DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
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April 26, 2016

GREAT BASIN SCIENTIFIC, INC.
CHUCK OWEN
DIRECTOR, REGULATORY AFFAIRS & QUALITY ASSURANCE
2441 S. 3850 WEST
SALT LAKE CITY, UT 84120

Re: K152470
Trade/Device Name: Great Basin Staph ID/R Blood Culture Panel
Regulation Number: 21 CFR 866.3365
Regulation Name: Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures
Regulatory Class: II
Product Code: PAM, OOI
Dated: February 22, 2016
Received: February 25, 2016

Dear Mr. Owen:

This letter corrects our substantially equivalent letter of March 25, 2016.

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

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

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must

comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

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

Sincerely yours,

Kristian M. Roth -S

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

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

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

510(k) Number *(if known)*
K152470

Device Name
Great Basin Staph ID/R Blood Culture Panel

Indications for Use *(Describe)*

The Great Basin Staph ID/R Blood Culture Panel is a qualitative, multiplex, nucleic acid-based in vitro diagnostic assay intended for the simultaneous identification of nucleic acid from Staphylococcus aureus, Staphylococcus lugdunensis and various Staphylococcus species to the genus level and the detection of the mecA gene for methicillin resistance directly from patient positive blood culture specimens. The test utilizes automated hot-start enabled polymerase chain reaction (PCR) for the amplification of specific DNA targets detected by hybridization probes immobilized on a silicon chip surface. The assay is performed directly on positive blood culture specimens identified as positive by continuous monitoring blood culture system that demonstrates the presence of organisms as determined by Gram stain to contain gram-positive cocci in clusters (GPCC) or gram-positive cocci in singles (GPC). The test may be performed using blood culture bottles. The Staph ID/R Blood Culture Panel identifies Staphylococcus aureus (SA), and Staphylococcus lugdunensis, and detects other Staphylococcus species without identification to species level.

The Portrait Staph ID/R Blood Culture Panel is indicated for use in conjunction with other clinical or laboratory findings to aid in the diagnosis of bacterial bloodstream infections; however, it is not used to monitor these infections. Sub-culturing positive blood cultures is necessary to recover viable organisms for further identification, susceptibility testing, or epidemiological typing to identify organisms in the blood culture that are not detected by the Great Basin Staph ID/R Blood Culture Panel. If detected, mecA may or may not be associated with Staphylococcus spp. detected or the agent responsible for the disease. Negative results for mecA antimicrobial resistance gene assays do not always indicate susceptibility, as other mechanisms of resistance to methicillin exist.

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

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

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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March 23, 2016

510(k) Summary: Great Basin Staph ID/R Blood Culture Panel

A. Submitted by:

Great Basin Corporation
2441 South 3850 West
Salt Lake City, Utah 84120
Phone: 801-990-1055
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Contact Information

Chuck Owen, Director of Regulatory Affairs
Phone: 385-215-3313
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B. Name of Device

Proprietary Name:	Great Basin Staph ID/R Blood Culture Panel
Common or Usual Names:	Staph ID/R Blood Culture Panel Staph Assay SIDR

C. Regulatory Information:

a.	Regulation Section:	21 CFR 866.3365 – Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures 21 CFR 862.2570 – Instrumentation for clinical multiplex test systems
b.	Classification:	Class II (Staph ID/R Blood Culture Panel; non-exempt) Class II (PA500 Portrait Analyzer System)
c.	Classification panel:	Microbiology Devices, OIVD (83) Microbiology
d.	Product Code:	PAM (Gram-positive bacteria and their resistance markers) OOI (Real-time nucleic acid amplification system)

D. Intended use(s)/Indications for Use:

The Great Basin Staph ID/R Blood Culture Panel is a qualitative, multiplex, nucleic acid-based *in vitro* diagnostic assay intended for the simultaneous identification of nucleic acid from *Staphylococcus aureus, Staphylococcus lugdunensis* and various *Staphylococcus* species to the genus level and the detection of the *mecA* gene for methicillin resistance directly from patient positive blood culture specimens. The test utilizes automated hot-start enabled polymerase chain reaction (PCR) for the

amplification of specific DNA targets detected by hybridization probes immobilized on a silicon chip surface. The assay is performed directly on positive blood culture specimens identified as positive by continuous monitoring blood culture system that demonstrates the presence of organisms as determined by Gram stain to contain gram-positive cocci in clusters (GPCC) or gram-positive cocci in singles (GPC). The test may be performed using blood culture bottles. The Staph ID/R Blood Culture Panel identifies *Staphylococcus aureus* (SA), and *Staphylococcus lugdunensis,* and detects other *Staphylococcus* species without identification to species level.

The Portrait Staph ID/R Blood Culture Panel is indicated for use in conjunction with other clinical or laboratory findings to aid in the diagnosis of bacterial bloodstream infections; however, it is not used to monitor these infections. Sub-culturing positive blood cultures is necessary to recover viable organisms for further identification, susceptibility testing, or epidemiological typing to identify organisms in the blood culture that are not detected by the Great Basin Staph ID/R Blood Culture Panel. If detected, *mecA* may or may not be associated with *Staphylococcus* spp. detected or the agent responsible for the disease. Negative results for *mecA* antimicrobial resistance gene assays do not always indicate susceptibility, as other mechanisms of resistance to methicillin exist.

E. Device Description:

Test Principle:

The Great Basin Staph ID/R Blood Panel on the PA500 Portrait™ Analyzer System utilizes automated hot-start enabled polymerase chain reaction (PCR) amplification technology to amplify specific nucleic acid sequences that are detected using species specific *Staphylococcal* DNA hybridization probes immobilized on a modified silicon chip surface.

Target genomic DNA is extracted from microbial cells and diluted to reduce potential inhibitors of the PCR reaction. During the PCR process, double-stranded DNA is separated and target nucleic acid sequences are amplified by thermal cycling. Biotin-labeled primers direct amplification of specific nucleic acid sequences within a variable region of the *tuf* gene for identification of coagulase-negative *Staphylococcus* species, within conserved region of the thermonuclease (*nuc*) gene for specific identification of *Staphylococcus aureus*, and the *mecA* gene for detecting oxacillin/methicillin resistance. Following the PCR process, biotin-labeled, amplified target DNA sequences are hybridized to an array of probes immobilized on the silicon chip surface, then incubated with anti-biotin antibody conjugated to the horseradish peroxidase enzyme (HRP). The unbound conjugate is removed by washing and tetramethylbenzidine (TMB) is added to produce a colored precipitate at the location of the probe/target sequence complex. The resulting signal is detected by the automated Portrait Optical Reader within the Portrait System.

Test Device:

The Great Basin PA500 Portrait Analyzer System is a fully automated system that includes the Portrait Analyzer, single-use Staph ID/R Blood Culture Panel Test Cartridges, and the Portrait data analysis software. The PA500 Portrait Analyzer System is designed to perform automated sample preparation, PCR, and optical chip-based detection with integrated data analysis in approximately 110 minutes.

F. Substantial Equivalence Information:

a. Predicate Device: Verigene Gram Positive Blood Culture Nucleic Acid Test (BC-GP)
b. Predicate 510(k) number: K113450

c. Comparison with Predicate

Item	Staph ID/R Blood Culture Panel	Predicate (K113450)
Manufacturer	Great Basin Scientific, Inc.	Nanosphere, Inc.
Trade Name	Portrait™ Staph ID/R Blood Culture Panel	Verigene Gram Positive Blood Culture Nucleic Acid Test (BC-GP)
510(k) Number	K152470	K113450
	Similarities	
Classification	Class II	same
Intended Use/Indications for Use	The Great Basin Staph ID/R Blood Culture Panel is a qualitative, multiplex, nucleic acid-based in vitro diagnostic assay intended for the simultaneous identification of nucleic acid from Staphylococcus aureus, Staphylococcus lugdunensis and various Staphylococcus species to the genus level and the detection of the mecA gene for methicillin resistance directly from patient positive blood culture specimens. The test utilizes automated hot-start enabled polymerase chain reaction (PCR) for the amplification of specific DNA targets detected by hybridization probes immobilized on a silicon chip surface. The assay is performed directly on positive blood culture specimens identified as positive by continuous monitoring blood culture system that demonstrates the presence of organisms as determined by Gram stain to contain gram-positive cocci in clusters (GPCC) or gram-positive cocci in singles (GPC). The test may be performed using blood culture bottles. The Staph ID/R Blood Culture Panel identifies Staphylococcus aureus (SA), and Staphylococcus lugdunensis, and detects other Staphylococcus species without identification to species level. The Staph ID/R Blood Culture Panel is indicated for use in conjunction with other clinical or laboratory findings to aid in the diagnosis of bacterial bloodstream infections; however, it is not used to monitor these infections. Sub-culturing positive blood cultures is necessary to recover viable organisms for further identification, susceptibility testing, or epidemiological typing to identify organisms in the blood culture that are not detected by the Great Basin Staph ID/R Blood Culture Panel. If detected, mecA may or may not be associated with Staphylococcus spp. detected or the agent responsible for the disease. Negative results for mecA antimicrobial resistance gene assays do not always indicate susceptibility, as other mechanisms of resistance to methicillin exist.	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 indicated for use in conjunction with other clinical and laboratory findings, such as culture, to aid in the diagnosis of bacterial bloodstream infections; however, it is not used to monitor bloodstream infections. BC-Gp detects the following bacterial genera and species: *Staphylococcus spp., Staphylococcus aureus, Staphylococcus epidermidis, and Staphylococcus lugdunensis, Streptococcus spp., Streptococcus pneumonia, Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus anginosus group*, *Entercoccus faecalis, Enterococcus faecium, Listeria spp*. In addition, BC-GP detects the *mecA* resistance marker, and the *vanA* and *vanB* resistance markers, inferring *vanA/vanB* mediated vancomycin resistance to either *E. faecalis* or *E. faecium*, or the mecA-mediated methicillin resistance to either *S. aureus* or *S. epidermidis*. BC-GP is indicated for use in conjunction with other clinical or laboratory findings to aid in the diagnosis of bacterial bloodstream infections; however, it is not 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 marker genes to a specific organism, or for epidemiological testing.
Qualitative/ Quantitative	Qualitative	same
Single-Use Test Cartridge	Disposable, single-use, self-contained fluidic test cartridge	same
Automated	Yes	same
Test Principle	Multiplex nucleic acid array-based detection	same
Sample Types	Direct blood culture positive by Gram stain for GPCC or GPC	same
Organism and Resistance Marker Detection	*S. aureus, S. lugdunensis, Staphylococcus spp., mec* A	*S. aureus, S. lugdunensis, S. epidermidis, Staphylococcus spp., mec* A
Controls	One Internal Processing Control (whole organism complete assay control)	Two Internal Processing Controls (whole organism complete assay control and single-stranded DNA Hybridization control)
Calibration	Not required	same
	Differences	
Intended Use/Indications for Use	Identification of *S. aureus, S. lugdunensis,* detection of other *Staphylococcus* spp. Detection of the *mecA* gene for methicillin resistance in all Staphylococcus organisms.	Tests for same *Staphylococcus* targets. Detection of *mecA* gene for methicillin reistance in *S. aureus* and *S. epidermidis* only. Tests for additional gram-positive bacteria including *Streptococcus spp., Streptococcus pneumonia, Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus anginosus group* , *Entercoccus faecalis, Enterococcus faecium*,*and Listeria spp* . Tests for additional resistance markers including the *vanA* and *vanB* resistance markers, inferring *vanA/vanB* mediated vancomycin resistance to either *E. faecalis* or *E. faecium* .
Test Principle/ Technology	Fully automated multiplex PCR and detection by target-specific capture oligonucleotides immobilized in a macroarray format onto a chip surface for probe-based end-point.	Fully automated multiplex DNA detection of specific nucleic acid sequences in a microarray format using target-specific capture and mediator oligonucleotides for probe-based end-point detection.
Instrument	PA500 Portrait Analyzer	Verigene Reader and Processor SP
Time to Result	110 minutes	150 minutes

