

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k152470

B. Purpose for Submission:

To obtain a substantial equivalence determination for the Great Basin Staph ID/R Blood Culture Panel.

C. Measurand:

Staphylococcus aureus, *Staphylococcus lugdunensis* and various *Staphylococcus* species to the genus level and the detection of the *mecA* gene for methicillin resistance.

D. Type of Test:

A multiplexed nucleic acid-based test intended for use with the Great Basin PA500 Portrait Analyzer instrument for the qualitative *in vitro* detection and identification of multiple bacterial nucleic acids and select genetic determinants of antimicrobial resistance from patient positive blood culture specimens. The Great Basin Staph ID/R Blood Culture Panel assay is performed directly on positive blood culture specimens determined by Gram stain to contain gram-positive cocci in clusters (GPCC) or gram-positive cocci in singles (GPC).

E. Applicant:

Great Basin Scientific, Inc.

F. Proprietary and Established Names:

Great Basin Staph ID/R Blood Culture Panel

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3365 - Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures

2. Classification:

Class II

3. Product codes:

PAM- Gram-positive bacteria and their resistance markers

OOI- Real time Nucleic acid amplification system

4. Panel:

83, (Microbiology)

H. Intended Use:

1. Intended use(s):

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.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

For use with The Great Basin PA500 Portrait™ Analyzer System.

I. Device Description:

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 a 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.

The single-use test cartridge contains blister packs, fluidic channels, processing chambers, and the assay chip coated with an array of sequence-specific detection probes. All reagents are contained within the integrated blister packs with the exception of the amplification enzymes that are lyophilized and placed into the Amplification Chamber. A positive blood culture specimen is placed into a sample port of the test cartridge for processing. Multiple fluidic channels move reagents from integrated blister packs to chambers where reagent mixing and sample processing occur. A waste chamber, self-contained and segregated within the test cartridge, collects and stores reagent waste.

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.

Materials provided in each kit:

- Staph ID/R Blood Culture Panel Test Cartridge Kit. Each test cartridge includes:
 - Blister Pack 1: Dilution Buffer (Tris buffer, salts, surfactant, BSA (bovine serum-albumin), antibacterial agents)
 - Blister Pack 2: Extraction Buffer (Enzymes, salts)
 - Blister Pack 3: Wash Solution (Saline Sodium Citrate (SSC) buffer, surfactant, preservative)
 - Blister Pack 4: Hybridization Buffer (SSC buffer, surfactant, preservative)
 - Blister Pack 5: Conjugate (Sodium citrate buffer, salts, fetal bovine serum (FBS), peroxidase conjugated monoclonal mouse antibody, preservative)
 - Blister Pack 6: Conjugate (Tetramethylbenzidine (TMB))
 - Chamber 1 (CC1): Stir bar, SPC (Lyophilized *Bacillus subtilis*)
 - Chamber 2 (CC2): Stir bar
 - Chamber 3 (Amp) Amplification Reagents (Tris buffer salts, sucrose, surfactant, nucleotides, primers, DNA polymerase)
 - Chamber 3 (Detect): Silicon chip with immobilized DNA probes

Materials required but not provided:

- PA500 Portrait™ Analyzer System and Operator Manual
- Calibrated, 50 µL, fixed-volume pipette
- 200µL sterile, barrier filter pipette tips

J. Substantial Equivalence Information:

1. Predicate device name(s):

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

2. Predicate 510(k) number(s):

k113450

3. Comparison with predicate:

Item	Device: Staph ID/R Blood Culture Panel	Predicate (k113450)
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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	<p>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 <i>Staphylococcus aureus</i>, <i>Staphylococcus lugdunensis</i> and various <i>Staphylococcus</i> species to the genus level and the detection of the <i>mecA</i> 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 <i>Staphylococcus aureus</i> (SA), and <i>Staphylococcus lugdunensis</i>, and detects other <i>Staphylococcus</i> species without identification to species level.</p> <p>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, <i>mecA</i> may or may not be associated with <i>Staphylococcus</i> spp. detected or the agent responsible for the disease. Negative results for <i>mecA</i> antimicrobial resistance gene assays do not always indicate susceptibility, as other mechanisms of resistance to methicillin exist.</p>	<p>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: <i>Staphylococcus</i> spp., <i>Staphylococcus aureus</i>, <i>Staphylococcus epidermidis</i>, and <i>Staphylococcus lugdunensis</i>, <i>Streptococcus</i> spp., <i>Streptococcus pneumoniae</i>, <i>Streptococcus pyogenes</i>, <i>Streptococcus agalactiae</i>, <i>Streptococcus anginosus</i> group, <i>Enterococcus faecalis</i>, <i>Enterococcus faecium</i>, <i>Listeria</i> spp. In addition, BC-GP detects the <i>mecA</i> resistance marker, and the <i>vanA</i> and <i>vanB</i> resistance markers, inferring <i>vanA/vanB</i> mediated vancomycin resistance to either <i>E. faecalis</i> or <i>E. faecium</i>, or the <i>mecA</i>-mediated methicillin resistance to either <i>S. aureus</i> or <i>S. epidermidis</i>. 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.</p>

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
Controls	One Internal Processing Control (whole organism complete assay control)	Same
Calibration	Not required	Same
Differences		
Intended Use/Indications for Use	Identification of <i>S. aureus</i> , <i>S. lugdunensis</i> , detection of other <i>Staphylococcus</i> spp. Detection of the <i>mecA</i> gene for methicillin resistance in all <i>Staphylococcus</i> organisms.	Tests for same <i>Staphylococcus</i> targets. Detection of <i>mecA</i> gene for methicillin resistance in <i>S. aureus</i> and <i>S. epidermidis</i> only. Tests for additional gram-positive bacteria including <i>Streptococcus</i> spp., <i>Streptococcus pneumoniae</i> , <i>Streptococcus pyogenes</i> , <i>Streptococcus agalactiae</i> , <i>Streptococcus anginosus</i> group, <i>Enterococcus faecalis</i> , <i>Enterococcus faecium</i> , and <i>Listeria</i> spp. Tests for additional resistance markers including the <i>vanA</i> and <i>vanB</i> resistance markers, inferring <i>vanA/vanB</i> mediated vancomycin resistance to either <i>E. faecalis</i> or <i>E. faecium</i> .
Test Principle/Technology	Fully automated multiplex PCR and detection by target-specific capture oligonucleotides immobilized in a microarray 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 SPTIME
Time to Result	110 minutes	150 minutes

K. Standard/Guidance Document Referenced (if applicable):

- Guidance for Industry and Food and Drug Administration Staff - Highly Multiplexed Microbiological/Medical Countermeasure In Vitro Nucleic Acid Based Diagnostic Devices, (November 9, 2012 August 27, 2014)
- Draft Guidance for Industry and FDA Staff - Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection of methicillin-

- resistant *Staphylococcus aureus* (MRSA) for Culture Based Devices (June 15, 2011)
- Draft Guidance for Industry and Food and Drug Administration Staff - Establishing the Performance Characteristics of Nucleic Acid-Based In vitro Diagnostic Devices for the Detection and Differentiation of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus* (SA) (January 5, 2011)
- Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests, FDA Guidance Document (March 13, 2007)
- User Protocol for Evaluation of Qualitative Test Performance, Clinical and Laboratory Standards Institute (CLSI) Approved Guideline – Second Edition, EP12-A2 (January 2008)
- Molecular Diagnostic Methods for Infectious Diseases, CLSI Approved Guideline, MM3-A2 (February 2006)
- Interference Testing in Clinical Chemistry, CLSI Approved Guideline EP7-A2 (November 2005)
- Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, FDA Guidance Document (May 11, 2005)
- Guidance for Industry, FDA Reviewers and Compliance on Off-The-Shelf Software Use in Medical Devices (September 9, 1999)
- General Principle of Software Validation; Final Guidance for Industry and FDA Staff (January 11, 2002)

L. 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.

M. Performance Characteristics:

1. Analytical performance:
 - 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 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 1×10^5 - 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 initial serial dilutions and testing for three strains were used to estimate the ideal range to test for all 22 strains. Correct results were determined by the call reported vs expected, e.g. *Staphylococcus aureus* ATCC strain BAA-1682 with *mecA* present was correct if detected in a ‘Positive, *Staphylococcus aureus*, *mecA* Present’ call. The results for the initial testing are summarized in the table below.

