June 6, 2025

Luminex Corporation
Rocio Rueda
Senior Regulatory Affairs Associate
4088 Commercial Avenue
Northbrook, Illinois 60062

Re: K243490
Trade/Device Name: LIAISON PLEX Gram-Positive Blood Culture Assay
Regulation Number: 21 CFR 866.3365
Regulation Name: Multiplex Nucleic Acid Assay For Identification Of Microorganisms And Resistance Markers From Positive Blood Cultures
Regulatory Class: Class II
Product Code: PAM
Dated: November 7, 2024
Received: November 12, 2024

Dear Rocio Rueda:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

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

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-

assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Noel J. Gerald -S

Noel J. Gerald, Ph.D.
Deputy Division Director
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K243490

Device Name
LIAISON PLEX® Gram-Positive Blood Culture Assay

Indications for Use *(Describe)*
The LIAISON PLEX® Gram-Positive Blood Culture Assay (BCP), 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. BCP 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 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 Assay contains targets for the detection of genetic determinants associated with resistance to methicillin (mecA/C) 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

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 the 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.

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

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

CONTINUE ON A SEPARATE PAGE IF NEEDED.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

510(k) Summary

This Summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

Preparation date: 04 April 2025

A. 510(k) Number:

K243490

B. Purpose for Submission:

Traditional 510(k), New Device

C. Measurand:

Nucleic acid sequences for the following organisms: *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*
Nucleic acid sequences for the following resistance markers: *mecA/mecC, vanA, vanB*

D. Type of Test:

Qualitative Multiplexed Direct Detection Hybridization Assay

E. Applicant:

Rocio Rueda, Luminex Corporation
4088 Commercial Avenue
Northbrook, IL 60062
(847) 400-9000

F. Proprietary and Established Names:

LIAISON PLEX® Gram-Positive Blood Culture Assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
PAM	II	21 CFR 866.3365 – Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures	83 (Microbiology)

H. Intended Use:

1. Intended use(s):

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]	Genera and Species
mecA/mecC	*Bacillus* spp.
vanA	*Enterococcus faecalis*
vanB	*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.

2. Indication(s) for use:
   Same as intended use.

3. Special conditions for use statement(s):
   For prescription use only.
   For *in vitro* diagnostic use only.

4. Special instrument requirements:
   For use with LIAISON PLEX® Systems.

I. 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 and microarray-based hybridization for the detection of target-specific nucleic acids. The test reagents are supplied as a single, disposable test cartridge. Target amplification is not performed as part of the BCP Assay workflow, as it is a non-amplified, direct detection test performed on the LIAISON PLEX® System.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   VERIGENE Blood Culture Gram-Positive (BC-GP) Nucleic Acid Test

2. Predicate 510(k) number(s):
   K122514

3. Comparison with predicate:
   The following tables compare the LIAISON PLEX® Gram-Positive Blood Culture Assay to the VERIGENE Blood Culture Gram-Positive (BC-GP) Nucleic Acid Test.

Comparison to Predicate Device	**Predicate Device:** **VERIGENE Blood Culture Gram-Positive (BC-GP) Nucleic Acid Test, K122514**	**Candidate Device:** **LIAISON PLEX® Gram-Positive Blood Culture Assay**
Product Code	PAM	PAM
Regulation Number	21 CFR 866.3365	21 CFR 866.3365

Comparison to Predicate Device	Predicate Device: **VERIGENE Blood Culture Gram-Positive (BC-GP) Nucleic Acid Test, K122514**	Candidate Device: **LIAISON PLEX® Gram-Positive Blood Culture Assay**
Organism Detected	Organisms: *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 Markers: *mecA, vanA, vanB*	Organisms: *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* Resistant markers: *mecA/mecC, vanA, vanB*
Measurand	Nucleic acid from Organisms detected	Same
Intended Use	The Verigene® Gram-Positive Blood Culture Nucleic Acid Test (BC-GP), performed using the sample-to-result Verigene System, is a qualitative multiplexed in vitro diagnostic test for the simultaneous detection and identification of potentially pathogenic gram-positive bacteria which may cause bloodstream infection (BSI). BC-GP is performed directly on blood culture bottles identified as positive by a continuous monitoring blood culture system and which contain gram-positive bacteria. BC-GP detects and identifies the following: **Bacterial Genera and Species** *Staphylococcus spp.* *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus lugdunensis* *Streptococcus spp.* *Streptococcus pneumoniae* *Streptococcus pyogenes* *Streptococcus agalactiae* *Streptococcus anginosus group* *Enterococcus faecalis* *Enterococcus faecium* *Listeria spp.* **Resistance Markers**[1] *mecA* *vanA* *vanB* [1]In mixed growth, BC-GP	The LIAISON PLEX® Gram-Positive Blood Culture Assay (BCP), 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. BCP 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 BCP Assay detects and identifies the following: **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*

Comparison to Predicate Device	Predicate Device: VERIGENE Blood Culture Gram-Positive (BC-GP) Nucleic Acid Test, K122514	Candidate Device: LIAISON PLEX® Gram-Positive Blood Culture Assay
	does not specifically attribute van-mediated vancomycin resistance to either *E. faecalis* or *E. faecium*, or *mecA*-mediated methicillin resistance to either *S. aureus* or *S. epidermidis*. BC-GP is indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial bloodstream infections; however, 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.	**Resistance Markers**[a] *mecA/mecC* *vanA* *vanB* [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 Assay 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, BCP 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 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.

Automated System (Sample to Answer)	Automated	Same
Instrumentation	VERIGENE	LIAISON PLEX®
Sample Types	Positive Blood Culture	Same

K. Standards/Guidance Documents Referenced:

- Class II Special Controls Guideline: Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures (May 2015)
- Electronic Submission Template for Medical Device 510(k) Submissions - Guidance for Industry and Food and Drug Administration Staff (October 2, 2023).
- Content of Premarket Submissions for Device Software Functions - Guidance for Industry and Food and Drug Administration Staff (June 14, 2023).
- Cybersecurity in Medical Devices: Quality System Considerations and Content of Premarket Submissions - Guidance for Industry and Food and Drug Administration Staff (September 23, 2023).
- Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests - Guidance for Industry and FDA Staff (March 13, 2007).
- CLSI. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition. CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
- CLSI. Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI document EP25-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
- 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

L. Test Principle:

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.

M. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Site-to-site Reproducibility

Site-to-site reproducibility of the LIAISON PLEX® BCP Assay was evaluated by testing LIAISON PLEX® BCP Assay cartridges across five non-consecutive days with at least two operators each at multiple sites; three external sites and one internal site. The reproducibility panel, blinded to operators, consisted of a total of 16 blood culture samples. 7 samples each contained representative on-panel organisms individually cultured at ring positivity, and eight hours after ring positivity, one sample was contrived with an off-panel organism (*Escherichia coli*), and the last sample was a negative blood culture matrix (NBM) specimen. The blinded reproducibility panel was tested in triplicate by each operator on each testing day for five non-consecutive testing days. The call agreement and 95% confidence intervals are presented in **Table 1**. Overall, results of site-to-site reproducibility evaluation demonstrated 99.7% reproducibility of the LIAISON PLEX® BCP Assay with a 95% CI of 99.3% to 99.9%.

