510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K243490

B Applicant

Luminex Corporation

C Proprietary and Established Names

LIAISON PLEX Gram-Positive Blood Culture Assay

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PAM	Class II	21 CFR 866.3365 - Multiplex Nucleic Acid Assay For Identification Of Microorganisms And Resistance Markers From Positive Blood Cultures	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

The purpose of this submission is to obtain a substantial equivalence determination for the LIAISION PLEX Gram Positive Blood Culture Assay

B Measurand:

Nucleic acids from *Bacillus spp.*, *Enterococcus faecalis*, *Enterococcus faecium*, *Listeria spp.*, *Staphylococcus spp.*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, *Streptococcus spp.*, *Streptococcus agalactiae*, *Streptococcus anginosus* group, *Streptococcus pneumoniae* and *Streptococcus pyogenes* organisms, and mecA/mecC, vanA, and vanB resistance markers.

C Type of Test:

A qualitative multiplexed nucleic acid test intended for use with the automated LIAISON PLEX instrument for the qualitative in vitro detection and identification of gram-positive bacteria in a positive blood culture media sample that demonstrates the presence of gram-positive bacteria as determined by Gram stain.

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The LIAISON PLEX Gram-Positive Blood Culture (BCP) Assay, performed using the automated, sample-to-result LIAISON PLEX System, is a qualitative multiplexed in vitro diagnostic test for the simultaneous detection and identification of selected gram-positive pathogens and/or selected genetic determinants associated with antimicrobial resistance in positive blood culture bottles.The LIAISON PLEX BCP Assay is performed directly on blood culture media using blood culture bottles identified as positive by a continuous monitoring blood culture system and which contain gram-positive bacteria as determined by Gram stain.

The LIAISON PLEX BCP Assay detects and identifies the following:

Gram Positive Resistance Markers (a):

mecA/mecC
vanA
vanB

Genera and Species:

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

(a) Negative results for antimicrobial resistance genes do not indicate bacterial susceptibility as there are multiple mechanisms that can contribute to resistance

The LIAISON PLEX BCP contains targets for the detection of genetic determinants associated with resistance to methicillin (mecA/mecC) and vancomycin (vanA and vanB) to aid in the identification of potentially antimicrobial-resistant organisms in positive blood culture samples. In mixed growth, the LIAISON PLEX BCP Assay does not specifically attribute vanA/vanB-mediated vancomycin resistance to either *E. faecalis* or *E. faecium*, or mecA/mecC-mediated methicillin resistance to either *Staphylococcus* spp., *S. aureus*, *S. epidermidis* or *S. lugdunensis*.

The antimicrobial resistance gene or marker detected may or may not be associated with the agent responsible for disease. Negative results for these select antimicrobial resistance gene and marker assays do not indicate susceptibility, as multiple mechanisms of methicillin and vancomycin resistance exist.

The LIAISON PLEX BCP Assay is indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial bloodstream infections (BSI). The LIAISON PLEX BCP Assay is not intended to monitor treatment of these infections. Sub-culturing of positive blood cultures is necessary to recover organisms for antimicrobial susceptibility testing (AST), for identification of organisms not detected by LIAISON PLEX BCP Assay, to detect mixed infections that may not be detected by the LIAISON PLEX BCP Assay, for association of antimicrobial resistance genes to a specific organism, or for epidemiological typing.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

For in vitro diagnostic use only

D Special Instrument Requirements:

LIAISON PLEX Instrument and LIAISON PLEX BCP Assay Software version 1.3

IV Device/System Characteristics:

A Device Description:

The LIAISON PLEX Gram-Positive Blood Culture Assay (BCP Assay) is an automated test for the detection and identification of nucleic acid from gram-positive bacteria in a positive blood culture media sample. The BCP Assay is performed directly on blood culture media using blood culture bottles identified as positive by a continuous monitoring blood culture system, and which contain gram-positive bacteria, as determined by a Gram stain.

The LIAISON PLEX System is a fully automated, bench-top "sample-to-answer" device that performs sample preparation, polymerase chain reaction (PCR) and microarray-based hybridization for the detection of target-specific nucleic acids. The test reagents are supplied as a single, disposable test cartridge, including disposable Assay Transfer Pipette Tips.

The LIAISON PLEX BCP Assay has 16 different reportable targets. Reporting of these targets is based on detection of one or more of the nucleic acid targets. Results can be:

- reviewed by the user on the touch-screen;
- exported to as USB flash drive; and,

- printed or submitted to a Laboratory Information System (LIS) for reporting.

B Principle of Operation:

The LIAISON PLEX Gram-Positive Blood Culture Assay (BCP Assay) is performed directly on blood culture media using blood culture bottles identified as positive by a continuous monitoring blood culture system, and which contain gram-positive bacteria, as determined by a Gram stain. The system consists of an instrument and a single-use, disposable test cartridge. The user loads an aliquot of the sample into the sample port of the LIAISON PLEX Gram-Positive Blood Culture Assay Cartridge. Next, the user sets up the sample order on the LIAISON PLEX System by first entering the sample information or scanning the barcode ID located on the sample tube, then scanning the barcode ID located on the test cartridge. Last, the user inserts the test cartridge into the processing module to initiate the test. The LIAISON PLEX System identifies the assay being run and automatically initiates the proper testing protocol to process the sample, analyze the data, and generate test results.

The LIAISON PLEX System automates the BCP Assay sample analysis through the following steps:

a) Sample Preparation: Nucleic acid extraction via mechanical and chemical cell lysis and magnetic bead-based nucleic acid isolation; PCR amplification is not used in this assay. By performing direct detection, the assay specifically detects the viable bacteria and minimizes interference from trace nucleic acids or non-viable bacteria that may be present at much lower levels and potentially lead to false positive results.

b) Hybridization: Extracted nucleic acid hybridize to target-specific capture DNA on a microarray format, and target-specific mediator and gold nanoparticle probe hybridize to captured nucleic acids; c) Signal Analysis: Gold nanoparticle probes bound specifically to target-containing spots in the microarray are silver-enhanced, and light scatter from the spots is measured and further analyzed to determine the presence (Detected) or absence (Not Detected) of a target.

The system completes all necessary steps for the assay. No user intervention is necessary once the run is initiated. Once the run is complete, the user removes the cartridge(s) and disposes of them in the appropriate waste container. Following a series of quality control checks a final call is made for the Target: Detected if the Target signal is above threshold and Not Detected otherwise. Failure of any quality control checks results in a “No Call” result.

C Instrument Description Information:

1. Instrument Name:

   LIAISON Plex Systems

2. Specimen Identification:

   Specimen identification information is entered either manually or via barcode.

3. Specimen Sampling and Handling:

   Blood culture media using blood culture bottles identified as positive by a continuous monitoring blood culture system and which contain gram-positive bacteria as determined by a Gram stain.

4. Calibration:

   LIAISON PLEX modules are calibrated during the manufacturing process; calibration is not performed by the user.

5. Quality Control:

   Internal Controls: LIAISON PLEX BCP Assay cartridge incorporates multiple controls to confirm proper test performance, including two internal processing controls (extraction and hybridization controls) that are designed to monitor effectiveness of the specimen processing and hybridization steps. The extraction control is present in the lysis tube of the assay cartridge and functions to assess extraction, nucleic acid isolation, and fragmentation steps of the assay workflow on the cartridge. The hybridization control is present in the sample buffer and added after nucleic acid extraction step and serves as an indicator of successful hybridization step.

   A "No Call" is reported based on the BCP Assay specific call logic for internal controls implemented in the assay file. Internal processing controls must generate a signal above the predetermined threshold in each internal reaction for the system to report a valid test result. It is recommended to repeat the test associated with a "No Call" result per the instructions for use.

   External Controls: External controls are not provided with the LIAISON Plex BCP Assay. However, six external control mixes were provided to the clinical study sites for daily testing during the prospective clinical study. External controls were tested on each day of testing, utilizing one external negative control and one of five external positive controls (tested on a rotating basis) representing all of the LIAISON Plex BCP targets.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Verigene Gram-Positive Blood Culture (BC-GP) Nucleic Acid Test

B Predicate 510(k) Number(s):
K122514

C Comparison with Predicate(s):

Device & Predicate Device(s):	K243490	K122514
Device Trade Name	LIAISON PLEX Gram Positive Blood Culture (BCP) Assay	Verigene Gram-Positive Blood Culture (BC-GP) Nucleic Acid Test

General Device Characteristic Similarities		
Intended Use/Indications For Use	The LIAISON PLEX Gram-Positive Blood Culture (BCP) Assay, performed using the automated, sample-to-result LIAISON PLEX System, is a qualitative multiplexed in vitro diagnostic test for the simultaneous detection and identification of selected gram-positive pathogens and/or selected genetic determinants associated with antimicrobial resistance in positive blood culture bottles. The LIAISON PLEX BCP Assay is performed directly on blood culture media using blood culture bottles identified as positive by a continuous monitoring blood culture system and which contain gram-positive bacteria as determined by Gram stain. The LIAISON PLEX BCP Assay detects and identifies the following: Resistance Markers[a] mecA/mecC vanA vanB Genera and Species *Bacillus* spp. *Enterococcus faecalis* *Enterococcus faecium* *Listeria* spp. *Staphylococcus* spp. *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus lugdunensis* *Streptococcus* spp. *Streptococcus agalactiae* *Streptococcus anginosus group* *Streptococcus pneumoniae* *Streptococcus pyogenes*	The Verigene Gram-Positive Blood Culture Nucleic Acid Test (BC-GP), performed using the sample-to-result Verigene System, is a qualitative multiplexed in vitro diagnostic test for the simultaneous detection and identification of potentially pathogenic gram-positive bacteria which may cause bloodstream infection (BSI). BC-GP is performed directly on blood culture bottles identified as positive by a continuous monitoring blood culture system and which contain gram-positive bacteria. BC-GP detects and identifies the following: Bacterial Genera and Species *Staphylococcus* spp. *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus lugdunensis* *Streptococcus* spp. *Streptococcus pneumoniae* *Streptococcus pyogenes* *Streptococcus agalactiae* *Streptococcus anginosus* group *Enterococcus faecalis* *Enterococcus faecium* *Listeria* spp. Resistance Markers1 mecA vanA vanB 1In mixed growth, BC-GP does not specifically attribute van-mediated vancomycin resistance to either *E. faecalis or E. faecium*, or mecA-mediated methicillin resistance to either *S. aureus* or *S. epidermidis.* BC-GP is indicated for use in conjunction with other

	[a]Negative results for antimicrobial resistance genes do not indicate bacterial susceptibility as there are multiple mechanisms that can contribute to resistance. The LIAISON PLEX BCP contains targets for the detection of genetic determinants associated with resistance to methicillin (mecA/mecC) and vancomycin (vanA and vanB) to aid in the identification of potentially antimicrobial-resistant organisms in positive blood culture samples. In mixed growth, the LIAISON PLEX BCP Assay does not specifically attribute vanA/vanB-mediated vancomycin resistance to either *E. faecalis* or *E. faecium*, or mecA/mecC-mediated methicillin resistance to either *Staphylococcus* spp., *S. aureus*, *S. epidermidis* or *S. lugdunensis.* The antimicrobial resistance gene or marker detected may or may not be associated with the agent responsible for disease. Negative results for these select antimicrobial resistance gene and marker assays do not indicate susceptibility, as multiple mechanisms of methicillin and vancomycin resistance exist. The LIAISON PLEX BCP Assay is indicated for use in conjunction with other clinical and laboratory findings to aid in the	clinical and 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 antimicrobial susceptibility testing (AST), for identification of organisms not detected by BC-GP, differentiation of mixed growth, for association of antimicrobial resistance marker genes to a specific organism, or for epidemiological typing.

	diagnosis of bacterial bloodstream infections (BSI). The LIAISON PLEX BCP Assay is not intended to monitor treatment of these infections. Sub-culturing of positive blood cultures is necessary to recover organisms for antimicrobial susceptibility testing (AST), for identification of organisms not detected by LIAISON PLEX BCP Assay, to detect mixed infections that may not be detected by the LIAISON PLEX BCP Assay, for association of antimicrobial resistance genes to a specific organism, or for epidemiological typing.	
Measurand	Target DNA sequences of gram-positive bacteria and antimicrobial resistance markers	Same
Specimen Type	Positive blood culture (PBC) samples that contain gram-positive bacteria.	Same
Sample Extraction	Chemical and mechanical lysis followed by a chaotrope-based nucleic acid isolation procedure	Same
Quality Controls	Two internal processing controls (extraction and hybridization controls). External controls are recommended to be run	Same
Test Interpretation Output	Detected/Not Detected	Same
General Device Characteristic Differences		
Organisms Detected	*Bacillus* spp. *Enterococcus faecalis* *Enterococcus faecium* *Listeria* spp. *Staphylococcus* spp. *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus lugdunensis* *Streptococcus* spp. *Streptococcus agalactiae* *Streptococcus anginosus* group	*Staphylococcus* spp. *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus lugdunensis* *Streptococcus* spp. *Streptococcus pneumoniae* *Streptococcus pyogenes* *Streptococcus agalactiae* *Streptococcus anginosus* group *Enterococcus faecalis* *Enterococcus faecium*