Performance of the Staph ID/R Blood Culture Panel on serial dilutions of three *Staphylococcus* strains for initial sensitivity study.

Species	ATCC #	Sample Input (CFU/mL)	Correct Staph ID/R Blood Culture Panel
<i>S. aureus</i>	BAA-1682	1.3×10^4	1/4
		4.1×10^4	1/4
		1.3×10^5	4/4
<i>S. epidermidis</i>	700562	1.4×10^4	0/3
		4.4×10^4	1/3
		1.4×10^5	4/4
<i>S. lugdunensis</i>	49576	1.4×10^4	0/4
		4.5×10^4	2/4
		1.4×10^5	4/4

In order to confirm an estimated LoD based on colony counting, an additional minimum of 20 replicates were tested for *S. aureus* BAA-1682, *S. epidermidis* 700562, and *S. lugdunensis* 49576 based on the results from on the initial sensitivity study. The 20 replicates initially tested for the three strains resulted in less than 19/20 correct calls, and were subsequently retested at a half-log increased concentration, and resulted in 100% correct calls. These experiments identified a target sample input range of 1×10^5 - 10^6 CFU/mL for future LoD studies. A minimum of 19/20 valid runs was required to identify the LoD for each strain tested.

The LoDs for 6 *S. aureus* strains \pm *mecA* are reported as 3.5 - 8.2×10^5 CFU/mL; the LoD for each *S. aureus* strain is listed in below.

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

Species	ATCC #	Sample Input (CFU/mL)	Correct Staph ID/R Blood Culture Panel
<i>S. aureus, mecA</i> +	BAA-1680	3.5 x10 ⁵	23/23
	BAA-1682	4.0 x10 ⁵	20/20
	BAA-1684	8.2 x10 ⁵	22/22
<i>S. aureus, mecA</i> -	25923	5.1 x10 ⁵	21/21
	6538	3.9 x10 ⁵	22/22
	11632	6.2 x10 ⁵	20/20*

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

The LoDs for 6 *S. epidermidis* strains ± *mecA* are reported as 2.2-7.1x10⁵ CFU/mL; the LoD for each *S. epidermidis* strain is listed in the table below.

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

Species	ATCC #	Sample Input (CFU/mL)	Correct Staph ID/R Blood
<i>S. epidermidis, mecA</i> +	35984	3.6 x10 ⁵	20/20
	51625	4.0 x10 ⁵	22/22
	700562	4.3 x10 ⁵	27/27
	700566	5.8 x10 ⁵	23/23
<i>S. epidermidis, mecA</i> -	700583	2.2 x10 ⁵	23/23
	12228	7.1 x10 ⁵	22/23

The LoDs for 3 *S. lugdunensis* strains without *mecA* are reported as 2.8-4.7x10⁵ CFU/mL;

the LoD for each *S. lugdunensis* strain is listed in below.

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

Species	ATCC #	Sample Input (CFU/mL)	Correct Staph ID/R Blood
<i>S. lugdunensis, mecA</i> -	43809	2.8 x10 ⁵	22/23
	49576	4.5 x10 ⁵	23/23
	7990	4.7 x10 ⁵	23/23

LoD's for 7 *Staphylococcus* species ± *mecA*, excluding *S. aureus*, *S. epidermidis*, and *S.*

lugdunensis, are reported as 2.0-5.3x10⁵ 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 the table below.

Performance of the Staph ID/R Blood Culture Panel on a minimum of 20 replicates of 7 *Staphylococcus* strains \pm *mecA* for establishing LoD.

Species	ATCC #	Sample Input (CFU/mL)	Correct Staph ID/R Blood
<i>S. simulans, mecA</i> -	27848	2.0×10^5	23/23
<i>S. haemolyticus,</i>	BAA-1693	3.1×10^5	19/20
<i>S. warneri, mecA</i> -	27830	4.0×10^5	22/22
<i>S. sciuri, mecA</i> -	29060	4.3×10^5	21/21**
<i>S. capitis, mecA</i> -	35661	1.5×10^5	20/20*
<i>S. pasteurii, mecA</i> -	51129	5.3×10^5	21/22
<i>S. hominis, mecA</i> -	27844	5.3×10^5	23/23*

*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 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 *Staphylococcus* stock cultures were prepared in TSB broth, incubated overnight, and measured by optical density to estimate the inoculum concentration. Broth cultures were combined with BACTEC Plus Aerobic/F media containing negative blood previously incubated 24 hours in a BACTEC Blood Culture System without alarm positivity. The *Staphylococcus* cultures were diluted to a sample input target range of approximately 2-3X LoD ($1-2.5 \times 10^6$ CFU/mL) determined by the *Staphylococcus* strain LoD studies. The CFU concentrations for each strain were estimated by optical density measurements and then confirmed by colony counting. This method led to some strains being tested at 4-7X LOD ($2.5-7 \times 10^6$ CFU/mL).

The Staph ID/R Blood Culture Panel correctly detected all of the additional *Staphylococcus* strains, *mecA* present or absent with a sample input range of 3.2×10^5 to 6.7×10^6 CFU/mL listed in the table below.

