticus pasteurianus, S. gordonii, S. infantarius subsp. coli, S. infantarius* subsp. *infantarius, S. infantis, S. intermedius, S. mitis, S. mitis/oralis, S. mutans, S. oralis, S. parasanguinis, S. peroris, S. pneumoniae, S. pyogenes, S. salivarius, S. sanguinis,* and *S. thoraltensis*

(5) The "S. anginosus group" includes: S. anginosus, S. constellatus, and S. intermedius.

(6) Evaluated *Listeria* spp. shown to be detected by **BC-GP** include: *L. innocua, L. ivanovii, L. monocytogenes, L. seeligeri,* and *L. welshimeri*

(7) On rare occasions, a *"Staphylococcus"* result may not be obtained when *S. aureus,S. epidermidis,* and/or *S. lugdunensis* is detected. When this occurs, a *S. aureus,S. epidermidis,* and/or *S. lugdunensis* result is still valid.

(8) On rare occasions, a *"Streptococcus."* result may not be obtained when *S. agalactiae, S. anginosus group, S. pneumoniae,* and/or *S. pyogenes* is detected. When this occurs, a *S. agalactiae, S. anginosus group, S. pneumoniae,* and/or *S. pyogenes* result is still valid.

2. Calls for Invalid Results - Error Calls and Recourse

Error calls related to an invalid test are listed in the table below**,** together with the appropriate recourse which should be taken by the user.

Error Call	***Reason***	***Recourse***
No Call – INT CTL 1	INT CTL 1 Not Detected. Processing and/or lysis/extraction Issues.	Repeat **BC-GP** from original blood culture specimen
No Call – INT CTL 2	INT CTL 2 Not Detected. Inhibition during the target hybridization procedure.	Repeat **BC-GP** from original blood culture specimen
No Call – INT CTL	INT CTL 1 and INT CTL 2 Not Detected. Processing and/or lysis/extraction Issues and Inhibition during target hybridization	Repeat **BC-GP** from original blood culture specimen
No Call – NO GRID	Reader unable to image Test Substrate	Ensure protective silver tape has been removed from back of Test Substrate. Ensure Test Substrate is seated properly in the substrate holder. Repeat image analysis by selecting 'Menu' and 'Enter Barcode' and then scanning the Substrate barcode. If the No-Call persists, repeat **BC-GP** from original blood culture specimen
No Call – VARIATION	Inability to obtain the test result because of high variability in the target-specific signals.	Repeat **BC-GP** from original blood culture specimen
No Call – BKGD		
No Call – NEG CTL		
Processing Error	Pre-analytical error. Internal checks within the Processor SP detected an unexpected event.	Power cycle Processor SP and repeat **BC-GP** from original blood culture specimen

QUALITY CONTROL

Quality control, as a component of an overall quality assurance program, consists of tests and procedures for monitoring and evaluating the analytical performance of a measurement system to ensure the reliability of patient test results.

A. Verigene System

The Verigene System uses a series of automated on-line quality measurements to monitor instrument functionality, software performance, fluidics, test conditions, reagent integrity, and procedural steps each time a test is performed. A series of automated on-line procedural checks guide the user through the testing process each time a test is performed. **BC-GP** test barcode and sample information are linked upon entry into the Verigene Reader to help prevent misreporting of results.

B. Assay Controls

BC-GP is performed using single-use disposable reagent trays and cartridges, in which all reagents are prepackaged to prevent reagent dispensing errors. Several levels of controls are built into **BC-GP** ensure that failures at any procedural step of **BC-GP** are identified during the procedure.

Internal Controls

An internal processing control, designated "INT CTL 1", comprises a non-target organism *Bacillus subtilis*, a gram-positive bacterium with an intact genome. It is automatically added to each sample in the processor immediately prior to Sample Extraction. The INT CTL 1 functions as a complete assay control, the primary

purpose of which is to monitor failures likely to be attributable to the sample preparation step (i.e., lysis and nucleic acid extraction); it also functions as non-target hybridization/detection control.

A second internal processing control, designated “INT CTL 2”, comprises an assay-specific single-stranded DNA target present in the Sample Hybridization Mix reagent and is added by the System to each sample as a means to monitor hybridization inhibition (due to sample- or process-related inhibitors or reagent failures).

For each test performed, both controls (INT CTL 1 and INT CTL 2) must yield correct results to enable reporting of a valid test result. For additional information regarding interpretation of results related to INT CTL 1 and/or INT CTL 2 failures, see the table entitled *Error Calls and Recourse* in the ***Interpretation of Results*** section

External Controls

It is highly recommended that known culture-confirmed blood culture specimens positive for each of the **BC-GP** panel organisms be tested routinely as defined by the user’s laboratory’s standard operating procedures on a rotating basis using 3-4 smaller groups of organisms, and/or under the following circumstances:

- Instrument installation, test validation, and when troubleshooting is necessary
- During performance verification for receipt of a new set/lot of consumables;
- When the integrity of consumables or the device is in question.

Frozen aliquots of blood cultures containing these organisms may be used for this purpose. When preparing QC material from a positive blood culture bottle, sterilize the bottle top by wiping with an alcohol wipe, invert the bottle 4-5 times to homogenize the specimen, draw fluid by using a 10 mL syringe (equipped preferably with a 16 gauge needle), and transfer to a secondary vessel. Vortex secondary vessel to homogenize specimen, dispense 500 µL aliquots into cryovials and store the aliquots at ≤-70°C.

Regardless of the choice of quality control materials, all external quality control requirements and testing should be performed in conformance with local, state, and federal regulations or accreditation organizations as applicable and should follow the user’s laboratory’s standard quality control procedures.

TROUBLESHOOTING

Refer to the Troubleshooting section of the Verigene System User’s Manual.

LIMITATIONS

- 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.
- Isolation on solid media is needed to differentiate mixed growth with other organisms and to identify positive blood cultures yielding a negative result.
- 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 detection of bacterial nucleic acid is dependent on proper specimen collection, handling, transport, storage, and preparation, including extraction. Failure to observe proper procedures in any of these steps could lead to incorrect results.
- False negative results may occur from improper specimen collection, handling or storage, technical error, sample mix-up, target concentration below the analytical sensitivity of the test, or below the concentration at bottle positivity, which might be caused by the growth of other organism(s).
- A negative result for *S. aureus, S. epidermidis,* or *mecA* should not be used as the sole basis for diagnosis, treatment or patient management decisions.
- A negative result for *vanA* or *vanB* in *E. faecium,* or *E. faecalis* should not be used as the sole basis for diagnosis, treatment or patient management decisions. Vancomycin resistance can be caused by genes other than *vanA* and *vanB*.
- Some anaerobic bacteria containing the *vanB* gene have been described in the literature and may give a positive *vanB* result in mixed cultures of *E. faecalis or E. faecium*.
- The clinical study included evaluation of BACTEC™ Plus Aerobic/F and BacT/ALERT FA FAN® Aerobic bottles only. All other bottle types were evaluated analytically in the Interfering Substances study (see Performance Characteristics section F.b).
- Pediatric patient specimens were not differentiated from adult patient specimens in the clinical study; therefore, the performance characteristics of the assay with specimens obtained from pediatric patients have not been determined.
- There is a risk of false negative results due to sequence variants in the bacterial targets of the assay.
- Methicillin-resistant *S. aureus* strains that carry the $mecA_{LGA251}$ gene (a novel *mecA* variant) were not evaluated; therefore the performance of **BC-GP** with these strains is unknown.
- Analytical inclusivity of the test was determined at bottle positivity. Analytical competitive inhibition studies were tested using organisms detected by **BC-GP** test. The performance of **BC-GP** in mixed cultures containing organisms other than the **BC-GP** panel organisms (e.g.; gram-negative rods, yeast) has not been evaluated and therefore is unknown.
- There is a risk of false positive results due to cross-contamination by target bacteria and their nucleic acids.
- The assay detects the presence of the *mec*A gene in a sample, but does not determine which *Staphylococcus* spp. (*S. aureus* and/or *S. epidermidis*) produced the gene.
- The assay detects the presence of the *vanA or vanB* gene in a sample, but does not determine which *Enterococcus* spp. (*E. faecalis* and/or *E. faecium*) produced the gene.
- The assay does not differentiate *Staphylococcus* spp. other than *Staphylococcus aureus, Staphylococcus epidermidis* and *Staphylococcus lugdunensis.* If, for example, *S. hominis* and/or *S. capitis* are present, the **BC-GP** result will be *"Staphylococcus"* detected.
- The assay does not differentiate *Streptococcus* spp. or groups other than *Streptococcus agalactiae, Streptococcus anginosus* group*, Streptococcus pneumoniae* and *Streptococcus pyogenes.* If, for example*, S. sanguinis* and/or *S. salivarius* are present, the **BC-GP** result will be *"Streptococcus"* detected.
- The following *Streptococcus* spp. were not evaluated; therefore, the performance of **BC-GP** with these organisms is unknown: *S. acidominimus, S. canis, S. criceti, S. downei, S. ferus, S. ictaluri, S. iniae, S. lactarius, S. lutetiensis, S. macacae, S. macedonicus, S. parauberis, S. pasteurianus, S. pluranimalium, S. porcinus, S. pseudopneumoniae, S. pseudoporcinus, S. ratti, S. suis, S. urinalis, S. cristatus, S. australis, S. sinensis, S. orisratti, S. oligofermentans, S. massiliensis, S. sobrinus, S. vestibularis, S.*

