September 25, 2025

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

Re: K251868
Trade/Device Name: Panther Fusion GI 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: June 17, 2025
Received: June 18, 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 11:15:10 -04'00'

Bryan Grabias, PhD
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)*
K251868

Device Name
Panther Fusion GI Bacterial Assay

Indications for Use *(Describe)*
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 co-infection with other organisms that are not detected by this test and may not be the sole or definitive cause of patient illness. Negative 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 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 16, 2025

II. DEVICE

Proprietary Name:	Panther Fusion GI 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 Bacterial Assay is the BioFire FilmArray Gastrointestinal (GI) Panel (K160459, K143005 & K140407).

IV. 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 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
Salmonella	*InvA* (Invasive antigen A)	FAM
Campylobacter	*glyA* (serine hydroxymethyl transferase)/*cadF* (outer membrane fibronectin-binding protein)	HEX
Shigella/EIEC	*ipaH* (Invasion plasmid antigen H)	ROX
STEC	*stx1* (Shigatoxin 1)/*stx2* (Shigatoxin 2)	RED647
Internal Control	Not Applicable	RED677

Assay Components

The assay components configuration for the Panther Fusion GI Bacterial Assay is analogous to the Panther Fusion Respiratory Assays. 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. 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 Multitest Swab Specimen Collection Kit (PRD-03546)

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

Box	Components Description
Refrigerated Box	Panther Fusion GI 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 Bacterial Assay Controls • Panther Fusion GI Bacterial Positive Control • Panther Fusion Negative Control

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

Aptima Assay Fluids Kit
Aptima Multitest Swab Specimen Collection Kit

Instrumentation

The Panther Fusion GI 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 Bacterial Assay.

V. 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 co-infection with other organisms that are not detected by this test and may not be the sole or definitive cause of patient illness. Negative 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 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 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 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

	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	and should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Positive results do not rule out co-infection with other organisms that are not detected by this test and may not be the sole or definitive cause of patient illness. Negative 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. 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).	*Salmonella*, *Shigella/EIEC*, *Campylobacter* (*C. coli*, *C. jejuni*), Shiga toxins 1 and 2
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 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 Bacterial Assay was determined by testing dilutions of processed negative Cary-Blair Stool (CBS) 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 4.

Table 4. Analytical Sensitivity

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	960
Salmonella bongori, 66:z41	109	2,180
Campylobacter coli	16	320
Campylobacter jejuni subsp. jejuni	25	500
Shigella sonnei	68	1,360
EIEC O29:NM	23	460
STEC O26:H11 (*stx1/stx2*)	106	2,120
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).

Inclusivity/Reactivity – Wet Testing

The inclusivity/reactivity of the Panther Fusion GI Bacterial Assay was determined by testing bacterial strains in the processed negative CBS matrix. Each strain was tested in triplicate at 3X LoD with 1 reagent lot in single or multi-analyte configuration. Table 5 shows the lowest concentration of each strain at which 100% positivity was observed.

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

Organism	ATCC# or Source	Strain/ serovar/ serotype/ antigenic properties	Test Concentration (3X LoD) (CFU/mL)	
			Aptima Multitest Tube	Preserved Stool
Salmonella bongori	43975[a]	CIP 82.33[a]	327	6,540
Salmonella enterica subsp. *enterica* (I)	13076	Enteritidis, CDC K--1891	144	2,880
	14028[a]	Typhimurium, CDC 6516--60[a]	144	2,880
	15791	Sloterdijk	144	2,880
	15611	Vellore, V1796	144	2,880
	11646	Illinois, CDC	144	2,880
	8391	Thompson, 2988	144	2,880
	19430	Typhi, NCTC 8385	144	2,880
	7378	Panama, Hochberg 2460	144	2,880
	6962	Newport, NCTC 129	144	2,880
	8388	Muenchen, 54	144	2,880
	8326	Heidelberg, 16	144	2,880
	9712	Saintpaul, 127	144	2,880
	8387	Montevideo, 623	144	2,880
	6539	Typhi, AMC	144	2,880
	9150	Paratyphi A	144	2,880
	10719	Paratyphi B, AMC 41-H-6	144	2,880
	13428	Paratyphi C, CDC 3310-52	144	2,880
	33062	Typhimurium, LJ211	144	2,880
	13311	Typhimurium, NCTC 74	144	2,880
	51956	Hadar, CDC 347	144	2,880
	51741	DUP-103	144	2,880
	10721	Javiana, ETS 146	144	2,880
	9239	Oranienburg, E1093	144	2,880
	51955	Virchow, CDC 41	144	2,880
	51957	Agona, CDC 873	144	2,880

