GI Bacterial Assay (Panther Fusion™ System)

For *in vitro* diagnostic use only

For U.S. Export Only

TABLE OF CONTENTS

General Information

Intended 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 preserved stool specimens collected from individuals exhibiting signs and symptoms of gastroenteritis.

This assay is intended to aid in the differential diagnosis of *Salmonella*, *Campylobacter*, *Shigella*/Enteroinvasive *E. coli* (EIEC) and Shigatoxigenic *Escherichia coli* (STEC) infections. The results of this assay should be used in conjunction with clinical presentation, laboratory findings, and epidemiological information and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out coinfection with other organisms that are not detected by this test and may not be the sole or definitive cause of patient illness. Negative results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test, or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease. This assay is designed for use on the Panther Fusion™ System.

Summary and Explanation of the Test

Acute diarrhea is a leading cause of outpatient visits, hospitalization, and lost quality of life in both domestic settings and among those traveling abroad. The global impact of foodborne disease is substantial with an estimated 600 million people becoming ill, resulting in 420,000 deaths annually.[1] The Centers for Disease Control and Prevention (CDC) has estimated 48 million cases of foodborne illness annually in the US leading to 128,000 hospitalizations and 3,000 deaths.[2] Acute diarrhea is associated with estimated healthcare costs upwards of $150 million.[3]

Infectious gastroenteritis can be caused by a variety of bacterial, viral, and parasitic organisms. Symptoms alone cannot be used to distinguish the cause of the infection, making rapid and accurate diagnostic tools essential for guiding treatment and patient management.

CDC estimates *Salmonella* causes about 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths in the United States every year. Food is the source for most of these illnesses.[4]

Shigella is estimated to cause nearly half a million illnesses each year in the United States, making it the third most common bacterial enteric disease. Shigellosis is not associated with a specific seasonality, which is likely a result of the importance of person-to-person transmission in the spread of this infection.[5]

Campylobacter causes an estimated 1.5 million illnesses each year in the United States. It is one of the most common causes of diarrheal illness in the United States. Active surveillance indicates that about 20 cases per 100,000 people are diagnosed each year. Many more cases go undiagnosed or unreported. Most cases are not part of recognized outbreaks, and more cases occur in summer than in winter.[6-7]

An estimated 265,000 STEC infections occur each year in the United States, with STEC O157 causing about 36% of these infections.[8] Public health experts rely on estimates rather than actual numbers of infections because not all STEC infections are diagnosed.

Principles of the Procedure

The Panther Fusion System fully automates specimen processing, including sample lysis, nucleic acid capture, amplification, and detection for the Panther Fusion GI Bacterial Assay. Nucleic acid capture and elution take place in a single tube on the Panther Fusion System. The eluate is transferred to the Panther Fusion System reaction tube containing the assay reagents. Multiplex real-time PCR is then performed for the eluted nucleic acid on the Panther Fusion System.

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).

> ***Note:*** *The Panther Fusion System adds the IC-B to the Panther Fusion Capture Reagent-B (FCR-B). After the IC-B is added to the FCR-B, it is referred to as wFCR-B (working FCR-B).*

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 cutoff 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

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. Carefully read this entire package insert and the *Panther™/Panther Fusion System Operator's Manual*.

C. The Panther Fusion Enhancer Reagent-B (FER-B) is corrosive, harmful if swallowed, and causes severe skin burns and eye damage.

D. Only personnel adequately trained on the use of this assay and in handling potentially infectious materials should perform these procedures. If a spill occurs, immediately disinfect using appropriate site procedures.

Laboratory Related

E. Use only supplied or specified disposable laboratory ware.

F. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.

G. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

Specimen Related

H. Handle all specimens as if infectious, using safe laboratory procedures such as those outlined in CDC/NIH Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29 Protection of Laboratory Workers from Occupationally Acquired Infections.[9]

I. Expiration dates listed on the Aptima Multitest tubes pertain to the transfer of sample into the tube and not to testing of the sample. Specimens collected/transferred any time prior to these expiration dates are valid for testing provided they are transported and stored in accordance with the appropriate package insert, even if these expiration dates have passed.

J. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

K. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of bacteria or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens.

Assay Related

L. Do not use the reagents and controls after the expiration date.

M. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements* and *Panther Fusion System Test Procedure* for more information.

N. Do not combine any assay reagents or fluids. Do not top off reagents or fluids; the Panther Fusion System verifies reagent levels.