thermophiles, S. alactolyticus, S. uberis and S. equisimilis.

- For *Listeria* spp. detection, *in silico* analysis suggests that *L. marthii* should be detected by the test; however, this species was not evaluated with analytical testing and therefore the performance of **BC-GP** for the detection of this organism is unknown. *L. grayi* should not be detected by the test, as suggested by *in silico* analysis and confirmed with analytical testing.
- Certain strains of *Streptococcus* that are genetically homologous to *S. pneumoniae,* but currently classified as *S. mitis*, will cross-react with **BC-GP** *Streptococcus pneumoniae* probes, which will cause a false positive "*Streptococcus pneumoniae detected*" result.
- *Lactococcus* spp. strains could cross-react with **BC-GP** *Streptococcus* spp. probes, which will cause a false positive *"Streptococcus" detected* result.
- *Aerococcus* spp. strains may cross-react with **BC-GP** *Staphylococcus* spp. probes, which will cause a false positive "*Staphylococcus"* detected result.
- *In-silico* analysis suggests that each of the following *Staphylococcus* spp. should be detected by the test; however, these species were not evaluated and the performance of **BC-GP** with these organisms is unknown: *S. carnosus, S. condimentii, S. delphini, S. equorum, S. felis, S. hyicus, S. gallinarum, S. kloosii, S. lentus, S. lutrae, S. nepalensis, S. pettenkoferi, S. piscifermentans, S. flueretii, S. pseudintermedius, S. simiae,* and *S. succinus.*
- Modified *S. aureus* (MOD-SA) strains that produce modified intrinsic PBP's with altered affinity for oxacillin were not evaluated; therefore the performance of **BC-GP** with these strains is unknown.
- Although theoretically possible based on complete homology of the *S. epidermidis* hsp60 gene with an *S. saccharolyticus* gene sequence (AF242289.1), the likelihood of clinically-relevant cross-reactivity of **BC-GP** with this organism is very low, given that two *S. saccharolyticus* strains (ATCC 14953 and NCIMB 701260) have been tested at anaerobic blood culture bottle positivity ($3.1x10^7$ and $1.5 x10^7$ CFU/mL, respectively), and have demonstrated no cross-reactivity with the detection of *S. epidermidis*.
- Vancomycin-resistant *Staphylococcus aureus* (VRSA) were not evaluated; therefore the performance of **BC-GP** with these strains is unknown. If **BC-GP** detects a positive *S. aureus* result for a specimen with VRSA, the *vanA* resistance gene will not be reported.
- In rare cases, *Listeria* spp. could cross-react with **BC-GP** *Staphylococcus* spp. probes, which will cause a false positive "*Staphylococcus"* detected result.
- *Abiotrophia defectiva* strains could cross-react with **BC-GP** *Staphylococcus* spp. and/or *Streptococcus* spp. probes, which will cause a false positive "*Staphylococcus* spp." and/or "*Streptococcus* spp." detected result.
- *Globicatella* spp. strains could cross-react with **BC-GP** *Listeria* spp. probes, which will cause a false positive "*Listeria*" detected result.
- *Staphylococcus intermedius* group, which is comprised of *S. delphini, S. intermedius*, and, *S. pseudintermedius*, could cross-react with **BC-GP** *Listeria* spp. probes, which will cause a false positive "*Listeria*" detected result.
- *Facklamia hominis* could cross-react with **BC-GP** *Staphylococcus* spp. probes (based on in silico analysis), which will cause a false positive "*Staphylococcus* spp." detected result.
- *Veillonella parvula* could cross-react with **BC-GP** *Staphylococcus* spp. probes (based on in silico analysis), which will cause a false positive "*Staphylococcus* spp." detected result.
- *Enterococcus avium* could cross-react with **BC-GP** *E. faecium* probes which will cause a false positive "*E. faecium*" detected result.

WARNINGS AND PRECAUTIONS– GENERAL

- **BC-GP** is for *in vitro* diagnostic use only.
- Federal law restricts this device to sale by or on the order of a physician, or to a clinical laboratory; its use is restricted to, by, or on the order of a physician.

- Never use any Tips, Trays, Tubes, or Test Cartridges which have been broken, cracked, punctured, previously used or anyway visibly damaged; using damaged material may lead to No Call or false results.
- Handle supplies, reagents, and kits with powder-free gloves at all times to avoid contamination and change gloves between removal of used disposables and loading of new disposables.
- Handle samples carefully. Open one tube or sample at a time to prevent sample contamination.
- Biological samples such as tissues, body fluids, and blood of humans and other animals are potentially infectious. When handling and/or transporting human specimens, follow all applicable regulations mandated by local, state/provincial, and federal agencies for the transport of etiologic agents.

WARNINGS AND PRECAUTIONS – INSTRUMENTS

A. General Instrument Safety
WARNING: Use this product only as specified in this document. Using this instrument in a manner not specified by Nanosphere may result in personal injury or damage to the instrument. Ensure that anyone who operates the instrument:
- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Reads and understands all applicable Material Safety Data Sheets (MSDS).

B. Electrical Shock Hazard
WARNING: Severe electrical shock can result from operating the instrument without its instrument covers or back panels in place. Do not remove instrument covers or panels. High-voltage contacts are exposed when instrument covers or panels are removed from the instrument. If service is required, contact Nanosphere Technical Support at 1-888- 837-4436.

C. Maintenance of the Verigene Reader and Verigene Processor *SP*
For routine and daily maintenance instructions, please refer to the Verigene System User's Manual.

WARNINGS AND PRECAUTIONS – REAGENTS AND TEST CARTRIDGES

A. Toxicity of Reagents

- Exposure to chemicals sealed inside the Test Cartridge is hazardous in case of skin contact and of ingestion. Protective disposable gloves, laboratory coats, and eye protection should be worn when handling specimens, Extraction Trays, Utility Trays, and Verigene Test Cartridges.
- See Material Safety Data Sheets (MSDS) for toxicity information. Material Safety Data Sheets (MSDS) are available upon request from Nanosphere, Inc.

B. Waste Disposal

- The Utility Tray contains residual lysis enzymes and a benign microorganism (*Bacillus subtilis*). It also contains a residual volume of the sample buffer which contains formamide, a teratogen. Dispose the Utility Tray in accordance with national, state, and local regulations.
- The Extraction Tray contains residual nucleic acids, extraction reagents, and residual sample. The lysing reagents (lysis enzymes and chaotropic salts) are expected to render the residual sample non-infectious; no studies to confirm non-infectivity have been performed. It is recommended to dispose the Extraction Tray in biohazardous waste.
- All of the waste reagents, including the purified DNA, are contained within the Test Cartridge. There is a very small amount of residual formamide (≤1% v/v). Dispose the Test Cartridge in accordance with national, state, and local regulations. An MSDS with more information is available for the Test Cartridge, Utility Tray and Extraction Tray at www.e-labeling.eu and at www.nanosphere.us.