BAA-2739	Mississippi, CDC 2012K-0487	144	2,880
13312	Choleraesuis, NCTC 5735	144	2,880
700136	Braenderup, NCTC 5750	144	2,880
15480	Dublin, HWS 51	144	2,880
CCUG 21280	*Sch*warzengrund	144	2,880

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

Organism	ATCC# or Source	Strain/ serovar/ serotype/ antigenic properties	Test Concentration (3X LoD) (CFU/mL)	
			Aptima Multitest Tube	Preserved Stool
Salmonella enterica subsp. *salamae* (II)	6959	NCTC 2206	144	2,880
	Univ of Calgary 2425	argC95	144	2,880
	700148	NCTC 10252	144	2,880
	43972	CIP 82.29	144	2,880
Salmonella enterica subsp. *arizonae* (IIIa)	12323	CDC 3153--55	144	2,880
	12324	CDC 1089-53	144	2,880
	13314	NCTC 8297	144	2,880
Salmonella enterica subsp. *diarizonae* (IIIb)	12325	CDC	144	2,880
	29226	CDC 656/75	144	2,880
	43973	CIP 82.31	144	2,880
Salmonella enterica subsp. houtenae (IV)	29932	16:z4,z23: -	144	2,880
Salmonella enterica subsp. indica (VI)	43976	CIP 102501	144	2,880
	Univ of Calgary 2430	pyrE20	144	2,880
Shigella dysenteriae (A)	13313	Type 1, NCTC 4837	204	4,080
	49555	Type 13, CDC 8008-79	204	4,080
	29028	Type 3, CDC 3596-74	204	4,080
	49551	Type 12, CDC 2243-66	204	4,080
	11835	AMC 43--A--1	204	4,080
	9361	Type 1, AMC 43-A-14	204	4,080
	12021	Type 8, CDC 2116-52	204	4,080
	12037	Type 9, CDC A-58:1646	204	4,080
	49547	Type 11, CDC 3883-66	204	4,080
	29903	Type 2a, 24570	204	4,080
	12022	Type 2b, CDC 3591-52	204	4,080
	9199	Type 1a, AMC 43-G-68	204	4,080

Shigella flexneri (B)	33948	612-003	204	4,080
	11836	Type 3, AMC 43-G-100	204	4,080
	12023	Type 4a, CDC 5380-52	204	4,080
	12025	Type 6, CDC 64	204	4,080
	700930	Type 2a, 2457T	204	4,080

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

Organism	ATCC# or Source	Strain/ serovar/ serotype/ antigenic properties	Test Concentration (3X LoD) (CFU/mL)	
			Aptima Multitest Tube	Preserved Stool
Shigella boydii (C)	8700	Type 2, NCTC 12985	204	4,080
	29928	Type10, C-10	204	4,080
	9207	Type 1, AMC 43-G-58	204	4,080
	BAA-1247	Type 20, SH-108	204	4,080
	12030	Type 10, CDC 6336-52	204	4,080
	12028	Type 8	204	4,080
	12031	Type 11, CDC 1624-54	204	4,080
	9905	Type 7, AMC 4006	204	4,080
Shigella sonnei (D)	9290	AMC 43-GG9	204	4,080
	29930[a]	WRAIR I virulent[a]	204	4,080
	11060	4628	204	4,080
	29031	CDC 45-75	204	4,080
	25931	NCDC 1120-66	204	4,080
Enteroinvasive *E. coli* (EIEC)	43893	Type O124:NM, CDC EDL 1284	69	1,380
	BAA-2190	Type O121, 98-3306	69	1,380
	49105	Type O15, 1/1/7482	69	1,380
	12806	Type O124:K72 (B17):H, CDC	69	1,380
	43892[a]	Type O29:NM, CDC EDL 1282[a]	69	1,380
Campylobacter jejuni	33560	CIP 702	75	1,500
	43432	Type O:4, MK7	75	1,500
	35920	BG 22	75	1,500
	43459	Type O:40, MPD570102	75	1,500
	29428	VPI H840	75	1,500
	33252	C3692	75	1,500
	33291[a]	AS-83-79[a]	75	1,500
	700819	NCTC 11168	75	1,500
	BAA-1062	RM 1221	75	1,500

