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

I Background Information:

A 510(k) Number

K251993

B Applicant

Hologic Inc.

C Proprietary and Established Names

Panther Fusion GI Expanded Bacterial 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
OOI	Class II	21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

To obtain substantial equivalence determination for the Panther Fusion Expanded GI Bacterial Assay on the Panther Fusion System using preserved Cary-Blair stool specimens.

B Measurand:

Target DNA sequences with the Panther Fusion GI Expanded Bacteria Panel:

- *Yersinia enterocolitica*,
- *Vibrio* (*V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*),
- *Escherichia coli* O157, and
- *Plesiomonas shigelloides*

C Type of Test:
The Panther Fusion GI Expanded Bacterial Assay is a qualitative multiplex real time polymerase chain reaction (PCR) assay for the amplification and detection of DNA from *Yersinia enterocolitica*, *Vibrio* (*V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*), *Escherichia coli* O157, and *Plesiomonas shigelloides.*

III Intended Use/Indications for Use:

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

B Indication(s) for Use:
The Panther Fusion GI Expanded Bacterial Assay is a multiplex real-time PCR in vitro diagnostic test for the rapid and qualitative detection and differentiation of *Yersinia enterocolitica*, *Vibrio* (*V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*), *Escherichia coli* O157, and *Plesiomonas shigelloides*. Nucleic acids are isolated and purified from Cary-Blair preserved stool specimens collected from individuals exhibiting signs and symptoms of gastroenteritis.

This assay is intended to aid in the differential diagnosis of *Yersinia enterocolitica*, *Vibrio* (*V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*), *Escherichia coli* O157, and *Plesiomonas shigelloides* infections. The results of this assay should be used in conjunction with clinical presentation, laboratory findings, and epidemiological information and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out coinfection with other organisms that are not detected by this test and may not be the sole or definitive cause of patient illness. Negative 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. This assay is designed for use on the Panther Fusion System.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:
Panther Fusion System

IV Device/System Characteristics:

A Device Description:
The Panther Fusion GI Expanded Bacterial Assay is a multiplex real-time PCR *in vitro* diagnostic test for the rapid and qualitative detection and differentiation of *Yersinia enterocolitica*, *Vibrio* (*V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*), *Escherichia coli* O157, and *Plesiomonas shigelloides*. Nucleic acids are isolated and purified from Cary-Blair preserved stool specimens collected from individuals exhibiting signs and symptoms of gastroenteritis.

The Panther Fusion System fully automates specimen processing, including sample lysis, nucleic acid capture, amplification, and detection for the Panther Fusion GI Expanded Bacterial Assay. Nucleic acid capture and elution takes place in a single tube on the Panther Fusion System. The eluate is transferred to the Panther Fusion System reaction tube containing the assay reagents.

Multiplex real-time PCR is then performed for the eluted nucleic acid on the Panther Fusion System.

B Principle of Operation:

Sample Collection and Transfer

Prior to processing and testing on the Panther Fusion System, Cary-Blair preserved stool (CBS) sample is transferred into an Aptima Multitest tube containing 2.9 mL of specimen transport media (STM) by using Aptima multi-test specimen collection kit swab. The amount of CBS transferred by a swab is approximately 150µL, equating to a 1:20 dilution of the CBS sample. The sample transport tube is placed on the Panther Fusion and 360µL of the sample is transferred into a multi-tube unit (MTU) to undergo lysis and target capture.

Lysis, Nucleic Acid Capture and Elution

Specimen transport media (STM) lyses the cells, releases target nucleic acid and protects them from degradation during storage. Additionally, specimens are subjected to further lysis with Panther Fusion Enhancer Reagent-B (FER-B). The Internal Control (IC) is added to each test specimen and controls via the working Panther Fusion Capture Reagent (wFCR). The IC in the reagent monitors specimen processing, amplification and detection. Capture oligonucleotides hybridize to nucleic acid in the test specimen. Hybridized nucleic acid is then separated from the specimen in a magnetic field. Wash steps remove extraneous components from the reaction tube. The elution step elutes purified nucleic acid. During the nucleic acid capture and elution step, total nucleic acid is isolated from specimens.

Elution Transfer and Multiplex PCR

During the elution transfer step, eluted nucleic acid is transferred to a Panther Fusion reaction tube already containing oil and reconstituted master mix. Target amplification occurs by PCR. Target specific forward and reverse primers and probes then amplify targets while simultaneously detecting and discriminating multiple target types via multiplex PCR. The Panther Fusion system compares the fluorescence signal to a predetermined cut-off to produce a qualitative result for the presence or absence of the analyte. The analytes and the channel used for their detection on the Panther Fusion system are summarized in the table below.

Table 1. Summary of analytes detected by the Panther Fusion GI Expanded Bacterial Assay and channels for their detection on the Panther Fusion system

Analyte	Gene Targeted	Instrument Channel
Yersinia enterocolitica	*InvA* (Invasive antigen A)	FAM
Vibrio parahaemolyticus	*gyrB* (Gyrase B)	HEX
Vibrio vulnificus	*gyrB* (Gyrase B)	HEX
Vibrio cholerae	*ompW* (Outer Membrane Protein W)	HEX
Escherichia coli O157	*rfbE* (Perosamine synthase-O-antigen)	ROX
Plesiomonas shigelloides	*hugA* (Heme utilization gene A)	RED647
Internal Control	Not Applicable	RED677

Kit Components

The reagents required to perform the Panther Fusion GI Expanded Bacterial Assay are packaged and sold separately. There are seven boxes containing nine reagents which are required for sample processing.

- **Box 1**: *Panther Fusion GI Expanded Bacterial Assay Cartridges – 96 Tests* (P/N PRD-07121). Contains eight Panther Fusion GI Expanded Bacterial Assay Cartridges - individually pouched in foil containing one desiccant (12 tests per cartridge). Storage at 2° – 8°C. Each well contains lyophilized, unit dose, assay specific, master mix required for PCR reaction.
- **Box 2**: *Panther Fusion Extraction Reagent-B – 960 Tests* (P/N PRD-06232). Contains:
    - Four Panther Fusion Capture Reagent-B bottles (240 tests per bottle). Storage at 15° – 30°C. buffered salt solution containing solid phase (magnetic particles) and non-infectious nucleic acids.
    - Four Panther Fusion Enhancer Reagent-B bottles (240 tests per bottle). Storage at 15° – 30°C. To enhance the disruption of bacterial particles and denaturation of nucleic acids that may be present in sample.
- **Box 3**: *Panther Fusion Internal Control-B - 960 Tests* (P/N PRD-06234). Contains four Panther Fusion Internal Control-B bottles. Storage at 2° – 8°C. Added to each reaction to determine validity of each individual reaction.
- **Box 4**: *Panther Fusion Reconstitution Buffer I– 1920 Tests* – (P/N PRD-04333). Storage at 15° – 30°C.
- **Box 5**: *Panther Fusion Elution Buffer – 2400 Tests* – (P/N PRD-04334). Storage at 15° – 30°C.
- **Box 6**: Panther Fusion Oil – 1920 Tests – (P/N PRD-04335). Storage at Storage at 15° – 30°C.
- **Box 7**: *Panther Fusion GI Expanded Bacterial Assay Controls – 5 Tests* (P/N PRD-07122). Storage at 2° – 8°C. Contains:
    - Five Panther Fusion GI Expanded Bacterial Positive Control tubes (single use). To validate the accuracy of the calibration curve
    - Five Panther Fusion GI Expanded Bacterial Negative Control tubes (single use). Controls for any potential contaminations of reagents or environment.

