September 25, 2025

Hologic Inc.
Patricia Villarreal
Regulatory Affairs Specialist
10210 Genetic Center Drive
San Diego, California 92121

Re: K251993
Trade/Device Name: Panther Fusion GI Expanded Bacterial Assay
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay
Regulatory Class: Class II
Product Code: PCH, OOI
Dated: July 26, 2025
Received: August 26, 2025

Dear Patricia Villarreal:

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

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

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device"

(https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

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

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

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

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

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Bryan M. Grabias -S

Digitally signed by Bryan M. Grabias -S
Date: 2025.09.25 11:10:47 -04'00'

Bryan Grabias, Ph.D.
Acting Branch Chief
Bacterial Respiratory and Medical Countermeasures Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

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

510(k) Number *(if known)*
K251993

Device Name
Panther Fusion GI Expanded Bacterial Assay

Indications for Use *(Describe)*
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.

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

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

CONTINUE ON A SEPARATE PAGE IF NEEDED.

510(k) SUMMARY

Panther Fusion GI Expanded Bacterial Assay

I. SUBMITTER: Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121

Contact Person: Patricia Villarreal
Regulatory Affairs Specialist
Patricia.Villarreal@hologic.com
Phone: 562.833.7906
Fax: N/A

Date Prepared: June 26, 2025

II. DEVICE

Proprietary Name:	Panther Fusion GI Expanded Bacterial Assay
Classification Name:	Gastrointestinal microorganism multiplex nucleic acid-based assay
Regulation Number:	21 CFR 866.3990 and 21 CFR 862.2570
Regulatory Class:	Class II
Product Code:	PCH and OOI

III. PREDICATE DEVICE

The predicate device for the Panther Fusion GI Expanded Bacterial Assay is the BioFire FilmArray Gastrointestinal (GI) Panel (K160459, K143005 & K140407).

IV. 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.

Sample processing: Prior to processing and testing on the Panther Fusion System, specimens are transferred to an Aptima Multitest tube containing specimen transport media (STM) that lyses the cells, releases target nucleic acid, and protects them from degradation during storage.

Nucleic acid capture and elution: An internal control (IC-B) is added automatically to each specimen via the working Panther Fusion Capture Reagent-B (wFCR-B) to monitor for interference during specimen processing, amplification, and detection caused by reagent failure or inhibitory substances. Specimens are first incubated in an alkaline reagent (FER-B) to enable cell lysis. Nucleic acid released during the lysis step hybridizes to magnetic particles in the wFCR-B. The capture particles are then separated from residual specimen matrix in a magnetic field by a series of wash steps with a mild detergent. The captured nucleic acid is then eluted from the magnetic particles with a reagent of low ionic strength (Panther Fusion Elution Buffer).

Multiplex PCR amplification and fluorescence detection: Lyophilized single unit dose reaction master mix is reconstituted with the Panther Fusion Reconstitution Buffer I and then combined with the eluted nucleic acid into a reaction tube. Panther Fusion Oil reagent is added to prevent evaporation during the PCR reaction.

Target-specific primers and probes then amplify targets via polymerase chain reaction while simultaneously measuring fluorescence of the multiplexed targets. The Panther Fusion System compares the fluorescence signal to a predetermined cut-off to produce a qualitative result for the presence or absence of each analyte.

The analytes and the channel used for their detection on the Panther Fusion System are summarized in the table below:

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

Assay Components

The assay components configuration for the Panther Fusion GI Expanded Bacterial Assay is analogous to the Panther Fusion Respiratory Assays. The reagents required to perform the Panther Fusion GI Expanded Bacterial Assay are packaged and sold separately. There are 7 boxes containing 9 reagents which are required for sample processing. 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)

Table 1: Reagents Required to Perform the Panther Fusion GI Expanded Bacterial Assay

Box	Components Description
Refrigerated Box	Panther Fusion GI Expanded Bacterial Assay Cartridges
Room Temperature Box	Panther Fusion Extraction Reagent-B • Panther Fusion Capture Reagent-B bottles • Panther Fusion Enhancer Reagent-B bottles
Refrigerated Box	Panther Fusion Internal Control-B
Room Temperature Box	Panther Fusion Reconstitution Buffer I
Room Temperature Box	Panther Fusion Elution Buffer
Room Temperature Box	Panther Fusion Oil
Refrigerated Box	Panther Fusion GI Expanded Bacterial Assay Controls • Panther Fusion GI Expanded Bacterial Positive Control • Panther Fusion Negative Control

Table 2: Ancillary and Collection Kits Required to Perform the Panther Fusion GI Expanded Bacterial Assay

Aptima Assay Fluids Kit
Aptima Multitest Swab Specimen Collection Kit

Instrumentation

The Panther Fusion GI Expanded Bacterial Assay has been designed for and validated on the Panther Fusion system. 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.