	Streptococcus pneumoniae *Streptococcus pyogenes*	*Listeria* spp.
Resistance Markers Detected	mecA/mecC vanA vanB	mecA vanA vanB
Storage Requirements	15-30°C (for cartridges only)	2-8°C (for cartridges only)
Required Instrumentation	LIAISON PLEX System	Verigene System, Reader and Processor SP
Time to Result	2.5 hours	~ 2 hours

VI Standards/Guidance Documents Referenced:

Document ID	Title
Class II Special Controls Guideline	Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures (May 2015)
Guidance document	Electronic Submission Template for Medical Device 510(k) Submissions - Guidance for Industry and Food and Drug Administration Staff (October 2, 2023).
Guidance document	Content of Premarket Submissions for Device Software Functions - Guidance for Industry and Food and Drug Administration Staff (June 14, 2023).
Guidance document	Cybersecurity in Medical Devices: Quality System Considerations and Content of Premarket Submissions - Guidance for Industry and Food and Drug Administration Staff (September 23, 2023).
Guidance document	Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests - Guidance for Industry and FDA Staff (March 13, 2007).
CLSI document EP25-A	Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
ISO 14971:2019	Medical devices -Application of risk management to medical devices
IEC 62366-1:2015 +A1:2020	Medical devices -Part 1: Application of usability engineering to medical devices
ISO 62304:2006	Medical device software -Software life-cycle processes
ISO 15223-1:2021:	Medical Devices -Symbols to be used with medical device labels, labeling and information to be supplied -Part 1: General requirements
IEC 61010-1 Ed. 3.1 2017-01	Safety requirements for electrical equipment for measurement, control, and laboratory use -Part 1: General requirements
EN 61010-2-101:2002/IEC 61010-2-101:2015	Safety requirements for electrical equipment for measurement, control and laboratory use -Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment
IEC 60601-1-2:2014 (Edition 4.0)	Medical electrical equipment -Part 1-2: General requirements for basic safety and essential performance -Collateral Standard: Electromagnetic disturbances -Requirements and tests
ISO 13485:2016/EN ISO 13485:2016	Medical devices -Quality Management System -Requirements for regulatory purposes
ISO 20916:2019	In vitro diagnostic medical devices. Clinical performance studies using specimens from human subjects. Good study practice
EN ISO 18113-1:2011	In vitro diagnostic medical devices -Information supplied by the manufacturer (labeling). Terms, definition and general requirements
EN ISO 18113-2:2011	In vitro diagnostic medical devices -Information supplied by the manufacturer (labeling) – Part 2: In vitro diagnostic reagents for professional use
EN ISO 18113-3:2011	In vitro diagnostic medical devices -Information supplied by the manufacturer (labeling) – Part 3: In vitro diagnostic instruments for professional use
EN ISO 23640:2015	In vitro diagnostic medical devices -Evaluation of stability of in vitro diagnostic reagents
IEC 61326-1:2012	Electrical equipment for measurement control and laboratory use -EMC requirements -Part 1: General requirements
EN 61326-2-6:2006/IEC 61326-2-6:2012	Electrical equipment for measurement control and laboratory use -EMC requirements -Part 2-6: Particular requirements -In vitro diagnostic (IVD) medical equipment

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility: A sample panel was prepared containing seven representative on-panel organisms prepared at ring-positive (RP) and ring-positive + 8 hours concentrations. Additionally, one negative sample contrived with an off-panel organism (*Escherichia coli*), and one negative blood culture matrix were also prepared. Assay reproducibility was evaluated in a study conducted across three different sites, for five days, with two runs and three replicates per run. A total of 90 replicates were evaluated for each tested analyte in each panel member.

   Results from the reproducibility study are summarized below (Table 1).

Table 1 Reproducibility Study Results

Organism ID	Reportable Targets	Sample Type	Agreement (%) with Expected results			% Agreement All sites [95% CI]
			Site1	Site 2	Site 3	
Staphylococcus aureus	*Staphylococcus spp.*	Panel 1 – RP	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%-100%]
		Panel 1 – RP+8 Hours	100% (30/30)	96.7% (29/30)[a]	100% (30/30)	(89/90) 98.9% [94.0%-99.8%]
Staphylococcus aureus	*Staphylococcus aureus*	Panel 1 – RP	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
		Panel 1 – RP+8 Hours	100% (30/30)	96.7% (29/30)[a]	100% (30/30)	(89/90) 98.9% [94.0%-99.8%]
Enterococcus faecalis	*Enterococcus faecalis*	Panel 2 – RP	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
		Panel 2 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
Bacillus subtilis	*Bacillus spp.*	Panel 3 – RP	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
		Panel 3 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
Listeria monocytogenes	*Listeria spp.*	Panel 4 – RP	100% (30/30)	100% (30/30)	96.7% (29/30)[c]	(89/90) 98.9% [94.0%-99.8%]
		Panel 4 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
Staphylococcus epidermidis	*Staphylococcus spp.*	Panel 5 – RP	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]

		Panel 5 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
Staphylococcus epidermidis	*Staphylococcus epidermidis*	Panel 5 – RP	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
		Panel 5 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
Streptococcus pneumoniae	*Streptococcus spp.*	Panel 6 – RP	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
		Panel 6 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
Streptococcus pneumoniae	*Streptococcus pneumonia*e.	Panel 6 – RP	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
		Panel 6 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
Streptococcus pyogenes	*Streptococcus spp.*	Panel 7 – RP	100% (30/30)	100% (30/30)	96.7% (29/30)[d]	(89/90) 98.9% [94.0%-99.8%]
		Panel 7 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
Streptococcus pyogenes	*Streptococcus pyogenes*	Panel 7 – RP	100% (30/30)	100% (30/30)	96.7% (29/30)[d]	(89/90) 98.9% [94.0%-99.8%]
		Panel 7 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
Escherichia coli	None	Contrived Negative	100% (60/60)	100% (60/60)	100% (60/60)	(180/180) 100% [97.9%-100%]
No Target	None	Negative Blood Matrix	100% (30/30)	100% (30/30)	96.7% (29/30)[b]	(89/90) 98.9% [94.0%-99.8%]
Staphylococcus aureus	*mecA/mecC*	Panel 1 – RP	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
		Panel 1 – RP+8 Hours	100% (30/30)	96.7% (29 [a]/30)	100% (30/30)	(89/90) 98.9% [94.0%-99.8%]
Enterococcus faecalis	*vanB*	Panel 2 – RP	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
		Panel 2 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]

Staphylococcus epidermidis	*mecA/mecC*	Panel 5 – RP	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]
		Panel 5 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	(90/90) 100% [95.9%- 100%]

[a] False positive *S. lugdunensis*, *S. anginosus* group, and *Streptococcus* spp. result in 1/30 replicates. Reportable targets were all detected.
[b] False positive *E. faecium* and *vanA* result in 1/30 replicates.
[c] False positive *S. lugdunensis* result in 1/30 replicates. Reportable targets were all detected.
[d] False positive *Staphylococcus* spp. result in 1/30 replicates. Reportable targets were all detected.

Precision and Repeatability: Within-laboratory precision was evaluated by testing a panel of samples containing two representative on-panel organisms at ring-positive and ring-positive +8 hours concentrations. Additionally, one contrived negative sample containing an off-panel organism and one negative blood culture sample were also evaluated. Samples were tested in one site, using one reagent lot. Percent agreement with expected results was calculated for each analyte at each tested concentration. Results are summarized in Table 2, below.

Table 2. Precision/Repeatability Study Results

Organism ID	Reportable Targets	Sample Type	Agreement (%) with Expected results	Overall 95% CI
Staphylococcus aureus	*Staphylococcus spp*	Panel 1 – RP	100% (30/30)	88.6% - 100%
		Panel 1 – RP+8 Hours	100% (30/30)	88.6% - 100%
Staphylococcus aureus	*Staphylococcus aureus*	Panel 1 – RP	100% (30/30)	88.6% - 100%
		Panel 1 – RP+8 Hours	100% (30/30)	88.6% - 100%
Enterococcus faecalis	*Enterococcus faecalis*	Panel 2 – RP	100% (30/30)	88.6% - 100%
		Panel 2 – RP+8 Hours	100% (30/30)	88.6% - 100%
Escherichia coli	None	Contrived Negative	100% (30/30)	88.6% - 100%
No Target	None	Negative Blood Matrix	100% (30/30)	88.6% - 100%
Resistance Marker genes				
Staphylococcus aureus	*mecA/mecC*	Panel 1 – RP	100% (30/30)	88.6% - 100%
		Panel 1 – RP+8 Hours	100% (30/30)	88.6% - 100%
Enterococcus faecalis	*vanB*	Panel 1 – RP	100% (30/30)	88.6% - 100%
		Panel 1 – RP+8 Hours	100% (30/30)	88.6% - 100%

Lot-to-Lot variability: Lot-to-Lot reproducibility was evaluated during five non-consecutive days using three lots of cartridges and four LIAISON PLEX Systems. A sample panel of six blood culture samples including two on-panel representative organisms at both ring-positive and ring positive +8 hours concentrations was evaluated. Additionally, one negative contrived sample

containing an off-panel organism and a negative blood culture sample were also tested. Each sample was tested in triplicate per cartridge lot on a given testing day. Between-lot percentage agreement was calculated as seen in Table 3, below.

Table 3 Lot-to-Lot Reproducibility Results

Organism ID	Reportable Targets	Sample Type	Reagent Lot	Results (%) agreement	Results (95% CI)
Staphylococcus aureus	*Staphylococcus spp*	Panel 1 – RP	Lot 1	100% (15/15)	79.6% - 100%
			Lot 2	100% (15/15)	
			Lot 3	100% (15/15)	
			Overall	100% (45/45)	92.1% – 100%
		Panel 1 – RP+8 Hours	Lot 1	100% (15/15)	79.6% - 100%
			Lot 2	100% (15/15)	
			Lot 3	100% (15/15)	
			Overall	100% (45/45)	92.1% – 100%
Staphylococcus aureus	*Staphylococcus aureus*	Panel 1 – RP	Lot 1	100% (15/15)	79.6% - 100%
			Lot 2	100% (15/15)	
			Lot 3	100% (15/15)	
			Overall	100% (45/45)	92.1% – 100%
		Panel 1 – RP+8 Hours	Lot 1	100% (15/15)	79.6% - 100%
			Lot 2	100% (15/15)	
			Lot 3	100% (15/15)	
			Overall	100% (45/45)	92.1% – 100%
Enterococcus faecalis	*Enterococcus faecalis*	Panel 2 – RP	Lot 1	100% (15/15)	79.6% - 100%
			Lot 2	100% (15/15)	
			Lot 3	100% (15/15)	
			Overall	100% (45/45)	92.1% – 100%
		Panel 2 – RP+8 Hours	Lot 1	100% (15/15)	79.6% - 100%
			Lot 2	100% (15/15)	
			Lot 3	100% (15/15)	
			Overall	100% (45/45)	92.1% – 100%
Escherichia coli	None	Contrived Negative	Lot 1	100% (15/15)	79.6% - 100%
			Lot 2	100% (15/15)	
			Lot 3	100% (15/15)	
			Overall	100% (45/45)	92.1% – 100%
No Target	None	Negative Blood Matrix	Lot 1	100% (15/15)	79.6% - 100%
			Lot 2	100% (15/15)	
			Lot 3	100% (15/15)	
			Overall	100% (45/45)	92.1% – 100%
Resistance Marker					
Staphylococcus aureus	*mecA/mecC*	Panel 1 – RP	Lot 1	100% (15/15)	79.6% - 100%
			Lot 2	100% (15/15)	
			Lot 3	100% (15/15)	
			Overall	100% (45/45)	92.1% – 100%
		Panel 1 – RP+8 Hours	Lot 1	100% (15/15)	79.6% - 100%
			Lot 2	100% (15/15)	
			Lot 3	100% (15/15)	
			Overall	100% (45/45)	92.1% – 100%
Enterococcus faecalis	*vanB*	Panel 2 – RP	Lot 1	100% (15/15)	79.6% - 100%
			Lot 2	100% (15/15)	
			Lot 3	100% (15/15)	

			Overall	100% (45/45)	92.1% – 100%
		Panel 2 – RP+8 Hours	Lot 1	100% (15/15)	79.6% - 100%
			Lot 2	100% (15/15)	
			Lot 3	100% (15/15)	
			Overall	100% (45/45)	92.1% – 100%

2. Linearity:

Not applicable as the candidate device is a qualitative assay.

