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

I Background Information:

A 510(k) Number

K251868

B Applicant

Hologic Inc.

C Proprietary and Established Names

Panther Fusion GI 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 Gastrointestinal (GI) Bacterial Assay on the Panther Fusion System

B Measurand:

Nucleic acids from *Salmonella, Shigella*/Enteroinvasive *Escherichia coli* (EIEC), *Campylobacter* (*C. coli*, *C. jejuni*) and Shiga-toxin producing *Escherichia coli* nucleic acids in addition to Shiga toxins 1 and 2 genes (undifferentiated).

C Type of Test:

Qualitative multiplex real-time polymerase chain reaction

III Intended Use/Indications for Use:

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

B Indication(s) for Use:
The Panther Fusion GI Bacterial Assay is a multiplex real-time PCR in vitro diagnostic test for the rapid and qualitative detection and differentiation of *Salmonella*, *Shigella*/Enteroinvasive *Escherichia coli* (EIEC), *Campylobacter* (*C. coli, C. jejuni*) nucleic acids and Shiga-toxin producing *Escherichia coli* Shiga toxins 1 and 2 (undifferentiated) genes. 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 *Salmonella*, *Campylobacter*, *Shigella*/Enteroinvasive *E. coli* (EIEC), and Shigatoxigenic *Escherichia coli* (STEC) 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 Bacterial Assay is a multiplex real-time PCR in vitro diagnostic test developed for use on the fully automated Panther Fusion system to detect nucleic acids from *Salmonella*, *Shigella*/ Enteroinvasive *Escherichia coli* (EIEC), *Campylobacter* (*C. coli, C. jejuni*) and Shiga-toxin producing *Escherichia coli* Shiga toxins 1 and 2 (undifferentiated) genes.

The Panther Fusion System fully automates specimen processing, including sample lysis, nucleic acid capture, amplification, and detection for the Panther Fusion GI Bacterial Assay. Nucleic acid capture and elution take 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: Stool specimens are collected from patients using commercially available collection kits and transferred into Cary Blair media. The Cary Blair preserved stool sample is then transferred into an Aptima Multitest tube containing 2.9 mL of specimen transport media by using Aptima multi-test specimen collection kit swab into the Cary Blair preserved stool. The amount of Cary-Blair Stool matrix transferred by a swab is approximately 150µL, equating to a 1:20 dilution of the Cary Blair preserved stool sample. The sample transport tube is placed on the Panther Fusion and 360µL of the sample is transferred into a MTU to undergo lysis and target capture.

Lysis, Nucleic Acid Capture and Elution: Specimen transport media 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.

Panther Fusion GI Bacterial Assay Kit Components: The reagents required to perform the Panther Fusion GI Bacterial Assay are packaged and sold separately. There are 7 boxes containing 9 reagents which are required for sample processing. A description of the components that are required to perform the Panther Fusion GI Bacterial Assay are detailed below. The Panther Fusion GI 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 Multi-test Swab Specimen Collection Kit (PRD-03546)

Stool specimens are collected from patients using commercially available collection kits and transferred into Cary Blair media. Stool specimens are then transferred to the Aptima Multi-test Tubes, which contain specimen transport media, by using the swab provided in the Aptima Multi-test Swab Specimen Collection Kit. This processing of the stool specimen prepares the sample for further testing on the Panther Fusion system.

The Panther Fusion Reconstitution Buffer I, Elution Buffer, and Oil Reagents are universally used for all Panther Fusion system assays.

C Instrument Description Information:

1. Instrument Name:

   Panther Fusion system

2. Specimen Identification:

   A barcode reader reads the barcodes assigned to the specimens.

3. Specimen Sampling and Handling:

   Specimen Collection and Transfer: 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 sample is then transferred into an Aptima Multi-test tube containing 2.9 mL of specimen transport media by using Aptima multi-test specimen collection kit swab into the Cary Blair preserved stool. The amount of Cary-Blair Stool matrix transferred by a swab is approximately 150µL, equating to a 1:20 dilution of the Cary Blair preserved stool sample. The sample transport tube is placed on the Panther Fusion and 360µL of the sample is transferred into a 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:

   The Panther Fusion system is configured to require that assay controls run at an administrator-specified interval of up to 30 days. Software on the Panther Fusion system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing.

   An internal control is added to each sample during automated specimen processing on the Panther Fusion system. During processing, the internal control acceptance criteria is automatically verified by the Panther Fusion system software.