V. 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.

VI. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH THE PREDICATE DEVICE

A comparison of the Panther Fusion GI Expanded Bacterial Assay to the predicate BioFire FilmArray Gastrointestinal (GI) Panel (K160459, K143005 & K140407) is summarized in **Table 3**.

Table 3: Comparison of Similarities and Differences Between the Predicate Device (BioFire FilmArray Gastrointestinal (GI) Panel) and the Subject Device (Panther Fusion GI Bacterial Assay)

	Predicate **BioFire FilmArray Gastrointestinal (GI) Panel**	**Subject** **Hologic Panther Fusion GI Expanded Bacterial Assay**
Classification	Class II	Class II
Regulation no. /Product code	21CFR 866.3990 PCH, OOI	21CFR 866.3990 PCH, OOI
Intended use	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*	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

	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	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.

	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)	Fully automated nucleic acid amplification, detection and result interpretation (multiplex PCR)
Analyte	RNA/DNA	DNA
Organism Detected	*Campylobacter (C. jejuni/C. coli/C.upsaliensis), Clostridium difficile (C.difficile) toxin A/B, Plesiomonas shigelloides, Salmonella, Vibrio (V.*	*Yersinia enterocolitica*, *Vibrio* (*V. parahaemolyticus*, *V. vulnificus*, *V. cholerae)*, *Escherichia coli* O157, and *Plesiomonas shigelloides*

	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 toxinproducing 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).	
Specimen Types	Human stool in Cary Blair transport medium	Human stool in Cary Blair transport medium
Sample Preparation	Automated sample processing	Automated sample processing
Intended Users	Professional use	Professional use
Amplification mode	Nested multiplex PCR	Real-time PCR
Detection mode	Fluorogenic double stranded DNA binding dye	Fluorogenic target-specific hybridization
Instrumentation	BioFire FilmArray Systems	Panther Fusion System
Time to Result	Approximately 1 hour	Approximately 2.4 hours
Controls	Two controls are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis.	Internal control in each sample. External control processed at periodic interval

VII. PERFORMANCE DATA

The following performance data (analytical and clinical) were provided in support of the substantial equivalence determination.

Brief Description of Analytical (Non-Clinical) Studies

The following analytical studies (non-clinical) were conducted to support the clearance of the Panther Fusion GI Expanded Bacterial Assay on the Panther Fusion System.

Analytical Sensitivity - Limit of Detection (LoD)

The analytical sensitivity (Limit of Detection or LoD) of the Panther Fusion GI Expanded Bacterial Assay was determined by testing dilutions of preserved negative Cary-Blair stool processed with STM (negative CBS matrix) spiked with bacterial cultures of *Yersinia* (2 strains), *Vibrio* (3 strains), *Plesiomonas* (2 strains), and STEC O157 (2 strains). A minimum of 24 replicates were tested with each of the 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. Analytical sensitivity is defined as the lowest concentration at which ≥95% of all replicates tested positive, as summarized in Table 4.

Table 4. Analytical Sensitivity

Strain	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

Inclusivity/Reactivity – Wet Testing

The inclusivity/reactivity of the Panther Fusion GI Expanded Bacterial Assay was determined by testing bacterial strains in negative CBS matrix. Each strain was tested in triplicate at 3X LoD with 1 reagent lot in single or multi-analyte configuration. For strains not detected at 3X LoD, additional testing at higher concentrations was performed until 100% positivity was observed. Table 5 shows the lowest concentration of each strain at which 100% positivity was observed.