Table 1. LIAISON PLEX® Gram-Positive Blood Culture Assay Site-to-Site Reproducibility Results Summary

Organism ID	Reportable Targets	Sample Type	Agreement (%) with Expected results				95% CI	
			Site1	Site 2	Site 3	Overall	Lower	Upper
Staphylococcus aureus	*Staphylococcus* spp. *S. aureus*	Panel 1 – RP	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
		Panel 1 – RP+8 Hours	100% (30/30)	96.7% (29/30)[a]	100% (30/30)	98.9% (89/90)	94.0%	99.8%
Enterococcus faecalis	*Enterococcus faecalis*	Panel 2 – RP	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
		Panel 2 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
Bacillus subtilis	*Bacillus* spp.	Panel 3 – RP	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
		Panel 3 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
Listeria monocytogenes	*Listeria* spp.	Panel 4 – RP	100% (30/30)	100% (30/30)	96.7% (29/30)[c]	98.9% (89/90)	94.0%	99.8%
		Panel 4 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
Staphylococcus epidermidis	*Staphylococcus* spp. *Staphylococcus epidermidis*	Panel 5 – RP	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
		Panel 5 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
Streptococcus pneumoniae	*Streptococcus* spp. *Streptococcus pneumoniae*	Panel 6 – RP	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
		Panel 6 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
Streptococcus pyogenes	*Streptococcus* spp. *Streptococcus pyogenes*	Panel 7 – RP	100% (30/30)	100% (30/30)	96.7% (29/30)[d]	98.9% (89/90)	94.0%	99.8%

		Panel 7 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
Escherichia coli	None	Contrived Negative	100% (60/60)	100% (60/60)	100% (60/60)	100% (180/180)	97.9%	100%
No Target	None	Negative Blood Matrix	100% (30/30)	100% (30/30)	96.7% (29/30)[b]	98.9% (89/90)	94.0%	99.8%
Resistance markers								
Staphylococcus aureus	*mecA/C*	Panel 1 – RP	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
		Panel 1 – RP+8 Hours	100% (30/30)	96.7% (29/30)	100% (30/30)	98.9% (89/90)	94.0%	99.8%
Enterococcus faecalis	*vanB*	Panel 2 – RP	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
		Panel 2 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
Staphylococcus epidermidis	mecA/mecC	Panel 5 – RP	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
		Panel 5 – RP+8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%

[a] False positive *S. lugdunensis*, *S. anginosus* group, and *Streptococcus* spp. result in 1/30 replicates. Reportable targets were fully 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 fully detected.

[d] False positive *Staphylococcus* spp. result in 1/30 replicates. Reportable targets were fully detected.

Precision/Repeatability

Within laboratory (operator-to-operator) precision/repeatability of the LIAISON PLEX® BCP Assay was evaluated based on the results generated by two operators testing samples (two on-panel organisms (*Staphylococcus aureus* and *Enterococcus faecalis)* at ring positivity and at 8 hours after ring positivity, contrived negative and negative blood culture samples) at the internal site (Site 1). Within laboratory precision/repeatability of the LIAISON PLEX® BCP Assay was 100%, and results are summarized in **Table 2**.

Table 2. LIAISON PLEX® Gram-Positive Blood Culture Assay Within-Laboratory Precision/Repeatability Results Summary

Organism ID	Reportable Targets	Sample Type	Agreement (%)	Overall 95% CI
Staphylococcus aureus	*Staphylococcus spp* | *S. 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 Markers				
Staphylococcus aureus	*mecA/C*	Panel 1 – RP	100% (30/30)	88.6% - 100%

		Panel 1 – RP+8 Hours	100% (30/30)	88.6% - 100%
Enterococcus faecalis	*vanB*	Panel 2 – RP	100% (30/30)	88.6% - 100%
		Panel 2 – RP+8 Hours	100% (30/30)	88.6% - 100%
Overall Agreement (All Targets/Sample Types)			100% (180/180)	97.9% - 100%

Lot-to-lot Reproducibility

Lot-to-lot reproducibility of the LIAISON PLEX® BCP Assay was evaluated by one operator over a minimum of five non-consecutive days across three lots of LIAISON PLEX® BCP Assay cartridges and four LIAISON PLEX® systems. The same reproducibility panel of six blood culture samples; two representative on-panel organisms (*Staphylococcus aureus* and *Enterococcus faecalis*) individually cultured at ring positivity, and eight hours after ring positivity, one negative sample contrived with an off-panel organism (*Escherichia coli*), and one negative blood culture matrix (NBM) sample, were prepared and tested for the evaluation of the lot-to-lot reproducibility. Each sample type was tested in triplicate per cartridge lot on a given testing day. Lot-to-lot reproducibility of the LIAISON PLEX® BCP Assay cartridges was 100%, and results are summarized in **Table 3**.

Table 3: LIAISON PLEX® Gram-Positive Blood Culture (BCP) Assay Lot-to-Lot Reproducibility Results

Organism ID	Reportable Targets	Sample Type	Reagent Lot	Results (%) agreement	Results (95% CI)
Staphylococcus aureus	*Staphylococcus* spp. *S. 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/C*	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%

b. Linearity/assay reportable range:

Not applicable. The LIAISON® PLEX Gram-Positive Blood Culture Assay is a qualitative assay.

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

Controls

Several controls are built into the assay and system to ensure identification of processing errors and to establish validity of test results.

Internal Controls

Each LIAISON PLEX® Gram-Positive Blood Culture Assay cartridge includes internal controls to ensure performance of sample preparation and detection. The internal extraction control is present in the lysis tube when the sample is added. Sample preparation is initiated and the extraction control assesses extraction, nucleic acid recovery, and detection. Finally, addition of a post-extraction hybridization control serves as an indicator of successful hybridization. Internal control results are reported as Pass or Fail on the printed reports (see **Table 4** for detailed explanations of each control result). Internal controls must generate a signal above threshold in each internal reaction for the system to report a valid test result.

Table 4. Interpretation of Controls on the LIAISON PLEX® Gram-Positive Blood Culture Assay Report

Internal Control Result	Explanation	Suggested Action
Pass	Test was completed and internal controls were successful, indicating that valid results were generated.	Review and report results
Fail	One or more internal control failed.	Repeat test with a new cartridge

External Controls

Positive and negative external controls should be tested with each new lot or shipment of reagents, or monthly, (whichever occurs first), or in accordance with updated local, regional, state, and/or federal guidelines. Verified negative blood matrix can be used as the negative control. Previously characterized positive samples or verified negative blood matrix spiked with well characterized organisms may be used as the external positive control. External controls should be used in accordance with laboratory protocols and in accordance with local, state, and federal accrediting organizations, as applicable.

Stability

Specimen Stability

Performance of the LIAISON PLEX® Gram-Positive Blood Culture (BCP) Assay was assessed using specimens tested in a fresh state (at bottle/ring positive and at bottle/ring positive + 8 hours) and after exposure to various storage conditions. Conditions tested included refrigerated storage (2° to 8°C) and room temperature storage (15°C to 30°C) to span the typical “fresh specimen” storage conditions across multiple time points. Positive specimens containing five target organisms representing a total of 9 reportable gram-positive targets

and select resistance markers were tested as well as negative blood matrix control specimen containing no target organisms. 100% detection of all reportable targets was observed across all timepoints. The negative sample demonstrated 0% positivity across all time-points and storage conditions tested. The results demonstrated that specimens may be stored under the following temperature conditions without impacting the performance of the LIAISON PLEX® BCP cartridge:

- Up to 72 hours (3 days) at Refrigerated (2-8°C) or Room Temperature (15-30°C) storage conditions.

Device Stability

A shelf-life study was conducted to evaluate the real-time stability of the LIAISON PLEX® BCP Assay at the recommended storage conditions of room temperature (15°C – 30°C). Real-time stability was assessed using three Positive Control panels which interrogate representative targets in the assay, and one Negative Control which consisted of negative blood matrix. Results of real-time stability for all tested lots demonstrated the LIAISON PLEX® BCP Assay is stable for at least 1 month when stored at 15°C – 30°C, and up to 3 months when stored at 15°C – 30°C for two of the three cartridge lots evaluated. Testing for the third lot is ongoing. Shelf-life will be extended based upon results of on-going stability testing.

An open box stability study was performed to evaluate the stability of the LIAISON PLEX® BCP Assay cartridges at room temperature once removed from their foil pouch. Testing was performed shortly after kits were manufactured and will be repeated at the end of the product shelf-life. Non-aged cartridges were tested at 0 hours (T0), 3 hours (T1), and 9 hours (T2) after removal from their pouch. Each time point included testing of three Positive Control panels which interrogate seven reportable targets in the assay, and one Negative Control which consisted of negative blood matrix. One unexpected result (false positive) for *Staphylococcus lugdunensis and Streptococcus* spp. occurred for a negative target. Expected results were observed at all other timepoints and conditions. Results of open-box stability indicate the cartridges are stable for up to nine hours after cartridges are removed from their foil pouches and stored at room temperature.

