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

I Background Information:

A 510(k) Number

K253722

B Applicant

Luminex Corporation

C Proprietary and Established Names

LIAISON PLEX Gastrointestinal *Flex* Assay

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PCH	Class II	21 CFR 866.3990 – Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay	MI-Microbiology
NSU	Class II	21 CFR 862.2570 – Instrumentation for clinical multiplex test systems	CH - Chemistry

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the LIAISON PLEX Gastrointestinal *Flex* Assay for the detection of microbial nucleic acids extracted from human stool specimens.

B Measurand:

Target nucleic acid sequences of the following gastrointestinal microorganisms:

- **Bacteria:** *Campylobacter* spp. (*C. coli, C. jejuni, C. lari, C. upsaliensis*), *Clostridioides difficile* (tcdA/tcdB), Enterotoxigenic *Escherichia coli* (ETEC) LT/ST), *Plesiomonas shigelloides*, *Salmonella* spp., Shiga-like toxin-producing *E. coli* (STEC) stx1, Shiga-like

toxin-producing *E. coli* (STEC) stx2, *Shigella*/Enteroinvasive *E. coli* (EIEC), *Vibrio cholerae*, *Vibrio* spp. (*V. parahaemolyticus*/*V. vulnificus*), *Yersinia enterocolitica*
- **Viruses:** Adenovirus F40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, Sapovirus I/II/IV/V
- **Parasites:** *Blastocystis* sp., *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Dientamoeba fragilis*, *Entamoeba histolytica*, *Giardia lamblia*, Microsporidia (*Encephalitozoon hellem*, *Encephalitozoon intestinalis*, *Enterocytozoon bieneusi*), *Strongyloides stercoralis*

C Type of Test:

Qualitative, multiplex nucleic acid amplification test (NAAT)

III Intended Use/Indications for Use:

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

B Indication(s) for Use:
The LIAISON PLEX Gastrointestinal *Flex* Assay is an automated and qualitative multiplexed test for simultaneous detection and identification of common pathogenic bacteria, viruses, and parasites from liquid or soft stool preserved in Cary-Blair or modified Cary-Blair medium and additionally for parasites, from liquid or soft stool preserved in alcohol-based formalin-free parasite fixative (e.g., EcoFix, Total-Fix) collected from individuals with signs and symptoms of gastrointestinal infection. The test is performed on the automated LIAISON PLEX System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific gastrointestinal microbial nucleic acid gene sequences associated with the pathogenic bacteria, viruses, and parasites:

Bacteria:
- *Campylobacter* spp. *(C. coli/C. jejuni/C. lari/C. upsaliensis)*
- *Clostridioides difficile (tcdA/tcdB)*
- Enterotoxigenic *Escherichia coli* (ETEC) *LT/ST*
- *Plesiomonas shigelloides*
- *Salmonella* spp.
- Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1*
- Shiga-like toxin-producing *Escherichia coli* (STEC) *stx2*
- *Shigella*/Enteroinvasive *Escherichia coli* (EIEC)
- *Vibrio cholerae*
- *Vibrio* spp. *(V. parahaemolyticus/V. vulnificus*)
- *Yersinia enterocolitica*

Viruses:
- Adenovirus F40/41
- Astrovirus
- Norovirus GI/GII
- Rotavirus A
- Sapovirus I/II/IV/V

Parasites:

- *Blastocystis* sp.
- *Cryptosporidium* spp.
- *Cyclospora cayetanensis*
- *Dientamoeba fragilis*
- *Entamoeba histolytica*
- *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*)
- Microsporidia (*Encephalitozoon hellem*/*Encephalitozoon intestinalis*/*Enterocytozoon bieneusi*)
- *Strongyloides stercoralis*

The LIAISON PLEX Gastrointestinal *Flex* Assay is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness, and results are meant to be used in conjunction with other clinical, laboratory, and epidemiological information; however, it is not to be used to monitor these infections.

Concomitant culture is necessary for organism recovery and further typing of bacterial agents.

This device is not intended to monitor *C. difficile* infection.

LIAISON PLEX Gastrointestinal *Flex* Assay results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out co-infection with other organisms that are not detected by this test and may not be the sole or definitive cause of patient illness. Negative LIAISON PLEX Gastrointestinal *Flex* Assay results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

C Special Conditions for Use Statement(s):

For prescription use only
For *in vitro* diagnostic use only

D Special Instrument Requirements:

LIAISON PLEX Gastrointestinal *Flex* Assay is to be used with the LIAISON PLEX System

IV Device/System Characteristics:

A Device Description:

The LIAISON PLEX Gastrointestinal *Flex* Assay is a test for the detection and identification of nucleic acids from enteric bacteria, viruses, and parasites in stool samples, and the assay is performed on the LIAISON PLEX System. The LIAISON PLEX System is a fully automated, bench-top "sample-to-answer" device that is capable of reverse transcription (RT) and polymerase chain reaction (PCR). The test system performs sample preparation, target amplification, microarray-based hybridization, and signal generation/analysis for the detection of target-specific nucleic acids.

The test reagents are supplied as a single, disposable test cartridge with a sample processing tube and a transfer pipette. The LIAISON PLEX Gastrointestinal *Flex* Assay Kit includes:

- 12 LIAISON PLEX Gastrointestinal *Flex* Assay Cartridges
- 15 LIAISON PLEX Gastrointestinal *Flex* Assay Sample Processing Tubes, 2.0 mL
- 1 bag of LIAISON PLEX Gastrointestinal *Flex* Assay Transfer Pipettes
  - Contains 15 individual Transfer Pipettes

Materials Required But Provided Separately:

- LIAISON PLEX System
  - LIAISON PLEX Chassis
  - One to six LIAISON PLEX Modules
- LIAISON PLEX Software
- LIAISON PLEX Gastrointestinal *Flex* Assay Software
- One hand-held barcode reader and stand
- Cartridge Removal Tool

Materials Recommended but Provided Separately:

- Sample Prep Tray

Materials Required but Not Provided:

- Specimen transport system: Cary-Blair, modified Cary-Blair, or alcohol-based formalin-free parasite fixative
- Vortex mixer
- 10% bleach
- Deionized water
- A FAT32-formatted USB drive

B Principle of Operation:

The LIAISON PLEX Gastrointestinal *Flex* Assay is an automated test for the simultaneous detection and identification of nucleic acids from enteric bacteria, viruses, and parasites in stool samples on the LIAISON PLEX System. The LIAISON PLEX Gastrointestinal *Flex* Assay is performed on liquid or soft stool samples preserved in Cary-Blair, modified Cary-Blair, and/or alcohol-based formalin-free parasite fixatives (parasite testing only). The user loads the mixed sample from the sample processing tube into the sample port of the LIAISON PLEX Gastrointestinal *Flex* Assay Cartridge. Next, the user sets up the sample order on the LIAISON PLEX System by entering the sample information or scanning the barcode ID located on the sample tube, then scanning the barcode ID located on the test cartridge. Lastly, the user inserts the test cartridge into the processing module to initiate the test. The LIAISON PLEX System identifies the assay being run and automatically initiates the proper testing protocol to process the sample, analyze the data, and generate test results.

The LIAISON PLEX System automates the LIAISON PLEX Gastrointestinal *Flex* Assay sample analysis through the following steps:

- Sample Extraction: Nucleic acid extraction via mechanical and chemical cell lysis and magnetic bead-based nucleic acid isolation of prepared stool samples.
- Target Amplification: Multiplex PCR- and RT-PCR-based amplification of the extracted nucleic acids to generate target-specific amplicons.

- Hybridization: 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;
- 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.

C Instrument Description Information:

1. Instrument Name:

   LIAISON PLEX

2. Specimen Identification:

   The user creates a new order and scans or manually enters the Sample ID and then scans the assay cartridge ID barcode with the hand-held barcode reader.

3. Specimen Sampling and Handling:

   Collect stool samples and place into Cary-Blair, modified Cary-Blair, or alcohol-based formalin-free parasite fixative (parasite testing only), as appropriate.

4. Calibration:

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

5. Quality Control:

   See Section VII.A.5 below for details.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Biofire Filmarray Gastrointestinal GI) Panel

B Predicate 510(k) Number(s):

K242367

C Comparison with Predicate(s):

Device & Predicate Device(s):	K253722 Subject Device	K242367 Predicate Device
Device Trade Name	LIAISON PLEX Gastrointestinal *Flex* Assay	Biofire Filmarray Gastrointestinal (GI) Panel

General Device Characteristic Similarities		
Intended Use/ Indications For Use	The LIAISON PLEX Gastrointestinal *Flex* Assay is an automated and qualitative multiplexed test for simultaneous detection and identification of common pathogenic bacteria, viruses, and parasites from liquid or soft stool preserved in Cary-Blair or modified Cary-Blair medium and additionally for parasites, from liquid or soft stool preserved in alcohol-based formalin-free parasite fixative (e.g., EcoFix, Total-Fix) collected from individuals with signs and symptoms of gastrointestinal infection. The test is performed on the automated LIAISON PLEX System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific gastrointestinal microbial nucleic acid gene sequences associated with the pathogenic bacteria, viruses, and parasites: **Bacteria:** • *Campylobacter* spp. *(C. coli/C. jejuni/C. lari/C. upsaliensis)* • *Clostridioides difficile (tcdA/tcdB)* • Enterotoxigenic *Escherichia coli* (ETEC) *LT/ST* • *Plesiomonas shigelloides* • *Salmonella* spp. • Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1* • Shiga-like toxin-producing *Escherichia coli* (STEC) *stx2* • *Shigella*/Enteroinvasive *Escherichia coli* (EIEC) • *Vibrio cholerae* • *Vibrio* spp. *(V. parahaemolyticus/V. vulnificus*) • *Yersinia enterocolitica* **Viruses:** • Adenovirus F40/41 • Astrovirus • Norovirus GI/GII • Rotavirus A • Sapovirus I/II/IV/V **Parasites:** • *Blastocystis* sp. • *Cryptosporidium* spp.	The BIOFIRE FILMARRAY Gastrointestinal (GI) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with BIOFIRE FILMARRAY Systems. The BIOFIRE GI Panel is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites directly from stool samples in Cary- Blair transport media obtained from individuals with signs and/or symptoms of gastrointestinal infection. The following bacteria (including several diarrheagenic *E. coli/Shigella* pathotypes), parasites, and viruses are identified using the BIOFIRE GI Panel: • *Campylobacter* (*C. jejuni/C. coli/C. upsaliensis*) • *Clostridium difficile* (*C. difficile*) toxin A/B • *Plesiomonas shigelloides* • *Salmonella* • *Vibrio* (*V. parahaemolyticus/V. vulnificus/ V. cholerae*), including specific identification of *Vibrio cholerae* • *Yersinia enterocolitica* • Enteroaggregative *Escherichia coli* (EAEC) • Enteropathogenic *Escherichia coli* (EPEC) • Enterotoxigenic *Escherichia coli* (ETEC) *lt/st* • Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1/stx2* (including specific identification of the *E. coli* O157 serogroup within STEC) • *Shigella*/ Enteroinvasive *Escherichia coli* (EIEC) • *Cryptosporidium* • *Cyclospora cayetanensis* • *Entamoeba histolytica* • *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*) • Adenovirus F 40/41 • Astrovirus • Norovirus GI/GII • Rotavirus A • Sapovirus (Genogroups I, II, IV, and V)

	• *Cyclospora cayetanensis* • *Dientamoeba fragilis* • *Entamoeba histolytica* • *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*) • Microsporidia (*Encephalitozoon hellem*/*Encephalitozoon intestinalis*/*Enterocytozoon bieneusi*) • *Strongyloides stercoralis* The LIAISON PLEX Gastrointestinal *Flex* Assay is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness, and results are meant to be used in conjunction with other clinical, laboratory, and epidemiological information; however, it is not to be used to monitor these infections. Concomitant culture is necessary for organism recovery and further typing of bacterial agents. This device is not intended to monitor *C. difficile* infection. LIAISON PLEX Gastrointestinal *Flex* Assay results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out co-infection with other organisms that are not detected by this test and may not be the sole or definitive cause of patient illness. Negative LIAISON PLEX Gastrointestinal *Flex* Assay results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.	The BIOFIRE GI Panel is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness and results are meant to be used in conjunction with other clinical, laboratory, and epidemiological data. Positive results do not rule out co-infection with organisms not included in the BIOFIRE GI Panel. The agent detected may not be the definite cause of the disease. Concomitant culture is necessary for organism recovery and further typing of bacterial agents. This device is not intended to monitor or guide treatment for *C. difficile* infection. Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *E. coli* O157, *Plesiomonas shigelloides*, *Yersinia enterocolitica*, *Astrovirus*, *and Rotavirus A* were established primarily with retrospective clinical specimens. Performance characteristics for *Entamoeba histolytica*, and *Vibrio* (*V. parahaemolyticus, V. vulnificus,* and *Vibrio cholerae*) were established primarily using contrived clinical specimens. Negative BIOFIRE GI Panel results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease. A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.
Test Interpretation	Automated test interpretation and report generation	Same
Measurand	Nucleic acid from organisms detected	Same
Cartridge/Pouch	Single use	Same

General Device Characteristic Differences		
Analytes	• *Campylobacter* spp. (*C. coli/C. jejuni/**C. lari**/C. upsaliensis*) • Shiga-like toxin-producing *Escherichia coli* (STEC) stx1 • Shiga-like toxin-producing *Escherichia coli* (STEC) stx2 • *Blastocystis* sp. • Microsporidia (*Encephalitozoon hellem/Encephalitozoon intestinalis/Enterocytozoon bieneusi*) • *Strongyloides stercoralis*	• *Campylobacter (C. jejuni/C. coli/C. upsaliensis*) • Enteroaggregative *Escherichia coli* (EAEC) • Enteropathogenic *Escherichia coli* (EPEC) • Shiga-like toxin-producing *Escherichia coli* (STEC) **stx1/stx2 (including specific identification of the *E. coli* O157 serogroup within STEC)**
Specimen Type	Liquid or soft stool in Cary-Blair / modified Cary-Blair; parasite targets also from alcohol-based formalin-free fixative.	Human stool sample collected in Cary Blair transport media
Technology	RT-PCR/PCR with microarray hybridization (nanoparticle/silver enhancement).	Nested multiplex PCR followed by high resolution melting analysis to confirm the identity of amplified product.
Instrument	LIAISON PLEX	BIOFIRE 2.0 System or BIOFIRE Torch System
Time to Result	Average Sample to Result time is 2 hours and 16 minutes	About 1 hour
Controls	Automated internal control for extraction/nucleic acid recovery/amplification/detection; synthetic DNA controls; hybridization control; results reported Pass/Fail/N/A. Internal control must pass or entire run is invalid.	Two controls are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis.

VI Standards/Guidance Documents Referenced:

- FDA Guidance – Class II Special Controls Guideline: Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assays for Detection and Identification of Microorganisms and Toxin Genes from Human Stool Specimens
- AAMI. Principles for medical device security – Risk Management. AAMI document TIR57:2016. Association for the Advancement of Medical Instrumentation; 2016.
- AAMI. Principles for medical device security – Postmarket risk management for device manufacturers. AAMI document TIR97:2019. Association for the Advancement of Medical Instrumentation; 2019.
- CLSI. Information Technology Security of In Vitro Diagnostic Instruments and Software Systems; Approved Standard – Second Edition. CLSI document AUTO11-A2. Wayne, PA: Clinical Laboratory Standards Institute; 2014.
- CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical Laboratory Standards Institute; 2019.
- CLSI. Interference Testing in Clinical Chemistry. 3rd Ed. CLSI Document EP07. Wayne, PA: Clinical Laboratory Standards Institute; 2018.
- CLSI. Evaluation of Qualitative, Binary Output Examination Performance; Approved Guideline – Third Edition. CLSI document EP12. Wayne, PA: Clinical Laboratory Standards Institute; 2023.

- CLSI. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI document EP17-A2. Wayne, PA: Clinical Laboratory Standards Institute; 2012.
- CLSI. Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Approved Guideline – Second Edition. CLSI document EP24-A2. Wayne, PA: Clinical Laboratory Standards Institute; 2011.
- CLSI. Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI document EP25-A. Wayne, PA: Clinical Laboratory Standards Institute; 2009.
- CLSI. Collection Transport Preparation and Storage of Specimens for Molecular Methods. 2nd Edition. CLSI Document MM13. Wayne, PA: Clinical Laboratory Standards Institute; 2020.
- CLSI. Verification and Validation of Multiplex Nucleic Acid Assays. 2nd Edition. CLSI Document MM17. Wayne, PA: Clinical Laboratory Standards Institute; 2018.
- ISTA. Packaged-Products for Parcel Delivery System Shipment 70 kg (150 lb) or Less. ISTA Document 3A. International Safe Transit Association. 2018.
- IEC 62366-1 Edition 1.1 2020-06 Consolidated Version; Medical devices – Part 1: Application of usability engineering to medical devices
- IEC 61010-1 Edition 3.1 2017-01 Consolidated Version; Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements
- IEC 60601-1-2 Edition 4.1 2020-09 Consolidated Version; Medical electrical equipment – Part 1-2: General requirements for basic safety and essential performance – Collateral Standard: Electromagnetic disturbances – Requirements and tests
- IEC 61326-1 Edition 3.0 2020-10; Electrical equipment for measurement, control and laboratory use – EMC requirements – Part 1: General requirements IEC 61326-2 Edition 3.0 2020-10; Electrical equipment for measurement, control and laboratory use – EMC requirements – Part 2-6: Particular requirements – In vitro diagnostic (IVD) medical equipment
- IEC 62304 Edition 1.1 2015-06 Consolidated Version; Medical device software – Software life cycle processes
- IEC TR 60878 Ed. 4.0 2022-11; Graphical symbols for electrical equipment in medical practice [Including: Corrigendum 1 (2023)]
- IEC TR 80001-2-2:2012. Application of risk management for IT Networks incorporating medical devices – Part 2-2: Guidance for the disclosure and communication of medical device security needs, risks and controls
- IEC TR 80001-2-8 Edition 1.0 206-05; Application of risk management for IT – networks incorporating medical devices – Part 2-8: Application guidance – Guidance on standards for establishing the security capabilities identified in IEC TR 80001-2-2
- ISO 14971:2019 Medical Devices – Application of risk management to medical devices
- ISO 15223-1: 2021-07 – Medical Devices- Symbols to be used with information to be supplied by the manufacturer – Part 1: General requirements
- UL ANSI 2900-1 First Edition 2017; Standard for Safety, Standard for Software Cybersecurity Network-Connectable Products, Part 1: General Requirements
- UL ANSI 2900-2-1 First Edition 2017; Standard for Safety, Software Cybersecurity for Network-Connectable Products, Part 2-1: Particular Requirements for Network Connectable Components of Healthcare and Wellness Systems
- EN ISO 18113-1 2011; In vitro diagnostic medical devices. Information supplied by the manufacturer (labelling). In vitro diagnostic instruments for professional use
- EN ISO 18113-2 2011; In vitro diagnostic medical devices — Information supplied by the manufacturer (labelling) Part 2: In vitro diagnostic reagents for professional use
- ISO 23640 2015; Medical devices. Symbols to be used with medical device labels, labeling and information to be supplied. General requirements
- CLSI MM13 2nd Edition, MM13Ed2E; Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods
- CLSI MM17 2nd Edition, MM17Ed2E; Verification and Validation of Multiplex Nucleic Acid Assays

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Within-Laboratory Precision/Repeatability
Within-laboratory precision and repeatability of the LIAISON PLEX Gastrointestinal *Flex* Assay was evaluated across days, operators, and replicates. A single assay kit lot was tested by two operators at one internal site. Testing included negative, low positive (1.5x LoD), and moderate positive (3.0x LoD) samples prepared in two separate sample matrices (Parasite Fixative Stool Matrix and Cary-Blair Stool Matrix). Negative and positive samples were prepared as described below.

Precision/Repeatability of the LIAISON PLEX Gastrointestinal *Flex* Assay was demonstrated across operators, days, and replicates with a ≥95% agreement with the expected result for stool samples prepared in Cary-Blair and for stool samples prepared in parasite fixative for all analytes, except for Rotavirus (93.3% at 1.5x LoD in Cary-Blair), *Shigella*/Enteroinvasive *E. coli* (EIEC) (90% at 1.5x LoD in Cary Blair), *Entamoeba histolytica* (93.3% at 1.5x LoD in parasite fixative), and Microsporidia (93.3% at 1.5x LoD in parasite fixative) at low positive concentrations. Results of the Precision/Repeatability study are summarized in **Table 1** and **Table 2**. All analytes evaluated were detected within performance expectations for qualitative agreement across the concentrations tested. Precision and Repeatability of the LIAISON PLEX Gastrointestinal Assay was demonstrated across operators, days, and replicates.