EXPECTED VALUES

Prevalence

In the Verigene **BC-GP** Test clinical study, over 1200 fresh, prospectively-collected blood culture specimens were obtained from five large hospitals geographically distributed across the United States. The number and percentage of positive cases (positivity rate) determined by the reference method, stratified by geographic region for each of the organisms and antimicrobial resistance markers detected by **BC-GP** are presented in **Table 1**. In routine practice, prevalence rates may vary depending on the institution, geographical location, and patient population.

Table 1: Prevalence of Organisms Detected by the Reference Method – Clinical Study Observations

Organism			*US Geographic Region/Division*(1)					
			Northeast / Mid-Atlantic	*Midwest / East North Central*			*South / West South Central*	*Total*
		State	NY	OH	IL	WI	TX	
		Total n=	*384*	*271*	*131*	*139*	*326*	*1251*
S. aureus (SA)		*POS n=*	102	82	31	42	68	325
		Prev.	26.6%	30.3%	23.7%	30.2%	20.9%	26.0%
S. epidermidis (SE)		*POS n=*	75	70	30	40	101	316
		Prev.	19.5%	25.8%	22.9%	28.8%	31.0%	25.3%
mecA		*POS n=*	103	98	30	44	114	389
	Prev.	*Total*	26.8%	36.2%	22.9%	31.7%	35.0%	31.1%
		SA/SE	58.2%	64.5%	49.2%	53.7%	67.5%	60.7%
Staphylococcus spp.		*POS n=*	268	209	84	100	253	914
		Prev.	69.8%	77.1%	64.1%	71.9%	77.6%	73.1%
S. lugdunensis		*POS n=*	2	1	0	1	4	8
		Prev.	0.5%	0.4%	0%	0.7%	1.2%	0.6%
E. faecium (EFC)		*POS n=*	13	9	1	8	5	36
		Prev.	3.4%	3.3%	0.8%	5.8%	1.5%	2.9%
E. faecalis (EFL)		*POS n=*	25	18	9	10	13	75
		Prev.	6.5%	6.6%	6.9%	7.2%	4.0%	6.0%
vanA		*POS n=*	14	9	2	7	5	37
	Prev.	*Total*	3.6%	3.3%	1.5%	5.0%	1.5%	3.0%
		EFC/EFL	36.8%	33.3%	20.0%	38.9%	27.8%	33.0%
vanB		*POS n=*	0	0	0	0	0	0
	Prev.	*Total*	0%	0%	0%	0%	0%	0%
		EFC/EFL	0%	0%	0%	0%	0%	0%
Streptococcus spp.		*POS n=*	52	24	29	12	39	156
		Prev.	13.5%	8.9%	22.1%	8.6%	12.0%	12.5%
S. agalactiae		*POS n=*	10	9	8	6	7	40
		Prev.	2.6%	3.3%	6.1%	4.3%	2.1%	3.2%
S. anginosus group		*POS n=*	2	0	1	0	6	9
		Prev.	0.5%	0%	0.8%	0%	1.8%	0.7%
S. pneumoniae		*POS n=*	11	2	5	0	7	25
		Prev.	2.9%	0.7%	3.8%	0%	2.1%	2.0%
S. pyogenes		*POS n=*	3	0	2	1	4	10
		Prev.	0.8%	0%	1.5%	0.7%	1.2%	0.8%
Listeria spp.		*POS n=*	2	0	0	0	1	3
		Prev.	0.5%	0%	0%	0%	0.3%	0.2%

(1) *Census Bureau Regions and Divisions with State FIPS Codes*; US Census Bureau. http://www.census.gov/geo/www/us_regdiv.pdf. (20 June 2010).

PERFORMANCE CHARACTERISTICS

The results of the eleven studies conducted to establish the performance characteristics of **BC-GP** are provided below.

A. Clinical Performance

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. Eligible study subjects included individuals receiving routine care requiring blood culture testing, from which were collected blood culture specimens positive for microbial growth (using BACTEC™ Plus Aerobic/F and BacT/ALERT FA FAN® Aerobic blood culture bottles) and identified as gram stain positive. A total of 1642 evaluable specimens were utilized to establish clinical performance of the test, 1426 of which were prospectively-collected (including 1251 fresh and175 frozen) specimens and 216 simulated specimens.

The clinical performance of **BC-GP** for the 15 organisms/targets detected are summarized below and stated for each target in **Table 2** for *Staphylococcus* spp.*, Micrococcus* spp. and *Listeria* spp.*,* **Table 3** for *Enterococcus* spp.*,* and **Table 4** for *Streptococcus* spp*.* **BC-GP** test results obtained during the study were compared with results from culture/conventional biochemical, cefoxitin disk, and/or bi-directional sequencing detection techniques, as appropriate and designated in each table. Test performance is also stratified by the manner by 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 tables. Additionally, simulated specimens were utilized for very rare organisms such as *S. lugdunensis*, *S. agalactiae*, *S. anginosus* group, *S. pyogenes*, and *Listeria* spp. These specimens were prepared from glycerol stocks grown on blood agar plates, from which individual colonies were used to inoculate standard aerobic blood culture media bottles containing whole blood, which were grown to positivity on continuous monitoring blood culture systems.

The percent positive and negative agreement, as well as the two-sided 95% confidence limits, observed for the detection of each of the organisms are provided in **Tables 2-4**.

Table 5 and **Table 6** provide the clinical performance of **BC-GP** linking *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), respectively. In the study protocol, *Staphylococcus* spp. (other than *S. aureus* and *S. epidermidis*) were not tested for methicillin resistance by cefoxitin disk analysis. However, 12 *Staphylococcus* spp. (as determined by the reference method) were positive for *S. epidermidis* and *mecA* by **BC-GP** and have been counted as false positives for *mecA* in **Table 2**. These 12 specimens are accounted for in the Negative Agreement calculation (see footnote (e) of **Table 2** and footnote (l) of **Table 5**)

In total, there were 98 mixed specimens that were detected either by **BC-GP**, the reference culture methods or both. **Table 7** lists the distinct mixed specimen combinations detected by **BC-GP** in the clinical study and **Table 8** lists the additional distinct mixed specimen combinations detected by the reference/comparator methods, but not detected by **BC-GP**.

Table 9 contains additional genus/group-level specific **BC-GP** performance data stratified by individual spp. within each genus; i.e.; *Staphylococcus* spp. (other than *SA, SE, S. lugdunensis), Streptococcus* spp. (other than *S. agalactiae, S. pyogenes, S. pneumoniae) Listeria* spp. and *Streptococcus anginosus* group.

Table 2: Summary of Clinical Test Performance versus Reference Method(s) – *Staphylococcus* and *Listeria*

Specimen Type		n=	% Agreement (95% CI) Positive	% Agreement (95% CI) Negative	Reference Method
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%[a] 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%[b] 296/318 (89.7-95.6)	98.9%[c] 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%[d] 375/398 (91.5-96.3)	98.2%[e] 1009/1028 (97.1-98.9)	
Simulated		216	-	100% 216/216 (98.3-100)	
***Staphylococcus* spp.**					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%[f] 925/944 (96.9-98.8)	99.4%[g] 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%[h] 19/20 (75.1-99.9)	100% 1406/1406 (99.7-99.9)	
Simulated		216	100% 20/20 (83.2-100)	99.5%[i] 195/196 (97.2-99.9)	
***Listeria* spp.**					
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)	