Fresh vs. Frozen Specimen Stability

A fresh vs. frozen specimen stability study was performed to evaluate the performance of the LIAISON PLEX® Gram-Positive Blood Culture (BCP) assay in specimens that have been prepared “fresh” (at two growth durations referred to as Bottle/Ring Positive and Bottle/Ring Positive + 8 hours) and subjected to a defined number of freeze/thaw (F/T) cycles as well as those experiencing a prolonged storage in frozen conditions. The study was performed using six representative organisms detected by the LIAISON PLEX® BCP Assay cultured individually in blood culture bottles. Performance testing using negative blood matrix served as a control test during the study. Material was tested under 5 different conditions – Material was tested at multiple freeze thaw cycles: 1st Freeze-thaw, 2nd Freeze-thaw, and 3rd Freeze-thaw, and was frozen for a minimum of 8 hours in between each freeze-thaw cycle. In the prolonged testing, material was frozen at time points of 15 days and 30 days. A total of 701 replicates were included in this study. The results

demonstrated 100% positivity for target positive samples at both growth durations and all freeze-thaw conditions. The negative blood matrix demonstrated 0% positivity of all reportable targets for the assay at all freeze-thaw conditions.

d. Growth and Detection Study

The growth and detection study was performed to evaluate detection of each organism at the time of culture positivity and 8 hours after culture positivity for the organisms listed in **Table 5**. Three bottles for each organism were grown to ring positive or 8 hours after ring positive and three replicates were tested for each bottle, for a total of 9 replicates tested per organism. The results, presented in **Table 5**, demonstrated 100% positivity for target positive samples and 0% positivity when tested with negative blood.

Table 5. LIAISON PLEX® Gram-Positive Blood Culture Assay Growth & Detection Results Summary

Organism Tested	Expected Targets	Ring Positivity		8 Hours After Ring Positivity	
		Per Bottle (CFU/mL)	Positive Agreement/ Total (% Detected)	Per Bottle (CFU/mL)	Positive Agreement/ Total (% Detected)
Bacillus subtilis ATCC 19659	*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 ATCC 700221	*Enterococcus faecium* | *vanA*	2.37E+08	9/9 (100%)	9.30E+08	12/12 (100%)[a]
		3.01E+08		7.80E+08	
		2.24E+08		9.30E+08	
Enterococcus faecalis ATCC 51575	*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 ATCC 15313	*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 ATCC BAA-2312	*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 ATCC 35984	*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 ATCC 49576	*Staphylococcus* spp.| *Staphylococcus lugdunensis*	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 ATCC 12386	*Streptococcus* spp.| *Streptococcus*	1.21E+09	9/9 (100%)	1.69E+09	9/9 (100%)
		1.32E+09		1.48E+09	

Organism Tested	Expected Targets	Ring Positivity		8 Hours After Ring Positivity	
		Per Bottle (CFU/mL)	Positive Agreement/ Total (% Detected)	Per Bottle (CFU/mL)	Positive Agreement/ Total (% Detected)
	agalactiae	1.12E+09		2.03E+09	
Streptococcus anginosus ATCC 33397	*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 ATCC 49619	*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 ATCC 700294	*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	
Organism Tested	Expected Targets	Ring Negative			
Negative Blood	None	0/3 (0%)			

[a] False Positive *Bacillus* spp. result in one replicate. Three additional replicates were tested and no additional FPs were observed.

e. Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the LIAISON PLEX® BCP Assay was evaluated by using a collection of 184 isolates, representing the genetic diversity of the analytes included as part of the LIAISON PLEX® BCP Assay. **Table 6** contains the summary of the organisms tested and the genus and species detected by the LIAISON PLEX® BCP Assay. **Table 7** contains the summary for the antimicrobial resistance marker reportable targets also detected by the assay.

Table 6: LIAISON PLEX® Gram-Positive Blood Culture (BCP) Inclusivity Summary (Microorganism Markers)

Reportable Target (Genus)	Reportable Target (Species)	Organism	# of strains	% Detected
Bacillus spp.	N/A	*Bacillus cereus*	4	100%
		Bacillus licheniformis	2	100%
		Bacillus subtilis	3	100%
		Bacillus thuringiensis	2	100%
N/A	*Enterococcus faecalis*	*Enterococcus faecalis*	9	100%
	Enterococcus faecium	*Enterococcus faecium*	9	100% [a]
Listeria spp.	N/A	*Listeria grayi*	2	100%
		Listeria innocua	2	100%
		Listeria ivanovii	2	100%
		Listeria monocytogenes	6	100%
		Listeria seeligeri	2	100%

Reportable Target (Genus)	Reportable Target (Species)	Organism	# of strains	% Detected
		Listeria welshimeri	2	100%
Staphylococcus spp.	*S. aureus*	*Staphylococcus aureus*	43	100%
	S. epidermidis	*Staphylococcus epidermidis*	8	100%
	S. lugdunensis	*Staphylococcus lugdunensis*	5	100%
	N/A	*Staphylococcus argenteus*	2	100% [b]
		Staphylococcus auricularis	2	100%
		Staphylococcus capitis	2	100%
		Staphylococcus caprae	1	100%
		Staphylococcus cohnii	2	100%
		Staphylococcus haemolyticus	2	100%
		Staphylococcus hominis	3	100%
		Staphylococcus intermedius	2	100%
		Staphylococcus muscae	1	100% [c]
		Staphylococcus pasteuri	1	100%
		Staphylococcus saccharolyticus	3	100%
		Staphylococcus saprophyticus	2	100%
		Staphylococcus schleiferi	1	100%
		Mammaliicoccus (Staphylococcus) sciuri	2	100%
		Staphylococcus simulans	2	100%
		Staphylococcus warneri	2	100%
		Staphylococcus xylosus	1	100%
Streptococcus spp.	*S. agalactiae*	*Streptococcus agalactiae*	5	100%
	S. anginosus Group	*Streptococcus anginosus*	2	100%
		Streptococcus constellatus	3	100%
		Streptococcus intermedius	2	100%
	S. pneumoniae	*Streptococcus pneumoniae*	5	100%
	S. pyogenes	*Streptococcus pyogenes*	5	100%
	N/A	*Streptococcus bovis*	3	100%
		Streptococcus dysgalactiae	2	100%
		Streptococcus equi	2	100%
		Streptococcus equinus	2	100%
		Streptococcus gallolyticus	3	100%
		Streptococcus gordonii	2	100%
		Streptococcus infantarius subsp. *coli*	1	100%
		Streptococcus infantis	2	100%
		Streptococcus mitis	2	100%
		Streptococcus mutans	2	88.9% [d]
		Streptococcus oralis	2	100%
		Streptococcus parasanguinis	2	100%

Reportable Target (Genus)	Reportable Target (Species)	Organism	# of strains	% Detected
		Streptococcus peroris	1	100%
		Streptococcus pseudopneumoniae	1	100%
		Streptococcus salivarius	1	100%
		Streptococcus 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 FPs were observed.
[b] *Staphylococcus argenteus* cross reacts with *S. aureus*.
[c] *Staphylococcus muscae* cross reacts with *Listeria* spp.
[d] One strain of *Streptococcus mutans,* ATCC 25175, was not detected as *Streptococcus* spp. in one of three initial replicates tested. Three additional replicates were run, giving an overall detection rate for *Streptococcus* spp. of 5/6 for that strain. *S. mutans* strain 31383 was fully detected in all replicates.

Table 7: LIAISON PLEX® Gram-Positive Blood Culture (BCP) Inclusivity Summary – Antimicrobial Resistance Markers

Reportable Target (Resistance Marker)	Organism	# of strains	% Detected
mecA/C	*Staphylococcus argenteus*	1	100%
mecA/C	*Staphylococcus aureus*	35	100%
	Staphylococcus epidermidis	5	100%
	Staphylococcus hominis	1	100%
	Mammaliicoccus (Staphylococcus) sciuri	2	100%
vanA	*Enterococcus faecalis*	3	100%
	Enterococcus faecium	3	100%[a]
vanB	*Enterococcus faecalis*	3	100%
	Enterococcus faecium	3	100%

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

Predicted (in silico) Reactivity (Inclusivity) Results

For all targets, in silico inclusivity analysis was performed using sequences available in the GenBank and WGS (whole genome shotgun) databases from March to April 2024. Alignments of the signal fragment for each target were generated using MAFFT (version 7.490). For all targets, the inclusivity analysis involved assessing the percent homology of each oligo sequence to its binding region on each target sequence retrieved from the public databases. The predicted inclusivity based on sequence homology is the percentage of sequences with at least 90% oligo identity. In determining oligo identity, the highest percent identity of each oligo in the same component was assessed and then the lowest percent identity of the two components (capture and mediator probes) is used to characterize the inclusivity of the sequence. *In silico* analysis results are summarized below in **Table 8**.