V Substantial Equivalence Information:

A Predicate Device Name(s):
BioFire FilmArray Gastrointestinal Panel

B Predicate 510(k) Number(s):

K160459

C Comparison with Predicate(s):

Device & Predicate Device(s):	K251868	K160459
Device Trade Name	Hologic Panther Fusion GI Bacterial Assay	BioFire FilmArray Gastrointestinal Panel
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Panther Fusion GI Bacterial Assay is a multiplex real-time PCR in vitro diagnostic test for the rapid and qualitative detection and differentiation of *Salmonella*, *Shigella*/ Enteroinvasive *Escherichia coli* (EIEC), *Campylobacter* (*C. coli*, *C. jejuni*) nucleic acids and Shiga-toxin producing *Escherichia coli* Shiga toxins 1 and 2 (undifferentiated) genes. 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 *Salmonella*, *Campylobacter*, *Shigella*/Enteroinvasive *E. coli* (EIEC), and Shigatoxigenic *Escherichia coli* (STEC) 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 coinfection 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 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.
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
Intended Use	Professional use	Same
General Device Characteristic Differences		
Analyte	DNA	RNA/DNA
Organism Detected	*Salmonella*, *Campylobacter*, *Shigella*/Enteroinvasive *E. coli* (EIEC), and Shigatoxigenic *Escherichia coli* (STEC) infections	*Campylobacter* (*C. jejuni/C. coli/C. upsaliensis)*, *Clostridium difficile* toxin A/B, *Plesiomonas shigelloides*, *Vibrio* (*V. parahaemolyticus*/*V. vulnificus*/*V. cholerae*) including specific identification of *Vibrio cholera*, *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, and Sapovirus

		(Genogroups I,II, IV, and V).
Amplification Mode	Real-time polymerase chain reaction	Nested multiplex polymerase chain reaction
Detection Mode	Fluorogenic target-specific hybridization	Fluorogenic double stranded DNA binding dye
Instrument	Panther Fusion system	BioFire FilmArray system
Time to Result	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. Reproducibility: A site-to-site reproducibility study evaluated contrived samples at three external US sites (Site A, Site B, and Site C). The study incorporated a range of factors that may introduce variability in assay results, including sites, days, replicates, cartridge lots,

operators, and Panther Fusion systems. For each site, testing was performed across five non-consecutive days with three replicates per day (for a total of 30 replicates per target and concentration at each site), three Panther Fusion systems (one instrument per site), and at least two operators on each testing day. A total of five sample mixes (including evaluation of analyte levels corresponding to 1.5x LoD and 3x LoD as well as negative samples containing no analyte) were prepared in Cary-Blair stool matrix and transferred to Aptima specimen transport medium prior to testing. For each mix, nine replicates were tested and evaluated, and data obtained at all three sites were compiled to calculate the agreement with expected results and the two-sided 95% Confidence Interval.

The percent agreement with expected results for all analytes was ≥95% for samples tested at 1.5x and 3x LoD, meeting the acceptance criteria for the study. All analytes evaluated were detected within performance expectations for qualitative agreement across all concentrations tested. The Reproducibility Study site-to-site qualitative results (i.e., agreement with expected results) are presented in Table 1 below.

Table 1: Site-Site Reproducibility Study Qualitative Results

Pathogen Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
Salmonella	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100.0%)
	1.5x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100.0%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100.0%)
Campylobacter	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100.0%)
	1.5x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100.0%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100.0%)
Shigella	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100.0%)
	1.5x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100.0%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100.0%)
STEC	3x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100.0%)

	1.5x LoD	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100.0%)
	None	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100.0%)

2. Precision: Within Laboratory reproducibility testing evaluating contrived samples was performed at one internal test site. The study incorporated a range of factors that may introduce variability in assay results, such as: operators, days, instruments and reagent lots. Testing was performed across nine non-consecutive days with two replicates per day (for a total of 162 replicates per panel member) and using three lots of GI Bacterial Assay reagents tested across three different Panther Fusion systems run by three operators.

   The percent agreement with expected results for all analytes was ≥95% for samples tested at both 1.5x and 3x LoD, meeting the acceptance criteria for the study. A summary of the reproducibility study results is presented in Table 2 below.

