510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K240627

B Applicant

Luminex Corporation

C Proprietary and Established Names

LIAISON PLEX Yeast Blood Culture Assay

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PEO	Class II	21 CFR 866.3365 - Multiplex Nucleic Acid Assay For Identification Of Microorganisms And Resistance Markers From Positive Blood Cultures	MI - Microbiology
NSU	Class II	21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

The purpose of this submission is to obtain a substantial equivalence determination for the LIAISION PLEX Yeast Blood Assay.

B Measurand:

Nucleic acids from the following organisms: *Candida albicans, Candida auris, Candida dubliniensis, Candida famata, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lipolytica, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Candida haemulonii/duobushaemulonii,* and *Cryptococcus neoformans/gattii*

C Type of Test:

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

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
The LIAISON PLEX Yeast Blood Culture (BCY) Assay is a qualitative nucleic acid multiplex in vitro diagnostic test intended for use on the LIAISON PLEX System for simultaneous detection and identification of multiple potentially pathogenic fungal organisms in positive blood culture. The LIAISON PLEX BCY Assay is performed directly on blood culture samples identified as positive by a continuous monitoring blood culture system and which contain fungal organisms as determined by Gram Stain.

The LIAISON PLEX BCY Assay detects and identifies the following fungal organisms:
Candida albicans
Candida auris
Candida dubliniensis
Candida famata
Candida glabrata
Candida guilliermondii
Candida kefyr
Candida krusei
Candida lipolytica
Candida lusitaniae
Candida parapsilosis
Candida tropicalis
Candida haemulonii / *duobushaemulonii*
Cryptococcus neoformans / *gattii*

The detection and identification of specific fungal nucleic acids from individuals exhibiting signs and/or symptoms of bloodstream infection aids in the diagnosis of bloodstream infection when used in conjunction with other clinical information. The results from LIAISON PLEX BCY Assay are intended to be interpreted in conjunction with Gram stain results and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a suspected bloodstream infection may be due to infection with pathogens that are not detected by this test. Positive results do not rule out co-infection with other organisms; the organism(s) detected by LIAISON PLEX BCY Assay may not be the definite cause of disease. Additional laboratory testing (e.g. sub-culturing of positive blood cultures for identification of organisms not detected by LIAISON PLEX BCY Assay, susceptibility testing and differentiation of mixed growth) and clinical presentation must be taken into consideration in the final diagnosis of bloodstream infection.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

For use with the LIAISON PLEX System

IV Device/System Characteristics:

A Device Description:

The LIAISON PLEX Yeast Blood Assay (BCY Assay) is an automated test for the detection and identification of nucleic acids from yeast in a positive blood culture media sample. The LIAISON PLEX BCY 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 a fungal organism, as determined by Gram stain. The LIAISON PLEX BCY Assay is performed on the LIAISON PLEX System. The LIAISON PLEX System is a fully automated, benchtop device that performs sample preparation, polymerase chain reaction (PCR), and microarray-based hybridization for the detection of target-specific nucleic acids. The LIAISON PLEX BCY Assay components required to perform the test include the following single-use, disposables:

- LIAISON PLEX BCY Assay Test Cartridge
- LIAISON PLEX BCY Assay Transfer Pipettes (or equivalent)

The test reagents are supplied in a single, disposable test cartridge. The LIAISON PLEX BCY Assay has 14 different reportable targets. Reporting of these targets is based on detection of one or more of the nucleic acid targets. Results can be:

- reviewed by the user on the touch screen,
- exported to a USB flash drive,
- printed, or submitted to a Laboratory Information System (LIS) for reporting.

The LIAISON PLEX System automates the BCY Assay sample analysis through the following steps: 1) Sample Preparation, 2) Amplification, and 3) Hybridization., and 4) Signal Analysis. The image analysis of the microarray provides light signal intensities from the target-specific capture spots, as well as the negative control, background, and imaging control spots.

B Principle of Operation:

The assay workflow is as follows:

1. The user adds the sample into the cartridge via a pipette and closes the cartridge sample port.
2. The user creates a new order and scans or manually enters the sample ID.
3. The user scans the assay cartridge barcode with the hand-held barcode reader.

4. The user inserts the assay cartridge into the open bay that the instrument identifies based on the number of runs, and then initiates the run.
5. This is repeated, as needed, for up to six cartridges.
6. Next, the system scans the barcode located on the top of the cartridge and automatically configures to perform the appropriate assay based on the unique barcode on the cartridge.

The following steps take place within the closed cartridge:

1. Sample Preparation - The sample is mixed with reagents and nucleic acid extraction occurs via mechanical and chemical cell lysis and magnetic bead-based nucleic acid isolation. The sample then goes through a series of washes to capture and purify the nucleic acid. The nucleic acid is then transferred into the PCR module.
2. Amplification - The LIAISON PLEX System initiates an amplification process, which heats and cools the PCR tubes for replication of the extracted nucleic acid to generate target specific amplicons.
3. Hybridization - Nucleic acid is transferred by the LIAISON PLEX System to the hybridization unit. Here, amplified DNA hybridizes to specific capture DNA arrayed on a glass slide in a microarray format and the bound target DNA, in turn, hybridizes with mediator and gold-nanoparticle probes. An image of the hybridization unit is taken and the LIAISON PLEX Software performs data analysis.
4. Signal Analysis - Gold nanoparticle probes bound specifically to target-containing spots in the microarray are silver-enhanced, and light scatter from the spots is measured and further analyzed to determine the presence (Detected) or absence (Not Detected) of a target.

The system completes all necessary steps for the assay. No user intervention is necessary once the run is initiated. Once the run is complete, the user removes the cartridge(s) and disposes of them in the appropriate waste container.

Following a series of quality control checks a final call is made for the Target: Detected if the Target signal is above threshold and Not Detected otherwise. Failure of any quality control checks results in a "No Call" result.

V Substantial Equivalence Information:

A Predicate Device Name(s):
ePlex Blood Culture Identification Panel Fungal Pathogen (BCID-FP) Panel

B Predicate 510(k) Number(s):
K182690

C Comparison with Predicate(s):

Device & Predicate Device(s):	K240627	K182690
Device Trade Name	LIAISON PLEX Yeast Blood Culture Assay	ePlex Blood Culture Identification Panel Fungal Pathogen (BCID-FP) Panel
General Device Characteristic		

Similarities		
Intended Use/Indications For Use	The LIAISON PLEX Yeast Blood Culture (BCY) Assay is a qualitative nucleic acid multiplex in vitro diagnostic test intended for use on the LIAISON PLEX System for simultaneous detection and identification of multiple potentially pathogenic fungal organisms in positive blood culture. The LIAISON PLEX BCY Assay is performed directly on blood culture samples identified as positive by a continuous monitoring blood culture system and which contain fungal organisms as determined by Gram Stain. The LIAISON PLEX BCY Assay detects and identifies the following fungal organisms: • *Candida albicans* • *Candida auris* • *Candida dubliniensis* • *Candida famata* • *Candida glabrata* • *Candida guilliermondii* • *Candida kefyr* • *Candida krusei* • *Candida lipolytica* • *Candida lusitaniae* • *Candida parapsilosis* • *Candida tropicalis* • *Candida haemulonii/ duobushaemulonii* • *Cryptococcus neoformans/gattii* The detection and identification of specific fungal nucleic acids from individuals exhibiting signs and/or symptoms of bloodstream infection aids in the diagnostic of bloodstream infection when used in conjunction with other clinical information. The	The GenMark ePlex Blood Culture Identification Fungal Pathogen (BCID-FP) Panel is a qualitative nucleic acid multiplex in vitro diagnostic test intended for use on GenMark's ePlex Instrument for simultaneous detection and identification of multiple potentially pathogenic fungal organisms in positive blood culture. The ePlex BCID-FP Panel is performed directly on blood culture samples identified as positive by a continuous monitoring blood culture system and which contains fungal organisms. The following fungal organisms are identified using the ePlex BCID-FP Panel: • *Candida albicans* • *Candida auris* • *Candida dubliniensis* • *Candida famata* • *Candida glabrata* • *Candida guilliermondii* • *Candida kefyr* • *Candida krusei* • *Candida lusitaniae* • *Candida parapsilosis* • *Candida tropicalis* • *Cryptococcus gattii* • *Cryptococcus neoformans* • *Fusarium* • *Rhodotorula* The detection and identification of specific fungal nucleic acids from individuals exhibiting signs and/or symptoms of bloodstream infection aids in the diagnosis of bloodstream infection when used in conjunction with other clinical information.

	results from the LIAISON PLEX BCY Assay are intended to be interpreted in conjunction with Gram stain results and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a suspected bloodstream infection may be due to infection with pathogens that are not detected by this test. Positive results do not rule out co-infection with other organisms; the organism(s) detected by LIAISON PLEX BCY Assay may not be the definite cause of disease. Additional laboratory testing (e.g., sub-culturing of positive blood cultures for identification of organisms not detected by LIAISON PLEX BCY Assay, susceptibility testing and differentiation of mixed growth) and clinical presentation must be taken into consideration in the final diagnosis of bloodstream infection.	The results from the ePlex BCID-FP Panel are intended to be interpreted in conjunction with Gram stain results and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a suspected bloodstream infection may be due to infection with pathogens that are not detected by this test. Positive results do not rule out co-infection with other organisms; the organism(s) detected by the ePlex BCID-FP Panel may not be the definite cause of disease. Additional laboratory testing (e.g., sub-culturing of positive blood cultures for identification of organisms not detected by ePlex BCID-FP Panel, susceptibility testing and differentiation of mixed growth) and clinical presentation must be taken into consideration in the final diagnosis of bloodstream infection.
Measurand	Same	DNA from detected organisms
Sample Types	Same	Blood Culture samples identified as positive by a continuous monitoring blood culture system and which contain fungal organisms.
Technological Principles	Same	Highly multiplexed nucleic acid PCR test on an array.
Level of Automation	Same	Automated test interpretation and reporting.
Controls	Same	Internal controls contained in the cartridge monitor performance of each step of the testing process.
General Device Characteristic Differences		
Target Analytes	Same except: - the LIAISON PLEX BCY has the additional targets *Candida lipolytica* and *Candida haemulonii/*	*Candida albicans, Candida auris, Candida dubliniensis, Candida famata, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae,*

	duobushaemulonii, - the LIAISON PLEX BCY targets do not include *Fusarium* or *Rhodotorula*	*Candida parapsilosis, Candida tropicalis, Cryptococcus gattii, Cryptococcus neoformans, Fusarium, Rhodotorula*
Instrumentation	LIAISON PLEX	ePlex Instrument
Time to Result	107 minutes	About 1.5 hours

VI Standards/Guidance Documents Referenced:

Standards

- ISTA 3A. Packaged-Products for Parcel Delivery System Shipment 70 kg (150 lbs.) or Less. (2018).
- ITSA 7D. Temperature Test for Transport Packaging.
- CLSI EP07. Interference Testing in Clinical Chemistry; Third Edition.
- CLSI EP25-A. Evaluation of Stability of *In Vitro* Diagnostic Reagents; Approved Guideline.
- CLSI EP17-A2. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition.
- CLSI EP37. Supplemental Tables for Interference Testing in Clinical Chemistry; First Edition.
- CLSI EP12-A2. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition.
- CLSI EP24-A2. Assessment of the Diagnostic Accuracy of Laboratory Testing Using Receiver Operating Characteristic Curves; Approved Guideline – Second Edition.
- ANSI AAMI ISO 14971:2019. Medical devices – Applications of risk management to medical devices.
- ISO 23640. In Vitro Diagnostic Medical Devices – Evaluation of Stability of In Vitro Diagnostic Reagents.
- ISO 15223-1. Medical Devices – Symbols to be Used with Information to be Supplied by the Manufacturer-Part 1: General Requirements. Fourth Edition (2021-07).
- ISO 18113-1:2011. *In vitro* diagnostic medical devices - Information supplied by the manufacturer (labeling). Terms, definition and general requirements.
- ISO 18113-2:2011. *In vitro* diagnostic medical devices - Information supplied by the manufacturer (labeling) – Part 2: In vitro diagnostic reagents for professional use.
- ISO 18113-3:2011. *In vitro* diagnostic medical devices - Information supplied by the manufacturer (labeling) – Part 3: In vitro diagnostic instruments for professional use.
- IEC 61010-1 Edition 3.1, Consolidated Version. Safety Requirements for Electrical Equipment for Measurement Control and Laboratory Use – Part 1: General Requirements, Including Corrigendum 1. (2017-01).
- IEC 61326-2-6 Edition 3.0. Electrical Equipment for Measurement Control and Laboratory Use – EMC Requirements – Part 2-6: Particular Requirements – In Vitro Diagnostic (IVD) Medical Equipment (2010-10).

- IEC 60601-1-2 Edition 4.0. Medial Electrical Equipment – Part 1-2: General Requirements for Basic Safety and Essential Performance – Collateral Standard: Electromagnetic Disturbances-Requirements and Tests (2014-02).
- ISO 3864-1. Graphic Symbols – Safety Colors and Safety Signs – Part 1. Design Principles for Safety Signs and Safety Markings (2011).

Special Controls

- Class II Special Controls Guideline. Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures (May 27, 2015)

Guidance Documents

- 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).