Table 1: Percent Agreement for Cary-Blair Stool Matrix Samples by Target

Panel Assay Target	% Agreement with Expected Result			
	Low Positive	Moderate Positive	Negative	All Concentrations (95% Confidence)
Adenovirus F40/41	96.7% (29/30)	100% (30/30)	100% (30/30)	98.9% (89/90) (94.0% - 99.8%)
Clostridioides difficile (tcdA/tcdB)	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
***Cryptosporidium* spp.**	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
Norovirus GI/GII	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
Rotavirus A	93.3% (28/30)	100% (30/30)	100% (30/30)	97.8% (88/90) (92.3% - 99.4%)
Shiga-like toxin-producing *E. coli* (STEC) *stx2*	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
***Shigella*/Enteroinvasive *E. coli* (EIEC)**	90.0% (27/30)	100% (30/30)	100% (30/30)	96.7% (87/90) (90.7% - 98.9%)
All Other Targets	100% (510/510)	100% (510/510)	100% (510/510)	100% (1530/1530) (99.7% - 100%)

Table 2: Percent Agreement for Parasite Fixative Stool Matrix Samples by Target

Panel Assay Target	% Agreement with Expected result			
	Low Positive	Moderate Positive	Negative	All Concentrations (95% Confidence)
***Blastocystis* sp.**				98.9% (89/90)

	96.7% (29/30)	100% (30/30)	100% (30/30)	(94.0% - 99.8%)
***Cryptosporidium* spp.**	96.7% (29/30)	100% (30/30)	100% (30/30)	98.9% (89/90) (94.0% - 99.8%)
Dientamoeba fragilis	96.7% (29/30)	100% (30/30)	100% (30/30)	98.9% (89/90) (94.0% - 99.8%)
Entamoeba histolytica	93.3% (28/30)	100% (30/30)	100% (30/30)	97.8% (88/90) (92.3% - 99.4%)
Giardia lamblia	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
Microsporidia	93.3% (28/30)	100% (30/30)	100% (30/30)	97.8% (88/90) (92.3% - 99.4%)
All Other Targets	100% (60/60)	100% (60/60)	100% (60/60)	100% (180/180) (97.9% - 100%)

Reproducibility

Site-to-site reproducibility of the LIAISON PLEX Gastrointestinal *Flex* Assay was evaluated across three sites. A single assay kit lot was tested by two operators at each of the three sites. Samples were randomized for each matrix and blinded to the operators, where each operator conducted nine tests per study day over five non-consecutive days at each site. Testing included negative, low positive (1.5x LoD), and moderate positive (3.0x LoD) samples in two sample matrices (Parasite Fixative Stool Matrix and Cary-Blair Stool Matrix). Negative samples were either negative stool matrix in Cary-Blair media or negative stool matrix in parasite fixative. Negative stool matrices were comprised of samples from multiple donors that had been pooled and confirmed negative for all targets on the LIAISON PLEX Gastrointestinal *Flex* Assay prior to use in the study. While positive Cary-Blair samples were designed to include analytes representative of each amplification pool and each target type included in the assay design (e.g., an RNA virus, a DNA virus, a gram-negative bacterium, a gram-positive bacterium, a toxin-producing bacterium, and a parasite), the positive parasite fixative samples were designed to include analytes representative of on-panel parasite targets.

Results of the site-to-site reproducibility study are summarized in **Table 3** (Cary-Blair) and **Table 4** (Parasite Fixative). Except for *Shigella*/Enteroinvasive *E. coli* (EIEC) at 1.5x LoD, the percent agreement with expected results was ≥95% for all analytes tested at 1.5x and 3x LoD, meeting the acceptance criteria for the study. The percent agreement for *Shigella*/Enteroinvasive *E. coli* (EIEC) at 1.5x LoD was 94.4%. All analytes evaluated were detected within performance expectations for qualitative agreement across all concentrations tested.

Table 3: Percent Agreement for Cary-Blair Stool Matrix Samples by Target (Site-to-Site)

Panel Assay Target	Sample Type	% Agreement with Expected Result			
		Site 1	Site 2	Site 3	All Sites (95% Confidence)
Adenovirus F40/41	**Low Positive**	96.7% (29/30)	100% (30/30)	100% (30/30)	98.9% (89/90) (94.0% - 99.8%)
	Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
	Negative	100% (30/30)	100% (30/30)	96.7% (29/30)	98.9% (89/90) (94.0% - 99.8%)
***Clostridioides difficile* (*tcdA/tcdB*)**	**Low Positive**	100% (30/30)	96.7% (29/30)	96.7% (29/30)	97.8% (88/90) (92.3% - 99.4%)
		100%	100%	96.7%	98.9% (89/90)

Panel Assay Target	Sample Type	% Agreement with Expected Result			
		Site 1	Site 2	Site 3	All Sites (95% Confidence)
	Moderate Positive	(30/30)	(30/30)	(29/30)	(94.0% - 99.8%)
	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
***Cryptosporidium* spp.**	**Low Positive**	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
	Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
Norovirus GI/GII	**Low Positive**	100% (30/30)	100% (30/30)	96.7% (29/30)	98.9% (89/90) (94.0% - 99.8%)
	Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
Rotavirus A	**Low Positive**	93.3% (28/30)	96.7% (29/30)	96.7% (29/30)	95.6% (86/90) (89.1% - 98.3%)
	Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
Shiga-like toxin-producing *E. coli* (STEC) *stx2*	**Low Positive**	100% (30/30)	96.7% (29/30)	100% (30/30)	98.9% (89/90) (94.0% - 99.8%)
	Moderate Positive	100% (30/30)	100% (30/30)	96.7% (29/30)	98.9% (89/90) (94.0% - 99.8%)
	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
***Shigella*/Enteroinvasive *E. coli* (EIEC)**	**Low Positive**	90.0% (27/30)	100% (30/30)	93.3% (28/30)	94.4% (85/90) (87.6% - 97.6%)
	Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
All Other Targets	**Negative**	100% (1530/1530)	100% (1530/1530)	100% (1530/1530)	100% (4590/4590) (99.9% - 100%)

Table 4: Percent Agreement for Parasite Fixative Stool Matrix Samples by Target (Site-to-Site)

Panel Assay Target	Sample Type	% Agreement with Expected result			
		Site 1	Site 2	Site 3	All Sites (95% Confidence)
***Blastocystis* sp.**	**Low Positive**	96.7% (29/30)	100% (30/30)	100% (30/30)	98.9% (89/90) (94.0% - 99.8%)
	Moderate Positive	100% (30/30)	100% (30/30)	96.7% (29/30)	98.9% (89/90) (94.0% - 99.8%)
	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
***Cryptosporidium* spp.**	**Low Positive**	96.7% (29/30)	100% (30/30)	100% (30/30)	98.9% (89/90) (94.0% - 99.8%)

Panel Assay Target	Sample Type	% Agreement with Expected result			
		Site 1	Site 2	Site 3	All Sites (95% Confidence)
	Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
Dientamoeba fragilis	Low Positive	96.7% (29/30)	100% (30/30)	93.3% (28/30)	96.7% (87/90) (90.7% - 98.9%)
	Moderate Positive	100% (30/30)	100% (30/30)	96.7% (29/30)	98.9% (89/90) (94.0% - 99.8%)
	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
Entamoeba histolytica	Low Positive	93.3% (28/30)	96.7% (29/30)	93.3% (28/30)	94.4% (85/90) (87.6% - 97.6%)
	Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
Giardia lamblia	Low Positive	100% (30/30)	100% (30/30)	93.3% (28/30)	97.8% (88/90) (92.3% - 99.4%)
	Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
Microsporidia	Low Positive	93.3% (28/30)	100% (30/30)	100% (30/30)	97.8% (88/90) (92.3% - 99.4%)
	Moderate Positive	100% (30/30)	100% (30/30)	96.7% (29/30)	98.9% (89/90) (94.0% - 99.8%)
	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
All Other Targets	Negative	100% (180/180)	100% (180/180)	100% (180/180)	100% (540/540) (99.3% - 100%)

Lot-to-Lot

Lot-to-Lot reproducibility of the LIAISON PLEX Gastrointestinal *Flex* Assay was evaluated across three lots of assay kits with one testing operator at one internal site. Positive and negative samples were prepared as described above. Lot reproducibility of the LIAISON PLEX Gastrointestinal *Flex* Assay was demonstrated across three lots of assay kits with a percent agreement ≥95% for all stool samples prepared in Cary-Blair and for stool samples prepared in parasite fixative, except for *Shigella*/Enteroinvasive *E. coli* (EIEC) (93.3% at 1.5x LoD in Cary Blair), *Entamoeba histolytica* (91.1% at 1.5x LoD in fixative). Confidence intervals were calculated using the Wilson Score Method. Results of the Lot-to-Lot Reproducibility study are summarized in **Tables 5** and **Table 6** below.

Table 5. Lot-to-Lot: Percent Agreement for Cary-Blair Stool Matrix Samples by Target

Panel Assay Target	Sample Type	% Agreement with Expected Result			
		Lot 1	Lot 2	Lot 3	All Lots (95% Confidence)
Adenovirus F40/41	Low Positive	93.3% (14/15)	100% (15/15)	93.3% (14/15)	95.6% (43/45) (85.2% - 98.8%)
					97.8% (44/45)

	Moderate Positive	93.3% (14/15)	100% (15/15)	100% (15/15)	(88.4% - 99.6%)
	Negative	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
Clostridioides difficile (tcdA/tcdB)	**Low Positive**	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Moderate Positive	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Negative	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
***Cryptosporidium* spp.**	**Low Positive**	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Moderate positive	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Negative	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
Norovirus GI/GII	**Low Positive**	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Moderate Positive	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Negative	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
Rotavirus A	**Low Positive**	93.3% (14/15)	100% (15/15)	93.3% (14/15)	95.6% (43/45) (85.2% - 98.8%)
	Moderate Positive	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Negative	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
Shiga-like toxin-producing *E.coli* (STEC) *stx2*	**Low Positive**	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Moderate Positive	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Negative	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
***Shigella*/Enteroinvasive *E.coli* (EIEC)**	**Low Positive**	93.3% (14/15)	100% (15/15)	86.7% (13/15)	93.3% (42/45) (82.1% - 97.7%)
	Moderate Positive	93.3% (14/15)	100% (15/15)	100% (15/15)	97.8% (44/45) (88.4% - 99.6%)
	Negative	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
All Other Targets	**Negative**	99.9% (764/765)	100% (765/765)	100% (765/765)	99.96% (2294/2295) (99.75% - 99.99%)

Table 6. Lot-to-Lot: Percent Agreement for Parasite Fixative Stool Matrix Samples

Panel Assay Target	**Sample Type**	**% Agreement with Expected Result**			
		Lot 1	**Lot 2**	**Lot 3**	**All Lots (95% Confidence)**
***Blastocystis* sp.**	**Low Positive**	100% (15/15)	100% (15/15)	93.3% (14/15)	97.8% (44/45) (88.4% - 99.6%)
	Moderate Positive	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Negative	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
***Cryptosporidium* spp.**	**Low Positive**	100% (15/15)	100% (15/15)	93.3% (14/15)	97.8% (44/45) (88.4% - 99.6%)
	Moderate Positive	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)

	Negative	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
Dientamoeba fragilis	**Low Positive**	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Moderate Positive	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Negative	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
Entamoeba histolytica	**Low Positive**	93.3% (14/15)	93.3% (14/15)	86.7% (13/15)	91.1% (41/45) (79.3% - 96.5%)
	Moderate Positive	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Negative	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
Giardia lamblia	**Low Positive**	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Moderate positive	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Negative	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
Microsporidia	**Low Positive**	100% (15/15)	100% (15/15)	93.3% (14/15)	97.8% (44/45) (88.4% - 99.6%)
	Moderate Positive	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
	Negative	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45) (92.1% - 100%)
All Other Targets	**Negative**	100% (90/90)	100% (90/90)	100% (90/90)	100% (270/270) (98.6% - 100%)

2. Linearity:
   Not applicable.

3. Analytical Specificity/Interference:

   Analytical Specificity (Cross-Reactivity)
   A study was performed to verify that the LIAISON PLEX Gastrointestinal *Flex* Assay does not detect nucleic acids off-panel organisms phylogenetically related to the on-panel organisms, as well as organisms typically present in stool samples. Organisms for the study panel were contrived in Cary-Blair Negative Stool Matrix at the highest available concentration and tested in triplicate with the LIAISON PLEX Gastrointestinal *Flex* Assay. When a non-specific positive result was observed, six additional replicates from a new preparation were tested to confirm potential cross-reactivity. Whole organisms and gBlocks with no observed cross-reactivity after wet testing are listed in **Table 7**. Organisms where cross-reactivity was observed through wet testing or predicted by *in silico* analysis (not wet tested) are reported in **Table 8** and **Table 9**, respectively. While *in silico* inclusivity analysis for all targets was performed with sequences available in the GenBank and WGS databases in July 2025*, in silico* exclusivity analysis was performed with sequences available from the GenBank nucleotide (nt) database as of January 10, 2025 for on-panel and off-panel organisms listed in **Table 9** below.

Table 7: Organisms with No Observed Cross-Reactivity to LIAISON PLEX Gastrointestinal *Flex* Assay During Wet Testing

Organism	Concentration Tested	Organism	Concentration Tested
Bacteria			
Abiotrophia defectiva	5.70E+06 CFU/mL	*Fusobacterium varium*	1.31E+08 CFU/mL
Acinetobacter baumannii	3.16E+08 CFU/mL	*Helicobacter fennelliae*	5.00E+04 CFU/mL
Acinetobacter lwoffii	6.30E+07 CFU/mL	*Helicobacter pylori*	4.98E+06 CFU/mL
Aeromonas hydrophila	1.76E+08 CFU/mL	*Klebsiella oxytoca*	2.69E+08 CFU/mL
Alcaligenes faecalis subsp. *faecalis*	1.09E+09 CFU/mL	*Klebsiella pneumoniae*	2.66E+08 CFU/mL
Bacillus cereus	2.41E+07 CFU/mL	*Lactobacillus acidophilus*	4.83E+07 CFU/mL
Bacteroides caccae	4.47E+08 CFU/mL	*Lactobacillus reuteri*	1.09E+08 CFU/mL
Bacteroides fragilis	7.60+E08 CFU/mL	*Lactococcus lactis*	3.02E+08 CFU/mL
Bacteroides stercoris	2.35E+08 CFU/mL	*Leminorella grimontii*	2.35E+08 CFU/mL
Bifidobacterium bifidum	1.91E+07 CFU/mL	*Listeria grayi*	2.90E+07 CFU/mL
Campylobacter concisus	1.40E+05 CFU/mL	*Listeria monocytogenes*	3.43E+08 CFU/mL
Campylobacter curvus	3.05E+06 CFU/mL	*Morganella morganii*	2.44E+08 CFU/mL
Campylobacter fetus	2.30E+08 CFU/mL	*Peptoniphilus asaccharolyticus*	8.35E+07 CFU/mL
Campylobacter gracilis	2.85E+05 CFU/mL	*Peptostreptococcus anaerobius*	3.15E+05 CFU/mL
Campylobacter hominis	2.25E+05 CFU/mL	*Porphyromonas asaccharolytica*	2.30E+06 CFU/mL
Campylobacter rectus	1.81E+07 CFU/mL	*Prevotella bivia*	1.14E+08 CFU/mL
Campylobacter showae	1.70E+06 CFU/mL	*Prevotella melaninogenica*	2.32E+07 CFU/mL
Candida albicans	1.21E+07 CFU/mL	*Prevotella (Hoylesella) oralis*	1.70E+07 CFU/mL
Cedecea davisae	1.40E+07 CFU/mL	*Proteus mirabilis*	3.24E+08 CFU/mL
Citrobacter amalonaticus	3.57E+08 CFU/mL	*Proteus penneri*	6.95E+07 CFU/mL
Citrobacter freundii	1.30E+08 CFU/mL	*Proteus vulgaris*	1.69E+08 CFU/mL
Citrobacter sedlakii	2.55E+07 CFU/mL	*Providencia alcalifaciens*	1.74E+08 CFU/mL
Clostridium bifermentans	6.05E+06 CFU/mL	*Providencia stuartii*	3.42E+08 CFU/mL
Clostridium bolteae	5.20E+05 CFU/mL	*Providencia rettgeri*	2.91E+08 CFU/mL
Clostridium butyricum	1.14E+07 CFU/mL	*Pseudomonas aeruginosa*	4.36E+08 CFU/mL
Clostridium difficile (Non-toxigenic)	3.19E+06 CFU/mL	*Pseudomonas putida*	8.45E+06 CFU/mL
Clostridium haemolyticum	2.60E+05 CFU/mL	*Ruminococcus bromii*	5.00E+05 CFU/mL
Clostridium methylpentosum	7.90E+06 CFU/mL	*Serratia liquefaciens*	4.93E+08 CFU/mL
Clostridium nexile	1.28E+06 CFU/mL	*Serratia marcescens*	1.82E+08 CFU/mL
Clostridium novyi	9.65E+05 CFU/mL	*Staphylococcus aureus*	2.71E+08 CFU/mL
Clostridium perfringens	1.77E+06 CFU/mL	*Staphylococcus epidermidis*	4.54E+08 CFU/mL
Clostridium scindens	1.20E+07 CFU/mL	*Streptococcus dysgalactiae*	6.75E+07 CFU/mL
Clostridium septicum	2.54E+06 CFU/mL	*Vibrio alginolyticus*	9.00E+06 CFU/mL
Clostridium sordellii	2.86E+06 CFU/mL	*Vibrio fluvialis*	3.60E+07 CFU/mL
Clostridium sporogenes	1.14E+07 CFU/mL	*Vibrio furnissii*	4.65E+07 CFU/mL
Desulfovibrio piger	1.50E+05 CFU/mL	*Vibrio harveyi*	3.80E+06 CFU/mL
Edwardsiella tarda	5.95E+08 CFU/mL	*Vibrio metschnikovii*	5.60E+06 CFU/mL
Enterobacter (Klebsiella) aerogenes	3.95E+07 CFU/mL	*Vibrio mimicus*	3.40E+07 CFU/mL
Enterobacter cloacae	5.70E+08 CFU/mL	*Vibrio natriegens*[1]	1.29E+07 CFU/mL

Organism	Concentration Tested	Organism	Concentration Tested
Enterococcus faecalis	2.09E+08 CFU/mL	*Yersinia bercovieri*	4.70E+07 CFU/mL
Enterococcus faecium	1.43E+08 CFU/mL	*Yersinia kristensenii* (gBlock)	1.50E+07 copies/mL
Escherichia coli	1.36E+08 CFU/mL	*Yersinia pseudotuberculosis*	2.84E+08 CFU/mL
Escherichia coli (EAEC)	4.47E+08 CFU/mL	*Yersinia rochesterensis* (gBlock)	1.50E+07 copies/mL
Escherichia coli (EPEC)	6.10E+08 CFU/mL	*Yersinia rohdei*	1.01E+07 CFU/mL
Escherichia fergusonii	2.95E+08 CFU/mL	*Yersinia ruckeri*	3.50E+07 CFU/mL
Escherichia hermannii	2.79E+08 CFU/mL		
Viruses			
Human adenovirus 31 (gBlock)	1.50E+07 copies/mL	Cytomegalovirus, AD-169	1.35E+03 TCID50/mL
Adenovirus Type 7A, Species B	9.75E+05 TCID50/mL	Echovirus Type 11	2.95E+06 TCID50/mL
Human mastadenovirus B (gBlock)	1.50E+07 copies/mL	Enterovirus Type 68, 09/2014 Isolate 4	1.23E+04 TCID50/mL
Adenovirus Type 4, Species E	8.50E+03 TCID50/mL	Hepatitis A virus, HM-175 (clone 1)	4.45E+05 TCID50/mL
Coxsackie virus Type A16	2.04E+06 TCID50/mL	Human mastadenovirus C (gBlock)	1.50E+07 copies/mL
Coxsackie virus B3	2.04E+06 TCID50/mL	Human mastadenovirus G (gBlock)	1.50E+07 copies/mL
Parasites			
Entamoeba dispar	1.25E+04 cells/mL	*Pentatrichomonas hominis*	1.30E+06 cells/mL
Entamoeba dispar (gBlock)	1.50E+07 copies/mL	*Pentatrichomonas hominis* (gBlock)	1.50E+07 copies/mL
Entamoeba moshkovskii	8.50E+04 cells/mL	*Trichomonas tenax*	1.10E+06 cells/mL
Cyclospora colobi (gBlock)	1.50E+07 copies/mL	*Trichomonas tenax* (gBlock)	1.50E+07 copies/mL

[1]*Vibrio cincinnatiensis was unavailable, Vibrio natriegens was used as a replacement.*

Table 8: Organisms with Observed Cross-Reactivity to LIAISON PLEX Gastrointestinal *Flex* Assay in Wet Testing

Reportable Target	Organism	Lowest Test Concentration with Positivity
Adenovirus F40/41	Adenovirus Type 31, Species A[1]	8.50E+03 TCID50/mL
	Adenovirus Type 26, Species D	5.85E+02 TCID50/mL
***Blastocystis* sp.**	*Cystoisospora belli* (gBlock)	1.50E+07 copies/mL
	Toxoplasma gondii	7.70E+04 cells/mL
Entamoeba histolytica	*Entamoeba nuttalli* (gBlock)[2]	1.50E+07 copies/mL
Giardia lamblia	*Giardia ardeae* (gBlock)[3]	1.50E+07 copies/mL
	Giardia cricetidarum (gBlock)[4]	1.50E+07 copies/mL
	Giardia microti (gBlock)[4]	1.50E+07 copies/mL
	Giardia muris[4]	6.25E+03 cysts/mL
	Giardia psittaci (gBlock)[3]	1.50E+07 copies/mL
Shiga-like toxin-producing *E.coli* (STEC) *stx1*	Adenovirus Type 1, Species C[1]	2.09E+05 TCID50/mL

[1] Organism was wet tested and produced a positive result, however, the organism was not predicted to cross-react by *in silico* analysis performed January 2025. The associated gBlock did not cross-react, suggesting one of the following: i) the cross-reacting area of the organism genome is not encompassed by the gBlock fragment, ii) the organism was mis-labeled, or iii) there was contamination.