3. Analytical Specificity/Interference:

Analytical Specificity/Cross-reactivity: To evaluate potential cross-reactivity of the LIAISON BCP assay, 92 off-panel organisms were evaluated by wet testing. The study included organisms phylogenetically related to the on-panel organisms, and organisms that are likely to be present in typical blood culture samples but are not detected by the candidate assay. All organisms were tested at the highest clinically relevant concentration. The evaluation determined that of the 9 tested organisms, 86 did not cross-react with the candidate assay's targets (Table 4). However, five organisms cross-reacted with target organisms in the candidate panel, and one organism showed positivity in 1/6 replicates tested, as seen in Table 5, below.

Table 4: Cross-Reactivity Study Results

Organism	Positivity	Organism	Positivity
Gram Positive			
Abitrophia defectiva	0%	*Erysipelothrix rhusiopathiae*	0%
Aerococcus viridans	0%	*Kocuria kristinae*	0%
Arcanobacterium bernardiae	0%	*Kytococcus sedentarius*, methicillin resistant	0%
Arcanobacterium haemolyticum	*0%*	*Lactobacillus acidophilus*	0%
Cutibacterium avidum	*0%*	*Lactobacillus crispatus*	0%
Propionibacterium freudenreichii	*0%*	*Lactobacillus rhamnosus*	0%
Enterococcus avium	***100%***	***Leuconostoc carnosum***	**100%**
Enterococcus casseliflavis, VRE, vanC	0%	*Leuconostoc mesenteroides*	0%
Enterococococcus dispar	0%	*Pediococcus acidilactici*	0%
Enterococcus durans	0%	*Pediococcus pentosaceus*	0%
Enterococcus flavescens	0%	*Peptostreptococcus anaerobius*	0%
Enterococcus gallinarum, vanC	0%	*Planococcus citreus*	0%
Enterococcus hirae	0%	*Planococcus kocurii*	0%
Enterococcus mundtii	0%	*Rothia dentocariosa*	0%
Enterococcus raffinosus	0%	*Rothia (Stomatococcus) mucilaginosa*	0%
Gram Negative			
Acinetobacter calcoaceticus	0%	*Haemophilus influenzae*	0%
Acinetobacter pittii	0%	*Herbaspirillum huttiense*	0%
Aggregatibacter aphrophilus	**100%**	*Kingella kingae*	0%
Bacteroides fragilis	0%	*Klebsiella oxytoca*	0%
Brevundimonas diminuta	0%	*Klebsiella pneumoniae*	0%
Burkholderia cepacia	0%	*Klebsiella variicola*	0%
Cardiobacterium hominis	0%	*Leclercia adecarboxylata*	0%
Cedecea lapagei	0%	*Moraxella catarrhalis*	0%

Citrobacter amalonaticus	0%	*Morganella morganii*	0%
Citrobacter freundii	0%	*Neisseria lactamica*	0%
Comamonas testosteroni	0%	*Neisseria meningitidis*	0%
Delftia acidovorans	0%	*Neisseria mucosa*	0%
Eikenella corrodens	0%	*Neisseria sicca*	0%
Elizabethkingia meningoseptica	0%	*Parabacteroides distasonis*	0%
Enteric Group 137	0%	*Pasteurella aerogenes*	0%
Enterobacter aerogenes	0%	*Prevotella bivia*	0%
Enterobacter cloacae	0%	*Proteus mirabilis*	0%
Enterobacter hormaechei	0%	***Proteus penneri***	**100%**
Escherichia albertii	0%	***Proteus vulgaris***	**100%**
Escherichia coli	0%	*Pseudomonas aeruginosa*	0%
Escherichia fergusonii	0%	*Pseudomonas alcaligenes*	0%
Escherichia hermannii	0%	*Raoultella ornithinolytica*	0%
Fusobacterium necrophorum	0%	*Salmonella enterica*	0%
Fusobacterium nucleatum	0%	*Serratia marcescens*	0%
Haemophilus haemolyticus	0%	*Stenotrophomonas maltophilia*	0%
Yeast/Fungi			
Aspergillus fumigatus	0%	*Cryptococcus neoformans*	0%
Candida albicans	0%	*Debaryomyces hansenii (Candida famata)*	0%
Nakaseomyces glabratus (*Candida glabrata*)	0%	*Kluyveromyces lactis*	0%
Pichia kudriavzevii (Candida krusei)	0%	*Saccharomyces cerevisiae*	0%
Candida parapsilosis	16.7%[a]	*Schizosaccharomyces pombe*	0%
Candida tropicalis	0%		

[a] Initial testing results showed 1 of 3 replicates detected as *S. lugdunensis*. An additional 3 replicates were tested (for a total of 6 replicates) and no additional false positives were seen.

Table 5. Cross-reactive Organisms Observed in Wet-testing.

Panel Target Organism	Predicted potential Cross-Reacting Organisms (false positive for the target organism)
Streptococcus spp.	- *Leuconostoc carnosum* - *Aggregatibacter aphrophilus* - *Proteus penneri* - *Proteus vulgaris*
Enterococcus faecium	- *Enterococcus avium*

In Silico Exclusivity (Cross-Reactivity): *In silico* analysis was conducted to predict potential cross-reactivity of the LIAISON BCP assay oligos through a BLAST comparison of the oligo sequences to the GenBank nucleotide sequence database. The analysis was performed with all capture and mediator probes in the assay, including the internal control oligos, for organisms that could not be wet tested. *In silico* exclusivity assessment of the oligo sequences incorporated in the assay were compared to on-panel and off-panel organisms listed in Table 6 Organisms identified as potentially cross-reactive as the *in-silico* analysis are summarized in Table 7, below.

Table 6. Organisms Evaluated for Cross-reactivity by *in silico* Analysis.

	Off-Panel Organisms

On Panel Organisms	Gram-Positive Bacteria	Gram-Negative Bacteria	Resistance Markers	Yeast/Virus/Parasites
Bacillus spp.	*Abiotrophia defectiva*	*Acinetobacter* spp.	AmpC	*Aspergillus flavus*
Enterococcus faecalis	*Actinomyces israelii*	*Actinobacillus hominis*	CMY	*Aspergillus fumigatus*
Enterococcus faecium	*Actinomyces naeslundii*	*Actinobacillus ureae*	CTX-M	*Aspergillus niger*
Listeria spp.	*Actinomyces odontolyticus*	*Aeromonas caviae*	IMP	*Aspergillus terreus*
Staphylococcus aureus	*Aerococcus sanguinicola*	*Aeromonas hydrophila*	KPC	*Blastomyces dermatitidis*
Staphylococcus epidermidis	*Aerococcus urinae*	*Aeromonas sobria*	MCR	*Candida albicans*
Staphylococcus lugdunensis	*Aerococcus viridans*	*Aggregatibacter actinomycetemcomitans*	NDM	*Candida auris*
Staphylococcus spp.	*Arcanobacterium bernardiae*	*Aggregatibacter aphrophilus*	ompK36	*Candida dubliniensis*
Streptococcus agalactiae	*Arcanobacterium haemolyticum*	*Bacteroides caccae*	OXA	*Candida famata*
Streptococcus anginosus group	*Arthrobacter psychrolactophilus*	*Bacteroides fragilis*	RAHN	*Candida glabrata*
Streptococcus pneumoniae	*Brochothrix thermosphacta*	*Bacteroides ovatus*	SHV	*Candida guilliermondii*
vanB	*Carnobacterium divergens*	*Bacteroides thetaiotaomicron*	SME	*Candida haemulonii*
	Carnobacterium maltaromaticum	*Bacteroides uniformis*	SPM	*Candida inconspicua*
	Clostridium perfringens	*Brevundimonas diminuta*		*Candida multis-gemmis*
	Clostridium ramosum (*Thomasclavelia ramosa*)	*Brevundimonas vesicularis*		*Candida nivariensis*
	Clostridium septicum	*Clostridium septicum*		*Candida norvegensis*
	Clostridium tertium	*Burkholderia mallei*		*Candida orthopsilosis*
	Clostridium tetani	*Burkholderia multivorans*		*Candida parapsilosis*
	Cutibacterium avidum	*Burkholderia pseudomallei*		*Candida sojae*
	Cutibacterium granulosum	*Campylobacter hominis*		*Candida tropicalis*
	Enterococcus avium	*Capnocytophaga ochracea*		*Candida duobushaemulonii*
	Enterococcus casseliflavus	*Cardiobacterium hominis*		*Candida viswanathii*
	Enterococcus cecorum	*Chlamydia trachomatis*		*Coccidioides immitis*
	Enterococcus dispar	*Chlamydophila pneumoniae*		*Coccidioides posadasii*
	Enterococcus durans	*Chromobacterium violaceum*		*Cryptococcus amylolentus*
	Enterococcus flavescens	*Citrobacter* spp.		*Cryptococcus gattii*

	Enterococcus gallinarum	*Comamonas testosteroni*		*Cryptococcus neoformans*
	Enterococcus hirae	*Delftia acidovorans*		*Cryptococcus uniguttulatus*
	Enterococcus mundtii	*Eikenella corrodens*		*Cutaneotrichosporon curvatum*
	Enterococcus raffinosus	*Elizabethkingia meningoseptica*		*Cyberlindnera fabianii*
	Erysipelothrix rhusiopathiae	*Enterobacter* spp.		*Geotrichum capitatum* (*Magnusiomyces capitatus*)
	Finegoldia magna	*Enterobacteriaceae*		*Histoplasma capsulatum*
	Gemella haemolysans	*Escherichia coli*		*Kluyveromyces lactis*
	Gemella morbillorum	*Fusobacterium necrophorum*		*Kodamaea ohmeri*
	Gemella morbillorum	*Fusobacterium necrophorum*		*Kodamaea ohmeri*
	Globicatella spp.	*Fusobacterium nucleatum*		*Lodderomyces elongisporus*
	Granulicatella adiacens	*Haemophilus influenzae*		*Magnusiomyces capitatus*
	Granulicatella elegans	*Haemophilus aegyptius*		*Millerozyma farinosa*
	Kocuria kristinae	*Haemophilus ducreyi*		*Naganishia albida*
	Kocuria rhizophila	*Haemophilus haemolyticus*		*Papiliotrema laurentii*
	Kytococcus sedentarius	*Haemophilus parahaemolyticus*		*Penicillium chrysogenum*
	Lactobacillus acidophilus	*Haemophilus parainfluenzae*		*Rhodotorula mucilaginosa*
	Lactobacillus rhamnosus	*Haemophilus quentini*		*Schizosaccharomyces pombe*
	Lactococcus garvieae	*Haemophilus sputorum*		*Talaromyces marneffei*
	Lactococcus lactis	*Herbaspirillum huttiense*		*Trichosporon asahii*
	Leuconostoc carnosum	*Kingella denitrificans*		*Wickerhamomyces anomalus*
	Leuconostoc citreum	*Kingella kingae*		BK Virus
	Leuconostoc mesenteroides	*Kingella negevensis*		Chikungunya Virus
	Macrococcus caseolyticus	*Kingella oralis*		Cytomegalovirus
	Micrococcus luteus	*Klebsiella oxytoca*		Dengue Virus
	Mycobacterium avium complex (MAC)	*Klebsiella pneumoniae*		Enterovirus
	Mycobacterium fortuitum	*Klebsiella variicola*		Epstein Barr Virus
	Mycobacterium mucogenicum	*Legionella pneumophila*		Hepatitis A virus
	Mycoplasma hominis	*Leptospira interrogans*		Hepatitis B virus
	Mycoplasma pneumoniae	*Moraxella catarrhalis*		Hepatitis C virus
	Nocardia farcinica	*Moraxella osloensis*		Human alphaherpesvirus 1
	Parvimonas micra	*Morganellaceae*		Human alphaherpesvirus 2
	Pediococcus acidilactici	*Mycobacterium tuberculosis*		Human betaherpesvirus 6
	Pediococcus pentosaceus	*Neisseria gonorrhoeae*		Human betaherpesvirus 7
	Peptostreptococcus anaerobius	*Neisseria lactamica*		Human Immunodeficiency Virus

	Planococcus citreus	*Neisseria meningitidis*		JC Virus
	Planococcus kocurii	*Neisseria mucosa*		Measles Virus
	Propionibacterium freudenreichii	*Neisseria sicca*		Mumps Virus
	Propionibacterium propionicum (*Arachnia propionica*)	*Parabacteroides distasonis*		Parvovirus B19
	Rhodococcus equi	*Parabacteroides merdae*		Rubella Virus
	Rothia dentocariosa	*Pasteurella aerogenes*		Varicella Zoster Virus
	Rothia mucilaginosa	*Pasteurella canis*		West Nile Virus
	Sarcina ventriculi	*Pasteurella multocida*		Zika Virus
	Solibacillus silvestris	*Pasteurella stomatis*		*Plasmodium falciparum*
	Ureaplasma parvum	*Prevotella bivia*		*Trypanosoma cruzi*
	Ureaplasma urealyticum	*Prevotella buccae*		
	Vagococcus fluvialis	*Prevotella denticola*		
	Weissella paramesenteroides	*Prevotella melaninogenica*		
		Prevotella oralis		
		Proteus spp.		
		Pseudomonas spp.		
		Psychrobacter cryohalolentis		
		Psychrobacter immobilis		
		Ralstonia mannitolilytica		
		Ralstonia pickettii		
		Salmonella spp.		
		Serratia spp.		
		Shigella spp.		
		Stenotrophomonas acidaminiphila		
		Stenotrophomonas maltophilia		
		Stenotrophomonas nitritireducens		
		Stenotrophomonas rhizophila		
		Treponema pallidum		
		Veillonella parvula		
		Vibrio alginolyticus		
		Vibrio parahaemolyticus		
		Vibrio vulnificus		

Table 7. Cross-reactive Organisms Predicted by *in silico* Analysis.