The Panther Fusion GI Expanded Bacterial Assay requires one ancillary kit and one specimen collection kit, neither of which are provided with the assay and can be acquired separately:

1) Aptima Assay Fluids Kit (303014)
2) Aptima Multitest Swab Specimen Collection Kit (PRD-03546)

Additionally, the Panther Fusion Reconstitution Buffer I, Elution Buffer, and Oil Reagents are universally used for all Panther Fusion system assays. The Elution Buffer and Oil Reagents may also be bundled and ordered under the Panther Fusion Universal Fluids Kit.

C Instrument Description Information:

1. Instrument Name:
   Panther Fusion

2. Specimen Identification:
   A barcode reader reads the barcodes assigned to the specimens.

3. Specimen Sampling and Handling:
   Stool specimens are collected from patients using commercially available collection kits and transferred into Cary-Blair media. The stool is diluted approximately 1:3 in Cary-Blair media following the instructions given by the Cary Blair manufactures. The Cary-Blair preserved stool (CBS) sample is then transferred into an Aptima Multitest tube containing 2.9 mL of specimen transport media (STM) by using Aptima multi-test specimen collection kit swab into the CBS. The amount of CBS transferred by a swab is approximately 150µL, equating to a 1:20 dilution of the CBS sample. The sample transport tube is placed on the Panther Fusion and 360 µL of the sample is transferred into a multi-tube unit (MTU) to undergo lysis and target capture.

4. Calibration:
   The instrument is calibrated by the manufacturer on-site as part of the installation procedure.

5. Quality Control:
   To generate valid results, the Panther Fusion GI Expanded Bacterial Assay uses internal and external controls:

   - *Internal control* (IC) is added to each test specimen and controls via the working Panther Fusion Capture Reagent (wFCR). The IC in the reagent monitors specimen processing, amplification and detection.
   - *Assay Controls*: Two types of controls are provided by Hologic to the user: one negative control; and one positive control that contains inactivated *Yersinia enterocolotica* cells and plasmids for other targets. One replicate of the negative assay control and one replicate of positive assay control must be tested each time a new lot of assay cartridges is loaded on the system or when the current set of valid controls for an active cartridge lot have expired.

   The Panther system is configured to require assay controls run at an administrator-specified interval of up to 30 days. During processing, the internal control acceptance criteria is automatically verified by the Panther Fusion system software. Detection of the internal control is not required for samples that are positive for *Yersinia enterocolitica*, *Vibrio* species, *Escherichia coli* O157, and *Plesiomonas shigelloides*. The internal control must be detected in all samples that are negative for all intended analytes. Samples that fail to meet that criteria will be reported as Invalid. Each sample with an Invalid result must be retested. Both types of controls must generate results within predefined specifications to be valid.

V Substantial Equivalence Information:

A Predicate Device Name(s):

FilmArray Gastrointestinal Panel (GI) for use with FilmArray Torch

B Predicate 510(k) Number(s):

K160459

C Comparison with Predicate(s):

Device & Predicate Device(s):	K251993	K160459
Device Trade Name	Hologic Panther Fusion GI Expanded Bacterial Assay	BioFire FilmArray Gastrointestinal (GI) Panel
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Panther Fusion GI Expanded Bacterial Assay is a multiplex real-time PCR *in vitro* diagnostic test for the rapid and qualitative detection and differentiation of *Yersinia enterocolitica*, *Vibrio* (*V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*), *Escherichia coli* O157, and *Plesiomonas shigelloides*. Nucleic acids are isolated and purified from Cary-Blair preserved stool specimens collected from individuals exhibiting signs and symptoms of gastroenteritis. This assay is intended to aid in the differential diagnosis of *Yersinia enterocolitica*, *Vibrio* (*V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*), *Escherichia coli* O157, and *Plesiomonas shigelloides* infections. The results of this assay should be used in conjunction with clinical presentation, laboratory findings, and epidemiological information and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out coinfection with other organisms that are not detected by this test and may not be the sole or definitive cause of patient illness. Negative 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. This assay is designed for use on the Panther Fusion System	The FilmArray Gastrointestinal (GI) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with FilmArray systems. The FilmArray 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 FilmArray 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) The FilmArray 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 FilmArray 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 FilmArray 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 noninfectious 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.
Technology	Fully automated nucleic acid amplification, detection and result interpretation (multiplex PCR)	Same
Specimen type	Human stool in Cary Blair transport medium	Same
Sample preparation	Automated sample processing	Same
General Device Characteristic Differences		
Analyte	DNA	DNA/RNA
Organisms detected	*Yersinia enterocolitica*, *Vibrio* (*V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*), *Escherichia coli* O157, and *Plesiomonas shigelloides*	*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)
Amplification mode	Real Time PCR	Nested multiple PCR
Detection Mode	Fluorogenic target-specific hybridization	Fluorogenic double stranded DNA binding dye
Instrumentation	Panther Fusion System	BioFire Film Array Systems
Time to Results	Approximately 2.4 hours	Approximately 1 hour
Controls	Internal control in each sample. External control processed at periodic interval	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:

Special Controls and Guidance Documents:

- 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 (November 2015)
- The 510(k) Program: Evaluating Substantial Equivalence in Premarket Notifications [510(k)], Guidance for Industry and Food and Drug Administration Staff, July 2014

Standards

- CLSI EP07 3rd Edition. Interference Testing in Clinical Chemistry.
- CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition.
- CLSI EP12 3rd Edition. Evaluation of Qualitative Binary Output Examination Performance.
- CLSI EP17-A2. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition.
- CLSI EP24-A2. Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Approved Guideline - Second Edition. (Replaces GP10-A)
- CLSI EP25. Evaluation of Stability of in vitro Medical Laboratory Test Reagents. 2nd Edition.
- CLSI MM03 3rd Edition. Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline. 2nd Edition. (Replaces MM03-A2).
- CLSI EP37. Supplemental Tables for Interference Testing in Clinical Chemistry. 1st Edition.
- ISO 15223-1 Medical devices - Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements. Fourth Edition 2021-07.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision: Within-laboratory precision for the Panther Fusion GI Expanded Bacterial Assay was evaluated at one internal site, using three lots of reagents on three instruments by three operators, and each operator performed two runs per day. Testing was conducted over nine days extended over 22 calendar days. Each sample was tested with three replicates in each run, for a total of 162 replicates per sample. The study included a panel of five samples: (1) negative sample, (2) low positive sample with *Yersinia enterocolitica* at 1.5x LoD, (3) moderate positive sample with *Yersinia enterocolitica* at 3xLoD, (4) low positive sample with *Vibrio*, STEC O157, and *Plesiomonas shigelloides* at 1.5xLoD, and (5) moderate positive sample with *Vibrio*, STEC O157, and *Plesiomonas shigelloides* at 3xLoD. All panel samples were generated in Cary-Blair Stool (CBS) matrix. All five samples were in 100% agreement with the expected results. Within-laboratory precision study results are summarized in table 2 below.

Table 2. Precision Study Results.