Table 2: Within Laboratory Reproducibility, Qualitative Results

Pathogen Tested	Concentration Tested	Expected Result	Agreement with Expected Result	
Salmonella	3X LoD	Detected	162/162	100% (97.7-100.0%)
	1.5X LoD	Detected	162/162	100% (97.7-100.0%)
	None	Not Detected	0/162	100% (97.7-100.0%)
Campylobacter	3X LoD	Detected	162/162	100% (97.7-100.0%)
	1.5X LoD	Detected	162/162	100% (97.7-100.0%)
	None	Not Detected	0/162	100% (97.7-100.0%)
Shigella	3X LoD	Detected	162/162	100% (97.7-100.0%)
	1.5X LoD	Detected	162/162	100% (97.7-100.0%)
	None	Not Detected	0/162	100% (97.7-100.0%)
STEC	3X LoD	Detected	162/162	100% (97.7-100.0%)
	1.5X LoD	Detected	162/162	100% (97.7-100.0%)
	None	Not Detected	0/162	100% (97.7-100.0%)

3. Linearity:

   Not applicable

4. Analytical Specificity/Interference:

Cross-Reactivity:

Cross reactivity and microbial interference studies tested 100 non-targeted microorganisms (Table 3). When organisms were not available, synthetic targets (invitro transcripts (IVTs)) were used to evaluate cross reactivity and interference. 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 $TCID_{50}$/mL or at the highest concentration that could be attained based on titer of the stock and IVTs were evaluated at 10^6 copies/mL. Table 3 provides a summary of the microorganisms and concentrations evaluated. Pools of the microorganisms were prepared in negative Cary-Blair Stool matrix (Cary-Blair Stool matrix in specimen transport media) with each pool containing 1-5 organisms. Each pool was tested without (negative test panels) and with GI Bacterial Assay analytes at 3X LoD (positive test panels) for cross reactivity and interference respectively. Testing was conducted on one Panther Fusion system, using one reagent lot, and at 3 replicates per panel.

Samples that did not yield the expected result upon initial testing were retested with each non-targeted organism corresponding to those specific panels individually at 3 replicates. If the expected result was not observed upon re-testing, the microorganism in question was tested at lower concentrations until the expected result was observed. Testing was conducted at an internal site.

Table 3: Microorganisms tested for Cross-Reactivity and Microbial Interference

Microorganism	Test Concentration	Microorganism	Test Concentration
Arcobacter cryaerophilus	10^6 CFU/mL	*Cronobacter sakazakii*	10^6 CFU/mL
Neisseria gonorrhoeae	10^6 CFU/mL	*Edwardsiella tarda*	10^6 CFU/mL
Streptococcus pyogenes	10^6 CFU/mL	*Egglerthella lenta*	10^6 rRNA copies/mL
Trabulsiella guamensis	10^6 CFU/mL	*Entercoccus faecalis*	10^6 CFU/mL
Faecalibacterium prausnitzii	10^6 rRNA copies/mL	*Enterobacter aerogenes*	10^6 CFU/mL
Escherichia coli (non-shigatoxigenic)	10^6 CFU/mL	*Enterobacter cloacae*	10^6 CFU/mL
Escherichia coli (non-shigatoxigenic O157)	10^6 CFU/mL	*Escherichia fergusonii*	10^6 CFU/mL
Giardia lamblia BG-A	10^6 CFU/mL	*Escherichia hermanii*	10^6 CFU/mL
Cyclospora	10^6 CFU/mL	*Escherichia vulneris*	10^6 CFU/mL
Cryptosporidium	10^6 CFU/mL	*Gardnerella vaginalis*	10^6 CFU/mL
Norovirus (Noro GII)	10^5 CFU/mL	*Helicobacter pylori*	10^6 CFU/mL
Astrovirus	10^5 CFU/mL	*Klebsiella oxytoca*	10^6 CFU/mL
Sapovirus (GII)	10^5 CFU/mL	*Klebsiella ozaenae*	10^6 CFU/mL
Enterovirus (Ent V)	10^5 CFU/mL	*Klebsiella pneumoniae*	10^6 CFU/mL
Rhinovirus	10^5 CFU/mL	*Lactobacillus acidophilus*	10^6 CFU/mL
Coronavirus 229E	10^5 $TCID_{50}$/mL	*Lactobacillus crispatus*	10^6 CFU/mL
Coxsakeivirus Type B4	10^5 $TCID_{50}$/mL	*Lactococcus lactis*	10^6 CFU/mL
Adenovirus Type 7A	10^5 $TCID_{50}$/mL	*Listeria grayi*	10^6 CFU/mL