Panel Target Organism	Predicted potential Cross-Reacting Organisms (False Positive for the Target Organism)
Enterococcus faecalis	- Some strains of *Enterococcus durans* - Three strains of *Streptococcus spp*. (AY123726.1, FJ577604.1, MK608388.1)
Enterococcus faecium	- One strain of *Enterococcus durans* (KT877992.1) - One strain of *Enterococcus faecalis* (CP092577.1)

	- Some strains *Enterococcus avium*
Staphylococcus aureus	- Some strains of *Staphylococcus argenteus* - One strain of *Staphylococcus schweitzeri* (LR134304.1)
Staphylococcus spp.	- Some strains of *Macrococcoides caseolyticum*.
Streptococcus anginosus Group	- Some strains of *Streptococcus spp.* (*S. lactarius*, *S. milleri*, *S. oralis*, *S. periodonticum*, *S. pneumoniae*, *S. rubneri*, *S. vaginalis*).
Streptococcus pneumoniae	- Strains of various *Streptococcus spp.* (*S. downii* (OQ283557.1), *S. mitis* (FN568063.1), *S. oralis* (CP065706.1, OQ786998.1))
Streptococcus spp.	- Strains of *Aggregatibacter aphrophilus* - Unspecified *Bacillus spp.* - *Enterococcus spp.* (*E. durans*, *E. gallinarum*) - *Glaesserella parasuis* - *Haemophilus spp.* (*H. ducreyi*, *H. influenzae*, *H. parahaemolyticus*, *H. parainfluenzae*), - *Lactococcus spp.* (*L. garvieae*, *L. lactis*) - *Leuconostoc carnosum* - *Moellerella wisconsensis* - *Morganella morganii* - *Pasteurella multocida* - *Proteus spp.* (*P. appendicitidis*, *P. columbae*, *P. faecis*, *P. penneri*, *P. terrae*, *P. vulgaris*), *Providencia spp.* (*P. alcalifaciens*, *P. hangzhouensis*, *P. heimbachae*, *P. huaxiensis*, P. *manganoxydans*, *P. rettgeri*, *P. rustigianii*, *P. stuartii*, *P. vermicola*, *P. zhijiangensis*) - *Serratia spp.* (*S. marcescens*, *S. symbiotica*) - *Xenorhabdus spp.* (*X. bovienii*, *X. hominickii*, *X. nematophila.)*
Listeria spp.	- *Staphylococcus muscae* (ATCC 49910).

Analytical Reactivity/Inclusivity: The analytical reactivity of the LIAISON BCP assay was evaluated using a collection of 209 isolates representing the genetic diversity of the analytes (Table 8) and antimicrobial resistance markers (Table 9) included in the panel.

Table 8. Analytical Reactivity (Inclusivity) Study Results Summary by Organism

Reportable Target (Genus)	Reportable Target (Species)	Organism	# of Strains	% Detected
Bacillus spp.	N/A	*B. cereus*	4	100%
		B. licheniformis	2	100%
		B. subtilis	3	100%
		B. thuringiensis	2	100%
Corynebacterium spp.	N/A	*C. amycolatum*	3	100%
		C. diphtheriae	3	100%
		C. flavescens	1	100%
		C. genitalium	1	100%

		C. glutamicum	2	100%
		C. jeikeium	2	100%
		C pseudodiphthericum	2	100%
		C. renale	2	100%
		C. striatum	3	100%
		C. urealyticum	1	100%
N/A	*Enterococcus faecalis*	*E. faecalis*	9	100%
	Enterococcus faecium	*E. faecium*	9	100% [a]
Listeria spp.	N/A	*L. grayi*	2	100%
		L. innocua	2	100%
		L. ivanovii	2	100%
		L. monocytogenes	6	100%
		L. seeligeri	2	100%
		L. welshimeri	2	100%
Staphylococcus spp.	*Staphylococcus aureus*	*S. aureus*	43	100%
	Staphylococcus epidermidis	*S. epidermidis*	8	100%
	Staphylococcus lugdunensis	*S. lugdunensis*	5	100%
	N/A	*S. argenteus*	2	100% [c]
		S. auricularis	2	100%
		S. capitis	2	100%
		S. caprae	1	100%
		S. cohnii	2	100%
		S. haemolyticus	2	100%
		S. hominis	3	100%
		S. intermedius	2	100%
		S. muscae	1	100% [b]
		S. pasteuri	1	100%
		S. saccharolyticus	3	100%
		S. saprophyticus	2	100%
		S. schleiferi	1	100%
		S. sciuri	2	100%
		S. stimulans	2	100%
		S. warneri	2	100%
		S. xylosus	1	100%
Streptococcus spp.	*Streptococcus agalactiae*	*S. agalactiae*	5	100%
	Streptococcus anginosus group	*S. anginosus*	2	100%
		S. constellatus	3	100%
		S. intermedius	2	100%
	Streptococcus pneumoniae	*S. pneumoniae*	5	100%
	S. pyogenens	*S. pyogenes*	5	100%
	N/A	*S. bovis*	3	100%
		S. dysagalactiae	2	100%
		S. equi	2	100%
		S. equinus	2	100%
		S. gallolyticus	3	100%
		S. gordonii	2	100%
		S. infantarius subsp. *coli*	1	100%
		S. infantis	2	100%
		S. mitis	2	100%
		S. mutans	2	88.9 [d]
		S. oralis	2	100%
		S. parasanguinis	2	100%
		S. peroris	1	100%
		S. pseudoneumoniae	1	100%
		S. salivarius	1	100%

		S. sanguinis	2	100%

[a] One replicate from one strain resulted in a false positive (FP) *S. lugdunensis* result, three additional replicates were run for that strain. No additional FP results were observed.
[b] *Staphylococcus muscae* cross reacts with *Listeria spp*.
[c] *Staphylococcus argenteus* cross reacts with *S. aureus*.
[d] One strain of *Streptococcus mutans*, ATCC 25175, did not detect *Streptococcus spp*. in one replicate of initial testing. It was run an additional 3 times, giving an overall detection rate of 5/6 for that strain. *S. mutans* strain 31383 was detected (3/3).

Table 9. Analytical Reactivity (Inclusivity) Results Summary by Resistance Marker

Reportable Target	Organism	# of Strains	% Detected
*mec*A/C	*Staphylococcus argenteus*	1	100%
*mec*A/C	*Staphylococcus aureus*	35	100%
	Staphylococcus epidermidis	5	100%
	Staphylococcus hominis	1	100%
	Staphylococcus sciuri	2	100%
*van*A	*Enterococcus faecalis*	3	100%
	Enterococcus faecium	3	100% [a]
*van*B	*Enterococcus faecalis*	3	100%
	Enterococcus faecium	3	100%

[a] One replicate from one strain resulted in a false positive (FP) *S. lugdunensis* result, 3 additional replicates were run for that strain. No additional FP's were observed.

In Silico Inclusivity: An *in-silico* analysis was conducted using sequences available in the GenBank and WGS databases from March to April 2024 to determine reactivity with organisms not included in the wet testing. Summary of the results can be found in Table 10.

Table 10. Inclusivity *in silico* Analysis Summary

Reportable Target	Inclusive Organism/Target	Total # Sequences in Alignment	# Sequences with percent Oligo Identity ≥90%	Predicted Inclusivity Percentage (%)
Bacillus spp. [a]	*B. cereus* group	6722	6718	100
	B. subtilis group	4320	4318	100
Enterococcus faecalis	*E. faecalis*	746	745	100
Enterococcus faecium	*E. faecium*	555	555	100
Listeria spp. [b]	*L. monocytogenes*	8812	8812	100
	Other *Listeria* spp.	899	876	97
Staphylococcus aureus	*S. aureus*	2307	2307	100
Staphylococcus epidermidis	*S. epidermidis*	250	250	100
Staphylococcus lugdunensis	*S. lugdunensis*	162	162	100
Staphylococcus spp. [c]	*Staphylococcus* spp.	13355	13354	100
Streptococcus agalactiae	*S. agalactiae*	454	454	100
Streptococcus anginosus Group	*S. anginosus*	356	356	100
	S. constellatus	70	70	100
	S. intermedius	127	127	100
	Unspecified species from *S. anginosus* Group	7	7	100
Streptococcus pneumoniae	*S. pneumoniae*	693	693	100
Streptococcus pyogenes	*S pyogenes*	732	732	100
Streptococcus spp. [d]	*Streptococcus* spp.	23589	23574	100

mecA/mecC [e]	mecA	2201	2158	98
	mecC	32	32	100
vanA [f]	vanA	570	551	97
vanB [f]	vanB	215	215	100

[a] Includes sequences for 26 species of *B. cereus* group and 15 species of *B. subtilis* group. Oligo designs may also detect *Bacillus* species that are not part of these two groups.
[b] Includes sequences for 28 *Listeria* species.
[c] Includes sequences for 44 *Staphylococcus* species.
[d] Includes sequences for 108 *Streptococcus* species.
[e] Includes sequences for *Staphylococcus* species only.
[f] Includes sequences for *E. faecalis* and *E. faecium* only.

Competitive Inhibition: To determine whether coinfection with another target panel organism may impact test results, eight samples containing bottle/ring positive concentrations of on-panel target organisms were combined with other on-panel analytes at bottle/ring positive +8 hours concentrations. The eight total on-panel organisms were selected to be representative of clinically relevant poly-microbial infections in blood culture specimens, as listed in Table 11, below. Expected results for competitive inhibition (co-infection) include 100% positivity for both on-panel organisms present.

Table 11. Competitive Inhibition Study Results

On-Panel High Level Analyte	Positivity	On-Panel Low Level Analyte	Positivity
Enterococcus faecalis	3/3	*Staphylococcus epidermidis*	3/3
	3/3	*Enterococcus faecium*	3/3
Enterococcus faecium	3/3	*Staphylococcus epidermidis*	3/3
	3/3	*Enterococcus faecalis*	3/3
Staphylococcus aureus	3/3	*Staphylococcus epidermidis*	3/3
	3/3	*Streptococcus agalactiae*	3/3
Staphylococcus epidermidis	3/3	*Enterococcus faecalis*	3/3
	3/3	*Enterococcus faecium*	3/3
	3/3	*Staphylococcus aureus*	3/3
	3/3	*Staphylococcus lugdunensis*	3/3
	3/3	*Streptococcus pneumoniae*	3/3
Staphylococcus lugdunensis	3/3	*Staphylococcus epidermidis*	3/3
Streptococcus agalactiae	3/3	*Staphylococcus aureus*	3/3
Streptococcus pneumoniae	3/3	*Staphylococcus epidermidis*	3/3

Microbial Interference: To evaluate whether microbial interference occurs, samples containing two on-panel organisms at bottle/ring positive concentrations were combined in pairs with three representative off-panel organisms at analyte concentration at bottle/ring positive +8 hours and tested in triplicate. The specific organism sets were selected to mimic potentially interfering micro-organisms that are commonly found in positive blood samples, but which detection is not intended by the LIAISON PLEX BCP Assay. Results are summarized Table 12 below. Expected results for microbial interference include 100% positivity for the on-panel organism in the presence of the off-panel organism.

Table 12. Microbial Interference Study Results

On-Panel Low Concentration	Positivity	Off-Panel High Concentration	Positivity
Staphylococcus epidermidis	3/3	*Escherichia coli*	0/3

	3/3	*Klebsiella pneumoniae*	0/3
	3/3	*Proteus mirabilis*	0/3
Enterococcus faecium	3/3	*Escherichia coli*	0/3
	3/3	*Klebsiella pneumoniae*	0/3
	3/3	*Proteus mirabilis*	0/3
N/A	N/A	*Escherichia coli*	0/3
	N/A	*Klebsiella pneumoniae*	0/3
	N/A	*Proteus mirabilis*	0/3

Interfering Substances: An interfering substances study was conducted to assess the performance of the LIAISON PLEX BCP Assay in the presence of potentially interfering non-microbial substances that may be present in blood culture specimens. A set of samples containing six representative on-panel target organisms were tested in the presence of six interfering substances across five replicates. A panel containing negative blood matrix was also tested to assess the risk of false positive results in the presence of potentially interfering substances. A positive control (specimen without interfering substances) for all targets was also tested to assess for detection capabilities No interference from any evaluated substance was observed (Table 13).