Panel	Description	Analyte	Agreed/Total	Agreement %[a]	Mean Ct	Between Lots		Between Instruments		Between Operators		Between Days		Between Runs		Within Run		Total	
						SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	Negative	Negative (Internal Control)	162/162	100	34.6	0.07	0.2	0.08	0.23	0.04	0.12	0.00	0.00	0.00	0.00	0.48	1.39	0.5	1.43
2	Low Pos (1.5X LoD)	*Yersinia*	162/162	100	33.7	0.03	0.08	0.09	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.41	1.23	0.42	1.26
3	Mod Pos (1.5X LoD)	*Yersinia*	162/162	100	33.7	0.12	0.35	0.07	0.21	0.01	0.04	0.00	0.00	0.17	0.52	0.23	0.69	0.32	0.95
4	Low Pos (1.5X LoD)	*Vibrio*	162/162	100	32.7	0.07	0.21	0.12	0.37	0.00	0.00	0.00	0.00	0.19	0.57	0.2	0.6	0.3	0.93
		STEC O157	162/162	100	32.4	0.02	0.08	0.04	0.13	0.00	0.00	0.00	0.00	0.11	0.34	0.28	0.87	0.31	0.95
		Plesiomonas	162/162	100	31.3	0.02	0.08	0.06	0.2	0.00	0.00	0.03	0.1	0.00	0.00	0.21	0.68	0.22	0.72
3	Mod Pos (3X LoD)	*Vibrio*	162/162	100	33.8	0.08	0.25	0.05	0.14	0.00	0.00	0.00	0.00	<0.01	0.03	0.25	0.73	0.26	0.78
		STEC O157	162/162	100	33.1	0.05	0.17	<0.01	0.03	0.01	0.03	0.06	0.17	0.00	0.00	0.19	0.56	0.2	0.61
		Plesiomonas	162/162	100	28	0.11	0.39	0.32	1.15	0.00	0.00	0.00	0.00	0.12	0.42	0.14	0.51	0.39	1.39

Ct = cycle threshold, CV = coefficient of variation, Mod = Moderate, N = sample size, Pos = positive, SD = standard deviation.
[a] Agreement to expected panel positivity result.

2. Reproducibility: Reproducibility for the Panther Fusion GI Expanded Bacterial Assay was evaluated at three U.S. clinical testing sites. Testing was performed using one Panther Fusion System at each site and the Panther Fusion GI expanded Bacterial Assay Controls. The study included the same sample panel tested during the within-laboratory precision. Testing was conducted by two operators per site over at least five days using one reagent lot. Each operator tested each panel member in replicates of three in each run for a total of 90 replicates per panel member. All sample panels showed 100% agreement with the expected results. Study results are summarized in tables below.

Table 3. Reproducibility Study: Overall Agreement

Target	Concentration[1]	Expected Result	Agreed/N	Agreement (%) 95% CI[2]
Yersinia	Negative	Negative	89/89	100 (95.9-100)
	Low Positive	Positive	90/90	100 (95.9-100)
	Mod Positive[3]	Positive	90/90	100 (95.9-100)
Vibrio	Negative	Negative	89/89	100 (95.9-100)
	Low Positive	Positive	90/90	100 (95.9-100)
	Mod Positive	Positive	90/90	100 (95.9-100)
O157	Negative	Negative	89/89	100 (95.9-100)
	Low Positive	Positive	90/90	100 (95.9-100)
	Mod Positive	Positive	90/90	100 (95.9-100)
Plesiomonas	Negative	Negative	89/89	100 (95.9-100)
	Low Positive	Positive	90/90	100 (95.9-100)
	Mod Positive	Positive	90/90	100 (95.9-100)

Mod = moderate.

[1]The concentration category of 'negative' only includes results from the negative panel member (i.e., matrix only).
[2]Score CI.
[3]There was one positive *Vibrio* result on the *Yersinia* moderate positive panel.

Table 4. Reproducibility Study: Signal Variability of the Panther Fusion GI Expanded Bacterial Assay.

				Between Site		Between Operator/Run[c]		Between Day		Within Run		Total	
Description	Analyte	N	Mean Ct	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Low Pos[a]	*Yersinia*	90	34.7	0.17	0.50	0.21	0.61	0.09	0.27	0.44	1.25	0.52	1.51
	Vibrio	90	33.7	0.16	0.49	0.08	0.25	0.00	0.00	0.26	0.77	0.32	0.95
	STEC O157	90	32.4	0.17	0.53	0.13	0.41	0.00	0.00	0.30	0.92	0.37	1.14
	Plesiomonas	90	33.9	0.16	0.47	0.06	0.17	0.00	0.00	0.32	0.94	0.36	1.06
Mod Pos[b]	*Yersinia*	90	33.8	0.35	1.03	0.19	0.58	0.07	0.21	0.37	1.08	0.55	1.61
	Vibrio	90	32.7	0.20	0.60	0.09	0.26	0.11	0.35	0.22	0.68	0.33	1.01
	STEC O157	90	31.4	0.24	0.75	0.08	0.27	0.07	0.21	0.26	0.81	0.36	1.16
	Plesiomonas	90	33.2	0.22	0.67	0.12	0.37	0.00	0.00	0.26	0.78	0.36	1.09

Ct = cycle threshold, CV = coefficient of variation, Mod = moderate, N = sample size, Pos = positive, SD = standard deviation.
Note: The analysis was performed using the SAS MIXED procedure, which applies a lower boundary of 0 to all variance components in the model by default. If a variance component is 0, SD, and %CV are displayed as 0.00
[a] Low Pos = All targets are 1.5X LoD.
[b] Mod Pos = All targets are 3X LoD.
[c] Between Operator may be confounded with Between Run; therefore, Between Operator and Between Run estimates are combined in Between Operator/Run.

Table 5. Reproducibility Study: Signal variability of the Panther Fusion GI Expanded Bacterial Assay Positive Control.

				Between Site		Between Operator		Between Day		Within Day		Total	
Control	Analyte	N	Mean Ct	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Pos	*Yersinia*	30	32.7	0.22	0.66	0.00	0.00	0.00	0.00	0.25	0.75	0.33	1.01
	Vibrio	30	33.4	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.86	0.29	0.86
	STEC O157	30	31.5	0.11	0.35	0.00	0.00	0.07	0.23	0.26	0.83	0.29	0.93
	Plesiomonas	30	32.9	0.05	0.16	0.08	0.23	0.12	0.37	0.24	0.74	0.29	0.87

Ct = cycle threshold, CV = coefficient of variation, N = sample size, Pos = positive, SD = standard deviation.
Note: The analysis was performed using the SAS MIXED procedure, which applies a lower boundary of 0 to all variance components in the model by default. If a variance component is 0, SD and %CV are displayed as 0.00.

3. Linearity:
Not applicable.

4. Analytical Specificity/Interference:

Cross-reactivity

Cross-reactivity and microbial interference for the Panther Fusion GI Expanded Bacterial Assay were evaluated in the presence of non-targeted microorganisms that are either phylogenetically related to the assay analytes or potentially found in clinical specimens. Panels, consisting of 109 bacteria, viruses, parasites, and yeast (listed in Table 6), were tested in negative CBS matrix in the absence and in the presence of GI Expanded Bacterial Assay analytes at 3x LoD. Except where noted, bacteria, yeast, and parasites were evaluated at 10^6 CFU/mL or 10^6 rRNA copies/mL or 10^6 cells/mL; viruses were evaluated at 10^5 TCID50/mL. If cross-reactivity or interference was observed in the initial testing, then the organism was tested at lower concentrations until the expected result was observed. No cross-reactivity or microbial interference was observed with any of the organisms tested on the Panther Fusion GI Expanded Bacterial Assay at the indicated concentrations.