[2] Primate pathogen which shows homology to *Entamoeba histolytica*.

[3] Avian pathogen which shows homology to *Giardia lamblia.*
[4] Rodent pathogen which shows homology to *Giardia lamblia.*

Table 9: Organisms Not Wet Tested but Predicted by *in silico* Analysis (January 2025) to Cross-React with LIAISON PLEX Gastrointestinal *Flex* Assay

Reportable Target	Predicted Cross-Reaction
Astrovirus	Strains of canine, feline, porcine/swine and California sea lion astroviruses
Rotavirus A	Strains of porcine rotavirus
Strongyloides stercoralis	*Strongyloides callosciureus* *Strongyloides cebus* *Strongyloides fuelleborni* *Strongyloides myopotami* *Strongyloides papillosus* *Strongyloides procyonis* *Strongyloides ransomi* *Strongyloides ratti* *Strongyloides robustus* *Strongyloides venezuelensis*
Vibrio cholerae	*Vibrio tarriae* (accession CP022353.1)[1]

[1]According to sponsor, Vibrio *tarriae* was considered a novel member of the *Cholerae clade*.

Interfering Substances

Nineteen endogenous and exogenous substances that may be present in stool specimens were evaluated for potential interference with the LIAISON PLEX Gastrointestinal *Flex* Assay. Various targets were tested at 3x LoD in the presence of negative stool matrix (prepared in either Cary-Blair or Parasite Fixative) and one potentially interfering substance. Also, negative samples with only interfering substances in stool matrix were prepared and tested. In each matrix, at least five replicates of positive and negative samples were tested in the presence of each potentially interfering substance. No interference was observed for the potentially interfering substances listed in **Table 10** at the indicated concentration.

Table 10: Potentially Interfering Substances Tested with No Observed Interference

Non-Microbial Interfering Substance	Concentration Tested
Triglyceride (Fecal Fat)	5% v/v
Cholesterol (Fecal Fat)	3% w/v
Human Hemoglobin	5% w/v
Human Whole Blood	25% v/v
Nystatin Suspension (Antifungal)	25% v/v
Phenylephrine (Preparation H)	10% w/v
Hydrocortisone (Preparation H)	12% w/v
Aluminum Hydroxide, Magnesium Hydroxide (Mylanta)	10% v/v
Mineral Oil	25% v/v
Sennosides (Ex-lax)	10% w/v
Bismuth subsalicylate (Pepto-Bismol)	10% v/v
Naproxen Sodium	2.1% w/v
Mucin	0.5% w/v
Benzalkonium Chloride	1% v/v
Ethanol	1% v/v

Calcium carbonate, Polymyxin B sulfate/Bacitracin zinc, and Loperamide Hydrochloride were identified as interfering substances at the initial concentration tested. When tested at the next lower input level, no interference was observed for any targets included in the assay. (See results in **Table 11** below).

Table 11: Potentially Interfering Substances Tested with Observed Interference

Product	Active Ingredient	Concentration of Product with Observed Interference	Concentration of Product with No Observed Interference
Tums Antacid	Calcium carbonate	10% w/v in Cary-Blair stool	5% w/v in Cary-Blair stool
Polysporin First Aid Antibiotic Ointment	Polymyxin B sulfate/Bacitracin zinc	10% w/v in Cary-Blair stool	5% w/v in Cary-Blair stool
Imodium AD Anti-Diarrheal Oral Solution	Loperamide Hydrochloride	10% v/v in Parasite Fixative stool	5% v/v in Parasite Fixative stool

Microbial Inhibition

A Microbial Interference study was designed to evaluate the ability of the LIAISON PLEX Gastrointestinal *Flex* Assay to detect low positive analytes in the presence of twelve potentially inhibitory non-panel microorganisms that are commonly found in stool specimens. The study was performed utilizing two LIAISON PLEX target groups prepared in negative stool matrix with either Cary-Blair or Parasite Fixative. For the first target panel, positive samples were prepared at 3x LoD with representative organisms (e.g., an RNA virus, a DNA virus, a gram-negative bacterium, a gram-positive bacterium, a toxin-producing bacterium, and a parasite) in Cary-Blair Negative Stool Matrix. The second target panel included analytes representative of parasite targets in Parasite Fixative Negative Stool Matrix. At least five replicates of positive and negative samples were tested in the presence of each potentially inhibitory microorganism at $\geq 10^6$ organisms/mL, or the highest available concentration. No interference was observed for any of the potentially inhibitory microorganisms tested in **Table 12** below.

Table 12: Potentially Inhibitory Microorganisms Tested with No Observed Interference

Bacteroides fragilis	*Escherichia coli*
Bifidobacterium bifidum	*Klebsiella pneumoniae*
Candida albicans	*Lactobacillus acidophilus*
Clostridium perfringens	*Prevotella bivia*
Klebsiella aerogenes	*Prevotella oralis*
Enterococcus faecalis	*Staphylococcus aureus*

Co-Infection

A Co-Infection study was designed to evaluate the ability of the LIAISON PLEX Gastrointestinal *Flex* Assay to detect low positive analytes in the presence of other targets at

high concentrations. Positive samples were prepared in Cary-Blair Negative Stool Matrix with prevalent co-infection targets, where one target was spiked at a low concentration (3x LoD) and the other target was spiked at a high concentration (100x LoD) and vice versa. Each combination was tested in three replicates. All targets in each co-infection combination were detected at 3x LoD and 100x LoD (**Table 13**).

Table 13: Percent Positivity of Each Target in Prevalent Co-Infection Combinations

Co-infection Combination	Target at 100x LoD	Target at 3x LoD	% Positivity of Target at 100x LoD	% Positivity of Target at 3x LoD
1-A	Adenovirus F40/41	Rotavirus A	100.0% (3/3)	100.0% (3/3)
1-B	Rotavirus A	Adenovirus F40/41	100.0% (3/3)	100.0% (3/3)
2-A	*Blastocystis* sp.	*Salmonella* spp.	100.0% (3/3)	100.0% (3/3)
2-B	*Salmonella* spp.	*Blastocystis* sp.	100.0% (3/3)	100.0% (3/3)
3-A	*Campylobacter* spp.	Shiga-like toxin-producing *E. coli* (STEC) *stx1*	100.0% (3/3)	100.0% (3/3)
3-B	Shiga-like toxin-producing *E. coli* (STEC) *stx1*	*Campylobacter* spp.	100.0% (3/3)	100.0% (3/3)
4-A	*Clostridioides difficile (tcdA/tcdB)*	*Blastocystis* sp.	100.0% (3/3)	100.0% (3/3)
4-B	*Blastocystis* sp.	*Clostridioides difficile (tcdA/tcdB)*	100.0% (3/3)	100.0% (3/3)
5-A	*Clostridioides difficile (tcdA/tcdB)*	*Campylobacter* spp.	100.0% (3/3)	100.0% (3/3)
5-B	*Campylobacter* spp.	*Clostridioides difficile (tcdA/tcdB)*	100.0% (3/3)	100.0% (3/3)
6-A	*Clostridioides difficile (tcdA/tcdB)*	Enterotoxigenic *E. coli* (ETEC) *LT/ST*	100.0% (3/3)	100.0% (3/3)
6-B	Enterotoxigenic *E. coli* (ETEC) *LT/ST*	*Clostridioides difficile (tcdA/tcdB)*	100.0% (3/3)	100.0% (3/3)
7-A	*Clostridioides difficile (tcdA/tcdB)*	Norovirus GI/GII	100.0% (3/3)	100.0% (3/3)
7-B	Norovirus GI/GII	*Clostridioides difficile (tcdA/tcdB)*	100.0% (3/3)	100.0% (3/3)
8-A	*Clostridioides difficile (tcdA/tcdB)*	*Salmonella* spp.	100.0% (3/3)	100.0% (3/3)
8-B	*Salmonella* spp.	*Clostridioides difficile (tcdA/tcdB)*	100.0% (3/3)	100.0% (3/3)
9-A	*Clostridioides difficile (tcdA/tcdB)*	Sapovirus I/II/IV/V	100.0% (3/3)	100.0% (3/3)
9-B	Sapovirus I/II/IV/V	*Clostridioides difficile (tcdA/tcdB)*	100.0% (3/3)	100.0% (3/3)
10-A	*Clostridioides difficile (tcdA/tcdB)*	Shiga-like toxin-producing *E. coli* (STEC) *stx1*	100.0% (3/3)	100.0% (3/3)

Co-infection Combination	Target at 100x LoD	Target at 3x LoD	% Positivity of Target at 100x LoD	% Positivity of Target at 3x LoD
10-B	Shiga-like toxin-producing *E. coli* (STEC) *stx1*	*Clostridioides difficile (tcdA/tcdB)*	100.0% (3/3)	100.0% (3/3)
11-A	*Clostridioides difficile (tcdA/tcdB)*	*Vibrio cholerae*	100.0% (3/3)	100.0% (3/3)
11-B	*Vibrio cholerae*	*Clostridioides difficile (tcdA/tcdB)*	100.0% (3/3)	100.0% (3/3)
12-A	*Giardia lamblia*	*Cryptosporidium* spp.	100.0% (3/3)	100.0% (3/3)
12-B	*Cryptosporidium* spp.	*Giardia lamblia*	100.0% (3/3)	100.0% (3/3)
13-A	Norovirus GI/GII	Adenovirus F40/41	100.0% (3/3)	100.0% (3/3)
13-B	Adenovirus F40/41	Norovirus GI/GII	100.0% (3/3)	100.0% (3/3)
14-A	Norovirus GI/GII	Astrovirus	100.0% (3/3)	100.0% (3/3)
14-B	Astrovirus	Norovirus GI/GII	100.0% (3/3)	100.0% (3/3)
15-A	Norovirus GI/GII	Rotavirus A	100.0% (3/3)	100.0% (3/3)
15-B	Rotavirus A	Norovirus GI/GII	100.0% (3/3)	100.0% (3/3)
16-A	Norovirus GI/GII	*Salmonella* spp.	100.0% (3/3)	100.0% (3/3)
16-B	*Salmonella* spp.	Norovirus GI/GII	100.0% (3/3)	100.0% (3/3)
17-A	Norovirus GI/GII	Sapovirus I/II/IV/V	100.0% (3/3)	100.0% (3/3)
17-B	Sapovirus I/II/IV/V	Norovirus GI/GII	100.0% (3/3)	100.0% (3/3)
18-A	Norovirus GI/GII	Shiga-like toxin-producing *E. coli* (STEC) *stx1*	100.0% (3/3)	100.0% (3/3)
18-B	Shiga-like toxin-producing *E. coli* (STEC) *stx1*	Norovirus GI/GII	100.0% (3/3)	100.0% (3/3)
19-A	Rotavirus A	Enterotoxigenic *E. coli* (ETEC) *LT/ST*	100.0% (3/3)	100.0% (3/3)
19-B	Enterotoxigenic *E. coli* (ETEC) *LT/ST*	Rotavirus A	100.0% (3/3)	100.0% (3/3)
20-A	Rotavirus A	Shiga-like toxin-producing *E. coli* (STEC) *stx1*	100.0% (3/3)	100.0% (3/3)
20-B	Shiga-like toxin-producing *E. coli* (STEC) *stx1*	Rotavirus A	100.0% (3/3)	100.0% (3/3)
21-A	*Salmonella* spp.	*Campylobacter* spp.	100.0% (3/3)	100.0% (3/3)
21-B	*Campylobacter* spp.	*Salmonella* spp.	100.0% (3/3)	100.0% (3/3)

Co-infection Combination	Target at 100x LoD	Target at 3x LoD	% Positivity of Target at 100x LoD	% Positivity of Target at 3x LoD
22-A	Shiga-like toxin-producing *E. coli* (STEC) *stx1*	*Shigella*/Enteroinvasive *E. coli* (EIEC)	100.0% (3/3)	100.0% (3/3)
22-B	*Shigella*/Enteroinvasive *E. coli* (EIEC)	Shiga-like toxin-producing *E. coli* (STEC) *stx1*	100.0% (3/3)	100.0% (3/3)
23-A	*Shigella*/Enteroinvasive *E. coli* (EIEC)	*Salmonella* spp.	100.0% (3/3)	100.0% (3/3)
23-B	*Salmonella* spp.	*Shigella*/Enteroinvasive *E. coli* (EIEC)	100.0% (3/3)	100.0% (3/3)

Carry-Over/Cross Contamination

The potential for carry-over/cross contamination using the LIAISON PLEX Gastrointestinal *Flex* Assay was assessed through testing high positive samples (containing representative assay targets) in an alternating series with negative samples (containing no targets) in a checkerboard design. On each instrument, negative samples were tested immediately following testing of high positive samples within the same module to evaluate possible carry-over. This alternating series was continued across five valid rounds of testing performed by two operators using one of two LIAISON PLEX instruments with six modules. The high positive sample was contrived in Cary-Blair Negative Stool Matrix containing *Salmonella enterica* (representative DNA target) at 10^6 CFU/mL and Rotavirus A (representative RNA target) at 10^6 copies/mL. The negative sample was prepared from Cary-Blair Negative Stool Matrix only. All high positive samples and negative samples yielded the expected results. No carry-over or cross contamination was observed.

4. Detection Limit and Assay Reportable Range:

Limit of Detection

A Limit of Detection (LoD) study was performed to determine the analytical sensitivity of the LIAISON PLEX Gastrointestinal *Flex* Assay. Testing included an initial estimation of the LoD followed by a final confirmation of each analyte's LoD. The LoD was estimated for each strain/sample using a 3-fold dilution series and four replicates per dilution (concentration). The preliminary LoD was identified as the lowest concentration where a 100% detection rate was observed. The LoD was confirmed for each strain by testing serial concentrations beginning at the preliminary LoD, with subsequent increases or decreases in three-fold increments until the lowest concentration demonstrating ≥95% detection across replicates was observed. During the confirmation phase of testing, twenty replicates were evaluated at each concentration across 3 assay kits. When multiple strains or samples per assay target call were tested, the highest LoD observed was defined as the LoD claim for the target. All assay targets were evaluated in Cary-Blair stool matrix. For parasite targets included in the assay, these were additionally evaluated in two parasite fixative stool matrices. **Table 14** below shows the LoD per strain, as well as the overall LoD reported for each LIAISON PLEX Gastrointestinal *Flex* Assay analyte.

Table 14: LIAISON PLEX Gastrointestinal *Flex* Assay Limit of Detection Summary by Strain/Sample

LIAISON PLEX GI Assay Target	Organism	Strain / Sample	Media	LoD per Strain	Percent Positivity at LoD	LoD per Target
Bacterial Targets						
***Campylobacter* spp.**	*Campylobacter coli*	ATCC 43482	Cary-Blair	5.65E+00 CFU/mL	95% (19/20)	4.57E+02 CFU/mL
	Campylobacter jejuni subsp. *jejuni*	ATCC 29428	Cary-Blair	4.57E+02 CFU/mL	100% (20/20)	
	Campylobacter lari	ATCC 35222	Cary-Blair	1.69E+01 CFU/mL	100% (20/20)	
	Campylobacter upsaliensis	ATCC 43953	Cary-Blair	5.08E+01 CFU/mL	100% (20/20)	
***Clostridioides difficile* (*tcdA/tcdB*)**	*Clostridioides difficile*	ATCC 43255	Cary-Blair	1.88E+00 CFU/mL	100% (20/20)	5.65E+00 CFU/mL
	Clostridioides difficile	ATCC BAA-1805	Cary-Blair	5.65E+00 CFU/mL	95% (19/20)	
Enterotoxigenic *E.coli* (ETEC) *LT/ST*	*Escherichia coli* (LT+, ST1A+, ST1B+)	ATCC 35401	Cary-Blair	4.57E+02 CFU/mL	100% (20/20)	4.57E+02 CFU/mL
	Escherichia coli (ST1B+)	ATCC 43896	Cary-Blair	4.57E+02 CFU/mL	100% (20/20)	
Plesiomonas shigelloides	*Plesiomonas shigelloides*	ATCC 51903	Cary-Blair	1.37E+03 CFU/mL	100% (20/20)	4.12E+03 CFU/mL
	Plesiomonas shigelloides	ATCC 14029	Cary-Blair	4.12E+03 CFU/mL	100% (20/20)	
***Salmonella* spp.**	*Salmonella enterica* subsp. *enterica* serovar Typhimurium	ATCC 13311	Cary-Blair	1.37E+03 CFU/mL	100% (20/20)	1.37E+03 CFU/mL
	Salmonella enterica subsp. *arizonae*	ATCC 13314	Cary-Blair	1.37E+03 CFU/mL	100% (20/20)	
Shiga-like toxin-producing *E.coli* (STEC) *stx1*	*Escherichia coli* (O157:H7), *STX1*	ATCC 43890	Cary-Blair	1.37E+03 CFU/mL	100% (20/20)	1.37E+03 CFU/mL
Shiga-like toxin-producing *E.coli* (STEC) *stx2*	*Escherichia coli* (O104:H4), *STX2*	ATCC BAA-2326	Cary-Blair	4.57E+02 CFU/mL	100% (20/20)	4.57E+02 CFU/mL