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

<i>Staphylococcus species</i> ; ATCC, CCUG, NRS, Clinical #	SCC <i>mec</i> , PFGE type / source	<i>Staphylococcus species</i> ; ATCC, CCUG, NRS, Clinical #	SCC <i>mec</i> , PFGE type / source
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<i>S. aureus</i> BAA-38	I, Denmark	<i>S. caprae</i> 35538	ATCC
<i>S. aureus</i> BAA-44	I, Iberian	<i>S. caprae</i> 51548	ATCC
<i>S. aureus</i> 700698	II, Japan	<i>S. chromogenes</i> 43764	ATCC
<i>S. aureus</i> BAA-41	II, USA100	<i>S. chohnii</i> subsp. <i>chohnii</i>	ATCC
<i>S. aureus</i> BAA-1681	II, USA100	<i>S. chohnii</i> subsp. <i>urealyticus</i> ²⁹⁹⁷²	ATCC
<i>S. aureus</i> BAA-1682	II, USA100	<i>S. condimentii</i> 4753	ATCC
<i>S. aureus</i> BAA-1761	II, USA100	<i>S. carnosus</i> 51365	ATCC
<i>S. aureus</i> NRS660	II, USA100	<i>S. delphini</i> 49171	ATCC
<i>S. aureus</i> BAA-1720	II, USA200	<i>S. epidermidis</i> 12228	ATCC
<i>S. aureus</i> BAA-1750	II, USA200	<i>S. epidermidis</i> 35983	ATCC
<i>S. aureus</i> BAA-1760	II, USA200	<i>S. epidermidis</i> 35984	ATCC
<i>S. aureus</i> NRS651	II, USA200	<i>S. epidermidis</i> 51625	ATCC
<i>S. aureus</i> BAA-39	III, Hungary	<i>S. epidermidis</i> 700562	ATCC
<i>S. aureus</i> 35592	III, ST239	<i>S. epidermidis</i> 700563	ATCC
<i>S. aureus</i> BAA-1680	IV, USA300	<i>S. epidermidis</i> 700565	ATCC
<i>S. aureus</i> BAA-1717	IV, USA300	<i>S. epidermidis</i> 700566	ATCC
<i>S. aureus</i> NRS643	IV, USA300	<i>S. epidermidis</i> 700567	ATCC
<i>S. aureus</i> NRS662	IV, USA300	<i>S. epidermidis</i> 700568	ATCC
<i>S. aureus</i> NRS688	IV, USA300	<i>S. epidermidis</i> 700576	ATCC
<i>S. aureus</i> NRS716	IV, USA300	<i>S. epidermidis</i> 700583	ATCC
<i>S. aureus</i> BAA-1683	IV, USA400	<i>S. equorum</i> 43958	ATCC
<i>S. aureus</i> BAA-1696	IV, USA400	<i>S. felis</i> 49163	ATCC
<i>S. aureus</i> BAA-1707	IV, USA400	<i>S. fleuretti</i> BAA-274	ATCC
<i>S. aureus</i> BAA-1752	IV, USA400	<i>S. gallinarum</i> 33539	ATCC
<i>S. aureus</i> BAA-1684	IV, USA500	<i>S. gallinarum</i> 49148	ATCC
<i>S. aureus</i> BAA-1689	IV, USA500	<i>S. haemolyticus</i> BAA-1693	ATCC
<i>S. aureus</i> BAA-1763	IV, USA500	<i>S. haemolyticus</i> 43253	ATCC
<i>S. aureus</i> NRS685	IV, USA500	<i>S. haemolyticus</i> 29968	ATCC
<i>S. aureus</i> BAA-1754	IV, USA600	<i>S. haemolyticus</i> 29970	ATCC
<i>S. aureus</i> BAA-1755	IV, USA700	<i>S. haemolyticus</i> 700564	ATCC
<i>S. aureus</i> BAA-1758	IV, USA800	<i>S. hominis</i> 25615	ATCC
<i>S. aureus</i> BAA-1768	IV, USA800	<i>S. hominis</i> 27844	ATCC
<i>S. aureus</i> NRS692	IV, USA800	<i>S. hominis</i> 51624	ATCC
<i>S. aureus</i> NRS675	IV, USA800	<i>S. hominis</i> 700586	ATCC
<i>S. aureus</i> BAA-1747	IV, USA1000	<i>S. hominis</i> subsp.	ATCC
<i>S. aureus</i> NRS483	IV, USA1000	<i>S. intermedius</i> 29663 ⁷⁰⁰²³⁷	ATCC
<i>S. aureus</i> NRS730	IV, USA1000	<i>S. intermedius</i> 49052	ATCC
<i>S. aureus</i> BAA-1764	IV, USA1100	<i>S. intermedius</i> 51874	ATCC
<i>S. aureus</i> NRS484	IV, USA1100	<i>S. kloosii</i> 43959	ATCC
<i>S. aureus</i> BAA-1766	V, USA700	<i>S. lentus</i> 29070	ATCC

<i>S. aureus</i> BAA-2094	V, WA-MRSA	<i>S. lugdunensis</i> 4436	CCM, Czech
<i>S. aureus</i> BAA-42	VI, USA800	<i>S. lugdunensis</i> 7990	ATCC, NCTC
<i>S. aureus (mecC)</i> BAA-2313	XI, CC130	<i>S. lugdunensis</i> 48413	ATCC
<i>S. aureus</i> BAA-1751	Untyped, USA600	<i>S. lugdunensis</i> 49576	CCUG, Sweden
<i>S. aureus</i> BAA-1771	Untyped, USA800	<i>S. lugdunensis</i> 700328	ATCC
<i>S. aureus</i> BAA-1718	NA, USA300	<i>S. lutrae</i> 700373	ATCC
<i>S. aureus</i> BAA-1749	NA, USA900	<i>S. massiliensis</i> 7895	ATCC
<i>S. aureus</i> BAA-1765	NA, USA1200	<i>S. muscae</i> 49912	ATCC
<i>S. aureus</i> BAA-40	Untyped, Lisbon	<i>S. nepalensis</i> 48992	ATCC
<i>S. aureus</i> BAA-1685	Untyped, ATCC	<i>S. pasteurii</i> 51129	ATCC
<i>S. aureus</i> BAA-1708	Untyped, ATCC	<i>S. pettenkoferi</i> 36	Indianapolis, Denys Lab
<i>S. aureus</i> BAA-1721	Untyped, UK	<i>S. piscifermentans</i> 51183	ATCC
<i>S. aureus</i> 6538	Untyped, ATCC	<i>S. pulvereri</i> 33938	ATCC
<i>S. aureus</i> 11632	Untyped, ATCC	<i>S. pseudintermedius</i> 49444	ATCC
<i>S. aureus</i> 12600	Untyped, ATCC	<i>S. saccharolyticus</i> 14953	ATCC
<i>S. aureus</i> 13150	Untyped, ATCC	<i>S. saprophyticus</i> BAA-750	ATCC
<i>S. aureus</i> 14775	Untyped, ATCC	<i>S. saprophyticus</i> 15305	ATCC
<i>S. aureus</i> 14776	Untyped, ATCC	<i>S. schleiferi</i> subsp.	ATCC
<i>S. aureus</i> 14993	Untyped, ATCC	<i>S. schleiferi</i> subsp. ⁴⁹⁵⁴⁵ <i>S. schleiferi</i> 43808	ATCC
<i>S. aureus</i> 25923	Untyped, ATCC	<i>S. sciuri</i> 29061	ATCC
<i>S. aureus</i> 29213	Untyped, ATCC	<i>S. sciuri</i> 29060	ATCC
<i>S. aureus</i> 29247	Untyped, ATCC	<i>S. sciuri</i> 700013	ATCC
<i>S. aureus</i> 43300	Untyped, ATCC	<i>S. sciuri</i> subsp. <i>carnaticus</i> 700058	ATCC
<i>S. aureus</i> 700699	Untyped, ATCC	<i>S. sciuri</i> subsp. <i>rodentium</i> 700063	ATCC
<i>S. aureus</i> BORSA MCW1	Untyped, Wisconsin, Ledebor Lab	<i>S. simulans</i> 27841	ATCC
<i>S. aureus</i> BORSA MCW2	Untyped, Wisconsin, Ledebor Lab	<i>S. simulans</i> 27848	ATCC
<i>S. aureus</i> BORSA 23737	Untyped, New Jersey, Kreiswirth Lab	<i>S. simiae</i> 7213	ATCC
<i>S. aureus</i> BORSA 23739	Untyped, New Jersey, Kreiswirth Lab	<i>S. succinus</i> subsp. <i>succinus</i> 700337	ATCC
<i>S. aureus</i> Empty Mec Cassette	Untyped, Iowa, Diekema lab	<i>S. vitulinus</i> 51162	ATCC
⁴⁵ <i>S. aureus</i> Empty Mec Cassette	Untyped, Iowa, Diekema lab	<i>S. warneri</i> 10209	ATCC
⁴⁶ <i>S. aureus</i> Empty Mec Cassette	Untyped, Iowa, Diekema lab	<i>S. warneri</i> 25614	ATCC
⁵⁰ <i>S. aureus</i> Empty Mec Cassette	Untyped, Iowa, Diekema lab	<i>S. warneri</i> 27836	ATCC
⁵¹ <i>S. auricularis</i> 33751	ATCC	<i>S. warneri</i> 49454	ATCC
<i>S. auricularis</i> 33753	ATCC	<i>S. xylosus</i> 35633	ATCC
<i>S. capitis</i> subsp. <i>capitis</i> 35661	ATCC	<i>S. xylosus</i> 49148	ATCC
<i>S. capitis</i> subsp. <i>ureolyticus</i> 49326	ATCC		