Table 13: Interfering Substances Study Results

Interfering Substance	Concentration	Target	Positivity
Unconjugated Bilirubin	20 mg/dL	*Staphylococcus aureus*	100%
		Staphylococcus epidermidis	100%
		Streptococcus pneumoniae	100%
		Streptococcus agalactiae	100%
		Enterococcus faecalis	100%
		Enterococcus faecium	100%
		Negative blood matrix	0%
Conjugated Bilirubin	20 mg/dL	*Staphylococcus aureus*	100%
		Staphylococcus epidermidis	100%
		Streptococcus pneumoniae	100%
		Streptococcus agalactiae	100%
		Enterococcus faecalis	100%
		Enterococcus faecium	100%
		Negative blood matrix	0%
Hemoglobin	14 g/L	*Staphylococcus aureus*	100%
		Staphylococcus epidermidis	100%
		Streptococcus pneumoniae	100%
		Streptococcus agalactiae	100%
		Enterococcus faecalis	100%
		Enterococcus faecium	100%
		Negative blood matrix	0%
Intralipid	3000 mg/dL	*Staphylococcus aureus*	100%
		Staphylococcus epidermidis	100%
		Streptococcus pneumoniae	100%
		Streptococcus agalactiae	100%
		Enterococcus faecalis	100%
		Enterococcus faecium	100%
		Negative blood matrix	0%
γ -Globulin	6 g/dL	*Staphylococcus aureus*	100%
		Staphylococcus epidermidis	100%
		Streptococcus pneumoniae	100%
		Streptococcus agalactiae	100%

		Enterococcus faecalis	100%
		Enterococcus faecium	100%
		Negative blood matrix	0%
Sodium polyanethol-sulfonate	0.25% w/v	*Staphylococcus aureus*	100%
		Staphylococcus epidermidis	100%
		Streptococcus pneumoniae	100%
		Streptococcus agalactiae	100%
		Enterococcus faecalis	100%
		Enterococcus faecium	100%
		Negative blood matrix	0%
No Interferent		*Staphylococcus aureus*	100%
		Staphylococcus epidermidis	100%
		Streptococcus pneumoniae	100%
		Streptococcus agalactiae	100%
		Enterococcus faecalis	100%
		Enterococcus faecium	100%
		Negative blood matrix	0%

4. Assay Reportable Range:

   Not applicable as the test device is a qualitative assay.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Sample Stability: A specimen stability study was conducted to establish the recommended storage conditions for positive blood cultures before testing with the LIAISON PLEX Gram-Positive Blood Culture (BCP) Assay. A representative panel of six organisms including aerobic/anaerobic gram-positive pathogens and resistance markers, as well as a negative blood matrix control were tested. Fresh samples containing ring positive, and ring positive +8 hours concentrations of organisms were stored at 2°C and 30°C and tested at T0, after 26 hours (T1), 74 hours (T2) and 8 days (T3). Results of the sample stability study are summarized in Table 14 and Table 15, below.

Table 14. Sample Stability Study Results - 2°C

Analyte	Condition	Percent Positivity per Tested Timepoint			
		T0	T1	T2	T3
Enterococcus faecalis	Ring Positive	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
vanB [1]	Ring Positive +8	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Enterococcus faecalis	Ring Positive	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
vanB [1]	Ring Positive +8	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Listeria monocytogenes	Ring Positive	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
	Ring Positive +8	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus spp.	Ring Positive	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus aureus		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
mecA/mecC		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus spp.	Ring Positive +8	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus aureus		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
mecA/mecC		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus spp.	Ring Positive	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus epidermidis		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
mecA/mecC		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus spp.	Ring Positive +8	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)

Staphylococcus epidermidis		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
mecA/mecC		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Streptococcus spp.	Ring Positive	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Streptococcus pyogenes		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Streptococcus spp.	Ring Positive +8	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Streptococcus pyogenes		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Negative Blood Matrix	NA	0% (0/4)	0% (0/3)	0% (0/3)	0% (0/3)

[1]Two bottles were grown and tested to assess vanB sample stability. Bottle 1 resulted in <100% positivity at T(0), 26 hours, and 74 hours at bottle/ring positive. Bottle 2 was grown and resulted in 100% positivity at all timepoints tested.

Table 15. Sample Stability Study Results - 30°C.

Analyte	Condition	Percent Positivity per Tested Timepoint			
		T0	T1	T2	T3
Enterococcus faecalis	Ring Positive	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
vanB [1]	Ring Positive +8	100% (20/20)[1]	100% (10/10)	100% (10/10)	100% (10/10)
Enterococcus faecalis	Ring Positive	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
vanB [1]	Ring Positive +8	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Listeria monocytogenes	Ring Positive	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
	Ring Positive +8	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus spp.	Ring Positive	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus aureus		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
mecA/mecC		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus spp.	Ring Positive +8	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus aureus		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
mecA/mecC		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus spp.	Ring Positive	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus epidermidis		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
mecA/mecC		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus spp.	Ring Positive +8	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Staphylococcus epidermidis		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
mecA/mecC		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Streptococcus spp.	Ring Positive	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Streptococcus pyogenes		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)

Streptococcus spp.	Ring Positive +8	100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Streptococcus pyogenes		100% (20/20)	100% (10/10)	100% (10/10)	100% (10/10)
Negative Blood Matrix	NA	0% (0/4)	0% (0/3)	0% (0/3)	0% (0/3)

[1]Two bottles were grown and tested to assess vanB sample stability. Bottle 1 resulted in <100% positivity at T(0), 26 hours, and 74 hours at bottle/ring positive. Bottle 2 was grown and resulted in 100% positivity at all timepoints tested.

Fresh vs. Frozen Study: To support the use of frozen samples in the clinical study, the performance of the LIAISON PLEX BCP Assay with fresh and frozen samples was evaluated by testing five on-panel organisms (at both ring positive and ring positive + 8 hours organism concentrations) as well as a negative blood matrix control. Specifically, seven condition were evaluated: Initial testing of fresh samples (T0), 1 freeze-thaw cycle (T1), 2 freeze-thaw cycles (T2), 3 freeze-thaw cycles (T3), 1 freeze-thaw cycle after over 8 hours of frozen storage (T4), 15 days (T5), and 1 month of frozen storage (T6). Samples were stored frozen (-80°) for a minimum of 8 hours in between each freeze-thaw cycle and allowed to equilibrate at RT before testing. Results presented in Table 16, demonstrated 100% positivity for target positive samples for all testing conditions and 0% positivity for negative blood culture sample.

Table 16. Fresh Vs. Frozen Study Results

Analyte	Condition	Percent Positivity per Tested Timepoint						
		T0 Fresh	**T1 1st F/T**	**T2 2nd F/T**	**T3 3rd F/T**	**T4 (≥ 8 hours)**	**T5 (15 days)**	**T6 (30 days)**
Enterococcus faecalis	Ring Positive	100% (3/3)	100% (12/12)	100% (10/10)	100% (10/10)	100% (12/12)	100% (10/10)	100% (10/10)
	Ring Positive +8	100% (3/3)	100% (12/12)	100% (10/10)	100% (10/10)	100% (12/12)	100% (10/10)	100% (10/10)
Listeria monocytogenes	Ring Positive	100% (3/3)	100% (12/12)	100% (10/10)	100% (10/10)	100% (12/12)	100% (10/10)	100% (10/10)
	Ring Positive +8	100% (3/3)	100% (12/12)	100% (10/10)	100% (10/10)	100% (12/12)	100% (10/10)	100% (10/10)
Staphylococcus aureus	Ring Positive	100% (3/3)	100% (12/12)	100% (10/10)	100% (10/10)	100% (12/12)	100% (10/10)	100% (10/10)
	Ring Positive +8	100% (3/3)	100% (12/12)	100% (10/10)	100% (10/10)	100% (12/12)	100% (10/10)	100% (10/10)
Staphylococcus epidermidis	Ring Positive	100% (3/3)	100% (12/12)	100% (10/10)	100% (10/10)	100% (12/12)	100% (10/10)	100% (10/10)
	Ring Positive +8	100% (3/3)	100% (12/12)	100% (10/10)	100% (10/10)	100% (12/12)	100% (10/10)	100% (10/10)
Streptococcus pyogenes	Ring Positive	100% (3/3)	100% (12/12)	100% (10/10)	100% (10/10)	100% (12/12)	100% (10/10)	100% (10/10)
	Ring Positive +8	100% (3/3)	100% (12/12)	100% (10/10)	100% (10/10)	100% (12/12)	100% (10/10)	100% (10/10)
Negative Blood Matrix	NA	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)

6. Detection Limit:

Growth and Detection Study: A growth and detection study was performed to establish the range of expected organism concentrations present in incubated blood culture bottles at ring positive and at 8 hours after ring positive conditions. Eleven representative organisms of the LIAISON PLEX BCP Assay reportable targets were incubated and monitored to ring positive and eight hours after ringing positive using a continuous monitoring blood culture system. Three bottles for each organism were grown and three replicates were tested for each bottle, for a total of 9 replicates tested per organism. Additionally, bacterial CFU/mL were quantified for each bottle. Results are summarized in Table 17, below.

Table 17. Growth and Detection Study Results

Organism Tested	Expected Target	Ring Positive		Ring Positive +8 hours	
		CFU/mL Per Bottle	Positive Agreement/Total (% Detected)	CFU/mL Per Bottle	Positive Agreement/Total (% Detected)
Bacillus subtilis	Bacillus spp.	1.29E+08	9/9 (100%)	3.57E+05	9/9 (100%)
		6.10E+08		4.97E+05	
		3.60E+08		2.90E+05	
Enterococcus faecium	*Enterococcus faecium* / vanA	2.37E+08	9/9 (100%)	9.30E+08	9/9 (100%)
		3.01E+08		7.80E+08	
		2.24E+08		9.30E+08	
Enterococcus faecalis	*Enterococcus faecalis*/ vanB	6.87E+08	9/9 (100%)	2.90E+09	9/9 (100%)
		9.27E+08		2.80E+09	
		9.83E+08		2.57E+09	
Listeria monocytogenes	*Listeria* spp.	7.57E+08	9/9 (100%)	6.40E+08	9/9 (100%)
		9.63E+08		4.33E+08	
		7.73E+08		8.17E+08	
Staphylococcus aureus	*Staphylococcus* spp. *Staphylococcus aureus* mecA/mecC	1.52E+07	9/9 (100%)	3.80E+08	9/9 (100%)
		2.34E+07		4.10E+08	
		1.88E+07		1.30E+08	
Staphylococcus epidermidis	*Staphylococcus* spp. *Staphylococcus epidermidis* mecA/mecC	1.55E+08	9/9 (100%)	1.40E+09	9/9 (100%)
		4.40E+08		1.48E+09	
		2.84E+08		1.52E+09	
Staphylococcus lugdunensis	*Staphylococcus* spp. *Staphylococcus lugdunensis* mecA/mecC	5.67E+08	9/9 (100%)	2.83E+08	9/9 (100%)
		7.43E+08		2.92E+08	
		8.90E+08		2.65E+08	
Streptococcus agalactiae	*Streptococcus* spp. *Streptococcus agalactiae*	1.21E+09	9/9 (100%)	1.69E+09	9/9 (100%)
		1.32E+09		1.48E+09	
		1.12E+09		2.03E+09	
Streptococcus anginosus	*Streptococcus* spp. *Streptococcus anginosus* group	7.57E+08	9/9 (100%)	3.30E+06	9/9 (100%)
		4.97E+08		6.13E+06	
		3.63E+08		1.90E+07	
Streptococcus pneumoniae	*Streptococcus* spp. *Streptococcus pneumoniae*	8.30E+08	9/9 (100%)	1.35E+07	9/9 (100%)
		8.40E+08		1.92E+07	
		9.80E+08		1.54E+08	
Streptococcus pyogenes	*Streptococcus* spp. *Streptococcus pyogenes*	3.43E+08	9/9 (100%)	7.60E+08	9/9 (100%)
		3.40E+08		5.00E+08	
		4.70E+08		4.10E+08	
		Ring Negative			
Negative Blood	None	0/3 (0%)			

7. Assay Cut-Off:

The specific parameters for the LIAISON PLEX BCP Assay are considered confidential and proprietary

8. Accuracy (Instrument):

Not applicable.

9. Carry-Over:

Carry Over and Cross Contamination: A study was conducted to evaluate the risk of carry-over and cross contamination occurring during normal use of the device when highly concentrated positive specimens are processed alongside negative specimens. Two operators tested 30 positive samples containing *Staphylococcus aureus* at concentrations of 1.90E+08 CFU/mL and 30 negative samples consisting of negative blood matrix. *S. aureus* was selected since its detection implies positivity of three analyte targets of the assay (i.e., *Staphylococcus* spp., *S. aureus*, and mecA/C). Testing was conducted with two LIAISON PLEX instruments containing six modules. Each operator was assigned to a single instrument throughout the four days of testing. The samples were loaded into cartridges alternating between positive and negative samples (in a checkerboard fashion), six specimens at a time using sample prep trays. The six cartridges were loaded into a LIAISON PLEX instrument in the same order they were loaded with sample. The order of positive and negative specimens was alternated across the ten runs of six cartridges to ensure that each blade of the instrument alternatively processed high positive and negative specimens. Results of the Carry-Over study are summarized in Table 18.