subsp. *Jejuni*	BAA-1234	RM3193	75	1,500
	33292	AS-84-79	75	1,500
	35918	BG 177	75	1,500
	43434	Type O:6, C6	75	1,500
	43435	Type O:7, DPH-1	75	1,500
	43449	Type O:23, MK 198	75	1,500
	43503	UA466	75	1,500
	43472	Type O:5, CFJ29	75	1,500
	43430	Type O:2, CJC-25	75	1,500

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

Organism	ATCC# or Source	Strain/ serovar/ serotype/ antigenic properties	Test Concentration (3X LoD) (CFU/mL)	
			Aptima Multitest Tube	Preserved Stool
Campylobacter coli	33559	CIP 7080[a]	48	960
	43488	Type O:56, RO 268	48	960
	43485	Type O:49, A1618	48	960
	43483	Type O:47, Ca 72	48	960
	43484	Type O:48, Ca 77	48	960
	43133	BG716	48	960
	43136	BG193	48	960
	43481	Type O:39, 80-102	48	960
	43482	Type O:46, VanH13	48	960
	49941	LRA 069.05.89	48	960
	BAA-372	D5708	48	960
	43135	BG192	48	960
	43478	Type O:28, 76-GA2	48	960
	BAA-1061	RM 2228	48	960
Shigatoxigenic *E.coli* (O157)	35150	O157:H7 (*stx1/stx2*), EDL 931[a]	60	1,200
	700377	O157:NM (*stx2*), CDC 92-3099	60	1,200
	700927	O157:H7:K- (*stx1/stx2*), EDL 933	60	1,200
	35150[a]	O157:H7 (*stx1/stx2*), EDL 931	60	1,200
	43894	O157:H7 (*stx1/stx2*), CDC EDL 932	60	1,200
	700378	O157:NM (*stx1/stx2*), CDC 92-3073	60	1,200
	43890	O157:H7 (*stx1*) CDC C984	60	1,200
	43895	O157:H7 (*stx1/stx2*), CDC EDL 933	60	1,200