Footnote		BC-GP Result	Reference Method Result	Mixed Specimen	Comments
a	1.	*EFL*	*EFL(vanS) & MRSA*	*X*	} Original blood culture Gram stain GPCCH; Low titer (1st quadrant growth) MRSA upon sub-culture
	2.	*EFC*	*EFC(vanS) & MRSA*	*X*	
	3.	*Staph spp.*	*SA*		
b	1.	*Staph spp.*	*MRSE*		
	2.	*Staph spp.*	*MRSE*		
	3.	*Not Detected*	*MRSE*		*Staph spp.* upon repeat BC-GP testing
	4.	*Staph spp.*	*MRSE*		
	5.	*Strep spp. & S. agalactiae*	*MRSE & S. agalactiae*	*X*	
	6.	*Staph spp.*	*MRSE& S. hominis*	*X*	
	7.	*Staph spp.*	*MRSE*		
	8.	*Staph spp.*	*MRSE*		
	9.	*Staph spp.*	*MRSE & S. hominis & S. auricularis*	*X*	
	10.	*Staph spp.*	*MRSE*		
	11.	*Staph spp.*	*SE & S. capitis*	*X*	
	12.	*Staph spp.*	*SE*		
	13.	*Not Detected*	*SE*		Not Detected result upon repeat BC-GP testing
	14.	*Staph spp.*	*SE & S. hominis*	*X*	
	15.	*Staph spp.*	*SE & S. hominis*	*X*	
	16.	*Staph spp.*	*SE*		
	17.	*EFL*	*EFL(vanS) & MRSE*	*X*	
	18.	*EFL (vanA)*	*EFL (vanR) & MRSE*	*X*	
	19.	*EFC (vanA)*	*EFC(vanR) & MRSE*	*X*	
	20.	*EFL (vanA)*	*EFL (vanR) & MRSE*	*X*	
	21.	*Strep spp. & S. pyogenes*	*SE (methR) & S. pyogenes*	*X*	
	22.	*Staph spp.*	*S. hominis & SE, (methR)*	*X*	
c	1.	*Staph spp. & SE & mecA*	*S. capitis*		
	2.	*Staph spp. & SE & mecA*	*Staph spp.* (CoNS) & *S. lugdunensis*	*X*	
	3.	*Staph spp. & SE & mecA*	*S. haemolyticus*		
	4.	*Staph spp. & SE & mecA*	*Staph spp.* (CoNS)		
	5.	*Staph spp. & SE & mecA*	*S. hominis*		
	6.	*Staph spp. & SE & mecA*	*S. haemolyticus*		
	7.	*Staph spp. & SE & mecA*	*S. intermedius*		
	8.	*Staph spp. & SE & mecA*	*S. hominis & S. capitis*	*X*	
	9.	*Staph spp. & SE & mecA*	*S. capitis*		
	10.	*Staph spp. & SE & mecA*	*Staph spp.* (CoNS)		
	11.	*Staph spp. & SE & mecA*	*S. haemolyticus*		
	12.	*Staph spp. & SE & mecA*	*S. hominis*		
d	1.	*Staph spp.*	*MRSE*		
	2.	*Staph spp.*	*MRSE*		
	3.	*Not Detected*	*MRSE*		*Staph* spp. upon repeat BC-GP testing
	4.	*Staph spp.*	*MRSE*		
	5.	*Strep spp. & S. agalactiae*	*MRSE & S. agalactiae*	*X*	
	6.	*Staph spp.*	*MRSE& S. hominis*	*X*	
	7.	*Staph spp.*	*MRSE*		
	8.	*Staph spp.*	*MRSE*		
	9.	*Staph spp.*	*MRSE & S. hominis & S. auricularis*	*X*	
	10.	*Staph spp.*	*MRSE*		
	11.	*Staph spp. & S. aureus*	*MRSA*		MSSA upon repeat cefoxitin disc and Vitek AST
	12.	*Staph spp. & S. aureus*	*MRSA*		MSSA upon repeat cefoxitin disc and Vitek AST
	13.	*Staph spp. & SE*	*MRSE*		
	14.	*Staph spp. & SE*	*MRSE*		
	15.	*Staph spp. & SE*	*MRSE*		
	16.	*EFL*	*EFL(vanS) & MRSE*	*X*	
	17.	*EFL (vanA)*	*EFL (vanR) & MRSE*	*X*	
	18.	*EFC (vanA)*	*EFC(vanR) & MRSE*	*X*	
	19.	*EFL (vanA)*	*EFL (vanR) & MRSE*	*X*	
	20.	*Strep spp. & S. pyogenes*	*SE (methR) & S. pyogenes*	*X*	
	21.	*Staph spp.*	*S. hominis & SE, (methR)*	*X*	
	22.	*EFL*	*EFL(vanS) & MRSA*	*X*	} Original blood culture Gram stain GPCCH; Low titer (1st quadrant growth) MRSA upon sub-culture
	23.	*EFC*	*EFC(vanS) & MRSA*	*X*	
e	1.	*Staph spp. & SE & mecA*	*S. capitis*		} Reference method did not detect *SE* or *SA*, therefore the presence or absence of methicillin resistance was not confirmed
	2.	*Staph spp. & SE & mecA*	*Staph spp.* (CoNS) & *S. lugdunensis*	*X*	
	3.	*Staph spp. & SE & mecA*	*S. haemolyticus*		
	4.	*Staph spp. & SE & mecA*	*Staph spp.* (CoNS)		
	5.	*Staph spp. & SE & mecA*	*S. hominis*		
	6.	*Staph spp. & SE & mecA*	*S. haemolyticus*		
	7.	*Staph spp. & SE & mecA*	*S. intermedius*		
	8.	*Staph spp. & SE & mecA*	*S. hominis & S. capitis*	*X*	
	9.	*Staph spp. & SE & mecA*	*S. capitis*		
	10.	*Staph spp. & SE & mecA*	*Staph spp.* (CoNS)		
	11.	*Staph spp. & SE & mecA*	*S. haemolyticus*		
	12.	*Staph spp. & SE & mecA*	*S. hominis*		
	13.	*Staph spp. & SA & mecA*	*SA*		
	14.	*Staph spp. & SA & mecA*	*SA & S. hominis*	*X*	
	15.	*Staph spp. & SE & mecA*	*SE*		
	16.	*Staph spp. & SE & mecA*	*SE & S. capitis*	*X*	
	17.	*Staph spp. & SE & mecA*	*SE & Staph spp.* (CoNS)	*X*	
	18.	*Staph spp. & SE & mecA*	*SE*		
	19.	*Staph spp. & SE & mecA*	*SE*		
f	1.	*Not Detected*	*SE*		*Staph spp.* upon repeat BC-GP testing
	2.	*Strep spp. & S. agalactiae*	*SE & S. agalactiae*	*X*	
	3.	*Not Detected*	*SE*		Not Detected result upon repeat BC-GP testing
	4.	No Call-VARIATION	*S. capitis*		No Call-VARIATION upon repeat BC-GP testing
	5.	*Not Detected*	*S. haemolyticus*		Not Detected result upon repeat BC-GP testing
	6.	*EFL*	*EFL & S. warneri & yeast spp.*	*X*	
	7.	*Not Detected*	*S. hominis*		Not Detected result upon repeat BC-GP testing
	8.	*EFL*	*EFL(vanS) & S. haemolyticus*	*X*	
	9.	*EFL*	*EFL(vanS) & MRSA*	*X*	
	10.	*EFL*	*EFL(vanS) & MRSE*	*X*	
	11.	*EFL*	*EFL(vanS) & S. haemolyticus*	*X*	
	12.	*EFC (vanA)*	*EFC(vanR) & S. warneri*	*X*	
	13.	*Not Detected*	*S. hominis*		*Staph spp.* upon repeat after Not Detected, Pre-AE, No Call-VARIATION
	14.	*EFL (vanA)*	*EFL (vanR) & MRSE*	*X*	
	15.	*EFC (vanA)*	*EFC(vanR) & S. capitis*	*X*	
	16.	*EFC (vanA)*	*EFC(vanR) & MRSE*	*X*	
	17.	*EFL (vanA)*	*EFL (vanR) & MRSE*	*X*	
	18.	*EFC*	*EFC(vanS) & MRSA*	*X*	
	19.	*Strep spp. & S. pyogenes*	*SE (methR) & S. pyogenes*	*X*	
g	1.	*Strep spp. & Staph spp.*	*S. mitis & Corynebacterium spp*	*X*	
	2.	*Staph spp. & EFL*	*EFL(vanS)*		
	3.	*Staph spp.*	*A. viridans*		
h		*Staph spp. & SE & mecA*	*S. lugdunensis & S. cohnii or S. warneri*	*X*	
i		*Staph spp. & S. lugdunensis*	*S. xylosus*		

Table 3: Summary of Clinical Test Performance versus Reference Method(s) – *Enterococcus* spp.