Table 18. Carry-Over/Cross Contamination Results

	Positive Control			**Negative Control**		
	***Staphylococcus* spp.**	***S. aureus***	**mecA/C**	***Staphylococcus* spp.**	***S. aureus***	**mecA/C**
Run 1, Operator 1	100%	100%	100%	0%	0%	0%
Run 2, Operator 1	100%	100%	100%	0%	0%	0%
Run 3, Operator 1	100%	100%	100%	0%	0%	0%
Run 4, Operator 1	100%	100%	100%	0%	0%	0%
Run 5, Operator 1	100%	100%	100%	0%	0%	0%
Run 1, Operator 2	100%	100%	100%	0%	0%	0%
Run 2, Operator 2	100%	100%	100%	0%	0%	0%
Run 3, Operator 2	100%	100%	100%	0%	0%	0%
Run 4, Operator 2	100%	100%	100%	0%	0%	0%
Run 5, Operator 2	100%	100%	100%	0%	0%	0%

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Please refer to the Clinical Studies Section below.

2. Bottle Type Equivalency Study: An equivalency study was conducted to demonstrate similar performance of the LIAISON PLEX BCP Assay among 12 different blood culture bottle types from three different blood culture systems (BacT/Alert, BACTEC, and VersaTREK). A minimum of two lots per media bottle type were used to grow blood culture samples containing representative on-panel BCP targets with resistance markers, off-panel (gram-negative) targets, and negative blood matrix (NBM). The organisms evaluated and the average titers of each organism grown in the 12 different types of media bottles are shown in Table 19 A total of 596 independent blood culture bottles were evaluated, which included 464 gram-positive blood cultures, 120 gram-negative blood cultures, and 12 negative blood matrix bottles (Table 20). The bioMerieux BACT/ALERT FA Plus blood culture bottle type (PN: 410851) was not tested in this study as the effectiveness of this bottle type is established through blood culture growth required for all other analytical studies, such as growth and detection and analytical reactivity/inclusivity verification testing.

Table 19. Organisms Evaluated in Bottle Type Equivalency Study

Tested Organism	Strain ID	Target Analyte	Bottle Concentration (CFU/mL)
Bacillus cereus	ATCC10702	*Bacillus* spp.	6.10E+07[b] - 4.40E+08
Bacillus subtilis	ATCC 19659	*Bacillus* spp.	4.90E+05 - 3.73E+08
Enterococcus faecalis	ATCC 51575	*Enterococcus faecalis* | *vanB*	8.63E+07 - 3.50E+09a
Enterococcus faecalis	Clinical Isolate CLCS VRE-1	*Enterococcus faecalis* | *vanA*	9.20E+06 - 6.50E+08
Enterococcus faecium	ATCC 700221	*Enterococcus faecium* | *vanA*	3.17E+07 - 9.77E+08
Enterococcus faecium	ATCC 51858	*Enterococcus faecium* | *vanB*	3.83E+06 - 5.50E+08
Listeria ivanovii	ATCC 700402	*Listeria* spp.	1.50E+08 - 1.40E+09
Listeria monocytogenes	ATCC 15313	*Listeria* spp.	6.60E+07 - 1.88E+09
Staphylococcus aureus	ATCC BAA-2312	*Staphylococcus* spp. | *S. aureus* | *mecA*/*C*	8.00E+06 - 1.29E+09
Staphylococcus aureus	CDC AR-0227	*Staphylococcus* spp. |*S. aureus* | *mecA*/*C*	1.43E+07 - 3.50E+09
Staphylococcus epidermidis	ATCC 35984	*Staphylococcus* spp. | *S. epidermidis* |*mecA*/*C*	6.93E+06 - 1.43E+09
Staphylococcus lugdunensis	ATCC 49576	*Staphylococcus* spp. | *S. lugdunensis*	2.20E+07 - 3.46E+09
Streptococcus agalactiae	ATCC 12386	*Streptococcus* spp. | *S. agalactiae*	2.70E+07 - 1.71E+09
Streptococcus anginosus	ATCC 33397	*Streptococcus* spp. | *S. anginosus* Group	1.82E+07 - 4.37E+09
Streptococcus constellatus	ATCC 27823	*Streptococcus* spp. | *S. anginosus* Group	4.00E+07 - 2.53E+09

Streptococcus pneumoniae	ATCC 49619	*Streptococcus* spp. | *S. pneumoniae*	2.70E+06 - 2.88E+09
Streptococcus pyogenes	ATCC 700294	*Streptococcus* spp. | *S. pyogenes*	2.80E+06 - 2.07E+09
Acinetobacter baumannii	IHMA 128307	No target detected	3.90E+07 - 9.10E+08
Escherichia coli	NCTC 13846	No target detected	6.70E+08 - 2.11E+09
Haemophilus influenzae	ATCC 9007	No target detected	2.30E+08 - 2.93E+09
Klebsiella oxytoca	IHMA 683079	No target detected	6.23E+07 - 1.56E+09
Klebsiella pneumoniae	IHMA 629630	No target detected	2.17E+08 - 1.19E+09
Klebsiella variicola	Clinical Isolate V0512	No target detected	2.60E+08 - 1.68E+09
Pseudomonas aeruginosa	IHMA 576602	No target detected	2.10E+07 - 9.70E+08
Proteus mirabilis	ATCC 12453	No target detected	1.10E+08 - 9.03E+08
Serratia marcescens	IHMA 1642209	No target detected	7.57E+08 - 2.87E+09
Citrobacter freundii	IHMA 549813	No target detected	3.93E+08 - 2.06E+09

Table 20. Blood Culture Bottle Type Equivalency Study Results

Blood Culture Bottle Manufacturer (System)	**Blood Culture Bottle Type [c]**	**Number of Inoculated Bottles**		**Negative Blood Matrix [d]**
Biomerieux BACT/ALERT 3D System	BACT/ALERT SA (Standard Aerobic)	20/20	10/10	1/1
	BACT/ALERT SN (Standard Anaerobic)	20/20	10/10	1/1
	BACT/ALERT FN Plus Plus (Anaerobic)	20/20	10/10	1/1
	BACT/ALERT PF Plus (Pediatric)	58/58	10/10	1/1
Becton Dickinson 9050 / FX40 [a]	BACTEC Standard (Aerobic)	20/20 [b]	10/10	1/1
	BACTEC Plus (Aerobic)	20/20	10/10	1/1
	BACTEC Standard (Anaerobic)	58/58	10/10	1/1
	BACTEC Plus (Anaerobic)	58/58	10/10	1/1
	BACTEC Peds Plus (Pediatric)	58/58	10/10	1/1
	BACTEC Lytic (Lytic Anaerobic)	20/20	10/10	1/1
Thermo Scientific a VersaTREK [a]	REDOX 1 EZ Draw (Aerobic)	56/56	10/10	1/1
	REDOX 2 EZ Draw (Anaerobic)	56/56	10/10	1/1
Bottles with Expected Results/Total Number Blood Bottles		464/464 [b]	120/120	12/12

[a] For gram-positive blood culture preparations, BACTEC and VersaTREK systems were not available and corresponding media bottles were placed in a standard laboratory incubator with a shaker for growth. For gram-positive blood culture growth, only VersaTREK bottles needed to be placed in a standard laboratory incubator with a shaker in lieu of VersaTREK system; BACT/ALERT and BACTEC blood bottles were grown using corresponding automated blood culture systems up to bottle ring positivity.

[b] One organism, *(E. faecalis* strain 51575) required use of an 8+ bottle to confirm the bottle matrix was not inhibitory.

[c] Biomerieux BACT/ALERT FA Plus blood culture bottle type (PN: 410851) was not tested in this study protocol as the effectiveness of this bottle type is established through blood culture growth required for other analytical studies including growth and detection and analytical reactivity/ inclusivity verification.
[d] Each unique bottle of Negative Blood Matrix was tested in replicates of three.

C Clinical Studies:

To establish the clinical performance of the LIAISON Plex BCP Assay, a total of 1045 samples were tested. Among these, 509 were samples prospectively collected (Arm 1) from four geographically different sites in the U.S., 162 were pre-selected, left over samples (Arm 2) that were blinded, randomized, and tested along with unique negative clinical specimens at four BCP testing sites. Table 21 below shows a summary of the demographic information for the 509 prospectively collected and 162 pre-selected specimens that were included in the study. A total of 255 contrived samples (Arm 3) were also evaluated for low prevalence analytes where an insufficient number of positive samples were obtained from prospective or retrospective samples. Contrived samples were prepared by inoculating isolated colonies of the specific Gram-Positive organisms in blood culture bottles containing whole blood from unique donors. A summary of the organisms used to prepare contrived samples is provided in Table 22, below. Contrived samples were blinded, randomized, and tested along with negative clinical specimens at four testing sites.

Sensitivity and specificity of the device was established by comparing the results of the LIAISON Plex Blood Culture Gram Positive Assay to culture followed by automated microbiological/biochemical identification using VITEK 2 instrument system for 13 analytes (i.e., *Enterococcus faecalis*, *Enterococcus faecium*, *Listeria spp*., *Staphylococcus spp*., *S. aureus*, *S. epidermidis*, *S. ludguneneis*, *Streptococcus spp*., *S. agalactiae*, *S. anginosus* group, S. *pneumoniae*, and *S. pyogenes*) and from PCR and bi-directional sequencing for 5 analytes (i.e., *Bacillus spp*., meA/mecC, vanA, vanB). The performance of the device and 95% CI were calculated for each arm of the study, as shown in Table 23, below. The LIAISON PLEX BCP Assay reports genus level for *Bacillus* spp., *Staphylococcus* spp., and *Streptococcus* spp. Positive percent agreement (PPA) for target organisms in all studies stratified at the species level is presented in Table 24, below. Additionally, the LIAISON PLEX BCP Assay reports the presence or absence of resistance markers only if an associated organism is also detected. PPA for each resistance marker stratified by eligible organism is listed in Table 25.

Table 21. Demographic Information of Clinical Study Participants

	Prospective (N-509)	Pre-Selected (N=162)
	Specimens (%)	Specimens (%)
Gender All Sites		
Male	295 (58.0%)	87 (53.7%)
Female	214 (42.0%)	71 (43.8%)
Gender Unknown	0 (0.0%)	4 (2.5%)
Total	509 (100.0%)	162 (100.0%)
Age (years)		
0-1	15 (2.9%)	4 (2.5%)
>1-5	0 (0.0%)	1 (0.6%)
>5-21	11 (2.2%)	5 (3.1%)
<21-65	266 (52.3%)	83 (51.2%)
>65	213 (41.8%)	57 (35.2%)

Age Unknown	4 (0.8%)	12 (7.4%)
Total	509 (100.0%)	162 (100.0%)
Subject Status		
Emergency Room	88 (17.3%)	0 (0.0%)
Hospitalized	391 (76.8%)	4 (2.5%)
Outpatient	2 (0.4%)	0 (0.0%)
Status Unknown	28 (5.5%)	158 (97.5%)
Total	509 (100.0%)	162 (100.0%)
Blood Culture Bottle Type		
BacT/ALERT FA Plus	148 (29.1%)	48 (29.6%)
BacT/ALERT FN Plus	93 (18.3%)	31 (19.1%)
BacT/ALERT PF Plus	4 (0.8%)	2 (1.2%)
BacT/ALERT SA Standard Aerobic	27 (5.3%)	0 (0.0%)
BacT/ALERT SN Standard Anaerobic	21 (4.1%)	0 (0.0%)
BD BACTEC Lytic Anaerobic	55 (10.8%)	2 (1.2%)
BD BACTEC Peds Plus	8 (1.6%)	0 (0.0%)
BD BACTEC Plus Aerobic	135 (26.5%)	2 (1.2%)
BD BACTEC Standard Aerobic	18 (3.5%)	42 (25.9%)
BD BACTEC Standard Anaerobic	0 (0.0%)	7 (4.3%)
Bottle Type Unknown	0 (0.0%)	28 (17.3%)
Bottle Type Total	509 (100.0%)	162 (100.0%)

Table 22. Organisms Evaluated with Contrived Samples

Organism	Resistance Marker (s)	Strain	# of Specimens Tested
Bacillus amyloliquefaciens	NA	ATCC 23350	10
Bacillus atrophaeus	NA	ATCC 6455	10
Bacillus cereus	NA	ATCC 11778	10
Bacillus licheniformis	NA	ATCC 14580	10
Bacillus thuringiensis	NA	ATCC 10792	10
Total *Bacillus* spp.			50
Enterococcus faecalis	vanB	ATCC 700802	10
		ATCC 51299	10
		64188262	10
Total *Enterococcus faecalis*			30
Enterococcus faecium	vanB	ATCC 51858	10
		JMI CS-712	10
Total *Enterococcus faecium*			20
Listeria grayi	NA	ATCC 25401	10
Listeria innocua	NA	ATCC 33090	10
Listeria ivanovii	NA	ATCC 19119	10
Listeria monocytogenes	NA	ATCC 19114	8
Listeria welshimeri	NA	ATCC 35897	11
Total *Listeria* spp.			
Staphylococcus lugdunensis	NA	ATCC 84497462	50