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

Organism	ATCC# or Source	Strain/ serovar/ serotype/ antigenic properties	Test Concentration (3X LoD) (CFU/mL)	
			Aptima Multitest Tube	Preserved Stool
Shigatoxigenic *E.coli*	51435	O91:H21 (*stx2*), B2F1	318	6,360
	700840	O111:H8 (*stx1/stx2*), B99BE001161	318	6,360
	51434	O91:H21 (*stx2*), H414-36/89	318	6,360
	BAA-181	O111:H8 (*stx1/stx2*), CDC 1999-3249	318	6,360
	BAA-180	O111:H8 (*stx1*), CDC 1999-3302	318	6,360
	BAA-176	O113:H21 (*stx2*), CDC 2001-3004	318	6,360
	BAA-177	O113:H21 (*stx1/stx2*), CDC 2000-3159	318	6,360
	BAA-182	O104:H21 (*stx2*), CDC 1994-3023	318	6,360
	BAA-1653[a]	O26:H11 (*stx1/stx2*), EH1534[a]	318	6,360
	BAA-2193	O45:H2 (*stx1*), 2000-3039	318	6,360
	BAA-2210	O103:H2 (*stx1*), 2003-3112	318	6,360
	BAA-2211	O145:H25 (*stx2*), 2003-3375	318	6,360
	BAA-2219	O121:H19 (*stx2*), 2002-3211	318	6,360
	BAA-2222	O145:Nonmotile (*stx1/stx2*), 2006-3142	318	6,360
	BAA-2326	O104:H4 (*stx2*), TY-2482	318	6,360
	BAA-2196	O26:H11 (*stx1/stx2*), 2003--3014	318	6,360
	BAA-2215	O103:H11 (*stx1*), 2006-3008	318	6,360
	BAA-2213	O103:H25 (stx1), 2005-3546	318	6,360
	BAA-178	O104:H21(*stx2*), CDC 1994-3024	318	6,360
	BAA-184	O111:H8 (*stx1*), CDC 2000-3025	318	6,360
	BAA-2217	O146 (*stx2*), 10C-3114	318	6,360
	BAA-179	O111:H8 (*stx1/stx2*), CDC 1997-3215	318	6,360
	BAA-2129	O145:H28 (*stx2*), TW07865	318	6,360
	BAA-1652	O145:H48 (*stx2*), EH1533	318	6,360
	BAA-2192	O145:Nonmotile (*stx1/stx2*), 99-3311	318	6,360

CFU = colony forming units.
[a] Strains used to establish LoD.

Inclusivity/Reactivity - *In silico* Analysis

The inclusivity of the Panther Fusion GI Bacterial Assay was evaluated using *in silico* inclusivity analysis for each analyte. In silico analysis was performed using analyte sequences available in NCBI database and 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 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.

Analytical Specificity: Cross Reactivity and Microbial Interference - Wet Testing

Analytical specificity (cross-reactivity) and microbial interference for the Panther Fusion GI 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 100 bacteria, viruses, parasites and yeast listed in Table 6 were tested in processed negative CBS matrix in the absence and in the presence of GI 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. No cross-reactivity or microbial interference was observed with any of the 100 organisms tested on the Panther Fusion GI Bacterial Assay at indicated concentrations.

Table 6. 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[a]	10^6 copies/mL	*Escherichia hermanii*	10^6 CFU/mL
Cyclospora[a]	10^6 copies/mL	*Escherichia vulneris*	10^6 CFU/mL

Cryptosporidium[a]	10^6 copies/mL	*Gardnerella vaginalis*	10^6 CFU/mL
Norovirus (Noro GII)[a]	10^5 copies/mL	*Helicobacter pylori*	10^6 CFU/mL
Astrovirus[a]	10^5 copies/mL	*Klebsiella oxytoca*	10^6 CFU/mL
Sapovirus (GII)[a]	10^5 copies/mL	*Klebsiella ozaenae*	10^6 CFU/mL
Enterovirus (Ent V)[a]	10^5 copies/mL	*Klebsiella pneumoniae*	10^6 CFU/mL
Rhinovirus[a]	10^5 copies/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[a]	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

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

Microorganism	Test Concentration	Microorganism	Test Concentration
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[b]	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 $TCID_{50}$/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 1E6 CFU/mL and 100% positivity was recovered for *Campylobacter* at ≤ 1E4 CFU/mL.

Coinfection/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 CBS matrix. The low concentration analyte was tested at 3X LoD against a high concentration analyte at 106 CFU/mL. Additionally, analytes were also tested in the absence of a second analyte. When analytes were tested at high concentration, all results for other analytes maintained expected positivity; no competitive interference was observed. Table 7 shows a summary of results observed in the competitive interference testing.