Table 6. Microorganisms tested for Cross-Reactivity and Microbial Interference

Microorganism	Test Concentration	Microorganism	Test Concentration
Arcobacter cryaerophilus	10^6 CFU/mL	*Entercoccus faecalis*	10^6 CFU/mL
Neisseria gonorrhoeae	10^6 CFU/mL	*Enterobacter aerogenes*	10^6 CFU/mL
Streptococcus pyogenes	10^6 CFU/mL	*Enterobacter cloacae*	10^6 CFU/mL
Trabulsiella guamensis	10^6 CFU/mL	*Escherichia fergusonii*	10^6 CFU/mL
Faecalibacterium prausnitzii	10^6 rRNA copies /mL	*Escherichia hermanii*	10^6 CFU/mL
Escherichia coli (non-shigatoxigenic)	10^6 CFU/mL	*Escherichia vulneris*	10^6 CFU/mL
Giardia lamblia BG-A[a]	10^6 copies/mL	*Gardnerella vaginalis*	10^6 CFU/mL
Cyclospora[a]	10^6 copies/mL	*Helicobacter pylori*	10^6 CFU/mL
Cryptosporidium[a]	10^6 copies/mL	*Klebsiella oxytoca*	10^6 CFU/mL
Norovirus (Noro GII)[a]	10^6 copies/mL	*Klebsiella ozaenae*	10^6 CFU/mL
Astrovirus[a]	10^6 copies/mL	*Klebsiella pneumoniae*	10^6 CFU/mL
Sapovirus (GII)[a]	10^5 copies/mL	*Lactobacillus acidophilus*	10^6 CFU/mL
Enterovirus (Ent V)[a]	10^5 copies/mL	*Lactobacillus crispatus*	10^6 CFU/mL
Rhinovirusa	10^5 copies/mL	*Lactococcus lactis*	10^6 CFU/mL
Coronavirus 229E	10^5 TCID50/mL	*Listeria grayi*	10^6 CFU/mL
Coxsakeivirus Type B4	10^5 TCID50/mL	*Listeria monocytogenes*	10^6 CFU/mL
Adenovirus Type 7A	10^5 TCID50/mL	*Morganella morganii*	10^6 CFU/mL
Rotavirusa	10^5 copies/mL	*Peptostreptococcus anaerobius*	10^6 CFU/mL
Anaerococcus tetradius	10^6 CFU/mL	*Peptostreptococcus micros*	10^6 rRNA copies /mL
Abiotrophia defectivia	10^6 CFU/mL	*Photobacterium damselae*	10^6 CFU/mL
Acinetobacter baumannii	10^6 CFU/mL	*Prevotella bivia*	10^6 CFU/mL
Acinetobacter lwoffii	10^6 CFU/mL	*Prevotella melaninogenica*	10^6 CFU/mL
Aeromonas hydrophila	10^6 CFU/mL	*Proteus mirabilis*	10^6 rRNA copies /mL
Alcaligenes faecalis	10^6 CFU/mL	*Proteus penneri*	10^6 CFU/mL
Campylobacter upsaliensis	10^6 CFU/mL	*Proteus vulgaris*	10^6 CFU/mL
Anaerococcus vaginalis	10^6 CFU/mL	*Providencia alcalifaciens*	10^6 CFU/mL
Arcobacter butzleri	10^6 CFU/mL	*Providencia rettgeri*	10^6 CFU/mL

Microorganism	Test Concentration	Microorganism	Test Concentration
Bacillus cereus	10^6 CFU/mL	*Providencia stuartii*	10^6 CFU/mL
Bacteriodes fragilis	10^6 CFU/mL	*Pseudomonas aeruginosa*	10^6 CFU/mL
Bacteroides thetaiotaomicron	10^6 CFU/mL	*Pseudomonas fluorescens*	10^6 CFU/mL
Bacteroides vulgatus	10^6 CFU/mL	*Serratia liquefaciens*	10^6 CFU/mL
Bifidobacterium adolescentis	10^6 CFU/mL	*Serratia marcescens*	10^6 CFU/mL
Bifidobacterium longum	10^6 rRNA copies /mL	*Staphylococcus aureus*	10^6 CFU/mL
Campylobacter fetus	10^6 CFU/mL	*Staphylococcus epidermidis*	10^6 CFU/mL
Campylobacter hyointestinalis	10^6 CFU/mL	*Stenotrophomonas maltophilia*	10^6 CFU/mL
Campylobacter rectus	10^6 CFU/mL	*Streptococcus anginosus*	10^6 CFU/mL
Campylobacter sputorum	10^6 CFU/mL	*Streptococcus dysgalactiae*	10^6 CFU/mL
Candida albicans	10^6 CFU/mL	*Yersinia bercovieri*	10^6 CFU/mL
Citrobacter freundii	10^6 CFU/mL	*Yersinia pseudotuberculosis*	10^6 CFU/mL
Citrobacter koseri	10^6 CFU/mL	*Yersinia rohdei*	10^6 CFU/mL
Clostridium difficile	10^6 CFU/mL	*Campylobacter lari*	10^6 CFU/mL
Clostridium perfringens	10^6 CFU/mL	*Entamoeba histolytica*	10^4 cells/mL
Clostridium ramosum	10^6 CFU/mL	*Megasphaeara elsdenii*	10^6 CFU/mL
Clostridium sordellii	10^6 CFU/mL	*Chlamydia trachomatis*	10^5 IFU/mL
Clostridium tertium	10^6 CFU/mL	*Leptotrichia buccalis*	10^6 CFU/mL
Collinsella aerofaciens	10^6 CFU/mL	*Cytomegalovirus*	10^5 TCID50/mL
Corynebacterium genitalium	10^6 CFU/mL	*Salmonella enterica*	10^6 CFU/mL
Cronobacter sakazakii	10^6 CFU/mL	*Campylobacter jejuni*	10^6 CFU/mL
Edwardsiella tarda	10^6 CFU/mL	*Shigella sonnei*	10^6 CFU/mL
Egglerthella lenta	10^6 rRNA copies /mL	*STEC - stx1*	10^6 CFU/mL
STEC - stx2	10^6 CFU/mL	*Vibrio mimicus*	10^6 CFU/mL
Vibrio fluvialis	10^6 CFU/mL	*Yersinia frederiksenii*	10^6 CFU/mL
Vibrio furnissii	10^6 CFU/mL	*Yersinia kristensenii*	10^6 CFU/mL
Vibrio metschnikovii	10^6 CFU/mL	*Vibrio alginolyticus*[b]	10^4 CFU/mL

CFU = colony forming units, IFU = inclusion forming units, rRNA copies = ribosomal ribonucleic acid copies, $TCID_{50}$ = Median Tissue Culture Infectious Dose.

[a] *In vitro* transcripts were used to evaluate cross-reactivity and microbial interference as cultured virus or whole genome purified nucleic acid are not readily available.

[b] Cross reactivity was observed at concentrations $\geq 10^5$ CFU/mL.