VII Performance Characteristics:

A Analytical Performance:

1. Reproducibility/Precision:

a. Site-to-Site Reproducibility

Reproducibility of the LIAISON PLEX BCY assay was evaluated at three different sites (one internal, two external) using replicates of four blinded panels (Table 1) representing a subset of targets detected by the assay. The four panels consisted of a negative sample, a negative sample contrived with an off-panel organism, a multi-analyte panel of targets at ring positive low positive concentrations, and a multi-analyte panel of targets at ring positive plus 8 hour blood culture moderate positive concentrations. “Ring Positive” represents when a blood culture bottle is indicated as positive when incubating in an automated blood culture system. “Ring Positive + 8 hour” represents when a blood culture bottle is allowed to incubate in an automated blood culture system for at least 8 hours beyond ring positivity (See Growth and Detection Study for more information). All panels were diluted into negative blood matrix. Negative blood matrix is media from a blood culture bottle spiked with whole blood and incubated for at least five days in an automated blood culture system and confirmed to be negative by the absence of growth on an appropriate nutrient agar plate. The study incorporated potential sources of variation introduced by site, day, and operator. Three replicates of each panel were evaluated once per day over five non-consecutive days for a total of 90 replicates per panel member (3 sites x 5 days x 2 Operators x 3 replicates = 90 replicates). A single lot

of LIAISON PLEX BCY assay cartridges was used for all testing. Results from the reproducibility study are shown in Table 2.

Table 1. Reproducibility Panel

Panel Name	Organisms	Expected Targets	Growth Type
Ring Positive	*Candida albicans* *Candida tropicalis* *Cryptococcus neoformans*	*Candida albicans* *Candida tropicalis* *Cryptococcus neoformans*	Ring Positive
Ring Positive +8	*Candida albicans* *Candida tropicalis* *Cryptococcus neoformans*	*Candida albicans* *Candida tropicalis* *Cryptococcus neoformans*	Ring Positive + 8 hours
Negative	Negative Blood Culture	None	Ring Negative
Negative contrived with off-panel organism	*Escherichia coli*	None	Ring Positive + 8 hours

Table 2. Reproducibility Study Summary

Organism	Target Type	Agreement with Expected Results				95% C.I.	
		Site 1	Site 2	Site 3	Overall	Lower	Upper
C. albicans	Ring Positive	100% (30/30)	96.7% (29/30)	100% (30/30)	98.9% (89/90)	94.0%	99.8%
	Ring Positive +8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
	Negative Blood Matrix	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
C. tropicalis	Ring Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
	Ring Positive +8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
	Negative Blood Matrix	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
C. neoformans	Ring Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
	Ring Positive +8 Hours	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
	Negative Blood Matrix	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%
E. coli	Off-panel Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.9%	100%

No false positives were observed in either of the two negative panels for any of the evaluated organisms. The Ring Positive concentration gave expected results 100% of the time for *Candida tropicalis* and *Candida neoformans* and 98.9% of the time for *Candida albicans*. The Ring Positive + 8 hour concentration yielded expected results 100% of the tested organisms. The results of the reproducibility study are acceptable.

b. Lot-to-Lot Reproducibility

Lot-to-lot reproducibility of the LIAISON PLEX BCY assay was evaluated using one operator at a single site testing three unique lots over five non-consecutive days. The same four panels used to evaluate site-to-site reproducibility were used in the lot-to-lot reproducibility assessments. Each panel member was tested in triplicate once per day on five different days generating a total of 45 replicates per panel member (1 Site x 1

Operator x 3 Lots x 5 Days X 1 Run per Day x 3 Replicates per Run). The agreement with expected results from the lot-to-lot reproducibility study is presented in Table 3.

Table 3. Lot-to-Lot Reproducibility Summary

Panel	Reportable Target	Agreement with Expected Results				95% C.I.	
		Lot 1	Lot 2	Lot 3	Overall	Lower	Upper
C. albicans	Ring Positive	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45)	94.0%	100%
	Ring Positive +8 hours	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45)	94.0%	100%
	Negative Blood Matrix	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45)	94.0%	100%
C. tropicalis	Ring Positive	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45)	94.0%	100%
	Ring Positive +8 hours	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45)	94.0%	100%
	Negative Blood Matrix	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45)	94.0%	100%
C. neoformans	Ring Positive	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45)	94.0%	100%
	Ring Positive +8 hours	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45)	94.0%	100%
	Negative Blood Matrix	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45)	94.0%	100%
Contrived Negative (*E. coli*)	N/A	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45)	94.0%	100%

Lot-to-lot Reproducibility testing demonstrated 100% positive agreement with expected results for evaluated LIAISON PLEX BCY assay targets (*Candida albicans*, *Candida tropicalis*, and *Cryptococcus neoformans*/*gattii*) at ring positivity and ring positivity + 8 hours, as well as 100% negative agreement with expected results for the negative sample with no spiked organism (i.e., negative blood matrix) and the contrived negative sample containing an off-panel analyte (i.e., *Escherichia coli*). The results of these studies are acceptable.

c. Within-Laboratory Precision

The within-laboratory precision/repeatability of the LIAISON PLEX BCY assay was assessed using two operators, two instruments, and a single lot of reagents over five non-consecutive days. The sample panel members described in the site-to-site reproducibility section above were used. Data from this study are presented in Table 4.

Table 4. Precision/Repeatability Summary

Reportable Target	Panel	Agreement with Expected results					95% C.I.	
		Operator 1		Operator 2		Overall	Lower	Upper
		Instrument 1	Instrument 2	Instrument 1	Instrument 2			
C. albicans	Ring Positive	100% (7/7)	100% (8/8)	100% (6/6)	100% (9/9)	100% (30/30)	88.7%	100%
	Ring Positive +8 hours	100% (8/8)	100% (7/7)	100% (5/5)	100% (10/10)	100% (30/30)	88.7%	100%
	Negative Blood Matrix	100% (7/7)	100% (8/8)	100% (8/8)	100% (7/7)	100% (30/30)	88.7%	100%

Reportable Target	Panel	Agreement with Expected results					95% C.I.	
		Operator 1		Operator 2		Overall	Lower	Upper
		Instrument 1	Instrument 2	Instrument 1	Instrument 2			
C. tropicalis	Ring Positive	100% (7/7)	100% (8/8)	100% (6/6)	100% (9/9)	100% (30/30)	88.7%	100%
	Ring Positive +8 hours	100% (8/8)	100% (7/7)	100% (5/5)	100% (10/10)	100% (30/30)	88.7%	100%
	Negative Blood Matrix	100% (7/7)	100% (8/8)	100% (8/8)	100% (7/7)	100% (30/30)	88.7%	100%
C. neoformans	Ring Positive	100% (7/7)	100% (8/8)	100% (6/6)	100% (9/9)	100% (30/30)	88.7%	100%
	Ring Positive +8 hours	100% (8/8)	100% (7/7)	100% (5/5)	100% (10/10)	100% (30/30)	88.7%	100%
	Negative Blood Matrix	100% (7/7)	100% (8/8)	100% (8/8)	100% (7/7)	100% (30/30)	88.7%	100%
Contrived Negative (*E. coli*)	N/A	100% (6/6)	100% (9/9)	100% (9/9)	100% (6/6)	100% (30/30)	88.7%	100%

Within-laboratory precision/repeatability testing demonstrated 100% positive agreement with expected results for the LIAISON PLEX BCY assay targets evaluated (*Candida albicans*, *Candida tropicalis*, and *Cryptococcus neoformans*/*gattii*) at ring positivity and ring positivity + 8 hours, as well as 100% negative agreement with expected results for the unspiked negative sample (i.e. negative blood matrix) in addition to the contrived negative sample containing off-panel analytes (i.e. *Escherichia coli*). The results of these studies are acceptable.

2. Linearity:

Not applicable, the assay is qualitative.

3. Analytical Specificity/Interference:

a. Analytical Specificity (Cross-Reactivity)

i. Laboratory Testing

This study evaluated the analytical specificity (cross-reactivity) of the LIAISON PLEX BCY assay in the presence of non-targeted microorganisms that may be found in clinical blood culture samples. A total of 77 off-panel organisms (40 off-panel bacterial species and 37 off-panel yeast species) were evaluated in the study. The potentially cross-reacting organisms were spiked into negative blood matrix for all targets on the assay and tested in triplicate in triplicate in the absence of the target organisms. Bacterial organisms were tested at concentrations ≥ 1.00E+08 CFU/mL (or equivalent) and yeast organisms were tested at ≥ 1.00E+07 CFU/mL (or equivalent), or the highest available concentration. Of the 40 bacterial species tested no cross-reactivity was observed at the concentrations tested (Table 5). One of three replicates was positive for the on-panel organisms

Candida albicans and *Candida dubliniensis* when the bacterium *Klebsiella pneumoniae* (ATCC-BAA 2146) was present. Additional testing with six replicates of *Klebsiella pneumoniae* did not result in any positive results which resulted in 11% positivity (1/9 total replicates) of *K. pneumoniae* at a concentration of 1.0E+08 CFU/mL.

Of the 37 yeast species tested, 35 organisms had no cross-reactivity with on-panel organisms (Table 6). *Trichosporon asteroides* (CBS-KNAW) generated positive results for on-panel organisms *Candida glabrata* (3/3 replicates) and *Candida haemulonii*/*duobushaemulonii* (2/3 replicates) during initial testing. Root cause analysis determined the stock material used to prepare the *T. asteroides* samples were contaminated with *C. glabrata* and *C. haemulonii*/*duobushaemulonii,* and additional testing with 3 replicates from a freshly prepared stock of *T. asteroides* did not produce any positive results. *Yarrowia deformans* (NRRL Y-63659) cross-reacted and had 100% detection for the BCY target *Candida lipolytica* when tested at a concentration of 1.0E+07 CFU/mL. The concentration of *Y. deformans* was lowered to 1.0E+04 CFU/mL and tested, cross-reactivity was not observed at this lower concentration. *Candida pseudohaemulonii* (CBS-KNAW 10004) cross-reacted and had 100% detection for the BCY target *Candida haemulonii/duobushaemulonii* when tested at 1.0E+07 CFU/mL. Six additional replicates of *C. pseudohaemulonii* were tested and demonstrated 100% detection of the on-panel target *C. haemulonii*/*duobushaemulonii.* The concentration of *C. pseudohaemulonii* was lowered to 1.0E+02 CFU/mL and tested, cross-reactivity was not observed at this lower concentration. During the first round of retesting of *C. pseudohaemulonii* 1/6 replicates was positive for the on-panel target *Candida lusitaniae.* Root cause analysis determined the use of *C. lusitaniae* prior to testing in the same area contributed to the positive result. Cross-reactive organisms identified in this assessment are noted in the device labeling.

Table 5. Bacterial Species Evaluated for Cross-Reactivity Results Summary

Organism	Concentration Tested (CFU/mL)	Source & Source ID	Positivity % (Organism)
Acinetobacter baumannii	1.0E+08	IHMA 128307	0% (0/3)
Acinetobacter Iwoffii	1.0E+08	ATCC 15309	0% (0/3)
Aerococcus viridans	1.0E+08	ATCC 700406	0% (0/3)
Bacillus cereus	1.0E+08	ATCC 14579	0% (0/3)
Bacteroides fragilis	1.0E+08	ATCC 25285	0% (0/3)
Bordetella pertussis	1.0E+08	ATCC 9797	0% (0/3)
Cutibacterium (Propionibacterium) acnes	1.0E+08	ATCC 33179	0% (0/3)
Citrobacter freundii	1.0E+08	ATCC 8090	0% (0/3)
Clostridium perfringens	1.0E+08	ATCC 13124	0% (0/3)
Corynebacterium striatum	1.0E+08	ATCC 43735	0% (0/3)
Enterobacter aerogenes	1.0E+08	ATCC 35029	0% (0/3)
Enterococcus avium	1.0E+08	ATCC 14025	0% (0/3)
Enterobacter cloacae	1.0E+08	ATCC 35030	0% (0/3)
Escherichia coli	1.0E+08	NCTC 13846	0% (0/3)
Enterococcus faecalis	1.0E+08	ATCC 29212	0% (0/3)

Organism	Concentration Tested (CFU/mL)	Source & Source ID	Positivity % (Organism)
Enterococcus faecium	1.0E+08	ATCC 700221	0% (0/3)
Eggerthella lenta	1.0E+08	ATCC 25559	0% (0/3)
Klebsiella oxytoca	1.0E+08	ATCC 43165	0% (0/3)
Klebsiella pneumoniae	1.0E+08	ATCC-BAA 2146	11% (*C. albicans*) (1/9)[1] 11% (*C. dubliniensis*) (1/9)[1]
Listeria monocytogenes	1.0E+08	ATCC 19115	0% (0/3)
Lactobacillus rhamnosus	1.0E+08	ATCC 53103	0% (0/3)
Micrococcus luteus	8.9E+06	ATCC 10054	0% (0/3)
Morganella morganii	1.0E+08	ATCC 25830	0% (0/3)
Pseudomonas aeruginosa	1.0E+08	IHMA 576602	0% (0/3)
Prevotella denticola	1.0E+08	BEI Resources HM-1173	0% (0/3)
Proteus mirabilis	1.0E+08	ATCC 12453	0% (0/3)
Streptococcus agalactiae	1.0E+08	ATCC 12401	0% (0/3)
Streptococcus anginosus	1.0E+08	ATCC 33397	0% (0/3)
Staphylococcus aureus	1.0E+08	ATCC 25923	0% (0/3)
Salmonella enterica	1.0E+08	ATCC 9993	0% (0/3)
Staphylococcus epidermidis	1.0E+08	ATCC 700567	0% (0/3)
Staphylococcus hominis	1.0E+08	ATCC 27844	0% (0/3)
Staphylococcus intermedius	1.0E+08	ATCC 29663	0% (0/3)
Staphylococcus lugdunensis	1.0E+08	ATCC 43809	0% (0/3)
Stenotrophomonas maltophilia	8.9E+07	ATCC 17666	0% (0/3)
Serratia marcescens	1.0E+08	NCTC 13920	0% (0/3)
Streptococcus mitis	1.0E+08	ATCC 15914	0% (0/3)
Streptococcus pneumoniae	1.0E+08	ATCC 6315	0% (0/3)
Streptococcus pyogenes	1.0E+08	ATCC 12964	0% (0/3)
Staphylococcus saprophyticus	1.0E+08	ATCC 15305	0% (0/3)

[1]After initial false positive observation (1/3 replicates) additional testing with six replicates of *Klebsiella pneumoniae* did not result in any positive results.