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)	
Prospective	Frozen	175	100% 21/21 (83.9-100)	100% 154/154 (97.6-100)	
Prospective	Total	1426	96.9%[j] 93/96 (91.1-99.4)	99.9%[k] 1329/1330 (99.6-99.9)	
Simulated		216	92.3%[j] 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)	
Prospective	Frozen	175	100% 32/32 (89.1-100)	100% 143/143 (97.5-100)	
Prospective	Total	1426	97.1%[l] 66/68 (89.8-99.6)	100% 1358/1358 (99.7-100)	
Simulated		216	100% 46/46 (92.3-100)	99.4%[m] 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)	
Prospective	Frozen	175	96.9% 31/32 (83.8-99.9)	97.9% 140/143 (94.0-99.6)	
Prospective	Total	1426	94.2%[n] 65/69 (85.8-98.4)	99.8%[o] 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)	
Prospective	Frozen	175	100% 3/3 (29.2-100)	100% 172/172 (97.9-100)	
Prospective	Total	1426	100% 3/3 (29.2-100)	100% 1423/1423 (99.7-100)	
Simulated		216	97.3%[p] 36/37 (85.8-99.9)	100% 179/179 (98.0-100)	

Footnote		BC-GP Result	Reference Method Result	Mixed	Comments
j	1.	*Not Detected*	*EFL (vanS)*		*EFL* result upon repeat BC-GP testing
	2.	*Not Detected*	*EFL (vanR)*		*Not Detected* upon repeat BC-GP testing
	3.	*Not Detected*	*EFL (vanS)*		*Not Detected* upon repeat BC-GP testing
	4.	*EFC (vanB)*	*EFL (vanR)*		Additional reference testing yielded *EFC (vanR)*
k		*Strep spp. & S. pyogenes & E. faecalis*	*S. pyogenes*	*X*	
l	1.	*Not Detected*	*EFC (vanR)*		*Not Detected* upon repeat BC-GP testing
	2.	*Not Detected*	*EFC (vanR)*		*Not Detected* upon repeat BC-GP testing
m		*EFC (vanB)*	*EFL (vanR)*		Additional reference testing yielded *EFC (vanR)*
n	1.	*Not Detected*	*EFC (vanR)*		*Not Detected* upon repeat BC-GP testing
	2.	*Not Detected*	*EFC (vanR)*		*Not Detected* upon repeat BC-GP testing
	3.	*Not Detected*	*EFL (vanR)*		*Not Detected* upon repeat BC-GP testing
	4.	*EFL & Strep spp. & S. agalactiae*	*EFL (vanR) & S. agalactiae*	*X*	Negative for *vanA* and *vanB* by Bi-Directional Sequencing
o	1.	*EFC & vanA*	*EFC (vanS)*		Positive for *vanA* by Bi-Directional Sequencing
	2.	*EFC & vanA*	*EFC (vanS)*		Positive for *vanA* by Bi-Directional Sequencing
	3.	*EFC & vanA*	*EFC (vanS)*		Positive for *vanA* by Bi-Directional Sequencing
p		*E. faecium*	*EFC (vanR)*		Positive for *vanB* by Bi-Directional Sequencing

Table 4: 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%[q] 206/220 (89.6-96.5)	99.6%[r] 1201/1206 (99.0-99.9)	
Simulated		216	100% 92/92 (96.1-100)	99.2%[r] 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%[s] 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)	
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%[t] 1411/1414 (99.4-99.9)	
Simulated		216	100% 23/23 (85.2-100)	99.5%[t] 192/193 (97.2-99.9)	

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%[u] 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%[v] 53/54 (90.1-99.9)	100% 162/162 (97.8-100)	

FN		BC-GP Result	Reference Method Result	Mixed	Comments
q	1.	Not Detected	S. salivarius & R. mucilaginosa	X	Not Detected, then *Streptococcus spp.* upon repeat BC-GP testing
	2.	Not Detected	S. mitis		*Streptococcus spp.* upon repeat BC-GP testing
	3.	Not Detected	Viridans Streptococci	X	Not Detected upon repeat BC-GP testing
	4.	Not Detected	S. salivarius & Leuconostoc mesteroides	X	Pre-AE, then No Call – NEG CTL as initial results
	5.	Not Detected	S. agalactiae		*Streptococcus spp. & S. agalactiae* upon repeat BC-GP testing
	6.	Not Detected	S. salivarius		Pre-AE as initial result *Streptococcus spp.* upon repeat BC-GP testing (after Not Detected)
	7.	Not Detected	S. mitis		*Streptococcus spp.* upon repeat BC-GP testing
	8.	No Call-INT CTL 1	S. pyogenes		No Call- *INT CTL* 1 upon repeat BC-GP testing
	9.	No Call- INT CTL 1	Streptococcus viridans group and GNR	X	No Call- *INT CTL* 1 upon repeat BC-GP testing
	10.	Not Detected	S. mutans		No Call – VARIATION upon repeat BC-GP testing
	11.	Not Detected	S. sanguinis		Not Detected upon repeat BC-GP testing
	12.	Staphylococcus spp.	S. hominis & S. salivarius	X	
	13.	Staphylococcus spp.	S. viridans group & S. hominis	X	
	14.	No Call- INT CTL 1	Streptococcus viridans group		No Call-INT CTL 1 result upon repeat BC-GP testing
r	1.	Streptococcus spp.	Lactococcus garviae		
	2.	Streptococcus spp.	Lactococcus garviae		
	3.	Streptococcus spp.	Lactococcus spp.		
	4.	Streptococcus spp.	G. adjacens		
	5.	EFL & Streptococcus spp.	EFL & Bacillus spp. (not anthracis) & GNR	X	Original blood culture Gram stain results – GPCCH, GPR, and GNR
	6.	Listeria spp. & Streptococcus spp. & S. anginosus group	Listeria monocytogenes	X	
s		Not Detected	S. agalactiae		*Streptococcus spp. & S. agalactiae* result upon repeat BC-GP testing
t	1.	Listeria spp. & Streptococcus spp. & S. anginosus group	Listeria monocytogenes	X	
	2.	Streptococcus spp. &S. anginosus group	Viridans Streptococci		
	3.	Streptococcus spp. & S. anginosus group & S. pneumoniae	S. pneumoniae	X	
	4.	Streptococcus spp. & S. anginosus group	Viridans Streptococci		
u	1.	Streptococcus spp. & S. pneumoniae	S. mitis		
	2.	Streptococcus spp. & S. pneumoniae	S. mitis		
	3.	Streptococcus spp. & S. pneumoniae	S. mitis		
	4.	Streptococcus spp. & S. pneumoniae	Viridans Streptococci		Repeat reference culture testing identified *S. mitis*
	5.	Streptococcus spp. & S. pneumoniae	S. mitis		
v	1.	Streptococcus spp.	S. pyogenes		
	2.	No Call- INT CTL 1	S. pyogenes		No Call- *INT CTL* 1 upon repeat BC-GP testing

Table 5: Detection of *mecA* Linked 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*	335(c)	97.5% 157/161(b)(d) (93.8-99.3)	98.8% 172/174(e) (95.9-99.9)
S. epidermidis (including 12 specimens identified as SE by BC-GP only)(h)	94.2%(a) 375/398(b) (91.5-96.3)	330(c)	92.0% 219/238(b)(f) (87.8-95.1)	81.5% 75/92(g)(h) (72.1-88.9)

(a) Positive Percent Agreement for combined mecA performance as stated in Table 2

(b) One specimen positive for S. aureus, S. epidermidis and mecA and counted once as a true positive in each of these categories.