G. Performance Data – Analytical Studies

a. Analytical Sensitivity

The limit of detection (LoD) of the Staph ID/R Blood Culture Panel to *Staphylococcus* with or without *mecA* was assessed and confirmed with twenty-two (22) different strains. For these studies, overnight incubations of each bacterial stock in Tryptic Soy Broth (TSB) were measured by optical density to estimate the inoculum, serially diluted and spiked into a BACTEC Plus Aerobic/F blood bottle containing negative blood. Spiked bottles were incubated until alarm positivity in a BACTEC Blood Culture System. Alarm positive blood cultures were gram stained, diluted, plated on agar plates and colonies were counted the following day. The enumerated blood culture bottles were diluted to a $1x10^5$-10^6 targeted sample input CFU/mL range in BACTEC Plus Aerobic/F media containing negative blood, tested on device and plated on agar plates to enumerate the actual sample input CFU/mL. The estimations of the sample input CFU/mL were revised to reflect the correct CFU/mL by this colony counting method.

The LoDs for six (6) *S. aureus* strains ± *mecA* are reported as 3.5-8.2x10^5 CFU/mL; the LoD for each *S. aureus* strain is listed in Table 1.

Table 1. Performance of the Staph ID/R Blood Culture Panel on a minimum of 20 replicates of six (6) *Staphylococcus aureus* strains ± *mecA* for establishing LoD.

Species	ATCC #	Sample Input (CFU/mL)	Correct Staph ID/R Blood Culture Panel Results
S. aureus, mecA+	BAA-1680	3.5E+05	23/23
	BAA-1682	4.0E+05	20/20
	BAA-1684	8.2E+05	22/22
S. aureus, mecA-	25923	5.1E+05	21/21
	6538	3.9E+05	22/22
	11632	6.2E+05	20/20*

*This set of test runs also contained 1 "Invalid" run

The LoDs for six (6) *S. epidermidis* strains ± *mecA* are reported as 2.2-7.1x10^5 CFU/mL; the LoD for each *S. epidermidis* strain is listed in Table 2.

Table 2. Performance of the Staph ID/R Blood Culture Panel on a minimum of 20 replicates of six (6) *Staphylococcus epidermidis* strains ± *mecA* for establishing LoD.

Species	ATCC #	Sample Input (CFU/mL)	Correct Staph ID/R Blood Culture Panel Results
S. epidermidis, mecA+	35984	3.6E+05	20/20
	51625	4.0E+05	22/22
	700562	4.3E+05	27/27
	700566	5.8E+05	23/23
S. epidermidis, mecA-	700583	2.2E+05	23/23
	12228	7.1E+05	22/23

The LoDs for three (3) *S. lugdunensis* strains without *mecA* are reported as 2.8-4.7x10^5 CFU/mL; the LoD for each *S. lugdunensis* strain is listed in Table 3.

Table 3. Performance of the Staph ID/R Blood Culture Panel on a minimum of 20 replicates of three (3) *Staphylococcus lugdunensis* strains ± *mecA* for establishing LoD.

Species	ATCC #	Sample Input (CFU/mL)	Correct Staph ID/R Blood Culture Panel Results
S. lugdunensis, mecA-	43809	2.8E+05	22/23
	49576	4.5E+05	23/23
	7990	4.7E+05	23/23

LoDs for seven (7) *Staphylococcus* species ± *mecA*, excluding *S. aureus, S. epidermidis,* and *S. lugdunensis*, are reported as 1.5-5.3x10^5 CFU/mL. The species were selected for inclusion in the LoD studies based on *tuf* sequence variation from *in silico* analysis of the target sequence. The LoD for each *Staphylococcus* strain is listed in Table 4.

Table 4. Performance of the Staph ID/R Blood Culture Panel on a minimum of 20 replicates of seven (7) *Staphylococcus* strains ± *mecA* for establishing LoD.

Species	ATCC #	Sample Input (CFU/mL)	Correct Staph ID/R Blood Culture Panel Results
S. capitis, mecA -	35661	1.5E+05	20/20*
S. haemolyticus, mecA +	BAA-1693	3.1E+05	19/20
S. hominis, mecA -	27844	5.3E+05	23/23*
S. pasteuri, mecA -	51129	5.3E+05	21/22
S. sciuri, mecA -	29060	4.3E+05	21/21**
S. simulans, mecA -	27848	2.0E+05	23/23
S. warneri, mecA -	27830	4.0E+05	22/22

*This set of test runs also contained 1 "Invalid" run

**This set of test runs also contained 2 "Invalid" runs

b. Analytical Reactivity (Inclusivity)

The analytical reactivity of the Staph ID/R Blood Culture Panel was tested against an additional 48 well characterized *S. aureus* strains from ATCC representing USA100-1200 and SCC*mecA* I-VI, XI types representative of temporal and geographical diversity. In addition, 104 untyped strains, representing *S. aureus, S. epidermidis, S. lugdunensis,* and other various *Staphylococcus* species were tested in the Staph ID/R Blood Culture Panel.

The Staph ID/R Blood Culture Panel correctly detected all of the additional *Staphylococcus* strains, *mecA* present or absent (Table 5).

Table 5. Analytical Reactivity (Inclusivity) Panel. *Staphylococcus* strains and results for inclusivity by the Staph ID/R Blood Culture Panel.

Staphylococcus species; **ATCC, CCUG, NRS, Clinical #**	**SCCmec, PFGE type / source**	**Sample Input (CFU/mL)**	**Correct Staph ID/R Blood Culture Panel Result**
S. aureus BAA-38	I, Denmark	$1.0x10^6$	2/2
S. aureus BAA-44	I, Iberian	$9.2x10^5$	2/2
S. aureus 700698	II, Japan	$1.1x10^6$	2/2
S. aureus BAA-41	II, USA100	$5.9x10^6$	2/2
S. aureus BAA-1681	II, USA100	$1.3x10^6$	2/2
S. aureus BAA-1682	II, USA100	$2.7x10^6$	2/2
S. aureus BAA-1761	II, USA100	$1.2x10^6$	2/2
S. aureus NRS660	II, USA100	$4.1x10^6$	2/2
S. aureus BAA-1720	II, USA200	$2.3x10^6$	2/2
S. aureus BAA-1750	II, USA200	$9.5x10^5$	2/2
S. aureus BAA-1760	II, USA200	$2.4x10^6$	2/2
S. aureus NRS651	II, USA200	$3.1x10^6$	2/2
S. aureus BAA-39	III, Hungary	$6.4x10^6$	2/2
S. aureus 35592	III, ST239	$1.0x10^6$	2/2
S. aureus BAA-1680	IV, USA300	$2.4x10^6$	2/2
S. aureus BAA-1717	IV, USA300	$3.5x10^6$	2/2
S. aureus NRS643	IV, USA300	$3.4x10^6$	2/2
S. aureus NRS662	IV, USA300	$5.8x10^6$	2/2
S. aureus NRS688	IV, USA300	$4.4x10^6$	2/2
S. aureus NRS716	IV, USA300	$4.4x10^6$	2/2
S. aureus BAA-1683	IV, USA400	$1.2x10^6$	2/2
S. aureus BAA-1696	IV, USA400	$4.1x10^6$	2/2
S. aureus BAA-1707	IV, USA400	$4.1x10^6$	2/2
S. aureus BAA-1752	IV, USA400	$4.5x10^6$	2/2
S. aureus BAA-1684	IV, USA500	$1.8x10^6$	2/2
S. aureus BAA-1689	IV, USA500	$2.5x10^6$	2/2
S. aureus BAA-1763	IV, USA500	$6.4x10^6$	2/2
S. aureus NRS685	IV, USA500	$3.5x10^6$	2/2
S. aureus BAA-1754	IV, USA600	$6.7x10^6$	2/2
S. aureus BAA-1755	IV, USA700	$6.9x10^6$	2/2
S. aureus BAA-1758	IV, USA800	$8.8x10^5$	2/2
S. aureus BAA-1768	IV, USA800	$8.8x10^5$	2/2
S. aureus NRS692	IV, USA800	$2.0x10^6$	2/2
S. aureus NRS675	IV, USA800	$3.1x10^6$	2/2
S. aureus BAA-1747	IV, USA1000	$1.5x10^6$	2/2
S. aureus NRS483	IV, USA1000	$6.2x10^6$	2/2
S. aureus NRS730	IV, USA1000	$2.6x10^6$	2/2

S. aureus BAA-1764	IV, USA1100	$9.8x10^5$	2/2
S. aureus NRS484	IV, USA1100	$4.4x10^6$	2/2
S. aureus BAA-1766	V, USA700	$4.4x10^6$	2/2
S. aureus BAA-2094	V, WA-MRSA	$1.0x10^6$	2/2
S. aureus BAA-42	VI, USA800	$4.2x10^6$	2/2
S. aureus (*mecC*) BAA-2313	XI, CC130	$4.0x10^6$	2/2
S. aureus BAA-1751	Untyped, USA600	$4.0x10^6$	2/2
S. aureus BAA-1771	Untyped, USA800	$4.7x10^6$	2/2
S. aureus BAA-1718	NA, USA300	$4.6x10^6$	2/2
S. aureus BAA-1749	NA, USA900	$2.5x10^6$	2/2
S. aureus BAA-1765	NA, USA1200	$1.1x10^6$	2/2
S. aureus BAA-40	Untyped, Lisbon	$6.5x10^6$	2/2
S. aureus BAA-1685	Untyped, ATCC	$4.8x10^6$	2/2
S. aureus BAA-1708	Untyped, ATCC	$2.4x10^6$	2/2
S. aureus BAA-1721	Untyped, UK	$9.0x10^5$	2/2
S. aureus 6538	Untyped, ATCC	$3.1x10^6$	2/2
S. aureus 11632	Untyped, ATCC	$1.4x10^6$	2/2
S. aureus 12600	Untyped, ATCC	$2.1x10^6$	2/2
S. aureus 13150	Untyped, ATCC	$9.0x10^5$	2/2
S. aureus 14775	Untyped, ATCC	$3.1x10^6$	2/2
S. aureus 14776	Untyped, ATCC	$4.1x10^6$	2/2
S. aureus 14993	Untyped, ATCC	$2.4x10^6$	2/2
S. aureus 25923	Untyped, ATCC	$4.5x10^6$	2/2
S. aureus 29213	Untyped, ATCC	$4.0x10^6$	2/2
S. aureus 29247	Untyped, ATCC	$3.0x10^6$	2/2
S. aureus 43300	Untyped, ATCC	$3.2x10^6$	2/2
S. aureus 700699	Untyped, ATCC	$3.6x10^6$	2/2
S. aureus BORSA MCW1	Untyped, Wisconsin, Ledeboer Lab	$1.9x10^6$	2/2
S. aureus BORSA MCW2	Untyped, Wisconsin, Ledeboer Lab	$3.6x10^6$	2/2
S. aureus BORSA 23737	Untyped, New Jersey, Kreiswirth Lab	$2.9x10^6$	2/2
S. aureus BORSA 23739	Untyped, New Jersey, Kreiswirth Lab	$3.4x10^6$	2/2
S. aureus Empty Mec Cassette 45	Untyped, Iowa, Diekema lab	$6.3x10^5$	2/2
S. aureus Empty Mec Cassette 46	Untyped, Iowa, Diekema lab	$2.7x10^6$	2/2
S. aureus Empty Mec Cassette 50	Untyped, Iowa, Diekema lab	$3.7x10^6$	2/2
S. aureus Empty Mec Cassette 51	Untyped, Iowa, Diekema lab	$2.2x10^6$	2/2