Table 6. Yeast Species Evaluated for Cross-Reactivity Results Summary

Organism	Concentration Tested (CFU/mL)	Source & Source ID	Positivity % (Organism)
Aspergillus fumigatus	1.0E+07	ATCC 1022	0% (0/3)
Acremonium kiliense	1.0E+07	ATCC 20337	0% (0/3)
Candida bracarensis	1.0E+07	JH 47	0% (0/3)
Candida carpophila	1.0E+07	CBS-KNAW 5256	0% (0/3)
Candida inconspicua	1.0E+07	NRRL Y-2029	0% (0/3)
Candida intermedia	1.0E+07	ATCC 14439	0% (0/3)
Candida metapsilosis	1.0E+07	NRRL Y-48470	0% (0/3)
Candida nivariensis	1.0E+07	CBS-KNAW 9983	0% (0/3)
Candida orthopsilosis	1.0E+07	ATCC 20504	0% (0/3)
Candida pseudohaemulonii	1.0E+07	CBS-KNAW 10004	100% (*C. haemulonii*) (9/9)

Organism	Concentration Tested (CFU/mL)	Source & Source ID	Positivity % (Organism)
			11% (*C. lusitaniae*) (1/9)
Candida pseudohaemulonii	1.0E+06	CBS-KNAW 10004	100% (*C. haemulonii*) (3/3)
Candida pseudohaemulonii	1.0E+05	CBS-KNAW 10004	100% (*C. haemulonii*) (3/3)
Candida pseudohaemulonii	1.0E+04	CBS-KNAW 10004	100% (*C. haemulonii*) (3/3)
Candida pseudohaemulonii	1.0E+03	CBS-KNAW 10004	33% (*C. haemulonii*) (1/3)
Candida pseudohaemulonii	1.0E+02	CBS-KNAW 10004	0% (0/3)
Candida rugosa	1.0E+07	NRRL Y-95	0% (0/3)
Candida sake	1.0E+07	NRRL Y-1622	0% (0/3)
Candida solani	1.0E+07	NRRL Y-2224	0% (0/3)
Candida utilis	1.0E+07	ATCC 9950	0% (0/3)
Exophiala lecanii-corni	1.0E+07	ATCC 12734	0% (0/3)
Filobasidium elegans	1.0E+07	NRRL Y-6486	0% (0/3)
Filobasidium globisporum	1.0E+07	NRRL Y-17828	0% (0/3)
Kluyveromyces lactis	1.0E+07	ATCC 24207	0% (0/3)
Kodamaea ohmeri	1.0E+07	NRRL Y-1932	0% (0/3)
Meyerozyma caribbica	1.0E+07	NRRL Y-27274	0% (0/3)
Malassezia furfur	1.0E+07	ATCC 14521	0% (0/3)
Malassezia globosa	1.0E+07	ATCC-MYA 4612	0% (0/3)
Metschnikowia pulcherrima	1.0E+07	ATCC 22032	0% (0/3)
Malassezia restricta	1.0E+07	ATCC-MYA 4611	0% (0/3)
Malassezia sympodialis	1.0E+07	ATCC 96803	0% (0/3)
Mucor velutinosus	1.0E+07	ATCC-MYA 4766	0% (0/3)
Pichia fermentans	1.0E+07	NRRL Y-1619	0% (0/3)
Pichia norvegensis	1.0E+07	NRRL YB-3904	0% (0/3)
Rhodotorula mucilaginosa	1.0E+07	ATCC 66034	0% (0/3)
Saccharomyces cerevisiae	1.0E+07	ATCC 18824	0% (0/3)
Scedosporium prolificans	1.0E+07	ATCC 64913	0% (0/3)
Sporidiobolus salmonicolor	1.0E+07	NRRL Y-5483	0% (0/3)
Sporothrix schenckii	1.0E+07	ATCC 58251	0% (0/3)
Trichosporon asteroides	1.0E+07	CBS-KNAW 6183	50% (*C. glabrata*) (3/6) 33% (*C. haemulonii*) (2/6)
Talaromyces marneffei	1.45E+06 spores/mL	ATCC 18224	0% (0/3)
Wickerhamomyces anomalus	1.0E+07	ATCC 10262	0% (0/3)
Yarrowia deformans	1.0E+07	NRRL Y-63659	100% (*C. lipolytica*) (9/9)
Yarrowia deformans	1.0E+06	NRRL Y-63659	100% (*C. lipolytica*) (3/3)

Organism	Concentration Tested (CFU/mL)	Source & Source ID	Positivity % (Organism)
Yarrowia deformans	1.0E+05	NRRL Y-63659	100% (*C. lipolytica*) (3/3)
Yarrowia deformans	1.0E+04	NRRL Y-63659	0% (0/3)

ii. *In silico* Testing

In silico analysis of assay specificity/exclusivity was performed by conducting a BLAST comparison of assay oligo sequences to the GenBank nt sequence database, as of December 28, 2023. Sequences for the organisms listed in Table 7 were evaluated for potential cross-reactivity. Sequences for all on-panel organisms were included to evaluate intra-panel cross-reactivity. *In silico* exclusivity analysis of potential non-specific amplification and detection indicates the following potential cross-reactivity against organisms listed in Table 7:

- The *Candida lipolytica* oligo designs are predicted to detect *Yarrowia deformans* strains resulting in false positive results.
- The *Candida haemulonii*/*duobushamulonii* oligo designs are predicted to detect *Candida pseudohaemulonii* strains resulting in false positive results.

Table 7. Potential Cross-Reactive Organisms Tested in *In Silico* Exclusivity Analysis

On-Panel Organisms	Off-Panel Organisms	
	Bacteria	**Fungi**
Candida albicans	*Acinetobacter baumanii*	*Acremonium kiliense*
Candida auris	*Acinetobacter Iwoffii*	*Aspergillus fumigatus*
Candida dubliniensis	*Aerococcus viridans*	*Candida bracarensis*
Candida duobushamulonii	*Bacillus cereus*	*Candida carpophila*
Candida famata	*Bacteroides fragilis*	*Candida inconspicua*
Candida glabrata	*Bordetella pertussis*	*Candida intermedia*
Candida guilliermondii	*Citrobacter freundii*	*Candida lambica*
Candida haemulonii	*Clostridium perfringens*	*Candida metapsilosis*
Candida kefyr	*Corynebacterium striatum*	*Candida nivariensis*
Candida krusei	*Cutibacterium (Propionibacterium) acnes*	*Candida norvegensis*
Candida lipolytica	*Eggerthella lenta*	*Candida orthopsilosis*
Candida lusitaniae	*Enterobacter aerogenes*	*Candida pelliculosa*
Candida parapsilosis	*Enterobacter cloacae*	***Candida pseudohaemulonii***
Candida tropicalis	*Enterococcus avium*	*Candida sake*
Cryptococcus gattii	*Enterococcus faecalis*	*Candida utilis*
Cryptococcus neoformans	*Enterococcus faecium*	*Diutina (Candida) rugosa*
	Escherichia coli	*Exophiala jeanselmei*
	Klebsiella oxytoca	*Filobasidium elegans*

On-Panel Organisms	Off-Panel Organisms	
	Bacteria	**Fungi**
	Klebsiella pneumoniae	*Filobasidium globisporum*
	Lactobacillus rhamnosus	*Fusarium solani*
	Listeria monocytogenes	*Kluyveromyces lactis*
	Micrococcus luteus	*Kodamaea ohmeri*
	Morganella morganii	*Malassezia furfur*
	Prevotella denticola	*Malassezia globose*
	Proteus mirabilis	*Malassezia restricta*
	Pseudomonas aeruginosa	*Malassezia sympodialis*
	Salmonella enterica (Typhi)	*Metschnikowia (Candida) pulcherrima*
	Serratia marcescens	*Meyerozyma caribbica (Candida fermentati)*
	Staphylococcus aureus	*Mucor velutinosus*
	Staphylococcus epidermidis	*Penicillium marneffei*
	Staphylococcus hominis	*Rhodotorula mucilaginosa*
	Staphylococcus intermedius	*Saccharomyces cerevisiae*
	Staphylococcus lugdunensis	*Scedosporium prolificans*
	Staphylococcus saprophyticus	*Sporidiobolus salmonicolor*
	Stenotrophomonas maltophilia	*Sporothrix schenckii*
	Streptococcus agalactiae	*Trichosporon spp.*
	Streptococcus anginosus	***Yarrowia deformans***
	Streptococcus mitis	**Protozoa**
	Streptococcus pneumoniae	*Plasmodium falciparum*
	Streptococcus pyogenes	*Trypanosoma cruzi*

Organisms in bold were cross reactive with on-panel targets during laboratory exclusivity testing.

Cross-reactivity of *Y. deformans* and *C. pseudohaemulonii* with LIAISON PLEX BCY assay target oligos identified in the *in silico* analyses was confirmed by wet testing in the Analytical Specificity study and identified cross-reactive organisms are noted in the device labeling.

b. Microbial Interference/Competitive Inhibition

To determine if non-target organisms can interfere with detection of on-panel organisms in the same sample, a microbial interference study was conducted. To simulate co-infection the study evaluated 33 pair-wise combinations of a low concentration (ring positive) on-panel target and potentially interfering off-panel organisms at high

concentration (1.0E+08 CFU/mL). To evaluate competitive inhibition pair-wise combinations of a low-concentration on-panel target and a second on-panel target at high concentration (ring positive + 8 hours) were also evaluated. The three on-panel targets selected for these evaluations are representative of the LIAISON PLEX BCY assay targets and chosen based on global prevalence. Off-panel organisms were also selected for their clinical prevalence and likelihood of being present in blood samples. Testing for all studies was performed in triplicate and results of the microbial interference study and competitive inhibition study are presented in Tables 8 and 9, respectively. Competitive Inhibition was not observed for the LIAISON PLEX BCY assay when low concentration on-panel organisms are tested in the presence of high concentration interfering microbes and other on-panel organisms and the results are acceptable.

Table 8. Microbial Interference Results Summary

Off-Panel High Concentration Interfering Microbe	On-Panel Low Concentration Target	Target Positivity
Escherichia coli (1.0E+08 CFU/mL)	*Candida albicans* (4.4E+05 CFU/mL)	100% (3/3)
	Candida glabrata (3.8E+06 CFU/mL)	100% (3/3)
	Candida parapsilosis (1.5E+07 CFU/mL)	100% (3/3)
Staphylococcus aureus (1.0E+08 CFU/mL)	*Candida albicans* (4.4E+05 CFU/mL)	100% (3/3)
	Candida glabrata (3.8E+06 CFU/mL)	100% (3/3)
	Candida parapsilosis (1.5E+07 CFU/mL)	100% (3/3)
Klebsiella pneumoniae (1.0E+08 CFU/mL)	*Candida albicans* (4.4E+05 CFU/mL)	100% (3/3)
	Candida glabrata (3.8E+06 CFU/mL)	100% (3/3)
	Candida parapsilosis (1.5E+07 CFU/mL)	100% (3/3)
Pseudomonas aeruginosa (1.0E+08 CFU/mL)	*Candida albicans* (4.4E+05 CFU/mL)	100% (3/3)
	Candida glabrata (3.8E+06 CFU/mL)	100% (3/3)
	Candida parapsilosis (1.5E+07 CFU/mL)	100% (3/3)
Enterococcus faecalis (1.0E+08 CFU/mL)	*Candida albicans* (4.4E+05 CFU/mL)	100% (3/3)
	Candida glabrata (3.8E+06 CFU/mL)	100% (3/3)
	Candida parapsilosis (1.5E+07 CFU/mL)	100% (3/3)
Staphylococcus epidermidis (1.0E+08 CFU/mL)	*Candida albicans* (4.4E+05 CFU/mL)	100% (3/3)
	Candida glabrata (3.8E+06 CFU/mL)	100% (3/3)
	Candida parapsilosis (1.5E+07 CFU/mL)	100% (3/3)
Enterococcus faecium (1.0E+08 CFU/mL)	*Candida albicans* (4.4E+05 CFU/mL)	100% (3/3)

Off-Panel High Concentration Interfering Microbe	On-Panel Low Concentration Target	Target Positivity
	Candida glabrata (3.8E+06 CFU/mL)	100% (3/3)
	Candida parapsilosis (1.5E+07 CFU/mL)	100% (3/3)
Acinetobacter baumannii (1.0E+08 CFU/mL)	*Candida albicans* (4.4E+05 CFU/mL)	100% (3/3)
	Candida glabrata (3.8E+06 CFU/mL)	100% (3/3)
	Candida parapsilosis (1.5E+07 CFU/mL)	100% (3/3)
Streptococcus pneumoniae (1.0E+08 CFU/mL)	*Candida albicans* (4.4E+05 CFU/mL)	100% (3/3)
	Candida glabrata (3.8E+06 CFU/mL)	100% (3/3)
	Candida parapsilosis (1.5E+07 CFU/mL)	100% (3/3)

Table 9. Competitive Inhibition Results Summary

On Panel High Concentration Target	High Concentration Target Positivity	On Panel Low Concentration Target	Low Concentration Target Positivity
Candida parapsilosis (5.5E+07 CFU/mL)	100% (3/3)	*Candida albicans* (4.4E+05 CFU/mL)	100% (3/3)
Candida parapsilosis (5.5E+07 CFU/mL)	100% (3/3)	*Candida glabrata* (3.8E+06 CFU/mL)	100% (3/3)
Candida glabrata (1.6E+08 CFU/mL)	100% (3/3)	*Candida parapsilosis* (1.5E+07 CFU/mL)	100% (3/3)
Candida glabrata (1.6E+08 CFU/mL)	100% (3/3)	*Candida albicans* (4.4E+05 CFU/mL)	100% (3/3)
Candida albicans (1.5E+07 CFU/mL)	100% (3/3)	*Candida glabrata* (3.8E+06 CFU/mL)	100% (3/3)
Candida albicans (1.5E+07 CFU/mL)	100% (3/3)	*Candida parapsilosis* (1.5E+07 CFU/mL)	100% (3/3)

c. Interfering Substances

An analytical study was performed to assess the potential inhibitory effects of 18 exogenous and endogenous substances that may be commonly found in blood samples (Table 10). Representative target organisms (*Candida albicans, Cryptococcus neoformans*, *Candida tropicalis*, *Candida glabrata, Candida guilliermondii, and Candida kefyr*) were tested at ring positive concentrations in negative blood matrix. A panel containing negative blood matrix was also tested to assess the risk of false positive results in the presence of potentially interfering substances. All organisms were tested using five replicates. No interference was observed except for the *C. albicans*/Fluconazole 25 mg/L combination. During initial testing of Fluconazole one replicate (1/5) did not detect *Candida albicans*. Dilution of Fluconazole from 25 mg/L to 8.3 mg/L resulted in 100% detection of *C. albicans*. Potential LIAISON PLEX BCY assay interference by Fluconazole at 25 mg/L is noted in the device labeling. These results from the interfering substances study are acceptable.