Table 8 – *In silico* Analysis Results

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

f. Analytical Specificity (Exclusivity)

Cross-Reactivity

The analytical specificity study was performed to evaluate cross-reactivity of the LIAISON PLEX® BCP Assay with "off-panel" organisms, including both phylogenetically related to the on-panel organisms and organisms likely to be present in typical blood culture samples. **Table 9** contains the summary for the genus and species reportable targets. Out

of the 103 off-panel species tested, 97 resulted in no cross-reactivity, 5 resulted in cross-reactivity with one reportable target, and 1 organism resulted in positivity for 1 out of 6 replicates tested. Organisms with cross-reactivity and the BCP target that was detected are listed in **Table 10.**

Table 9: LIAISON PLEX® BCP Assay Analytical Specificity (Cross Reactivity) Summary

Organism	Positivity	Organism	Positivity
Gram Positive			
Abitrophia defectiva	0%	*Enterococcus flavescens*	0%
Aerococcus viridans	0%	*Enterococcus gallinarum, vanC*	0%
Arcanobacterium bernardiae	0%	*Enterococcus hirae*	0%
Arcanobacterium haemolyticum	0%	*Enterococcus mundtii*	0%
Corynebacterium amycolatum	0%	*Enterococcus raffinosus*	0%
Corynebacterium diphtheriae	0%	*Erysipelothrix rhusiopathiae*	0%
Corynebacterium flavescens	0%	*Kocuria kristinae*	0%
Corynebacterium genitalium	0%	*Kytococcus sedentarius, methicillin resistant*	0%
Corynebacterium glutamicum	0%	*Lactobacillus acidophilus*	0%
Corynebacterium jeikeium	0%	*Lactobacillus crispatus*	0%
Corynebacterium pseudodiptherticum	0%	*Lactobacillus rhamnosus*	0%
Corynebacterium renale	0%	*Leuconostoc carnosum*	100%
Corynebacterium striatum	0%	*Leuconostoc mesenteroides*	0%
Corynebacterium urealyticum	0%	*Pediococcus acidilactici*	0%
Cutibacterium acnes	0%	*Pediococcus pentosaceus*	0%
Cutibacterium avidum	0%	*Peptostreptococcus anaerobius*	0%
Propionibacterium freudenreichii	0%	*Planococcus citreus*	0%
Enterococcus avium	100%	*Planococcus kocurii*	0%
Enterococcus casseliflavis, VRE, *vanC*	0%	*Rothia dentocariosa*	0%
Enterocococcus dispar	0%	*Rothia (Stomatococcus) mucilaginosa*	0%
Enterococcus durans	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%
Capnocytophaga ochracea	0%	*Leclercia adecarboxylata*	0%
Cardiobacterium hominis	0%	*Moraxella catarrhalis*	0%
Cedecea lapagei	0%	*Morganella morganii*	0%
Citrobacter amalonaticus	0%	*Neisseria lactamica*	0%

Organism	Positivity	Organism	Positivity
Citrobacter freundii	0%	*Neisseria meningitidis*	0%
Comamonas testosteroni	0%	*Neisseria mucosa*	0%
Delftia acidovorans	0%	*Neisseria sicca*	0%
Eikenella corrodens	0%	*Parabacteroides distasonis*	0%
Elizabethkingia meningoseptica	0%	*Pasteurella aerogenes*	0%
Enteric Group 137	0%	*Prevotella bivia*	0%
Enterobacter aerogenes	0%	*Proteus mirabilis*	0%
Enterobacter cloacae	0%	*Proteus penneri*	100%
Enterobacter hormaechei	0%	*Proteus vulgaris*	100%
Escherichia albertii	0%	*Pseudomonas aeruginosa*	0%
Escherichia coli	0%	*Pseudomonas alcaligenes*	0%
Escherichia fergusonii	0%	*Raoultella ornithinolytica*	0%
Escherichia hermannii	0%	*Salmonella enterica*	0%
Fusobacterium necrophorum	0%	*Serratia marcescens*	0%
Fusobacterium nucleatum	0%	*Stenotrophomonas maltophilia*	0%
Haemophilus haemolyticus	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 10: LIAISON PLEX® BCP Assay Analytical Specificity (Cross Reactivity) Summary

Cross-Reactive Organism	BCP Target Detected
Enterococcus avium	*Enterococcus faecium*
Leuconostoc carnosum	*Streptococcus* spp.
Aggregatibacter aphrophilus	*Streptococcus* spp.
Proteus penneri	*Streptococcus* spp.
Proteus vulgaris	*Streptococcus* spp.

In Silico Cross-Reactivity

For *in silico* exclusivity assessment of the oligo sequences incorporated in the assay designs against on-panel and off-panel organisms listed in **Table 11**, based on analysis of sequences available in the GenBank nt database as of July 27, 2024, the following potential cross-reactivity is predicted:

- Some strains of *Enterococcus durans* and 3 strains of *Streptococcus* species (AY123726.1, FJ577604.1, MK608388.1) are predicted to produce false positive *Enterococcus faecalis* results.
- One strain of *Enterococcus durans* (KT877992.1), 1 strain of *Enterococcus faecalis* (CP092577.1) and some strains *Enterococcus avium* are predicted to produce false positive *Enterococcus faecium* results.
- *Staphylococcus aureus* oligo designs are predicted to detect some strains of *Staphylococcus argenteus* and one strain of *Staphylococcus schweitzeri* (LR134304.1).
- *Staphylococcus* spp. oligo designs are predicted to detect some strains of *Macrococcoides caseolyticum*.
- Some strains of *Streptococcus* species (*S. lactarius*, *S. milleri*, *S. oralis*, *S. periodonticum*, *S. pneumoniae*, *S. rubneri*, *S. vaginalis*) are predicted to produce false positive *Streptococcus anginosus* Group results.
- *Streptococcus pneumoniae* oligo designs are predicted to detect a few strains of various Streptococcus species (*S. downii, S. mitis, S. oralis*).
- Some strains of *Aggregatibacter aphrophilus*, unspecified *Bacillus* species, *Enterococcus* species (*E. durans*, *E. gallinarum*), *Glaesserella parasuis*, *Haemophilus* species (*H. ducreyi, H. influenzae, H. parahaemolyticus, H. parainfluenzae*), *Lactococcus* species (*L. garvieae, L. lactis*), *Leuconostoc carnosum, Moellerella wisconsensis*, *Morganella morganii*, *Pasteurella multocida*, *Proteus* species (*P. appendicitidis, P. columbae, P. faecis, P. penneri, P. terrae, P. vulgaris*), *Providencia* species (*P. alcalifaciens, P. hangzhouensis, P. heimbachae, P. huaxiensis, P. manganoxydans, P. rettgeri, P. rustigianii, P. stuartii, P. vermicola, P. zhijiangensis*), *Serratia* species (*S. marcescens, S. symbiotica*) and *Xenorhabdus* species (*X. bovienii, X. hominickii, X. nematophila*) are predicted to produce false positive *Streptococcus* spp. results.
- *Listeria* spp. oligo designs are predicted to detect some strains of *Staphylococcus muscae*.