Table 5. Inclusivity/Reactivity Summary for the GI Expanded Bacterial Assay Analytes

Organism	ATCC# or Source	Strain/ serovar/ serotype/ antigenic properties	Test Concentration (3X LoD) (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
	33817	329 [CDC B3547], Biotype 2	33	660
	BAA-86	CDC 9505-95	33	660
	CCUG 38297	N/A	33	660

Vibrio vulnificus	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

Table 5. Inclusivity/Reactivity Summary for the GI Expanded Bacterial Assay Analytes (continued)

Organism	ATCC# or Source	Strain/ serovar/ serotype/ antigenic properties	Test Concentration (3X LoD) (CFU/mL)	
			Aptima Multitest Tube	Preserved Stool
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
	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 # 428[a]	N/A	1,197	23,940
	AR Bank # 429[a]	N/A	1,197	23,940
	AR Bank # 430[a]	N/A	1,197	23,940
	14030	CDC 16408 [Ferguson and Henderson C27, RH 864], O:17	195	3,900

	51903	GNI 14[c]	195	3,900
Plesiomonas shigelloides	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.

Inclusivity/Reactivity - *In silico* Analysis

The inclusivity of the Panther Fusion GI Expanded Bacterial Assay was evaluated using *in silico* inclusivity analysis for each 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.

Analytical Specificity: Cross Reactivity and Microbial Interference - Wet Testing

Analytical specificity (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

Table 6. Microorganisms Tested for Cross-Reactivity and Microbial Interference (continued)

Microorganism	Test Concentration	Microorganism	Test Concentration
Rhinovirus[a]	10^5 copies/mL	*Lactococcus lactis*	10^6 CFU/mL
Coronavirus 229E	10^5 $TCID_{50}$/mL	*Listeria grayi*	10^6 CFU/mL
Coxsakeivirus Type B4	10^5 $TCID_{50}$/mL	*Listeria monocytogenes*	10^6 CFU/mL
Adenovirus Type 7A	10^5 $TCID_{50}$/mL	*Morganella morganii*	10^6 CFU/mL
Rotavirus[a]	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
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 $TCID_{50}$/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

Table 6. Microorganisms Tested for Cross-Reactivity and Microbial Interference (continued)

Microorganism	Test Concentration	Microorganism	Test Concentration
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.

Coinfection/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 3X LoD 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. The highest concentration of competing analyte at which the low concentration analyte maintained a 100% positivity is shown in Table 7. When the analytes were tested at high concentration, all results for other analytes maintained expected positivity; no competitive interference was observed.

Table 7. Summary of Coinfection Results

Analyte 1		**Analyte 2**		***Yersinia*** **% Pos**	***Vibrio*** **% Pos**	**STEC O157 % Pos**	***Plesiomonas*** **% Pos**
Name	**3X LoD (CFU/mL)[a]**	**Name**	**High Conc (CFU/mL)[1]**				
Negative	NA	Negative	NA	0%	0%	0%	0%
Yersinia	282	None	0	100%	0%	0%	0%
		Vibrio[b]	10^4	100%	100%	0%	0%
		STEC O157	10^6	100%	0%	100%	0%
		Plesiomonas	10^6	100%	0%	0%	100%
Vibrio	282	None	0	0%	100%	0%	0%
		Yersinia	10^6	100%	100%	0%	0%
		STEC O157	10^6	0%	100%	100%	0%
		Plesiomonas	10^6	0%	100%	0%	100%

STEC O157	1,197	None	0	0%	0%	100%	0%
		Yersinia	10^6	100%	0%	100%	0%
		Vibrio[b]	10^4	0%	100%	100%	0%
		Plesiomonas	10^6	0%	0%	100%	100%
Plesiomonas	195	None	0	0%	0%	0%	100%
		Yersinia	10^6	100%	0%	0%	100%
		Vibrio[b]	10^6	0%	100%	0%	100%
		STEC O157	10^6	0%	0%	100%	100%
None	0	*Yersinia*	10^6	100%	0%	0%	0%
		Vibrio	10^6	0%	100%	0%	0%
		STEC O157	10^6	0%	0%	100%	0%
		Plesiomonas	10^6	0%	0%	0%	100%

CFU = colony forming units, Pos = positive.

[a] Analyte concentration in Aptima Multitest tube.

[b] Less than 100% positive results were observed for analyte 1 with *Vibrio* at $\geq 10^5$ CFU/mL.

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 8. No impact on the performance of the Panther Fusion GI Expanded Bacterial Assay was observed for any of the substances at the concentrations tested.