In addition to these studies, a subset of (8) *S. aureus* strains representing SCC*mecA*

subtypes I-V, (1) *mecC* strain, (4) Borderline Oxacillin Resistant *S. aureus* (BORSA), (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 the table below:

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

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

<i>S. epidermidis</i>	700576	NA	Unknown	MR SE	>1	<i>Staphylococcus</i> species OTHER than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> Present
<i>S. epidermidis</i>	51625	NA	Unknown	MR SE	1	<i>Staphylococcus</i> species OTHER than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> Present
<i>S. epidermidis</i>	12228	NA	Unknown	MS SE	≤0.25	<i>Staphylococcus</i> species OTHER than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> Absent
<i>S. epidermidis</i>	700583	NA	Unknown	MS SE	≤0.25	<i>Staphylococcus</i> species OTHER than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> Absent
<i>S. hominis</i>	700586	NA	Unknown	MR Staph ssp	>1	<i>Staphylococcus</i> species OTHER than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> Present
<i>S. lugdunensis</i>	49576	NA	Unknown	MS - Lug	≤0.25	<i>S. lugdunensis mecA</i> Absent
<i>S. lugdunensis</i>	700328	NA	Unknown	MS - Lug	≤0.25	<i>S. lugdunensis mecA</i> Absent
<i>S. lugdunensis</i>	NCTC 7990	NA	Unknown	MS - Lug	≤0.25	<i>S. lugdunensis mecA</i> Absent
<i>S. lugdunensis</i>	43809	NA	Unknown	MS - Lug	≤0.25	<i>S. lugdunensis mecA</i> 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 *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 mollicute 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 1×10^8 CFU/mL or higher. Alarm positive blood cultures were not obtained for a subset of organisms. For 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 2 replicates was tested in the Staph ID/R Blood Culture Panel for each of the bacterial and fungal strains evaluated and these data are summarized in the table below.

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 (ATT, CCUG,	Exclusivity Species	Strain (ATT, CCUG, Clinical)
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	Clinical)		
Gram Positive Bacteria			
<i>Actinomyces odontolyticus</i> (1)	17929	<i>Listeria monocytogenes</i>	15313
<i>Abiotrophia defectiva</i>	49176	<i>Listeria seeligeri</i>	35967
<i>Aerococcus urinae</i>	51268	<i>Macrococcus caseolyticus</i> (1,2)	13548
<i>Arcanobacterium haemolyticum</i> (2)	BAA-1784	<i>Micrococcus luteus</i>	10240
<i>Bacillus cereus</i>	14579	<i>Micrococcus lylae</i>	27567
<i>Corynebacterium diphtheriae</i>	11051	<i>Mycobacterium avium</i>	700898
<i>Corynebacterium jeikeium</i>	43734	<i>Pediococcus damnosus</i>	29358
<i>Enterococcus avium</i>	14025	<i>Pediococcus pentosaceus</i>	33316
<i>Enterococcus casseliflavus</i>	700327	<i>Peptostreptococcus anaerobius</i>	27337
<i>Enterococcus durans</i>	6056	<i>Planococcus citreus</i>	14404
<i>Enterococcus faecalis</i>	29212	<i>Planococcus kocurii</i>	43650
<i>Enterococcus faecalis</i>	19433	<i>Propionibacterium acnes</i>	11827
<i>Enterococcus faecalis, van A</i>	1MC	<i>Rhodococcus equi</i>	6939
<i>Enterococcus faecalis, van B</i>	51575	<i>Rothia dentocariosa</i>	BAA-907
<i>Enterococcus faecium</i>	19434	<i>Rothia mucilaginoso</i>	49040
<i>Enterococcus faecium</i>	6057	<i>Streptococcus agalactiae</i>	BAA-611
<i>Enterococcus faecium, van A</i>	700221	<i>Streptococcus agalactiae</i>	13813
<i>Enterococcus gallinarium</i>	700425	<i>Streptococcus angunosis</i>	NCTC 10713
<i>Enterococcus gallinarium</i>	49573	<i>Streptococcus constellatus</i>	27823
<i>Enterococcus hirae</i>	8043	<i>Streptococcus dysagalactiae</i>	43078
<i>Enterococcus raffinosus</i>	49464	<i>Streptococcus equi</i>	9528
<i>Gemella morbillorum</i>	27824	<i>Streptococcus gallolyticus</i>	9809
<i>Globicatella sanguinis</i>	51174	<i>Streptococcus gallolyticus</i>	49475
<i>Kocuria kristinae</i>	BAA-752	<i>Streptococcus mitis</i>	6249
<i>Kocuria rosea</i>	186	<i>Streptococcus mutans</i>	25175
<i>Kytococcus schroeteri</i>	BAA-2410	<i>Streptococcus mutans</i> (2,3)	35668
<i>Lactobacillus acidophilus</i>	4356	<i>Streptococcus parasanguinis</i>	15909
<i>Lactococcus lactis</i>	11454	<i>Streptococcus pneumoniae</i>	ARUP
<i>Lactococcus lactis</i>	40932	<i>Streptococcus pyogenes</i>	49399
<i>Leuconostoc mesenteroides subsp. mesenteroides</i>	8293	<i>Streptococcus pyogenes</i>	12344
<i>Leuconostoc mesenteroides subsp. mesenteroides</i>	19254	<i>Streptococcus pyogenes</i>	4543
<i>Listeria grayi</i>	19120	<i>Streptococcus sanguinis</i>	10556
<i>Listeria innocua</i>	33090	<i>Streptococcus thoraltensis</i>	700865
<i>Listeria ivanovii</i>	19119	<i>Streptococcus uberis</i> (1)	9927
Gram Negative Bacteria			
<i>Acinetobacter baumannii</i>	19606	<i>Kluyvera intermedia</i> (1)	33421
<i>Acinetobacter calcoaceticus</i>	23055	<i>Moraxella catarrhalis</i> (1)	23246
<i>Acinetobacter haemolyticus</i>	19002	<i>Morganella morganii</i>	25829
<i>Acinetobacter lwoffii</i>	17925	<i>Neisseria gonorrhoeae</i>	19424
<i>Bacteriodes fragilis</i>	23745	<i>Neisseria meningitides</i> (1)	13077
<i>Bordetella pertussis</i>	9797	<i>Neisseria subflava</i> (1)	49275
<i>Burkholderia cepacia</i>	25416	<i>Oligella urethralis</i>	17960
<i>Citrobacter amalonaticus</i>	25405	<i>Proteus mirabilis</i> (1,4)	25933
<i>Citrobacter freundii</i>	8090	<i>Proteus vulgaris</i>	6896
<i>Citrobacter koseri</i>	27156	<i>Providencia rettgeri</i>	9250

<i>Enterobacter aerogenes</i>	15038	<i>Providencia rustigianii</i>	13159
<i>Enterobacter cloacae</i>	13047	<i>Pseudomonas aeruginosa</i>	10145
<i>Escherichia coli</i> (1)	BAA-199	<i>Pseudomonas putida</i>	49128
<i>Escherichia coli</i>	4157	<i>Salmonella enterica</i>	14028
<i>Fusobacterium nucleatum</i>	25586	<i>Salmonella typhimurium</i> (1)	13311
<i>Haemophilus haemolyticus</i>	33390	<i>Serratia liquefaciens</i> (2,5)	27592
<i>Hafnia alvei</i>	13337	<i>Serratia marcescens</i>	13880
<i>Klebsiella oxytoca</i>	13182	<i>Shigella sonnei</i>	29930
<i>Klebsiella pneumoniae</i>	700603	<i>Stenotrophomonas maltophilia</i>	13637
<i>Klebsiella pneumoniae</i>	BAA-1705	<i>Yersinia enterocolitica</i>	9610
Yeast			
<i>Candida albicans</i>	18804	<i>Candida parapsilosis</i>	14054
<i>Candida glabrata</i>	66032	<i>Candida tropicalis</i>	ARUP 2
<i>Candida krusei</i>	24210	<i>Cryptococcus neoformans</i>	90112
Mollicutes			
<i>Mycoplasma pneumonia</i> (6)	15531		

- (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 27 ‘invalid’ calls and 6 ‘*Staphylococcus* POSITIVE’ calls, all noted in the table above.