Table 10. List of Potentially Interfering Substances

Interfering Substance	Testing Concentration
Unconjugated Bilirubin	20 mg/dL
Conjugated Bilirubin	20 mg/dL
Hemoglobin	14 g/L
γ-globulin	6 g/dL
Sodium Polyanetholsulfonate	0.25% w/v
Amoxicillin clavulanate	3.5 µg/mL
Amphotericin B	2 µg/mL
Caspofungin	5 µg/mL
Ceftriaxone	0.23 mg/mL
Ciprofloxacin	3 mg/L
Fluconazole	25 mg/L[1]
	8.3 mg/L[1]
Flucytosine	90 µg/mL
Gentamicin sulfate	3 µg/mL
Heparin	0.9 U/mL

Interfering Substance	Testing Concentration
Imipenem	83 µg/mL
Tetracycline	5 mg/L
Vancomycin	30 mg/L
Intralipid/Triglycerides	3000 mg/dL
Controls	N/A
0.9% NaCl Solvent Blank	N/A

[1]During initial testing Fluconazole had (4/5) positivity for *C. albicans* at a concentration of 25 mg/L, retesting at 8.3 mg/L resulted in 100% detection.

4. Assay Reportable Range:

   Not applicable, this is a qualitative assay.

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

   a. Assay Controls

      i. Internal Controls:
      Each BCY Assay cartridge includes internal controls that monitor performance of each step of the testing process, including sample preparation, amplification, and detection of targets. The internal control is added to the sample automatically prior to initiation of sample preparation. A post-amplification hybridization control serves as a proxy for hybridization success. Internal control results are reported as Pass or Fail on printed result reports (Table 11).

Table 11. Internal Control Results

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 controls failed.	Repeat test with a new cartridge

ii. Recommended External Control:

External controls are not provided with the BCY Assay but are recommended in the package insert. Positive and negative external controls should be tested with each new lot of reagents or monthly, whichever occurs first. Blood culture medium can be used as the negative control. Previously characterized positive samples or blood culture medium spiked with well characterized organisms can be used as the external positive control. External controls should be run in accordance with laboratory protocols and accrediting organizations, as applicable.

b. Sample Stability

A sample stability study was performed to establish the recommended storage conditions for positive blood cultures prior to testing with the LIAISON PLEX BCY assay. Two representative panels, each containing three LIAISON PLEX BCY assay target organisms (Panel A - *Candida albicans, Cryptococcus neoformans*, and *Candida tropicalis* and Panel B - *Candida glabrata, Candida guilliermondii, and Candida kefyr*) were prepared at concentrations approximating ring positivity and were stored at -80 ±15°C, 2-8°C, and 33-37°C temperature over various time points (Table 12). Additionally, sample stability was compared at ring positivity and 12 hours after ring positivity. Panel organisms were selected to represent both genera of yeast detected by the LIAISON PLEX BCY assay and a diverse fungal target panel. Twenty replicates were tested at the beginning of the study using freshly made panels (time point T0). Ten replicates were tested for each subsequent temperature and time point. The results of the stability study are presented in Tables 13-16 below.

Table 12. Sample Stability Storage Conditions and Time Points

Storage Condition	**Time Points**							
Incubator (33-37°C)	T0	25 hours	49 hours	73 hours	--	--	--	--
Refrigerated (2-8°C)	T0	25 hours	49 hours	73 hours	5 days	7 days	14 days	30 days
Frozen (-80 ±15°C)	T0	7 days	14 days	30 days	--	--	--	--
Bottle Incubator	Ring Positive	12 hours post-Ring Positive						

Table 13. 33-37°C Incubator Stability Storage Results Summary

Time Point	**Target (% Positive)**					
	C. albicans	***C. tropicalis***	***C. neoformans***	***C. glabrata***	***C. guilliermondii***	***C. kefyr***
T0 n= 20	100%	100%	100%	100%	100%	100%
25 hours n=10	100%	100%	100%	100%	100%	100%
49 hours n=10	100%	100%	100%	100%	100%	100%
73 hours n=10	100%	100%	100%	100%	100%	100%

Table 14. 2-8°C Refrigerated Stability Storage Results Summary

Time Point	Target (% Positive)					
	C. albicans	***C. tropicalis***	***C. neoformans***	***C. glabrata***	***C. guilliermondii***	***C. kefyr***
T0 n= 20	100%	100%	100%	100%	100%	100%
25 hours n=10	100%	100%	100%	100%	100%	100%
49 hours n=10	100%	100%	100%	100%	100%	100%
73 hours n=10	100%	100%	100%	100%	100%	100%
5 days n=10	100%	100%	100%	100%	100%	100%
7 days n=10	100%	100%	100%	100%	100%	100%
14 days n=10	100%	100%	100%	100%	100%	100%
30 days n=10	100%	100%	100%	100%	100%	100%

Table 15. -80 ±15°C Incubator Stability Storage Results Summary

Time Point	Target (% Positive)					
	C. albicans	***C. tropicalis***	***C. neoformans***	***C. glabrata***	***C. guilliermondii***	***C. kefyr***
T0 n= 20	100%	100%	100%	100%	100%	100%
7 days n=10	100%	100%	100%	100%	100%	100%
14 days n=10	100%	100%	100%	100%	100%	100%
30 days n=10	100%	100%	100%	100%	100%	100%

Table 16. Ring Positive and 12 Hours Post-Ring Positive Stability Storage Results Summary

Time Point	Target (% Positive)	
	C. neoformans	***C. guilliermondii***
Ring Positive n= 20	100%	100%
12 hrs Post Ring Positive n=10	100%	100%

The results of the sample stability study support the positive blood culture stability claims for the LIAISON PLEX BCY assay at the following conditions:

- Up to 48 hours at 33-37°C
- Up to 14 days at 2-8°C or ≤-70°C
- Samples can also be tested when incubated up to 12 hours after ring positivity in a continuously monitoring blood culture device.

c. Freeze-Thaw Study

The performance of the LIAISON PLEX BCY assay with fresh and frozen samples was evaluated by testing ten organisms at the following concentrations and replicates in negative blood matrix: three replicates at ring positive (RP), three replicates at ring

positive + 8 hours, and six replicates at ring positive -10-fold dilution (representative of a near-LoD sample concentration). Representative organisms were selected to encompass all three PCR pools of the LIAISON PLEX BCY assay cartridge and represented the majority of the reportable targets detectable by the assay. Each target organism was evaluated at three conditions, fresh, first freeze thaw, and second freeze thaw. The positive agreement with the fresh condition at each freeze-thaw cycle demonstrated 100% positive agreement at each condition (Table 17).

Table 17. Freeze-Thaw Contrived Sample Stability Results Summary

Organism	Testing Condition	Concentration (CFU/mL)	Target Positivity
Candida auris	RP Fresh[1]	1.45E+07	100% (3/3)
	RP Frozen 1st thawed		100% (3/3)
	RP Frozen 2nd thawed		100% (3/3)
	RP + 8 Fresh[1]	5.30E+07	100% (3/3)
	RP + 8 Frozen 1st thawed		100% (3/3)
	RP + 8 Frozen 2nd thawed		100% (3/3)
	RP -10 fold Fresh	2.90E+06	100% (6/6)
	RP -10 fold Frozen 1st thawed		100% (6/6)
	RP -10 fold Frozen 2nd thawed		100% (6/6)
Candida dubliniensis	RP Fresh[1]	3.20E+07	100% (3/3)
	RP Frozen 1st thawed		100% (3/3)
	RP Frozen 2nd thawed		100% (3/3)
	RP + 8 Fresh[1]	4.20E+07	100% (3/3)
	RP + 8 Frozen 1st thawed		100% (3/3)
	RP + 8 Frozen 2nd thawed		100% (3/3)
	RP -10 fold Fresh	1.16E+06	100% (6/6)
	RP -10 fold Frozen 1st thawed		100% (6/6)
	RP -10 fold Frozen 2nd thawed		100% (6/6)
Candida guilliermondii	RP Fresh[1]	3.60E+07	100% (3/3)
	RP Frozen 1st thawed		100% (3/3)
	RP Frozen 2nd thawed		100% (3/3)
	RP + 8 Fresh[1]	3.40E+07	100% (3/3)
	RP + 8 Frozen 1st thawed		100% (3/3)
	RP + 8 Frozen 2nd thawed		100% (3/3)
	RP -10 fold Fresh	3.10E+06	100% (6/6)
	RP -10 fold Frozen 1st thawed		100% (6/6)
	RP -10 fold Frozen 2nd thawed		100% (6/6)
Candida haemulonii	RP Fresh[1]	9.70E+06	100% (3/3)
	RP Frozen 1st thawed		100% (3/3)
	RP Frozen 2nd thawed		100% (3/3)
	RP + 8 Fresh[1]	4.30E+07	100% (3/3)
	RP + 8 Frozen 1st thawed		100% (3/3)

Organism	Testing Condition	Concentration (CFU/mL)	Target Positivity
	RP + 8 Frozen 2nd thawed		100% (3/3)
	RP -10 fold Fresh	1.29E+06	100% (6/6)
	RP -10 fold Frozen 1st thawed		100% (6/6)
	RP -10 fold Frozen 2nd thawed		100% (6/6)
Candida kefyr	RP Fresh[1]	1.20E+07	100% (3/3)
	RP Frozen 1st thawed		100% (3/3)
	RP Frozen 2nd thawed		100% (3/3)
	RP + 8 Fresh[1]	2.34E+07	100% (3/3)
	RP + 8 Frozen 1st thawed		100% (3/3)
	RP + 8 Frozen 2nd thawed		100% (3/3)
	RP -10 fold Fresh	4.20E+05	100% (6/6)
	RP -10 fold Frozen 1st thawed		100% (6/6)
	RP -10 fold Frozen 2nd thawed		100% (6/6)
Candida krusei	RP Fresh[1]	1.24E+07	100% (3/3)
	RP Frozen 1st thawed		100% (3/3)
	RP Frozen 2nd thawed		100% (3/3)
	RP + 8 Fresh[1]	4.00E+07	100% (3/3)
	RP + 8 Frozen 1st thawed		100% (3/3)
	RP + 8 Frozen 2nd thawed		100% (3/3)
	RP -10 fold Fresh	7.20E+05	100% (6/6)
	RP -10 fold Frozen 1st thawed		100% (6/6)
	RP -10 fold Frozen 2nd thawed		100% (6/6)
Candida lusitaniae	RP Fresh[1]	2.23E+07	100% (3/3)
	RP Frozen 1st thawed		100% (3/3)
	RP Frozen 2nd thawed		100% (3/3)
	RP + 8 Fresh[1]	1.32E+08	100% (3/3)
	RP + 8 Frozen 1st thawed		100% (3/3)
	RP + 8 Frozen 2nd thawed		100% (3/3)
	RP -10 fold Fresh	4.00E+06	100% (6/6)
	RP -10 fold Frozen 1st thawed		100% (6/6)
	RP -10 fold Frozen 2nd thawed		100% (6/6)
Candida parapsilosis	RP Fresh[1]	1.54E+07	100% (3/3)
	RP Frozen 1st thawed		100% (3/3)
	RP Frozen 2nd thawed		100% (3/3)
	RP + 8 Fresh[1]	3.90E+07	100% (3/3)
	RP + 8 Frozen 1st thawed		100% (3/3)
	RP + 8 Frozen 2nd thawed		100% (3/3)
	RP -10 fold Fresh	6.10E+05	100% (6/6)
	RP -10 fold Frozen 1st thawed		100% (6/6)
	RP -10 fold Frozen 2nd thawed		100% (6/6)

Organism	Testing Condition	Concentration (CFU/mL)	Target Positivity
Candida tropicalis	RP Fresh[1]	1.20E+07	100% (3/3)
	RP Frozen 1st thawed		100% (3/3)
	RP Frozen 2nd thawed		100% (3/3)
	RP + 8 Fresh[1]	3.30E+07	100% (3/3)
	RP + 8 Frozen 1st thawed		100% (3/3)
	RP + 8 Frozen 2nd thawed		100% (3/3)
	RP -10 fold Fresh	7.00E+05	100% (6/6)
	RP -10 fold Frozen 1st thawed		100% (6/6)
	RP -10 fold Frozen 2nd thawed		100% (6/6)
Cryptococcus neoformans	RP Fresh[1]	3.40E+06	100% (3/3)
	RP Frozen 1st thawed		100% (3/3)
	RP Frozen 2nd thawed		100% (3/3)
	RP + 8 Fresh[1]	5.00E+06	100% (3/3)
	RP + 8 Frozen 1st thawed		100% (3/3)
	RP + 8 Frozen 2nd thawed		100% (3/3)
	RP -10 fold Fresh	2.70E+05	100% (6/6)
	RP -10 fold Frozen 1st thawed		100% (6/6)
	RP -10 fold Frozen 2nd thawed		100% (6/6)

Results from the freeze-thaw study are acceptable and demonstrate that the LIAISON PLEX BCY assay can detect organisms in samples that have been frozen and thawed up to two times.

d. Carryover

An analytical study was performed to assess potential carryover or cross-contamination in the LIAISON PLEX BCY assay by testing high positive and negative samples in an alternating pattern on the LIAISON PLEX instrument. The high positive sample consisted of *Candida lusitaniae* grown to ring positive + eight hours at a concentration of 1.3E+08 CFU/mL; the negative control samples were negative blood matrix. The study was performed by two operators, each using one of two LIAISON PLEX instruments, each containing six modules. Alternating high positive and negative samples were evaluated per the format summarized in Table 18. A total of 30 high positive and 30 negative samples were evaluated. All high positive samples produced positive results, while all negative samples were negative. These results support there is no evidence of carryover from samples tested with the LIAISON PLEX BCY assay.