S. auricularis 33751	ATCC	$3.2x10^5$	2/2
S. auricularis 33753	ATCC	$3.2x10^5$	2/2
S. capitis subsp. capitis 35661	ATCC	$7.2x10^5$	2/2
S. capitis subsp. ureolyticus 49326	ATCC	$2.2x10^6$	2/2
S. caprae 35538	ATCC	$9.2x10^5$	2/2
S. caprae 51548	ATCC	$1.3x10^6$	2/2
S. chromogenes 43764	ATCC	$4.8x10^6$	2/2
S. chohnii subsp. cohnii 29972	ATCC	$4.3x10^6$	2/2
S. chohnii subsp. urealyticus 49328	ATCC	$9.3x10^5$	2/2
S. condimenti 4753	ATCC	$1.2x10^6$	2/2
S. carnosus 51365	ATCC	$4.9x10^6$	2/2
S. delphini 49171	ATCC	$1.7x10^5$	2/2
S. epidermidis 12228	ATCC	$1.2x10^6$	2/2
S. epidermidis 35983	ATCC	$1.8x10^6$	2/2
S. epidermidis 35984	ATCC	$4.6x10^6$	2/2
S. epidermidis 51625	ATCC	$1.2x10^6$	2/2
S. epidermidis 700562	ATCC	$2.0x10^6$	2/2
S. epidermidis 700563	ATCC	$2.7x10^6$	2/2
S. epidermidis 700565	ATCC	$1.1x10^6$	2/2
S. epidermidis 700566	ATCC	$6.6x10^5$	2/2
S. epidermidis 700567	ATCC	$3.9x10^6$	2/2
S. epidermidis 700568	ATCC	$9.4x10^5$	2/2
S. epidermidis 700576	ATCC	$8.5x10^5$	2/2
S. epidermidis 700583	ATCC	$3.5x10^5$	2/2
S. equorum 43958	ATCC	$4.2x10^6$	2/2
S. felis 49163	ATCC	$1.1x10^6$	2/2
S. fleuretti BAA-274	ATCC	$1.3x10^6$	2/2
S. gallinarum 33539	ATCC	$2.2x10^6$	2/2
S. gallinarum 49148	ATCC	$2.5x10^6$	2/2
S. haemolyticus BAA-1693	ATCC	$3.5x10^6$	2/2
S. haemolyticus 43253	ATCC	$1.2x10^6$	2/2
S. haemolyticus 29968	ATCC	$4.8x10^6$	2/2
S. haemolyticus 29970	ATCC	$3.9x10^6$	2/2
S. haemolyticus 700564	ATCC	$3.5x10^6$	2/2
S. hominis 25615	ATCC	$3.2x10^6$	2/2
S. hominis 27844	ATCC	$1.6x10^6$	2/2
S. hominis 51624	ATCC	$3.0x10^6$	2/2
S. hominis 700586	ATCC	$2.4x10^6$	2/2
S. hominis subsp. novobiosepticus 700237	ATCC	$2.2x10^6$	2/2
S. intermedius 29663	ATCC	$1.1x10^6$	2/2
S. intermedius 49052	ATCC	$1.0x10^6$	2/2

S. intermedius 51874	ATCC	$2.5x10^6$	2/2
S. kloosii 43959	ATCC	$2.4x10^6$	2/2
S. lentus 29070	ATCC	$6.8x10^5$	2/2
S. lugdunensis 4436	CCM, Czech	$2.9x10^6$	2/2
S. lugdunensis 7990	ATCC, NCTC	$3.0x10^6$	2/2
S. lugdunensis 43809	ATCC	$2.0x10^6$	2/2
S. lugdunensis 48413	CCUG, Sweden	$4.3x10^6$	2/2
S. lugdunensis 49576	ATCC	$8.2x10^5$	2/2
S. lugdunensis 700328	ATCC	$3.0x10^6$	2/2
S. lutrae 700373	ATCC	$8.4x10^5$	2/2
S. massiliensis 7895	ATCC	$1.8x10^6$	2/2
S. muscae 49912	ATCC	$1.4x10^6$	2/2
S. nepalensis 48992	ATCC	$3.3x10^6$	2/2
S. pasteuri 51129	ATCC	$5.1x10^6$	2/2
S. pettenkoferi 36	Indianapolis, Denys Lab	$2.0x10^6$	2/2
S. piscifermentans 51183	ATCC	$2.4x10^6$	2/2
S. pulvereri 33938	ATCC	$1.2x10^6$	2/2
S. pseudintermidus 49444	ATCC	$1.7x10^6$	2/2
S. saccharolyticus 14953	ATCC	$7.3x10^5$	2/2
S. saprophyticus BAA-750	ATCC	$2.1x10^6$	2/2
S. saprophyticus 15305	ATCC	$1.7x10^6$	2/2
S. schleiferi subsp. coagulans 49545	ATCC	$1.7x10^6$	2/2
S. schleiferi subsp. schleiferi 43808	ATCC	$1.3x10^6$	2/2
S. sciuri 29061	ATCC	$5.5x10^5$	2/2
S. sciuri 29060	ATCC	$1.1x10^6$	2/2
S. sciuri 700013	ATCC	$2.9x10^6$	2/2
S. sciuri subsp. carnaticus 700058	ATCC	$2.1x10^6$	2/2
S. sciuri subsp. rodentium 700063	ATCC	$1.8x10^6$	2/2
S. simulans 27841	ATCC	$5.3x10^5$	2/2
S. simulans 27848	ATCC	$5.7x10^5$	2/2
S. simiae 7213	ATCC	$1.2x10^6$	2/2
S. succinus subsp. succinus 700337	ATCC	$1.6x10^5$	2/2
S. vitulinus 51162	ATCC	$1.1x10^6$	2/2
S. warneri 10209	ATCC	$2.4x10^6$	2/2
S. warneri 25614	ATCC	$1.1x10^6$	2/2
S. warneri 27836	ATCC	$4.2x10^6$	2/2
S. warneri 49454	ATCC	$1.4x10^6$	2/2
S. xylosus 35633	ATCC	$1.7x10^6$	2/2
S. xylosus 49148	ATCC	$3.5x10^6$	2/2

In addition to these studies, a subset of eight (8) *S. aureus* strains representing SCC*mecA* subtypes I-V, one (1) *mecC* strain, four (4) Borderline Oxacillin Resistant *S. aureus* (BORSA), four (4) Empty Cassette *S. aureus* variants, and multiple *S. epidermidis and S. lugdunensis* strains were selected to be part of a "Challenge Panel". The challenge panel was tested for oxacillin MIC using BD Phoenix ID. The results from the MIC determination and card results are listed in Table 6.

Table 6. Analytical Reactivity (Inclusivity) Challenge Panel. *Staphylococcus* strains tested for oxacillin MIC and for inclusivity by the Staph ID/R Blood Culture Panel.

Staphylococcus species	Strain	SCC*mec* Type	PFGE/Type Strain	Significance	Oxacillin MIC (µg/mL)	Staph ID/R Blood Culture Panel Result
S. aureus	BAA-38	I	Unknown	MRSA	>2	*S. aureus*, *mecA* Present
S. aureus	700699	II	Genome sequenced	MRSA	>2	*S. aureus*, *mecA* Present
S. aureus	BAA-1682	II	USA100	MRSA	>2	*S. aureus*, *mecA* Present
S. aureus	BAA-1681	II	USA100	MRSA	2	*S. aureus*, *mecA* Present
S. aureus	33592	III	ST239	MRSA	>2	*S. aureus*, *mecA* Present
S. aureus	BAA-1680	IV	USA300	MRSA	>2	*S. aureus*, *mecA* Present
S. aureus	BAA-1684	IV	USA500	MRSA	>2	*S. aureus*, *mecA* Present
S. aureus	BAA-2094	V	WA-MRSA	MRSA	1	*S. aureus*, *mecA* Present
S. aureus	BAA-2313	XI (*mecC*)	CC130	*mecC* - MRSA	2	*S. aureus*, *mecA* Absent
S. aureus	20723.046	NA	Unknown	Empty Cassette	0.5	*S. aureus*, *mecA* Absent
S. aureus	20723.051	NA	Unknown	Empty Cassette	0.5	*S. aureus*, *mecA* Absent
S. aureus	20723.045	NA	Unknown	Empty Cassette	≤0.25	*S. aureus*, *mecA* Absent
S. aureus	20723.050	NA	Unknown	Empty Cassette	≤0.25	*S. aureus*, *mecA* Absent
S. aureus	23736	NA	Unknown	BORSA	>2	*S. aureus*, *mecA* Absent
S. aureus	23739	NA	Unknown	BORSA	>2	*S. aureus*, *mecA* Absent
S. aureus	23737	NA	Unknown	BORSA	1	*S. aureus*, *mecA* Absent
S. aureus	MCW1	NA	Unknown	BORSA	0.5	*S. aureus*, *mecA* Absent
S. aureus	MCW2	NA	Unknown	BORSA	≤0.25	*S. aureus*, *mecA* Absent
S. aureus	12600	NA	serotype 3	MSSA	0.5	*S. aureus*, *mecA* Absent
S. aureus	14993	NA	Unknown	MSSA	0.5	*S. aureus*, *mecA* Absent
S. aureus	11632	NA	Unknown	MSSA	≤0.25	*S. aureus*, *mecA* Absent
S. aureus	6538	NA	Unknown	MSSA	≤0.25	*S. aureus*, *mecA* Absent
S. aureus	25923	NA	Unknown	MSSA	≤0.25	*S. aureus*, *mecA* Absent
S. epidermidis	35984	NA	Genome sequenced	MRSE	>1	*Staphylococcus* species OTHER than *S. aureus* or *S. lugdunensis*, *mecA* Present
S. epidermidis	35983	NA	Unknown	MRSE	>1	*Staphylococcus* species OTHER than *S. aureus* or *S. lugdunensis*, *mecA* Present
S. epidermidis	700562	NA	Unknown	MRSE	>1	*Staphylococcus* species OTHER than *S. aureus* or *S. lugdunensis*, *mecA* Present
S. epidermidis	700565	NA	Unknown	MRSE	>1	*Staphylococcus* species OTHER than *S. aureus* or *S. lugdunensis*, *mecA* Present
S. epidermidis	700567	NA	Unknown	MRSE	>1	*Staphylococcus* species OTHER than *S. aureus* or *S. lugdunensis*, *mecA* Present
S. epidermidis	700566	NA	Unknown	MRSE	>1	*Staphylococcus* species OTHER than *S. aureus* or *S. lugdunensis*, *mecA* Present
S. epidermidis	700576	NA	Unknown	MRSE	>1	*Staphylococcus* species OTHER than *S. aureus* or *S. lugdunensis*, *mecA* Present
S. epidermidis	51625	NA	Unknown	MRSE	1	*Staphylococcus* species OTHER than *S. aureus* or *S. lugdunensis*, *mecA* Present
S. epidermidis	12228	NA	Unknown	MSSE	≤0.25	*Staphylococcus* species OTHER than *S. aureus* or *S. lugdunensis*, *mecA* Absent
S. epidermidis	700583	NA	Unknown	MSSE	≤0.25	*Staphylococcus* species OTHER than *S. aureus* or *S. lugdunensis*, *mecA* Absent
S. hominis	700586	NA	Unknown	MR Staph ssp	>1	*Staphylococcus* species OTHER than *S. aureus* or *S. lugdunensis*, *mecA* Present
S. lugdunensis	49576	NA	Unknown	MS - Lug	≤0.25	*S. lugdunensis*, *mecA* Absent
S. lugdunensis	700328	NA	Unknown	MS - Lug	≤0.25	*S. lugdunensis*, *mecA* Absent
S. lugdunensis	NCTC 7990	NA	Unknown	MS - Lug	≤0.25	*S. lugdunensis*, *mecA* Absent
S. lugdunensis	43809	NA	Unknown	MS - Lug	≤0.25	*S. lugdunensis*, *mecA* Absent