Table 23. Clinical Study Performance Summary

Analyte	Study	Sensitivity/PPA			Specificity/NPA		
		TP/ (TP+FN)	(%)	95% CI (%)	TN/ (TN+FP)	(%)	95% CI (%)
Bacillus spp.	Prospective	1/1	100	20.7 – 100	506/507	99.8	98.9 – 100

	Archived	17/19	89.5	68.6 – 97.1	173/174	99.4	96.8 – 99.9
	Combined	**18/20**	**90.0**	**69.9 – 97.2**	**679/681[1]**	**99.7**	**98.9 – 99.9**
	Contrived	**50/50**	**100**	**92.9 - 100**	**268/268**	**100**	**98.6 - 100**
Enterococcus faecalis	Prospective	38/38	100	90.8 - 100	468/468	100	99.2 - 100
	Archived	2/2	100	34.2 - 100	147/147	100	97.5 – 100
	Combined	**40/40**	**100**	**91.2 - 100**	**615/615**	**100**	**99.4 - 100**
	Contrived	**30/30**	**100**	**88.6 - 100**	**288/288**	**100**	**98.7 - 100**
Enterococcus faecium	Prospective	16/16	100	80.6 - 100	489/489	99.8	98.9 – 100
	Archived	20/20	100	83.9 - 100	128/129	99.2	95.7 – 99.9
	Combined	**36/36**	**100**	**90.4 - 100**	**617/619[2]**	**99.7**	**98.8 – 99.9**
	Contrived	**20/20**	**100**	**83.9 - 100**	**298/298**	**100**	**98.7 - 100**
Listeria spp.	Prospective	0/0	NA	NA	506/506	100	99.2 – 100
	Archived	5/5	100	56.6 - 100	144/144	100	97.4 – 100
	Combined	**5/5**	**100**	**56.6 - 100**	**650/650**	**100**	**99.4 - 100**
	Contrived	**49/49**	**100**	**92.7 - 100**	**269/269**	**100**	**98.6 - 100**
Staphylococcus spp.	Prospective	316/322	98.1	96 – 99.1	182/184	98.9	96.1 – 99.7
	Archived	20/20	100	83.9 - 100	129/129	100	97.1 – 100
	Combined	**336/342[3]**	**98.2**	**96.2 – 99.2**	**311/313[4]**	**99.4**	**97.7 – 99.8**
	Contrived	**50/50**	**100**	**92.9 - 100**	**268/268**	**100**	**98.6 - 100**
Staphylococcus aureus	Prospective	160/161	99.4	96.6 – 99.9	344/345	99.7	98.4 – 99.9
	Archived	0/0	NA	NA	149/149	100	97.5 – 100
	Combined	**160/161**	**99.4**	**96.6 – 99.9**	**493/494[5]**	**99.8**	**98.9 - 100**
	Contrived	**0/0**	**NA**	**NA**	**318/318**	**100**	**98.8 - 100**
Staphylococcus epidermidis	Prospective	93/96[7]	96.9	91.2 – 98.9	405/410	98.8	97.2 – 99.5
	Archived	0/0	NA	NA	148/149	99.3	96.3 – 99.9
	Combined	**93/96[6]**	**96.9**	**91.2 – 98.9**	**553/559[7]**	**98.9**	**97.7 – 99.5**
	Contrived	**0/0**	**NA**	**NA**	**318/318**	**100**	**98.8 - 100**
Staphylococcus lugdunensis	Prospective	6/6	100	61 – 100	500/500	100	99.2 - 100
	Archived	20/20	100	83.9 - 100	129/129	100	97.1 – 100
	Combined	**26/26**	**100**	**87.1 -100**	**629/629**	**100**	**99.4 - 100**
	Contrived	**50/50**	**100**	**92.9 - 100**	**267/268**	**99.6**	**97.9 - 99.9**
Streptococcus spp.	Prospective	97/98	99	94.4 - 99.8	406/408	99.5	98.2 - 99.9
	Archived	78/80	97.5	91.3 - 99.3	69/69	100	94.7 - 100
	Combined	**175/178**	**98.3**	**95.2 - 99.4**	**475/477[8]**	**99.6**	**98.5 - 99.9**
	Contrived	**0/0**	**NA**	**NA**	**317/318**	**99.7**	**98.2 - 99.9**
Streptococcus agalactiae	Prospective	21/21	100	84.5 - 100	485/485	100	99.2 - 100
	Archived	17/17	100	81.6 - 100	132/132	100	97.2 - 100
	Combined	**38/38**	**100**	**90.8 - 100**	**617/617**	**100**	**99.4 - 100**
	Contrived	**0/0**	**NA**	**NA**	**318/318**	**100**	**98.8 - 100**
***Streptococcus anginosus* group**	Prospective	8/9	88.9	56.5 - 98	496/497	99.8	98.9 - 100
	Archived	25/26	96.2	81.1 - 99.3	123/123	100	97 - 100
	Combined	**33/35**	**94.3**	**81.4 - 98.4**	**619/620[9]**	**99.8**	**99.1 - 100**
	Contrived	**0/0**	**NA**	**NA**	**318/318**	**100**	**98.8 - 100**
Streptococcus pneumoniae	Prospective	11/11	100	74.1 - 100	494/495	99.8	98.9 - 100
	Archived	21/21	100	84.5 - 100	128/128	100	97.1 - 100
	Combined	**32/32**	**100**	**89.3 - 100**	**622/623**	**99.8**	**99.1 - 100**
	Contrived	**0/0**	**NA**	**NA**	**318/318**	**100**	**98.8 - 100**
Streptococcus pyogenes	Prospective	21/21	100	84.5 - 100	485/485	100	99.2 - 100
	Archived	16/16	100	80.6 - 100	133/133	100	97.2 - 100
	Combined	**37/37**	**100**	**90.6 - 100**	**618/618**	**100**	**99.4 - 100**
	Contrived	**0/0**	**NA**	**NA**	**318/318**	**100**	**98.8 - 100**
Resistance Markers[10]							
mecA/mecC	Prospective	155/157	98.7	95.5 - 99.6	154/161	95.7	91.3 - 97.9
	Archived	3/3	100	43.9 - 100	18/18	100	82.4 - 100

	Combined	**158/160**	**98.8**	**95.6 - 99.7**	**172/179**	**96.1**	**92.1 - 98.1**
	Contrived	**0/0**	**NA**	**NA**	**50/50**	**100**	**92.9 - 100**
vanA	Prospective	8/8	100	67.6 - 100	47/47	100	92.4 - 100
	Archived	22/22	100	85.1 - 100	1/1	100	20.7 - 100
	Combined	**30/30**	**100**	**88.6 - 100**	**48/48**	**100**	**92.6 - 100**
	Contrived	**0/0**	**NA**	**NA**	**50/50**	**100**	**92.9 - 100**
vanB	Prospective	1/1	100	20.7 - 100	54/54	100	93.4 - 100
	Archived	0/0	NA	NA	23/23	100	85.7 - 100
	Combined	**1/1**	**100**	**20.7 - 100**	**77/77**	**100**	**95.2 - 100**
	Contrived	**50/50**	**100**	**92.9 - 100**	**0/0**	**NA**	**NA**

[1]Out of two *Bacillus* spp. FPs, one was positive by Standard of Care MALDI-ToF assay.
[2]Out of two *Enterococcus faecium* FPs, one was positive by Standard of Care molecular and biochemical assays.
[3]Out of six *Staphylococcus* spp. FNs, one was negative by BDS and one was negative by Standard of Care MALDI-ToF assay.
[4]Out of two *Staphylococcus* spp. FPs, one was positive by BDS and one was positive by Standard of Care MALDI-ToF and molecular assays
[5]The one *Staphylococcus aureus* FP was positive by BDS and Standard of Care molecular assay.
[6]Out of three *Staphylococcus epidermidis* FNs, one was negative by BDS.
[7]Out of six *Staphylococcus epidermidis* FPs, three were positive by both BDS and Standard of Care MALDI-ToF assay, one was positive by both BDS and Standard of Care molecular assay and one was positive by Standard of Care MALDI-ToF assay and not tested by BDS.
[8]Out of two *Streptococcus* spp. FPs, one was positive by BDS and one was positive by Standard of Care molecular assay.
[9]The one *Streptococcus anginosus* group FP was positive by both BDS and Standard of Care molecular assay.
[10]The LIAISON PLEX BCP assay will report the presence or absence of resistance markers only if an applicable organism is also detected, therefore the total number of evaluable samples for each resistance marker is dependent on the number of applicable organisms enrolled.

Table 24. PPA Summary Results for Genus Level Targets Stratified by Species

Organism	**Prospective (Arm 1)**		**Pre-selected (Arm 2)**		**Contrived (Arm 3)**	
	Sensitivity/PPA	**95% CI**	**Sensitivity/PPA**	**95% CI**	**Sensitivity/PPA**	**95% CI**
***Bacillus* spp.**	**100 % (1/1)**	**20.7 - 100**	**89.5% (17/19)**	**68.6 – 97.1**	**100% (50/50)**	**92.9 - 100**
B. amyloliquefaciens	NA	NA	NA	NA	100% (10/10)	72.2% - 100
B. atrophaeus	NA	NA	NA	NA	100% (10/10)	72.2% - 100
B. cereus	NA	NA	NA	NA	100% (10/10)	72.2% - 100
B. licheniformis	NA	NA	NA	NA	100% (10/10)	72.2% - 100
B. thruringiensis	NA	NA	NA	NA	100% (10/10)	72.2% - 100
***Listeria* spp.**	**NA**	**NA**	**100% (5/5)**	**56.6 - 100**	**100% (49/49)**	**92.7% - 100**
L. grayi	NA	NA	NA	NA	100% (10/10)	72.2% - 100
L. innocua	NA	NA	NA	NA	100% (10/10)	72.2% - 100
L. inanovii	NA	NA	NA	NA	100% (10/10)	72.2% - 100
L. monocytogenes	NA	NA	100% (5/5)	56.6 - 100	100% (8/8)	67.6% - 100
L. welshimeri	NA	NA	NA	NA	100% (11/11)	74.1% - 100
***Staphylococcus* spp.**	**98.1% (316/322)**	**96% - 99.1%**	**100% (20/20)**	**83.9 - 100**	**100% (50-50)**	**92.9% - 100%**

S. aureus	99.4% (160/161)	96.6 – 99.9	NA	NA	NA	NA
S. epidermidis	96.9% (93/96)	91.2 – 98.9	NA	NA	NA	NA
S. lugdunensis	100% (6/6)	61 - 100	100% (20/20)	83.9 - 100	100% (50/50)	92.9 - 100
S. arlettae	100% (1/1)	20.7 - 100	NA	NA	NA	NA
S. auricularis	66.7% (2/3)	20.8 – 93.9	NA	NA	NA	NA
S. capitis	100% (9/9)	70.1 - 100	NA	NA	NA	NA
S. caprae	100% (1/1)	20.7 - 100	NA	NA	NA	NA
S. haemolyticus	100% (6/6)	61 - 100	NA	NA	NA	NA
S. hominis	96.3% (31/32)	84.3 – 99.4	NA	NA	NA	NA
S. pseudintermedius	100% (1/1)	20.7 - 100	NA	NA	NA	NA
S. saccharolyticus	0% (0/1)	0-79.3	NA	NA	NA	NA
S. saprophyticus	100% (1/1)	20.7 - 100	NA	NA	NA	NA
S. simulans	100 (1/1)	20.7 - 100	NA	NA	NA	NA
S. vitulinus	0% (0/1)	0-79.3	NA	NA	NA	NA
S. warneri	100 (3/3)	43.9 - 100	NA	NA	NA	NA
Staphylococcus spp. Unknown	87.5% (7/8)	52.9 – 97.8	NA	NA	NA	NA
***Streptococcus* spp.**	**99% (97/98)**	**94.4 – 99.8**	**97.5% (78/80)[1]**	**91.3 – 99.3**	**NA**	**NA**
S. agalactiae	100% (21/21)	84.5 - 100	100% (17/17)	81.6 - 100	NA	NA
S. anginosus	88.9% (8/9)	56.5 - 98	96.2% (25/26)	81.1 – 99.3	NA	NA
S. pneumoniae	100% (11/11)	74.1 - 100	100% (21/21)	84.5 - 100	NA	NA
S. pyogenes	100% (21/21)	84.5 - 100	100% (16/16)	80.6 - 100	NA	NA
S. dysgalactiae	100% (15/15)	79.6 - 100	NA	NA	NA	NA
S. gallolyticus	100% (1/1)	20.7 - 100	NA	NA	NA	NA
S. gordonii	100% (4/4)	51 - 100	NA	NA	NA	NA
S. parasanguinis	100% (1/1)	20.7 - 100	NA	NA	NA	NA
S. salivarius	100% (2/2)	34.2 - 100	NA	NA	NA	NA
S. sanguinis	100% (2/2)	34.2 - 100	NA	NA	NA	NA
Streptococcus spp. Unknown	100% (11/11)	74.1 - 100	NA	NA	NA	NA

[1] For one specimen the assay returned a true positive call for *Streptococcus anginosus* without detecting *Streptococcus* spp. As a result, there are two total FNs for the *Streptococcus* spp. target, but only one listed when stratified by species.