LIAISON PLEX GI Assay Target	Organism	Strain / Sample	Media	LoD per Strain	Percent Positivity at LoD	LoD per Target
***Shigella*/Enteroinvasive *E.coli* (EIEC)**	*Shigella boydii*	ATCC 25930	Cary-Blair	1.52E+02 CFU/mL	95% (19/20)	4.57E+02 CFU/mL
	Shigella flexneri	ATCC 25929	Cary-Blair	4.57E+02 CFU/mL	100% (20/20)	
Vibrio cholerae	*Vibrio cholerae* (O:1)	ATCC 39315	Cary-Blair	4.12E+03 CFU/mL	100% (20/20)	4.12E+03 CFU/mL
	Vibrio cholerae (O:139)	ATCC 51394	Cary-Blair	4.12E+03 CFU/mL	100% (20/20)	
***Vibrio* spp.**	*Vibrio parahaemolyticus*	ATCC 49398	Cary-Blair	1.37E+03 CFU/mL	100% (20/20)	1.37E+03 CFU/mL
	Vibrio vulnificus	ATCC 27562	Cary-Blair	4.57E+02 CFU/mL	100% (20/20)	
Yersinia enterocolitica	*Yersinia enterocolitica*	ATCC 23715	Cary-Blair	1.37E+03 CFU/mL	95% (19/20)	1.37E+03 CFU/mL
	Yersinia enterocolitica	ATCC 700822	Cary-Blair	1.37E+03 CFU/mL	100% (20/20)	
Viral Targets						
Adenovirus F40/41	Human adenovirus 40	ATCC VR-931	Cary-Blair	4.57E+02 copies/mL	95% (19/20)	4.57E+02 copies/mL
	Human adenovirus 41	ATCC VR-930	Cary-Blair	4.57E+02 copies/mL	100% (20/20)	
Astrovirus	Astrovirus Type 1	ATCC VR-1936	Cary-Blair	1.00E+06 copies/mL	100% (20/20)	1.00E+06 copies/mL
	Astrovirus Type 2	ATCC VR-1943	Cary-Blair	1.11E+05 copies/mL	100% (20/20)	
Norovirus GI/GII	Norovirus GI	Zeptometrix 0830086CFL	Cary-Blair	2.75E+04 copies/mL	100% (20/20)	3.70E+04 copies/mL
	Norovirus GII	Zeptometrix 0830087CFL	Cary-Blair	3.70E+04 copies/mL	100% (20/20)	
Rotavirus A	Rotavirus A	ATCC VR-2550	Cary-Blair	1.23E+04 copies/mL	100% (20/20)	1.23E+04 copies/mL
	Rotavirus A	ATCC VR-2551	Cary-Blair	4.57E+02 copies/mL	100% (20/20)	
Sapovirus I/II/IV/V	Sapovirus GI	Clinical Specimen LMNX-2829	Cary-Blair	1.23E+04 copies/mL	100% (20/20)	1.23E+04 copies/mL
	Sapovirus GII	Clinical Specimen LMNX-2964	Cary-Blair	1.23E+04 copies/mL	95% (19/20)	
Parasitic Targets						

LIAISON PLEX GI Assay Target	Organism	Strain / Sample	Media	LoD per Strain	Percent Positivity at LoD	LoD per Target
***Blastocystis* sp.**	*Blastocystis hominis*	ATCC 50177	Cary-Blair	1.69E+01 cells/mL	100% (20/20)	Cary-Blair: 4.57E+02 cells/mL EcoFix: 1.52E+02 cells/mL Total-Fix: 5.08E+01 cells/mL
			EcoFix	1.52E+02 cells/mL	100% (20/20)	
			Total-Fix	5.65E+00 cells/mL	95% (19/20)	
	Blastocystis hominis	ATCC 50608	Cary-Blair	4.57E+02 cells/mL	100% (20/20)	
			EcoFix	1.52E+02 cells/mL	100% (20/20)	
			Total-Fix	5.08E+01 cells/mL	100% (20/20)	
***Cryptosporidium* spp.**	*Cryptosporidium parvum*	Waterborne P102C	Cary-Blair	1.37E+03 oocysts/mL	100% (20/20)	Cary-Blair: 4.12E+03 oocysts/mL EcoFix: 4.12E+03 oocysts/mL Total-Fix: 4.12E+03 oocysts/mL
			EcoFix	4.12E+03 oocysts/mL	100% (20/20)	
			Total-Fix	1.37E+03 oocysts/mL	95% (19/20)	
	Cryptosporidium meleagridis	TU1867	Cary-Blair	4.12E+03 oocysts/mL	100% (20/20)	
			EcoFix	1.37E+03 oocysts/mL	100% (20/20)	
			Total-Fix	4.12E+03 oocysts/mL	100% (20/20)	
	Cryptosporidium muris	Waterborne P104	Cary-Blair	4.12E+03 oocysts/mL	100% (20/20)	
			EcoFix	1.37E+03 oocysts/mL	100% (20/20)	
			Total-Fix	4.12E+03 oocysts/mL	100% (20/20)	
Cyclospora cayetanensis	*Cyclospora cayetanensis*	Clinical Specimen LMNX-3415	Cary-Blair	4.12E+03 copies/mL	100% (20/20)	Cary-Blair: 4.12E+03 copies/mL EcoFix: Not Found[1] Total-Fix: 4.12E+03 copies/mL
	Cyclospora cayetanensis	Clinical Specimen LMNX-3862	Cary-Blair	7.57E+02 copies/mL	100% (20/20)	
			EcoFix	Not Found[1]	N/A	
			Total-Fix	4.12E+03 copies/mL	95% (19/20)	
Dientamoeba fragilis	*Dientamoeba fragilis*	D.fragilis-1	Cary-Blair	1.52E+02 copies/mL	100% (20/20)	Cary-Blair: 4.57E+02 copies/mL EcoFix: 4.57E+02 copies/mL Total-Fix: 4.57E+02 copies/mL
			EcoFix	1.52E+02 copies/mL	100% (20/20)	
			Total-Fix	1.52E+02 copies/mL	100% (20/20)	
	Dientamoeba fragilis	D.fragilis-2	Cary-Blair	4.57E+02 copies/mL	100% (20/20)	

LIAISON PLEX GI Assay Target	Organism	Strain / Sample	Media	LoD per Strain	Percent Positivity at LoD	LoD per Target
			EcoFix	4.57E+02 copies/mL	100% (20/20)	
			Total-Fix	4.57E+02 copies/mL	100% (20/20)	
Entamoeba histolytica	*Entamoeba histolytica*	ATCC 30459	Cary-Blair	6.97E-02 cells/mL	100% (20/20)	Cary-Blair: 1.88E+00 cells/mL EcoFix: 1.88E+00 cells/mL Total-Fix: 1.88E+00 cells/mL
			EcoFix	6.97E-02 cells/mL	100% (20/20)	
			Total-Fix	6.97E-02 cells/mL	95% (19/20)	
	Entamoeba histolytica	ATCC 50524	Cary-Blair	1.88E+00 cells/mL	100% (20/20)	
			EcoFix	1.88E+00 cells/mL	100% (20/20)	
			Total-Fix	1.88E+00 cells/mL	100% (20/20)	
Giardia lamblia	*Giardia intestinalis*	ATCC 30888	Cary-Blair	5.08E+01 cells/mL	95% (19/20)	Cary-Blair: 1.52E+02 cells/mL EcoFix: 1.52E+02 cells/mL Total-Fix: 1.52E+02 cells/mL
			EcoFix	1.52E+02 cells/mL	95% (19/20)	
			Total-Fix	5.08E+01 cells/mL	95% (19/20)	
	Giardia lamblia	ATCC PRA-254	Cary-Blair	1.52E+02 cells/mL	100% (20/20)	
			EcoFix	1.52E+02 cells/mL	100% (20/20)	
			Total-Fix	1.52E+02 cells/mL	100% (20/20)	
Microsporidia	*Encephalitozoon hellem*	ATCC 50451	Cary-Blair	2.08E+03 copies/mL	95% (19/20)	Cary-Blair: 2.08E+03 copies/mL EcoFix: 1.87E+04 copies/mL Total-Fix: 6.25E+03 copies/mL
			EcoFix	1.87E+04 copies/mL	100% (20/20)	
			Total-Fix	6.25E+03 copies/mL	100% (20/20)	
	Encephalitozoon intestinalis	ATCC 50651	Cary-Blair	3.97E+02 copies/mL	95% (19/20)	
			EcoFix	3.57E+03 copies/mL	100% (20/20)	
			Total-Fix	1.19E+03 copies/mL	100% (20/20)	
	Enterocytozoon bieneusi	Clinical Specimen LMNX-2885	Cary-Blair	1.37E+03 copies/mL	100% (20/20)	
	Enterocytozoon bieneusi	Clinical Specimen LMNX-4140	Cary-Blair	1.37E+03 copies/mL	100% (20/20)	
			EcoFix	4.12E+03 copies/mL	100% (20/20)	

LIAISON PLEX GI Assay Target	Organism	Strain / Sample	Media	LoD per Strain	Percent Positivity at LoD	LoD per Target
			Total-Fix	1.37E+03 copies/mL	100% (20/20)	
Strongyloides stercoralis	*Strongyloides stercoralis*	Clinical Specimen LMNX-3433[2]	Cary-Blair	2.03E+04 copies/mL	95% (19/20)	Cary-Blair: 2.03E+04 copies/mL EcoFix: 2.72E+04 copies/mL Total-Fix: 2.16E+04 copies/mL
			EcoFix	2.72E+04 copies/mL	95% (19/20)	
			Total Fix	2.16E+04 copies/mL	100% (20/20)	

[1] To further assess analytical sensitivity in EcoFix relative to Total-Fix, an additional clinical raw stool specimen (LMNX-4486) containing *Cyclospora cayetanensis* was identified and used for additional evaluation. At neat (undiluted) concentration, detection was 19/20 (95%) in Eco-Fix and 20/20 (100%) in Total-Fix. The LoD per target for both EcoFix and Total-Fix was reported as 9.95E+02 copies/mL. At a three-fold dilution in Negative Stool Matrix, the detection rate of the same specimen was 15/19 (79%) in EcoFix and 18/20 (90%) in Total-Fix. The analytical data from parallel matrix testing and the contrived clinical study results provide supportive evidence that detection of *Cyclospora cayetanensis* in EcoFix is comparable to that observed in Total-Fix at concentrations near the limit of detection.

[2] In the original LoD study, testing with this clinical specimen yielded substantial variability in the stock concentration across matrices Results demonstrated that the variability in measured copies/mL in the original study was present even during the quantification stage pre-LoD. Therefore, testing was repeated with independently prepared material. Results of new LoD testing showed detection at similar copies/mL, which are included in the table above.

Inclusivity

Analytical Reactivity (Inclusivity) of the LIAISON PLEX Gastrointestinal *Flex* Assay was assessed with a collection of 243 strains and clinical samples, representing the genetic diversity of the analytes targeted by the assay. While fifty of the strains/clinical samples were used to determine the LoD for each assay target, the remaining 193 strains/clinical samples were diluted in Cary-Blair Negative Stool Matrix to a final concentration of 3x LoD or the highest available concentration where stock concentrations were limiting. Each diluted strain/clinical sample was tested in triplicate. In cases where 100% positivity was not achieved at 3x LoD, higher concentrations of each analyte (up to 1000x LoD) were tested until 100% positivity was achieved.

Of the 243 strains/clinical samples tested, four had unknown concentrations. All four of the samples with unknown concentrations demonstrated 100% positivity in wet testing. Of the 239 strains/clinical samples tested at known concentrations, fifty demonstrated ≥95% positivity at 1x LoD (strains used previously to determine LoD). Of the remaining 189 strains/clinical samples tested at known concentrations: 168 demonstrated 100% positivity at ≤3x LoD, five (5) demonstrated 100% positivity at 10x LoD, thirteen (13) demonstrated 100% positivity at 100x LoD, and three (3) demonstrated 100% positivity at 1000x LoD. Results of the Analytical Reactivity study are shown in **Table 15** through **Table 18** below.

Table 15: Summary of Analytical Reactivity Study Results

Organism	Number of Strains/Clinical Samples						
	Total Tested	≥95% Positivity at 1x LoD	100% Positivity at ≤3x LoD	100% Positivity at 10x LoD	100% Positivity at 100x LoD	100% Positivity at 1000x LoD	100% Positivity at Unknown Concentration
Bacteria	147	22	111	3	11	---	---
Viruses	55	10	39	2	2	1	1[1,2]
Parasites	41	18	18	---	---	2	3[2]
Total	243	50	168	5	13	3	4

[1]Sample tested neat

[2]Sample concentration unknown

Table 16: Analytical Reactivity (Inclusivity) Bacterial Strains

Target Call	Species/ Species Type	Serotype/ Toxin	Source/	Strain/ Clinical Sample (LoD strains are in bold font)	Test Level (xLoD)	Test Concentration (CFU/mL)	% Positivity
Campylobacter spp.	*Campylobacter coli*	N/A	N/A	**ATCC 43482**	1x	5.65E+00	95% (19/20)
	Campylobacter coli			ATCC 43488	100x	5.65E+02	100% (3/3)
	Campylobacter coli			ATCC 43483	100x	5.65E+02	100% (3/3)
	Campylobacter coli			ATCC BAA-371	100x	5.65E+02	100% (3/3)
	Campylobacter coli			ATCC 43489	100x	5.65E+02	100% (3/3)
	Campylobacter jejuni (Including subsp.*jejuni and* subsp.*doylei)*			ATCC 49349	3x	1.37E+03	100% (3/3)
	Campylobacter jejuni (Including subsp.*jejuni and* subsp.*doylei)*			**ATCC 29428**	1x	4.57E+02	100% (20/20)
	Campylobacter jejuni (Including subsp.*jejuni and* subsp.*doylei)*			ATCC 43479	3x	1.37E+03	100% (3/3)
	Campylobacter jejuni (Including subsp.*jejuni and* subsp.*doylei)*			ATCC 43474	3x	1.37E+03	100% (3/3)
	Campylobacter jejuni (Including subsp.*jejuni and* subsp.*doylei)*			ATCC 43465	3x	1.37E+03	100% (3/3)
	Campylobacter upsaliensis			ATCC 43954	100x	5.08E+03	100% (3/3)
	Campylobacter upsaliensis			ATCC BAA-1059	3x	1.52E+02	100% (3/3)
	Campylobacter upsaliensis			**ATCC 43953**	1x	5.08E+01	100% (20/20)
	Campylobacter upsaliensis			ATCC 49816	3x	1.52E+02	100% (3/3)
	Campylobacter upsaliensis			ATCC 49815	3x	1.52E+02	100% (3/3)
	Campylobacter lari			**ATCC 35222**	1x	1.69E+01	100% (20/20)
	Campylobacter lari			ATCC 43675	100x	1.69E+03	100% (3/3)
	Campylobacter lari			ATCC 35223	100x	1.69E+03	100% (3/3)
	Campylobacter lari			ATCC BAA-1060	100x	1.69E+03	100% (3/3)
	Campylobacter lari			ATCC 35221	100x	1.69E+03	100% (3/3)
Clostridioides difficile (*tcdA/tcdB*)	*Clostridioides difficile*	0, A+, B+		**ATCC 43255 (CCUG19126, VPI 10463)**	1x	1.88E+00	100% (20/20)
	Clostridioides difficile	0, A+, B+		ATCC 9689 (90556-M6S)	3x	1.70E+01	100% (3/3)
	Clostridioides difficile	0, A+, B+		ATCC 700792 (14797-2)	3x	1.70E+01	100% (3/3)
	Clostridioides difficile	0, A+, B+		ATCC 17858 (1253)	3x	1.70E+01	100% (3/3)
	Clostridioides difficile	IIIb, A+, B+		**ATCC BAA-1805 (NAP I strain)**	1x	5.65E+00	95% (19/20)

Target Call	Species/ Species Type	Serotype/ Toxin	Source/	Strain/ Clinical Sample (LoD strains are in bold font)	Test Level (xLoD)	Test Concentration (CFU/mL)	% Positivity
	Clostridioides difficile	0, A+, B+		ATCC BAA-1382 (630)	3x	1.70E+01	100% (3/3)
	Clostridioides difficile	0, A+, B+		ATCC 51695 (BDMS 18 AN)	3x	1.70E+01	100% (3/3)
	Clostridioides difficile	0, A+, B+		ATCC 43600 (2149)	3x	1.70E+01	100% (3/3)
	Clostridioides difficile	0, A+, B+		ATCC 43599 (2022)	3x	1.70E+01	100% (3/3)
	Clostridioides difficile	0, A+, B+		ATCC 43596 (545)	3x	1.70E+01	100% (3/3)
	Clostridioides difficile	0, A+, B+		ATCC 43594 (W1194)	3x	1.70E+01	100% (3/3)
	Clostridioides difficile	0, A+, B+		ATCC 17857 (870)	3x	1.70E+01	100% (3/3)
	Clostridioides difficile	VIII, A-, B+		ATCC 43598 (1470)	3x	1.70E+01	100% (3/3)
	Clostridioides difficile	X, A-, B+		CCUG 20309 (Also known as CCUG 8864)	3x	1.70E+01	100% (3/3)
	Clostridioides difficile	N/A		ATCC 43597	100x	5.65E+02	100% (3/3)
	Clostridioides difficile			ATCC BAA-1803	3x	1.70E+01	100% (3/3)
	Clostridioides difficile			ATCC BAA-1875	3x	1.70E+01	100% (3/3)
	Clostridioides difficile			ATCC BAA-1814	3x	1.70E+01	100% (3/3)
	Clostridioides difficile			ATCC BAA-1870	3x	1.70E+01	100% (3/3)
	Clostridioides difficile			ATCC BAA-1812	3x	1.70E+01	100% (3/3)
	Clostridioides difficile			ATCC BAA-1808	3x	1.70E+01	100% (3/3)
	Clostridioides difficile			ATCC BAA-1874	3x	1.70E+01	100% (3/3)
Enterotoxigenic *E.coli* (ETEC) *LT/ST*	Enterotoxigenic *Escherichia coli* (ETEC, *LT/ST*)	LT+, ST1A+, ST1B+		**ATCC 35401**	1x	4.57E+02	100% (20/20)
	Enterotoxigenic *Escherichia coli* (ETEC, *LT/ST*)	LT+, ST1A+		CI Penn State 14.1385	3x	1.37E+03	100% (3/3)
	Enterotoxigenic *Escherichia coli* (ETEC, *LT/ST*)	ST1A+		CI Penn State 10.0049	3x	1.37E+03	100% (3/3)
	Enterotoxigenic *Escherichia coli* (ETEC, *LT/ST*)	ST1B+		**ATCC 43896**	1x	4.57E+02	100% (20/20)
	Enterotoxigenic *Escherichia coli* (ETEC, *LT/ST*)	LT+		ATCC 43886	3x	1.37E+03	100% (3/3)
Plesiomonas shigelloides	*Plesiomonas shigelloides*	N/A		ATCC 51572	3x	1.24E+04	100% (3/3)
	Plesiomonas shigelloides			Zeptometrix 0801899	3x	1.24E+04	100% (3/3)
	Plesiomonas shigelloides			**ATCC 51903**	1x	1.37E+03	100% (20/20)
	Plesiomonas shigelloides			**ATCC 14029**	1x	4.12E+03	100% (20/20)

Target Call	Species/ Species Type	Serotype/ Toxin	Source/	Strain/ Clinical Sample (LoD strains are in bold font)	Test Level (xLoD)	Test Concentration (CFU/mL)	% Positivity
	Plesiomonas shigelloides			CI OSUPS-1	3x	1.24E+04	100% (3/3)
Salmonella spp.	*Salmonella enterica* subsp. *enterica*	I	*Agona*	ATCC 51957	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Anatum*	ATCC 9270	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Bareilly*	ATCC 9115	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Berta*	ATCC 8392	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Braenderup*	ATCC BAA-664	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Enteritidis*	ATCC 13076	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Heidelberg*	ATCC 8326	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*I4,[5],12:i-*	ATCC 700720	10x	1.37E+04	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Infantis*	ATCC 51741	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Javiana*	ATCC BAA-1593	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Mississippi*	ATCC BAA-2739	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Montevideo*	ATCC 8387	10x	1.37E+04	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Muenchen*	ATCC BAA-1594	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Newport*	ATCC 27869	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Oranienburg*	ATCC 9239	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Paratyphi B*	ATCC 10719	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Saintpaul*	ATCC 9712	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Thompson*	ATCC BAA-3141	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Typhi*	ATCC 9993	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Typhimurium*	**ATCC 13311**	1x	1.37E+03	100% (20/20)
	Salmonella enterica subsp. *enterica*		*Choleraesuis*	ATCC 7001	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Dublin*	ATCC 39184	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *enterica*		*Paratyphi A*	ATCC 11511	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *salamae*	II	N/A	ATCC 700148	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *arizonae*	IIIa		**ATCC 13314**	1x	1.37E+03	100% (20/20)
	Salmonella enterica subsp. *diarizonae*	IIIb		ATCC 12325	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *houtenae*	IV		ATCC 43974	3x	4.11E+03	100% (3/3)
	Salmonella enterica subsp. *Indica*	VI		ATCC 43976	3x	4.11E+03	100% (3/3)