O. Avoid microbial and nuclease contamination of reagents.

P. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

Q. Do not use the assay cartridge if the storage pouch is compromised or if the assay cartridge foil is not intact. Contact Hologic Technical Support if either occurs.

R. Do not use the fluid packs if the foil seal is leaking. Contact Hologic Technical Support if this occurs.

S. Handle the assay cartridges with care. Do not drop or invert assay cartridges. Avoid prolonged exposure to ambient light.

T. Some reagents of this kit are labeled with hazard information.

Note: *For more information on any hazard and precautionary statements that may be associated with reagents refer to the Safety Data Sheet Library at www.hologicsds.com. For more information on the symbols, refer to the symbol legend on http://www.hologic.com/package-inserts.*

Canada Hazard Information

Panther Fusion Enhancer Reagent-B (FER-B)

Lithium Hydroxide, Monohydrate 5 - 10%

DANGER

H302 - Harmful if swallowed
H314 - Causes severe skin burns and eye damage
P264 - Wash face, hands and any exposed skin thoroughly after handling
P270 - Do not eat, drink or smoke when using this product
P330 - Rinse mouth
P501 - Dispose of contents/container to an approved waste disposal plant
P260 - Do not breathe dusts or mists
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P301 + P330 + P331 - IF SWALLOWED: Rinse mouth. Do NOT induce vomiting
P303 + P361 + P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower
P304 + P340 - IF INHALED: Remove person to fresh air and keep comfortable for breathing
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P321 - Specific treatment (see supplemental first aid instructions in the SDS)
P363 - Wash contaminated clothing before reuse
P405 - Store locked up
P301+ P317 - IF SWALLOWED: Get medical help
P316 - Get emergency medical help immediately

Reagent Storage and Handling Requirements

A. The following table provides storage and handling requirements for this assay.

Reagent	Unopened Storage	On Board/ Open Stability[a]	Opened Storage
Panther Fusion GI Bacterial Assay Cartridge	2°C to 8°C	60 days	2°C to 8°C[b]
Panther Fusion Capture Reagent-B (FCR-B)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Enhancer Reagent-B (FER-B)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Internal Control-B (IC-B)	2°C to 8°C	(In wFCR-B)	Not applicable
Panther Fusion Elution Buffer	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Oil	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Reconstitution Buffer I	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion GI Bacterial Positive Control	2°C to 8°C	Single use vial	Not applicable-single use
Panther Fusion Negative Control	2°C to 8°C	Single use vial	Not applicable-single use

When reagents are removed from the Panther Fusion System, return them immediately to their appropriate storage temperatures.

[a] On-board stability starts at the time the reagent is placed on the Panther Fusion System for the Panther Fusion GI Bacterial Assay cartridge, FCR-B, FER-B, and IC-B. On-board stability for the Panther Fusion Reconstitution Buffer I, Panther Fusion Elution Buffer, and Panther Fusion Oil Reagent starts when the reagent pack is first used.

[b] If removed from the Panther Fusion System, store the assay cartridge in an air-tight container with desiccant at the recommended storage temperature.

B. Working Panther Fusion Capture Reagent-B (wFCR-B) and Panther Fusion Enhancer Reagent-B (FER-B) are stable for 60 days when capped and stored at 15ºC to 30ºC. Do not refrigerate.

C. Controls are stable until the date indicated on the vials.

D. Discard any unused reagents that have surpassed their on board stability.

E. Avoid cross-contamination during reagent handling and storage.

F. **Do not freeze reagents.**

Specimen Collection and Storage

Specimens – Clinical material collected from patient and placed in an appropriate transport system. For the Panther Fusion GI Bacterial Assay, this includes raw stool preserved in Cary-Blair transport media.

Samples – Represents a more generic term to describe any material for testing on the Panther Fusion System including specimens, specimens transferred into an Aptima Multitest tube and controls.

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during specimen handling steps. For example, discard used material without passing over open tubes.*

A. Specimen types include stool samples preserved in Cary-Blair transport media.

Collect raw stool following appropriate standard stool collection and handling procedures. Transfer raw stool specimens into Cary-Blair transport media according to manufacturer's instructions.

B. Specimen Processing

1. Mix Cary-Blair preserved specimen thoroughly to ensure homogeneity immediately prior to transfer into the Aptima Multitest tube.
2. Prior to testing on the Panther Fusion System, transfer specimen to an Aptima Multitest tube.
   a. Partially peel open the swab package. Remove the swab. Do not touch the soft tip or lay the swab down. If the soft tip is touched, the swab is laid down, or the swab is dropped, use a new Aptima™ Multitest Swab Specimen Collection Kit. Completely submerge the soft tip of the swab in Cary-Blair preserved stool specimen.