Inclusivity/Reactivity

The inclusivity/reactivity of the Panther Fusion GI Expanded Bacterial Assay was determined by testing multiple bacterial strains for each of the intended analytes in negative CBS matrix. Each strain was tested in triplicate at 3x LoD with 1 reagent lot in single or multi-analyte configuration. *Vibrio vulnificus* ATCC# CCUG 38297 strain was not detected at 3x LoD and additional testing at higher concentrations was performed until 100% positivity was observed at 5x LoD. Table 7 shows the lowest concentration of each strain at which 100% positivity was observed.

Table 7. Summary of inclusivity/reactivity study results.

Organism	ATCC# or Source	Strain/ serovar/ serotype/ antigenic properties	Test Concentration (3xLoD) (CFU/mL)	
			Aptima Multitest Tube	Preserved Stool
Yersinia enterocolitica	BEI NR-207	CDC 497-70, O:8	282	5,640
	BEI NR-212	NCTC 11175, O:3	282	5,640
	23715	Billups-1803-68, O:8	282	5,640
	49397	1375, O:8[c]	282	5,640
	NCTC 10463	P 77, O:5, 27	282	5,640
	CCUG 4588	Type 2, O:9	282	5,640
	CCUG 8050	N/A	282	5,640
	CCUG 8232	Type 5, O:1, 2, 3 | O:2, 3 | O:3/XI	282	5,640
	CCUG 8234	Type 4	282	5,640
	55075	O:9	282	5,640
	27729	WA, Type 1, O:8	282	5,640
Vibrio parahaemolyticus	BEI NR-21990	48057, O4: K12	270	5,400
	BEI NR-21992	KXV 755, O4: K41	270	5,400
	BAA-242	VP250, O1:KUT	270	5,400
	27969	FC 1011	270	5,400
	BAA-241	VP232, O4:K68	270	5,400
	33845	117 [CDC KC830]	270	5,400
	43996	NCTC 10884 [70/116655]	270	5,400
	33846	205 [9302]	270	5,400
	49529	MDL 3875-7-83, O4:K12	270	5,400
	CCUG 34902	N/A	270	5,400
	CCUG 67711	N/A	270	5,400
	33847	279 [11590]	270	5,400
Vibrio vulnificus	33817	329 [CDC B3547], Biotype 2	33	660
	BAA-86	CDC 9505-95	33	660
	CCUG 38297	N/A	33	660
	CCUG 47321	N/A	33	660
	29306	CDC A1402 [P. Baumann 328]	33	660
	43382	VVL1	33	660
	29307	CDC A8694	33	660
	CCUG 38297	N/A[b]	55	1,110
Vibrio cholerae	BEI NR-147	N16961, O:1	99	1,980
	BEI NR-148	CVD 101, O:1	99	1,980
	BEI NR-149	Nanking 32/123, O:2	99	1,980
	BEI NR-152	Nanking 32/124 (NCTC 8042), O:7	99	1,980
	14033	NCTC 8457 [R. Hugh 1092], O1, Inaba	99	1,980
	9459	AMC 20-A-10 [R. Hugh 583], Inaba	99	1,980
	CCUG 2573	NAG/NCV	99	1,980
	CCUG 2569	NAG/NCV	99	1,980
	CCUG 4070	Non O-1	99	1,980
	CCUG 21589	18	99	1,980
	CCUG 56875	N/A	99	1,980
	CCUG 53725	O1/O139	99	1,980
	CCUG14542	NA	99	1,980
	9458	AMC 20-A-41 [R. Hugh 582], Ogawa	99	1,980
	25870	569B	99	1,980
STEC O157: H7	43890	CDC C984 [CDC 3526-87], H7	159	3,180

Organism	ATCC# or Source	Strain/ serovar/ serotype/ antigenic properties	Test Concentration (3xLoD) (CFU/mL)	
			Aptima Multitest Tube	Preserved Stool
	43895	CDC EDL 933, H7	159	3,180
	43894	CDC EDL 932, H7	159	3,180
	700927	EDL 933, H7:K-	159	3,180
STEC O157: NM	700375	CDC 94-G7771, NM	1,197	23,940
	700377	CDC 92-3099, NM	1,197	23,940
	700378	CDC 92-3073, NM	1,197	23,940
	AR Bank # 427[a]	N/A	1,197	23,940
	AR Bank # 428a	N/A	1,197	23,940
	AR Bank # 429a	N/A	1,197	23,940
	AR Bank # 430a	N/A	1,197	23,940
Plesiomonas shigelloides	14030	CDC 16408 [Ferguson and Henderson C27, RH 864], O:17	195	3,900
	51903	GNI 14[c]	195	3,900
	51572	CIP 69.35 [2886]	195	3,900
	CCUG 7041A	O17: H2	195	3,900
	CCUG 9221	O17	195	3,900
	CCUG 14309	O17: H2	195	3,900
	CCUG 14597	N/A	195	3,900

CFU = colony forming units.

[a] These strains were evaluated using the higher LoD of the 2 serotypes which is the NM serotype.

[b] For this strain 100% positivity was observed at ~ 5X LoD. *In silico* analysis showed 100% homology to amplification region.

[c] Strains used to establish LoD.

The inclusivity/reactivity of the Panther Fusion GI Expanded Bacterial Assay was also evaluated *in silico* for each intended analyte. *In silico* analysis was performed using analyte sequences available in the NCBI database and the whole genome shotgun sequence database. For each analyte, corresponding oligonucleotide sequences (primers and probes) were evaluated against the database sequences. Any sequences with insufficient lengths (not covering the entire amplicon region) were excluded from the analysis.

Based on *in silico* analysis of all sequences available up to May 30, 2023, in the databases, the Panther Fusion GI Expanded Bacterial Assay is predicted to detect 99.9% of 1,054 Y*ersinia Enterocolitica*, 99.5% of 1,337 *Vibrio parahaemolyticus*, 99.1% of 1,180 *Vibrio vulnificus*, 98.0% of 1,189 *Vibrio cholerae*, 100% of 2,004 STEC O157, and 91.5% of 47 *Plesiomonas shigelloides* sequences evaluated.

Competitive interference

Competitive interference in the Panther Fusion GI Expanded Bacterial Assay was evaluated in triplicate using pairs of assay analytes at low/high concentrations in negative CBS matrix. The low concentration analyte was tested at 3xLoD against a high concentration analyte at

10^6 CFU/mL. Additionally, analytes were also tested in the absence of a second analyte. If less than 100% positivity was observed for the low concentration analyte, the high concentration analyte was diluted until a concentration was reached where 100% positivity was achieved for the low concentration analyte. This was observed when high concentrations of *Vibrio* (10^6 and 10^5 CFU/mL) and low concentrations for Yersinia or STEC O157 were present. The highest concentration of competing analyte at which the low concentration analyte maintained a 100% positivity is shown in Table 8. When the analytes were tested at high concentration, all results for other analytes maintained expected positivity.

Table 8. Summary of Competitive Interference study results.