Rotavirus	10^5 copies/mL	*Listeria monocytogenes*	10^6 CFU/mL
Anaerococcus tetradius	10^6 CFU/mL	*Morganella morganii*	10^6 CFU/mL
Yersinia enterecolitica	10^6 CFU/mL	*Peptostreptococcus anaerobius*	10^6 CFU/mL
Vibrio parahaemolyticus	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	*Plesiomonas shigelloides*	10^6 CFU/mL
Acinetobacter lwoffii	10^6 CFU/mL	*Prevotella bivia*	10^6 CFU/mL
Aeromonas hydrophila	10^6 CFU/mL	*Prevotella melaninogenica*	10^6 CFU/mL
Alcaligenes faecalis	10^6 CFU/mL	*Proteus mirabilis*	10^6 rRNA copies/mL
Campylobacter upsaliensis	10^6 CFU/mL	*Proteus penneri*	10^6 CFU/mL
Anaerococcus vaginalis	10^6 CFU/mL	*Proteus vulgaris*	10^6 CFU/mL
Arcobacter butzleri	10^6 CFU/mL	*Providencia alcalifaciens*	10^6 CFU/mL
Bacillus cereus	10^6 CFU/mL	*Providencia rettgeri*	10^6 CFU/mL
Bacteriodes fragilis	10^6 CFU/mL	*Providencia stuartii*	10^6 CFU/mL
Bacteroides thetaiotaomicron	10^6 CFU/mL	*Pseudomonas aeruginosa*	10^6 CFU/mL
Bacteroides vulgatus	10^6 CFU/mL	*Pseudomonas fluorescens*	10^6 CFU/mL
Bifidobacterium adolescentis	10^6 CFU/mL	*Serratia liquefaciens*	10^6 CFU/mL
Bifidobacterium longum	10^6 rRNA copies/mL	*Serratia marcescens*	10^6 CFU/mL
Campylobacter fetus	10^6 CFU/mL	*Staphylococcus aureus*	10^6 CFU/mL
Campylobacter hyointestinalis	10^6 CFU/mL	*Staphylococcus epidermidis*	10^6 CFU/mL
Campylobacter rectus	10^6 CFU/mL	*Stenotrophomonas maltophilia*	10^6 CFU/mL
Campylobacter sputorum	10^6 CFU/mL	*Streptococcus anginosus*	10^6 CFU/mL
Candida albicans	10^6 CFU/mL	*Streptococcus dysgalactiae*	10^6 CFU/mL
Citrobacter freundii	10^6 CFU/mL	*Yersinia bercovieri*	10^6 CFU/mL
Citrobacter koseri	10^6 CFU/mL	*Yersinia pseudotuberculosis*	10^6 CFU/mL
Clostridium difficile	10^6 CFU/mL	*Yersinia rohdei*	10^6 CFU/mL
Clostridium perfringens	10^6 CFU/mL	*Campylobacter lari*	10^6 CFU/mL
Clostridium ramosum	10^6 CFU/mL	*Entamoeba histolytica*	10^4 cells/mL
Clostridium sordellii	10^6 CFU/mL	*Megasphaeara elsdenii*	10^6 CFU/mL
Clostridium tertium	10^6 CFU/mL	*Chlamydia trachomatis*	10^5 IFU/mL
Collinsella aerofaciens	10^6 CFU/mL	*Leptotrichia buccalis*	10^6 CFU/mL
Corynebacterium genitalium	10^6 CFU/mL	Cytomegalovirus	10^5 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] In the interference testing, a 100% positivity was observed for *Salmonella*, *Shigella*, and STEC at 10^6 CFU/mL and 100% positivity was recovered for *Campylobacter* at $\leq 10^4$ CFU/mL.

The presence of non-targeted microorganisms potentially found in clinical specimens or phylogenetically related to the assay analytes did not demonstrate cross-reactivity with or interfere with detection of GI Bacterial Assay analytes.

The inclusivity/reactivity of the Panther Fusion GI Bacterial Assay was also evaluated *in silico* for each intended analyte. *In silico* analysis was performed using analyte sequences available in the Genbank 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 Bacterial Assay is predicted to detect 100% of 121 *Salmonella bongori*, 99.03% of 2,365 *Salmonella enterica*, 96.43% of 392 *Campylobacter jejuni*, 99.09% of 1,104 *Campylobacter coli*, 100% of 1,080 *Shigella sonnei*, 100% of 1,164 *Shigella flexneri*, 100% of 192 *Shigella dysenteriae*, 100% of 364 *Shigella boydii*, 98.71% of 387 STEC-expressing *stx1*and 97.35% of 1,019 STEC-expressing *stx2* sequences evaluated.