Table 8. Substances Tested for Interference

Substance Type	Generic Name	Active Ingredient(s)	Test Concentration[a, b, c]
Antibiotics	Amoxicillin	Amoxicillin	0.7 µg/mL
	Ampicillin	Ampicillin	0.9 µg/mL
	Doxycycline	Doxycycline	0.2 µg/mL
	Metronidazole	Metronidazole	1.5 µg/mL
	Neosporin®	Polymyxin B sulfate, bacitracin zinc, neomycin sulfate	1.3% w/v
Anti-microbial and anti-fungal	BZK Antiseptic Towelettes	Benzalkonium chloride	1.3% v/v
	Nystatin	Nystatin	1.3% v/v
Laxatives and stool softeners	Dulcolax® suppository	Bisacodyl	75 ng/mL
	Colace®	Docusate sodium	3.0 µg/mL
	Fleet® mineral oil enema	Mineral oil	1.3% v/v
	Ex-Lax®	Sennosides	0.8 µg/mL
	Miralax®	Polyethylene glycol 3350	0.1 mg/mL
	Milk of Magnesia	Magnesium hydroxide, Aluminum hydroxide	1.3% v/v
	Visicol®	Sodium phosphate	53 ng/mL
Anti-diarrheal	Imodium	Loperamide hydrochloride	0.1 µg/mL
Anti-Itch	Vagisil®	Benzocaine	1.3% w/v
	Preparation H®	Hydrocortisone	1.3% w/v
Anti-inflammatory	Phenylephrine hydrochloride (for hemorrhoids)	Phenylephrine hydrochloride	0.4 ng/mL
	Mesalazine (Rx only, for Crohns disease/ ulcerative colitis)	Salicylic acid	0.4 µg/mL
	Aleve®	Naproxen sodium	4.5 µg/mL
Antacid	Pepto-Bismol®	Bismuth subsalicylate	1.3% v/v
	Tums®	Calcium carbonate	55 µg/mL
Radiopaque contrast material	Barium sulfate	Barium sulfate	0.1 mg/mL
Lubricants and skin protectants	K-Y® Personal Lubricant Jelly Glycerin	Glycerin	1.3% w/v
	Vaseline® Original 100% Pure Petroleum Jelly White	Petrolatum	1.3% w/v
	Desitin®	Zinc oxide	1.3% w/v

Spermicide	Options Conceptrol®Vaginal Contraceptive Gel	Nonoxynol-9	1.3% w/v

Table 8. Substances Tested for Interference (continued)

Substance Type	Generic Name	Active Ingredient(s)	Test Concentration[a, b, c]
Endogenous	Cholesterol	Cholesterol	50 µg/mL
	Fatty acids	Palmitic acid	16 µg/mL
	Fatty acids	Stearic acid	34 µg/mL
	Triglycerides, total (Fecal fat, Intralipid)	Triglycerides	1.3% v/v
	Human Bile	Bilirubin, conjugated	5.0 µg/mL
	Urine	Human Urine	1.3% v/v
	Human Whole Blood	Blood/hemoglobin	1.3% v/v
	Mucin	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.

Stool specimens prepared in various preservative media were evaluated for potential impact on the Panther Fusion GI Expanded Bacterial Assay performance. The preservative media evaluated include 7 different types of Cary-Blair transport media from different vendors and preservative media containing fixatives shown in Table 9. All media were tested with GI Expanded Bacterial Assay analytes at 3X LoD. Comparable performance was seen with all Cary-Blair media. Interference was observed when specimens were processed in media containing fixative.

Table 9. Stool Preservative Media Tested for Interference

Cary-Blair Media
Culture & Sensitivity (C&S) Medium
Cary Blair Transport Medium w/ Indicator
Para-Pak® C&S
Para-Pak® Enteric Plus
Cardinal Health™ C&S Stool Transport Vial
Protocol Cary Blair Medium
Enteric Transport Media (ETM)
Fixative Media (interference was observed)
Fisher® 10% Buffered Formalin
Para-Pak® 10% Buffered Formalin
Para-Pak® LV-PVA

Carryover Contamination

Panther Fusion GI Bacterial Assay and GI Expanded Bacterial Assay belong to the same family of assays that 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.

Within Laboratory Precision/Repeatability

Panther Fusion GI Expanded Bacterial Assay within laboratory precision was evaluated with a 5-member panel consisting of assay analytes in negative CBS matrix. The 5-member panel included 1 negative, 2 single analyte (Yersinia), and 2 multi-analyte (with Vibrio, STEC O157, and Plesiomonas) panel members. The panels were tested by 3 operators on 2 runs per day, using 3 reagent lots on 3 Panther Fusion Systems over 9 days.