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

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

One ‘*Staphylococcus* POSITIVE’ call out of 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 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*.

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 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. 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 $1-2.5 \times 10^6$ 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.

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. Results are shown in the table below:

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

"Off-Panel" Microorganisms Species, Sample Input $\geq 10^8$ CFU/mL; TCC/NCTC strain #	Species; ATCC Strain #; Sample Input (CFU/mL)				
	<i>S. aureus</i> BAA-1682 $0.7-1.2 \times 10^6$	<i>S. aureus</i> 11632 $0.6-1.6 \times 10^6$	<i>S.</i> <i>epidermidis</i> 700562 $0.8-0.9 \times 10^6$	<i>S.</i> <i>epidermidis</i> 700583 $0.9-2.2 \times 10^6$	<i>S.</i> <i>lugdunensis</i> 49576 $1-1.2 \times 10^6$
Gram Positive Bacteria					
<i>Corynebacterium jeikeium</i> 43734	2/2	2/2	2/2	2/2	2/2
<i>Enterococcus faecalis</i> 19433	2/2	2/2	2/2"	2/2	2/2
<i>Enterococcus faecium</i> 19434	2/2	2/2	2/2"	2/2	2/2
<i>Listeria monocytogenes</i> 19115	2/2	2/2	2/2"	2/2"	2/2
<i>Micrococcus luteus</i> 10240	2/2	2/2	2/2	2/2	2/2
<i>Propionibacterium acnes</i> 11827	2/2	2/2	2/2	2/2	2/2
<i>Streptococcus agalactiae</i> 13813	2/2	2/2	2/2*	2/2	2/2
<i>Streptococcus anginosus</i> 10713	2/2	2/2	2/2	2/2	2/2"
<i>Streptococcus pneumoniae</i> 27336	2/2	2/2	2/2	2/2	2/2
<i>Streptococcus pyogenes</i> 49399	2/2	2/2	2/2"	2/2	2/2

Gram Negative Bacteria					
<i>Escherichia coli</i> 4157	2/2	2/2	2/2	2/2"	2/2*
<i>Klebsiella pneumoniae</i> 700603	2/2	2/2	2/2	2/2	2/2
<i>Pseudomonas aeruginosa</i> 10145	2/2	2/2	2/2	2/2	2/2
Yeast					
<i>Candida albicans</i> 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

Staphylococcus Microbial Interference: 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 $1-2.5 \times 10^6$ 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.

The studies assessed the detection of 5 ATCC *Staphylococcus* test strains: *S. aureus* (*mecA*+) BAA-1682 ($0.7-1.2 \times 10^6$ CFU/mL) *S. aureus* (*mecA*-) 11632 ($0.6-1.6 \times 10^6$ CFU/mL), *S. epidermidis* (*mecA*+) 700562 ($0.2-0.9 \times 10^6$ CFU/mL), *S. epidermidis* (*mecA*-) strain 700583 ($0.9-2.2 \times 10^6$ CFU/mL), and *S. lugdunensis* (*mecA*-) strain 49576 ($1-1.2 \times 10^6$ CFU/mL). A minimum of 2 replicate Staph ID/R Blood Culture Panels were performed for each combination of competing and test organisms.

Staphylococcus interference was observed for *S. aureus* with *S. epidermidis* and *S. caprae* at initial concentrations tested (5.9×10^5 CFU/mL), but the interference was resolved upon re-testing at higher concentrations (1.5×10^6 CFU/mL, within 2-3X LoD). There were 13 cases of interference with *S. epidermidis* at initial concentrations ($2.2-2.5 \times 10^5$ 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 ($8.5-9.4 \times 10^5$ CFU/mL, within 2-3X LoD). Two cases of interference were observed with *S. lugdunensis*: one case with *S. epidermidis*, one case with *S. hominis*. Both cases resolved at higher concentrations of *S. lugdunensis* (1.2×10^6 , within 2-3X LoD). Results are shown in the table below:

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

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

e. Interfering Substances:

The Staph ID/R Blood Culture Panel was evaluated for interference by a panel of 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 ($1-2.5 \times 10^6$ 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 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. A minimum of two replicate assays were 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 below.

Interfering Substances Panel (BACTEC Plus with Resin)

Substance Input Concentration into Plus Aerobic Media (with resin)	Species, ATCC strain, Sample Input (CFU/mL)										
	<i>S. aureus, mecA +</i>		<i>S. aureus, mecA -</i>		<i>S. lugdunensis, mecA -</i>		<i>S. epidermidis, mecA +</i>		<i>S. epidermidis, mecA -</i>		<i>E. faecalis (Neg)</i>
	BAA-1680 0.9-1.8x10 ⁶	BAA-1682 1.4- 1.6x10 ⁶	11632 1.2-2.2x10 ⁶	6538 1.8-2.2x10 ⁶	43809 1.1-1.7x10 ⁶	49576 0.9-1.8x10 ⁶	51625 0.3-1.5x10 ⁶	700562 0.3-0.8x10 ⁶	12228 0.9-1.2x10 ⁶	700583 0.3-1.9x10 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 Polyethanolsulfonate (≥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

Interfering Substances Panel (BACTEC Standard without Resin)

Substance Input Concentration into Standard Aerobic Media (without resin)	Species, ATCC strain, Sample Input (CFU/mL)										
	<i>S. aureus, mecA+</i>		<i>S. aureus, mecA-</i>		<i>S. lugdunensis, mecA-</i>		<i>S. epidermidis, mecA+</i>		<i>S. epidermidis, mecA-</i>		<i>E. faecalis</i> (Neg)
	BAA-1680 0.9-1.8x10 ⁶	BAA-1682 1.4-1.6x10 ⁶	11632 1.2-2.2x10 ⁶	6538 1.8-2.2x10 ⁶	43809 1.1-1.7x10 ⁶	49576 0.9-1.8x10 ⁶	51625 0.3-1.5x10 ⁶	700562 0.3-0.8x10 ⁶	12228 0.9-1.2x10 ⁶	700583 0.3-1.9x10 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

Testing of specimen with the above described potential interfering substances produced the expected positive and negative results indicating that none of the

endogenous or exogenous test substances compete or interfere with obtaining accurate test results with the Staph ID/R Blood Culture Panel.

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* (3.0×10^7 CFU/mL) and $>10^8$ *E. faecalis* (5.8×10^9 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.

No false positive results were observed during consecutive testing of high positive samples alternating with negative samples, demonstrating that recommended sample handling and testing practices are effective in preventing false positive results due to carryover or cross-contamination between samples.

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. 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* species (*S. aureus*, *S. epidermidis*, and *S. lugdunensis*), expected

positive results were obtained for 534/540 runs (98.8%) and expected negative results were obtained for 1345/1350 (99.6%) results.