All strains showed expected oxacillin MIC results, including the BORSA strains, which showed a range of oxacillin resistance (0.25-2 µg/mL) as expected for strains resistant by alternative mechanisms other than *mecA*. In addition, all empty cassette strains, which lack *mecA*, were sensitive to oxacillin (0.5-0.25 µg/mL). Samples were correctly detected as detected as *mecA* Present or Absent in the Staph ID/R Blood Culture Panel. The Staph ID/R did not detect *mecA* for *mecC* strain BAA-2313, empty cassette and BORSA strains as expected.

c. Analytical Specificity (Exclusivity)

Studies were performed to assess the potential cross-reactivity of the Staph ID/R Blood Culture Panel with 116 off-panel microflora (bacterial, yeast, and mycoplasma strains). BACTEC Plus Aerobic/F or Anaerobic/F media (for anaerobic strains) containing negative blood were inoculated with isolates and incubated in a BACTEC Blood Culture System until alarm positivity. Alarm positive samples were incubated for additional time in the Blood Culture System consistent with specimen stability studies to obtain a target microorganism bottle load $\geq 10^8$ CFU/mL. The positive alarm bottles were Gram stained, diluted, plated and counted to confirm all organisms were tested at $1x10^8$ CFU/mL or higher. For two (2) organisms, alarm positive bottles samples were substituted with genomic DNA at a final concentration of $\geq 10^8$ copies/mL. Genomic DNA was spiked into a matrix of negative blood and BACTEC Plus Aerobic/F media.

A minimum of two (2) replicates was tested in the Staph ID/R Blood Culture Panel for each of the bacterial, fungal and mycoplasma strains evaluated and these data are summarized in Table 7.

Table 7. Analytical Specificity (Exclusivity) Panel. Non-*Staphylococcus* microorganisms or DNA from micro-organisms tested for exclusivity by the Staph ID/R Blood Culture Panel.

Exclusivity Species	Strain (ATCC, CCUG, Clinical)	Sample Input in CFU/mL or copies/mL (gDNA)	Correct Staph ID/R Blood Panel Result
Gram Positive Bacteria			
Actinomyces odontolyticus	17929	$1.7x10^9$	2/2 (1)
Abiotrophia defectiva	49176	$1.4x10^8$	2/2
Aerococcus urinae	51268	$6.1x10^8$	2/2
Arcanobacterium haemolyticum	BAA-1784	$6.0x10^8$	10/11 (2)
Bacillus cereus	14579	$2.4x10^9$	2/2
Corynebacterium diphtheriae	11051	$1.5x10^9$	2/2
Corynebacterium jeikeium	43734	$6.0x10^8$	2/2
Enterococcus avium	14025	$1.4x10^9$	2/2
Enterococcus casseliflavus	700327	$1.7x10^9$	2/2
Enterococcus durans	6056	$7.8x10^8$	2/2
Enterococcus faecalis	29212	$1.7x10^9$	2/2
Enterococcus faecalis	19433	$1.4x10^9$	2/2
Enterococcus faecalis, van A	1MC	$3.1x10^8$	2/2
Enterococcus faecalis, van B	51575	$1.5x10^9$	2/2
Enterococcus faecium	19434	$4.0x10^8$	2/2
Enterococcus faecium	6057	$8.2x10^8$	2/2
Enterococcus faecium, van A	700221	$2.6x10^8$	2/2
Enterococcus gallinarum	700425	$4.1x10^8$	2/2
Enterococcus gallinarum	49573	$6.4x10^8$	2/2
Enterococcus hirae	8043	$6.9x10^8$	2/2
Enterococcus raffinosus	49464	$9.9x10^8$	2/2
Gemella morbillorum	27824	$1.0x10^9$	2/2
Globicatella sanguinis	51174	$6.2x10^8$	2/2

Kocuria kristinae	BAA-752	$1.7x10^9$	2/2
Kocuria rosea	186	$2.5x10^8$	2/2
Kytococcus schroeteri (oxacillin resistant)	BAA-2410	$1.8x10^8$	2/2
Lactobacillus acidophilus	4356	$4.8x10^8$	2/2
Lactococcus lactis	11454	$6.9x10^8$	2/2
Lactococcus lactis	40932	$2.1x10^9$	2/2
Leuconostoc mesenteroides subsp. mesenteroides	8293	$1.1x10^9$	2/2
Leuconostoc mesenteroides subsp. mesenteroides	19254	$9.7x10^8$	2/2
Leuconostoc pseudomesenteroides	12291	$1.7x10^9$	2/2
Listeria grayi	19120	$1.1x10^9$	2/2
Listeria innocua	33090	$1.3x10^8$	2/2
Listeria ivanovii	19119	$1.5x10^9$	2/2
Listeria monocytogenes	15313	$4.9x10^8$	2/2
Listeria seeligeri	35967	$1.1x10^9$	2/2
Macrococcus caseolyticus	13548	$8.3x10^8$	12/13 (1,2)
Micrococcus luteus	10240	$2.5x10^8$	2/2
Micrococcus lylae	27567	$5.8x10^8$	2/2
Mycobacterium avium	700898	$1.9x10^8$ (gDNA)	2/2
Pediococcus damnosus	29358	$1.2x10^9$	2/2
Pediococcus pentosaceus	33316	$2.9x10^8$	2/2
Peptostreptococcus anaerobius	27337	$2.3x10^8$	2/2
Planococcus citreus	14404	$9.2x10^8$	2/2
Planococcus kocurii	43650	$8.7x10^8$	2/2
Propionibacterium acnes	11827	$2.4x10^8$	2/2
Rhodococcus equi	6939	$6.8x10^8$	2/2
Rothia dentocariosa	BAA-907	$2.3x10^8$	2/2
Rothia mucilaginosa	49040	$9.6x10^8$	2/2
Streptococcus agalactiae	BAA-611	$8.7x10^8$	4/4
Streptococcus agalactiae	13813	$7.6x10^8$	2/2
Streptococcus angunosis	NCTC 10713	$7.0x10^8$	2/2
Streptococcus constellatus	27823	$1.1x10^9$	2/2
Streptococcus dysagalactiae	43078	$4.8x10^8$	2/2
Streptococcus equi	9528	$6.8x10^8$	2/2
Streptococcus gallolyticus	9809	$1.9x10^9$	2/2
Streptococcus gallolyticus	49475	$2.3x10^9$	2/2
Streptococcus mitis	6249	$1.0x10^8$	2/2
Streptococcus mutans	25175	$4.2x10^8$	2/2
Streptococcus mutans	35668	$5.1x10^8$	12/13 (2,3)
Streptococcus parasanguinis	15909	$2.2x10^8$	2/2
Streptococcus pneumoniae	ARUP	$1.6x10^8$	2/2
Streptococcus pyogenes	49399	$1.5x10^8$	2/2
Streptococcus pyogenes	12344	$1.0x10^8$	2/2
Streptococcus pyogenes	4543	$1.1x10^9$	2/2
Streptococcus sanguinis	10556	$9.0x10^8$	2/2
Streptococcus thoraltensis	700865	$1.1x10^9$	2/2
Streptococcus uberis	9927	$1.3x10^8$	2/2 (1)

Gram Negative Bacteria			
Acinetobacter baumannii	19606	$9.0x10^8$	2/2
Acinetobacter calcoaceticus	23055	$6.5x10^8$	2/2
Acinetobacter haemolyticus	19002	$3.0x10^8$	2/2
Acinetobacter lwoffi	17925	$1.4x10^9$	2/2
Bacteriodes fragilis	23745	$7.6x10^8$	2/2
Bordetella pertussis	9797	$1.2x10^9$	2/2
Burkholderia cepacia	25416	$4.4x10^8$	2/2
Citrobacter amalonaticus	25405	$3.3x10^8$	2/2
Citrobacter freundii	8090	$1.1x10^9$	2/2
Citrobacter koseri	27156	$1.0x10^9$	2/2
Enterobacter aerogenes	15038	$1.1x10^9$	2/2
Enterobacter cloacae	13047	$1.0x10^9$	2/2
Escherichia coli	BAA-199	$1.5x10^9$	2/2 (1)
Escherichia coli	4157	$1.1x10^9$	2/2
Fusobacterium nucleatum	25586	$6.7x10^8$	2/2
Haemophilus haemolyticus	33390	$1.4x10^9$	2/2
Hafnia alvei	13337	$1.6x10^9$	2/2
Klebsiella oxytoca	13182	$1.7x10^9$	2/2
Klebsiella pneumoniae	700603	$2.3x10^9$	2/2
Klebsiella pneumoniae	BAA-1705	$2.2x10^9$	2/2
Kluyvera intermedia	33421	$4.4x10^8$	2/2 (1)
Moraxella catarrhalis	23246	$2.1x10^9$	2/2 (1)
Morganella morganii	25829	$1.2x10^9$	2/2
Neisseria gonorrhoeae	19424	$2.3x10^9$	2/2
Neisseria meningitidis	13077	$1.8x10^9$	2/2 (1)
Neisseria subflava	49275	$3.5x10^7$	2/2 (1)
Oligella urethralis	17960	$3.1x10^8$	2/2
Proteus mirabilis	25933	$1.3x10^9$	11/13 (1,4)
Proteus vulgaris	6896	$2.8x10^8$	2/2
Providencia rettgeri	9250	$1.5x10^8$	2/2
Providencia rustigianii	13159	$7.3x10^8$	2/2
Pseudomonas aeruginosa	10145	$1.0x10^9$	2/2
Pseudomonas putida	49128	$1.2x10^9$	2/2
Salmonella enterica	14028	$2.1x10^9$	2/2
Salmonella typhimurium	13311	$1.3x10^9$	2/2 (1)
Serratia liquefaciens	27592	$6.0x10^8$	14/15 (2,5)
Serratia marcescens	13880	$4.4x10^8$	2/2
Shigella sonnei	29930	$7.6x10^8$	2/2
Stenotrophomonas maltophilia	13637	$1.2x10^9$	2/2
Yersinia enterocolitica	9610	$1.6x10^9$	2/2

Yeast			
Candida albicans	18804	$3.5x10^9$	2/2
Candida glabrata	66032	$2.2x10^9$	2/2
Candida krusei	24210	$9.3x10^8$	2/2
Candida parapsilosis	14054	$6.0x10^8$	2/2
Candida tropicalis	ARUP 2	$1.5x10^9$	2/2
Cryptococcus neoformans	90112	$1.1x10^9$	2/2
Mycoplasma			
Mycoplasma pneumoniae	15531	$2.7x10^8$ (gDNA)	2/2 (6)

(1) This set of test runs also contained 1 "Invalid" run
(2) This set of test runs contained 1 false positive result
(3) This set of test runs also contained 10 "Invalid" runs
(4) This set of test runs contained 2 false positive results
(5) This set of test runs also contained 5 "Invalid" runs
(6) This set of test runs also contained 2 "Invalid" runs

The vast majority of strains tested '*Staphylococcus* NEGATIVE,' indicating no cross-reactivity or interference with internal controls. The exceptions were twenty-seven (27) 'invalid' calls and six (6) '*Staphylococcus* POSITIVE' calls, all noted in Table 7.