Competitive Interference:

Competitive interference in the Panther Fusion GI Bacterial Assay was evaluated in triplicate using pairs of assay analytes at low/high concentrations in processed negative Cary-Blair Stool matrix. The low concentration analyte was tested at 3X LoD against a high concentration analyte at 10^6 CFU/mL. Table 4 shows a summary of results observed in the competitive interference testing.

Table 4: Summary of Competitive Interference Study Results

Analyte 1	CFU/mL[a]	Analyte 2	CFU/mL[a]	*Salmonella* % Pos	*Campylobacter* % Pos	*Shigella* % Pos	STEC % Pos
Salmonella	327	None	-	100%	0%	0%	0%
		Campylobacter	10^6	100%	100%	0%	0%
		Shigella	10^6	100%	0%	100%	0%
		STEC	10^6	100%	0%	0%	100%
Campylobacter	75	None	-	0%	100%	0%	0%
		Salmonella	10^6	100%	100%	0%	0%
		Shigella	10^6	0%	100%	100%	0%
		STEC	10^6	0%	100%	0%	100%
Shigella	204	None	-	0%	0%	0%	100%
		Salmonella	10^6	100%	0%	0%	100%
		Campylobacter	10^6	0%	100%	0%	100%
		STEC	10^6	0%	0%	100%	100%
STEC	318	None	-	0%	0%	0%	100%
		Salmonella	10^6	100%	0%	0%	100%

		Campylobacter	10^6	0%	100%	0%	100%
		STEC	10^6	0%	0%	100%	100%
None	-	*Salmonella*	10^6	100%	0%	0%	0%
		Campylobacter	10^6	0%	100%	0%	0%
		Shigella	10^6	0%	0%	100%	0%
		STEC	10^6	0%	0%	0%	100%
Negative	N/A	Negative	-	N/A	0%	0%	0%

CFU = colony forming units, Pos = positive

[a] Analyte concentration in Aptima Multitest tube

All on panel targets present at near LoD concentrations were successfully detected and no competitive interference was observed.

Interference:

Potential inhibitory effects of endogenous and exogenous substances that may be present in a specimen were evaluated with the Panther Fusion GI Bacterial Assay. Clinically relevant concentrations of potentially interfering substances were added to processed negative Cary-Blair Stool matrix and tested in the absence and in the presence of GI Bacterial Assay analytes at 3X LoD. Tests were performed in triplicate. The substances and test concentrations are shown in Table 5. No impact on the performance of the Panther Fusion GI Bacterial Assay was observed for any of the substances at the concentrations tested. Additionally, ParaPak 10% Buffered Formalin and ParaPak LV-PVA showed interference for analytes detected with the device.

Table 5: Substances Tested for Interference

Substance Type	Active Ingredient(s)	Test Concentration[abc]
Antibiotics	Amoxicillin	0.7 µg/mL
	Ampicillin	0.9 µg/mL
	Doxycycline	0.2 µg/mL
	Metronidazole	1.5 µg/mL
	Polymyxin B sulfate, bacitracin zinc, neomycin sulfate	1.3% w/v
Anti-microbial and Anti-fungal	Benzalkonium chloride	1.3% v/v
	Nystatin	1.3% v/v
Laxatives and Stool Softeners	Bisacodyl	75 ng/mL
	Docusate sodium	3.0 µg/mL
	Mineral oil	1.3% v/v
	Sennosides	0.8 µg/mL
	Polyethylene glycol 3350	0.1 mg/mL
	Magnesium hydroxide, Aluminum hydroxide	1.3% v/v
	Sodium phosphate	53 ng/mL
Anti-diarrheal	Loperamide hydrochloride	0.1 µg/mL
Anti-itch	Benzocaine	1.3% v/v

	Hydrocortisone	1.3% v/v
Anti-inflammatory	Phenylephrine hydrochloride	0.4 ng/mL
	Salicylic acid	0.4 µg/mL
	Naproxen sodium	4.5 µg/mL
Antacid	Bismuth subsalicylate	1.3% v/v
	Calcium carbonate	55 µg/mL
Radiopaque contrast material	Barium sulfate	0.1 mg/mL
Lubricants and skin protectants	Glycerin	1.3% w/v
	Petrolatum	1.3% w/v
	Zinc oxide	1.3% w/v
Spermicide	Nonoxynol-9	1.3% w/v
Endogenous	Cholesterol	50 µg/mL
	Palmitic acid	16 µg/mL
	Stearic acid	34 µg/mL
	Triglycerides	1.3% v/v
	Bilirubin, conjugated	5.0 µg/mL
	Human urine	1.3% v/v
	Blood/hemoglobin	1.3% v/v
	Purified mucin protein	0.05% w/v

[a] Analyte concentration in Aptima Multitest tube
[b] v/v: volume by volume
[c] w/v: weight by volume

5. Assay Reportable Range:

   Not applicable.