A summary of results (percent agreement with the expected result) for each analyte is provided in the following tables:

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
<i>S. aureus</i> , <i>mecA</i> present	<i>S. aureus</i> (<i>mecA</i> +), 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	
	<i>S. aureus</i> (<i>mecA</i> +) 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 <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> present	<i>S. epidermidis</i> (<i>mecA</i> +), 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	
	<i>S. epidermidis</i> (<i>mecA</i> +), 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	
<i>S. lugdunensis</i> , <i>mecA</i> absent	<i>S. lugdunensis</i> (<i>mecA</i> -), 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	
	<i>S. lugdunensis</i> (<i>mecA</i> -), 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	<i>S. aureus</i> , <i>S. epidermidis</i> , <i>S. lugdunensis</i> , 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	<i>E. faecalis</i> , (<i>mecA</i> -), 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*

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
<i>S. aureus</i> , <i>mecA</i> present	<i>S. aureus</i> (<i>mecA</i> +), 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	
	<i>S. aureus</i> (<i>mecA</i> +), 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	
<i>Staphylococcus</i> species OTHER than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> present	<i>S. epidermidis</i> (<i>mecA</i> +), 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	
	<i>S. epidermidis</i> (<i>mecA</i> +), 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	
<i>mecA</i> Present; no organism associated	<i>S. aureus</i> (<i>mecA</i> +), <i>S. epidermidis</i> (<i>mecA</i> +) 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	
<i>mecA</i> Absent; no organism associated	<i>S. lugdunensis</i> (<i>mecA</i> -), <i>E. faecalis</i> (<i>mecA</i> -) 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

For detection of the *mecA* gene with no associated organism, positive result (*mecA* Present) were detected in 359/360 (99.7%) runs and negative results (*mecA* absent) were detected in 270/270 (100.0%) runs. For *S. aureus* (*mecA*+), positive *mecA* results were obtained for 180/180 (100.0%) runs and negative *mecA* results were obtained for 450/450 (100.0%) 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 (100.0%) runs.

Summary of discrepant results from Reproducibility Studies

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

h. Linearity/assay reportable range:

Not applicable

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

Internal Controls: To ensure that the reported Portrait Analyzer result is correct, controls have been built into the system to minimize the risk of errors during assay processing. These controls are designed to monitor reagent performance and to indicate correct performance of each Test Cartridge. Each Test Cartridge has integrated control features that are automatically performed with every Staph ID/R Blood Culture Panel. The following internal controls are included:

- **Fiducial Controls:** Establish orientation of the silicon chip for image analysis.
- **Amplification Threshold Control (AC):** The AC is used to threshold amplification such that potentially contaminating levels of *Staphylococci* are not amplified in the test. Two synthetic double-stranded DNA templates or plasmids are added to the test. One has homology with the primer regions specific for the *tuf* gene used in the Staph ID/R Blood Culture Panel, but with unique intervening sequence so that the amplified product is not recognized by probes on the chip surface. The shared primer binding site allows for the *tuf* gene specific primers to be used for both the amplification of *Staphylococci* present in a positive blood culture and the AC. The second AC has homology with the primer region for the *nuc* gene used in the Staph

ID/R Blood Culture Panel, but with unique intervening sequence and works similarly to the AC directed at the *tuf* gene. The ACs are lyophilized within a small chamber preceding the Extraction Chamber of the Staph ID/R Blood Culture Panel Test Cartridge. Upon sample loading the AC is rehydrated and during the PCR step is amplified. The ACs can compete with *Staphylococcal* genomic DNA for binding to the *tuf* and *nuc* gene primers, but has been optimized to do so only at levels below alarm positive blood cultures. At high *Staphylococcal* concentrations, the AC may be negative due to competitive inhibition of the amplification reagents. Note that the AC control is not detected by any probe(s) on the chip surface.

- **Sample Processing Control (SPC):** The SPC controls for all analytical steps in the procedure, including DNA extraction from organisms present in the specimen, amplification of target DNA sequences, hybridization, and detection on the chip surface. The SPC is a strain of *Bacillus subtilis* cells that has been genetically engineered with an integration vector designed to contain shared sequence with the *tuf* gene primers used in the Staph ID/R Blood Culture Panel, but with unique intervening sequence. The shared primer binding site allows for the *tuf* gene specific primers to be used for both the amplification of *Staphylococci* present in a positive blood culture and the SPC. The SPC is lyophilized within a small chamber preceding the Extraction Chamber of the Staph ID/R Blood Culture Panel Test Cartridge. After sample loading the SPC is extracted, amplified and the unique intervening sequence is detected by a target-specific capture probe on the chip surface. At high *staphylococcal* concentrations, the SPC may be negative due to competitive inhibition of the amplification reagents.

Recommended External Controls: External controls are not provided with the Staph ID/R Blood Culture Panel, but are recommended in the package insert. External positive and negative controls are intended to monitor for correct procedural technique and reagent integrity. The External Positive Control is intended to monitor for substantial reagent failure. The External Negative Control is intended to confirm non-reactivity. External controls should be used in accordance with the appropriate accrediting organization requirements, as applicable.

External Controls used in clinical studies: External positive and negative controls were incorporated in the clinical trial to monitor for correct procedural technique and reagent integrity. The external positive controls were intended to monitor for substantial reagent failure. The external negative control using a non-*Staphylococcus* Gram-positive cocci (*E. faecalis*) was intended to confirm non-reactivity. Each species in the daily QC blood culture panel were grown to bottle positivity in 2 separate preparations and aliquoted into single use frozen aliquots for investigational use during the prospective and analytical studies.

Daily QC testing was performed by dividing the strains into 2 panels and each panel was tested on alternating days. Daily QC testing was performed every day a

prospective, reproducibility or low prevalence study sample was tested with a Staph ID/R Blood Culture Panel Test Cartridge. Valid, correct results were required from the daily QC panel to include the test sample data for that day. In cases with an invalid or erroneous result, repeat testing with a valid, correct result was required to include the test sample data for that day. The daily QC panel results from all prospective sites from prospective, reproducibility and low prevalence studies are listed in the table below:

Species	Site #1	Site #2	Site #3	Site #4	Total
<i>S. aureus, mecA+</i>	32/33 ⁽¹⁾	21/21	16/16	2/2	71/72
<i>S. epidermidis, mecA-</i>	32/32	21/21	16/16	2/2	71/71
<i>S. lugdunensis, mecA-</i>	30/30	19/19	16/16	1/1	66/66
<i>S. epidermidis, mecA+</i>	29/29 ⁽²⁾	19/19 ⁽²⁾	16/16 ⁽²⁾	1/1	65/65
<i>E. faecalis, mecA-</i> (Negative)	61/62 ^(1,2)	40/41 ^(1,3)	32/32 ⁽²⁾	3/3	136/138

(1) This set of test run also contained 1 miscall

(2) This set of test runs also contained 1 "Invalid" run

(3) This set of test runs also contained 3 "invalid" runs

Eight (8) invalid test runs and 3 miscalls for a total of 11 discrepant results, were obtained in the Daily QC Panel from all clinical sites during prospective, reproducibility and low prevalence studies.

Specimen Stability:

A specimen stability study was conducted to support the claims of the sample storage on the bench for alarm positive blood culture bottles. Conditions include detection immediately after blood culture "bottle ring" and after incubation "On-board" up to 8 hours post alarm on the BACTEC Blood Culture System (35°-37°C). Additionally testing included, incubation once the bottle is removed from the blood culture system, termed "Off-board", for up to 18 hours at room temperature (15°-30°C), up to 72 hours at 2°-8°C and the combination of room temperature for up to 12 hours followed by up to 72 hours at 2°-8°C

For sample storage on the bench studies, 4 different *Staphylococcus* strains were tested: methicillin resistant *S. aureus* ATCC BAA-1682, methicillin resistant *S. epidermidis* ATCC 700562, methicillin sensitive *S. lugdunensis* ATCC 49576, methicillin resistant *S. haemolyticus* BAA-1693. An off-panel strain (Staph ID/R Blood Culture Panel "Negative"), *E. faecalis* ATCC 29212, was also included in the study. All strains were tested in three bottles in triplicate at each time point for a total of 9 replicates per condition.