Target Call	Species/ Species Type	Serotype/ Toxin	Source/	Strain/ Clinical Sample (LoD strains are in bold font)	Test Level (xLoD)	Test Concentration (CFU/mL)	% Positivity
	Salmonella bongori	N/A		ATCC 43975	3x	4.11E+03	100% (3/3)
Shiga-like toxin-producing *E. coli* (STEC) *stx1*	*Escherichia coli,* O157	O157:H7		**ATCC 43890**	1x	1.37E+03	100% (20/20)
	Escherichia coli, Non-O157	O26:H11		CI MSU DEC10C	3x	4.11E+03	100% (3/3)
	Escherichia coli, Non-O157	O26:NM		CI MSU TW07948	3x	4.11E+03	100% (3/3)
	Escherichia coli, Non-O157	O45:H2		CI MSU TW10121	3x	4.11E+03	100% (3/3)
	Escherichia coli, Non-O157	O103:H2		CI TW08101	3x	4.11E+03	100% (3/3)
	Escherichia coli, Non-O157	O111:H8		ATCC BAA-184	3x	4.11E+03	100% (3/3)
	Escherichia coli, Non-O157	O118:H2		CI CDC 2009C-4091	3x	4.11E+03	100% (3/3)
	Escherichia coli, Non-O157	O145:NM		ATCC BAA-2130	3x	4.11E+03	100% (3/3)
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	*Escherichia coli,* O157	O157:H7		ATCC 43894	3x	4.11E+03	100% (3/3)
	Escherichia coli, O157	O157:H7		ATCC 35150	3x	4.11E+03	100% (3/3)
	Escherichia coli, O157	O157:H7		ATCC 43895	3x	4.11E+03	100% (3/3)
	Escherichia coli, Non-O157	O26:H11		ATCC BAA-2196	3x	4.11E+03	100% (3/3)
	Escherichia coli, Non-O157	O111:H8		ATCC BAA-179	3x	4.11E+03	100% (3/3)
	Escherichia coli, Non-O157	O111:NM		CI TN DOH N10E001241	3x	4.11E+03	100% (3/3)
	Escherichia coli, Non-O157	O113:H21		ATCC BAA-177	3x	4.11E+03	100% (3/3)
Shiga-like toxin-producing *E. coli* (STEC) *stx2*	*Escherichia coli,* O157	O157:NM		CI TN DOH 61361	3x	1.37E+03	100% (3/3)
	Escherichia coli, Non-O157	O104:H21		ATCC BAA-178	3x	1.37E+03	100% (3/3)
	Escherichia coli, Non-O157	O104:H4		**ATCC BAA-2326**	1x	4.57E+02	100% (20/20)
	Escherichia coli, Non-O157	O111:H2		CI TW06296	3x	1.37E+03	100% (3/3)
	Escherichia coli, Non-O157	O121:H19		ATCC BAA-2219	3x	1.37E+03	100% (3/3)
Shigella/ Enteroinvasive *E.coli* (EIEC)	*Shigella boydii*	N/A		ATCC 12031	3x	4.56E+02	100% (3/3)
	Shigella boydii			ATCC 12028	3x	4.56E+02	100% (3/3)
	Shigella boydii			ATCC 12030	10x	1.52E+03	100% (3/3)
	Shigella boydii			ATCC 12035	3x	4.56E+02	100% (3/3)
	Shigella boydii			**ATCC 25930**	1x	1.52E+02	95% (19/20)
	Shigella dysenteriae			ATCC 9361	3x	1.37E+03	100% (3/3)
	Shigella dysenteriae			ATCC 12037	3x	1.37E+03	100% (3/3)
	Shigella dysenteriae			ATCC 49549	3x	1.37E+03	100% (3/3)

Target Call	Species/ Species Type	Serotype/ Toxin	Source/	Strain/ Clinical Sample (LoD strains are in bold font)	Test Level (xLoD)	Test Concentration (CFU/mL)	% Positivity
	Shigella dysenteriae			ATCC 49556	3x	1.37E+03	100% (3/3)
	Shigella dysenteriae			ATCC 29027	3x	1.37E+03	100% (3/3)
	Shigella flexneri			ATCC 12022	3x	1.37E+03	100% (3/3)
	Shigella flexneri			**ATCC 25929**	1x	4.57E+02	100% (20/20)
	Shigella flexneri			ATCC 29903	3x	1.37E+03	100% (3/3)
	Shigella flexneri			ATCC 49070	3x	1.37E+03	100% (3/3)
	Shigella flexneri			ATCC 700930	3x	1.37E+03	100% (3/3)
	Shigella sonnei			ATCC 9290	3x	1.37E+03	100% (3/3)
	Shigella sonnei			ATCC 25931	3x	1.37E+03	100% (3/3)
	Shigella sonnei			ATCC 29029	3x	1.37E+03	100% (3/3)
	Shigella sonnei			ATCC 29030	3x	1.37E+03	100% (3/3)
	Shigella sonnei			ATCC 11060	3x	1.37E+03	100% (3/3)
	Enteroinvasive *Escherichia coli* (EIEC)			ATCC 43892	3x	1.37E+03	100% (3/3)
	Enteroinvasive *Escherichia coli* (EIEC)			ATCC 43893	3x	1.37E+03	100% (3/3)
	Enteroinvasive *Escherichia coli* (EIEC)			CI Penn State 86.0937	3x	1.37E+03	100% (3/3)
	Enteroinvasive *Escherichia coli* (EIEC)			CI Penn State 85.1797	3x	1.37E+03	100% (3/3)
	Enteroinvasive *Escherichia coli* (EIEC)			CI Penn State 85.1792	3x	1.37E+03	100% (3/3)
Vibrio cholerae	*Vibrio cholerae*	O1		ATCC 14100	3x	1.24E+04	100% (3/3)
	Vibrio cholerae			ATCC 25870	3x	1.24E+04	100% (3/3)
	Vibrio cholerae			**ATCC 39315**	1x	4.12E+03	100% (20/20)
	Vibrio cholerae	O139		**ATCC 51394**	1x	4.12E+03	100% (20/20)
	Vibrio cholerae	Non-O1/ Non-O139		ATCC 25874	3x	1.24E+04	100% (3/3)
Vibrio spp.	*Vibrio parahaemolyticus*	N/A		ATCC 17802	3x	4.11E+03	100% (3/3)
	Vibrio parahaemolyticus			ATCC 35118	3x	4.11E+03	100% (3/3)
	Vibrio parahaemolyticus			**ATCC 49398**	1x	1.37E+03	100% (20/20)
	Vibrio parahaemolyticus			ATCC 49529	3x	4.11E+03	100% (3/3)
	Vibrio parahaemolyticus			ATCC BAA-238	3x	4.11E+03	100% (3/3)
	Vibrio vulnificus			**ATCC 27562**	1x	4.57E+02	100% (20/20)
	Vibrio vulnificus			ATCC 29307	3x	1.37E+03	100% (3/3)

Target Call	Species/ Species Type	Serotype/ Toxin	Source/	Strain/ Clinical Sample (LoD strains are in bold font)	Test Level (xLoD)	Test Concentration (CFU/mL)	% Positivity
	Vibrio vulnificus			ATCC 29306	3x	1.37E+03	100% (3/3)
	Vibrio vulnificus			ATCC 33817	3x	1.37E+03	100% (3/3)
	Vibrio vulnificus			ATCC BAA-86	100x	4.57E+04	100% (3/3)
Yersinia enterocolitica	*Yersinia enterocolitica*	O:3		**ATCC 700822**	1x	1.37E+03	100% (20/20)
	Yersinia enterocolitica	O:5,27		CI CDC B5725	3x	4.11E+03	100% (3/3)
	Yersinia enterocolitica	O:8		ATCC 9610	3x	4.11E+03	100% (3/3)
	Yersinia enterocolitica			**ATCC 23715**	1x	1.37E+03	95% (19/20)
	Yersinia enterocolitica	O:9		ATCC 700823	3x	4.11E+03	100% (3/3)
	Yersinia enterocolitica	N/A		ATCC 49397	3x	4.11E+03	100% (3/3)

Table 17: Analytical Reactivity (Inclusivity) Viral Strains

Target Call	Species/Species Type	Serotype / Toxin	Part Number (LoD strains are in bold font)	Test Level (xLoD)	Test Concentration	% Positivity
Adenovirus F40/41	Adenovirus 40	N/A	**ATCC VR-931**	1x	4.57E+02 copies/mL	100% (20/20)
	Adenovirus 40		Zeptometrix 0810084CF	3x	1.37E+03 copies/mL	100% (3/3)
	Adenovirus 40		Virapur Tak 40	10x	4.57E+03 copies/mL	100% (3/3)
	Adenovirus 41		**ATCC VR-930**	1x	4.57E+02 copies/mL	95% (19/20)
	Adenovirus 41		Zeptometrix 0810085CF	3x	1.37E+03 copies/mL	100% (3/3)
	Adenovirus 41		Virapur Tak 41	3x	1.37E+03 copies/mL	100% (3/3)
	Adenovirus 41		LMNX-2729	3x	1.37E+03 copies/mL	100% (3/3)
	Adenovirus 41		LMNX-2734	3x	1.37E+03 copies/mL	100% (3/3)
Astrovirus	Astrovirus, Type 1	Type 1	**ATCC VR-1936**	1x	1.00E+06 copies/mL	100% (20/20)
	Astrovirus, Type 2	Type 2	**ATCC VR-1943**	1x	1.11E+05 copies/mL	100% (20/20)
	Astrovirus, Type 3	Type 3	ATCC VR-1944	3x	3.00E+06 copies/mL	100% (3/3)
	Astrovirus, Type 4	Type 4	ATCC VR-1945	3x	3.00E+06 copies/mL	100% (3/3)
	Astrovirus, Type 5	Type 5	ATCC VR-1947	3x	3.00E+06 copies/mL	100% (3/3)
	Astrovirus, Type 6	Type 6	ATCC VR-1948	3x	3.00E+06 copies/mL	100% (3/3)
	Astrovirus, Type 7	Type 7	ATCC VR-1949	3x	3.00E+06 copies/mL	100% (3/3)

Target Call	Species/Species Type	Serotype / Toxin	Part Number (LoD strains are in bold font)	Test Level (xLoD)	Test Concentration	% Positivity
	Astrovirus, Type 8	Type 8	Zeptometrix 0810277CF	3x	4.20E+04 TCID50/mL	100% (3/3)
Norovirus GI/GII	Norovirus GI	GI.1	LMNX-3117	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GI	GI.2	PGI01-0263	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GI	GI.3	LMNX-3229	100x	2.75E+06 copies/mL	100% (3/3)
	Norovirus GI	GI.5	LMNX-2947	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GI	GI.6	PGI02-0617	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GI	GI.7	LMNX-3000	1000x	2.75E+07 copies/mL	100% (3/3)
	Norovirus GI	N/A	**Zeptometrix 0830086CFL**	1x	2.75E+04 copies/mL	100% (20/20)
	Norovirus GII	GII.1	LMNX-916	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GII	GII.2	LMNX-2951	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GII	GII.3	PGI05-0019	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GII	GII.4	LMNX-928	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GII		LMNX-1151	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GII		LMNX-2936	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GII		LMNX-3509	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GII	GII.6	LMNX-915	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GII	GII.7	LMNX-704	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GII	GII.8	LMNX-3005	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GII	GII.9	LMNX-3582	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GII	GII.12	LMNX-2963	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GII	GII.17	PGI01-0570	3x	1.11E+05 copies/mL	100% (3/3)
	Norovirus GII	N/A	**Zeptometrix 0830087CFL**	1x	3.70E+04 copies/mL	100% (20/20)
Rotavirus A	Rotavirus Group A		**ATCC VR-2550**	1x	1.23E+04 copies/mL	100% (20/20)
	Rotavirus Group A	N/A	**ATCC VR-2551**	1x	4.57E+02 copies/mL	100% (20/20)
	Rotavirus Group A		ATCC VR-2272	3x	3.69E+04 copies/mL	100% (3/3)
	Rotavirus Group A		ATCC VR-2104	3x	3.69E+04 copies/mL	100% (3/3)
	Rotavirus Group A		CI MCRI G6P5 #476	3x	3.69E+04 copies/mL	100% (3/3)
	Rotavirus Group A		CI MCRI G8P10 #475	3x	3.69E+04 copies/mL	100% (3/3)

Target Call	Species/Species Type	Serotype / Toxin	Part Number (LoD strains are in bold font)	Test Level (xLoD)	Test Concentration	% Positivity
	Rotavirus Group A		CI MCRI G12P4 #262	3x	3.69E+04 copies/mL	100% (3/3)
Sapovirus I/II/IV/V	Sapovirus, GI	GI.1	**LMNX-2829**	1x	1.23E+04 copies/mL	100% (20/20)
	Sapovirus, GI	GI.2	LMNX-3419	100x	1.23E+06 copies/mL	100% (3/3)
	Sapovirus, GI	GI.3	LMNX-746	Neat	Unknown	100% (3/3)
	Sapovirus, GI	GI.6	LMNXC-1747	3x	3.69E+04 copies/mL	100% (3/3)
	Sapovirus, GII	GII.1	**LMNX-2964**	1x	1.23E+04 copies/mL	95% (19/20)
	Sapovirus, GII	GII.2	LMNX-2945	3x	3.69E+04 copies/mL	100% (3/3)
	Sapovirus, GII	GII.3	LMNX-080	3x	3.69E+04 copies/mL	100% (3/3)
	Sapovirus, GII	GII.5	LMNX-2967	3x	3.69E+04 copies/mL	100% (3/3)
	Sapovirus, GIV	GIV.1	LMNX-155	3x	3.69E+04 copies/mL	100% (3/3)
	Sapovirus, GV	GV.1	LMNX-3118	3x	3.69E+04 copies/mL	100% (3/3)
	Sapovirus, GV	GV.1	LMNX-3206	10x	1.23E+05 copies/mL	100% (3/3)

Table 18: Analytical Reactivity (Inclusivity) Parasite Strains

Target Call	Species/ Species Type	Part Number (LoD strains are in bold font)	Test Level (xLoD)	Test Concentration	% Positivity
Blastocystis sp.	*Blastocystis hominis*	**ATCC 50177**	1x	1.69E+01 cells/mL	100% (20/20)
	Blastocystis hominis	**ATCC 50608**	1x	4.57E+02 cells/mL	100% (20/20)
	Blastocystis hominis	ATCC 50587	3x	1.37E+03 copies/mL	100% (3/3)
	Blastocystis hominis	ATCC 50754	3x	1.37E+03 copies/mL	100% (3/3)
	Blastocystis hominis	ATCC 50609	3x	1.37E+03 copies/mL	100% (3/3)
Cryptosporidium spp.	*Cryptosporidium parvum*	**Waterborne P102C**	1x	1.37E+03 oocysts/mL	100% (20/20)
	Cryptosporidium parvum	UA180108	3x	4.11E+03 oocysts/mL	100% (3/3)
	Cryptosporidium parvum	ATCC 87760	Unknown	2.00E-01 relative to stock	100% (3/3)
	Cryptosporidium parvum	ATCC 87762	Unknown	2.00E-01 relative to stock	100% (3/3)

Target Call	Species/ Species Type	Part Number (LoD strains are in bold font)	Test Level (xLoD)	Test Concentration	% Positivity
	Cryptosporidium parvum	ATCC 87765	Unknown	1.00E-01 relative to stock	100% (3/3)
	Cryptosporidium hominis	TU502	3x	1.24E+04 oocysts/mL	100% (3/3)
	Cryptosporidium muris	**Waterborne P104**	1x	4.12E+03 oocysts/mL	100% (20/20)
	Cryptosporidium meleagridis	**TU1867**	1x	4.12E+03 oocysts/mL	100% (20/20)
Cyclospora cayetanensis	*Cyclospora cayetanensis*	**LMNX-3415**	1x	4.12E+03 copies/mL	100% (20/20)
	Cyclospora cayetanensis	**LMNX-3862**	1x	7.57E+02 copies/mL	100% (20/20)
	Cyclospora cayetanensis	LMNX-2562	1.2x Neat	4.81E+03 copies/mL	100% (3/3)
Dientamoeba fragilis	*Dientamoeba fragilis*	**D.fragilis-1**	1x	1.52E+02 copies/mL	100% (20/20)
	Dientamoeba fragilis	**D.fragilis-2**	1x	4.57E+02 copies/mL	100% (20/20)
	Dientamoeba fragilis	LMNX-2693	3x	1.37E+03 copies/mL	100% (3/3)
	Dientamoeba fragilis	LMNX-2896	3x	1.37E+03 copies/mL	100% (3/3)
	Dientamoeba fragilis	LMNX-3679	3x	1.37E+03 copies/mL	100% (3/3)
Entamoeba histolytica	*Entamoeba histolytica*	ATCC 30015	3x	5.65E+00 cells/mL	100% (3/3)
	Entamoeba histolytica	**ATCC 30459**	1x	6.97E-02 cells/mL	100% (20/20)
	Entamoeba histolytica	ATCC 30458	3x	5.65E+00 cells/mL	100% (3/3)
	Entamoeba histolytica	ATCC 50412	3x	5.65E+00 cells/mL	100% (3/3)
	Entamoeba histolytica	**ATCC 50524**	1x	1.88E+00 cells/mL	100% (20/20)
Giardia lamblia	*Giardia lamblia/ Giardia duodenalis/ Giardia intestinalis*	ATCC 30957	3x	1.52E+03 cells/mL	100% (3/3)
	Giardia lamblia/ Giardia duodenalis/ Giardia intestinalis	**ATCC 30888**	1x	5.08E+01 cells/mL	95% (19/20)
	Giardia lamblia/ Giardia duodenalis/ Giardia intestinalis	ATCC 50582	3x	1.52E+03 cells/mL	100% (3/3)
	Giardia lamblia/ Giardia duodenalis/ Giardia intestinalis	ATCC PRA-242	3x	1.52E+03 cells/mL	100% (3/3)

Target Call	Species/ Species Type	Part Number (LoD strains are in bold font)	Test Level (xLoD)	Test Concentration	% Positivity
	Giardia lamblia/ Giardia duodenalis/ Giardia intestinalis	**ATCC PRA-254**	1x	1.52E+02 cells/mL	100% (20/20)
Microsporidia	*Encephalitozoon hellem*	**ATCC 50451**	1x	1.52E+02 cells/mL	95% (19/20)
	Encephalitozoon hellem	ATCC 50504	3x	4.56E+02 cells/mL	100% (3/3)
	Encephalitozoon hellem	Waterborne P103H	1000x	1.52E+05 spores/mL	100% (3/3)
	Encephalitozoon intestinalis	**ATCC 50651**	1x	5.08E+01 cells/mL	95% (19/20)
	Encephalitozoon intestinalis	Waterborne P103I	1000x	5.08E+04 spores/mL	100% (3/3)
	Enterocytozoon bieneusi	**LMNX-2885**	1x	1.37E+03 copies/mL	100% (20/20)
	Enterocytozoon bieneusi	**LMNX-4140**	1x	1.37E+03 copies/mL	100% (20/20)
	Enterocytozoon bieneusi	LUM-920-240	3x	4.11E+03 copies/mL	100% (3/3)
Strongyloides stercoralis	*Strongyloides stercoralis*	**LMNX-3433**	1x	2.02E+04 copies/mL	95% (19/20)
	Strongyloides stercoralis	GI_FLEX-012	1.8x	3.69E+04 copies/mL	100% (3/3)

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

Internal Control

Each LIAISON PLEX Gastrointestinal *Flex* Assay Cartridge incorporates multiple controls to confirm proper test performance. The internal controls assess specimen preparation, reverse-transcription, amplification, and hybridization steps of the test system. Internal control results are reported as Pass, Fail, or N/A on the printed reports (see **Table 19** for a brief explanation of each control result). Internal controls must generate a signal above the threshold in each internal reaction for the system to report a valid test result.

Table 19. Interpretation of Controls on the LIAISON PLEX Gastrointestinal *Flex* Assay Report

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

Internal Control Result	Explanation	Suggested Action
N/A	The run failed for a reason other than internal control failure. Such failures may be a result of high background on the microarray, or negative control, or other internal processing errors.	Repeat test with a new cartridge.

External Control
Positive and negative external controls are tested with each new lot or shipment of reagents, or monthly, (whichever occurs first), or in accordance with updated local, regional, state, and/or federal guidelines, as applicable.

Traceability
Cartridge barcode ensures assay identification. Internal controls monitor extraction, amplification, and hybridization. External controls are recommended per lot and per laboratory protocol.

RUN Controls
LIAISON PLEX Gastrointestinal *Flex* Assay Daily Run Controls were processed to confirm performance of the assay and to identify potential issues with reagents, instrumentation, or operator technique during testing. Cary-Blair media was used as a negative run control. Four multi-analyte panels encompassing representative assay targets were used as positive run controls. Positive panels were tested in rotation to cover all assay targets. At each testing site, validity of at least 1 positive and 1 negative run control was confirmed on at least 1 LIAISON PLEX instrument prior to performing data analysis on a given test date (**Table 20** below).