Table 25. Sensitivity/PPA of Resistance Markers by Organism

Organism	Prospective (Arm 1)		Pre-Selected (Arm 2)		Contrived (Arm3)	
	Sensitivity/PPA	95% CI	Sensitivity/ PPA	95% CI	Sensitivity/ PPA	95% CI
mecA/mecC						
Staphylococcus aureus	100% (64/64)	*94.3% - 100%*	NA	NA	NA	NA
Staphylococcus epidermidis	97% (65/67)	89.8% - 99.2%	NA	NA	NA	NA
Staphylococcus lugdunensis	100% (4/4)	51% - 100%	100% (3/3)	43.8% - 100%	NA	NA
Staphylococcus capitis	100% (2/2)	34.2% - 100%	NA	NA	NA	NA
Staphylococcus haemolyticus	100% (6/6)	61.0% - 100%	NA	NA	NA	NA
Staphylococcus hominis	100% (12/12)	75.7% – 100%	NA	NA	NA	NA
Spp. unknown	100% (2/2)	34.2% - 100%	NA	NA	NA	NA
Overall	98.70% (155/157)	95.4% - 99.6%	100% (3/3)	43.8% - 100%	NA	NA
vanA						
Enterococcus faecalis	NA	NA	100% (2/2)	34.2% - 100%	NA	NA
Enterococcus faecium	100% (8/8)	67.6% - 100%	100% (20/20)	83.9% - 100%	NA	NA
Overall	100% (8/8)	67.6% - 100%	100% (22/22)	85.1% - 100%	NA	NA
vanB						
Enterococcus faecalis	NA	NA	NA	NA	100% (30/30)	88.6% - 100%
Enterococcus faecium	100% (1/1)	20.7% - 100%	NA	NA	100% (20/20)	83.9% - 100%
Overall	100% (1/1)	20.7% - 100%	NA	NA	100% (50/50)	92.9% - 100%

A supplemental comparison of the performance of the *mecA/mecC* and *vanA/vanB* resistance genes to phenotypic antimicrobial susceptibility testing (AST) was performed. Results by relevant organism for the β-lactam antibiotics, oxacillin and cefazolin, are shown in Table 26 and for vancomycin in Table 27. In total, 263 clinical isolates had AST results for one or more antibiotics.

Table 26. Clinical Performance of *mecA/mecC* Compared to Phenotypic AST Results

On-Panel Organism	cefazolin		oxacillin	
	TP/(TP+FN) (%)	TN/(TN+FP) (%)	TP/(TP+FN) (%)	TN/(TN+FP) (%)
Staphylococcus spp.	23/25 (92.0%)	22/22 (100.0%)	71/72 (98.6%)	84/85 (98.8%)
Staphylococcus aureus	17/17 (100.0%)	20/20 (100.0%)	49/49 (100.0%)	76/76 (100.0%)
Staphylococcus epidermidis	5/6 (83.3%)	0/0 (NA)	17/18 (94.4%)	2/2 (100.0%)
Staphylococcus lugdunensis	0/0 (NA)	0/0 (NA)	4/4 (100.0%)	2/2 (100.0%)

Table 27. Clinical Performance of *vanA/vanB* Compared to Phenotypic AST Results

On-Panel Organism	vancomycin	
	TP/(TP+FN) (%)	TN/(TN+FP) (%)
Enterococcus faecalis	0/0 (NA)	29/29 (100.0%)
Enterococcus faecium	9/9 (100.0%)	8/8 (100.0%)

D Clinical Cut-Off:

Not Applicable.

E Expected Values/Reference Range:

The LIAISON PLEX Blood Culture Gram-Positive BCP Assay prospective clinical study was conducted to evaluate the clinical performance of the device using positive blood culture samples. The expected values for each target organism in the prospective samples are presented by site, age, and Co-infection Combination in Tables 28, 29, and 30 respectively.

Table 28. Expected Values for Prospective Samples by Clinical Site.

	Site 01 (N=241)		Site 02 (N=149)		Site 03 (N=66)		Site 04 (N=52)		Total (N=508)	
Target	# Pos	%	# Pos	%	# Pos	%	# Pos	%	# Pos	%
Bacillus spp.	1	0.4% (1/241)	0	0.0% (0/149)	1	1.5% (1/66)	0	0.0% (0/52)	2	0.4% (2/508)
Enterococcus faecalis	23	9.6% (23/240)	7	4.7% (7/149)	3	4.5% (3/66)	5	9.8% (5/51)	38	7.5% (38/506)
Enterococcus faecium	12	5.0% (12/240)	5	3.4% (5/149)	0	0.0% (0/66)	0	0.0% (0/51)	17	3.4% (17/506)
Listeria spp.	0	0.0% (0/240)	0	0.0% (0/149)	0	0.0% (0/66)	0	0.0% (0/51)	0	0.0% (0/506)
Staphylococcus aureus	92	38.3% (92/240)	38	25.5% (38/149)	13	19.7% (13/66)	18	35.3% (18/51)	161	31.8% (161/506)
Staphylococcus epidermidis	37	15.4% (37/240)	38	25.5% (38/149)	13	19.7% (13/66)	10	19.6% (10/51)	98	19.4% (98/506)
Staphylococcus lugdunensis	6	2.5% (6/240)	0	0.0% (0/149)	0	0.0% (0/66)	0	0.0% (0/51)	6	1.2% (6/506)
Staphylococcus spp.	153	63.8% (153/240)	99	66.4% (99/149)	33	50.0% (33/66)	33	64.7% (33/51)	318	62.8% (318/506)
Streptococcus agalactiae	7	2.9% (7/240)	6	4.0% (6/149)	5	7.6% (5/66)	3	5.9% (3/51)	21	4.2% (21/506)
Streptococcus anginosus group	4	1.7% (4/240)	4	2.7% (4/149)	1	1.5% (1/66)	0	0.0% (0/51)	9	1.8% (9/506)
Streptococcus pneumoniae	7	2.9% (7/240)	1	0.7% (1/149)	3	4.5% (3/66)	1	2.0% (1/51)	12	2.4% (12/506)
Streptococcus pyogenes	8	3.3% (8/240)	6	4.0% (6/149)	5	7.6% (5/66)	2	3.9% (2/51)	21	4.2% (21/506)
Streptococcus spp.	44	18.3% (44/240)	23	15.4% (23/149)	22	33.3% (22/66)	10	19.6% (10/51)	99	19.6% (99/506)
mecA/mecC	74	48.4% (74/153)	53	53.5% (53/99)	19	57.6% (19/33)	16	48.5% (16/33)	162	50.9% (162/318)

vanA	6	17.1% (6/35)	2	16.7% (2/12)	0	0.0% (0/3)	0	0.0% (0/5)	8	14.5% (8/55)
vanB	1	2.9% (1/35)	0	0.0% (0/12)	0	0.0% (0/3)	0	0.0% (0/5)	1	1.8% (1/55)

Table 29. Expected Values for Prospective Samples by Age Group

	0-1 years (N=15)		1-5 years (N=0)		6-21 years (N=11)		22-65 years (N=266)		>65 years (N=212)		Unknown years (N=4)		Overall (N=508)	
Target	**# Pos**	**%**	**# Pos**	**%**	**# Pos**	**%**	**# Pos**	**%**	**# Pos**	**%**	**# Pos**	**%**	**# Pos**	**%**
Bacillus spp.	0	0.0% (0/15)	0	0.0% (0/0)	0	0.0% (0/11)	2	0.8% (2/266)	0	0.0% (0/212)	0	0.0% (0/4)	2	0.4% (2/508)
Enterococcus faecalis	1	6.7% (1/15)	0	0.0% (0/0)	0	0.0% (0/11)	12	4.5% (12/264)	25	11.8% (25/212)	0	0.0% (0/4)	38	7.5% (38/506)
Enterococcus faecium	1	6.7% (1/15)	0	0.0% (0/0)	0	0.0% (0/11)	8	3.0% (8/264)	8	3.8% (8/212)	0	0.0% (0/4)	17	3.4% (17/506)
Listeria spp.	0	0.0% (0/15)	0	0.0% (0/0)	0	0.0% (0/11)	0	0.0% (0/264)	0	0.0% (0/212)	0	0.0% (0/4)	0	0.0% (0/506)
Staphylococcus aureus	3	20.0% (3/15)	0	0.0% (0/0)	0	0.0% (0/11)	95	36.0% (95/264)	63	29.7% (63/212)	0	0.0% (0/4)	161	31.8% (161/506)
Staphylococcus epidermidis	5	33.3% (5/15)	0	0.0% (0/0)	2	18.2% (2/11)	49	18.6% (49/264)	40	18.9% (40/212)	2	50.0% (2/4)	98	19.4% (98/506)
Staphylococcus lugdunensis	0	0.0% (0/15)	0	0.0% (0/0)	0	0.0% (0/11)	4	1.5% (4/264)	2	0.9% (2/212)	0	0.0% (0/4)	6	1.2% (6/506)
Staphylococcus spp.	9	60.0% (9/15)	0	0.0% (0/0)	5	45.5% (5/11)	173	65.5% (173/264)	129	60.8% (129/212)	2	50.0% (2/4)	318	62.8% (318/506)
Streptococcus agalactiae	2	13.3% (2/15)	0	0.0% (0/0)	0	0.0% (0/11)	10	3.8% (10/264)	9	4.2% (9/212)	0	0.0% (0/4)	21	4.2% (21/506)
Streptococcus anginosus group	0	0.0% (0/15)	0	0.0% (0/0)	0	0.0% (0/11)	4	1.5% (4/264)	4	1.9% (4/212)	1	25.0% (1/4)	9	1.8% (9/506)
Streptococcus pneumoniae	0	0.0% (0/15)	0	0.0% (0/0)	1	9.1% (1/11)	9	3.4% (9/264)	2	0.9% (2/212)	0	0.0% (0/4)	12	2.4% (12/506)

Streptococcus pyogenes	0	0.0% (0/15)	0	0.0% (0/0)	0	0.0% (0/11)	12	4.5% (12/264)	8	3.8% (8/212)	1	25.0% (1/4)	21	4.2% (21/506)
Streptococcus spp.	4	26.7% (4/15)	0	0.0% (0/0)	3	27.3% (3/11)	51	19.3% (51/264)	39	18.4% (39/212)	2	50.0% (2/4)	99	19.6% (99/506)
mecA/mecC	5	55.6% (5/9)	0	0.0% (0/0)	1	20.0% (1/5)	87	50.3% (87/173)	68	52.7% (68/129)	1	50.0% (1/2)	162	50.9% (162/318)
vanA	0	0.0% (0/2)	0	0.0% (0/0)	0	0.0% (0/0)	4	20.0% (4/20)	4	12.1% (4/33)	0	0.0% (0/0)	8	14.5% (8/55)
vanB	0	0.0% (0/2)	0	0.0% (0/0)	0	0.0% (0/0)	0	0.0% (0/20)	1	3.0% (1/33)	0	0.0% (0/0)	1	1.8% (1/55)

Table 30. Expected Values for Each Co-Infection for Prospective Samples by Age Group

	0-1 years (N=0)		**1-5 years (N=0)**		**6-21 years (N=0)**		**22-65 years (N=2)**		**>65 years (N=1)**		**Unknown years (N=4)**		**Overall (N=3)**	
Co-Infection	**# Pos**	**%**	**# Pos**	**%**	**# Pos**	**%**	**# Pos**	**%**	**# Pos**	**%**	**# Pos**	**%**	**# Pos**	**%**
Staphylococcus aureus | *Staphylococcus epidermidis* | *Staphylococcus spp.* | *mecA/mecC*	0	0.0% (0/0)	0	0.0% (0/0)	0	0.0% (0/0)	1	33.3% (1/3)	0	0.0% (0/212)	0	0.0% (0/4)	1	33.3% (1/3)
Staphylococcus aureus | *Staphylococcus epidermidis* | *Staphylococcus spp.*	0	0.0% (0/0)	0	0.0% (0/0)	0	0.0% (0/0)	1	33.3% (1/3)	0	0.0% (0/0)	0	0.0% (0/4)	1	33.3% (1/3)
Staphylococcus epidermidis | *Staphylococcus spp.* | *Streptococcus pneumoniae* | *Streptococcus spp.*	0	0.0% (0/0)	0	0.0% (0/0)	0	0.0% (0/0)	0	0.0% (0/0)	1	33.3% (1/3)	0	0.0% (0/4)	1	33.3% (1/3)

F Other Supportive Instrument Performance Characteristics Data:

Not Applicable.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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