Table 7. Summary of Coinfection Results

Analyte 1		Analyte 2		*Salmonella* % Pos	*Campylobacter* % Pos	*Shigella* % Pos	STEC % Pos
Name	**3X LoD (CFU/mL)[a]**	**Name**	**High Conc (CFU/mL)[a]**				
Negative	N/A	Negative	N/A	0%	0%	0%	0%
Salmonella	327	None	0	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	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%	0%
		Salmonella	10^6	100%	0%	100%	0%
		Campylobacter	10^6	0%	100%	100%	0%
		STEC	10^6	0%	0%	100%	100%
STEC	318	None	0	0%	0%	0%	100%
		Salmonella	10^6	100%	0%	0%	100%
		Campylobacter	10^6	0%	100%	0%	100%
		Shigella	10^6	0%	0%	100%	100%
None	0	*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%

CFU = colony forming units, Pos = positive.

[a] Analyte concentration in Aptima Multitest tube.

Interference

Potential inhibitory effects of endogenous and exogenous substances that may be present in a specimen were evaluated in the Panther Fusion GI Bacterial Assay. Clinically relevant concentrations of potentially interfering substances were added to processed negative CBS 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 8. No impact on the performance of the Panther Fusion GI 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

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 Bacterial Assay performance. The preservative evaluated include 10 different types of Cary-Blair transport media from different vendors and preservative media containing fixatives shown in Table 9. All media were tested with Panther Fusion GI Bacterial Assay analytes at 3X LoD. Comparable performance was seen with all Cary-Blair media. Comparable 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	Protocol Cary Blair Medium
Cary Blair Transport Medium w/ Indicator	Enteric Transport Media (ETM®)
Para-Pak® C&S	Puritan® Cary-Blair Medium 2mL
Para-Pak® Enteric Plus	Puritan® Cary-Blair Medium 5mL
Cardinal Health™ C&S Stool Transport Vial	Copan® FecalSwab® Collection, Transport and Preservation System
Fixative media (interference was observed)	
Fisher® 10% Buffered Formalin	
Para-Pak® 10% Buffered Formalin	
Para-Pak® LV-PVA	

Carryover Contamination

The carryover contamination rate of the assay was evaluated using a checkerboard design with negative and positive panels made in processed negative CBS matrix. A total of 270 negatives interspersed with 270 positives samples (spiked with *Salmonella* at 10^6 CFU/mL or 9,714 X LoD) were tested across 5 runs on 2 Panther Fusion Instruments. The Panther Fusion GI Bacterial Assay demonstrated a 0% carryover rate.

Within Laboratory Precision/Repeatability

Panther Fusion GI Bacterial Assay within laboratory precision was evaluated with a 3-member panel consisting of assay analytes in processed negative CBS matrix. The 3-member panel included 1 negative and 2 multi-analyte (with *Salmonella*, *Campylobacter*, *Shigella*, and STEC) 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	Low Pos (1.5X LoD)	*Salmonella*	162/162	100	36	0.12	0.33	0.00	0.00	0.07	0.19	0.18	0.5	0.22	0.61	0.51	1.41	0.6	1.66
		Campylobacter	162/162	100	35.1	0.06	0.17	0.04	0.11	0.04	0.12	0.03	0.08	0.19	0.55	0.31	0.87	0.37	1.06
		Shigella	162/162	100	36.4	0.00	0.00	0.23	0.62	0.00	0.00	0.09	0.24	0.00	0.00	0.47	1.29	0.53	1.45
		STEC	162/162	100	34.3	0.07	0.2	0.00	0.00	0.00	0.00	0.04	0.11	0.05	0.13	0.35	1.02	0.36	1.05
2	Negative	Negative (Internal Control)	162/162	100	28	0.04	0.15	0.33	1.16	0.00	0.00	0.00	0.00	0.15	0.52	0.11	0.39	0.38	1.34
3	Mod Pos (3X LoD)	*Salmonella*	162/162	100	35.1	0.22	0.62	0.00	0.00	0.00	0.00	0.06	0.16	0.26	0.74	0.39	1.11	0.52	1.48
		Campylobacter	162/162	100	34.3	0.08	0.24	0.04	0.11	<0.01	<0.01	0.00	0.00	0.14	0.4	0.24	0.7	0.29	0.85
		Shigella	162/162	100	35.4	0.12	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.39	1.09	0.41	1.14
		STEC	162/162	100	33.3	0.08	0.24	0.00	0.00	0.00	0.00	<0.01	<0.01	0.08	0.23	0.28	0.85	0.3	0.91

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 Bacterial Assay reproducibility was evaluated at 3 US sites using 1 negative panel member and 2 panel members positive for all 4 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 *Salmonella, Campylobacter, Shigella,* and STEC (Table 11).