Table 20. Summary of Run Control Results

Run Control ID	Total Cartridge	"PASS"	"FAIL"			System Failure	Rejected Cartridge
			False Positive	False Negative	No Call		
GI Control NEG	159	150	0	0	5	3	1
GI Positive Control 01	41	40	0	1[a]	0	0	0
GI Positive Control 02	39	38	0	0	1	0	0
GI Positive Control 03	38	37	0	0	1	0	0
GI Positive Control 04	38	35	0	1[b]	2	0	0
Overall Total	315	300	0	2	9	3	1

[a] All targets "Not Detected"
[b] Four out of seven targets "Not Detected"

The overall invalid result rate for all sites for the Daily Run Control testing was 4.1% (13/315). Nine (9) invalid results were due to No Call result, three (3) invalid results were due to a System failure, and one (1) invalid result was due to a cartridge that was rejected due to a detached sample port closure. For all invalid results, repeat testing was performed on the same day. The overall invalid rate is outlined in **Table 21.**

Table 21. Invalid Results

Invalid Result Type	Overall
No Call	9
System Failure	3
Rejected Cartridge	1
Total Invalid Results	4.1% (13/315)

Specimen Stability

a. Fresh Cary-Blair Specimen Stability

A study was conducted to demonstrate stability of organisms targeted by the LIAISON PLEX Gastrointestinal *Flex* Assay using contrived samples prepared at 3x LoD and 5x LoD concentrations in stool samples stored in Cary-Blair. Samples were evaluated after storage at 4°C (refrigerated) and 25°C (room temperature) at multiple time points over the course of eight days to support fresh specimen stability claims. The positive sample panel [e.g., Adenovirus F40/41, *Clostridioides difficile*, *Cryptosporidium spp*., Norovirus GI/GII, Rotovirus A, Shiga-like toxin-producing *E. coli* (STEC) stx1, Shiga-like toxin-producing *E. coli* (STEC) stx2, *Shigella*/Enteroinvasive *E. coli* (EIEC)] tested was designed to include analytes representative of each amplification pool and each target type included in the assay design. Two concentrations of targets were prepared (3x and 5x LoD) to test the stability of the sample through extraction, amplification, and detection after the sample was exposed to storage conditions. At each time point, three replicates were tested for each concentration. The detection rate was 100% for all targets at each time point, except for the following with detection rates ≤90%:

- 4°C: Adenovirus 40/41 (5x LoD: Day 3)
- 25°C: Rotavirus A [(3x LoD: Day 2, Day 3, and Day 8) and 5x LoD: Day 5]

An additional study was conducted for Rotavirus A with a new preparation of samples and 19 individual Rotavirus A positive clinical samples tested in Cary-Blair. Results demonstrated 100% Rotavirus detection following seven days of storage at 25°C. A limitation was added to indicate potential impact on detection of Rotavirus A at/near the LoD when stored at 25°C for ≥48 hours. Results from the stability study support the sample handling and storage claims described in the device labeling.

b. Fresh Parasite Fixative Specimen Stability

A stability study was conducted in parasite fixative to evaluate the detection of targets at 25°C for the LIAISON Plex Gastrointestinal *Flex* Assay. Samples were tested at multiple time points over 34 days with analytes spiked at 3x LoD and 5x LoD in both Eco-Fix Negative Stool Matrix and Total-Fix Negative Stool Matrix. A sample panel was prepared to include representative parasite targets (*Blastocystis sp*., *Cryptosporidium* spp., *Entamoeba histolytica*, *Giardia lamblia*, Microsporidia). A 100% detection rate was observed for all targets at all time points up to and including the 34-day timepoint. Results from the stability study support the sample handling and storage claims.

c. Frozen Specimen Stability

A study was conducted to evaluate the stability of LIAISON PLEX Gastrointestinal *Flex* Assay targets when frozen at -65°C to -95°C in stool and Cary-Blair medium. Samples were

tested at multiple time points over 99 days. Both positive and negative samples were evaluated. Positive samples were designed to include panel analytes [e.g., Adenovirus F40/41, *Clostridioides difficile*, *Cryptosporidium spp*., Norovirus GI/GII, Rotavirus A, Shiga-like toxin-producing *E. coli* (STEC) stx2, *Shigella*/Enteroinvasive *E. coli* (EIEC)] and tested in replicates at three concentrations—1.5x LoD, 3x LoD, and 10x LoD. Aliquots were stored frozen and thawed before testing. When an expected target was not detected, additional replicates were tested and percent positivity was calculated from all valid replicate results. A positive rate of 100% was observed for all analytes at time points up to and including 99 days, except for the following:

- Adenovirus F40/41: 90% [3x LoD (fresh) and 3x LoD (Day 90)]
- Rotavirus A: 33% [1.5x LoD (Day 33)]

Rotavirus A detection was 100% for all remaining time points from Day 34 to Day 99. All negative samples yielded the expected result and no false positives were observed. Results from the stability study supported use of frozen specimens (in Cary-Blair only) for testing with the LIAISON PLEX Gastrointestinal *Flex* Assay up to 30 days.

d. Fresh vs Frozen Contrived Stability

Stability of frozen stool samples stored in Cary-Blair was evaluated across three freeze-thaw cycles for use with the LIAISON PLEX Gastrointestinal *Flex* Assay by comparing performance to specimens tested *"fresh"* prior to freezing. Twenty of the twenty-four targets detected by the LIAISON PLEX Gastrointestinal (GI) Flex Assay were evaluated as part of the Fresh vs. Frozen study. Three panels of target combinations were prepared for testing in Cary-Blair Negative Stool Matrix at 1.5x LoD, 3x LoD, and 10x LoD. Aliquots for each panel were stored at -65°C to -95°C. After an overnight freeze cycle, 60 replicates (20 at each of three target concentrations) were tested for each panel. Additional aliquots were subjected to two or three total freeze-thaw cycles followed before testing. The specimens were stable through three freeze-thaw cycles with an overall detection rate for each target ≥ 95.4%. Negative results were 99.2% in agreement with the expected result.

6. Assay Cut-Off:

The LIAISON PLEX Gastrointestinal *Flex* Assay includes established threshold and parameter values for assay targets defined in the assay protocol file.

B Comparison Studies:

1. Method Comparison with Predicate Device:

N/A

2. Matrix Comparison:

Cary Blair Equivalency Study

A study was conducted to evaluate the performance of the LIAISON PLEX Gastrointestinal *Flex* Assay with Cary-Blair transport media from different vendors. The following transport media were assessed:

- Thermo Scientific Cary-Blair with Indicator Single Vial
- C&S Medium Transport Vials
- Para-Pak Enteric Plus

- Para-Pak C&S
- Puritan Cary-Blair Medium

A positive sample panel was designed to include analytes representative of each amplification pool and each target type included in the assay design [e.g., Adenovirus F40/41, *Clostridioidies difficile*(tcdA/tcdB), *Cryptosporidium* spp, Norovirus GI/GII, Rotavirus A, Shiga-like toxin-producing *E.coli* (STEC) stx2, and *Shigella*/Enteroinvasive *E.coli* (EIEC)]. Analytes were tested in each Cary-Blair medium at approximately 3x LoD. For negative samples, a single pool of confirmed negative raw stool sample was prepared in each Cary-Blair medium. Positive and negative (no analytes) samples were tested with a minimum of 20 replicates for each transport medium under evaluation. All positive Cary-Blair panels showed ≥ 95% positivity for all analytes tested. The negative call rate was ≥99.8% for negative samples across all targets.

C Clinical Studies:

1. Clinical Sensitivity:
   A multi-site clinical study established the diagnostic accuracy of the LIAISON PLEX Gastrointestinal *Flex* Assay. The clinical performance of the LIAISON PLEX Gastrointestinal *Flex* Assay was evaluated using clinical specimens prospectively collected between January 2024 and July 2025 from seven geographically diverse clinical sites within the United States. The clinical study utilized residual, de-identified specimens collected from pediatric and adult patients exhibiting clinical signs and symptoms of gastrointestinal infection. The demographic information is summarized in **Table 22**.

Table 22. LIAISON PLEX Gastrointestinal *Flex* Assay Summary of the General Demographic Information

	Prospective (N =2233)	**Pre-selected (N=333)**
	Specimens (%)	**Specimens (%)**
Gender		
Female	1228 (55.0%)	169 (50.8%)
Male	1004 (45.0%)	154 (46.2%)
Gender Unknown	1 (0.0%)	10 (3.0%)
Total	2233 (100.0%)	333 (100.0%)
Age (years)		
<=5	128 (5.7%)	38 (11.4%)
6-18	282 (12.6%)	34 (10.2%)
19-40	458 (20.5%)	74 (22.2%)
41-60	489 (21.9%)	91 (27.3%)
61+	827 (37.0%)	74 (22.2%)
Age Unknown	49 (2.2%)	22 (6.6%)
Total	2233 (100.0%)	333 (100.0%)
Media Type		
Cary-Blair	1665 (74.6%)	309 (92.8%)
EcoFix	203 (9.1%)	14 (4.2%)
TotalFix	365 (16.3%)	10 (3.0%)

	Prospective (N =2233)	Pre-selected (N=333)
	Specimens (%)	**Specimens (%)**
Total	2233 (100.0%)	333 (100.0%)
Patient Location		
Emergency Room	299 (13.4%)	12 (3.6%)
Hospitalized	651 (29.2%)	29 (8.7%)
Outpatient	467 (20.9%)	54 (16.2%)
Status Unknown	816 (36.5%)	238 (71.5%)
Total	2233 (100.0%)	333 (100.0%)

A total of 2240 unique prospective specimens that met the pre-defined inclusion criteria were collected across all sites and enrolled in the study. Clinical testing runs and re-runs were performed according to the Instructions for Use using the LIAISON PLEX Gastrointestinal *Flex* Assay on the LIAISON PLEX System by trained operators at all sites. Prospective specimen (Arm 1) testing occurred between August 2024 and July 2025. Of the 2240 specimens enrolled in the prospective arm of the study, 7 (0.3%) specimens were disqualified and removed from further analysis. Of the seven (7) specimens, four (4) specimens were duplicate patient specimens and three (3) specimens did not have comparator results available. Following exclusions, 2233 prospective specimens were included in the data analysis.

For targets that exhibited low prevalence rates in the prospective study, the prospective specimen set was supplemented with 333 pre-selected, remnant, de-identified specimens (Arm 2) sourced from 17 sites/vendors in the United States, one (1) vendor in France, and one (1) vendor in Germany. The pre-selected specimens were identified by Standard of Care (SoC) results and confirmed by comparator method testing prior to enrollment in the study. To minimize bias, pre-selected specimens were tested in a randomized, blinded manner along with negative specimens at multiple sites. Pre-selected specimen collection dates ranged from September 2017 through February 2026, while (Arm 2) testing occurred between August 2025 and March 2026.

Targets such as *Blastocystis* sp., *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Giardia lamblia*, *Dientamoeba fragilis*, *Plesiomonas shigelloides,* Shiga-like toxin-producing *E.coli* (STEC) *stx2, Vibrio cholerae, Vibrio* spp., *Entamoeba histolytica,* Microsporidia*, Strongyloides stercoralis,* and *Yersinia enterocolitica* exhibited low prevalence rates in the prospective study, and insufficient pre-selected specimens were available for enrollment in the study. Therefore, contrived samples were prepared and tested for these analytes. Additional specimens were also contrived for the parasite targets, as needed, to ensure a sufficient number of specimens in both Cary-Blair and fixative media were evaluated. A total of 827 specimens were contrived and tested as part of Arm 3 (contrived specimens). To minimize bias, contrived specimens were blinded, randomized, and tested along with Arm 2 positive and/or negative clinical specimens at four external testing sites and one internal testing site from July 2025 to March 2026. Results from the prospective, pre-selected, and contrived specimens were analyzed separately.

All clinical specimens were compared to an FDA cleared molecular assay, PCR followed by bi-directional sequencing (BDS) assays, or a combination of the two according to the

algorithm described in **Table 23** below. In the case of Shiga-like toxin-producing *Escherichia coli* (STEC), PCR/BDS was also used to distinguish between stx1 and stx2.

Table 23. Comparator Method Algorithm

LIAISON PLEX Gastrointestinal *Flex* Assay Target	Comparator Method
Bacteria	
Clostridioides difficile (*tcdA/tcdB*)	FDA-cleared molecular assay
Enterotoxigenic *Escherichia coli* (ETEC) LT/ST	FDA-cleared molecular assay with positive confirmation by PCR/BDS
Campylobacter spp. (*C. coli*/*C. jejuni*/*C. lari/C.upsaliensis*)	
Salmonella spp.	
Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1*	
Shiga-like toxin-producing *Escherichia coli* (STEC) *stx2*	
Shigella/Enteroinvasive *Escherichia coli* (EIEC)	
Vibrio cholerae	
Plesiomonas shigelloides	PCR/BDS
Vibrio spp. (*V.parahaemolyticus/V.vulnificus*)	
Yersinia enterocolitica	
Viruses	
Adenovirus F (40/41)	FDA-cleared molecular assay with positive confirmation by PCR/BDS
Norovirus GI/GII	
Rotavirus A	
Astrovirus	PCR/BDS
Sapovirus I/II/IV/V	
Parasites	
Cryptosporidium spp.	For Cary-Blair specimens: FDA-cleared molecular assay with positive confirmation by PCR/BDS For Fixative specimens: PCR/BDS
Entamoeba histolytica	
Giardia lamblia (also known as *G. intestinalis* and *G. duodenalis*)	
Blastocystis sp.	PCR/BDS
Cyclospora cayetanensis	
Dientamoeba fragilis	
Microsporidia (*Encephalitozoon hellem/Encephalitozoon intestinalis/Enterocytozoon bieneusi*)	
Strongyloides stercoralis	

The overall invalid rate across prospective, pre-selected, and contrived arms of testing was 3.8% (129/3393) after the initial run. Of the 129 specimens with initial invalid results, 128 (3.8%) specimens were re-tested, and 113 (3.3%) specimens generated valid LIAISON PLEX Gastrointestinal *Flex* Assay results after a single retest. Fifteen (0.4%) specimens remained

invalid on repeat, and one (0.03%) specimen was not re-tested due to insufficient volume, resulting in an overall success rate of 99.5% (3377/3393).

For each target, the diagnostic performance of the LIAISON PLEX Gastrointestinal *Flex* Assay was determined using Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA), along with the associated Wilson score 95% confidence intervals (95% CI) as compared to the comparator method. The results for prospective and pre-selected specimen analyses are summarized in **Table 24** through **Table 26**. The performance of the LIAISON PLEX Gastrointestinal *Flex* Assay with contrived specimens is presented separately in **Table 27** and **Table 28**.

Discordant results for applicable targets were resolved by testing with assays not included in the comparator testing analysis. Methods of discordant resolution included PCR/BDS, an FDA-cleared molecular method, or standard of care results, as applicable. Results of discordant analysis are presented as footnotes to the tables, when performed.

Table 24. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of Prospective (Arm 1) and Pre-Selected Data Set (Arm 2)

Pathogen Target		Positive Percent Agreement			Negative Percent Agreement		
		TP / (TP+FN)	PPA (%)	95% CI	TN / (TN+FP)	NPA (%)	95% CI
Bacteria							
Clostridioides difficile (tcdA/tcdB)	Prospective	40/41[1]	97.6%	87.4% - 99.6%	202/204[2]	99%	96.5% - 99.7%
	Pre-selected	0/0	N/A	N/A	0/0	N/A	N/A
***Campylobacter* spp.**	Prospective	36/40[3]	90%	76.9% - 96%	1400/1402[4]	99.9%	99.5% - 100%
	Pre-selected	8/8	100%	67.6% - 100%	290/291[5]	99.7%	98.1% - 99.9%
Enterotoxigenic *E. coli* (ETEC) LT/ST	Prospective	8/10[6]	80%	49% - 94.3%	1419/1431[7]	99.2%	98.5% - 99.5%
	Pre-selected	22/22	100%	85.1% - 100%	278/279[8]	99.6%	98% - 99.9%
Plesiomonas shigelloides	Prospective	6/6	100%	61% - 100%	1654/1654	100%	99.8% - 100%
	Pre-selected	10/10	100%	72.2% - 100%	292/292	100%	98.7% - 100%
***Salmonella* spp.**	Prospective	29/29	100%	88.3% - 100%	1405/1405	100%	99.7% - 100%
	Pre-selected	5/5	100%	56.6% - 100%	294/294	100%	98.7% - 100%
Shiga-like toxin-producing *E. coli* (STEC) *stx1*	Prospective	10/10	100%	72.2% - 100%	1429/1432[9]	99.8%	99.4% - 99.9%
	Pre-selected	21/21	100%	84.5% - 100%	279/279	100%	98.6% - 100%
Shiga-like toxin-producing *E. coli* (STEC) *stx2*	Prospective	4/4	100%	51% - 100%	1437/1438[10]	99.9%	99.6% - 100%
	Pre-selected	11/11	100%	74.1% - 100%	287/289[11]	99.3%	97.5% - 99.8%
***Shigella*/ Enteroinvasive *E. coli* (EIEC)**	Prospective	19/19	100%	83.2% - 100%	1423/1423	100%	99.7% - 100%
	Pre-selected	15/15	100%	79.6% - 100%	284/284	100%	98.7% - 100%
Vibrio cholerae	Prospective	0/0	N/A	N/A	1440/1441[12]	99.9%	99.6% - 100%
	Pre-selected	0/0	N/A	N/A	299/299	100%	98.7% - 100%
***Vibrio* spp.**	Prospective	0/0	N/A	N/A	1659/1660[13]	99.9%	99.7% - 100%
	Pre-selected	7/8[14]	87.5%	52.9% - 97.8%	294/294	100%	98.7% - 100%
Yersinia enterocolitica	Prospective	5/8[15]	62.5%	30.6% - 86.3%	1649/1652[16]	99.8%	99.5% - 99.9%
	Pre-selected	38/39[17]	97.4%	86.8% - 99.5%	263/263	100%	98.6% - 100%
Viruses							

Astrovirus	Prospective	26/27[18]	96.3%	81.7% - 99.3%	1633/1633	100%	99.8% - 100%
	Pre-selected	7/7	100%	64.6% - 100%	294/295[19]	99.7%	98.1% - 99.9%
Adenovirus F (40/41)	Prospective	9/9	100%	70.1% - 100%	1430/1435[20]	99.7%	99.2% - 99.9%
	Pre-selected	21/21	100%	84.5% - 100%	276/278[21]	99.3%	97.4% - 99.8%
Norovirus GI/GII	Prospective	117/117	100%	96.8% - 100%	1307/1333[22]	98%	97.2% - 98.7%
	Pre-selected	2/2	100%	34.2% - 100%	297/297	100%	98.7% - 100%
Rotavirus A	Prospective	26/26	100%	87.1% - 100%	1411/1417[23]	99.6%	99.1% - 99.8%
	Pre-selected	6/6	100%	61% - 100%	293/293	100%	98.7% - 100%
Sapovirus I/II/IV/V	Prospective	21/29[24]	72.4%	54.3% - 85.3%	1621/1631[25]	99.4%	98.9% - 99.7%
	Pre-selected	11/11	100%	74.1% - 100%	281/290[26]	96.9%	94.2% - 98.4%
Parasites							
***Blastocystis* sp.**	Prospective	45/47[27]	95.7%	85.8% - 98.8%	2083/2173[28]	95.9%	94.9% - 96.6%
	Pre-selected	10/10	100%	72.2% - 100%	292/316[29]	92.4%	88.9% - 94.8%
***Cryptosporidium* spp.**	Prospective	13/17[30]	76.5	52.7% - 90.4%	1985/1985	100%	99.8% - 100%
	Pre-selected	29/29	100%	88.3% - 100%	294/294	100%	98.7% - 100%
Cyclospora cayetanensis	Prospective	2/2	100%	34.2% - 100%	2217/2218[31]	100%	99.7% - 100%
	Pre-selected	27/30[32]	90%	74.4% - 96.5%	295/296[33]	99.7%	98.1% - 99.9%
Dientamoeba fragilis	Prospective	14/16[34]	87.5%	64% - 96.5%	2203/2204[34]	100%	99.7% - 100%
	Pre-selected	13/14[34]	92.9%	68.5% - 98.7%	311/312[34]	99.7%	98.2% - 99.9%
Entamoeba histolytica	Prospective	2/2	100%	34.2% - 100%	1998/1998	100%	99.8% - 100%
	Pre-selected	1/1	100%	20.7% - 100%	322/322	100%	98.8% - 100%
Giardia lamblia	Prospective	9/9	100%	70.1% - 100%	1996/1998[35]	99.9%	99.6% - 100%
	Pre-selected	20/21[36]	95.2%	77.3% - 99.2%	302/302	100%	98.7% - 100%
Microsporidia	Prospective	4/5[37]	80%	37.6% - 96.4%	2214/2215[37]	100%	99.7% - 100%
	Pre-selected	8/8	100%	67.6% - 100%	318/318	100%	98.8% - 100%
Strongyloides stercoralis	Prospective	0/0	N/A	N/A	2220/2220	100%	99.8% - 100%
	Pre-selected	9/10[38]	90%	59.6% - 98.2%	316/316	100%	98.8% - 100%

[1]The prospective *Clostridioides difficile* False Negative specimen was negative by the Standard of Care molecular panel.