Table 18. Carryover Study Design

Operator	LIAISON PLEX Instrument	Test Round	Module					
			1	2	3	4	5	6
1	1	1	POS	NEG	POS	NEG	POS	NEG
		2	NEG	POS	NEG	POS	NEG	POS

		3	POS	NEG	POS	NEG	POS	NEG
		4	NEG	POS	NEG	POS	NEG	POS
		5	POS	NEG	POS	NEG	POS	NEG
2	2	1	NEG	POS	NEG	POS	NEG	POS
		2	POS	NEG	POS	NEG	POS	NEG
		3	NEG	POS	NEG	POS	NEG	POS
		4	POS	NEG	POS	NEG	POS	NEG
		5	NEG	POS	NEG	POS	NEG	POS

6. Detection Limit:

   a. Limit of Detection

   A limit of detection (LoD) study was performed to assess the analytical sensitivity of the LIAISON PLEX BCY assay. Two strains for each of the 14 target organisms were evaluated in negative blood matrix. The preliminary LoD for each strain was determined using a 6-point 3-fold dilution series and four replicates of each dilution. The preliminary LoD for each target was defined as the lowest concentration at which 100% of replicates were positive for the intended target. The LoD was confirmed using 20 replicates of a 3-point 3-fold dilution series around the preliminary LoD for each strain. The confirmed LoD was defined as the lowest concentration at which ≥95% (19/20) of replicates tested positive for the intended reportable target. If ≥95% (19/20) of replicates at a particular concentration tested positive, a sample dilution with a three-fold lower concentration was tested until the detection rate was <95%. The confirmed LoDs for the LIAISON PLEX BCY assay targets are presented in Table 19.

Table 19. Limit of Detection Results Summary

Reported Target	Organism	Source & ID	Concentration (CFU/mL)
Candida albicans	*Candida albicans*	ATCC 10231	3.14E+04
		ATCC 14053	2.83E+05
Candida auris	*Candida auris*	CBS-KNAW 10913	3.50E+03
		CBS-KNAW 12766	3.50E+03
Candida dubliniensis	*Candida dubliniensis*	ATCC-MYA 578	3.16E+03
		ATCC-MYA 577	3.16E+03
Candida famata	*Candida famata*	ATCC 20278	7.77E+02
		ATCC 60229	6.99E+03
Candida glabrata	*Candida glabrata*	ATCC 15545	9.99E+03
		ATCC 15126	1.00E+04
Candida guilliermondii	*Candida guilliermondii*	ATCC 22017	3.33E+03
		ATCC 34134	1.00E+04
Candida haemulonii / duobushaemulonii	*Candida haemulonii*	CBS-KNAW 7375	3.51E+03
	Candida duobushaemulonii	CBS-KNAW 7798	3.51E+03
Candida kefyr	*Candida kefyr*	ATCC 8553	3.33E+03
		ATCC 4135	3.33E+03
Candida krusei	*Candida krusei*	ATCC 6258	9.83E+03
		ATCC 28870	2.95E+04
Candida lipolytica	*Candida lipolytica*	ATCC 20460	3.18E+04
		ATCC 20177	3.19E+04
Candida lusitaniae	*Candida lusitaniae*	ATCC 42720	1.11E+04
		ATCC 34449	3.33E+04
Candida parapsilosis	*Candida parapsilosis*	ATCC 28474	2.85E+04
		ATCC 28475	9.50E+03
Candida tropicalis	*Candida tropicalis*	ATCC 13803	3.51E+03
		ATCC 201380	3.51E+03
Cryptococcus neoformans / gattii	*Cryptococcus neoformans*	ATCC 208821	9.01E+04
	Cryptococcus gattii	ATCC-MYA 4871	9.00E+04

b. Analytical Reactivity (Inclusivity)

The inclusivity of the LIAISON PLEX BCY assay was evaluated using contrived samples containing fungal isolates that were selected to represent phylogenetic, geographic, and temporal diversity of the organisms detected by the panel. The inclusivity panel was comprised of 80 strains representing all 14 reportable targets. Assay performance at bacterial concentrations representative of bottle positivity (ring positive) was assessed with five strains. Bottle positivity concentrations for each strain were determined in the Growth & Detection study (see section 6.d.). The inclusivity panel strains and the concentration for which they were detected are presented in Table 20.

Table 20. Inclusivity Results Summary

Assay Target	Organism Genus and Species	Source ID	Concentration (CFU/mL)	Target Positivity
Candida albicans	*Candida albicans*	ATCC 90028	4.4E+05	100% (3/3)
		ATCC 24433	4.4E+05	100% (3/3)
		ATCC 90029	4.4E+05	100% (3/3)
		ATCC MYA-4780	4.4E+05	100% (3/3)
		Zeptometrix 801504	4.4E+05	100% (3/3)
Candida auris	*Candida auris*	CBS-KNAW 12373	1.5E+07	100% (3/3)
		CBS-KNAW 12767	1.5E+07	100% (3/3)
		CBS-KNAW 12768	1.5E+07	100% (3/3)
		CDC AR-0383	1.5E+07	100% (3/3)
		Zeptometrix 804386	1.5E+07	100% (3/3)
Candida dubliniensis	*Candida dubliniensis*	ATCC MYA-582	1.5E+07	100% (3/3)
		ATCC MYA-579	1.5E+07	100% (3/3)
		ATCC 44508	1.5E+07	100% (3/3)
		ATCC MYA-2975	1.5E+07	100% (3/3)
		Zeptometrix 801915	1.5E+07	100% (3/3)
Candida famata	*Debaryomyces hansenii*[1]	ATCC 9365	3.2E+06	100% (3/3)
		ATCC 10619	3.2E+06	100% (3/3)
		ATCC 36239	3.2E+06	100% (3/3)
		CBS-KNAW 1961	3.2E+06	100% (3/3)
		CBS-KNAW 14266	3.2E+06	100% (3/3)
Candida glabrata	*Candida glabrata*	ATCC MYA-2950	3.8E+06	100% (3/3)
		ATCC 34138	3.8E+06	100% (3/3)
		ATCC 66032	3.8E+06	100% (3/3)
		ATCC 90876	3.8E+06	100% (3/3)
		Zeptometrix 801535	3.8E+06	100% (3/3)
Candida guilliermondii	*Meyerozyma guilliermondii*	ATCC 56822	1.9E+07	100% (3/3)
		ATCC 14242	1.9E+07	100% (3/3)
		ATCC 6260	1.9E+07	100% (3/3)
		ATCC 90197	1.9E+07	100% (3/3)
	Candida guilliermondii	Zeptometrix 801602	1.9E+07	100% (3/3)
Candida haemulonii/duobushaemulonii	*Candida haemulonii*	CDC AR-0395	9.4E+06	100% (3/3)
	Candida haemulonis	CBS-KNAW 10970	9.4E+06	100% (3/3)
		CBS-KNAW 10973	9.4E+06	100% (3/3)
	Candida haemulonii var. vulnera	CBS-KNAW 12437	9.4E+06	100% (3/3)
		CBS-KNAW 12438	9.4E+06	100% (3/3)
	Candida duobushaemulonii	CBS-KNAW 6915	9.4E+06	100% (3/3)
		CBS-KNAW 7098	9.4E+06	100% (3/3)
		CDC AR-0392	9.4E+06	100% (3/3)
		CDC AR-0394	9.4E+06	100% (3/3)
		CDC AR-0391	9.4E+06	100% (3/3)
Candida kefyr	*Candida kefyr*	ATCC 2512	6.3E+06	100% (3/3)
		ATCC 66028	6.3E+06	100% (3/3)

Assay Target	Organism Genus and Species	Source ID	Concentration (CFU/mL)	Target Positivity
		ATCC 204093	6.3E+06	100% (3/3)
		ATCC 4135	6.3E+06	100% (3/3)
		Zeptometrix 801837	6.3E+06	100% (3/3)
Candida krusei	*Candida krusei*	ATCC 32196	1.2E+07	100% (3/3)
		ATCC 14243	1.2E+07	100% (3/3)
		ATCC 22985	1.2E+07	100% (3/3)
		ATCC 34135	1.2E+07	100% (3/3)
		Zeptometrix 801536	1.2E+07	100% (3/3)
Candida lipolytica	*Candida lipolytica*	ATCC 8662	2.4E+06	100% (3/3)
	Yarrowia lipolytica	ATCC 201242	2.4E+06	100% (3/3)
		ATCC 90812	2.4E+06	100% (3/3)
		ATCC MYA-2613	2.4E+06	100% (3/3)
		ATCC 32338	2.4E+06	100% (3/3)
Candida lusitaniae	*Candida lusitaniae*	ATCC 200953	2.2E+07	100% (3/3)
		CBS-KNAW 4870	2.2E+07	100% (3/3)
		ATCC 66035	2.2E+07	100% (3/3)
	Clavispora lusitaniae	CBS-KNAW 6936	2.2E+07	100% (3/3)
		Zeptometrix 801603	1.9E+07[2]	100% (3/3)
Candida parapsilosis	*Candida parapsilosis*	ATCC 34136	1.5E+07	100% (3/3)
		ATCC 96137	1.5E+07	100% (3/3)
		ATCC 200954	1.5E+07	100% (3/3)
		ATCC 38291	1.5E+07	100% (3/3)
		Zeptometrix 801537	1.5E+07	100% (3/3)
Candida tropicalis	*Candida tropicalis*	ATCC MYA-2734	1.2E+07	100% (3/3)
		ATCC 1369	1.2E+07	100% (3/3)
		ATCC 90874	1.2E+07	100% (3/3)
		ATCC 18807	1.2E+07	100% (3/3)
		Zeptometrix 801538	1.2E+07	100% (3/3)
Cryptococcus neoformans/gattii	*Cryptococcus neoformans var. grubii*	ATCC 14116	1.8E+06	100% (3/3)
	Cryptococcus neoformans	ATCC 90112	1.8E+06	100% (3/3)
		ATCC MYA-4564	1.8E+06	100% (3/3)
		ATCC MYA-4566	1.8E+06	100% (3/3)
		Zeptometrix 801803	1.8E+06	100% (3/3)
	Cryptococcus gattii	ATCC MYA-4563	1.8E+06	100% (3/3)
		ATCC MYA-4873	1.8E+06	100% (3/3)
		ATCC MYA-4560	1.8E+06	100% (3/3)
		ATCC MYA-4094	1.8E+06	100% (3/3)
		Zeptometrix 801917	1.8E+06	100% (3/3)

[1]*Debaryomyces hansenii* is a synonymous taxon name for *Candida famata*.

[2] The stock concentration of *Clavispora lusitaniae* (Zeptometrix 801603) was not concentrated enough to achieve the representative bottle positivity concentration. This preparation was prepared as stock material diluted 1:1 in negative blood matrix which is allowed per protocol directive.

The results from this study demonstrate the LIAISON PLEX BCY assay is capable of detecting multiple clinically relevant strains of each target organism.

c. *In silico* Analytical Reactivity (Inclusivity)

The inclusivity of the LIAISON PLEX BCY assay was further assessed using *in silico* analysis of the oligonucleotides (i.e., forward primer(s), reverse primer(s), capture and mediator probe(s)) for all assay targets in relation to sequences available in the NCBI GenBank nt database. Sequence alignments were generated using a publicly available sequence alignment program. The *in silico* analysis determined the percent homology of each oligo sequence to its binding region on each target sequence retrieved from public databases. A match (i.e., predicted reactivity) was based on the following criteria, >90% homology between the oligo and reference sequences. To determine oligo homology, the highest percent homology of each oligo in the same component was assessed and then the lowest percent identity of the four components (forward primer, reverse primer, mediator, and capture) was used to characterize the inclusivity of the sequence. A summary of *in-silico* analysis results is provided below in Table 21.