Table 11: Potential Cross-Reactive Organisms assessed in the *In Silico* Exclusivity Analysis

On-Panel Organisms	Off-Panel Organisms			
	Gram-Positive Bacteria	Gram-Negative Bacteria	Resistance Markers	Yeasts / Viruses / 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*

On-Panel Organisms	Off-Panel Organisms			
	Gram-Positive Bacteria	Gram-Negative Bacteria	Resistance Markers	Yeasts / Viruses / Parasites
Streptococcus pneumoniae	*Brochothrix thermosphacta*	*Bacteroides ovatus*	SHV	*Candida guilliermondii*
Streptococcus pyogenes	*Carnobacterium divergens*	*Bacteroides thetaiotaomicron*	SME	*Candida haemulonii*
Streptococcus spp.	*Carnobacterium maltaromaticum*	*Bacteroides uniformis*	SPM	*Candida inconspicua*
mecA	*Cellulomonas turbata*	*Bacteroides vulgatus*	TEM	*Candida kefyr*
mecC	*Cellulosimicrobium cellulans*	*Bacteroides xylanisolvens*	*vanC*	*Candida krusei*
vanA	*Clostridioides difficile*	*Bordetella bronchiseptica*	*vanD*	*Candida lipolytica*
vanB	*Clostridium bifermentans*	*Bordetella parapertussis*	*vanM*	*Candida lusitaniae*
	Clostridium clostridioforme	*Bordetella pertussis*	VIM	*Candida metapsilosis*
	Clostridium perfringens	*Brevundimonas diminuta*		*Candida multis-gemmis*
	Clostridium ramosum (Thomasclavelia ramosa)	*Brevundimonas vesicularis*		*Candida nivariensis*
	Clostridium septicum	*Burkholderia cepacia*		*Candida norvegensis*
	Clostridium tertium	*Burkholderia mallei*		*Candida orthopsilosis*
	Clostridium tetani			
	Corynebacterium spp.	*Burkholderia multivorans*		*Candida parapsilosis*
	Cutibacterium acnes			
	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*
	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 crispatus	*Haemophilus parasuis*		*Saccharomyces cerevisiae*

On-Panel Organisms	Off-Panel Organisms			
	Gram-Positive Bacteria	**Gram-Negative Bacteria**	**Resistance Markers**	**Yeasts / Viruses / Parasites**
	Lactobacillus rhamnosus *Lactococcus garvieae* *Lactococcus lactis* *Leuconostoc carnosum* *Leuconostoc citreum* *Leuconostoc mesenteroides* *Macrococcus caseolyticus* *Micrococcus luteus* *Mycobacterium avium* complex (MAC) *Mycobacterium fortuitum* *Mycobacterium mucogenicum* *Mycoplasma hominis* *Mycoplasma pneumoniae* *Nocardia farcinica* *Parvimonas micra* *Pediococcus acidilactici* *Pediococcus pentosaceus* *Peptostreptococcus anaerobius* *Planococcus citreus* *Planococcus kocurii* *Propionibacterium freudenreichii* *Propionibacterium propionicum (Arachnia propionica)* *Rhodococcus equi* *Rothia dentocariosa* *Rothia mucilaginosa* *Sarcina ventriculi* *Solibacillus silvestris* *Ureaplasma parvum* *Ureaplasma urealyticum* *Vagococcus fluvialis* *Weissella paramesenteroides*	*Haemophilus quentini* *Haemophilus sputorum* *Herbaspirillum huttiense* *Kingella denitrificans* *Kingella kingae* *Kingella negevensis* *Kingella oralis* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Klebsiella variicola* *Legionella pneumophila* *Leptospira interrogans* *Moraxella catarrhalis* *Moraxella osloensis* *Morganellaceae* *Mycobacterium tuberculosis* *Neisseria gonorrhoeae* *Neisseria lactamica* *Neisseria meningitidis* *Neisseria mucosa* *Neisseria sicca* *Parabacteroides distasonis* *Parabacteroides merdae* *Pasteurella aerogenes* *Pasteurella canis* *Pasteurella multocida* *Pasteurella stomatis* *Prevotella bivia* *Prevotella buccae* *Prevotella denticola* *Prevotella melaninogenica* *Prevotella oralis* *Proteus* spp. *Pseudomonas* spp. *Psychrobacter cryohalolentis* *Psychrobacter immobilis* *Ralstonia mannitolilytica* *Ralstonia pickettii*		*Schizosaccharomyces pombe* *Talaromyces marneffei* *Trichosporon asahii* *Wickerhamomyces anomalus* BK Virus Chikungunya Virus Cytomegalovirus Dengue Virus Enterovirus Epstein Barr Virus Hepatitis A virus Hepatitis B virus Hepatitis C virus Human alphaherpesvirus 1 Human alphaherpesvirus 2 Human betaherpesvirus 6 Human betaherpesvirus 7 Human Immunodeficiency Virus JC Virus Measles Virus Mumps Virus Parvovirus B19 Rubella Virus Varicella Zoster Virus West Nile Virus Zika Virus *Plasmodium falciparum* *Trypanosoma cruzi*

On-Panel Organisms	Off-Panel Organisms			
	Gram-Positive Bacteria	Gram-Negative Bacteria	Resistance Markers	Yeasts / Viruses / Parasites
		Salmonella spp. *Serratia* spp. *Shigella* spp. *Stenotrophomonas acidaminiphila* *Stenotrophomonas maltophilia* *Stenotrophomonas nitritireducens* *Stenotrophomonas rhizophila* *Treponema pallidum* *Veillonella parvula* *Vibrio alginolyticus* *Vibrio parahaemolyticus* *Vibrio vulnificus*		

g. Interference

Competitive Inhibition / Co-Infection and Microbial Interference

The competitive inhibition and microbial interference study was executed to evaluate the performance of the LIAISON PLEX® BCP Assay in the presence of clinically significant levels of potential co-infections of on-panel organisms and potentially interfering (off-panel) microbes. To evaluate potential co-infections, varying ratios of two on-panel target organisms including a low titer (concentration at bottle/ring positive) and a high titer specimen (concentration at bottle/ring positive +8 hours) were tested in combination, as listed in **Table 12.** The on-panel target organisms were chosen to be representative of clinically relevant poly-microbial infections in blood culture specimens. To assess microbial interference, three off-panel microbes were combined in pairs with each of three representative on-panel target organisms, as listed in **Table 13**. These specific organism sets were chosen to mimic potentially interfering micro-organisms that are commonly found in positive blood culture samples but are not designed to be detected by the LIAISON PLEX® BCP Assay.

Results are shown in **Tables 12** and **Table 13.** On-panel targets were detected with 100% positivity for all combinations tested, both in the presence of competitive on-panel targets and in the presence of competitive off-panel pathogens.

Table 12: LIAISON PLEX® BCP Assay Competitive Inhibition Summary

On-Panel High Titer Target	Positivity	On-Panel Low Titer Target	Positivity
Enterococcus faecalis	100%	*Staphylococcus epidermidis*	100%
	100%	*Enterococcus faecium*	100%
Enterococcus faecium	100%	*Staphylococcus epidermidis*	100%
	100%	*Enterococcus faecalis*	100%
Staphylococcus aureus	100%	*Staphylococcus epidermidis*	100%
	100%	*Streptococcus agalactiae*	100%
Staphylococcus epidermidis	100%	*Enterococcus faecalis*	100%
	100%	*Enterococcus faecium*	100%
	100%	*Staphylococcus aureus*	100%
	100%	*Staphylococcus lugdunensis*	100%
	100%	*Streptococcus pneumoniae*	100%
Staphylococcus lugdunensis	100%	*Staphylococcus epidermidis*	100%
Streptococcus agalactiae	100%	*Staphylococcus aureus*	100%
Streptococcus pneumoniae	100%	*Staphylococcus epidermidis*	100%

Table 13: LIAISON PLEX® BCP Assay Microbial Interference Summary

On-Panel Low Titer Target	Positivity	Off-Panel High Titer Target	Positivity
Staphylococcus epidermidis	100%	*Escherichia coli*	0%
	100%	*Klebsiella pneumoniae*	0%
	100%	*Proteus mirabilis*	0%
Enterococcus faecium	100%	*Escherichia coli*	0%
	100%	*Klebsiella pneumoniae*	0%
	100%	*Proteus mirabilis*	0%
N/A	N/A	*Escherichia coli*	0%
	N/A	*Klebsiella pneumoniae*	0%
	N/A	*Proteus mirabilis*	0%

Interfering Substances

The interfering substances study was performed to evaluate the performance of the LIAISON PLEX® BCP Assay in the presence of non-microbial (endogenous and exogenous) interfering substances which may be present in blood culture specimens. Six representative "on-panel" organisms were individually tested to assess effectiveness of target detection in the presence of six typically occurring interfering substances. Each interfering substance was tested across five replicates for each organism. In addition, five replicates of a negative control was tested alongside the positive specimens to assess impact of the same interfering agents in specimens containing no target. A positive control (specimen without interfering substances) for all four targets was also tested to assess for detection capabilities.