The panel members are described in Table 10, along with a summary of the agreement with the expected results, mean Ct, variability analysis between reagent lots, operators, instruments, days, between and within runs, and overall (total).

Table 10. Ct Variability Analysis Summary

Panel	Description	Analyte	Agreed/N	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.

Reproducibility

Panther Fusion GI Expanded Bacterial Assay reproducibility was evaluated at 3 US sites using 1 negative panel member and 4 panel members positive for 1 or 3 targets. Testing was performed for 5 days by 6 operators (2 at each site) using 1 lot of assay reagents. Each run included 3 replicates of each panel member.

A negative panel member was created using a matrix comprised of stool specimens negative for all assay targets preserved in Cary-Blair media processed into STM. Positive panel members were created by spiking 1.5X LoD (low positive) or 3X LoD (moderate positive) concentrations of the target analytes into the negative matrix.

The agreement with expected results was 100% for all panel members components for *Yersinia, Vibrio*, STEC O157, and *Plesiomonas* (Table 11).

Table 11. Agreement of Panther Fusion GI Expanded Bacterial Assay Results with Expected Results

		Agreement with Expected Results	
Description	**Analyte**	**N**	**% (95% CI)**
Neg	Internal Control	89/89	100 (95.9-100)
Low Pos[a]	*Yersinia*[c]	90/90	100 (95.9-100)
	Vibrio[c]	90/90	100 (95.9-100)
	STEC O157	90/90	100 (95.9-100)
	Plesiomonas[c]	90/90	100 (95.9-100)
Mod Pos[b]	*Yersinia*[c d]	90/90	100 (95.9-100)
	Vibrio[c]	90/90	100 (95.9-100)
	STEC O157	90/90	100 (95.9-100)
	Plesiomonas[c]	90/90	100 (95.9-100)

CI = score confidence interval, Mod = moderate, N = sample size, Neg = negative, Pos = positive.
[a] Low Pos = All targets are 1.5X LoD.
[b] Mod Pos = All targets are 3X LoD.
[c] *Yersinia enterocolitica*, *Vibrio parahaemolyticus*, STEC O157, and *Plesiomonas shigelloides* strains were used to build the positive panels.
[d] One (1) false positive *Vibrio* result was obtained for a moderate positive *Yersinia* panel member.

Signal variability was measured as %CV of the Ct values. The total signal variability was ≤1.61% (SD ≤0.55) for all panel components (Table 12). For the sources of variation except the 'within run' factor, %CV values were ≤1.03% for all panel components. The signal variability was ≤1.01% (SD ≤0.33) for the Panther Fusion GI Expanded Bacterial Assay positive controls (Table 13).

Table 12. Signal Variability of the Panther Fusion GI Expanded Bacterial Assay by Target and Concentration

				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 13. Signal Variability of the Panther Fusion GI Expanded Bacterial Assay Positive Controls

				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.

Brief Description of Clinical Studies

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 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 resolution testing, if applicable. Positive (PPA) and negative (NPA) percent agreement, with corresponding 2-sided 95% Score CIs, were calculated relative to comparator results, by target and by specimen category.

A total of 1,548 prospective specimens and 251 retrospective specimens were enrolled in the study; 94 specimens were excluded from the performance analyses (for example, duplicate individuals, invalid Panther Fusion or comparator results for all targets). An additional 189 contrived specimens were assessed 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 specimens, 182 retrospective specimens, and 189 contrived specimens); not all were evaluable for all analytes. Demographic information for the 1,705 evaluable prospective and retrospective specimens is provided in Table 14.

Table 14. Summary of 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)
	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)

N = population size.

Performance characteristics for detection of *Yersinia, Vibrio*, STEC O157, and *Plesiomonas* are shown in Table 15 through Table 18.

Table 15. Clinical Performance - Yersinia spp.