Analyte 1		**Analyte 2**					
Name	**3xLOD (CFU/mL)[a]**	**Name**	**High Conc (CFUmL)**	***Yersinia* % Pos**	***Vibrio* % Pos**	**STEC O157 % Pos**	***Plesiomonas* % Pos**
Negative	NA	Negative	NA	0%	0%	0%	0%
Yersinia	282	None	0	**100%**	0%	0%	0%
		Vibrio	1.0E+6	0%	**100%**	0%	0%
		Vibrio	1.0E+5	66%	**100%**	0%	0%
		Vibrio	1.0E+4	**100%**	**100%**	0%	0%
		Vibrio	1.0E+3	**100%**	**100%**	0%	0%
		STEC O157	1.0E+6	**100%**	0%	**100%**	0%
		Plesiomonas	1.0E+6	**100%**	0%	0%	**100%**
Vibrio	270	None	0	0%	**100%**	0%	0%
		Yersinia	1.0E+6	**100%**	**100%**	0%	0%
		STEC O157	1.0E+6	0%	**100%**	**100%**	0%
		Plesiomonas	1.0E+6	0%	**100%**	0%	**100%**
STEC O157	1197	None	0	0%	0%	**100%**	0%
		Yersinia	1.0E+6	**100%**	0%	**100%**	0%
		Vibrio	1.0E+6	0%	**100%**	0%	0%
		Vibrio	1.0E+5	0%	**100%**	33%	0%
		Vibrio	1.0E+4	0%	**100%**	**100%**	0%
		Vibrio	1.0E+3	0%	**100%**	**100%**	0%
		Plesiomonas	1.0E+6	0%	0%	**100%**	**100%**
Plesiomonas	195	None	0	0%	0%	0%	**100%**
		Yersinia	1.0E+6	**100%**	0%	0%	**100%**
		Vibrio	1.0E+6	0%	**100%**	0%	**100%**
		STEC O157	1.0E+6	0%	0%	**100%**	**100%**
None	0	*Yersinia*	1.0E+6	**100%**	0%	0%	0%
		Vibrio	1.0E+6	0%	**100%**	0%	0%
		STEC O157	1.0E+6	0%	0%	**100%**	0%
		Plesiomonas	1.0E+6	0%	0%	0%	**100%**

CFU = colony forming units, Pos = positive.
[a] Analyte concentration in Aptima Multitest tube.

Interference

Potential inhibitory effects of endogenous and exogenous substances that may be present in a specimen were evaluated in the Panther Fusion GI Expanded Bacterial Assay. Clinically relevant concentrations of potentially interfering substances were added to negative CBS matrix and tested in the absence and in the presence of GI Expanded Bacterial Assay analytes at 3x LoD. Tests were performed in triplicate. The substances and test concentrations are shown in Table 9 below. Interference was observed with Mucin at concentrations higher than

0.1% (*w:v*), generating false negative results for *Vibrio*, *Yersinia* and *Plesiomonas*. ParaPak 10% Buffered Formalin showed interference in the detection of *Vibrio* and *Yersinia*, and ParaPak LV-PVA also showed interference for all analytes detected with the device.

Table 9. Summary of Interference study results.

Pool #	Substance Type	Generic Name	% Positivity: Negative Panels	% Positivity: Positive Panels
Neg Control	**NA**	Negative CBS matrix	0.0%	NA
Pos Control A		*Yersinia* and *V.prahaemolyticus* in CBS Matrix	NA	100%
Pos Control B	**NA**	STEC O157 and *P.shigelloides* in CBS Matrix	NA	100%
1	**Antibiotics**	Amoxicillin	0.0%	100%
		Ampicillin		
		Doxycycline		
		Metronidazole		
2		Neosporin		
	Anti-microbial and anti- fungal	BZK Antiseptic Towelettes	0.0%	100%
		Nystatin		
3	**Laxatives and Stool Softeners**	Dulcolax suppository	0.0%	100%
		Colace		
		Fleet mineral oil enema		
		Ex-Lax		
4		Miralax	0.0%	100%
		Milk of Magnesia		
		Visicol		
5	**Anti-diarrheal**	Imodium	0.0%	100%
6	**Anti-Itch**	Vagisil	0.0%	100%
		Preparation H		
7	**Anti-Inflammatory**	Phenylephrine hydrochloride (for hemorrhoids)	0.0%	100%
		Mesalazine (Rx only, for Crohns disease/ ulcerative colitis)		
		Aleve		
8	**Antacid**	Pepto-Bismol	0.0%	100%
		Tums		
9	**Radiopaque contrast material**	Barium Sulfate	0.0%	100%
10	**Lubricants and Skin Protectants**	K-Y®Personal Lubricant Jelly Glycerin	0.0%	100%
		Vaseline Original 100% Pure Petroleum Jelly White		
		Desitin		
11	**Spermicide**	Options Conceptrol®Vaginal Contraceptive Gel	0.0%	100%
12	**Endogenous**	Cholesterol	0.0%	100%
		Fatty acids		
		Fatty acids		
		Triglycerides, total (Fecal fat, Intralipid)		
13		Human Bile	0.0%	100%
		Urine		
14		Human Whole Blood	0.0%	100%
15		Mucin (1.3% w/v)	0.0% (66% IC valid)	33% *Yersinia*, STEC O157, and 0% *Vibrio* and *Plesiomonas*

Pool #	Substance Type	Generic Name	% Positivity: Negative Panels	% Positivity: Positive Panels
15a		Mucin (1% w/v)	0.0%	0% *Yersinia*, *Vibrio*, 33% *Plesiomonas* and 67 % STEC O157
15b		Mucin (0.5% w/v)	0.0%	0% *Yersinia, Vibrio,* 33% *Plesiomonas* and 67 % STEC O157
15c		Mucin (0.1% w/v)	0.0%	100%
15d		Mucin (0.05% w/v)	0.0%	100%
16	**Fixation Buffer**	Fisher 10% Buffered Formalin	0.0%	100%
17		Parapak 10% Buffered Formalin	0.0%	0% *Yersinia* and *Vibrio*
18		ParaPak LV-PVA	0.0% (0% IC valid)	0%
19	**Solvent Control**	Ethanol	0.0%	100%
20		Cary- Blair Media	0.0%	100%

5. Assay Reportable Range:
   Not applicable.

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

   Sample stability in Cary-Blair transport medium.
   The stability of Panther Fusion GI Expanded Bacterial assay analytes was determined when specimens are stored as Cary-Blair preserved stool (CBS) under various storage durations and conditions, prior to transfer to an Aptima Multitest tube for testing. Stability testing panels were prepared by spiking bacterial cells into 10 unique negative stools from individual donors. GI Expanded Bacterial Assay analytes were tested in single analyte format at 3x, 10x and/or 20x LoD concentrations. Aliquots of the spiked CBS samples were tested at baseline (immediately after preparation) and then stored at 4°C, 15°C, and 30°C. Stored aliquots are tested after 72 hours or 96 hours. A total of 2 replicates/stool were tested per analyte, concentration, and storage temperature condition. A negative condition (no target spike) was also tested using 14 unique negative stools (2 replicates/stool) at baseline and after 96 hours. Specimen stability for each analyte was determined as the longest stability duration at which ≥ 90% positivity was maintained. The study results support the following storage recommendations in Cary-Blair medium:
   - 2° to 8°C for up to 96 hours.
   - 15° to 30°C for up to 72 hours.