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

   Fresh vs Frozen:
   To establish equivalent performance between fresh and frozen samples and support use of frozen specimens in the clinical validation study, a comparison study was performed. For this study, 10 unique Cary-Blair Stool matrix pools were used to prepare the negative and contrived positive panels. Contrived positive panels were spiked with one GI Bacterial analyte at final concentrations of approximately 5X LoD, 2X LoD, and 0.99X LoD.

   Baseline testing of freshly prepared contrived panels was performed at day 0. The freeze/thaw (F/T) cycling was performed by shifting samples between -70°C (freeze) and room temperature (15-30°C, thaw). Up to two F/T cycles were performed. All testing used one lot of each reagent with samples evaluated on two Panther Fusion Systems. A total of ≥60 replicates were tested for each analyte consisting of 10 negative panel replicates and 50 positive panel replicates at each time point.

   For the negative panel, results were in 100% agreement for *Salmonella*, *Shigella*, and STEC at all time points. The panel showed 1 false positive result for *Campylobacter* at T0, but 100% negative results were observed for *Campylobacter* at T1 and T2.

Freezing and thawing did not negatively impact *Salmonella*, *Shigella*, or STEC positivity for up to 2 freeze/thaw cycles. However, *Campylobacter* positivity decreased after 1 freeze/thaw cycle. Therefore, it is not recommended to freeze stool samples in Cary-Blair media prior to testing with the Panther Fusion GI Bacterial Assay.

Specimen Stability in Cary Blair Transport Medium:
Stability testing panels were prepared by spiking bacterial cells into 10 unique negative stools from individual donors. GI bacterial assay analytes were tested in single analyte format at 3X, 10X, and/or 20X LoD. Aliquots of the spiked CB stools were tested at baseline (immediately after preparation) and after storage at 4°C, 15°C and 30°C. Stored aliquots were tested after 72 hours or 96 hours. A total of 20 replicates (2 replicates/stool) were tested per analyte, concentration, and storage temperature condition. Sixteen unique negative stools were tested in a total of 32 replicates (2 replicates/stool) at baseline and after 96 hrs.

Specimen stability for each analyte was determined as the longest stability duration at which ≥90% positivity was maintained. Conditions not meeting the ≥ 90% positivity criteria during initial testing were re-tested using the corresponding processed specimen (Cary-Blair Stool matrix in specimen transport media). If a condition was re-tested and still did not meet the ≥ 90% positivity criteria at the >72hr timepoint, the corresponding stability condition was not tested further at the >96hr timepoint. The totality of the data provided support the indicated claims in the device labeling.

Sample Transport Medium Processed Specimen Stability:
Stability testing panels were prepared by spiking bacterial cells into 10 unique negative CB stools from individual donors. GI bacterial assay analytes were tested in single analyte format at 3X, 10X and/or 20X LoD concentrations. Processed specimen stability was assessed using panels that were placed on primary stability (in Cary-Blair stool) for at least 72 hours. Primary aliquots of analyte-spiked Cary-Blair stools were stored at 4ºC, 15ºC, and 30ºC and tested at >72 hours and >96 hours. These time points, at which samples were transferred from Cary-Blair Stool matrix into the Aptima lysis tube, serve as the baseline for Processed specimen stability. Once in the Aptima lysis tube, samples were stored at 30ºC for ≥ 7 days (for 6 days stability), at 4ºC for ≥ 31 days (for 30 days stability), and at -20ºC for ≥ 91 days (for 90 days stability). To assess Processed stability storage at room temperature (15-30ºC), testing was only performed at 30ºC to evaluate the worst-case scenario. The totality of the data provided support the indicated claims in the device labeling.

7. Detection Limit:

The Limit of Detection (LoD) of the Panther Fusion GI Bacterial Assay was determined by testing dilutions of processed negative Cary-Blair Stool matrix spiked with bacterial cultures of *Salmonella* (2 strains), *Campylobacter* (2 strains), *Shigella*/EIEC (2 strains) and STEC (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 multianalyte configurations. Analytical sensitivity is defined as the lowest concentration at which ≥95% of all replicates tested positive, as summarized in Table 6.