      Note: *Submerge only the soft tip of the swab 1 time in the liquid part, ensuring the pink shaft is not submerged.*

   b. Uncap the Aptima Multitest tube containing the transport medium. If the contents of the tube are spilled, use a new Aptima Multitest Swab Specimen Collection Kit. Place the swab in the tube and gently swirl the swab in the tube for 5 seconds to release material. Leave the swab in the tube.

   c. Carefully break the swab shaft at the score line against the side of the tube and discard the top portion of the swab shaft.

   d. Affix the provided or new penetrable cap to the tube.
3. Storing specimens before testing
   a. After collection, the Cary-Blair preserved specimens can be stored at 2°C to 8°C for up to 72 hours before transfer to the Aptima Multitest tube.

      Note: *Campylobacter is affected by storage temperature and time. If samples are not stored appropriately, they may have reduced recovery and lose their positive results.*

b. Specimen in the Aptima Multitest tube may be stored under 1 of the following conditions:
   - 15°C to 30°C for up to 6 days or
   - 2°C to 8°C for up to 30 days or
   - ≤ -20°C for up to 3 months

Note: *Do not exceed 1 freeze-thaw cycle. Multiple freeze-thaw cycles may lead to sample degradation.*

Note: *It is recommended that specimens transferred to the Aptima Multitest tube are stored capped and upright in a rack.*

C. Specimen Storage after Testing

1. Samples that have been assayed should be stored upright in the rack under 1 of the following conditions:
   - 15°C to 30°C for up to 6 days or
   - 2°C to 8°C for up to 30 days or
   - ≤ -20°C for up to 3 months

Note: *Do not exceed 1 freeze-thaw cycle. Multiple freeze-thaw cycles may lead to sample degradation.*

2. The samples should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped, remove the penetrable cap and place a new non-penetrable cap on the specimen tubes. If samples need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping, specimen transport tubes must be kept upright for 5 minutes to bring all of the liquid down to the bottom of the tube. Avoid splashing and cross-contamination. Do not centrifuge.

Specimen Transport

Maintain specimen storage conditions during transport as described under *Specimen Collection and Storage*.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther Fusion System

The Panther Fusion System is an integrated nucleic acid testing system that fully automates all steps necessary to perform various Panther Fusion assays from sample processing through amplification, detection, and data reduction.

Reagents and Materials Provided for Panther Fusion GI Bacterial Assay

Assay Packaging

Components	Part No.	Storage
Panther Fusion GI Bacterial Assay Cartridge 96 Tests Panther Fusion GI Bacterial Assay cartridge, 12 tests, 8 per box	PRD-07113	2°C to 8°C
Panther Fusion Internal Control-B 960 Tests Panther Fusion Internal Control-B tube, 4 per box	PRD-06234	2°C to 8°C
Panther Fusion GI Bacterial Assay Controls Panther Fusion GI Bacterial Positive Control tube, 5 per box Panther Fusion Negative Control tube, 5 per box	PRD-07116	2°C to 8°C
Panther Fusion Extraction Reagent-B 960 Tests Panther Fusion Capture Reagent-B bottle, 240 tests, 4 per box Panther Fusion Enhancer Reagent-B bottle, 240 tests, 4 per box	PRD-06232	15°C to 30°C
Panther Fusion Elution Buffer 2400 Tests Panther Fusion Elution Buffer pack, 1200 tests, 2 per box	PRD-04334	15°C to 30°C
Panther Fusion Reconstitution Buffer I 1920 Tests Panther Fusion Reconstitution Buffer I, 960 Tests, 2 per box	PRD-04333	15°C to 30°C
Panther Fusion Oil Reagent 1920 Tests Panther Fusion Oil Reagent, 960 tests, 2 per box	PRD-04335	15°C to 30°C

Individually Packaged Items

Items	Part No.
Panther Fusion Tube Trays, 1008 Tests, 18 trays per box	PRD-04000
Aptima Multitest Specimen Collection Kit, pack of 50	PRD-03546

Materials Required and Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Panther System	303095
Panther Fusion System	PRD-04172
Panther System Continuous Fluid and Waste (Panther Plus)	PRD-06067
Aptima™ Assay Fluids Kit (Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther System Run Kit contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects[a]	303096 (5000 tests)
Tips, 1000 µL, filtered, liquid-sensing, conductive, and disposable: Not all products are available in all regions. Contact your representative for region-specific information.	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128 MME-04110
Aptima penetrable caps (optional)	105668
Replacement non-penetrable caps (optional)	103036A
Replacement extraction reagent bottle caps	CL0040
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution **Note:** Refer to the *Panther/Panther Fusion System Operator's Manual* for instructions on preparing diluted sodium hypochlorite solution.	—
Disposable powderless gloves	—

[a] Needed only for Aptima assays that use TMA technology.