Specimen Origin	N	TP	FP	TN	FN	Prevalence[a] (%)	PPA % (95% CI)[b]	NPA % (95% CI)[b]
Prospective (Fresh)	1,507	10	9[c]	1,487	1[d]	0.7	90.9 (62.3, 98.4)	99.4 (98.9, 99.7)
Retrospective (Frozen)	182	15	3[e]	164	0	N/A[f]	100 (79.6, 100)	98.2 (94.9, 99.4)
Contrived (Frozen)	189	63	0	126	0	N/A[f]	100 (94.3, 100)	100 (97.0, 100)

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] Study prevalence reported based on comparator testing.

[b] Score CI.

[c] 6 of 9 discordant false positive prospective specimens were positive for *Yersinia* by the alternate NAAT.

[d] The discordant false negative prospective specimen was negative for *Yersinia* by the alternate NAAT.

[e] The 3 discordant false positive retrospective specimens were positive for *Yersinia* by the alternate NAAT.

[f] Calculation of prevalence is not applicable.

Table 16. Clinical Performance - Vibrio spp.

Specimen Origin	N	TP	FP	TN	FN	Prevalence[a] (%)	PPA % (95% CI)[b]	NPA % (95% CI)[b]
Prospective (Fresh)	1,507	1	0	1,505	1[c]	0.1	50.0 (9.5, 90.5)	100 (99.7, 100)
Retrospective (Frozen)	182	9	6[d]	167	0	N/A[f]	100 (70.1, 100)	96.5 (92.6, 98.4)
Contrived (Frozen)	189	63	1[e]	125	0	N/A[f]	100 (94.3, 100)	99.2 (95.6, 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] Study prevalence reported based on comparator testing.

[b] Score CI.

[c] The discordant false negative prospective specimen was positive for *Vibrio* by the alternate NAAT.

[d] All 6 discordant false positive retrospective specimens were positive for *Vibrio* by the alternate NAAT.

[e] The discordant false positive contrived specimen was negative for *Vibrio* by the alternate NAAT.

[f] Calculation of prevalence is not applicable.

Table 17. Clinical Performance – STEC O157

Specimen Origin	N	TP	FP	TN	FN	Prevalence[a] (%)	PPA % (95% CI)[b]	NPA % (95% CI)[b]
Prospective (Fresh)	1,522	1	2[c]	1,519	0	0.1	100 (20.7, 100)	99.9 (99.5, 100)
Retrospective (Frozen)	182	3	1[d]	178	0	N/A[g]	100 (43.9, 100)	99.4 (96.9, 99.9)
Contrived (Frozen)	189	62	1[e]	125	1[f]	N/A[g]	98.4 (91.5, 99.7)	99.2 (95.6, 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] Study prevalence reported based on comparator testing.

[b] Score CI.

[c] 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.

[d] The discordant false positive retrospective specimen was positive for STEC O157 by the alternate NAAT.

[e] The discordant false positive contrived specimen was negative for STEC O157 by the alternate NAAT.

[f] The discordant false negative contrived specimen was not retested by the alternate NAAT.

[g] Calculation of prevalence is not applicable.

Table 18. Clinical Performance - Plesiomonas

Specimen Origin	N	TP	FP	TN	FN	Prevalence[a] (%)	PPA % (95% CI)[b]	NPA % (95% CI)[b]
Prospective (Fresh)	1,507	1	1[c]	1,505	0	0.1	100 (20.7, 100)	99.9 (99.6, 100)
Retrospective (Frozen)	182	8	1[d]	173	0	N/A[f]	100 (67.6, 100)	99.4 (96.8, 99.9)
Contrived (Frozen)	189	62	0	126	1[e]	N/A[f]	98.4 (91.5, 99.7)	100 (97.0, 100)

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] Study prevalence reported based on comparator testing.
[b] Score CI.
[c] The discordant false positive prospective specimen was positive for *Plesiomonas* by the alternate NAAT.
[d] The discordant false positive retrospective specimen was positive for *Plesiomonas* by the alternate NAAT.
[e] The discordant false negative contrived specimen was not retested by the alternate NAAT.
[f] Calculation of prevalence is not applicable.

No coinfections were detected by the Panther Fusion GI Expanded Bacterial Assay or by the comparator methods in prospective and retrospective specimens.

VIII. CONCLUSIONS

The analytical and clinical study results demonstrate that the Panther Fusion GI Expanded Bacterial Assay on the Panther Fusion system performs comparably to the predicate device that is currently marketed for the same intended use. Hardware and software verification and validation demonstrate that the Panther Fusion GI Expanded Bacterial Assay on the Panther Fusion system will perform as intended.