One (1) 'invalid' call out of two (2) tests was observed for a single strain of the following species: *Actinomyces odontolyticus*, *Escherichia coli*, *Kluyvera intermedia*, *Moraxella catarrhalis*, *Neisseria meningitides, Neisseria subflava*, *Proteus mirabilis*, *Salmonella typhimurium*, and *Streptococcus uberis*. Each invalid case resolved upon re-testing as '*Staphylococcus* NEGATIVE'.

Two (2) 'invalid' calls out of two (2) tests were observed for *Mycoplasma pneumoniae* upon initial testing. Two (2) valid calls out of two (2) tests were obtained upon re-testing as '*Staphylococcus* NEGATIVE'.

One (1) '*Staphylococcus* POSITIVE' call out of two (2) tests was observed for a single strain of the following species: *Arcanobacterium haemolyticum*, *Macrococcus caseolyticus*, *Streptococcus mutans*, *Proteus mirabilis*, *Serratia liquefaciens*. Each positive result resolved upon re-testing as '*Staphylococcus* NEGATIVE' with a minimum of six (6) repeat tests, indicating the positive results previously obtained were likely a single contamination event in one card. One or more invalid calls were observed upon re-testing for the following species: *Macrococcus caseolyticus*, *Streptococcus mutans* and *Serratia liquefaciens* as noted in Table 7.

d. Microbial Interference

Off-Panel Microbial Interference: As a follow up to the previous exclusivity and inclusivity studies, the Staph ID/R Blood Culture Panel was further evaluated for the ability to detect low level *Staphylococcus* species in the presence of fourteen (14) "off-panel" microorganism strains that should not be detected. The "off-panel" strains represent Gram Positive, Gram Negative, Yeast and likely skin contaminants. BACTEC Plus Aerobic/F or Anaerobic/F Bottles containing blood were inoculated with competing "off-panel" microorganisms. The "off-panel" strains were grown to high concentrations by incubating 8 hours past bottle ring 'On-board', consistent with incubation time and temperatures tested in the specimen stability study. The bottle contents were confirmed by Gram stain and serial dilutions plated on agar and counted the following day to

confirm a concentration of >10^8 CFU/mL. Bottles were stored at 4°C and tested within 72 hours in combination with *Staphylococcus* TSB cultures at approximately 1-2.5x10^6 CFU/mL for each strain. The concentration of the *Staphylococcus* strains were verified by plating serial dilutions on agar and performing colony counts the following day. Results for the studies are shown in Table 8.

Table 8. Microbial Interference Panel (Off-panel): Non-*Staphylococcus* microbial strains tested for microbial interference in detecting five (5) *Staphylococcus* strains by the Staph ID/R Blood Culture Panel.

"Off-Panel" Microorganisms Species, Sample Input ≥ 10^8 CFU/mL; ATCC/NCTC strain #	Species; ATCC Strain #; Sample Input (CFU/mL)				
	S. aureus **BAA-1682** **0.7-1.2x10^6**	***S. aureus*** **11632** **0.6-1.6x10^6**	***S. epidermidis*** **700562** **0.8-0.9x10^6**	***S. epidermidis*** **700583** **0.9-2.2x10^6**	***S. lugdunensis*** **49576** **1-1.2x10^6**
Gram Positive Bacteria					
Corynebacterium jeikeium 43734	2/2	2/2	2/2	2/2	2/2
Enterococcus faecalis 19433	2/2	2/2	2/2"	2/2	2/2
Enterococcus faecium 19434	2/2	2/2	2/2"	2/2	2/2
Listeria monocytogenes 19115	2/2	2/2	2/2"	2/2"	2/2
Micrococcus luteus 10240	2/2	2/2	2/2	2/2	2/2
Propionibacterium acnes 11827	2/2	2/2	2/2	2/2	2/2
Streptoccocus agalactiae 13813	2/2	2/2	2/2*	2/2	2/2
Streptococcus anginosus 10713	2/2	2/2	2/2	2/2	2/2"
Streptococcus pneumoniae 27336	2/2	2/2	2/2	2/2	2/2
Streptococcus pyogenes 49399	2/2	2/2	2/2"	2/2	2/2
Gram Negative Bacteria					
Escherichia coli 4157	2/2	2/2	2/2	2/2"	2/2*
Klebsiella pneumoniae 700603	2/2	2/2	2/2	2/2	2/2
Pseudomonas aeruginosa 10145	2/2	2/2	2/2	2/2	2/2
Yeast					
Candida albicans 18804	2/2	2/2	2/2	2/2	2/2

*This set of test runs also contained 1 "Invalid" run

"This set of test runs initially miscalled, but called correctly with a higher CFU/mL input of the low level target

For the 'valid' runs tested, the potentially interfering 'off-panel' microorganisms did not interfere with the detection of the Staphylococcus strains, resulting in 'POSITIVE' calls as expected. In some cases, a miscall was observed, and the low-target *Staphylococcus* strains were re-tested at a higher concentration and resulted in a positive result as expected. The re-tested concentrations are included in the table and were within the 2-3X LoD range for each species.

***Staphylococcus* Microbial Interference:** Twelve (12) *Staphylococcus* species expected to be co-detected with the low level *Staphylococcus* species. BACTEC Plus Aerobic/F bottles containing blood were inoculated with *Staphylococcus* isolates. The bacteria were grown to high concentrations by incubating 8 hours past bottle ring 'On-board', consistent with incubation time and temperatures tested in the specimen stability study. The bottle contents were confirmed by Gram stain and serial dilutions plated on agar and counted the following day to confirm a concentration of >10^8 CFU/mL for competing *Staphylococcus* strains. Bottles were stored at 4°C and tested within 72 hours in combination with *Staphylococcus* TSB cultures at approximately 1-2.5x10^6 CFU/mL for each strain. The concentration of the *Staphylococcus* strains were verified

by plating serial dilutions on agar and performing colony counts the following day. Results for the studies are shown in Table 9.

Table 9. Microbial Interference Panel (*Staphylococcus*): *Staphylococcus* microbial strains tested for microbial interference in detecting five (5) different low level *Staphylococcus* strains by the Staph ID/R Blood Culture Panel.

Microbial Interference *Staphylococcus* species, Sample Input ≥ 10^8 CFU/mL; ATCC, Clinical #	Species; ATCC Strain #; Sample Input (CFU/mL)				
	S. aureus **BAA-1682** **0.7-1.2x10^6**	***S. aureus*** **11632** **0.6-1.6x10^6**	***S. epidermidis*** **700562** **0.2-0.9x10^6**	***S. epidermidis*** **700583** **0.9-2.2x10^6**	***S. lugdunensis*** **49576** **1-1.2x10^6**
S. aureus BAA-1682, *mecA+*	--	2/2	2/2"	2/2"	2/2
S. aureus 11632, *mecA* -	2/2	--	2/2"	2/2"	2/2
S. epidermidis 700562, *mecA+*	2/2	2/2	--	2/2"	2/2
S. epidermidis 700583, *mecA*-	2/2"	2/2"	2/2"	--	2/2"
S. lugdunensis 49576, *mecA* -	2/2	2/2	2/2"	2/2"	--
S. capitis 35661, *mecA* -	2/2	2/2	2/2"	2/2	2/2
S. caprae 35538, *mecA* -	2/2"	1/2*	2/2	2/2	2/2
S. hominis 27844, *mecA* -	2/2	2/2	2/2"	2/2	2/2"
S. haemolyticus BAA-1693, *mecA* +	2/2	2/2*	2/2"	2/2"	2/2
S. pettenkoferii Denys 38, *mecA* +	2/2	2/2	2/2	2/2	2/2
S. simulans 27848, *mecA* -	2/2	2/2	2/2"	2/2	2/2
S. warneri 27830, *mecA* -	2/2	2/2	2/2	2/2	2/2

*This set of test runs also contained 1 "Invalid" run

"This set of test runs initially miscalled, but called correctly with a higher CFU/mL input of the low level target

Staphylococcus interference was observed for *S. aureus* with *S. epidermidis* and *S. caprae* at initial concentrations tested (5.9E+05 CFU/mL), but the interference was resolved upon re-testing at higher concentrations (1.5E+06 CFU/mL, within 2-3X LoD). There were 13 cases of interference with *S. epidermidis* at initial concentrations (2.2-2.5E+05 CFU/mL) when tested against *S. aureus, S. epidermidis*, *S. lugdunensis*, *S. capitis, S. hominis, S. haemolyticus*, and *S. simulans*. Higher concentrations of *S. epidermidis* resolved the interference (8.5-9.4E+05 CFU/mL, within 2-3X LoD). Two (2) cases of interference were observed with *S. lugdunensis*: one (1) case with *S. epidermidis*, one (1) case with *S. hominis*. Both cases resolved at higher concentrations of *S. lugdunensis* (1.2E+06 CFU/mL, within 2-3X LoD).

e. Interfering Substances (Chemical Interference)

The Staph ID/R Blood Culture Panel was evaluated for interference by a panel of sixteen (16) different substances. Substances were spiked into BACTEC Plus Aerobic/F (with resin) or Standard Aerobic/F (without resin) media containing negative blood incubated 24 hours in a BACTEC Blood Culture Device. Target *Staphylococcus* cells were combined with the substances at low positive concentrations at approximately 2-3X LoD (1-2.5x10^6 CFU/mL). The CFU concentrations for each strain were estimated by optical density measurements and then confirmed by colony counting. The studies assessed the detection of the same ten (10) *Staphylococcus* ATCC strains used for analytical sensitivity and microbial interference: *S. aureus*, *mecA*+ strains BAA-1680 and BAA-1682, *S. aureus, mecA*+ strains 11632 and 6538, *S. epidermidis*, *mecA*+ strains 700562 and 51625, *S. epidermidis, mecA*- strains 700583 and 12228, and *S. lugdunensis, mecA*-

strains 49576 and 43809. ATCC strain, *E. faecalis, mecA*- 29212, an off-panel "Negative" in the Staph ID/R Blood Culture Panel, was also included in the study to assess chemical interference with the sample processing control and all downstream detection steps.