(c) Prospectively-collected specimens only

	No Observed	*BC-GP Result*	*No Observed*	*Reference Method Result*	*Comments*
(d) n=4	*1*	*EFL*	*1*	*EFL(vanS) & MRSA*	Original blood culture Gram stain GPCCH; Low titer (1st quadrant growth) MRSA upon sub-culture
	1	*EFC*	*1*	*EFC(vanS) & MRSA*	
	2	*Staph spp. & S. aureus*	*2*	*MRSA*	MSSA upon repeat cefoxitin disc and Vitek AST
(e) n=2	*1*	*Staph spp. & SA & mecA*		*SA*	
	1	*Staph spp. & SA & mecA*		*SA & S. hominis*	
(f) n=19	*9*	*Staph spp.**	*6*	*MRSE*	
			2	*MRSE & S. hominis,*	
			1	*MRSE & S. hominis & S. auricularis*	
	3	*Staph spp. & SE*	*3*	*MRSE*	
	1	*Staph spp. & S. agalactiae**	*1*	*MRSE & S. agalactiae*	
	1	*Not Detected*	*1*	*MRSE*	*Staph spp.* upon repeat BC-GP testing
	2	*EFL (vanA)*	*2*	*EFL (vanR) & MRSE*	
	1	*EFL*	*1*	*EFL (vanS) & MRSE*	
	1	*Strep spp. & S. pyogenes*	*1*	*SE (methR) & S. pyogenes*	
	1	*EFC (vanA)*	*1*	*EFC (vanR) & MRSE*	
(g) n=5	*5*	*Staph spp. & SE & mecA*	*3*	*SE*	
			1	*SE & S. capitis,*	
			1	*SE & Staph spp. (CoNS)*	
(h) n=12	*12*	*Staph spp. & SE & mecA*	*2*	*S. capitis§*	
			3	*S. haemolyticus§*	
			1	*Staph spp. (CoNS) & S. lugdunensis§*	
			2	*S. hominis§*	
			2	*Staph spp. (CoNS) §*	
			1	*S. intermedius§*	
			1	*S. hominis & S. capitis§*	

**BC-GP test did not detect SA or SE, therefore the presence or absence of mecA could not be confirmed.*

§Staphylococcus spp. (other than SA and SE) not tested by cefoxitin disk analysis, therefore presence or absence of methicillin resistance could not be confirmed

Table 6: 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%[a] 80/84[b] (88.3-98.7)	109[d]	85.7% 12/14[b][e] (57.2-98.2)	100% 95/95 (96.2-100)
E. faecium		114[d]	97.2% 69/71[b][f] (90.2-99.7)	93.0% 40/43[g] (80.9-98.5)
E. faecalis	*vanB* 97.5%[c] 39/40 (86.8-99.9)	109[d]	100% 7/7 (59.0-100)	100% 102/102 (96.5-100)
E. faecium		114[d]	97.0% 32/33[h] (84.2-99.9)	100% 81/81 (95.6-100)

(a) Positive Percent Agreement for combined *vanA* performance as stated in Table 3
(b) One specimen positive for *E. faecalis, E. faecium and vanA* and counted once as a true positive in each of these categories
(c) Positive Percent Agreement for combined *vanB* performance as stated in Table 3
(d) Combined prospectively-collected and contrived specimens

	No Observed	*BC-GP Result*	*No Observed*	*Reference Method Result*
(e)	1	*Not Detected* (upon repeat testing)	1	*EFL (vanR)*
n=2	1	*EFL & Strep spp. & S. agalactiae*	1	*EFL (vanR) & S. agalactiae*
(f)	2	*Not Detected*	2	*EFC (vanR)*
(g)	3	*EFC & vanA*	3	*EFC (vanS)*
(h)	1	*E. faecium*	1	*EFC (vanR)*

Table 7: Clinical Mixed Specimen Combinations Detected by **BC-GP**

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 spp.*	3	0	
E. faecalis	*Streptococcus spp.*	1	1	*Streptococcus spp.*
E. faecium and vanA	*Staphylococcus spp.*	1	0	
Listeria	*S. anginosus group*	1	1	*S. anginosus group*
S. lugdunensis	*Listeria spp.*	1	0	
Staphylococcus spp.	*E. faecalis*	2	1	*Staphylococcus spp.*
Staphylococcus spp. and S. epidermidis and mecA+	*E. faecalis*	1	0	
Staphylococcus spp. and S. aureus	*Staphylococcus spp. and S. epidermidis and mecA+*	1	0	
Streptococcus spp. and S. agalactiae	*Staphylococcus spp. and S. aureus*	1	0	
Streptococcus spp. and S. pneumoniae	*Streptococcus spp. and S. anginosus group*	1	1	*S. anginosus group*
Streptococcus spp. and S. pyogenes	*E. faecalis*	1	1	*E. faecalis*
Streptococcus spp.	*Staphylococcus spp.*	2	1	*Staphylococcus spp.*
TOTAL		*17*	*6*	

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

Table 8: 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 spp. (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)*
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 spp. (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 spp.*
S. epidermidis (methS)	*S. warneri*		1	0	
S. epidermidis	*Staphylococcus spp. (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 spp. (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 spp.*		1	0	
S. salivarius	*R. mucilaginosa*		1	0	
Viridans Streptococcus	*S. mitis*		1	0	
S. agalactiae	*Proteus spp.*		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.

Table 9: 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)
Staphylococcus warneri	90.0% 18/20 (68.3-98.8)	100% 2/2 (15.8-100)
Staphylococcus xylosus	100% 2/2 (15.8-100)	100% 2/2 (15.8-100)
Staphylococcus spp. (CONS)	100% 29/29 (88.1-100)	NT

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 sanguinis	75% 3/4 (19.4-99.4)	100% 3/3 (29.2-100)
Streptococcus thoraltensis	NT	100% 1/1 (2.5-100)
Streptococcus viridans	83.3% 20/24 (62.6-95.3)	NT
Streptococcus uberis	100% 1/1 (2.5-100)	NT

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

*NT = "Not Tested" ** Analytical Reactivity (Inclusivity) (Section D)

The initial and final no-call and pre-analysis error (pre-AE) rates observed during the clinical trial were as stated in **Table 10**:

Table 10: Percent No-Call and Pre-AE

No-Call Rate(95% CI)		***Pre-AE Rate(95% CI)***		***Total No-Calls and Pre-AEs(95% CI)***	
*Initial**	*Final*	*Initial**	*Final*	*Initial**	*Final*
4.7% 77/1642 (3.7-5.8)	1.1% 18/1642 (0.7-1.7)	1.6% 24/1642 (0.9-2.2)	0.1% 2/1642 (0.0-0.4)	6.2% 101/1642 (5.0-7.4)	1.2% 20/1642 (0.8-1.9)

*Initial No-Calls and Pre-AEs were repeated according to Section C.2. of this package insert.

B. Precision and Reproducibility

The precision and reproducibility of **BC-GP** 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 (BP) (Concentration 1 - approx. 10^7-10^8 CFU/mL) and b) bottle positivity plus an additional 8 hours (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 *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 (*EFL*/*vanA* and SLUG, 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 SLUG 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.

C. Analytical Sensitivity (Limit of Detection)

Analytical sensitivity (LODs) for **BC-GP** was determined as follows:

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

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

Sixteen (16) strains of *Streptococcus* spp., *Staphylococcus* spp. (other than MRSA/SA/MRSE/SE), and *Listeria* spp. ranged from (CFU/mL):

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

The analytical sensitivity or limit of detection (LOD) 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 comprised 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 4. 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.

D. Analytical Reactivity (Inclusivity)

Analytical reactivity was determined for **BC-GP** by testing the organisms described below and listed in this section; 100% reactivity was observed with the organisms and strains tested, except for *Listeria grayi*, two strains of which were not detected. The BORSA strains were characterized as *mec*A negative and expectantly identified by **BC-GP** as *mec*A negative; however, they demonstrated an oxacillin minimum inhibitory concentration (MIC value) of 4 - 8µg/mL

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

E. faecium and *E. faecalis* (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*, and 45 *Staphylococcus* strains (of which four were *Staphylococcus lugdunensis).*

Separately, *in silico* analysis was performed by aligning the assay probes for each of the above strains against available GenBank sequence entries to ensure that **BC-GP** is able to detect these strains. All strains were grown to bottle positivity in blood culture bottles in automated blood culture instruments. Samples were tested for purity and counted to estimate concentration (CFU/mL) and tested in duplicate with **BC-GP**.