For preparation of these samples, the strain cultures were prepared in TSB broth, incubated overnight, and the CFU concentrations for each strain were estimated by optical density measurements. Three BACTEC Plus Aerobic/F blood bottles

containing negative blood were inoculated with each respective strain at approximately 30-100 CFU/bottle and incubated until alarm positivity in a BACTEC Blood Culture System. Alarm positive bottles were pulled at bottle ring or incubated on the Blood Culture System past positivity for 8 hours +/- 0.5 hour "On-board". Samples were removed from each bottle and stored "Off-board" at the various temperatures for the times indicated in table below. All samples were Gram stained, serially diluted and plated for colony counts to determine the CFU/mL concentration at each time point and storage condition. Three tests were performed for each of 3 bottles, resulting in 9 total test runs for each condition. The data for each strain and time point are shown in the table below.

Specimen Stability Study Sample Input

On-board	Off-board		Species tested with Correct Staph ID/R Blood Panel				
	Temperature	Time (hours)	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. lugdunensis</i>	<i>E. faecalis</i>	<i>S. haemolyticus</i>
Bottle Ring	N	0	9/9*	9/9	9/9	9/9	9/9
	2	≤7	9/9	9/9	9/9	9/9	9/9
	1	≤1	9/9*	9/9	9/9*	9/9	9/9
	3	≤1	9/9	9/9	9/9	9/9	9/9
	30, then 2-8	<12, then <72	9/9	9/9	9/9	9/9	9/9
Bottle Ring + 8 Hours	N	0	9/9*	9/9	9/9*	9/9	9/9**
	2	≤7	9/9	9/9	9/9	9/9	9/9
	1	≤1	9/9	9/9	9/9	9/9*	9/9
	3	≤1	9/9	9/9	9/9	9/9	9/9
	30, then 2-8	<12, then <72	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

The Staph ID/R Blood Culture Panel results for all the conditions and time points are in 100% concordance with the expected results. The stability studies resulted in 8 'invalid' calls out of a total of 458 cards, for a rate of 2%, consistent with the prospective study invalid rate. At each time point, under the three storage conditions, *S. aureus* ATCC BAA-1682, *S. epidermidis* ATCC 700562, *S. lugdunensis* ATCC 49576, *S. haemolyticus* BAA-1693 and *E. faecalis* ATCC 29212 resulted in the expected 'Positive' or 'Negative' call, demonstrating sufficient stability of *Staphylococcus* in the BACTEC Plus Aerobic/F bottles in support of the claims of the sample stability for up to 18 hours at room temperature (15°-30°C), 8 hours at 35°-37°C and 72 hours at 2°-8°C.

The study was also informative in determining the conditions that result in the highest and lowest CFU/mL bacterial load in each bottle based on colony counts. As expected, bottle ring resulted in the lowest bacterial load across all strains tested: 7.2×10^6 – 7.7×10^8 CFU/mL. Also as expected, 8 hours incubation past bottle ring on the BACTEC Blood Culture Device increased bacterial load levels (2.3×10^7 - 3.4×10^9 CFU/mL), with Bottle Ring + 8 hours "On-board" + 18 hours (30°C) "Off-board" the consensus highest bacterial load condition for all strains: 1.1×10^8 – 3.1×10^9 CFU/mL.

j. Evaluation of Blood Culture Bottle Types:

To determine the effect of blood culture bottle type on Staph ID/R Blood Culture Panel, 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 (2×10^6 - 1×10^8 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 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. The results are shown in the table below:

Evaluation of Blood Culture Types:

Bottle Type	<i>Staphylococcus</i> Species, ATCC Strain; Sample Input (CFU/mL), Correct Staph ID/R Blood Panel							
	<i>S. aureus</i> (<i>mecA</i> +) BAA-1680 1.9×10^7	<i>S. aureus</i> (<i>mecA</i> +) BAA-1682 1.2×10^7	<i>S. aureus</i> (<i>mecA</i> -) 11632 7.5×10^7	<i>S. lugdunensis</i> (<i>mecA</i> -) 49576 1.9×10^8	<i>S. epidermidis</i> (<i>mecA</i> -) 12228 2.9×10^7	<i>S. epidermidis</i> (<i>mecA</i> +) 51625 6.5×10^7	<i>S. capitis</i> (<i>mecA</i> -) 35661 2.3×10^6	<i>E. faecalis</i> (<i>mecA</i> -) 29212 2.1-4.5x10 ⁶
BACTEC Standard 10 Aerobic/F	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9
BACTEC Standard Anaerobic	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

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 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 9 ‘Invalid’ call runs that are noted in the table above, all with *E. faecalis* 29212. When tested with *E. faecalis* 29212, 1 invalid run was observed for BACTEC PEDS Plus/F, 1 invalid run for BacT/Alert SA Aerobic, and 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 9 cards resulted in an additional 3 invalid runs for BACTEC PEDS Plus/F, 1 invalid run for BacT/Alert SA Aerobic, and 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%).

k. *Fresh versus Frozen Study:*

A study was performed to assess the tolerance of the Staph ID/R Blood Culture Panel to correctly identify cultures stored frozen at -20°C. In this study, 5 different strains used for the Staph ID/R Blood Culture QC Panel were tested: *S. aureus, mecA+* ATCC 33592, *S. epidermidis, mecA+* ATCC 700562, *S. epidermidis, mecA-* ATCC 12228, *S. lugdunensis mecA-*, ATCC 49576, and *E. faecalis* ATCC 29212 (Negative). In addition, 4 strains for fresh vs frozen Reference Site testing were used in the study: *S. aureus, mecA+* ATCC BAA-1682, *S. epidermidis, mecA+* ATCC 700562, *S. lugdunensis mecA-*, ATCC 49576, and *E. faecalis* ATCC 29212 (Negative).

For preparation of these samples, isolate cultures were prepared in TSB broth, incubated overnight and the CFU concentrations for each strain were estimated by optical density measurements. BACTEC Plus Aerobic/F blood bottles containing

negative blood were inoculated with each culture at approximately 30-100 CFU/bottle and incubated until alarm positivity in a BACTEC Blood Culture System. Alarm positive bottles were pulled at bottle ring or incubated past positivity < 8 hours, consistent with timeframes used during the specimen stability studies. Alarm positive blood cultures were gram stained, diluted, plated on agar plates and colonies counted the following day to confirm concentrations of each bottle.

Samples were tested in the Staph ID/R Blood Culture Panel after bottle ring, but before freezing, and aliquots from the bottles were stored frozen at -20°C. The frozen aliquots were thawed and tested before 1 month and after 5 months storage and tested in the Staph ID/R Blood Culture Panel.

The Staph ID/R Blood Culture Panel results for the fresh vs frozen sample stability study were 100% concordant with expected results for all strains tested. Frozen storage did not impact performance in the Staph ID/R Blood Culture Panel.