Table 21. *In silico* Inclusivity Results Summary

d.

Reportable Target	Inclusive Organism/Target	Total # Sequences in Alignment	# Sequences with Percent Oligo Identity ≥ 90%	Predicted Inclusivity Percentage (%)
Candida albicans	*Candida albicans*	181 *	181	100
Candida auris	*Candida auris*	286	286	100
Candida dubliniensis	*Candida dubliniensis*	13 *	13	100
Candida famata	*Candida famata*	32 *	32	100
Candida glabrata	*Candida glabrata*	206	206	100
Candida guilliermondii	*Candida guilliermondii*	24 *	24	100
Candida haemulonii/ duobushamulonii	*Candida haemulonii*	17 *	17	100
	Candida duobushamulonii	9 *	9	100
Candida kefyr	*Candida kefyr*	30 *	30	100
Candida krusei	*Candida krusei*	48 *	48	100
Candida lipolytica	*Candida lipolytica*	82 *	82	100
Candida lusitaniae	*Candida lusitaniae*	75 *	75	100
Candida parapsilosis	*Candida parapsilosis*	59 *	59	100
Candida tropicalis	*Candida tropicalis*	86 *	85	99
Cryptococcus neoformans/gattii	*Cryptococcus neoformans*	3243	3243	100
	Cryptococcus gattii	1236	1236	100

* Includes WGS (whole genome shotgun) sequences.

All 80 strains evaluated by laboratory testing were detected with 100% positivity. *In-silico* analyses performed demonstrate the LIAISON PLEX BCY assay exhibits ≥99% inclusivity to sequences available in the GenBank nt database as of December 30, 2023. The results of the analytical reactivity/inclusivity studies are acceptable.

d. Growth and Detection Study

A growth and detection study was conducted to establish the range of expected organism concentrations present in incubated blood cultures at bottle positivity (i.e., ring positive) and eight hours after ring positive. Fourteen organisms representing the LIAISON PLEX BCY assay reportable targets were cultivated to ring positive and eight hours after ring positivity using a continuous monitoring blood culture system. Three unique blood bottles were used to assess ring positive growth and ring positive + eight hours for a total of six bottles per organism. For each growth condition, all three blood bottles were removed from the incubator within two hours of being identified as ring positive or between 8 and 12 hours after Ring Positivity and counted in CFU/mL. In addition to CFU/mL quantitation, a minimum of one ring positive bottle and one ring positive + eight hours bottle from each target organism were tested in triplicate on the LIAISON PLEX BCY assay. For several organisms a minimum of one ring positive bottle and one ring positive + eight hours bottle was frozen at -80°C prior to testing on the LIAISON PLEX BCY assay. Results from the growth and detection study are presented in Table 22 and represent approximate organism levels that may be observed in a clinical setting. All quantified bottle positivity concentrations are equivalent or greater than the established limit of detection for each the targets of the LIAISON PLEX BCY assay and the results are acceptable.

Table 22. Ring Positivity Growth and Detection Results Summary

Organism	Representative Strain	Testing Condition	Concentration (CFU/mL)	Target Positivity
Candida albicans	*Candida albicans*	RP Fresh	2.21E+04	100%
		RP Frozen	4.30E+05	100%
		RP Frozen	8.60E+05	100%
		RP + 8 Fresh	1.83E+06	100%
		RP + 8 Frozen	1.54E+07	100%
		RP + 8 Frozen	6.90E+06	100%
Candida auris	*Candida auris*	RP Fresh	1.88E+07	100%
		RP Fresh	1.45E+07	100%
		RP Fresh	2.02E+07	100%
		RP + 8 Fresh	5.30E+07	100%
		RP + 8 Frozen	4.20E+07	100%
		RP + 8 Frozen	4.80E+07	100%
Candida dubliniensis	*Candida dubliniensis*	RP Fresh	1.47E+07	100%
		RP Fresh	1.98E+07	100%
		RP Fresh	3.20E+07	100%
		RP + 8 Fresh	4.20E+07	100%
		RP + 8 Fresh	4.20E+07	100%
		RP + 8 Fresh	5.10E+07	100%
Candida famata	*Debarymoyces fabryi*	RP Fresh	7.30E+06	100%
		RP Frozen	4.90E+06	100%
		RP Frozen	3.20E+06	100%
		RP + 8 Fresh	1.20E+07	100%

Organism	Representative Strain	Testing Condition	Concentration (CFU/mL)	Target Positivity
		RP + 8 Frozen	1.80E+07	100%
		RP + 8 Frozen	5.90E+06	100%
Candida glabrata	*Candida glabrata*	RP Fresh	8.00E+06	100%
		RP Frozen	3.80E+06	100%
		RP Frozen	6.10E+06	100%
		RP + 8 Fresh	1.44E+08	100%
		RP + 8 Frozen	1.36E+08	100%
		RP + 8 Frozen	1.60E+08	100%
Candida guilliermondii	*Meyerozyma guilliermondii*	RP Fresh	1.91E+07	100%
		RP Fresh	3.60E+07	100%
		RP Fresh	1.86E+07	100%
		RP + 8 Fresh	3.60E+07	100%
		RP + 8 Fresh	3.80E+07	100%
		RP + 8 Fresh	3.40E+07	100%
Candida haemulonii/ duobushaemulonii	*Candida haemulonis var. haemulonis*	RP Fresh	9.70E+06	100%
		RP Fresh	9.40E+06	100%
		RP Fresh	1.07E+07	100%
		RP + 8 Fresh	3.20E+07	100%
		RP + 8 Fresh	3.50E+07	100%
		RP + 8 Fresh	4.30E+07	100%
Candida kefyr	*Candida kefyr*	RP Fresh	8.20E+06	100%
		RP Fresh	6.30E+06	100%
		RP Fresh	1.20E+07	100%
		RP + 8 Fresh	2.34E+07	100%
		RP + 8 Fresh	2.07E+07	100%
		RP + 8 Fresh	2.15E+07	100%
Candida krusei	*Candida krusei*	RP Fresh	1.24E+07	100%
		RP Frozen	1.66E+07	100%
		RP Frozen	2.09E+07	100%
		RP + 8 Fresh	4.00E+07	100%
		RP + 8 Fresh	4.20E+07	100%
		RP + 8 Fresh	3.50E+07	100%
Candida lipolytica	*Yarrowia lipolytica*	RP Fresh	2.44E+06	100%
		RP Frozen	2.50E+06	100%
		RP Frozen	2.42E+06	100%
		RP + 8 Frozen	3.90E+06	100%
		RP + 8 Fresh	4.60E+06	100%
		RP + 8 Fresh	3.80E+06	100%

Organism	Representative Strain	Testing Condition	Concentration (CFU/mL)	Target Positivity
Candida lusitaniae	*Candida lusitaniae*	RP Fresh	2.23E+07	100%
		RP Frozen	5.10E+07	100%
		RP Frozen	3.50E+07	100%
		RP + 8 Fresh	1.33E+08	100%
		RP + 8 Fresh	1.22E+08	100%
		RP + 8 Fresh	1.32E+08	100%
Candida parapsilosis	*Candida parapsilosis*	RP Fresh	1.54E+07	100%
		RP Fresh	1.61E+07	100%
		RP Fresh	1.76E+07	100%
		RP + 8 Fresh	4.10E+07	100%
		RP + 8 Fresh	3.90E+07	100%
		RP + 8 Fresh	5.50E+07	100%
Candida tropicalis	*Candida tropicalis*	RP Fresh	1.31E+07	100%
		RP Fresh	1.20E+07	100%
		RP Fresh	1.35E+07	100%
		RP + 8 Fresh	3.00E+07	100%
		RP + 8 Fresh	3.90E+07	100%
		RP + 8 Fresh	3.30E+07	100%
Cryptococcus neoformans/gattii	*Cryptococcus neoformans var. grubii*	RP Fresh	1.84E+06	100%
		RP Fresh	3.40E+06	100%
		RP Fresh	2.09E+06	100%
		RP + 8 Fresh	5.00E+06	100%
		RP + 8 Frozen	5.50E+06	100%
		RP + 8 Frozen	4.40E+06	100%
Negative Blood Matrix	NA	Fresh	NA	0%

RP - Ring Positive; RP + 8 - Ring Positive + 8 hours

7. Assay Cut-Off:

The LIAISON PLEX BCY assay determines the presence/absence of each target analyte (and control) by comparing the mean signal intensity of capture spots to pre-defined thresholds. In addition to the capture spot thresholds, a set of assay-dependent parameters are used to evaluate the validity of the run. To determine the threshold and parameter values, a sequential, multi-step approach was employed.

In the first step, a threshold training set consisting of 1293 samples from analytical testing was used to estimate the thresholds and assay-dependent parameters. Proposed threshold and assay-dependent parameter values were determined by their ability to distinguish between true and false target calls while minimizing the no call rate. The proposed thresholds were determined by the negative signal distribution, where no target is present in the samples.

In the second step of threshold determination, the performance of the proposed parameter and threshold values from step 1 were evaluated using the threshold test set, which consisted of 92 samples from analytical testing which did not overlap with the threshold training set. The true call vs. no call rate for performance parameters and controls, and reportable target

sensitivity and specificity were tabulated. Final assay thresholds were established using ROC analysis.

B Comparison Studies:

1. Method Comparison with Predicate Device:
   Not applicable

2. Matrix Comparison:

   Bottle Type Equivalency Study
   This study was designed to evaluate equivalence of 12 different blood culture bottle matrices from three different blood culture systems on LIAISON PLEX BCY assay performance. Ten percent of bottles from two lots of each bottle type were screened for contamination prior to inclusion in the study. After screening, each bottle matrix was inoculated with either of two positive contrived panels, bacterial strains (*Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*; selected for their clinical prevalence in blood infections) or incubated without inoculation for 5-7 days to make negative blood matrix (NBM) (Table 23). Five replicates of each organism were tested in each of two bottle lots. Yeasts for each positive contrived sample were prepared at a concentration approximating blood culture bottle positivity in a clinical sample. Study results are presented in Table 24.

Table 23. Positive Control and Bacterial Concentrations

Control/Sample Type	Organism Name	Concentration (CFU/mL)
Positive Control 1	*Candida albicans*	4.40E+05
	Candida tropicalis	1.20E+07
	Cryptococcus neoformans	1.84E+06
Positive Control 2	*Candida glabrata*	3.80E+06
	Candida guilliermondii	1.86E+07
	Candida kefyr	6.30E+06
All Bacteria		1.00E+08

Table 24. Sample Matrix Equivalency (Bottle Evaluation) Results Summary

Manufacturer	Bottle Manufacturer Part Number	Bottle Name	Study Outcome
Thermo Scientific	7106-44	VersaTrek Redox 1	No interference detected
	7107-44	VersaTrek Redox 2	No interference detected
Becton, Dickinson, and Company (BD)	442022	Bactec Plus Anaerobic/F	No interference detected
	442024	Bactec Plus Standard Anaerobic/F	2 of 3 lots tested gave false positive results for *Candida tropicalis*
	442021	Bactec Lytic/10 Anaerobic/F	No interference detected

	442023	Bactec Plus Aerobic/F	No interference detected
	442020	Bactec Peds Plus	No interference detected
	442027	Bactec Standard/10 Aerobic	No interference detected
BioMérieux	259789	BACT/Alert SA	No interference detected
	410852	BACT/Alert FN Plus	No interference detected
	259790	BACT/Alert SN	No interference detected
	410853	BACT/Alert PF Plus	No interference detected

Eleven bottle types tested demonstrated no detectable interference for any of the targets evaluated. Two lots of the Bactec Plus Standard Anaerobic/F bottle type gave false positive results for *Candida tropicalis* during the screen phase of the study. An additional lot was screened, and negative results were observed for all on-panel organisms, but showed a low, sub-threshold signal for *C. tropicalis.* Subsequent testing in the bottle type equivalency study showed *C. tropicalis* contamination was detected in negative blood matrix testing (1/5 replicates). No growth occurred on the check plates and root cause analysis identified the bottle media was contaminated with *C. tropicalis* nucleic acids. The 12 bottle types evaluated in the bottle type equivalency study demonstrated correct positive and negative agreement with the exception of the Bactec Plus Standard Anaerobic/F bottle type which resulted in one *C. tropicalis* false positive. The results of this study are acceptable.

C Clinical Studies:

1. Prospective Clinical Study:
   The clinical performance of the LIAISON PLEX BCY assay to detect and identify target fungal pathogens from blood samples identified as 1) positive by a continuous monitoring blood culture system, and 2) a Gram stain indicative of a fungal organism, was assessed in a multi-site study using prospective samples. Device testing occurred at four geographically diverse sites within the U.S. representative of the clinical laboratory intended use environment. Prospective samples meeting study inclusion criteria were collected between June 2023 and October 2023. Samples were stored either refrigerated (2-8°C) if tested within 24 hours, or frozen (≤ -70°C) if not tested within 24 hours of collection. Due to the low prevalence of prospective positive fungal blood cultures, the study was supplemented with retrospective and contrived samples. LIAISON PLEX BCY assay clinical performance was compared with standard of care culture followed by MALDI-TOF mass spectrometry results. Discordant results analysis for applicable targets were performed by an FDA-cleared molecular assay testing and reported as footnotes in the clinical performance tables.

   A total of 3447 prospective blood samples identified as positive by a continuously monitored blood culture system. Of these, 70 samples were identified as exhibiting yeast morphology following Gram stain and were enrolled in the study. One sample was excluded following enrollment due to inconclusive Gram stain results leaving 69 clinical samples for subsequent analysis of the prospective clinical data set. Prospective samples were tested either fresh

(Category I samples) (38/69, 55.1%) or frozen (Category II samples) (31/69, 44.9%). Patient demographic information for the 69 prospective samples is presented in Table 25.