Similarly to previous studies, a minimum of two replicate assays was performed for each *Staphylococcus* strain using each substance in a background of BACTEC Plus Aerobic bottles (with resin) or Standard Aerobic bottles (without resin), see Tables 10 and 11.

Table 10. Interfering Substances Panel (BACTEC Plus with Resin). Staph ID/R Blood Culture Panel performance evaluation for chemical interference in detecting ten (10) different *Staphylococcus* strains and one (1) off-panel *E. faecalis* strain.

Substance Input Concentration into Plus Aerobic Media (with resin)	Species, ATCC strain, Sample Input (CFU/mL)										
	S. aureus, mecA +		*S. aureus, mecA*-		*S. lugdunensis, mecA* -		*S. epidermidis, mecA* +		*S. epidermidis, mecA*-		*E. faecalis* (Neg)
	BAA-1680 $0.9\text{-}1.8x10^6$	BAA-1682 $1.4\text{-}1.6x10^6$	11632 $1.2\text{-}2.2x10^6$	6538 $1.8\text{-}2.2x10^6$	43809 $1.1\text{-}1.7x10^6$	49576 $0.9\text{-}1.8x10^6$	51625 $0.3\text{-}1.5x10^6$	700562 $0.3\text{-}0.8x10^6$	12228 $0.9\text{-}1.2x10^6$	700583 $0.3\text{-}1.9x10^6$	29212 $2.4x10^7$
Whole Blood in ACD (≥35% v/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Whole Blood in EDTA (≥40% v/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Whole Blood in Heparin (≥40% v/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Whole Blood in Sodium Citrate (≥35% v/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Human Plasma (≥40%, v/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Sodium Polyanetholsulfonate (≥0.20% w/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Hemoglobin (≥10 mg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
γ-Globulin (≥40 mg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Triglycerides (≥10 mg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2*
White Blood Cells (≥40% v/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Platelets (≥40% v/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Unconjugated Bilirubin (≥0.075 mg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Conjugated Bilirubin (≥0.075 mg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Vancomycin (≥50 µg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Ciprofloxacin (≥7.5 µg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Cefoxitin (≥125 µg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2

*This set of test runs also contained 1 "Invalid" run

In Table 10, the substances did not interfere with detection of strains at the concentrations listed, resulting in Positive or Negative calls as expected.

Table 11. Interfering Substances Panel (BACTEC Standard without Resin). Staph ID/R Blood Culture Panel performance evaluation for chemical interference in detecting ten (10) different *Staphylococcus* strains and one (1) off-panel *E. faecalis* strain.

Substance Input Concentration into Standard Aerobic Media (without resin)	Species, ATCC strain, Sample Input (CFU/mL)										
	S. aureus, mecA+		*S. aureus, mecA-*		*S. lugdunensis, mecA-*		*S. epidermidis, mecA+*		*S. epidermidis, mecA-*		*E. faecalis* (Neg)
	BAA-1680 $0.9\text{-}1.8\text{x}10^6$	BAA-1682 $1.4\text{-}1.6\text{x}10^6$	11632 $1.2\text{-}2.2\text{x}10^6$	6538 $1.8\text{-}2.2\text{x}10^6$	43809 $1.1\text{-}1.7\text{x}10^6$	49576 $0.9\text{-}1.8\text{x}10^6$	51625 $0.3\text{-}1.5\text{x}10^6$	700562 $0.3\text{-}0.8\text{x}10^6$	12228 $0.9\text{-}1.2\text{x}10^6$	700583 $0.3\text{-}1.9\text{x}10^6$	29212 $2.4\text{x}10^7$
Whole Blood in ACD (≥35% v/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Whole Blood in EDTA (≥40% v/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Whole Blood in Heparin (≥40% v/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Whole Blood in Sodium Citrate (≥35% v/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Human Plasma (≥40%, v/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Sodium Polyanetholsulfonate (≥0.20% w/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Hemoglobin (≥10 mg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
γ-Globulin (≥40 mg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Triglycerides (≥10 mg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
White Blood Cells (≥40% v/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Platelets (≥40% v/v)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Unconjugated Bilirubin (≥0.075 mg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Conjugated Bilirubin (≥0.075 mg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2*
Vancomycin (≥50 µg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Ciprofloxacin (≥7.5 µg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Cefoxitin (≥125 µg/mL)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2

*This set of test runs also contained 1 "Invalid" run

As summarized in Table 11, the chemical substances tested did not interfere with detection of the majority of strains, resulting in Positive or Negative calls as expected.

f. Carry-over/Cross-Contamination Study

A study was performed to assess the cross-contamination of the Staph ID/R Blood Culture Panel by alternatively testing high titer *S. aureus*, *mecA*+ ATCC strain BAA-1682 and off-target negative *E. faecalis* ATCC strain 29212. BACTEC Plus Aerobic/F blood bottle containing negative blood were inoculated with strain isolates and incubated until alarm positivity in a BACTEC Blood Culture System. Alarm positive samples were incubated past positivity consistent with timeframes used during the specimen stability studies to obtain a high titer. Alarm positive blood cultures were Gram stained, diluted, plated on agar plates and colonies were counted the following day to confirm target concentrations $>10^7$ for *S. aureus* ($3.0x10^7$ CFU/mL) and $>10^8$ *E. faecalis* ($5.8x10^9$ CFU/mL). Aliquots from the high titer blood culture bottles were stored at -20°C until testing. Carry-over/cross-contamination was tested by running a series of alternating runs of high titer positive and negative samples on multiple Portrait Analyzers.

In conclusion, all of the 'calls' were in concordance with expected 'calls'. Therefore, there was no evidence of contamination in any of the tests.

g. Reproducibility

A multicenter, blinded, reproducibility study was performed to determine reproducibility of the Staph ID/R Blood Culture Panel. Testing occurred at three sites using a panel of seven simulated blood culture specimens, each spiked with a single organism. Specimens were prepared in a matrix of whole blood and blood culture media. Half of the replicates for the three *Staphylococcus* positive samples were consistent with the level of organism present at the time of positivity (low) and half were at a concentration similar to that observed after 8 hours of positivity (high). For the one off-panel organism (*E. faecalis*; Staph ID/R negative) the concentration was "high."

The study incorporated several variables including six different operators at three sites (two operators/site), five different cartridge lots, and 89 different Portrait Analyzers (15 at site 1, and 12 at site 3, 62 at site 4). Over the course of 10 weeks, samples were tested on 12 different days, for a total of 90 replicates per analyte per concentration.

Valid results were attained for 630 of 642 (98.1%) runs (see Table 12). For the detection of *Staphylococcus* positivity (Test result "Positive"), expected positive results were obtained for 540/540 runs (100%), and expected *Staphylococcus* negative results (Test result "Negative") were obtained in 87/90 runs (96.7%). For the detection of specific *Staphylococcus*, expected positive results were obtained for 538/540 runs (99.6%) and expected negative results were obtained for 1341/1350 (99.3%) results (Table 12).

Table 12. Summary of Reproducibility Study.

Staph ID/R Blood Culture Panel Result	Species, Bacteria Load (Low or High), Sample Input (CFU/mL)	Test Site	Detected	Not Detected	%Agreement with Expected Result
S. aureus, *mecA* present	*S. aureus* (*mecA* +), Low; $4.0x10^6$	1	30/30	0/30	90/90; 100%
		3	30/30	0/30	
		4	30/30	0/30	
		Total	**90/90**	**0/90**	
	S. aureus (*mecA* +) High; $4.0x10^7$	1	30/30	0/30	90/90; 100%
		3	30/30	0/30	
		4	30/30	0/30	
		Total	**90/90**	**0/90**	
	Negative	1	1/150 (1)	149/150	448/450; 99.6%
		3	1/150 (2)	149/150	
		4	0/150	150/150	
		Total	**2/450**	**448/450**	
Staphylococcus species OTHER than *S. aureus* or *S. lugdunensis*, *mecA* present	*S. epidermidis* (*mecA* +), Low; $8.5x10^6$	1	30/30	0/30	90/90; 100%
		3	30/30	0/30	
		4	30/30	0/30	
		Total	**90/90**	**0/90**	
	S. epidermidis (*mecA* +), High; $7.0x10^7$	1	30/30	0/30	90/90; 100%
		3	30/30	0/30	
		4	30/30	0/30	
		Total	**90/90**	**0/90**	
	Negative	1	2/150 (3)	148/150	446/450; 99.1%
		3	0/150	150/150	
		4	2/150 (4)	148/150	
		Total	**4/450**	**446/450**	
S. lugdunensis, *mecA* absent	*S. lugdunensis* (*mecA* -), Low; $6.0x10^7$	1	28/30 (3)	2/30	88/90; 97.8%
		3	30/30	0/30	
		4	30/30	0/30	
		Total	**88/90**	**2/90**	
	S. lugdunensis (*mecA* -), High; $5.1x10^8$	1	30/30	0/30	90/90; 100%
		3	30/30	0/30	
		4	30/30	0/30	
		Total	**90/90**	**0/90**	
	Negative	1	2/150 (5,6)	148/150	447/450; 99.3%
		3	1/150 (5)	149/150	
		4	0/150	150/150	
		Total	**3/450**	**447/450**	
Staphylococcus Positive	*S. aureus* , *S. epidermidis* , *S. lugdunensis*, Low and High	1	180/180	0/180	540/540; 100%
		3	180/180	0/180	
		4	180/180	0/180	
		Total	**540/540**	**0/540**	
Staphylococcus Negative	*E. faecalis*, (*mecA* -), High; $1.1x10^9$	1	1/30 (6)	29/30	87/90; 96.7%
		3	0/30	30/30	
		4	2/30 (4)	28/30	
		Total	**3/90**	**87/90**	

(1) Sample detected as "*Staphylococcus aureus* in mixed Staph infection (NOT *S. lugdunensis*)"
(2) One S. *lugdunensis* specimen additionally detected *S. aureus*
(3) Two *S. lugdunensis* specimens detected as *Staphylococcus OTHER than S. aureus or S. lugdunensis*
(4) Two *E. faecalis* specimens detected as *Staphylococcus* OTHER than *S. aureus* or *S. lugdunensis*
(5) One specimen detected *S. aureus* correctly, but additionally detected *S. lugdunensis*
(6) One *E. faecalis* specimen detected as *S. lugdunensis*

For detection of the *mecA* gene with no associated organism, positive results (*mecA* Present) were detected in 359/360 (99.7%) runs and negative results (*mecA* absent) were detected in 270/270 (100%) runs (see Table 13). For *S. aureus* (*mecA*+), positive *mecA* results were obtained for 180/180 (100%) runs and negative *mecA* results were obtained for 450/450 (100%) runs. For *S. epidermidis* (*mecA*+), positive *mecA* results were obtained for 179/180 (99.4%) runs and negative *mecA* results were obtained for 450/450 (100%) runs.

Table 13. Summary of *mecA* results from Reproducibility Studies.