Optional Materials

Material	Cat. No.
Benchtop Vortex (VWR Analog Vortex Mixer 120V, Cat. No. 10153-838) or equivalent	—

Panther Fusion System Test Procedure

Note: *Refer to the Panther/Panther Fusion System Operator's Manual for additional procedural information.*

A. Work Area Preparation

1. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.

B. Reagent Preparation

1. Remove the bottles of IC-B, FCR-B, and FER-B from storage.
2. Mix FCR-B by gently swirling until full resuspension of the beads. Avoid creating foam during this step.
3. Open the bottles of IC-B, FCR-B, and FER-B, and discard the caps. Open the TCR door on the upper bay of the Panther Fusion System.
4. Place the IC-B, FCR-B, and FER-B bottles in the appropriate positions on the TCR carousel.
5. Close the TCR door.

Note: *The Panther Fusion System adds the IC-B to the FCR-B. After the IC-B is added to the FCR-B, it is referred to as wFCR-B (working FCR-B). If the wFCR-B and FER-B are removed from the system, use new caps and immediately store according to the proper storage conditions.*

C. Specimen Handling

1. Visually confirm that each specimen tube contains a single pink Aptima collection swab in the Aptima Multitest tube. If the Aptima Multitest tube contains no swab, multiple swabs, or a swab not provided by Hologic, the transfer of stool in Cary-Blair media should be repeated using a new Aptima Multitest Swab Specimen Collection Kit.
2. Verify the appearance of the sample in the Aptima Multitest tube.
   a. If the specimen is homogeneous, proceed with testing.
   b. If solids or mucoidal materials are observed, note that these can interfere with the test.

Note: *If any invalid flags are observed when processing specimens (e.g., CLT, icrfu, ebh or ebl), samples in the Aptima Multitest tube may be vortexed after replacing with a new penetrable cap for 30 to 60 seconds at maximum speed on a standard bench top vortex prior to retesting.*

Note: *Prepare specimens per the Specimen Processing instructions in the Specimen Collection and Storage section before loading specimens onto the Panther Fusion System.*

D. System Preparation

For instructions on setting up the Panther Fusion System including loading samples, reagents, assay cartridges and universal fluids, refer to the *Panther/Panther Fusion System Operator's Manual.*

Procedural Notes

A. Controls

1. The Panther Fusion GI Bacterial Positive Control and the Panther Fusion Negative Control can be loaded in any rack position, in any Sample Bay lane on the Panther Fusion System.
2. Once the control tubes are pipetted and processed for the Panther Fusion GI Bacterial Assay, they are valid for up to 30 days (control frequency configured by an administrator) unless control results are invalid or a new assay cartridge lot is loaded.
3. Each control tube can be tested once.
4. Patient specimen pipetting begins when 1 of the following 2 conditions is met:
   a. Valid results for the controls are registered on the system.
   b. A pair of controls is currently in process on the system.

Quality Control

A run or specimen result may be invalidated by the Panther Fusion System if problems occur while performing the assay. Specimens with invalid results must be retested.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One (1) replicate of the negative assay control and positive assay control must be tested each time a new lot of assay cartridges is loaded on the Panther Fusion System or when the current set of valid controls for an active cartridge lot have expired.

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

During processing, criteria for acceptance of the assay controls are automatically verified by the Panther Fusion System. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther Fusion System.

If the assay controls pass all validity checks, they are considered valid for the administrator-specified time interval. When the time interval has passed, the assay controls are expired by the Panther Fusion System and a new set of assay controls will be required prior to starting any new samples.

If any one of the assay controls fails the validity checks, the Panther Fusion System automatically invalidates the affected samples and a new set of assay controls will be required prior to testing any new samples.