As seen in **Table 14**, 100% target detection was observed for all six targets without interferent and in the presence of all six interfering substances. 0% target detection was observed with the negative sample with and without interfering substances.

Table 14: LIAISON PLEX® BCP Assay Interfering Substances Summary

	Organism	Interfering Substance & Tested Concentration						
		Unconjugated Bilirubin	Conjugated Bilirubin	Hemoglobin	Intralipid	γ-globulin	Sodium polyanethol-sulfonate	No interferent
		20 mg/dL	20 mg/dL	14 g/L	3000 mg/dL	6 g/dL	0.25% w/v	
% Positivity	*Staphylococcus aureus*	100%	100%	100%	100%	100%	100%	100%
	Staphylococcus epidermidis	100%	100%	100%	100%	100%	100%	100%
	Streptococcus pneumoniae	100%	100%	100%	100%	100%	100%	100%
	Streptococcus agalactiae	100%	100%	100%	100%	100%	100%	100%
	Enterococcus faecalis	100%	100%	100%	100%	100%	100%	100%
	Enterococcus faecium	100%	100%	100%	100%	100%	100%	100%
	Negative Blood Matrix	0%	0%	0%	0%	0%	0%	0%

Carry-Over/Cross Contamination

This study was performed 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 high concentration positive samples consisting of *Staphylococcus aureus* at a final concentration of 1.90E+08 CFU/mL, and 30 negative samples consisting of Negative Blood Matrix. *Staphylococcus aureus* was selected as it is a representative organism with three expected positive target detections on the LIAISON PLEX® BCP Assay.

Testing was performed on two LIAISON PLEX® instruments containing 6 modules over the course of four days. The samples were loaded into cartridges alternating between positive and negative samples (checkerboard fashion), six specimens at a time using sample prep trays. The results, presented in **Table 15**, demonstrate 100% agreement between expected and observed results, indicating that no cross contamination occurred within runs and no carry-over was observed across runs.

Table 15. Overall Results

Overall Percent Agreement between Expected and Observed Results	100%
***Staphylococcus aureus* Positivity in High Positive Control Replicates**	100%
***Staphylococcus aureus* Positivity in Negative Control Replicates**	0%

h. Assay Cut-off

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

2. Comparison Studies:

a. Method comparison with predicate device:

Refer to Section 3 Clinical Performance.

b. Matrix Comparison: Testing of Blood Culture Bottle Types / Matrix Equivalency

The performance of LIAISON PLEX® Gram-Positive Blood Culture (BCP) Assay was evaluated across 12 additional types of commercially available blood culture media bottles using target organisms representative of all "on-panel" BCP targets with resistance markers, representative off-panel (gram-negative) targets, and negative blood matrix (NBM). The results, presented in **Table 16,** demonstrate that all 12 bottle (media) types are compatible with the LIAISON PLEX® Gram-Positive Blood Culture (BCP) Assay. BACT/ALERT® FA Plus blood culture bottle type is established in the clinical evaluation and for all other analytical studies, such as, growth and detection and analytical reactivity/ inclusivity verification testing and was not included in testing performed for the media equivalency study. **Table 17** presents the range of CFU/mL by organism across all bottle types tested in the study.

Table 16: LIAISON PLEX® Gram-Positive Blood Culture (BCP) Assay Media Equivalency/ Universal Blood Culture Bottle Result Summary

Manufacturer System	Blood Culture Bottle Manufacturer	Blood Culture Bottle Type[c]		Unique Bottles		
				Seeded Organisms		Negative Blood Matrix[d]
				Gram-positive Bacteria	Gram-negative Bacteria	
				Expected Results		
bioMérieux BACT/ALERT® 3D System	**bioMérieux BACT/ALERT®**	Aerobic	BACT/ALERT® SA	20/20	10/10	1/1
		Anaerobic	BACT/ALERT®	20/20	10/10	1/1

			SN			
			BACT/ALERT® FN Plus	20/20	10/10	1/1
		Pediatric	BACT/ALERT® PF Plus	58/58	10/10	1/1
Becton Dickinson 9050 / FX40	**Becton Dickinson[a] BACTEC™**	Aerobic	BACTEC™ Standard	20/20 [b]	10/10	1/1
			BACTEC™ Plus	20/20	10/10	1/1
		Anaerobic	BACTEC™ Standard	58/58 [b]	10/10	1/1
			BACTEC™ Plus	58/58	10/10	1/1
		Pediatric	BACTEC™ Peds Plus	58/58	10/10	1/1
		Lytic Anaerobic	BACTEC™ Lytic	20/20	10/10	1/1
N/A	**Thermo Scientific[a] VersaTREK™**	Aerobic	REDOX™ 1 EZ Draw™	56/56	10/10	1/1
		Anaerobic	REDOX™ 2 EZ Draw™	56/56	10/10	1/1
Bottles with Expected Results /Total Number Blood Bottles				464/464 [b] (100%)	120/120 (100%)	12/12 (100%)

[a] For gram-negative blood culture preparation, 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 a ring positivity + 8 bottle to confirm the bottle matrix was not inhibitory.
[c] bioMérieux BACT/ALERT® FA Plus blood culture bottle type (PN: 410851) was not tested in this study protocol as the effectiveness of this is bottle type was 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.

Table 17: Range of CFU/mL by Organism Across All Bottle Types Tested

Gram-Positive Organisms	Strain ID	Expected Result	Bottle Titer Range (CFU/mL)
Bacillus cereus	ATCC 10702	*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+09[a]
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
Corynebacterium diphtheriae	ATCC 27010	No target detected	2.80E+06 - 1.31E+09
Corynebacterium striatum	ATCC 43735	No target detected	4.30E+07 - 1.17E+09
Cutibacterium acnes	ATCC 6919	No target detected	2.83E+07 - 5.90E+09
Gram-negative	**Strain ID**	**Expected Result**	**Bottle Titer Range (CFU/mL)**
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

[a] Two bottles tested at ring positive with titers of 8.63E+07 and 1.46E+08 were not fully detected. Bottles grown to ring positivity + 8 hours were used to confirm that the results were not due to bottle matrix.
[b] No viable counts were achieved for *B. cereus* in two of the BD BACTEC™ Standard Anaerobic media bottles.

3. Clinical Performance:

A multi-site clinical study established the diagnostic accuracy of the LIAISON PLEX® Gram-Positive Blood Culture (BCP) Assay for the detection and identification of pathogenic gram-positive organisms in positive blood culture. The clinical performance of the LIAISON PLEX® BCP Assay was evaluated using clinical specimens prospectively collected between April 2024 and August 2024 from four geographically diverse clinical sites within the United States. The clinical study utilized remnant, de-identified blood culture specimens collected from patients exhibiting clinical signs and symptoms of bloodstream infection, evidenced by positive identification by a continuous monitoring blood culture system.

A total of 562 unique prospectively collected specimens that met the pre-determined inclusion criteria were enrolled in the study. Clinical runs and re-runs using the LIAISON PLEX® BCP Assay were tested on the LIAISON PLEX® System by trained operators at four clinical sites. For targets that exhibited low prevalence rates in the prospective study, the prospective specimen set was supplemented with 163 pre-selected left-over, de-identified specimens sourced from ten vendors in the United States and one site in Italy. The pre-selected specimens were identified by Standard of Care (SoC) testing and confirmed as positive by VITEK 2 and/or PCR/BDS according to the reference method algorithm prior to enrollment in the study. To minimize bias, pre-selected specimens were tested across four external and one internal site in a randomized, blinded manner along with negative specimens.

Out of the 562 specimens enrolled in the prospective arm of the study, 53 prospective specimens were excluded from the analysis (one (1) duplicate patient enrollment, one (1) due to sample handling errors and 51 with incomplete reference testing results due to lack of clinical isolates, mixed growth, insufficient growth, or no growth). The remaining 509 specimens were included in the analysis.