Table 25. Demographic Data for Prospectively Collected Specimens

Gender	Site 01	Site 02	Site 03	Site 04	All Sites
Male	24(57.1%)	6(54.5%)	5(45.5%)	1(20.0%)	36(52.2%)
Female	17(40.5%)	5(45.5%)	6(54.5%)	4(80.0%)	32(46.4%)
Gender Unknown	1(2.4%)	0(0.0%)	0(0.0%)	0(0.0%)	1(1.4%)
Total	42(100%)	11(100%)	11(100%)	5(100%)	69(100%)
Age (years)					
0-1	2(4.8%)	0(0.0%)	0(0.0%)	1(20.0%)	3(4.3%)
>1-5	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
>5-21	0(0.0%)	1(9.1%)	0(0.0%)	0(0.0%)	1(1.4%)
>21-65	21(50.0%)	9(81.8%)	8(72.7%)	3(60.0%)	41(59.4%)
>65	18(42.9%)	1(9.1%)	3(27.3%)	1(20.0%)	23(33.3%)
Age Unknown	1(2.4%)	0(0.0%)	0(0.0%)	0(0.0%)	1(1.4%)
Total	42(100%)	11(100%)	11(100%)	5(100%)	69(100%)
Subject Status					
Emergency Room	0(0.0%)	1(9.1%)	4(36.4%)	0(0.0%)	5(7.2%)
Hospitalized	0(0.0%)	10(90.9%)	7(63.6%)	5(100%)	22(31.9%)
Status Unknown	42(100%)	0(0.0%)	0(0.0%)	0(0.0%)	42(60.9%)
Total	42(100%)	11(100%)	11(100%)	5(100%)	69(100%)
Blood Culture Bottle type					
BD BACTEC Lytic Anaerobic	0(0.0%)	3(27.3%)	0(0.0%)	0(0.0%)	3(4.3%)
BD BACTEC Plus Aerobic	0(0.0%)	8(72.7%)	6(54.5%)	0(0.0%)	14(20.3%)
BD BACTEC Standard Aerobic	0(0.0%)	0(0.0%)	5(45.5%)	0(0.0%)	5(7.2%)
BacT/ALERT FA Plus	40(95.2%)	0(0.0%)	0(0.0%)	0(0.0%)	40(58.0%)
BacT/ALERT FN Plus	1(2.4%)	0(0.0%)	0(0.0%)	0(0.0%)	1(1.4%)
BacT/ALERT PF Plus	1(2.4%)	0(0.0%)	0(0.0%)	1(20.0%)	2(2.9%)
BacT/ALERT SA Standard Aerobic	0(0.0%)	0(0.0%)	0(0.0%)	3(60.0%)	3(4.3%)
BacT/ALERT SN Standard Anaerobic	0(0.0%)	0(0.0%)	0(0.0%)	1(20.0%)	1(1.4%)
Bottle Type Total	42(100%)	11(100%)	11(100%)	5(100%)	69(100%)

Of the 69 clinical samples included in the prospective study analysis, 67 (97.1%) generated valid LIAISON PLEX BCY assay results (i.e., Detected or Not Detected) on the first attempt. Two samples (2.9%) with initial invalid results generated valid LIAISON PLEX BCY assay results after a single retest for a final success rate of 100% (69/69).

The positive percent agreement (PPA) was calculated by dividing the number of true positive (TP) results by the sum of TP and false negative (FN) results, while negative percent agreement (NPA) was calculated by dividing the number of true negative (TN) results by the

sum of TN and false positive (FP) results. A TP result was defined as a sample where the detected LIAISON PLEX BCY assay result matched the detected comparator method result, while a TN result was one where a negative LIAISON PLEX BCY assay result matched a negative comparator method result. The two-sided 95% confidence interval was also calculated. Table 26 provides a summary of the LIAISON PLEX BCY assay prospective clinical study performance.

Table 26. Prospective Sample Clinical Performance of the LIAISON PLEX BCY Assay

Pathogen Target		Positive Percent Agreement			Negative Percent Agreement		
		TP / (TP+FN)	(%)	95% CI	TN / (TN+FP)	(%)	95% CI
Candida albicans	Fresh	10/10	100%	72.2%-100%	27/28	96.4%	82.3%-99.4%
	Frozen	7/7	100%	64.6%-100%	24/24	100%	86.2%-100%
	Overall	17/17	100%	81.6%-100%	51/52[1]	98.1%	89.9%-99.7%
Candida auris	Fresh	3/3	100%	43.9%-100%	35/35	100%	90.1%-100%
	Frozen	1/1	100%	20.7%-100%	30/30	100%	88.6%-100%
	Overall	4/4	100%	51.0%-100%	65/65	100%	94.4%-100%
Candida dubliniensis	Fresh	0/0	NA	NA	38/38	100%	90.8%-100%
	Frozen	0/0	NA	NA	31/31	100%	89.0%-100%
	Overall	0/0	NA	NA	69/69	100%	94.7%-100%
Candida famata	Fresh	0/0	NA	NA	38/38	100%	90.8%-100%
	Frozen	0/0	NA	NA	31/31	100%	89.0%-100%
	Overall	0/0	NA	NA	69/69	100%	94.7%-100%
Candida glabrata	Fresh	11/11	100%	74.1%-100%	27/27	100%	87.5%-100%
	Frozen	14/14	100%	78.5%-100%	17/17	100%	81.6%-100%
	Overall	25/25	100%	86.7%-100%	44/44	100%	92.0%-100%
Candida guilliermondii	Fresh	0/0	NA	NA	38/38	100%	90.8%-100%
	Frozen	0/0	NA	NA	31/31	100%	89.0%-100%
	Overall	0/0	NA	NA	69/69	100%	94.7%-100%
Candida haemulonii / *Candida duobushaemulonii*	Fresh	0/0	NA	NA	38/38	100%	90.8%-100%
	Frozen	0/0	NA	NA	31/31	100%	89.0%-100%
	Overall	0/0	NA	NA	69/69	100%	94.7%-100%
Candida kefyr	Fresh	0/0	NA	NA	38/38	100%	90.8%-100%
	Frozen	1/1	100%	20.7%-100%	30/30	100%	88.6%-100%
	Overall	1/1	100%	20.7%-100%	68/68	100%	94.7%-100%
Candida krusei	Fresh	2/2	100%	34.2%-100%	36/36	100%	90.4%-100%
	Frozen	1/1	100%	20.7%-100%	30/30	100%	88.6%-100%
	Overall	3/3	100%	43.9%-100%	66/66	100%	94.5%-100%
Candida lipolytica	Fresh	0/0	NA	NA	38/38	100%	90.8%-100%
	Frozen	0/0	NA	NA	31/31	100%	89.0%-100%
	Overall	0/0	NA	NA	69/69	100%	94.7%-100%
Candida lusitaniae	Fresh	1/1	100%	20.7%-100%	37/37	100%	90.6%-100%
	Frozen	1/1	100%	20.7%-100%	30/30	100%	88.6%-100%
	Overall	2/2	100%	34.2%-100%	67/67	100%	94.6%-100%
Candida parapsilosis	Fresh	9/9	100%	70.1%-100%	28/29	96.6%	82.8%-99.4%
	Frozen	2/2	100%	34.2%-100%	29/29	100%	88.3%-100%
	Overall	11/11	100%	74.1%-100%	57/58[2]	98.3%	90.9%-99.7%
Candida tropicalis	Fresh	2/2	100%	34.2%-100%	35/36	97.2%	85.8%-99.5%
	Frozen	4/4	100%	51.0%-100%	25/27	92.6%	76.6%-97.9%
	Overall	6/6	100%	61.0%-100%	60/63[3]	95.2%	86.9%-98.4%
Cryptococcus neoformans/	Fresh	0/0	NA	NA	38/38	100%	90.8%-100%
	Frozen	0/0	NA	NA	31/31	100%	89.0%-100%

Pathogen Target		Positive Percent Agreement			Negative Percent Agreement		
		TP / (TP+FN)	(%)	95% CI	TN / (TN+FP)	(%)	95% CI
Cryptococcus gattii	Overall	0/0	NA	NA	69/69	100%	94.7%-100%

[1]The one *Candida albicans* False Positive sample was also positive by an FDA-cleared molecular assay.
[2]The one *Candida parapsilosis* False Positive sample was also positive by an FDA-cleared molecular assay.
[3]One out of the three *Candida tropicalis* False Positive samples was also positive by an FDA-cleared molecular assay.

The PPA of the LIAISON PLEX BCY assay was 100% across all analytes in prospectively collected samples. The limited number of positive blood culture samples which contained a fungal organism as determined by Gram stain required supplementation with archived (Category III samples) and contrived (Category IV samples) to obtain more robust performance estimates. The NPA for all organisms was >98% in prospectively collected samples with the exception of *Candida tropicalis* which had an NPA of 95.2%. Discordant analysis testing provided evidence for the presence of *C. tropicalis* in one out of three *C. tropicalis* false positive results.

The LIAISON PLEX BCY assay reported multiple organism detections (co-infections) in a total of six prospective samples. This represents 8.7% (6/69) of all prospective samples. All six co-infections contained two organisms. Out of the six samples with multiple detections, 16.7% (1/6) was concordant with the reference method and 83.3% (5/6) contained one organism that was not detected by the reference method (Table 27).

Table 27. Coinfections Reported in the Prospective Study

Analyte 1	Analyte 2	Overall (N=69)	
		#Pos	(%)
Candida albicans	*Candida parapsilosis*	1	1.45% (1/69)
Candida albicans	*Candida tropicalis*	1	1.45% (1/69)
Candida auris	*Candida tropicalis*	1	1.45% (1/69)
Candida glabrata	*Candida tropicalis*	2	2.90% (2/69)
Candida kefyr	*Candida tropicalis*	1	1.45% (1/69)

2. Retrospective Sample Evaluation:
Several targets detected by the LIAISON PLEX BCA assay were not encountered during the prospective clinical study in sufficient numbers to demonstrate assay performance. Therefore, the prospective clinical study was supplemented with additional testing using retrospective positive samples characterized previously by a standard of care molecular assay. To minimize bias, negative retrospective samples (N=13) were tested concurrently with positive retrospective samples (N=50) obtained from six sources within the U.S. for a total of 63 retrospective samples evaluated. All samples were randomized and blinded by an individual not directly involved in the study testing. A summary of the demographic information available for retrospective samples is presented in Table 28.

Table 28. Demographic Data for Retrospective Samples

Gender	# Samples (%)
Male	24(38.1%)
Female	25(39.7%)
Gender Unknown	14(22.2%)
Total	**63(100%)**
Age (years)	
0-1	0(0.0%)
>1-5	1(1.6%)
>5-21	0(0.0%)
>21-65	27(42.9%)
>65	19(30.2%)
Age Unknown	16(25.4%)
Total	**63(100%)**
Subject Status	
Hospitalized	2(3.2%)
Status Unknown	61(96.8%)
Total	**63(100%)**
Blood Culture Bottle Type	
BD BACTEC Lytic Anaerobic	2(3.2%)
BD BACTEC Plus Aerobic	17(27.0%)
BD BACTEC Standard Aerobic	29(46.0%)
Bottle Type Unknown	15(23.8%)
Total	**63(100%)**

The performance of the LIAISON PLEX BCY assay was determined by comparing to an FDA-cleared molecular assay for all retrospective samples and summarized in Table 29. All retrospective samples generated a valid result on the first attempt.

Table 29. Retrospective Sample Clinical Performance of the LIAISON PLEX BCY Assay

Pathogen Target	Positive Percent Agreement			Negative Percent Agreement		
	TP / (TP+FN)	(%)	95% CI	TN / (TN+FP)	(%)	95% CI
Candida albicans	17/17	100%	81.6%-100%	46/46	100%	92.3%-100%
Candida auris	0/0	NA	NA	63/63	100%	94.3%-100%
Candida dubliniensis	0/0	NA	NA	63/63	100%	94.3%-100%
Candida famata	0/0	NA	NA	63/63	100%	94.3%-100%
Candida glabrata	21/21	100%	84.5%-100%	42/42	100%	91.6%-100%
Candida guilliermondii	0/0	NA	NA	63/63	100%	94.3%-100%
Candida haemulonii / C. duobushaemulonii	0/0	NA	NA	63/63	100%	94.3%-100%
Candida kefyr	0/0	NA	NA	63/63	100%	94.3%-100%
Candida krusei	1/1	100%	20.7%-100%	62/62	100%	94.2%-100%
Candida lipolytica	0/0	NA	NA	63/63	100%	94.3%-100%
Candida lusitaniae	0/0	NA	NA	63/63	100%	94.3%-100%
Candida parapsilosis	6/6	100%	61.0%-100%	57/57	100%	93.7%-100%
Candida tropicalis	0/0	NA	NA	63/63	100%	94.3%-100%
Cryptococcus neoformans/ Cryptococcus gattii	5/5	100%	56.6%-100%	58/58	100%	93.8%-100%

3. Contrived Sample Evaluation:

Due to the low prevalence of some of the LIAISON PLEX BCY assay target organisms encountered during prospective and retrospective sample collection, contrived samples were used to supplement clinical performance. Contrived samples were prepared from multiple strains of each of the yeast organisms detected by the assay (Table 30). Blood culture bottles containing whole blood from unique patients were inoculated with low inoculum concentrations of individual isolates and grown until flagged positive (ring positive) by a continuous monitoring blood culture system. Samples were collected within eight hours of ring positivity and stored frozen until testing. Fifty contrived samples were evaluated for each LIAISON PLEX BCY assay target. A total of 750 contrived samples and 79 negative samples were randomized, blinded, and interspersed at the four clinical sites for testing with prospective and retrospective samples.