Fresh vs. Frozen Sample Stability Study (Staph ID/R Blood Culture Panel)

Sample	Species, <i>mecA</i> status	ATCC Strain #	Sample Input (CFU/mL)	Fresh (Correct)	Frozen, <1 month (Correct)	Frozen, ≥ 5 months (Correct)
QC1	<i>S. aureus</i> , <i>mecA</i> +	33592	1.7x10 ⁸	2/2	2/2	2/2
QC2	<i>S. epidermidis</i> , <i>mecA</i> -	12228	3.9x10 ⁷	2/2	2/2	2/2
QC3	<i>S. lugdunensis</i> , <i>mecA</i> -	49576	3.0x10 ⁶	2/2	2/2	2/2
QC4	<i>S. epidermidis</i> , <i>mecA</i> +	700562	6.9x10 ⁷	2/2	2/2	2/2
QC5	<i>E. faecalis</i> , <i>mecA</i> -	29212	3.2x10 ⁸	2/2	2/2	2/2
RP1	<i>S. aureus</i> , <i>mecA</i> +	BAA-1682	5.2x10 ⁶	2/2	2/2	2/2
RP2	<i>S. epidermidis</i> , <i>mecA</i> +	700562	1.9x10 ⁷	2/2	2/2	2/2
RP3	<i>S. lugdunensis</i> , <i>mecA</i> -	49576	4.9x10 ⁷	2/2	2/2	2/2
RP4	<i>E. faecalis</i> , <i>mecA</i> -	29212	1.1x10 ⁹	2/2	2/2	2/2

A parallel study was performed to assess the tolerance of the Staph ID/R Blood Culture Panel reference methods to correctly identify cultures stored frozen at -20°C. Reference site validation samples were shipped at room temperature or frozen and tested in the same assays used during prospective studies (i.e., BD Phoenix ID for species identification and cefoxitin disc diffusion for methicillin resistance). The samples shipped at room temperature were tested within 18 hours of bottle ring, consistent with the specimen stability study conditions. Frozen aliquots were stored frozen (-20°C), consistent with conditions used for the prospective study, and thawed when ready to be tested. Frozen aliquots were thawed and tested at 1, 30, 60, 90 and 120 days.

2. Comparison studies:

a. *Method comparison with predicate device:*

N/A

b. *Matrix comparison:*

N/A

3. Clinical Studies:

A method comparison study was conducted at three external, geographically-diverse clinical study sites in the U.S. during an eight month period in 2014–2015 to evaluate the comparative performance of the Staph ID/R Blood Culture Panel assay with the PA500 Portrait™ Analyzer System to applicable conventional biochemical and culture reference microbiology methods. 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 and compared to traditional laboratory reference methods (i.e., culture followed by testing blood culture isolates with conventional biochemical, BD Phoenix, and cefoxitin disc diffusion testing). Cefoxitin disc diffusion tests were used as the reference method for confirming *mecA* mediated resistance. 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 samples were tested originally in the clinical trial, while the remaining 69 archived frozen specimens were tested after the prospective clinical trial

Clinical evaluation sites in the United States

Clinical Site 1 - 3		City, State	Geographic Area	Institution Type	Principal Investigator
1	Indiana University School of Medicine (IU)	Indianapolis, IN	Midwest	Hospital Lab	Gerald Denys, PhD
2	Primary Children's Medical Center (PCMC)	Salt Lake City, UT	West	Hospital Lab	Judy Daly, PhD
3	TriCore Reference Laboratories	Albuquerque, NM	Southwest	Reference Laboratory	Stephen Young, PhD
Additional Collection Site for PCMC		City, State	Geographic Area	Institution Type	Principal Investigator

ARUP Laboratories	Salt Lake City, UT	West	Reference Laboratory	Marc Couturier, PhD
Microbiology Reference Site				
Medical College of Wisconsin	Milwaukee, WI	Midwest	Reference Laboratory	Nate Ledebor, PhD

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.

The following tables provide the clinical performance of the Staph ID/R Blood Culture Panel for organism identification of overall *Staphylococcus* species, *S. aureus*, *S. epidermidis*, *S. lugdunensis*, and *Staphylococcus* species undetermined (i.e., not *S. aureus*, or *S. lugdunensis*) as determined by the reference methods. For each organism identification, Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of the Staph ID/R Blood Culture Panel assay are calculated based upon the % agreement to the reference methods.

Demographic Summary for Prospective Arm of the Staph ID/R Blood Culture Panel

Clinical Evaluation

Prospective Study Specimens: Total Specimens - 831			
Sex		Number of Specimens	
Male		454	
Female		377	
Patient Age	Prospective	Supplemental	Overall
≤ 1 year	87	5	92
1 - 18 years	60	1	61
19 - 35 years	69	14	83
36 - 55 years	153	15	168
56 - 70 years	203	18	221
≥ 71 years	190	16	206

Positive percent agreement (PPA) was calculated as $100\% \times (TP/TP + FN)$. True positive (TP) indicates that both Staph ID/R Blood Culture Panel and the reference/comparator method had a positive result for a specific analyte, and false

negative (FN) indicates that the Staph ID/R Blood Culture Panel result was negative while the reference/comparator method was positive. Clinical specificity or negative percent agreement (NPA) was calculated as $100\% \times (TN / (TN + FN))$. True negative (TN) indicates that both Staph ID/R Blood Culture Panel and the reference/comparator method had a negative result for a specific analyte, and false positive (FP) indicates that the Staph ID/R Blood Culture Panel result was positive while the reference/comparator method was negative. The exact binomial two-sided 95% confidence interval was calculated. The results are summarized in the following tables.

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

All sites combined		% Agreement			
		TP/TP + FN	PPA 95% CI	TN/TN + TP	NPA 95% CI
Detection of <i>Staphylococcus aureus</i>	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 <i>Staphylococcus lugdunensis</i>	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 <i>Staphylococcus</i> species OTHER than <i>S. aureus</i> or <i>S. lugdunensis</i>	Prospective	444/449	98.9% 97.4 - 99.5%	307/316	97.2% 94.7 - 98.5%
Detection of <i>mecA</i> with <i>Staphylococcus aureus</i>	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 <i>mecA</i> with <i>Staphylococcus lugdunensis</i>	Prospective	0/0	N/A	762/762	100.0% 99.5 - 100%
Detection of <i>mecA</i> with <i>Staphylococcus</i> species OTHER than <i>S. aureus</i> or <i>S. lugdunensis</i>	Prospective	243/262	92.7% 88.1 - 97.1%	481/500	96.2% 92.4 - 98.0%

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

Species	% Agreement (95% CI)			
	Prospective		Simulated	
<i>S. arlettae</i>	-	-	3/3	100% 43.9-100%
<i>S. auricularis</i>	-	-	3/3	100% 43.9-100%
<i>S. capitis</i>	32/35	91.4% 77.6-97.0%	-	-
<i>S. carnosus</i>	1/1	100% 20.7-100%	-	-
<i>S. cohnii</i>	2/2	100% 34.2-100%	3/3	100% 43.9-100%
<i>S. equorum</i>	2/2	100% 34.2-100%	-	-
<i>S. haemolyticus</i>	12/15	80.0% 54.8-93.0%	3/3	100% 43.9-100%
<i>S. hominis</i>	83/97	85.6% 77.2-91.2%	-	-
<i>S. intermedius</i>	-	-	3/3	100% 43.9-100%
<i>S. pettenkoferi</i>	7/7	100% 64.6-100%	-	-
<i>S. saprophyticus</i>	4/8	50.0% 21.5-78.5%	-	-
<i>S. schleiferi</i>	0/2	0.0% 0-0.7%	3/3	100% 43.9-100%
<i>S. sciuri</i>	-	-	3/3	100% 43.9-100%
<i>S. simulans</i>	2/2	100% 34.2-100%	3/3	100.0% 43.9-100%
<i>S. species</i>	2/2	100% 34.2-100%	-	-
<i>S. warneri</i>	8/8	100% 67.6-100%	3/3	100% 43.9-100%
<i>S. xylosus</i>	-	-	3/3	100% 43.9-100%

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

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

N. Instrument Name:

The Great Basin PA500 Portrait™ Analyzer System

O. System Descriptions:

1. Modes of Operation:

See Device Description (Section I) above

2. Software

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

Yes X or No _____

3. Specimen Identification:

N/A

4. Specimen Sampling and Handling:

N/A

5. Calibration:

N/A

6. Quality Control:

See Section M (i) above

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

Not Applicable

Q. Proposed Labeling:

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

R. Conclusion:

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