For targets that exhibited low prevalence rates in the prospective study, the prospective specimen set was supplemented with 163 pre-selected (Arm 2) and 225 contrived specimens (Arm 3). Of these, one pre-selected specimen was excluded (sample handling error), leaving 162 pre-selected and 225 contrived specimens included in the analysis.

Table 18 provides a summary of the general demographic information for the 509 prospectively collected and 162 pre-selected specimens that were included in the study analysis.

Table 18: LIAISON PLEX® BCP Assay Summary of the General Demographic Information

	Prospective (N=509)	Preselected (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%)

The clinical performance of the LIAISON PLEX® BCP Assay was compared to culture followed by automated microbiological/biochemical identification using VITEK 2, PCR followed by BDS, or a combination according to the algorithm described in **Table 19** below.

Table 19: LIAISON PLEX® BCP Assay Summary of Comparator Method

LIAISON PLEX® BCP Assay Target	Comparator Method
Enterococcus faecalis	Culture followed by Automated microbiological/biochemical identification using VITEK 2
Enterococcus faecium	
Listeria spp.	
Staphylococcus spp.	
Staphylococcus aureus	
Staphylococcus epidermidis	
Staphylococcus lugdunensis	
Streptococcus spp.	
Streptococcus agalactiae	
Streptococcus anginosus group	
Streptococcus pneumoniae	
Streptococcus pyogenes	
Bacillus spp.	PCR followed by bi-directional sequencing
mecA/mecC	
vanA	
vanB	

Of the 671 clinical specimens collectively included in the prospective and pre-selected study analysis (Arms 1 and 2 combined), 646 samples (96.3%) generated valid LIAISON PLEX BCP Assay results (i.e., Detected or Not Detected) on the first attempt. There were 25 specimens (3.7%) with an invalid result on the initial run. Of the 25 specimens retested, 24 specimens generated valid BCP results after a single retest for a final testing success rate of 99.9% (670/671).

Prospective and Pre-selected Clinical Evaluation

Clinical Performance (Sensitivity, Specificity, and 95% confidence interval) of the LIAISON PLEX® BCP Assay compared to the reference method is summarized in **Table 20** for combined prospective and pre-selected specimens.

Table 20. LIAISON PLEX® Gram-Positive Blood Culture Assay Performance with Prospective and Pre-selected Specimens - Arm 1 and Arm 2

Pathogen Target		Sensitivity/PPA			Specificity/NPA		
		TP / (TP+FN)	Sensitivity /PPA (%)	95% CI	TN / (TN+FP)	Specificity /NPA (%)	95% CI
Bacteria							
Bacillus spp.	Prospective	1/1	100%	20.7% - 100%	506/507	99.8%	98.9% - 100%
	Pre-selected	17/19	89.5%	68.6% - 97.1%	127/127	100%	97.1% - 100%
	Combined	18/20	90%	69.9% - 97.2%	633/634[1]	99.8%	99.1% - 100%

Enterococcus faecalis	Prospective	38/38	100%	90.8% - 100%	468/468	100%	99.2% - 100%
	Pre-selected	2/2	100%	34.2% - 100%	125/125	100%	97% - 100%
	Combined	40/40	100%	91.2% - 100%	593/593	100%	99.4% - 100%
Enterococcus faecium	Prospective	16/16	100%	80.6% - 100%	489/490	99.8%	98.9% - 100%
	Pre-selected	20/20	100%	83.9% - 100%	106/107	99.1%	94.9% - 99.8%
	Combined	36/36	100%	90.4% - 100%	595/597[2]	99.7%	98.8% - 99.9%
Listeria spp.	Prospective	0/0	NA%	NA	506/506	100%	99.2% - 100%
	Pre-selected	5/5	100%	56.6% - 100%	122/122	100%	96.9% - 100%
	Combined	5/5	100%	56.6% - 100%	628/628	100%	99.4% - 100%
Staphylococcus spp.	Prospective	316/322	98.1%	96% - 99.1%	182/184	98.9%	96.1% - 99.7%
	Pre-selected	20/20	100%	83.9% - 100%	107/107	100%	96.5% - 100%
	Combined	336/342[3]	98.2%	96.2% - 99.2%	289/291[4]	99.3%	97.5% - 99.8%
Staphylococcus aureus	Prospective	160/161	99.4%	96.6% - 99.9%	344/345	99.7%	98.4% - 99.9%
	Pre-selected	0/0	NA %	NA	127/127	100%	97.1% - 100%
	Combined	160/161	99.4%	96.6% - 99.9%	471/472[5]	99.8%	98.8% - 100%
Staphylococcus epidermidis	Prospective	93/96	96.9%	91.2% - 98.9%	405/410	98.8%	97.2% - 99.5%
	Pre-selected	0/0	NA	NA	126/127	99.2%	95.7% - 99.9%
	Combined	93/96[6]	96.9%	91.2% - 98.9%	531/537[7]	98.9%	97.6% - 99.5%
Staphylococcus lugdunensis	Prospective	6/6	100%	61% - 100%	500/500	100%	99.2% - 100%
	Pre-selected	20/20	100%	83.9% - 100%	107/107	100%	96.5% - 100%
	Combined	26/26	100%	87.1% - 100%	607/607	100%	99.4% - 100%
Streptococcus spp.	Prospective	97/98	99%	94.4% - 99.8%	406/408	99.5%	98.2% - 99.9%
	Pre-selected	78/80	97.5%	91.3% - 99.3%	47/47	100%	92.4% - 100%
	Combined	175/178	98.3%	95.2% - 99.4%	453/455[8]	99.6%	98.4% - 99.9%
Streptococcus agalactiae	Prospective	21/21	100%	84.5% - 100%	485/485	100%	99.2% - 100%
	Pre-selected	17/17	100%	81.6% - 100%	110/110	100%	96.6% - 100%
	Combined	38/38	100%	90.8% -	595/595	100%	99.4% - 100%

				100%			
Streptococcus anginosus group	Prospective	8/9	88.9%	56.5% - 98%	496/497	99.8%	98.9% - 100%
	Pre-selected	25/26	96.2%	81.1% - 99.3%	101/101	100%	96.3% - 100%
	Combined	33/35	94.3%	81.4% - 98.4%	597/598[9]	99.8%	99.1% - 100%
Streptococcus pneumoniae	Prospective	11/11	100%	74.1% - 100%	494/495	99.8%	98.9% - 100%
	Pre-selected	21/21	100%	84.5% - 100%	106/106	100%	96.5% - 100%
	Combined	32/32	100%	89.3% - 100%	600/601	99.8%	99.1% - 100%
Streptococcus pyogenes	Prospective	21/21	100%	84.5% - 100%	485/485	100%	99.2% - 100%
	Pre-selected	16/16	100%	80.6% - 100%	111/111	100%	96.7% - 100%
	Combined	37/37	100%	90.6% - 100%	596/596	100%	99.4% - 100%
Resistance Marker Genes[10]							
mecA/mecC	Prospective	155/157	98.7%	95.5% - 99.6%	154/161	95.7%	91.3% - 97.9%
	Pre-selected	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%
vanA	Prospective	8/8	100%	67.6% - 100%	47/47	100%	92.4% - 100%
	Pre-selected	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%
vanB	Prospective	1/1	100%	20.7% - 100%	54/54	100%	93.4% - 100%
	Pre-selected	0/0	NA	NA	23/23	100%	85.7% - 100%

[1]The one *Bacillus* spp. FP 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.

Contrived Specimen Testing

For low-prevalence targets that did not reach the minimum number of positives from the combined prospective and pre-selected populations, a total of 225 specimens were contrived and tested as part of Arm 3. To minimize bias, contrived specimens were blinded, randomized, and tested along with negative clinical specimens at three external testing sites and one internal testing site between June 2024 to September 2024. Results from contrived specimens were analyzed separately from the prospective and pre-selected data sets.