Staph ID/R Blood Culture Panel Result	Species, Bacteria Load (Low or High), Sample Input (CFU/mL)	Test Site	Detected	Not Detected	%Agreement with Expected Result
S. aureus, mecA present	*S. aureus* (*mec*A+), Low; $4.0x10^6$	Site 1	30/30	0/30	90/90; 100%
		Site 3	30/30	0/30	
		Site 4	30/30	0/30	
		Total	**90/90**	**0/90**	
	S. aureus (*mec*A+) High; $4.0x10^7$	Site 1	30/30	0/30	90/90; 100%
		Site 3	30/30	0/30	
		Site 4	30/30	0/30	
		Total	**90/90**	**0/90**	
	Negative	Site 1	0/150	150/150	450/450; 100%
		Site 3	0/150	150/150	
		Site 4	0/150	150/150	
		Total	**0/450**	**450/450**	
Staphylococcus species OTHER than *S. aureus* or *S. lugdunensis, mecA* present	*S. epidermidis* (*mec*A+), Low; $8.5x10^6$	Site 1	30/30	0/30	90/90; 100%
		Site 3	30/30	0/30	
		Site 4	30/30	0/30	
		Total	**90/90**	**0/90**	
	S. epidermidis (*mec*A+), High; $7.0x10^7$	Site 1	29/30 (1)	1/30	89/90; 98.9%
		Site 3	30/30	0/30	
		Site 4	30/30	0/30	
		Total	**89/90**	**1/90**	
	Negative	Site 1	0/150	150/150	450/450; 100%
		Site 3	0/150	150/150	
		Site 4	0/150	150/150	
		Total	**0/450**	**450/450**	
mecA Present; no organism associated	*S. aureus* (*mec*A+), *S. epidermidis* (*mecA*+) Low and High	Site 1	119/120	1/120	359/360; 99.7%
		Site 3	120/120	0/120	
		Site 4	120/120	0/120	
		Total	**359/360**	**1/360**	
mecA Absent; no organism associated	*S. lugdunensis* (*mec*A -), *E. faecalis* (*mecA* -) Low and High	Site 1	0/90	90/90	270/270; 100%
		Site 3	0/90	90/90	
		Site 4	0/90	90/90	
		Total	**0/270**	**270/270**	

(1) One *Staphylococcus species OTHER than S. aureus or S. lugdunensis, mecA* present reported as *mecA* absent

In the study, ten (10) discrepant results were obtained (see Table 14).

Table 14. Summary of discrepant results from Reproducibility Studies.

Correct Result	Staph ID/R Blood Culture Panel Result	Discrepant Result	Site	Operator	Test Day
Staphylococcus lugdunensis, mecA absent	*Staphylococcus* OTHER than *S. aureus or S. lugdunensis, mecA* absent	*Staph. species*	1	1	3
Staphylococcus lugdunensis, mecA absent	*Staphylococcus* OTHER than *S. aureus or S. lugdunensis, mecA* absent	*Staph. species*	1	2	4
Negative, *mecA* N/A	*Staphylococcus lugdunensis, mecA* absent	*S. lugdunensis*	1	2	5
Staphylococcus aureus, mecA present	*Staphylococcus aureus ; Staphylococcus lugdunensis, mecA* present	*S. lugdunensis*	1	2	5
Staphylococcus OTHER than *S. aureus or S. lugdunensis, mecA* absent	*Staphylococcus* OTHER than *S. aureus or S. lugdunensis, mecA* absent	*mecA* absent	1	2	5
Staphylococcus OTHER than *S. aureus or S. lugdunensis, mecA* absent	*Staphylococcus aureus* in mixed Staph infection (NOT *S. lugdunensis*), *mecA* present	*S. aureus*	1	2	5
Staphylococcus aureus, mecA present	*Staphylococcus aureus ; Staphylococcus lugdunensis, mecA* present	*S. lugdunensis*	3	1	1
Staphylococcus lugdunensis, mecA absent	*Staphylococcus aureus ; Staphylococcus lugdunensis, mecA* absent	*S. aureus*	3	2	2
Negative, *mecA* N/A	*Staphylococcus* OTHER than *S. aureus or S. lugdunensis, mecA* absent	*Staph. species*	4	2	4
Negative, *mecA* N/A	*Staphylococcus* OTHER than *S. aureus or S. lugdunensis, mecA* absent	*Staph. species*	4	2	5

h. Evaluation of Blood Culture Bottle Types

To determine the effect of blood culture bottle type on Staph ID/R Blood Culture Panel, thirteen (13) unique bottle types were tested in the presence of target and non-target organisms. *Staphylococcus* isolates or *E. faecalis* (negative) at bottle ring load levels ($2x10^6$-$1x10^8$ CFU/mL) consistent with the specimen stability studies. All bottles were tested with the highest volume of negative blood recommended by the manufacturer (e.g., if the recommended blood volume was 8-10 mL, 10 mL of blood was spiked into the bottle). Bottles with blood were pre-incubated 18 hours at 35-37°C in a BACTEC Blood Culture System or in a shaking incubator prior to testing. Bacteria isolates were incubated >18 hrs in TSB, and the CFU concentrations for each strain were estimated by optical density measurements, confirmed by serial dilution and colony counting.

The studies assessed the detection of seven (7) *Staphylococcus* ATCC strains used for analytical sensitivity: *S. aureus, mecA*+ BAA-1680, *S. aureus, mecA*+ 1682, *S. aureus, mecA*- 11632, *S. epidermidis, mecA*+ 51625, *S. epidermidis, mecA*- 12228, *S. lugdunensis, mecA*- 49576, *S. capitis, mecA*- 35661. The studies also assessed performance of the Staph ID/R Blood Culture Panel in the presence of *E. faecalis* 29212 (Negative).

The following bottle types were tested in the study: BACTEC (Standard 10 Aerobic/F, Standard Anaerobic 10/F, Plus Aerobic/F, Plus Anaerobic/F, Lytic 10/F, and PEDS Plus/F), BacT/Alert (SA Standard Aerobic, SN Standard Anaerobic, FA FAN Aerobic, FN FAN Anaerobic, and PF Pediatric FAN), and Versa Trek (Redox 1 and Redox 2). Three bottles of each bottle type were used for each strain, and the samples tested three

times in the Staph ID/R Blood Culture Panel for a total of 9 runs for each strain and bottle type combination.

For the 'valid' runs tested, all of the potential blood bottle types were compatible with the Staph ID/R Blood Culture Panel, with no false negative results (see Table 15).

	***Staphylococcus* Species, ATCC Strain; Sample Input (CFU/mL), Correct Staph ID/R Blood Panel Results**							
Bottle Type	***S. aureus* (*mecA* +) BAA-1680 $1.9x10^7$**	***S. aureus* (*mecA* +) BAA-1682 $1.2x10^7$**	***S. aureus* (*mecA* -) 11632 $7.5x10^7$**	***S. lugdunensis* (*mecA* -) 49576 $1.9x10^8$**	***S. epidermidis* (*mecA* -) 12228 $2.9x10^7$**	***S. epidermidis* (*mecA* +) 51625 $6.5x10^7$**	***S. capitis* (*mecA*-) 35661 $2.3x10^6$**	***E. faecalis* (*mecA* -) 29212 $2.1\text{-}4.5x10^7$**
BACTEC Standard 10 Aerobic/F	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9
BACTEC Standard Anaerobic 10/F	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9
BACTEC Plus Aerobic/F	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9
BACTEC Plus Anaerobic/F	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9
BACTEC Lytic 10/F	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9
BACTEC PEDS Plus/F	9/9	9/9	9/9	9/9	9/9	9/9	9/9	14/14****
BacT/Alert SA Aerobic	9/9	9/9	9/9	9/9	9/9	9/9	9/9	16/16**
BacT/Alert SN Anaerobic	9/9	9/9	9/9	9/9	9/9	9/9	9/9	16/17*"
BacT/Alert FA FAN Aerobic	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9
BacT/Alert FN FAN Anaerobic	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9
BacT/Alert PF Pediatric FAN	9/9	9/9	9/9	9/9	9/9	9/9	9/9	17/17**
Versa Trek Redox 1	9/9	9/9	8/9'	9/9	9/9	9/9	9/9	9/9
Versa Trek Redox 2	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9

*This set of test runs also contained 1 "Invalid" run

**This set of test runs also contained 2 "Invalid" runs

****This set of test runs also contained 4 "Invalid" runs

'This set of test runs contained 1 false positive result for *mecA*

"This set of test runs contained 1 false positive *Staphylococcus,* species undetermined result

Table 15. Evaluation of blood culture bottle types study results.

A false positive "*S. aureus*, *mecA* Present" result was observed in one test run from one Versa Trek Redox 1 bottle for *S. aureus, mecA*- 11632. The discrepant result is thought to be a contamination event, since all other samples (8/9) gave the correct "*S. aureus* present, *mecA* Absent" call, including 2/3 correct results from the same bottle with the discrepant call. All test results did result in a correct *S. aureus* result, suggesting that the bottle type did not interfere with the assay.

A false positive "*Staphylococcus* Positive, Staphylococcus species OTHER than S. aureus or S. lugdunensis, *mecA* absent" result was observed for *E. faecalis* 29212 in one test run from one BacT/Alert SN Anaerobic bottle. The discrepant result is also thought to be a random contamination event, because the sixteen (16) other "valid" test runs returned a correct "Staphylococcus Negative" result, including 2/3 correct initial results and 3/3 correct re-test results from the same bottle with the discrepant call.

The only other exceptions observed during this study were nine (9) 'Invalid' call runs that are noted in Table 15, all with *E. faecalis* 29212. When tested with *E. faecalis* 29212, one (1) invalid run was observed for BACTEC PEDS Plus/F, one (1) invalid run for BacT/Alert SA Aerobic, and one (1) invalid run for BacT/Alert PF Pediatric FAN bottle types. In all of these cases, extra test runs were performed to evaluate any possible

interference with the assay in a Negative sample (evaluating SPC only). Re-test with nine (9) cards resulted in an additional three (3) invalid runs for BACTEC PEDS Plus/F, one (1) invalid run for BacT/Alert SA Aerobic, and one (1) invalid run for BacT/Alert SN Anaerobic (retested due to false positive contamination result). Overall, it appears that the bottle types with invalid results have an elevated invalid rate (5-28%) compared to the prospective study (2%).

H. Performance Data – Prospective Clinical Studies

Specimens for the clinical study were collected prospectively at three geographically diverse U.S. sites. Eligible study subjects included individuals receiving routine care requiring blood culture testing. Blood culture specimens were collected from the patients and incubated on the BACTEC continuous monitoring blood culture system. Bottles that were flagged positive by the instrument were Gram stained and then bottles confirmed to contain gram-positive cocci in clusters (GPCC) or gram-positive cocci in singles (GPC) were then tested with the Staph ID/R Blood Culture Panel. A total of 853 samples were collected for all three sites combined. Twenty-two (22) specimens were excluded from the Staph ID/R Blood Culture Panel clinical study dataset. The remaining 831 clinical specimens met the inclusion criteria and were used in the prospective study to evaluate the performance of the Staph ID/R Blood Culture Panel. A total of 762 prospective specimens were tested in the clinical trial, while the remaining 69 archived frozen specimens were tested after the prospective clinical trial.