   Sample stability in Multitest tubes (containing Sample Transport Medium)
   The stability of Panther Fusion GI Expanded Bacterial assay analytes after transferring to the Aptima Multitest tube (processed) was established when stored under various storage durations and conditions. To assess processed specimen stability, samples were first subjected to primary specimen stability in Cary-Blair summarized in the above section, and then processed into the Aptima Multitest tubes. Once in the Aptima lysis tube, samples were stored at 30°C for ≥ 7 days (for 6 days stability), at 4°C for ≥ 31 days (for 30 days stability), and at -20°C for ≥ 91 days (for 90 days stability). To assess processed stability storage at room temperature (15 – 30°C) testing was only performed at 30°C to evaluate the worst-case scenario. A total 2 replicates/stool were tested per analyte, concentration, and storage temperature condition. A negative condition (no target spike) was also tested using 14 unique

negative stools (2 replicates/stool) at baseline and after 96 hours. The study results support the following storage conditions after collection:

- CB samples stored at 2 – 8°C for up to 96 hours or at 15 – 30°C for up to 72 hours can be transferred to the Aptima tubes and stored at:
    - 15-30°C for up to 6 days,
    - 2-8°C for up to 30 days, or
    - -20°C for up to 90 days

Fresh versus Frozen stability of Cary-Blair preserved stools (CBS)
The impact of freezing and multiple freeze/thaw (F/T) cycles on the stability of CBS specimens positive for GI Expanded Bacterial assay analytes relative to corresponding fresh specimens was performed. For this study 10 unique CBS pools were used to prepare the negative and contrived positive panels. Contrived positive panels were spiked with one GI Expanded Bacterial analyte at final concentrations of approximately 5xLoD, 2xLoD, and 0.99xLoD. Up to two freeze/thaw cycles were tested. This study demonstrated that freezing and thawing up to 2 cycles at the concentrations tested does not negatively impact the detection of *Plesiomonas shigelloides* and STEC O157 positivity. However, *Yersinia enterocolitica* and *Vibrio* positivity decreased after 1 F/T cycle. Therefore, it is not recommended to freeze stool samples in Cary Blair prior to testing with the Panther Fusion GI Expanded Bacterial Assay.

7. Detection Limit:
The Limit of Detection (LoD) of the Panther Fusion GI Expanded Bacterial Assay was determined by testing dilutions of negative CBS matrix spiked with bacterial cultures of *Yersinia enterocolitica* (2 strains), *Vibrio* (3 strains, one strain per organism detected), *Plesiomonas shigelloides* (2 strains), and STEC O157 (2 strains). A minimum of 24 replicates were tested with each of 3 reagent lots. The LoD for each analyte was determined by Probit analysis for each reagent lot and was confirmed with an additional 24 replicates using a single reagent lot in single analyte and multi- analyte configuration. LoD is defined as the lowest concentration at which ≥95% of all replicates tested positive, as summarized in Table 10 below.

Table 10. Limit of detection (LoD) for each analyte detected by the Panther Fusion GI Expanded Bacterial Assay.

Strains	LoD Concentration (CFU/mL)[a]	
	Aptima Multitest Tube	Preserved Stool
Yersinia enterocolitica, 33114	91	1,820
Yersinia enterocolitica, 1375, O:8	94	1,880
Vibrio parahaemolyticus, EB101	90	1,800
Vibrio vulnificus, B9629	10	200
Vibrio cholerae, 8021	33	660
STEC O157:H7, EDL 931	53	1,060
O157:NM, CDC 92-3073	357	7,140
Plesiomonas shigelloides, CDC 3085-55	65	1,300
Plesiomonas shigelloides, GNI 14	34	680

CFU = colony forming units.
[a] Analyte concentrations in Aptima Multitest tube are ~ 20X dilute compared to preserved stool (~150µL preserved stool in ~3 mL.

8. Assay Cut-Off:
   Not Applicable

9. Accuracy (Instrument):
   Not applicable

10. Carry-Over:
    Panther Fusion GI Bacterial Assay and GI Expanded Bacterial Assay belong to the same family of assays, and both utilize Cary Blair Stool as the sample type and follow identical assay processing steps. Carryover contamination was evaluated using Panther Fusion GI Bacterial Assay as a representative assay and demonstrated a 0% carryover rate. Please refer to K251868.

B Comparison Studies:

1. Method Comparison with Predicate Device:
   Not applicable.

2. Transport Media Equivalency:
   Equivalency between the Cary Blair (CB) transport media from different vendors for use with the Panther Fusion GI Expanded Bacterial assay was conducted using 7 unique CB transport medium. GI Expanded Bacterial Assay analytes were tested in each of the CB medium at approximately 3x LoD. Positive target panels were prepared in a multi-analyte format containing 2 analytes:

   - Positive CB Panel 1: contains *Yersinia enterocolitica* and *Vibrio* group.
   - Positive CB panel 2: contains STEC O157 and *Plesiomonas shigelloides.*

   For each transport medium under evaluation, positive (with GI Expanded Bacterial analytes) and negative (no analytes) panels were tested at a minimum of 20 replicates. Testing was performed with one lot of Panther Fusion GI Expanded Bacterial Assay reagents and one Panther Fusion instrument. Overall, a ≥ 95% positivity for all analytes was observed for all CB media tested, demonstrating equivalency when tested with the Panther Fusion GI Expanded Bacterial assay.

Table 11. Summary of Transport Media Equivalency Study results.

Panel type	Transport Media	% Positivity: Negative Panels	% Positivity: Positive Panels
Negative CB Panel	Cardinal Health C&S	0.0%	NA
	ETM	0.0%	
	MCC C&S	0.0%	
	Para-Pak Enteric Plus	0.0%	
	Para-Pak C&S	0.0%	
	Protocol CB Medium	0.0%	
	Remel CB w/ Indicator	0.0%	
Positive CB Panel 1	Cardinal Health C&S	NA	100%
	ETM		100%
	MCC C&S		≥95%
	Para-Pak Enteric Plus		100%

	Para-Pak C&S		100%
	Protocol CB Medium		≥95%
	Remel CB w/ Indicator		100%
Positive CB Panel 2	Cardinal Health C&S	NA	100%
	ETM		≥95%
	MCC C&S		100%
	Para-Pak Enteric Plus		100%
	Para-Pak C&S		≥95%
	Protocol CB Medium		100%
	Remel CB w/ Indicator		≥95%

C Clinical Studies:

1. Clinical Sensitivity:
   Not applicable.

2. Clinical Specificity:
   Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

A multicenter study was conducted using remnant stool specimens in Cary-Blair preservative medium collected as part of routine patient care at 10 US clinics. Samples were collected from pediatric or adult patients suspected of acute gastroenteritis. All specimens were tested with the Panther Fusion GI Expanded Bacterial Assay and with comparator assays: a PCR plus bidirectional sequencing (run in duplicate) for STEC O157 and an FDA-cleared Nucleic Acid Amplification Test (NAAT) for all other targets. An alternate FDA-cleared NAAT was used for discordant analysis, if applicable. Positive (PPA) and negative (NPA) percent agreement, with corresponding two-sided 95% Confidence Intervals (CI) were calculated relative to comparator results, by target and by specimen category.

A total of 1,548 prospective specimens and 251 retrospective archived specimens were enrolled in the study; 94 specimens were excluded from the performance analyses (e.g., duplicate individuals or invalid Panther Fusion or comparator results for all targets). An additional 189 contrived specimens were tested to supplement the prospective and retrospective data for all targets. Of the 1,919 specimens tested in valid Panther Fusion GI Expanded Bacterial Assay runs, 36 (1.9%) had initial invalid results. Upon retest, 25 of the 36 specimens yielded valid results, for a total of 11 (0.6%) specimens with final invalid results. The final data set consisted of 1,894 evaluable specimens (1,523 prospective samples, 182 retrospective samples, and 189 contrived samples). Demographic information for the 1,705 evaluable prospective and retrospective archived specimens is provided in Table 12.