Table 6: Analyte Limits of Detection

Strain	LoD Concentration (CFU/mL[a])	
	Aptima Multitest Tube	Preserved Stool
S.enterica subsp. enterica, serovar Typhimurium, I, 4,5,12:i:1,2	48	980
Salmonella bongonri, 66Z41	109	2180
Campylobacter coli	16	320
Campylobacter jejuni subsp. *jejuni*	25	500
Shigella sonnei	68	1360
EIEC Q29:NM	23	460
STEC O26:H11 (stx1/stx2)	106	2120
STEC O157:H7 (stx1/stx2)	20	400

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:

    The carryover contamination rate of the assay was evaluated using a checkerboard design with negative and positive panels made in processed negative Cary-Blair Stool matrix. A total of 270 negatives interspersed with 270 positive samples (spiked with *Salmonella* at 10^6 CFU/mL or 9,714 X LoD) were tested across five runs on two Panther Fusion Instruments. The Panther Fusion GI Bacterial Assay demonstrated a 0% carryover rate.

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 (Table X). Panels of all GI Bacterial Assay analytes (*Salmonella*, *Shigella*/EIEC, *Campylobacter* (*C. coli*, *C. jejuni*), Shigatoxigenic *Escherichia coli*) were tested in each of the CB medium at approximately 3x LoD.

For each transport medium under evaluation, positive (with GI 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. All positive CB panels showed ≥ 95% positivity for all GI Bacterial analytes except for the positive CB panel with Cardinal Health C&S medium.

Positive CB panel with Cardinal Health C&S medium showed ≥ 95% positivity for *Campylobacter*, *Shigella* and STEC, but 2 false negative results were observed for *Salmonella* with 90% positivity during initial testing. The same panel was remade and re-tested with an additional 20 replicates. Upon retest, ≥ 95% positivity was observed for all GI Bacterial targets.

Table 7. 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	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 8 US clinics from pediatric or adult patients suspected of acute gastroenteritis. All specimens were tested with the Panther Fusion GI Bacterial Assay and with a comparator FDA-cleared Nucleic Acid Amplification Test (NAAT). An alternate FDA-cleared NAAT was used for discordant resolution testing, if

applicable. Positive (PPA) and negative (NPA) percent agreement, with corresponding 2-sided 95% Confidence Intervals (CI), were calculated relative to comparator results, by target and by specimen category. A total of 1548 prospective specimens and 261 retrospective specimens were enrolled in the study; 69 specimens were excluded from the performance analyses (e.g., duplicate individuals, invalid Panther Fusion or comparator results for all targets). An additional 126 contrived specimens were assessed to supplement the prospective and retrospective data for the target *stx1/stx2*. Of the 1896 specimens tested in valid Panther Fusion GI Bacterial Assay runs, 41 (2.2%) had initial invalid results. Upon retest, 33 of the 41 specimens yielded valid results, for a total of 1888 (99.6%) specimens with final valid results. The final data set consisted of 1866 evaluable specimens; not all were evaluable for all analytes. Demographic information for the 1740 evaluable prospective and retrospective specimens is provided in Table 8.

Table 8: Summary of Subject Demographics

		Total N (%)	Prospective N (%)	Retrospective N (%)
Total Specimens		1740	1521	219
Sex	Female	909 (52.2)	794 (52.2)	115 (52.5)
	Male	831 (47.8)	727 (47.8)	104 (47.5)
Age	0-28 days	7 (0.4)	7 (0.5)	0 (0)
	29 days to <2 years	70 (4.0)	67 (4.4)	3 (1.4)
	2-5 years	53 (3.0)	50 (3.3)	3 (1.4)
	6-11 years	73 (4.2)	66 (4.3)	7 (3.2)
	12-17 years	73 (4.2)	71 (4.7)	2 (0.9)
	18-21 years	53 (3.0)	45 (3.0)	8 (3.7)
	22-64 years	849 (48.8)	723 (47.5)	126 (57.5)
	≥65 years	562 (32.3)	492 (32.3)	70 (32.0)

N = population size

Performance characteristics for detection of *Salmonella*, *Campylobacter*, *Shigella*/EIEC, and stx1/stx2 are shown in Table 9 through Table 12 below.

Table 9: Clinical Performance – *Salmonella* spp.

Specimen Origin	N	TP	FP	TN	FN	PPA (95% CI) [a]	NPA % (95% CI) [a]
Prospective (fresh)	1520	33	2[b]	1484	1[c]	97.1% (85.1%-99.5%)	99.9% (99.5%-100.0%)
Retrospective (frozen)	219	20	2[d]	197	0	100.0% (83.9%-100.0%)	99.0% (96.4%-99.7%)

CI = confidence interval, FN = false negative, FP = false positive, N = sample size, NPA = negative percent agreement, PPA = positive percent agreement, TN = true negative, TP = true positive.
a Score CI.
b The 2 discordant false positive prospective specimens were positive for *Salmonella* by the alternate NAAT.
c The discordant false negative prospective specimen was negative for *Salmonella* by the alternate NAAT.
d The 2 discordant retrospective false positive specimens were positive for *Salmonella* by the alternate NAAT.