Out of the 225 specimens included in the contrived study analysis, 211 specimens (93.8%) generated valid LIAISON PLEX BCP Assay results (i.e., Detected or Not Detected) on the first attempt. There were 14 specimens with an invalid result on the initial run. Of the 14 specimens retested, all 14 specimens generated a valid result after a single retest for a final testing success rate of 100% (225/225).

A summary of the contrived specimen set is provided in **Table 21**.

Table 21: Summary of Contrived Specimens

Organism	Resistance Marker(s)	Strain	# of Independent 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
***Bacillus* spp. Total**			50
Enterococcus faecalis	*vanB*	ATCC 700802	10
		ATCC 51299	10
		64188262	10
***Enterococcus faecalis* Total**			**30**
Enterococcus faecium	*vanB*	ATCC 51858	10
		JMI CS-712	10
***Enterococcus faecium* Total**			**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
***Listeria* spp. Total**			**49**
Staphylococcus lugdunensis	NA	ATCC 84497462	50
***Staphylococcus lugdunensis* Total**			**50**

Clinical Performance (Sensitivity, Specificity, and 95% confidence interval) of the LIAISON PLEX® BCP Assay compared to the reference method is summarized in **Table 22** for contrived specimens.

Table 22: Sensitivity/PPA and Specificity/NPA of Contrived Data Set – Arm 3

Pathogen Target	Sensitivity/PPA			Specificity/NPA		
Analyte	**TP / (TP+FN)**	**Sensitivity/PPA (%)**	**95% CI**	**TN / (TN+FP)**	**Specificity/NPA (%)**	**95% CI**
Bacteria						
Bacillus spp.	50/50	100%	92.9% - 100%	175/175	100%	97.9% - 100%
Enterococcus faecalis	30/30	100%	88.6% - 100%	195/195	100%	98.1% - 100%
Enterococcus faecium	20/20	100%	83.9% - 100%	205/205	100%	98.2% - 100%
Listeria spp.	49/49	100%	92.7% - 100%	176/176	100%	97.9% - 100%
Staphylococcus spp.	50/50	100%	92.9% - 100%	175/175	100%	97.9% - 100%
Staphylococcus aureus	0/0	NA	NA	225/225	100%	98.3% - 100%
Staphylococcus epidermidis	0/0	NA	NA	225/225	100%	98.3% - 100%
Staphylococcus lugdunensis	50/50	100%	92.9% - 100%	175/175	100%	97.9% - 100%
Streptococcus spp.	0/0	NA	NA	225/225	100%	98.3% - 100%
Streptococcus agalactiae	0/0	NA	NA	225/225	100%	98.3% - 100%
Streptococcus anginosus group	0/0	NA	NA	225/225	100%	98.3% - 100%
Streptococcus pneumoniae	0/0	NA	NA	225/225	100%	98.3% - 100%
Streptococcus pyogenes	0/0	NA	NA	225/225	100%	98.3% - 100%
Resistance Marker Genes[1]						
mecA/mecC	0/0	NA	NA	50/50	100%	92.9% - 100%
vanA	0/0	NA	NA	50/50	100%	92.9% - 100%
vanB	50/50	100%	92.9% - 100%	0/0	NA	NA

[1]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.

LIAISON PLEX BCP Assay Performance for Genus-Level Targets by Species

The LIAISON PLEX BCP Assay reports genus-level results for *Bacillus* spp*., Staphylococcus* spp*. and Streptococcus* spp. Sensitivity/PPA for each of these target organisms stratified at the species level is presented in **Table 23** below. Specimens with reference results of low discrimination between two species of the same genus that were not further resolved are listed as "spp. (unknown)".

Table 23: Sensitivity/PPA of 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%
Bacillus amyloliquefaciens	NA	NA	NA	NA	100% (10/10)	72.2% - 100%
Bacillus atrophaeus	NA	NA	NA	NA	100% (10/10)	72.2% - 100%
Bacillus cereus	NA	NA	NA	NA	100% (10/10)	72.2% - 100%
Bacillus licheniformis	NA	NA	NA	NA	100% (10/10)	72.2% - 100%
Bacillus thuringiensis	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%
Listeria grayi	NA	NA	NA	NA	100% (10/10)	72.2% - 100%
Listeria innocua	NA	NA	NA	NA	100% (10/10)	72.2% - 100%
Listeria ivanovii	NA	NA	NA	NA	100% (10/10)	72.2% - 100%
Listeria monocytogenes	NA	NA	100% (5/5)	56.6% - 100%	100% (8/8)	67.6% - 100%
Listeria 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%
Staphylococcus aureus	99.4% (160/161)	96.6% - 99.9%	NA	NA	NA	NA
Staphylococcus epidermidis	96.9% (93/96)	91.2% - 98.9%	NA	NA	NA	NA
Staphylococcus lugdunensis	100% (6/6)	61% - 100%	100% (20/20)	83.9% - 100%	100% (50/50)	92.9% - 100%
Staphylococcus arlettae	100% (1/1)	20.7% - 100%	NA	NA	NA	NA
Staphylococcus auricularis	66.7% (2/3)	20.8% - 93.9%	NA	NA	NA	NA
Staphylococcus capitis	100% (9/9)	70.1% - 100%	NA	NA	NA	NA
Staphylococcus caprae	100% (1/1)	20.7% - 100%	NA	NA	NA	NA
Staphylococcus haemolyticus	100% (6/6)	61% - 100%	NA	NA	NA	NA
Staphylococcus hominis	96.3% (31/32)	84.3% - 99.4%	NA	NA	NA	NA

Staphylococcus pseudintermedius	100% (1/1)	20.7% - 100%	NA	NA	NA	NA
Staphylococcus saccharolyticus	0% (0/1)	0% - 79.3%	NA	NA	NA	NA
Staphylococcus saprophyticus	100% (1/1)	20.7% - 100%	NA	NA	NA	NA
Staphylococcus simulans	100% (1/1)	20.7% - 100%	NA	NA	NA	NA
Staphylococcus vitulinus	0% (0/1)	0% - 79.3%	NA	NA	NA	NA
Staphylococcus 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
Streptococcus agalactiae	100% (21/21)	84.5% - 100%	100% (17/17)	81.6% - 100%	NA	NA
Streptococcus anginosus	88.9% (8/9)	56.5% - 98%	96.2% (25/26)	81.1% - 99.3%	NA	NA
Streptococcus pneumoniae	100% (11/11)	74.1% - 100%	100% (21/21)	84.5% - 100%	NA	NA
Streptococcus pyogenes	100% (21/21)	84.5% - 100%	100% (16/16)	80.6% - 100%	NA	NA
Streptococcus dysgalactiae	100% (15/15)	79.6% - 100%	NA	NA	NA	NA
Streptococcus gallolyticus	100% (1/1)	20.7% - 100%	NA	NA	NA	NA
Streptococcus gordonii	100% (4/4)	51% - 100%	NA	NA	NA	NA
Streptococcus parasanguinis	100% (1/1)	20.7% - 100%	NA	NA	NA	NA
Streptococcus salivarius	100% (2/2)	34.2% - 100%	NA	NA	NA	NA
Streptococcus 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.

The clinical performance for each resistance marker stratified by eligible organism (identified by the reference method for clinical specimens or the inoculated organism for contrived specimens) is listed in **Tables 24-26**.

Table 24: Performance of *mecA/mecC*, Stratified by Organism

Organism	Prospective (Arm 1)		Pre-selected (Arm 2)		Contrived (Arm 3)	
	Sensitivity/PPA	95% CI	Sensitivity/PPA	95% CI	Sensitivity/ PPA	95% CI
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

Table 25: Performance of *vanA*, Stratified by Organism

Organism	Prospective (Arm 1)		Pre-selected (Arm 2)		Contrived (Arm 3)	
	Sensitivity/ PPA	95% CI	Sensitivity/PPA	95% CI	Sensitivity/ PPA	95% CI
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

Table 26: Performance of *vanB*, Stratified by Organism

Organism	Prospective (Arm 1)		Pre-selected (Arm 2)		Contrived (Arm 3)	
	Sensitivity/PPA	95% CI	Sensitivity/PPA	95% CI	Sensitivity/PPA	95% CI
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%

N. Proposed Labeling:

The labeling provided in the submission satisfies the requirements of 21 CFR 809.10.

O. Conclusion:

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