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

Description	Analyte	Agreement with Expected Results	
		N	% (95% CI)
Neg	Internal Control	90/90	100 (95.9-100)
Low Pos[a]	*Salmonella*[c]	90/90	100 (95.9-100)
	Campylobacter[c]	90/90	100 (95.9-100)
	Shigella/EIEC[c]	90/90	100 (95.9-100)
	STEC[c]	90/90	100 (95.9-100)
Mod Pos[b]	*Salmonella*[c]	90/90	100 (95.9-100)
	Campylobacter[c]	90/90	100 (95.9-100)
	Shigella/EIEC[c]	90/90	100 (95.9-100)
	STEC[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] *Salmonella bongori*, *Campylobacter jejuni*. *Shigella sonnei*, and STEC serotype O26 were used to build the positive panels.

Signal variability was measured as %CV of the Ct values. The total signal variability was ≤2.03% (SD ≤0.74) for all panel components (Table 12). For the sources of variation except the 'withinrun' factor, %CV values were ≤1.00% for all panel components. The signal variability was ≤0.77% (SD ≤0.25) for the Panther Fusion GI Bacterial Assay positive controls (Table 13).

Table 12. Signal Variability of the Panther Fusion GI 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]	*Salmonella*	90	36.4	0.00	0.00	0.36	1.00	0.12	0.32	0.63	1.74	0.74	2.03
	Campylobacter	90	35.1	0.16	0.45	0.05	0.14	0.09	0.25	0.32	0.91	0.37	1.05
	Shigella/EIEC	90	36.3	0.08	0.22	0.03	0.08	0.00	0.00	0.48	1.32	0.49	1.34
	STEC	90	34.3	0.00	0.00	0.06	0.18	0.04	0.11	0.31	0.92	0.32	0.94
Mod Pos[b]	*Salmonella*	90	35.2	0.16	0.47	0.00	0.00	0.14	0.39	0.43	1.23	0.48	1.37
	Campylobacter	90	34.2	0.15	0.43	0.04	0.13	0.11	0.31	0.30	0.88	0.35	1.03
	Shigella/EIEC	90	35.2	0.19	0.55	0.10	0.30	0.00	0.00	0.34	0.96	0.40	1.14
	STEC	90	33.3	0.08	0.23	0.00	0.00	0.07	0.20	0.25	0.74	0.27	0.80

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 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	*Salmonella*	30	30.5	0.12	0.40	0.00	0.00	0.11	0.35	0.15	0.49	0.22	0.73
	Campylobacter	30	31.4	0.04	0.13	0.04	0.13	0.09	0.29	0.05	0.17	0.12	0.38
	Shigella/EIEC	30	31.9	0.16	0.50	0.00	0.00	0.13	0.42	0.13	0.41	0.25	0.77
	STEC	30	31.8	0.00	0.00	0.01	0.04	0.11	0.33	0.11	0.35	0.15	0.49

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 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% Score CIs, 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 (eg, 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 (1521 prospective and 219 retrospective specimens) is provided in Table 14.

Table 14. 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 Group	0 to 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 to 5 years	53 (3.0)	50 (3.3)	3 (1.4)
	6 to 11 years	73 (4.2)	66 (4.3)	7 (3.2)
	12 to 17 years	73 (4.2)	71 (4.7)	2 (0.9)
	18 to 21 years	53 (3.0)	45 (3.0)	8 (3.7)
	22 to 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 15 through Table 18.

Table 15. Clinical Performance - Salmonella spp.