Table 12. Summary of Study Subject Demographics.

		Total N (%)	Prospective N (%)	Retrospective N (%)
	Total Specimens	1,705	1,523	182
Sex	Female	888 (52.1)	793 (52.1)	95 (52.2)
	Male	817 (47.9)	730 (47.9)	87 (47.8)
Age Group	0 to 28 days	7 (0.4)	7 (0.5)	0 (0)
	29 days to <2 years	74 (4.3)	67 (4.4)	7 (3.8)
	2 to 5 years	55 (3.2)	50 (3.3)	5 (2.7)

	Total N (%)	Prospective N (%)	Retrospective N (%)
6 to 11 years	68 (4.0)	66 (4.3)	2 (1.1)
12 to 17 years	73 (4.3)	71 (4.7)	2 (1.1)
18 to 21 years	47 (2.8)	44 (2.9)	3 (1.6)
22 to 64 years	825 (48.4)	724 (47.5)	101 (55.5)
≥65 years	556 (32.6)	494 (32.4)	62 (34.1)

Performance characteristics for *Yersinia enterocolitica*, *Vibrio* (*V. cholerae*, *V. parahaemoliticus*, and *V. vulnificus*), STEC O157, and *Plesiomonas shigelloides* are presented by study cohort in tables below. No coinfections were detected by the Panther Fusion GI Expanded Bacterial Assay or by the comparator methods in prospective of retrospectively collected specimens.

Table 13. Summary of Clinical Performance – *Yersinia enterocolitica*

		PROSPECTIVE		RETROSPECTIVE		CONTRIVED	
		Comparator		Comparator		Comparator	
		Positive	Negative	Positive	Negative	Positive	Negative
Panther Fusion GI Expanded Bacterial Test	Positive	10	9[a]	15	3[c]	63	0
	Negative	1[b]	1487	0	164	0	126
	Total	11	1496	15	167	63	126
	PPA (95%CI)	90.9% (62.3% - 98.4%)		100% (79.6% - 100%)		100% (94.2% - 100%)	
	NPA (95%CI)	99.4% (98.9% - 99.7%)		98.2% (94.8% - 99.4%)		100% (97.0% - 100%)	

[a] 6 of 9 discordant false positive prospective specimens were positive for Yersinia by the alternate NAAT.
[b] The discordant false negative prospective specimen was negative for *Yersinia* by the alternate NAAT.
[c] The 3 discordant false positive retrospective specimens were positive for Yersinia by the alternate NAAT.

Table 14. Summary of Clinical Performance – *Vibrio* spp.

		PROSPECTIVE		RETROSPECTIVE		CONTRIVED	
		Comparator		Comparator		Comparator	
		Positive	Negative	Positive	Negative	Positive	Negative
Panther Fusion GI Expanded Bacterial Test	Positive	1	0	9	6[b]	63	1[c]
	Negative	1[a]	1505	0	167	0	125
	Total	2	1505	9	173	63	126
	PPA (95%CI)	50.0% (9. 5% - 90.5%)		100% (70.1% - 100%)		100% (94.3% - 100%)	
	NPA (95%CI)	100% (99.7% - 100%)		96.5% (92.6% - 98.4%)		99.2% (95.6% - 99.9%)	

[a] The discordant false negative prospective specimen was positive for Vibrio by the alternate NAAT.
[b] All 6 discordant false positive retrospective specimens were positive for Vibrio by the alternate NAAT.
[c] The discordant false positive contrived specimen was negative for Vibrio by the alternate NAAT.

Table 15. Summary of Clinical Performance – STEC O157

		PROSPECTIVE		RETROSPECTIVE		CONTRIVED	
		Comparator		Comparator		Comparator	
		Positive	Negative	Positive	Negative	Positive	Negative
Panther Fusion GI Expanded Bacterial Test	Positive	1	2[a]	3	1[b]	62	1[c]
	Negative	0	1519	0	178	1[d]	125
	Total	1	1521	3	179	63	126
	PPA (95%CI)	100% (20.7% - 100%)		100% (43.9% - 100%)		98.4% (91.54% - 99.7%)	
	NPA (95%CI)	99.9% (99.5% - 100%)		99.4% (96.9% - 99.9%)		99.2% (95.6% - 99.9%)	

[a] 1 of 2 discordant false positive prospective specimens was negative for STEC O157 by the alternate NAAT. The other discordant was positive for O157 but negative for stx1/stx2 by the alternate NAAT.
[b] The discordant false positive retrospective specimen was positive for STEC O157 by the alternate NAAT.
[c] The discordant false positive contrived specimen was negative for STEC O157 by the alternate NAAT
[d] The discordant false negative contrived specimen was not retested by the alternate NAAT

Table 16. Summary of Clinical Performance – *Plesiomonas shigelloides*

		PROSPECTIVE		RETROSPECTIVE		CONTRIVED	
		Comparator		Comparator		Comparator	
		Positive	Negative	Positive	Negative	Positive	Negative
Panther Fusion GI Expanded Bacterial Test	Positive	1	1[a]	8	1[b]	62	0
	Negative	0	1505	0	173	1[c]	126
	Total	1	1506	8	174	63	126
	PPA (95%CI)	100% (20.7% - 100%)		100% (67.6% - 100%)		98.4% (91.5% - 99.7%)	
	NPA (95%CI)	99.9% (99.6% - 99.9%)		99.4% (96.8% - 99.9%)		100% (97.0% - 100%)	

[a] The discordant false positive prospective specimen was positive for *Plesiomonas* by the alternate NAAT.
[b] The discordant false positive retrospective specimen was positive for *Plesiomonas* by the alternate NAAT.
[c] The discordant false negative contrived specimen was not retested by the alternate NAAT.

D Clinical Cut-Off:
Not applicable

E Expected Values/Reference Range:
In the Panther Fusion GI Expanded Bacterial Assay clinical study, reportable results from evaluable specimens were obtained from four geographically diverse sites and compared to the comparator method. The number and percentage of positive cases by target and site as determined by the Panther Fusion GI Expanded Bacterial Assay during the prospective segment of the clinical study are presented in table 17 below.

Table 17. Prevalence Values Observed during the Clinical Study

	% Positivity (# positive/# evaluable)			
Collection site	***Yersinia***	***Vibrio***	**O157**	***Plesiomonas***
Total evaluable for target, N	1507	1507	1522	1507
All Sites	1.3 (19/1507)	0.1 (1/1507)	0.2 (3/1522)	0.1 (2/1507)
Site 1	2.3 (10/441)	0 (0/441)	0.2 (1/447)	0 (0/441)
Site 2	1.0 (5/489)	0.2 (1/489)	0.2 (1/498)	0 (0/489)
Site 3	0.8 (4/482)	0 (0/482)	0.2 (1/482)	0.2 (1/482)
Site 4	0 (0/95)	0 (0/95)	0 (0/95)	1.1 (1/95)

F Other Supportive Instrument Performance Characteristics Data:
Not applicable

VIII Proposed Labeling:

The labeling supports or 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.