In addition, 102 Staph ID/R Blood Culture Panel assays were performed on a 'Low Prevalence' panel of contrived or 'simulated' blood culture specimens, consisting of low prevalence Staphylococcus species and gram-positive negatives. These specimens were prepared by spiking blood culture bottles containing whole blood with bacterial suspensions of bacterial isolates. Prepared blood culture bottles were then grown to positivity on the BACTEC blood culture system until flagged positive. Gram stain was performed to verify the presence of gram-positive cocci in clusters (GPCC) or gram-positive cocci in singles (GPC) and then testing was performed with the Staph ID/R Blood Culture Panel. Results from the studies of all three clinical sites combined are summarized in Table 16.

Table 16. Summary of Clinical Performance of Staph ID/R Blood Culture Panel versus Reference Method(s) – Prospective and Simulated/Supplemental Blood Cultures.

All sites combined		% Agreement			
		TP/TP + FN	PPA 95% CI	TN/TN + TP	NPA 95% CI
Detection of *Staphylococcus aureus*	Prospective	211/214	**98.6%** 96.0 - 99.5%	548/551	**99.5%** 98.4 - 99.8%
	Simulated	0	**N/A**	102/102	**100.0%** 96.4 - 100%
	Overall	211/214	**98.6%** 96.0 - 99.5%	650/653	**99.5%** 98.7 - 99.8%
Detection of *Staphylococcus lugdunensis*	Prospective	3/3	**100.0%** 43.9 - 100%	761/762	**99.9%** 99.3 - 99.9%
	Simulated	30/30	**100.0%** 88.7 - 100%	72/72	**100.0%** 94.9 - 100%
	Overall	33/33	**100.0%** 89.6 - 100%	833/834	**99.9%** 99.3 - 99.9%
Detection of *Staphylococcus* species OTHER than *S. aureus* or *S. lugdunensis*	Prospective	444/449	**98.9%** 97.4 - 99.5%	307/316	**97.2%** 94.7 - 98.5%
Detection of *mecA* with *Staphylococcus aureus*	Prospective	68/72	**94.4%** 86.6 - 97.8%	682/690	**98.8%** 97.7 - 99.4%
	Frozen	35/35	**100.0%** 90.1 - 100.0%	34/34	**100.0%** 89.9 - 100.0%
	Overall	103/107	**96.3%** 90.8 - 98.5%	716/724	**98.9%** 97.8 - 99.4%
Detection of *mecA* with *Staphylococcus lugdunensis*	Prospective	0/0	**N/A**	762/762	**100.0%** 99.5 - 100%
Detection of *mecA* with *Staphylococcus* species OTHER than *S. aureus* or *S. lugdunensis*	Prospective	243/262	**92.7%** 88.1 - 97.1%	481/500	**96.2%** 92.4 - 98.0%

Table 17 lists all polymicrobial specimens from the prospective performance data determined by the Staph ID/R Blood Culture Panel and/or Reference Method(s).

Table 17. Polymicrobials – Mixed Specimen Combinations Detected by Staph ID/R Blood Culture Panel and Reference Method(s).

		Staph ID/R Result		Reference Result					Discrepant Result Description	
Site	Sample ID	Species Identification	*mecA* Result	Organism 1	Cefoxitin Result	Organism 2	Cefoxitin Result	Organism 3	Species Identification	*mecA* Result
Polymicrobial for both Staph ID/R Blood Culture Panel AND Reference Results										
Site 2 (Daly)	DALY125	*S. aureus* in mixed Staph infection (NOT *S. lugdunensis*)	Present	*S. aureus*	Sensitive	*S. epidermidis*	Resistant		Correct species call	Correct *mecA* call
Polymicrobial for Reference Results										
Site 1, IU (Denys)	DNYS031	*Staph.* species OTHER than *S. aureus* or *S. lugdunensis*	Absent	*S. aureus*	Sensitive	*S. epidermidis*		*S. hominis*	FN for *S. aureus*; Correct for Staph call	Correct *mecA* call
	DNYS033	*Staph.* species OTHER than *S. aureus* or *S. lugdunensis*	Absent	*S. capitis*	Sensitive	*S. epidermidis*	No growth		Correct species call	Correct *mecA* call
	DNYS047	*Staph.* species OTHER than *S. aureus* or *S. lugdunensis*	Present	*S. capitis*	Sensitive	*S. epidermidis*	Resistant		Correct species call	Correct *mecA* call
	DNYS071	*Staph.* species OTHER than *S. aureus* or *S. lugdunensis*	Absent	*S. epidermidis*	Sensitive	*S. haemolyticus*	Sensitive		Correct species call	Correct *mecA* call
	DNYS123	*Staph.* species OTHER than *S. aureus* or *S. lugdunensis*	Present	*S. hominis*	Resistant	*S. epidermidis*	Resistant		Correct species call	Correct *mecA* call
	DNYS191	*Staph.* species OTHER than *S. aureus* or *S. lugdunensis*	Present	*S. hominis*	Resistant	*S. epidermidis*	Resistant		Correct species call	Correct *mecA* call
	DNYS202	*Staph.* species OTHER than *S. aureus* or *S. lugdunensis*	Absent	*S. epidermidis*	Sensitive	*S. epidermidis*	Resistant		Correct species call	FN *mecA*
	DNYS288	*Staph.* species OTHER than *S. aureus* or *S. lugdunensis*	Absent	*S. capitis*	Sensitive	*S. pettenkoferi*	Resistant		Correct species call	FN *mecA*
	DNYS297	*Staph.* species OTHER than *S. aureus* or *S. lugdunensis*	Present	*S. hominis*	Resistant	*S. capitis*	Sensitive		Correct species call	Correct *mecA* call
Site 3, TriCore (Young)	YNG109	*Staph.* species OTHER than *S. aureus* or *S. lugdunensis*	Absent	*S. epidermidis*	Sensitive	*S. capitis*	Sensitive		Correct species call	Correct *mecA* call
	YNG129	*Staph.* species OTHER than *S. aureus* or *S. lugdunensis*	Absent	*S. epidermidis*	Sensitive	*S. warneri*	Sensitive		Correct species call	Correct *mecA* call
	YNG172	*Staph.* species OTHER than *S. aureus* or *S. lugdunensis*	Present	*S. epidermidis*	Resistant	*S. haemolyticus*	Sensitive		Correct species call	Correct *mecA* call
	YNG197	*Staph.* species OTHER than *S. aureus* or *S. lugdunensis*	Absent	*S. hominis*	Sensitive	*S. capitis*	Sensitive		Correct species call	Correct *mecA* call
Polymicrobial for Staph ID/R Blood Culture Panel - 'mixed Staph infections'										
Site 1, IU (Denys)	DNYS026	*S. aureus* in mixed Staph infection (NOT *S. lugdunensis*)	Present	*S. hominis*	Resistant				FP for *S. aureus*; Correct for Staph call	Correct *mecA* call
	DNYS045	*S. aureus* in mixed Staph infection (NOT *S. lugdunensis*)	Present	*S. aureus*	Sensitive				Correct for *S. aureus*; FP for mixed	FP *mecA*
Site 3, TriCore (Young)	YNG207	*S. aureus* in mixed Staph infection (NOT *S. lugdunensis*)	Present	*S. epidermidis*	Resistant				FP for *S. aureus*; Correct for Staph call	Correct *mecA* call
	YNG299	*S. aureus* in mixed Staph infection (NOT *S. lugdunensis*)	Absent	*S. epidermidis*	Sensitive				FP for *S. aureus*; Correct for Staph call	Correct *mecA* call

Table 18 summarizes the prospective and 'Low Prevalence' simulated performance data for *Staphylococcus* genus-level analyte in the Staph ID/R Blood Culture Panel, i.e., the 'Staphylococcus OTHER than S. aureus or S. lugdunensis' analyte. The performance data are stratified by individual *Staphylococcus* species as determined by the Reference Method(s).

Table 18. Summary of *Staphylococcus* Genus-level Analyte for "*Staphylococcus* OTHER than *S. aureus* or *S. lugdunensis* versus Reference Method(s).

	% Agreement (95% CI)			
Species	**Prospective**		**Simulated**	
S. arlettae	-	-	3/3	**100%** 43.9-100%
S. auricularis	-	-	3/3	**100%** 43.9-100%
S. capitis	35/35	**100%** 90.1-100%	-	-
S. carnosus	1/1	**100%** 20.7-100%	-	-
S. cohnii	2/2	**100%** 34.2-100%	3/3	**100%** 43.9-100%
S. equorum	2/2	**100%** 34.2-100%	-	-
S. haemolyticus	15/15	**100%** 79.6-100%	3/3	**100%** 43.9-100%
S. hominis	96/97	**97.9%** 92.8-99.4%	-	-
S. intermedius	-	-	3/3	**100%** 43.9-100%
S. pettenkoferi	7/7	**100%** 64.6-100%	-	-
S. saprophyticus	8/8	**100%** 67.6-100%	-	-
S. schleiferi	2/2	**100.0%** 67.6-100%	3/3	**100%** 43.9-100%
S. sciuri	-	-	3/3	**100%** 43.9-100%
S. simulans	2/2	**100%** 34.2-100%	3/3	**100.0%** 43.9-100%
S. species	2/2	**100%** 34.2-100%	-	-
S. warneri	8/8	**100%** 67.6-100%	3/3	**100%** 43.9-100%
S. xylosus	-	-	3/3	**100%** 43.9-100%

I. Invalid and Abort Rates

Invalid rates and run abort rates for the prospective clinical studies are found in Table 19. The overall initial Invalid rate in the prospective clinical studies was 1.39%. Valid results were achieved after a single retest for all of the Invalid runs, resulting in a final Invalid rate of 0%. For run aborts, the overall abort rate for the prospective clinical studies was 3.30%. All of these specimens were later run successfully resulting in a final abort rate of 0.00%. All run aborts are referred as "Test Incomplete" by the Portrait Staph ID/R Blood Culture Panel software.

Table 19. Invalids and Aborts with Prospective Clinical Specimens.

Clinical Site	# Runs	# of Initial Invalids	Initial Invalid Rate	# of Final Invalids	Final Invalid Rate	# of Initial Aborts	Initial Abort Rate	# of Final Aborts	Final Abort Rate
Site 1, IU (Denys)	332	4	1.20%	0	0.00%	10	3.01%	0	0.00%
Site 2, PCMC (Daly)	157	4	2.55%	0	0.00%	6	3.82%	0	0.00%
Site 3, TriCore (Young)	300	3	1.00%	0	0.00%	10	3.33%	0	0.00%
Overall	**789**	**11**	**1.39%**	**0**	**0.00%**	**26**	**3.30%**	**0**	**0.00%**

No Call rates (invalid + abort rates) for the prospective clinical studies are found in Table 20. The overall initial No Call Rate was 4.69%. All Invalid runs and run aborts were later run successfully, resulting in a final No Call rate of 0.00%.

Table 20. Prospective Clinical Specimens with "No Call" (Invalids + Aborts).

Clinical Site	# of Runs	Initial No Call (Invalids + Aborts)	Initial No Call Rate (Invalids + Aborts)	Final No Call (Invalids + Aborts)	Final No Call Rate (Invalids + Aborts)
Site 1, IU (Denys)	332	14	4.22%	0	0.00%
Site 2, PCMC (Daly)	157	10	6.37%	0	0.00%
Site 3, TriCore (Young)	300	13	4.33%	0	0.00%
Overall	**789**	**37**	**4.69%**	**0**	0.00%

J. Conclusion

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