[2]The two prospective *Clostridioides difficile* False Positive specimens were negative by the Standard of Care molecular panel.

[3]One of the four prospective *Campylobacter* spp. False Negative specimens was negative by the Standard of Care molecular panel. An additional two specimens were negative by Standard of Care culture. The remaining specimen was negative when tested with an FDA-cleared molecular panel.

[4]One of the two prospective *Campylobacter* spp. False Positive specimens was positive by the Standard of Care molecular panel. The other prospective specimen was negative when tested with an FDA-cleared molecular panel.

[5]The pre-selected *Campylobacter* spp. False Positive specimen was positive when tested with an FDA-cleared molecular panel.

[6]One of the two prospective Enterotoxigenic *E. coli* (ETEC) LT/ST False Negative specimens was negative by Standard of Care FDA-cleared molecular panel. Both specimens were positive when tested with a second FDA-cleared molecular panel.

[7]Two of the twelve prospective Enterotoxigenic *E. coli* (ETEC) LT/ST False Positive specimens were positive by the Standard of Care molecular panel, and nine additional specimens were positive when tested with an FDA-cleared molecular panel.

[8]The pre-selected Enterotoxigenic *E. coli* (ETEC) LT/ST False Positive specimen was negative by the Standard of Care molecular panel.

[9]The three prospective Shiga-like toxin-producing E. coli (STEC) stx1 False Positive specimens were stx1/2 positive when tested with an FDA-cleared molecular panel and stx1 positive by PCR/BDS.

[10]The prospective Shiga-like toxin-producing *E. coli* (STEC) stx2 False Positive specimen was stx1/2 positive when tested with an FDA-cleared molecular panel and stx1 positive by PCR/BDS.

[11]One of the two pre-selected Shiga-like toxin-producing *E. coli* (STEC) stx2 False Positive specimens was stx1/2 positive when tested with an FDA-cleared molecular panel. The remaining specimen was stx1/2 negative by Standard of Care molecular panel.

[12]The prospective *Vibrio cholerae* False Positive specimen was positive by PCR/BDS.

[13]The prospective *Vibrio* spp. False Positive specimen was positive when tested with an FDA-cleared molecular panel.

[14]The pre-selected *Vibrio* spp. False Negative specimen was negative when tested with an FDA-cleared molecular panel.

[15]One of the three prospective *Yersinia enterocolitica* False Negative specimens was negative by the Standard of Care molecular panel. Another was negative by Standard of Care culture and positive when tested with an FDA-cleared molecular panel. No evaluation of discordant results was performed for the third specimen.

[16]One of the three prospective *Yersinia enterocolitica* False Positive specimens was positive by the Standard of Care molecular panel. Another specimen was positive when tested with an FDA-cleared molecular panel. The third was negative by Standard of Care culture and when tested with an FDA-cleared molecular panel.
[17]No evaluation of discordant results was performed for the pre-selected *Yersinia enterocolitica* False Negative specimen.
[18]The prospective Astrovirus False Negative specimen was negative when tested with an FDA-cleared molecular panel.
[19]The pre-selected Astrovirus False Positive specimen was negative by Standard of Care molecular panel.
[20]The five prospective Adenovirus False Positive specimens were negative by PCR/BDS.
[21]The two pre-selected Adenovirus False Positive specimens were negative when tested with an FDA-cleared molecular panel.
[22]Eight of the twenty-six prospective Norovirus GI/GII False Positive specimens were positive by PCR/BDS. Two additional specimens were positive by the Standard of Care molecular panel. Six additional specimens were positive when tested with an FDA-cleared molecular panel. The remaining ten specimens were negative by PCR/BDS.
[23]Two of the six Rotavirus False Positive specimens were positive by PCR/BDS. An additional three specimens were positive when tested with an FDA-cleared molecular panel. The remaining specimen was negative by PCR/BDS.
[24]Three of the eight prospective Sapovirus I/II/IV/V False Negative specimens were negative when tested with an FDA-cleared molecular panel and one was negative by the Standard of Care molecular panel. Two additional specimens were positive by the Standard of Care molecular panel. The remaining two specimens were positive when tested with an FDA-cleared molecular panel.
[25]One of the ten prospective Sapovirus I/II/IV/V False Positive specimens was positive by the Standard of Care molecular panel. An additional five specimens were positive when tested with an FDA-cleared molecular panel. One specimen was negative by the Standard of Care molecular panel and one additional specimen was negative when tested with an FDA-cleared molecular panel. No evaluation of discordant results was performed for the remaining two specimens.
[26]Two of the nine pre-selected Sapovirus I/II/IV/V False Positive specimens were positive when tested with an FDA-cleared molecular panel. Two additional specimens were negative by Standard of Care molecular panel, four were negative when tested with an FDA-cleared molecular panel and no evaluation of discordant results was performed for the remaining specimen.
[27]No evaluation of discordant results was performed for the prospective *Blastocystis* sp. False Negative specimens.
[28]Twenty-seven prospective *Blastocystis* sp. False Positive specimens were negative by Standard of Care O&P testing. No evaluation of discordant results was performed for the remaining sixty-three prospective *Blastocystis* sp. False Positive specimens.
[29]Two pre-selected *Blastocystis* sp. False Positive specimens were negative by Standard of Care O&P testing. No evaluation of discordant results was performed for the remaining twenty-two pre-selected Blastocystis sp. False Positive specimens.
[30]Two of the four prospective *Cryptosporidium* spp. False Negative specimens were negative by the Standard of Care molecular panel, one additional specimen was negative by Standard of Care O&P testing and the remaining specimen was positive by Standard of Care O&P testing and an FDA-cleared molecular panel.
[31]The prospective *Cyclospora cayetanensis* False Positive specimen was positive by Standard of Care molecular panel.
[32]One of the three pre-selected *Cyclospora cayetanensis* False Negative specimens was positive by Standard of Care molecular panel, and the remaining two were positive by Standard of Care O&P testing.
[33]The pre-selected *Cyclospora cayetanensis* False Positive specimen was positive by Standard of Care O&P testing.
[34]No evaluation of discordant results was performed for *Dientamoeba fragilis*.
[35]Both prospective *Giardia lamblia* False Positive specimens were negative by PCR/BDS.
[36]No evaluation of discordant results was performed for the pre-selected *Giardia lamblia* False Negative specimen.
[37]No evaluation of discordant results was performed for Microsporidia.
[38]No evaluation of discordant results was performed for *Strongyloides stercoralis*.

Table 25. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of Parasite Targets by Media Type of Prospective Data Set (Arm 1)

Pathogen Target		**Positive Percent Agreement**			**Negative Percent Agreement**		
Analyte	**Media Type**	**TP / (TP+FN)**	**PPA (%)**	**95% CI**	**TN / (TN+FP)**	**NPA (%)**	**95% CI**
***Blastocystis* sp.**	Cary-Blair	31/33	93.9%	80.4% - 98.3%	1561/1627	95.9%	94.9% - 96.8%
	Fixative	14/14	100%	78.5% - 100%	522/546	95.6%	93.5% - 97%
	Combined	**45/47[1]**	**95.7%**	**85.8% -98.8%**	**2083/2173[2]**	**95.9%**	**94.9% - 96.6%**
***Cryptosporidium* spp.**	Cary-Blair	10/11	90.9%	62.3% - 98.4%	1431/1431	100%	99.7% - 100%
	Fixative	3/6	50%	18.8% - 81.2%	554/554	100%	99.3% - 100%
	Combined	**13/17[3]**	**76.5%**	**52.7% -90.4%**	**1985/1985**	**100%**	**99.8% - 100%**
Cyclospora cayetanensis	Cary-Blair	1/1	100%	20.7% - 100%	1659/1659	100%	99.8% - 100%
	Fixative	1/1	100%	20.7% - 100%	558/559	99.8%	99% - 100%
	Combined	**2/2**	**100%**	**34.2% - 100%**	**2217/2218[4]**	**100%**	**99.7% - 100%**
Dientamoeba fragilis	Cary-Blair	10/12	83.3%	55.2% - 95.3%	1647/1648	99.9%	99.7% - 100%
	Fixative	4/4	100%	51% - 100%	556/556	100%	99.3% - 100%

Pathogen Target		Positive Percent Agreement			Negative Percent Agreement		
Analyte	**Media Type**	**TP / (TP+FN)**	**PPA (%)**	**95% CI**	**TN / (TN+FP)**	**NPA (%)**	**95% CI**
	Combined	**14/16[5]**	**87.5%**	**64% - 96.5%**	**2203/2204[5]**	**100%**	**99.7% - 100%**
Entamoeba histolytica	Cary-Blair	1/1	100%	20.7% - 100%	1439/1439	100%	99.7% - 100%
	Fixative	1/1	100%	20.7% - 100%	559/559	100%	99.3% - 100%
	Combined	**2/2**	**100%**	**34.2% - 100%**	**1998/1998**	**100%**	**99.8% - 100%**
Giardia lamblia	Cary-Blair	7/7	100%	64.6% - 100%	1439/1440	99.9%	99.6% - 100%
	Fixative	2/2	100%	34.2% - 100%	557/558	99.8%	99% - 100%
	Combined	**9/9**	**100%**	**70.1% - 100%**	**1996/1998[6]**	**99.9%**	**99.6% - 100%**
Microsporidia	Cary-Blair	3/4	75%	30.1% - 95.4%	1655/1656	99.9%	99.7% - 100%
	Fixative	1/1	100%	20.7% - 100%	559/559	100%	99.3% - 100%
	Combined	**4/5[7]**	**80%**	**37.6% -96.4%**	**2214/2215[7]**	**100%**	**99.7% - 100%**
Strongyloides stercoralis	Cary-Blair	0/0	N/A	N/A	1660/1660	100%	99.8% - 100%
	Fixative	0/0	N/A	N/A	560/560	100%	99.3% - 100%
	Combined	**0/0**	**N/A**	**N/A**	**2220/2220**	**100%**	**99.8% - 100%**

[1]No evaluation of discordant results was performed for *Blastocystis* sp. False Negative specimens.
[2]Twenty-seven prospective *Blastocystis* sp. False Positive specimens were negative by Standard of Care O&P testing. No evaluation of discordant results was performed for the remaining sixty-three prospective *Blastocystis* sp. False Positive specimens.
[3]Two of the four prospective *Cryptosporidium* spp. False Negative specimens were negative by the Standard of Care molecular panel, one additional specimen was negative by Standard of Care O&P testing and the remaining specimen was positive by Standard of Care O&P testing and an FDA-cleared molecular panel.
[4]The prospective *Cyclospora cayetanensis* False Positive specimen was positive by Standard of Care molecular panel.
[5]No evaluation of discordant results was performed for *Dientamoeba fragilis*.
[6]Both prospective *Giardia lamblia* False Positive specimens were negative by PCR/BDS.
[7]No evaluation of discordant results was performed for Microsporidia.

Table 26. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of Parasite Targets by Media Type of Pre-selected Data Set (Arm 2)

Pathogen Target		Positive Percent Agreement			Negative Percent Agreement		
Analyte	**Media Type**	**TP / (TP+FN)**	**PPA (%)**	**95% CI**	**TN / (TN+FP)**	**NPA (%)**	**95% CI**
***Blastocystis* sp.**	Cary-Blair	6/6	100%	61% - 100%	273/296	92.2%	88.6% - 94.8%
	Fixative	4/4	100%	51% - 100%	19/20	95%	76.4% - 99.1%
	Combined	**10/10**	**100%**	**72.2% - 100%**	**292/316[1]**	**92.4%**	**88.9% - 94.8%**
***Cryptosporidium* spp.**	Cary-Blair	29/29	100%	88.3% - 100%	270/270	100%	98.6% - 100%
	Fixative	0/0	N/A	N/A	24/24	100%	86.2% - 100%
	Combined	**29/29**	**100%**	**88.3% - 100%**	**294/294**	**100%**	**98.7% - 100%**
Cyclospora cayetanensis	Cary-Blair	19/21	90.5%	71.1% - 97.3%	281/281	100%	98.7% - 100%
	Fixative	8/9	88.9%	56.5% - 98%	14/15	93.3%	70.2% - 98.8%
	Combined	**27/30[2]**	**90%**	**74.4% - 96.5%**	**295/296[3]**	**99.7%**	**98.1% - 99.9%**
Dientamoeba fragilis	Cary-Blair	12/13	92.3%	66.7% - 98.6%	288/289	99.7%	98.1% - 99.9%
	Fixative	1/1	100%	20.7% - 100%	23/23	100%	85.7% - 100%
	Combined	**13/14[4]**	**92.9%**	**68.5% - 98.7%**	**311/312[4]**	**99.7%**	**98.2% - 99.9%**
Entamoeba histolytica	Cary-Blair	1/1	100%	20.7% - 100%	298/298	100%	98.7% - 100%
	Fixative	0/0	N/A	N/A	24/24	100%	86.2% - 100%

Pathogen Target		Positive Percent Agreement			Negative Percent Agreement		
Analyte	**Media Type**	**TP / (TP+FN)**	**PPA (%)**	**95% CI**	**TN / (TN+FP)**	**NPA (%)**	**95% CI**
	Combined	**1/1**	**100%**	**20.7% - 100%**	**322/322**	**100%**	**98.8% - 100%**
Giardia lamblia	Cary-Blair	20/21	95.2%	77.3% - 99.2%	278/278	100%	98.6% - 100%
	Fixative	0/0	N/A	N/A	24/24	100%	86.2% - 100%
	Combined	**20/21[5]**	**95.2%**	**77.3% - 99.2%**	**302/302**	**100%**	**98.7% - 100%**
Microsporidia	Cary-Blair	8/8	100%	67.6% - 100%	294/294	100%	98.7% - 100%
	Fixative	0/0	N/A	N/A	24/24	100%	86.2% - 100%
	Combined	**8/8**	**100%**	**67.6% - 100%**	**318/318**	**100%**	**98.8% - 100%**
Strongyloides stercoralis	Cary-Blair	2/3	66.7%	20.8% - 93.9%	299/299	100%	98.7% - 100%
	Fixative	7/7	100%	64.6% - 100%	17/17	100%	81.6% - 100%
	Combined	**9/10[6]**	**90%**	**59.6% - 98.2%**	**316/316**	**100%**	**98.8% - 100%**

[1]Two pre-selected *Blastocystis* sp. False Positive specimens were negative by Standard of Care O&P testing. No evaluation of discordant results was performed for the remaining twenty-two pre-selected *Blastocystis* sp. False Positive specimens.
[2]One of the three pre-selected *Cyclospora cayetanensis* False Negative specimens was positive by Standard of Care molecular panel, and the remaining two were positive by Standard of Care O&P testing.
[3]The pre-selected *Cyclospora cayetanensis* False Positive specimen was positive by Standard of Care O&P testing.
[4]No evaluation of discordant results was performed for *Dientamoeba fragilis*.
[5]No evaluation of discordant results was performed for the pre-selected Giardia lamblia False Negative specimen.
[6]No evaluation of discordant results was performed for *Strongyloides stercoralis*.

Table 27. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of Contrived Data Set (Arm 3) Bacterial Targets

Pathogen Target	Positive Percent Agreement			Negative Percent Agreement		
Analyte	**TP / (TP+FN)**	**PPA (%)**	**95% CI**	**TN / (TN+FP)**	**NPA (%)**	**95% CI**
Plesiomonas shigelloides	47/48	97.9%	89.1% -99.6%	428/428	100%	99.1% - 100%
Shiga-like toxin-producing *E. coli* (STEC) *stx2*	50/50	100%	92.9% - 100%	426/426	100%	99.1% - 100%
Vibrio cholerae	49/50	98%	89.5% -99.6%	426/426	100%	99.1% - 100%
***Vibrio* spp.**	48/50	96%	86.5% -98.9%	426/426	100%	99.1% - 100%
Yersinia enterocolitica	49/50	98%	89.5% -99.6%	426/426	100%	99.1% - 100%

Table 28. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of Contrived Data Set (Arm 3) Parasite Targets

Pathogen Target		Positive Percent Agreement			Negative Percent Agreement		
Analyte	**Media Type**	**TP / (TP+FN)**	**PPA (%)**	**95% CI**	**TN / (TN+FP)**	**NPA (%)**	**95% CI**
***Blastocystis* sp.**	Cary-Blair	0/0	N/A	N/A	470/476	98.7%	97.3% - 99.4%
	Fixative	50/50	100%	92.9% - 100%	294/298	98.7%	96.6% - 99.5%
	Combined	**50/50**	**100%**	**92.9% - 100%**	**741/751**	**98.7%**	**96.6% - 99.5%**
	Cary-Blair	0/0	N/A	N/A	476/476	100%	99.2% - 100%

Pathogen Target		Positive Percent Agreement			Negative Percent Agreement		
Analyte	**Media Type**	**TP / (TP+FN)**	**PPA (%)**	**95% CI**	**TN / (TN+FP)**	**NPA (%)**	**95% CI**
***Cryptosporidium* spp.**	Fixative	49/50	98%	89.5% - 99.6%	298/298	100%	98.7% - 100%
	Combined	49/50	98%	89.5% - 99.6%	**774/774**	**100%**	**99.5% - 100%**
Cyclospora cayetanensis	Cary-Blair	29/29	100%	88.3% - 100%	447/447	100%	99.1% - 100%
	Fixative	27/30	90%	74.4% - 96.5%	318/318	100%	98.8% - 100%
	Combined	**56/59**	**94.9%**	**86.1% -98.3%**	**765/765**	**100%**	**99.5% - 100%**
Dientamoeba fragilis	Cary-Blair	30/30	100%	88.6% - 100%	446/446	100%	99.1% - 100%
	Fixative	30/30	100%	88.6% - 100%	318/318	100%	98.8% - 100%
	Combined	**60/60**	**100%**	**94% - 100%**	**764/764**	**100%**	**99.5% - 100%**
Entamoeba histolytica	Cary-Blair	30/30	100%	88.6% - 100%	446/446	100%	99.1% - 100%
	Fixative	57/60	95%	86.3% - 98.3%	288/288	100%	98.7% - 100%
	Combined	**87/90**	**96.7%**	**90.7% -98.9%**	**734/734**	**100%**	**99.5% - 100%**
Giardia lamblia	Cary-Blair	30/30	100%	88.6% - 100%	446/446	100%	99.1% - 100%
	Fixative	29/30	96.7%	83.3% - 99.4%	317/318	99.7%	98.2% - 99.9%
	Combined	**59/60**	**98.3%**	**91.1% -99.7%**	**763/764**	**99.9%**	**99.3% - 100%**
Microsporidia	Cary-Blair	29/30	96.7%	83.3% - 99.4%	446/446/	100%	99.1% - 100%
	Fixative	30/30	100%	88.6% - 100%	318/318	100%	98.8% - 100%
	Combined	**59/60**	**98.3%**	**91.1% -99.7%**	**764/764**	**100%**	**99.5% - 100%**
Strongyloides stercoralis	Cary-Blair	25/25	100%	86.7% - 100%	451/451	100%	99.2% - 100%
	Fixative	50/50	100%	92.9% - 100%	298/298	100%	98.7% - 100%
	Combined	**75/75**	**100%**	**95.1% - 100%**	**749/749**	**100%**	**99.5% - 100%**

2. Clinical Specificity:

   See above.

3. Clinical Cut-Off

   N/A

4. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   N/A

D Expected Values/Reference Range:

LIAISON PLEX Gastrointestinal *Flex* Assay positive results (expected values) for each individual target are summarized for specimens included in the prospective arm of the clinical study (**Table 29** and **Table 30**).