Of the 829 contrived samples evaluated, 803 (96.9%) generated valid LIAISON PLEX BCY assay results on the first attempt. Invalid results were obtained for 26 (3.1%) samples at first attempt. Twenty-four of these samples generated a valid result after retest leading to a final success rate of 99.7% (827/829). The results of contrived sample testing with the LIAISON PLEX BCY assay are presented in Table 31.

Table 30. Contrived Sample Summary

Sample Name	Strain or Source PN	Culture Collection Manufacturer	# of Samples tested
Candida albicans	NCCLS 11	ATCC	10
	3147		10
	AR-0761	CDC	10
	AR-0762		10
	NSBCY0205	NSBCY	10
Candida albicans Total			50
Candida glabrata	NCCLS 84	ATCC	10
	NSBCY0235	North Shore University Health System	10
	303542	ATCC	10
	AmMS 231		10
	DLS0084334	Discovery Life Science	10
Candida glabrata Total			50
Candida auris	KCTC 17809	Westerdijk Institute	10
	VPCI669/P/12		10
	CBS 10913		10
	VPCI671/P/12		10
	MYA-5003	ATCC	10
Candida auris Total			50
Candida dubliniensis	H1		10
	H12		10
	Wu272		10

Sample Name	Strain or Source PN	Culture Collection Manufacturer	# of Samples tested
	75/043	ATCC	10
	MAD045	Tampa General Hospital	10
Candida dubliniensis Total			50
Candida famata	ATCC 60229	ATCC	10
	ATCC 20278		10
	CBS 14260	Westerdijk Institute	10
	CBS 14261		10
	CBS 14994		10
Candida famata Total			50
Candida guilliermondii	CBS 6021	ATCC	10
	848		10
	ATCC 7350, CBS 566, DBVPG 6140, IFO 10279, IGC 2730, JCM 1539, NRRL Y-324		10
	ATCC 14242		10
	AR-0590	CDC	10
Candida guilliermondii Total			50
Candida kefyr	[BI CZAS 652/6, CCY 29-8-9	ATCC	10
	AmMS 226		13
	14 Type B		14
	CBS 607		13
Candida kefyr Total			50
Candida krusei	M 298	ATCC	10
	ATCC 14243		10
	ATCC 749, CBS 573, CCRC 20514, IFO 1064, IFO 1395, JCM 1609, NBRC 1395, NRRL Y-413, NRRL Y-7179		10
	MAD 062	N/A	10
	UTMB0817	UTMB	10
Candida krusei Total			50
Yarrowia /Candida lipolytica	NRRL Y-1094 [ATCC 9948, CCY 29-26-47, FMJ 1278, IFO 1209]	ATCC	10

Sample Name	Strain or Source PN	Culture Collection Manufacturer	# of Samples tested
	PO1f		10
	CP 602		10
	CBS 6125	Westerdijk Institute	10
	CBS 6317		10
Yarrowia /Candida lipolytica			50
	Y534	ATCC	10
	AmMS 233		10
	45090		10
	MAD158	Tampa General Hospital	10
Candida lusitaniae	*CBS 4870*	*Westerdijk* Institute	10
Candida lusitaniae Total			50
	API 90 01 105	ATCC	10
	FDA PCI M-59		10
Candida tropicalis	AR-0345	CDC	10
	MAD069.2	Tampa General Hospital	10
	DLS0082284	Discovery Life Science	10
Candida tropicalis Total			50
	CDC 317	ATCC	10
	CL524		10
Candida parapsilosis	UTMB074	UTMB	10
	AR-0339	CDC	10
	MAD004	Tampa General Hospital	10
Candida parapsilosis Total			50
	RV 48370	ATCC	10
	A1M R272		10
Cryptococcus gattii	WM779		10
	NR-49162	BEI	10
	NR 50422		10
	613	ATCC	10
	WM629		10
Cryptococcus neoformans	LACNY0148	Laboratory Alliance of Central New York	10
Cryptococcus neoformans var. grubii	3756	ATCC	10
	H99		10
Cryptococcus neoformans/Cryptococcus gattii Total			100
Candida duobushaemulonii	CBS 7798	Westerdijk Institute	10
	AR-0394	CDC	10
Candida haemulonis	CBS 10970		10

Sample Name	Strain or Source PN	Culture Collection Manufacturer	# of Samples tested
Candida haemulonii var. vulnera	*CBS 12436*	Westerdijk Institute	10
Candida pseudohaemulonii	CBS 10004		10
Candida haemulonii / C. duobushaemulonii Total			50

Table 31. Contrived Sample Clinical Performance of the LIAISON PLEX BCY Assay

Pathogen Target	Positive Percent Agreement			Negative Percent Agreement		
Analyte	**TP / (TP+FN)**	**(%)**	**95% CI**	**TN / (TN+FP)**	**(%)**	**95% CI**
Candida albicans	50/50	100%	92.9%-100%	777/777	100%	99.5%-100%
Candida auris	50/50	100%	92.9%-100%	777/777	100%	99.5%-100%
Candida dubliniensis	50/50	100%	92.9%-100%	777/777	100%	99.5%-100%
Candida famata	50/50	100%	92.9%-100%	776/777[1]	99.9%	99.3%-100%
Candida glabrata	49/49	100%	92.7%-100%	778/778	100%	99.5%-100%
Candida guilliermondii	50/50	100%	92.9%-100%	777/777	100%	99.5%-100%
Candida haemulonii / C. duobushaemulonii	50/50	100%	92.9%-100%	777/777	100%	99.5%-100%
Candida kefyr	50/50	100%	92.9%-100%	777/777	100%	99.5%-100%
Candida Krusei	50/50	100%	92.9%-100%	777/777	100%	99.5%-100%
Candida lipolytica	50/50	100%	92.9%-100%	777/777	100%	99.5%-100%
Candida lusitaniae	50/50	100%	92.9%-100%	777/777	100%	99.5%-100%
Candida parapsilosis	50/50	100%	92.9%-100%	776/777[2]	99.9%	99.3%-99.9%
Candida tropicalis	49/49	100%	92.7%-100%	775/778[3]	99.6%	98.9%-100%
Cryptococcus neoformans/ Cryptococcus gattii	100/100	100%	96.3%-100%	727/727	100%	99.5%-100%

[1]The *C. famata* False Positive sample was also negative by an FDA-cleared molecular assay.
[2]The C. *parapsilosis* False Positive sample was also negative by an FDA-cleared molecular assay.
[3]The three C. *tropicalis* False Positive samples were also negative by an FDA-cleared molecular assay.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The LIAISON PLEX BCY assay prospective clinical study was conducted to evaluate clinical performance of the device using positive blood culture samples. A total of 3447 blood culture samples identified as positive by a continuous monitoring blood culture system were collected across four geographically diverse clinical sites within the U.S. between June and October of 2023. Prospective samples were tested with the LIAISON PLEX BCY assay either fresh or frozen. Of the 3447 samples, 69 samples met inclusion criteria and exhibited yeast morphology

after Gram stain and were enrolled in the study. The expected values of each target organism in the 69 prospective samples are presented by site and age in Tables 32 and 33, respectively.

Table 32. Expected Values by Clinical Site (Prospective Samples)

Target	Site 01 (N=42)		Site 02 (N=11)		Site 03 (N=11)		Site 04 (N=5)		Total (N=69)	
	#Pos	(%)	#Pos	(%)	#Pos	(%)	#Pos	(%)	#Pos	(%)
Candida albicans	12	28.6% (12/42)	3	27.3% (3/11)	2	18.2% (2/11)	1	20.0% (1/5)	18	26.1% (18/69)
Candida auris	2	4.8% (2/42)	2	18.2% (2/11)	0	0.0% (0/11)	0	0.0% (0/5)	4	5.8% (4/69)
Candida dubliniensis	0	0.0% (0/42)	0	0.0% (0/11)	0	0.0% (0/11)	0	0.0% (0/5)	0	0.0% (0/69)
Candida famata	0	0.0% (0/42)	0	0.0% (0/11)	0	0.0% (0/11)	0	0.0% (0/5)	0	0.0% (0/69)
Candida glabrata	15	35.7% (15/42)	4	36.4% (4/11)	5	45.5% (5/11)	1	20.0% (1/5)	25	36.2% (25/69)
Candida guilliermondii	0	0.0% (0/42)	0	0.0% (0/11)	0	0.0% (0/11)	0	0.0% (0/5)	0	0.0% (0/69)
Candida haemulonii/ C. duobushaemulonii	0	0.0% (0/42)	0	0.0% (0/11)	0	0.0% (0/11)	0	0.0% (0/5)	0	0.0% (0/69)
Candida kefyr	1	2.4% (1/42)	0	0.0% (0/11)	0	0.0% (0/11)	0	0.0% (0/5)	1	1.4% (1/69)
Candida krusei	3	7.1% (3/42)	0	0.0% (0/11)	0	0.0% (0/11)	0	0.0% (0/5)	3	4.3% (3/69)
Candida lipolytica	0	0.0% (0/42)	0	0.0% (0/11)	0	0.0% (0/11)	0	0.0% (0/5)	0	0.0% (0/69)
Candida lusitaniae	1	2.4% (1/42)	0	0.0% (0/11)	1	9.1% (1/11)	0	0.0% (0/5)	2	2.9% (2/69)
Candida parapsilosis	6	14.3% (6/42)	2	18.2% (2/11)	2	18.2% (2/11)	2	40.0% (2/5)	12	17.4% (12/69)
Candida tropicalis	3	7.1% (3/42)	2	18.2% (2/11)	3	27.3% (3/11)	1	20.0% (1/5)	9	13.0% (9/69)
Cryptococcus neoformans/ Cryptococcus gattii	0	0.0% (0/42)	0	0.0% (0/11)	0	0.0% (0/11)	0	0.0% (0/5)	0	0.0% (0/69)

Table 33. Expected Values by Age Group (Prospective Samples)

Target	0-1 year (N=3)		>2-5 years (N=0)		>6-21 years (N=1)		>22-65 years (N=41)		> 65 years (N=23)		Unknown years (N=1)		Overall (N=69)	
	#Pos	(%)	#Pos	(%)	#Pos	(%)	#Pos	(%)	#Pos	(%)	#Pos	(%)	#Pos	(%)
Candida albicans	1	33.3% (1/3)	0	0.0% (0/0)	0	0.0% (0/1)	13	31.7% (13/41)	4	17.4% (4/23)	0	0.0% (0/1)	18	26.1% (18/69)
Candida auris	0	0.0% (0/3)	0	0.0% (0/0)	0	0.0% (0/1)	3	7.3% (3/41)	1	4.3% (1/23)	0	0.0% (0/1)	4	5.8% (4/69)
Candida dubliniensis	0	0.0% (0/3)	0	0.0% (0/0)	0	0.0% (0/1)	0	0.0% (0/41)	0	0.0% (0/23)	0	0.0% (0/1)	0	0.0% (0/69)
Candida famata	0	0.0% (0/3)	0	0.0% (0/0)	0	0.0% (0/1)	0	0.0% (0/41)	0	0.0% (0/23)	0	0.0% (0/1)	0	0.0% (0/69)
Candida glabrata	1	33.3% (1/3)	0	0.0% (0/0)	1	100% (1/1)	10	24.4% (10/41)	12	52.2% (12/23)	1	100% (1/1)	25	36.2% (25/69)

Candida guilliermondii	0	0.0% (0/3)	0	0.0% (0/0)	0	0.0% (0/1)	0	0.0% (0/41)	0	0.0% (0/23)	0	0.0% (0/1)	0	0.0% (0/69)
Candida haemulonii/ C. duobushaemulonii	0	0.0% (0/3)	0	0.0% (0/0)	0	0.0% (0/1)	0	0.0% (0/41)	0	0.0% (0/23)	0	0.0% (0/1)	0	0.0% (0/69)
Candida kefyr	0	0.0% (0/3)	0	0.0% (0/0)	0	0.0% (0/1)	0	0.0% (0/41)	1	4.3% (1/23)	0	0.0% (0/1)	1	1.4% (1/69)
Candida krusei	0	0.0% (0/3)	0	0.0% (0/0)	0	0.0% (0/1)	2	4.9% (2/41)	1	4.3% (1/23)	0	0.0% (0/1)	3	4.3% (3/69)
Candida lipolytica	0	0.0% (0/3)	0	0.0% (0/0)	0	0.0% (0/1)	0	0.0% (0/41)	0	0.0% (0/23)	0	0.0% (0/1)	0	0.0% (0/69)
Candida lusitaniae	0	0.0% (0/3)	0	0.0% (0/0)	0	0.0% (0/1)	1	2.4% (1/41)	1	4.3% (1/23)	0	0.0% (0/1)	2	2.9% (2/69)
Candida parapsilosis	1	33.3% (1/3)	0	0.0% (0/0)	0	0.0% (0/1)	10	24.4% (10/41)	1	4.3% (1/23)	0	0.0% (0/1)	12	17.4% (12/69)
Candida tropicalis	0	0.0% (0/3)	0	0.0% (0/0)	0	0.0% (0/1)	5	12.2% (5/41)	4	17.4% (4/23)	0	0.0% (0/1)	9	13.0% (9/69)
Cryptococcus neoformans/ Cryptococcus gattii	0	0.0% (0/3)	0	0.0% (0/0)	0	0.0% (0/1)	0	0.0% (0/41)	0	0.0% (0/23)	0	0.0% (0/1)	0	0.0% (0/69)

VIII Proposed Labeling:

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

IX Conclusion:

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