Table 10: Clinical Performance – *Campylobacter* spp.

Specimen Origin	N	TP	FP	TN	FN	PPA (95% CI) [a]	NPA % (95% CI) [a]
Prospective (fresh)	1520	39	2 [b]	1478	1 [c]	97.5% (87.1%-99.6%)	99.9% (99.5%-100.0%)
Retrospective (frozen)	219	18	4 [d]	197	0	100.0% (82.4%-100.0%)	99.0% (95.0%-99.2%)

CI = confidence interval, FN = false negative, FP = false positive, N = sample size, NPA = negative percent agreement, PPA = positive percent agreement, TN = true negative, TP = true positive.
a Score CI.
b The 2 discordant false positive prospective specimens were negative for *Campylobacter* by the alternate NAAT.
c The discordant false negative prospective specimen was negative for *Campylobacter* by the alternate NAAT.
d 3 of 4 discordant false positive retrospective specimens were positive for *Campylobacter* by the alternate NAAT.

Table 11: Clinical Performance – *Shigella*/EIEC

Specimen Origin	N	TP	FP	TN	FN	PPA (95% CI) [a]	NPA % (95% CI) [a]
Prospective (fresh)	1521	27	0	1494	0	100.0% (87.5%-100.0%)	100.0% (99.7%-100.0%)
Retrospective (frozen)	219	19	1 [b]	199	0	100.0% (83.2%-100.0%)	99.5% (97.2%-99.9%)

CI = confidence interval, FN = false negative, FP = false positive, N = sample size, NPA = negative percent agreement, PPA = positive percent agreement, TN = true negative, TP = true positive.
a Score CI.
b The discordant false positive retrospective specimen was positive for *Shigella*/EIEC by the alternate NAAT.

Table 12: Clinical Performance – Shiga Toxin 1 and 2 (stx1 and stx2)

Specimen Origin	N	TP	FP	TN	FN	PPA (95% CI) [a]	NPA % (95% CI) [a]
Prospective (fresh)	1520	7	5 [b]	1508	0	100.0% (64.6%-100.0%)	99.7% (99.2%-99.9%)
Retrospective (frozen)	219	39	8 [c]	172	0	100.0% (91.0%-100.0%)	95.6% (91.5%-97.7%)
Contrived (frozen)	126	63	0	63	0	100.0% (94.3%-100.0%)	100.0% (94.3%-100.0%)

CI = confidence interval, FN = false negative, FP = false positive, N = sample size, NPA = negative percent agreement, PPA = positive percent agreement, TN = true negative, TP = true positive.
a Score CI.
b The 5 discordant false positive prospective specimens were positive for *stx1/stx2* by the alternate NAAT.
c The 8 discordant false positive retrospective specimens was positive for *stx1/stx2* by the alternate NAAT.

D. Clinical Cut-Off:

Not applicable.

E. Expected Values/Reference Range:

In the Panther Fusion GI 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 Bacterial Assay during the prospective segment of the clinical study are presented in table 13 below.

Table 13. Prevalence Values Observed during the Clinical Study

	% Positivity (# positive/# evaluable)			
Collection site	***Salmonella***	***Campylobacter***	***Shigella*/EIEC**	***stx 1/2***
Total evaluable for target, N	1520	1520	1521	1520
All Sites	2.3 (35/1520)	2.7 (41/1520)	1.8 (27/1521)	0.8 (12/1520)
Site 1	2.0 (9/449)	3.6 (16/449)	1.8 (8/450)	0.7 (3/449)
Site 2	1.0 (5/494)	1.4 (7/494)	1.8 (9/494)	0.8 (4/494)
Site 3	3.7 (18/482)	3.3 (16/482)	1.7 (8/482)	0.8 (4/482)
Site 4	3.2 (3/95)	2.1 (2/95)	2.1 (2/95)	1.1 (1/95)

EIEC = Enteroinvasive *E. coli*, stx 1/2 = Shiga toxin-producing *E. coli* toxins 1 and 2.

F. Other Supportive Instrument Performance Characteristics Data:

Not applicable.

VIII Proposed Labeling:

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

IX Conclusion:

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