Staphylococcus

Methicillin-Resistant Staphylococcus aureus (MRSA) Strains

NRS642	NRS721	NRS686	NRS707	NRS675	NRS687	NRS736	ATCC BAA-39	ATCC BAA-1707	CDC ART2011006
NRS661	NRS735	NRS652	NRS732	NRS692	NRS688	NRS739	ATCC BAA-1762	CDC ART2011012	CDC ART2011008
NRS666	NRS738	NRS676	NRS714	NRS647	NRS693	NRS678	ATCC-BAA-1688	CDC ART2011004	CDC ART2011015
NRS669	NRS651	NRS730	NRS734	NRS654	NRS702	NRS685	ATCC BAA-44	CDC ART2011005	CDC ART2011013
NRS670	NRS701	NRS703	NRS384	NRS655	NRS716	NRS745	ATCC BAA-42	CDC ART2011007	CDC ART2011021
NRS673	NRS722	NRS724	NRS691	NRS657	NRS725	ATCC BAA-38	ATCC 33591	CDC ART2011014	CDC ART2011020
NRS681	NRS740	NRS643	NRS708	NRS659	NRS726	ATCC 70698	ATCC 33593	CDC ART2011010	CDC ART2011018
NRS696	NRS648	NRS662	NRS689	NRS667	NRS729	ATCC BAA-41	ATCC 43300	CDC ART2011001	CDC ART2011017
NRS705	NRS715	NRS683	NRS653	NRS677	NRS731	ATCC 70699	ATCC BAA-40	CDC ART2011019	
NRS719	NRS645	NRS694	NRS668	NRS684	NRS733	ATCC BAA-1720	ATCC BAA-43	CDC ART2011009	

BORSA Strains

SBK-01	SBK-03	SBK-06	SBK-09
SBK-02	SBK-05	SBK-07	SBK-10

S. epidermidis (MSSE)

ATCC 12228	MCW CS-168	MCW CS-77	ATCC 70583
MCW CS-106	MCW CS-74	HER 1292	

MRSE

ATCC 35983	ATCC 51625	ATCC 70567
ATCC 35984	ATCC 70565	MCW CS-70

Staphylococcus aureus (MSSA) Strains

ATCC BAA-1761	ATCC BAA-1749	ATCC 29213	ATCC 6538P	OSU CS-55	CDC ART2011003
ATCC BAA-1765	ATCC BAA-1759	ATCC 29247	OSU CS-10	OSU CS-129	CDC ART2011011
ATCC BAA-1721	ATCC 25923	ATCC 33594	OSU CS-76	OSU CS-84	CDC ART2011016

S. auricularis

ATCC 33750
ATCC 33753

S. capitis

ATCC 27840
MCW CS-78

S. caprae

ATCC 51548
MCW CS-112

S. cohnii

ATCC 29974
NCIMB 13637

S. haemolyticus

ATCC 29970	ATCC 31874	NARSA 62	
ATCC 43253	ATCC 29969	NARSA 69	
BAA-1693	NARSA 50	NARSA 116	

S. hominis

ATCC 27844
ATCC 700236
MCW CS-6

S. lugdunensis

HFH-FZ17
HFH-FZ19
HFH-FZ16
HFH-FZ20

S. intermedius

ATCC 29663
ATCC 49052

S. muscae

ATCC 49910

S. saccharolyticus

ATCC 14953
NCIMB 701260
WAL 15188
WAL 15161

S. pasteuri

ATCC 51128
ATCC 51129

S. schleiferi

ATCC 43808
ATCC 49545

S. sciuri

ATCC 29060
ATCC 29061

S. warneri

ATCC 27836
MCW CS-49

S. simulans

ATCC 27581
ATCC 27848

S. xylosus

ATCC 29971
ATCC 49148

S. saprophyticus

ATCC 15305
ATCC 35552

Enterococcus

Enterococcus faecalis Strains, vancomycin sensitive

ATCC 10100	ATCC 19433	ATCC 33186	ATCC 49332	ATCC 51188	MCW CS-40
ATCC 12399	ATCC 29200	ATCC 4200	ATCC 49474	CMC CS N-25b	MCW CS-65
ATCC 19083	ATCC 29212	ATCC 49149	ATCC 49533	CMC CS N-26b	

Enterococcus faecalis Strains, vancomycin resistant, vanA

			vanB
CL CS 8/19-1	CL CS 8/19-7	LC CS-E013B	ATCC 51299
CL CS 8/19-2	CL CS 8/19-8	LC CS-E014B	ATCC 51575
CL CS 8/19-5	CMC CS J-56	MCW CS-38	ATCC 700802
CL CS 8/19-6	CMC CS N-12		

Enterococcus faecium Strains, vancomycin sensitive

ATCC 19434	ATCC 27273	ATCC 49224	ATCC 6569	MCW CS-32
ATCC 19953	ATCC 31282	ATCC 49624	ATCC 9756	MCW CS-50
ATCC 23828	ATCC 35667	ATCC 51558	ATCC BAA-472	ATCC 700221

Enterococcus faecium Strains, vancomycin resistant, vanA

				vanB	
CL CS 8/19-3	KAL CS-1132	KAL CS-651	MCW CS-173	JMI CS-2231	JMI CS-3251
CL CS 8/19-4	KAL CS-1275	KAL CS-653	MCW CS-27	JMI CS-6818	JMI CS-13556
CMC CS K-24	KAL CS-297	KAL CS-661	MCW CS-53	JMI CS-1697	JMI CS-10004
CMC CS M-95	KAL CS-359	KAL CS-721	MCW CS-63	JMI CS-712	
CMC CS N-25	KAL CS-45	KAL CS-806	ATCC 51858		
CMC CS N-26	KAL CS-47	KAL CS-807			
KAL CS-1047	KAL CS-601	LC CS-E004			

Streptococcus

Streptococcus agalactiae Strains

ATCC 12386	ATCC 13813	CLCS-2	CLCS-32	CLCS-66	HFH-FZ13	NANBL-0003	NANBL-0033	NANBL-0019	NANBL-0499
ATCC 12401	ATCC 49446	CLCS 8/19 CS(-3)	CLCS-34	HFH-FZ11	HFH-FZ14	NANBL-0017	NANBL-0185	NANBL-0405	NANBL-0642
ATCC 12973	CLCS 8/19 CS(-1)	CLCS-17	CLCS-40	HFH-FZ12	HFH-FZ15	NANBL-0029	NANBL-0223	NANBL-0481	NCIMB 701523

Streptococcus pneumoniae Strains

ATCC 6315	ATCC BAA-1656	ATCC BAA-475	MCW CS-200	CMC 2009-134	NANBL-0835	CMC 30661	CMC 30706	MCW CS-130
ATCC 6321	ATCC 10015	ATCC BAA-659	MCW CS-81	CMC 2009-148	CMC 2011-24	CMC 30684	CMC 30723	OSU CS-19
ATCC 700673	ATCC 10357	OSU CS-77	MCW CS-93	CMC 2010-61	CMC 2011-37	NANBL-0839	CMC 70672	OSU CS-25
ATCC 700674	ATCC 49619	ATCC BAA-661	CMC 10371	CMC 2010-62	CMC 2011-38	NCIMB 13286	CMC 70675	OSU CS-60
ATCC 700678	ATCC 6301	CLCS-27	CMC 10389	NANBL-0055	CMC 2011-49	OSU CS-12	IHMA 191929	
ATCC 8335	ATCC BAA-1657	CLCS-50	CMC 10421	NANBL-0248	CMC 2011-6	OSU CS-13	IHMA 30355	
ATCC 8338	ATCC BAA-1667	MCW CS-141	CMC 2008-136	NANBL-0274	CMC 30646	CMC 30695	IHMA 311144	