Specimen Origin	N	TP	FP	TN	FN	Prevalence[a] (%)	PPA % (95% CI)[b]	NPA % (95% CI)[b]
Prospective (Fresh)	1520	33	2[c]	1484	1[d]	2.2	97.1 (85.1, 99.5)	99.9 (99.5, 100)
Retrospective (Frozen)	219	20	2[e]	197	0	N/A[f]	100 (83.9, 100)	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] Study prevalence reported based on comparator testing.
[b] Score CI.
[c] The 2 discordant false positive prospective specimens were positive for *Salmonella* by the alternate NAAT.
[d] The discordant false negative prospective specimen was negative for *Salmonella* by the alternate NAAT.
[e] The 2 discordant retrospective false positive specimens were positive for *Salmonella* by the alternate NAAT.
[f] Calculation of prevalence is not applicable.

Table 16. Clinical Performance - Campylobacter spp.

Specimen Origin	N	TP	FP	TN	FN	Prevalence[a] (%)	PPA % (95% CI)[b]	NPA % (95% CI)[b]
Prospective (Fresh)	1520	39	2[c]	1478	1[d]	2.6	97.5 (87.1, 99.6)	99.9 (99.5, 100)
Retrospective (Frozen)	219	18	4[e]	197	0	N/A[f]	100 (82.4, 100)	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] Study prevalence reported based on comparator testing.
[b] Score CI.
[c] The 2 discordant false positive prospective specimens were negative for *Campylobacter* by the alternate NAAT.
[d] The discordant false negative prospective specimen was negative for *Campylobacter* by the alternate NAAT.
[e] 3 of 4 discordant false positive retrospective specimens were positive for *Campylobacter* by the alternate NAAT.
[f] Calculation of prevalence is not applicable.

Table 17. Clinical Performance - Shigella/EIEC

Specimen Origin	N	TP	FP	TN	FN	Prevalence[a] (%)	PPA % (95% CI)[b]	NPA % (95% CI)[b]
Prospective (Fresh)	1521	27	0	1494	0	1.8	100 (87.5, 100)	100 (99.7, 100)
Retrospective (Frozen)	219	19	1[c]	199	0	N/A[d]	100 (83.2, 100)	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] Study prevalence reported based on comparator testing.
[b] Score CI.
[c] The discordant false positive retrospective specimen was positive for *Shigella*/EIEC by the alternate NAAT.
[d] Calculation of prevalence is not applicable.

Table 18. Clinical Performance - Shiga Toxins 1 and 2 (stx1/stx2)

Specimen Origin	N	TP	FP	TN	FN	Prevalence[a] (%)	PPA % (95% CI)[b]	NPA % (95% CI)[b]
Prospective (Fresh)	1520	7	5[c]	1508	0	0.5	100 (64.6, 100)	99.7 (99.2, 99.9)
Retrospective (Frozen)	219	39	8[d]	172	0	N/A[e]	100 (91.0, 100)	95.6 (91.5, 97.7)
Contrived (Frozen)	126	63	0	63	0	N/A[e]	100 (94.3, 100)	100 (94.3, 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 5 discordant false positive prospective specimens were positive for stx1/stx2 by the alternate NAAT.
[d] The 8 discordant false positive retrospective specimens was positive for stx1/stx2 by the alternate NAAT.
[e] Calculation of prevalence is not applicable.

The 14 coinfections detected by the Panther Fusion GI Bacterial Assay are described in Table 19. Nine (9) coinfections were also detected by the comparator NAAT.

Table 19. Coinfections Detected in Prospective and Retrospective Specimens

Coinfections	Detected by Panther Fusion GI Bacterial Assay (n)	Confirmed by Comparator (n)
Salmonella, *Campylobacter*	1	0
Salmonella, *Shigella*/EIEC	1	0
Salmonella, *stx1/stx2*	1	0
Campylobacter, *Shigella*/EIEC	5	4
Campylobacter, *stx1/stx2*	5	4
Shigella/EIEC, *stx1/stx2*	1	1

VIII. CONCLUSIONS

The analytical and clinical study results demonstrate that the Panther Fusion GI 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 Bacterial Assay on the Panther Fusion system will perform as intended.