Table 29. LIAISON PLEX Gastrointestinal *Flex* Assay Expected Values for Prospective Specimens by Age Group (Cary-Blair Specimens)

	≤ 5 years (N=99)		6-18 years (N=229)		19-40 years (N=320)		41-60 years (N=353)		61+ years (N=618)		Unknown years (N=46)		Overall (N=1665)[1]	
Target	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**
Bacterial Targets														
Clostridioides difficile (tcdA/tcdB)	17	17.2% (17/99)	44	19.3% (44/228)	36	11.3% (36/319)	53	15.1% (53/351)	107	17.3% (107/617)	12	26.1% (12/46)	269	16.2% (269/1660)
***Campylobacter* spp.**	2	2% (2/99)	7	3.1% (7/228)	11	3.4% (11/319)	7	2% (7/351)	13	2.1% (13/617)	1	2.2% (1/46)	41	2.5% (41/1660)
Enterotoxigenic *E. coli* (ETEC) LT/ST	1	1% (1/99)	5	2.2% (5/228)	4	1.3% (4/319)	6	1.7% (6/351)	5	0.8% (5/617)	0	0% (0/46)	21	1.3% (21/1660)
Plesiomonas shigelloides	0	0% (0/99)	1	0.4% (1/228)	0	0% (0/319)	2	0.6% (2/351)	2	0.3% (2/617)	1	2.2% (1/46)	6	0.4% (6/1660)
***Salmonella* spp.**	3	3% (3/99)	6	2.6% (6/228)	5	1.6% (5/319)	8	2.3% (8/351)	6	1% (6/617)	2	4.3% (2/46)	30	1.8% (30/1660)
Shiga-like toxin-producing *E. coli* (STEC) *stx1*	2	2% (2/99)	2	0.9% (2/228)	4	1.3% (4/319)	3	0.9% (3/351)	2	0.3% (2/617)	0	0% (0/46)	13	0.8% (13/1660)
Shiga-like toxin-producing *E. coli* (STEC) *stx2*	2	2% (2/99)	1	0.4% (1/228)	0	0% (0/319)	1	0.3% (1/351)	1	0.2% (1/617)	0	0% (0/46)	5	0.3% (5/1660)
***Shigella*/Enteroinvasive *E. coli* (EIEC)**	2	2% (2/99)	4	1.8% (4/228)	2	0.6% (2/319)	5	1.4% (5/351)	6	1% (6/617)	0	0% (0/46)	19	1.1% (19/1660)
Vibrio cholerae	1	1% (1/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
***Vibrio* spp.**	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	1	0.2% (1/617)	0	0% (0/46)	1	0.1% (1/1660)
Yersinia enterocolitica	1	1% (1/99)	1	0.4% (1/228)	1	0.3% (1/319)	1	0.3% (1/351)	4	0.6% (4/617)	0	0% (0/46)	8	0.5% (8/1660)
Viral Targets														
Astrovirus	4	4% (4/99)	2	0.9% (2/228)	7	2.2% (7/319)	5	1.4% (5/351)	7	1.1% (7/617)	1	2.2% (1/46)	26	1.6% (26/1660)
Adenovirus F (40/41)	5	5.1% (5/99)	3	1.3% (3/228)	3	0.9% (3/319)	3	0.9% (3/351)	2	0.3% (2/617)	0	0% (0/46)	16	1% (16/1660)
Norovirus GI/GII	16	16.2% (16/99)	12	5.3% (12/228)	40	12.5% (40/319)	21	6% (21/351)	55	8.9% (55/617)	9	19.6% (9/46)	153	9.2% (153/1660)
Rotavirus A	9	9.1% (9/99)	6	2.6% (6/228)	4	1.3% (4/319)	5	1.4% (5/351)	8	1.3% (8/617)	0	0% (0/46)	32	1.9% (32/1660)
Sapovirus I/II/IV/V	1	1% (1/99)	13	5.7% (13/228)	6	1.9% (6/319)	4	1.1% (4/351)	4	0.6% (4/617)	3	6.5% (3/46)	31	1.9% (31/1660)
Parasite Targets														
***Blastocystis* sp.**	5	5.1% (5/99)	20	8.8% (20/228)	30	9.4% (30/319)	21	6% (21/351)	20	3.2% (20/617)	1	2.2% (1/46)	97	5.8% (97/1660)
***Cryptosporidium* spp.**	0	0% (0/99)	2	0.9% (2/228)	5	1.6% (5/319)	3	0.9% (3/351)	0	0% (0/617)	0	0% (0/46)	10	0.6% (10/1660)
Cyclospora cayetanensis	0	0% (0/99)	0	0% (0/228)	1	0.3% (1/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)

	≤ 5 years (N=99)		6-18 years (N=229)		19-40 years (N=320)		41-60 years (N=353)		61+ years (N=618)		Unknown years (N=46)		Overall (N=1665)[1]	
Target	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**
Dientamoeba fragilis	2	2% (2/99)	6	2.6% (6/228)	2	0.6% (2/319)	0	0% (0/351)	1	0.2% (1/617)	0	0% (0/46)	11	0.7% (11/1660)
Entamoeba histolytica	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	1	0.2% (1/617)	0	0% (0/46)	1	0.1% (1/1660)
Giardia lamblia	1	1% (1/99)	0	0% (0/228)	4	1.3% (4/319)	2	0.6% (2/351)	1	0.2% (1/617)	0	0% (0/46)	8	0.5% (8/1660)
Microsporidia	1	1% (1/99)	0	0% (0/228)	1	0.3% (1/319)	1	0.3% (1/351)	1	0.2% (1/617)	0	0% (0/46)	4	0.2% (4/1660)
Strongyloides stercoralis	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	0	0% (0/1660)

[1]Five specimens with invalid results on the LIAISON PLEX Gastrointestinal *Flex* assay were excluded from the analysis.

Table 30. LIAISON PLEX Gastrointestinal *Flex* Assay Expected Values for Prospective Specimens by Age (Fixative Specimens)

	≤ 5 years (N=29)		6-18 years (N=53)		19-40 years (N=138)		41-60 years (N=136)		61+ years (N=209)		Unknown years (N=3)		Overall (N=568)[1]	
Target	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**	**# Pos**	**(%)**
Parasite Targets														
***Blastocystis* sp.**	5	17.9% (5/28)	5	9.4% (5/53)	12	8.8% (12/136)	9	6.8% (9/133)	7	3.4% (7/208)	0	0% (0/2)	38	6.8% (38/560)
***Cryptosporidium* spp.**	0	0% (0/28)	0	0% (0/53)	3	2.2% (3/136)	0	0% (0/133)	0	0% (0/208)	0	0% (0/2)	3	0.5% (3/560)
Cyclospora cayetanensis	0	0% (0/28)	0	0% (0/53)	0	0% (0/136)	0	0% (0/133)	2	1% (2/208)	0	0% (0/2)	2	0.4% (2/560)
Dientamoeba fragilis	1	3.6% (1/28)	0	0% (0/53)	1	0.7% (1/136)	0	0% (0/133)	2	1% (2/208)	0	0% (0/2)	4	0.7% (4/560)
Entamoeba histolytica	0	0% (0/28)	0	0% (0/53)	1	0.7% (1/136)	0	0% (0/133)	0	0% (0/208)	0	0% (0/2)	1	0.2% (1/560)
Giardia lamblia	0	0% (0/28)	1	1.9% (1/53)	2	1.5% (2/136)	0	0% (0/133)	0	0% (0/208)	0	0% (0/2)	3	0.5% (3/560)
Microsporidia	0	0% (0/28)	0	0% (0/53)	1	0.7% (1/136)	0	0% (0/133)	0	0% (0/208)	0	0% (0/2)	1	0.2% (1/560)
Strongyloides stercoralis	0	0% (0/28)	0	0% (0/53)	0	0% (0/136)	0	0% (0/133)	0	0% (0/208)	0	0% (0/2)	0	0% (0/560)

[1]Eight specimens with invalid results on the LIAISON PLEX Gastrointestinal *Flex* assay were excluded from the analysis.

The LIAISON PLEX Gastrointestinal *Flex* Assay expected values for each co-infection combination per age group are summarized in **Table 31** and **Table 32** below.

Table 31. LIAISON PLEX Gastrointestinal *Flex* Assay Expected Values for Each Co-Infection Combination for Prospective Specimens by Age Group (Cary-Blair Specimens)

	<=5 years (N=99)		6-18 years (N=229)		19-40 years (N=320)		41-60 years (N=353)		61+ years (N=618)		Age Unknown (N=46)		Total (N=1665)[1]	
Co-infection	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**
Adenovirus F40/41 | *Clostridioides difficile* (*tcdA/tcdB*)	1	1% (1/99)	1	0.4% (1/228)	1	0.3% (1/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	3	0.2% (3/1660)
Adenovirus F40/41 | *Clostridioides difficile* (*tcdA/tcdB*) | Rotavirus A	1	1% (1/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Adenovirus F40/41 | Norovirus GI/GII	1	1% (1/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	1	0.2% (1/617)	0	0% (0/46)	2	0.1% (2/1660)
Adenovirus F40/41 | *Shigella*/Enteroinvasive *E. coli* (EIEC)	0	0% (0/99)	1	0.4% (1/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Astrovirus | *Clostridioides difficile* (*tcdA/tcdB*)	1	1% (1/99)	1	0.4% (1/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	2	0.1% (2/1660)
Astrovirus | Norovirus GI/GII	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	1	0.2% (1/617)	0	0% (0/46)	1	0.1% (1/1660)
Astrovirus | Sapovirus I/II/IV/V	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	1	2.2% (1/46)	1	0.1% (1/1660)
Astrovirus | Shiga-like toxin-producing *E. coli* (STEC) *stx2*	1	1% (1/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Blastocystis sp. | *Campylobacter* spp.	0	0% (0/99)	0	0% (0/228)	2	0.6% (2/319)	0	0% (0/351)	1	0.2% (1/617)	0	0% (0/46)	3	0.2% (3/1660)
Blastocystis sp. | *Clostridioides difficile* (*tcdA/tcdB*)	0	0% (0/99)	3	1.3% (3/228)	4	1.3% (4/319)	4	1.1% (4/351)	0	0% (0/617)	0	0% (0/46)	11	0.7% (11/1660)
Blastocystis sp. | *Clostridioides difficile* (*tcdA/tcdB*) | Norovirus GI/GII	0	0% (0/99)	0	0% (0/228)	1	0.3% (1/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Blastocystis sp. | *Clostridioides difficile* (*tcdA/tcdB*) | *Salmonella* spp.	0	0% (0/99)	0	0% (0/228)	1	0.3% (1/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Blastocystis sp. | *Cryptosporidium* spp.	0	0% (0/99)	1	0.4% (1/228)	2	0.6% (2/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	3	0.2% (3/1660)
Blastocystis sp. | *Cryptosporidium* spp. | *Dientamoeba fragilis*	0	0% (0/99)	1	0.4% (1/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Blastocystis sp. | *Cryptosporidium* spp. | Enterotoxigenic *E. coli* (ETEC) LT/ST | Shiga-like toxin-producing *E. coli* (STEC) *stx1*	0	0% (0/99)	0	0% (0/228)	1	0.3% (1/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Blastocystis sp. | *Cryptosporidium* sp. | *Giardia lamblia*	0	0% (0/99)	0	0% (0/228)	1	0.3% (1/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Blastocystis sp. | *Dientamoeba fragilis*	0	0% (0/99)	2	0.9% (2/228)	1	0.3% (1/319)	0	0% (0/351)	1	0.2% (1/617)	0	0% (0/46)	4	0.2% (4/1660)
Blastocystis sp. | *Dientamoeba fragilis* | Rotavirus A	0	0% (0/99)	1	0.4% (1/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)

	<=5 years (N=99)		6-18 years (N=229)		19-40 years (N=320)		41-60 years (N=353)		61+ years (N=618)		Age Unknown (N=46)		Total (N=1665)[1]	
Co-infection	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**
Blastocystis sp. | Enterotoxigenic *E. coli* (ETEC) LT/ST | Shiga-like toxin-producing *E. coli* (STEC) *stx1* | Shiga-like toxin-producing *E. coli* (STEC) *stx2*	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	1	0.3% (1/351)	1	0.2% (1/617)	0	0% (0/46)	2	0.1% (2/1660)
Blastocystis sp. | *Giardia lamblia*	0	0% (0/99)	0	0% (0/228)	1	0.3% (1/319)	1	0.3% (1/351)	0	0% (0/617)	0	0% (0/46)	2	0.1% (2/1660)
Blastocystis sp. | Norovirus GI/GII	0	0% (0/99)	1	0.4% (1/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Blastocystis sp. | *Salmonella* spp.	1	1% (1/99)	2	0.9% (2/228)	0	0% (0/319)	0	0% (0/351)	2	0.3% (2/617)	0	0% (0/46)	5	0.3% (5/1660)
Blastocystis sp. | Sapovirus I/II/IV/V	0	0% (0/99)	1	0.4% (1/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Blastocystis sp. | Shiga-like toxin-producing *E. coli* (STEC) *stx1*	0	0% (0/99)	0	0% (0/228)	1	0.3% (1/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Blastocystis sp. | *Shigella*/Enteroinvasive *E. coli* (EIEC)	0	0% (0/99)	0	0% (0/228)	2	0.6% (2/319)	1	0.3% (1/351)	1	0.2% (1/617)	0	0% (0/46)	4	0.2% (4/1660)
Blastocystis sp. | *Vibrio* spp.	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	1	0.2% (1/617)	0	0% (0/46)	1	0.1% (1/1660)
Blastocystis sp. | *Yersinia enterocolitica*	1	1% (1/99)	1	0.4% (1/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	2	0.1% (2/1660)
Campylobacter spp. | *Clostridioides difficile* (*tcdA/tcdB*)	0	0% (0/99)	0	0% (0/228)	1	0.3% (1/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Campylobacter spp. | *Clostridioides difficile* (*tcdA/tcdB*) | Sapovirus I/II/IV/V	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	1	2.2% (1/46)	1	0.1% (1/1660)
Campylobacter spp. | Norovirus GI/GII | *Shigella*/Enteroinvasive *E. coli* (EIEC) | *Vibrio cholerae*	1	1% (1/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Clostridioides difficile (*tcdA/tcdB*) | Enterotoxigenic *E. coli* (ETEC) LT/ST	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	1	0.3% (1/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Clostridioides difficile (*tcdA/tcdB*) | *Giardia lamblia*	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	1	0.3% (1/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Clostridioides difficile (*tcdA/tcdB*) | Norovirus GI/GII	3	3% (3/99)	2	0.9% (2/228)	5	1.6% (5/319)	1	0.3% (1/351)	7	1.1% (7/617)	2	4.3% (2/46)	20	1.2% (20/1660)
Clostridioides difficile (*tcdA/tcdB*) | Norovirus GI/GII | Sapovirus I/II/IV/V	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	1	2.2% (1/46)	1	0.1% (1/1660)
Clostridioides difficile (*tcdA/tcdB*) | Rotavirus A	0	0% (0/99)	0	0% (0/228)	1	0.3% (1/319)	0	0% (0/351)	1	0.2% (1/617)	0	0% (0/46)	2	0.1% (2/1660)

	<=5 years (N=99)		6-18 years (N=229)		19-40 years (N=320)		41-60 years (N=353)		61+ years (N=618)		Age Unknown (N=46)		Total (N=1665)[1]	
Co-infection	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**	**# POS**	**%**
Clostridioides difficile (*tcdA/tcdB*) | *Salmonella* spp.	1	1% (1/99)	0	0% (0/228)	0	0% (0/319)	1	0.3% (1/351)	0	0% (0/617)	1	2.2% (1/46)	3	0.2% (3/1660)
Clostridioides difficile (*tcdA/tcdB*) | Sapovirus I/II/IV/V	0	0% (0/99)	4	1.8% (4/228)	1	0.3% (1/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	5	0.3% (5/1660)
Clostridioides difficile (*tcdA/tcdB*) | *Yersinia enterocolitica*	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	1	0.3% (1/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Cryptosporidium spp. | Sapovirus I/II/IV/V	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	1	0.3% (1/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Enterotoxigenic *E. coli* (ETEC) LT/ST | Norovirus GI/GII | *Plesiomonas shigelloides*	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	1	0.3% (1/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Enterotoxigenic *E. coli* (ETEC) LT/ST | Rotavirus A	1	1% (1/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Enterotoxigenic *E. coli* (ETEC) LT/ST | Shiga-like toxin-producing *E. coli* (STEC) *stx1*	0	0% (0/99)	0	0% (0/228)	1	0.3% (1/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Enterotoxigenic *E. coli* (ETEC) LT/ST | *Shigella*/Enteroinvasive *E. coli* (EIEC)	0	0% (0/99)	2	0.9% (2/228)	0	0% (0/319)	0	0% (0/351)	1	0.2% (1/617)	0	0% (0/46)	3	0.2% (3/1660)
Giardia lamblia | Rotavirus A | Shiga-like toxin-producing *E. coli* (STEC) *stx1*	1	1% (1/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Norovirus GI/GII | *Plesiomonas shigelloides* | Sapovirus I/II/IV/V	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	1	0.2% (1/617)	0	0% (0/46)	1	0.1% (1/1660)
Norovirus GI/GII | Rotavirus A	3	3% (3/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	1	0.2% (1/617)	0	0% (0/46)	4	0.2% (4/1660)
Norovirus GI/GII | *Salmonella* spp.	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	1	2.2% (1/46)	1	0.1% (1/1660)
Norovirus GI/GII | Sapovirus I/II/IV/V	1	1% (1/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Norovirus GI/GII | Shiga-like toxin-producing *E. coli* (STEC) *stx1*	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	1	0.3% (1/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Norovirus GI/GII | *Yersinia enterocolitica*	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	1	0.2% (1/617)	0	0% (0/46)	1	0.1% (1/1660)
Shiga-like toxin-producing *E. coli* (STEC) *stx1* | Shiga-like toxin-producing *E. coli* (STEC) *stx2*	1	1% (1/99)	0	0% (0/228)	0	0% (0/319)	0	0% (0/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)
Shiga-like toxin-producing *E. coli* (STEC) *stx1* | *Shigella*/Enteroinvasive *E. coli* (EIEC)	0	0% (0/99)	0	0% (0/228)	0	0% (0/319)	1	0.3% (1/351)	0	0% (0/617)	0	0% (0/46)	1	0.1% (1/1660)

[1]Five specimens with invalid results on the LIAISON PLEX Gastrointestinal *Flex* Assay are excluded from the analysis.

Table 32. LIAISON PLEX Gastrointestinal *Flex* Assay Expected Values for Each Co-Infection Combination for Prospective Specimens by Age Group (Fixative Specimens)

	<=5 years (N=29)		6-18 years (N=53)		19-40 years (N=138)		41-60 years (N=136)		61+ years (N=209)		Age Unknown (N=3)		Total (N=568)[1]	
Co-infection	**#POS**	**%**	**#POS**	**%**	**#POS**	**%**	**#POS**	**%**	**#POS**	**%**	**#POS**	**%**	**#POS**	**%**
***Blastocystis* sp. | *Cryptosporidium* spp.**	0	0% (0/28)	0	0% (0/53)	2	1.5% (2/136)	0	0% (0/133)	0	0% (0/208)	0	0% (0/2)	2	0.4% (2/560)
Blastocystis* sp. | *Dientamoeba fragilis	0	0% (0/28)	0	0% (0/53)	0	0% (0/136)	0	0% (0/133)	1	0.5% (1/208)	0	0% (0/2)	1	0.2% (1/560)
Entamoeba histolytica* | *Giardia lamblia	0	0% (0/28)	0	0% (0/53)	1	0.7% (1/136)	0	0% (0/133)	0	0% (0/208)	0	0% (0/2)	1	0.2% (1/560)
***Giardia lamblia* | Microsporidia**	0	0% (0/28)	0	0% (0/53)	1	0.7% (1/136)	0	0% (0/133)	0	0% (0/208)	0	0% (0/2)	1	0.2% (1/560)

[1]Eight specimens with invalid results on the LIAISON PLEX Gastrointestinal *Flex* assay are excluded from the analysis.

E Other Supportive Instrument Performance Characteristics Data:

Electrical safety and electromagnetic compatibility (EMC) testing were previously performed, and the system was found to be acceptable.

Software and cybersecurity documentation were reviewed and found to be acceptable.

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.