S. anginosus Strains

ATCC 33397	CLCS-57	HFH-FZ31
ATCC 700231	CLCS-58	MCW CS-114
CLCS-31	HFH-FZ28	
MCW CS-19	HFH-FZ29	

S. bovis

ATCC 33317
ATCC 35034
NANBL-0002
NANBL-0817

S. constellatus

NANBL-0061
ATCC 70277
ATCC 702226

S. dysgalactiae

ATCC 12394
ATCC 35666

S. dysgalactiae subsp equisimilis

NANBL-0494
NANBL-0763
NANBL-0773

S. gallolyricus

HFH-FZ30
ATCC 9809
ATCC 49475

S. gallolyticus pasteurianus

ATCC 43144

S. gordonii

ATCC 10558
ATCC 35557

S. equi

ATCC 9528
ATCC 43079

S. equinus

ATCC 702757
ATCC 9812

S. infantarius

ATCC BAA-103 *(subsp. coli)*
ATCC BAA-102 *(subsp. Infantarius)*

S. mitis Strains

ATCC 15914	NANBL-0086
ATCC 49456	NANBL-0088
NANBL-0084	NANBL-0094
NANBL-0090	

S. mitis/oralis

MCW CS-122	OSU CS-159	MCW CS-134
NANBL-0310	OSU CS-226	OSU CS-99
NANBL-0654	OSU CS-90	MCW CS-135
OSU CS-107		

S. infantis

ATCC 700779
ATCC BAA-2089

S. intermedius

ATCC 9895
CLCS-59

S. mutans

ATCC 25175
ATCC 31383

S. oralis

ATCC 35037
ATCC 55229

S. pyogenes Strains			
NANBL-0581	CLCS-62	HFH-FZ8	NANBL-0583
ATCC 14289	HFH-FZ10	HFH-FZ9	NANBL-0595
ATCC 19615	HFH-FZ6a	NANBL-0065	NANBL-0597
CLCS-61	HFH-FZ7	NANBL-0387	NANBL-0656

S. parasanguinis
MCW CS-154
MCW CS-51
NANBL-0781
OSU CS-105

S. salivarius
ATCC 25975
ATCC 7073
OSU CS-161
OSU CS-170
OSU CS-367

S. sanguinis
ATCC 10056
MCW CS-11
NANBL-0633
ATCC BAA-1455

S. peroris
ATCC 700780

S. thoraltensis
MHA 298377

Listeria

Listeria monocytogenes Strains		
ATCC 15313	ATCC 7644	HFH-FZ27
ATCC 19115	HFH-FZ26	NCIMB 13449

*Listeria grayi**
ATCC 2540
ATCC 700545

L. innocua
ATCC 33090

L. ivanovii
ATCC 700402

L. seeligeri
ATCC 35967

L. welshimeri
ATCC 35897

**Not Detected*

E. Analytical Specificity (Exclusivity)

Analytical specificity was assessed using organisms phylogenetically related to panel organisms detected by **BC-GP** as well as those present as contaminants in blood culture specimens. The exclusivity samples were divided into two distinct panels of organisms. **BC-GP** 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 *Enterococcus avium,* which was identified as *E. faecium, Lactococcus lactis*, which was identified as *Streptococcus* spp., *Abiotrophia defective*, which was identified as both *Staphylococcus* spp.and *Streptococcus* spp., *Globicatella* spp., which was identified as *Listeria* spp., and *Staphylococcus intermedius* group, which is comprised of *S. delphini, S, intermedius*, and *S. pseudintermedius*, which identified as Listeria.

The first panel consisted of 130 "non-**BC-GP** panel" organisms which would not have been expected to be detected by **BC-GP**:

- Eighty-two (82) 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*); and
- Six (6) yeast strains.

The second panel consisted of over 450 "**BC-GP** panel" organisms, which in total comprised the analytical inclusivity study samples (see Section D, above for more information). 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); and
- *Listeria* strains (12).

F. Interfering Substances

The potential inhibitory effects of substances that may be encountered in blood and associated with the blood culturing process were tested with **BC-GP** at biologically or experimentally relevant concentrations. The interference testing was conducted in two parts: (a) by adding potential interferents present in patient blood specimens directly into blood cultures containing the bacterial strains and (b) by growing the strains in various blood culture media to bottle positivity, testing the resulting samples with **BC-GP** in both cases.

a. Exogenous Interferents

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 challenged with hemoglobin (14 g/L) , triglycerides (3000 mg/dL), conjugated and unconjugated bilirubin (20 mg/dL), gamma-globulin (6 g/dL), and Sodium Polyanetholesulfonate (SPS, 0.25% w/v) at concentrations approximately one log higher than reference levels (except SPS). None of the added interferents were found to have an impact on the performance of **BC-GP**.

b. Evaluation of Additional Blood Culture Bottles

In separate studies, representative bacterial strains were grown in a range of aerobic and anaerobic blood culture bottles/media (listed below) to bottle positivity and the cultures were tested to evaluate the suitability of these additional bottle types for use with **BC-GP** (in addition to those utilized to establish clinical performance of the test; i.e.; BACTEC Plus Aerobic /F and BacT/ALERT FA Fan Aerobic). All of the additional bottle types demonstrated correct results for all tests performed with two exceptions: (1) BACTEC Lytic/10 Anaerobic/F bottles for which 2 of 21 MRSE replicates were positive for *Staphylococcus* spp., but negative for *Staphylococcus epidermidis* and *mecA,* and (2) VersaTREK REDOX 2 EX Draw Anaerobic bottles for which 1 of 7 *Listeria monocytogenes* replicates was positive for *Staphylococcus* spp. in addition to *Listeria* spp.

All of the bottle types demonstrated an acceptable level of analytical performance when tested with **BC-GP.** The blood culture bottles evaluated include:

BACTEC™	***BacT/ALERT®***	***VersaTREK®***
Plus/Aerobic/F	SA Standard Aerobic	REDOX 1 EZ Draw® Aerobic
Plus/Anaerobic/F	FA FAN Aerobic	REDOX 2 EZ Draw® Anaerobic
Standard/10 Aerobic/F	PF Pediatric FAN	
Peds PlusTM	SN Anaerobic	
Standard Anaerobic/F	FN FAN® Anaerobic	
Lytic/10 Anaerobic/F		

G. Competitive Inhibition

Competitive inhibition was assessed using organisms detected by **BC-GP** combined in pairs (binary) with a set of related organisms commonly associated with blood stream infections; i.e.; the impact of an organism at high titer on the detection of a second organism was assessed. Therefore, competitive inhibition representing mixed bacteremia with one organism was evaluated by testing these samples at a high titer in the presence of a second organism at a low titer (at LOD) and vice versa. More specifically, 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. at their respective LOD (low titer) were combined in pairs with opposing high titer strains of these organisms (e.g.; *S. epidermidis* (low titer) / *S. agalactiae* (high titer), *S. aureus* (low titer) / *S. anginosus* (high titer), *S. pneumonia* (low titer) / *E. faecalis* (high titer), and so on) and tested with **BC-**

GP test. No evidence of competitive inhibition was observed for either the samples at the LOD or for the high titer samples.

H. Cutoff Verification

Retrospective clinical samples (65) were tested with **BC-GP** to verify the cut off values of a three-tiered filter algorithm determined with known reference strains and by using logistic fit and ROC statistics. Taking into consideration all the decisions, 2340 data points were utilized to verify the cut-off values.

I. Carryover/Cross-Contamination Study

A study was performed to assess the potential for carryover/cross-contamination with **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 **BC-GP**: Sample Extraction and Verigene Hybridization.

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TEST KIT LABELING

The contents of a Test Kit may use symbols defined below on labels.

REF	Catalog number
	Use by YYYY-MM-DD
LOT	Batch code
SN	Serial number
IVD	*In vitro* diagnostic medical device
	Manufacturer
	Upper Limit - Temperature limitation

	Upper and Lower Limit – Temperature limitation
	Consult instructions for use
	Key-code; Use this key-code to obtain instructions for use at www.e-labeling.eu
	Irritant
	Health Hazard
	Flammable
	Corrosive
	Environmentally Damaging
	Toxic

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