Internal Control

An internal control is added to each sample during the extraction process. During processing, the internal control acceptance criteria is automatically verified by the Panther Fusion System software. Detection of the internal control is not required for samples that are positive for *Salmonella*, *Campylobacter*, *Shigella*/EIEC, and/or STEC. The internal control must be detected in all samples that are negative for all of the intended analytes; samples that fail to meet that criteria will be reported as Invalid. Each sample with an Invalid result must be retested.

The Panther Fusion System is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther/Panther Fusion System Operator's Manual*.

Interpretation of Results

The Panther Fusion System automatically determines the test results for samples and controls. Results for *Salmonella*, *Campylobacter*, *Shigella*/EIEC, and STEC detection are reported separately. A test result may be negative, positive, or invalid.

The first valid result is the result that should be reported. Samples with invalid results should be retested. If the result is invalid upon retest, a new specimen should be collected.

Table 1 shows the possible results reported in a valid run with corresponding result interpretations.

Table 1: Result Interpretation

Salmonella **Result**	***Campy*** **Result**	***Shigella*/EIEC** **Result**	***Stx1/Stx2*** **Result**	**IC Result**	**Interpretation**
Neg	Neg	Neg	Neg	Valid	*Salmonella*, *Campylobacter*, *Shigella*/EIEC, and STEC not detected.
POS	Neg	Neg	Neg	Valid	*Salmonella* detected.
Neg	POS	Neg	Neg	Valid	*Campylobacter* detected.
Neg	Neg	POS	Neg	Valid	*Shigella*/EIEC detected.
Neg	Neg	Neg	POS	Valid	STEC detected.
POS	POS	Neg	Neg	Valid	*Salmonella* and *Campylobacter* detected.
POS	Neg	POS	Neg	Valid	*Salmonella* and *Shigella*/EIEC detected.
POS	Neg	Neg	POS	Valid	*Salmonella* and STEC detected.
Neg	POS	POS	Neg	Valid	*Campylobacter* and *Shigella*/EIEC detected.
Neg	POS	Neg	POS	Valid	*Campylobacter* and STEC detected.
Neg	Neg	POS	POS	Valid	*Shigella*/EIEC and STEC detected.
POS	POS	POS	Neg	Valid	*Salmonella*, *Campylobacter*, and *Shigella*/ EIEC detected. Infections with 3 bacteria are rare. Retest to confirm result.
POS	POS	Neg	POS	Valid	*Salmonella*, *Campylobacter*, and STEC detected. Infections with 3 bacteria are rare. Retest to confirm result.
POS	Neg	POS	POS	Valid	*Salmonella*, *Shigella*/EIEC, and STEC detected. Infections with 3 bacteria are rare. Retest to confirm result.
Neg	POS	POS	POS	Valid	*Campylobacter*, *Shigella*/EIEC, and STEC detected. Infections with 3 bacteria are rare. Retest to confirm result.
POS	POS	POS	POS	Valid	*Salmonella*, *Campylobacter*, *Shigella*/EIEC, and STEC detected. Infections with 4 bacteria are rare. Retest to confirm result.
Invalid	Invalid	Invalid	Invalid	Invalid	Invalid. There was an error in the generation of the result; retest specimen.

Neg = negative, POS = positive.

Note: POS result will be accompanied by cycle threshold (Ct) values. POS/HT represents a high titer result and will not have a Ct reported.

Limitations

A. Use of this assay is limited to personnel who are trained in this procedure. Failure to follow these instructions may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.

D. Dehydrated Cary-Blair medium powders and Cary-Blair media in solid configuration with high agarose content were not evaluated and may not be compatible with the assay sample processing steps.

E. The performance of this test has only been validated with human stool collected in liquid Cary-Blair transport medium, according to the media manufacturers' instructions.

F. This product should not be used to test stool samples in fixative.

Analytical Performance

Analytical Sensitivity

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 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 configurations. Analytical sensitivity is defined as the lowest concentration at which ≥95% of all replicates tested positive, as summarized in Table 2.

Table 2: 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	2180
Campylobacter coli	16	320
Campylobacter jejuni subsp. jejuni	25	500
Shigella sonnei	68	1360
EIEC O29:NM	23	460
STEC O26:H11 (*stx1/stx2*)	106	2120
STEC O157:H7 (*stx1/stx2*)	20	400

CFU = colony forming units.

[a] Analyte concentrations in Aptima Multitest tube are ~20X dilute compared to preserved stool (~150 µL preserved stool in ~3 mL STM).

Inclusivity/Reactivity - Wet Testing

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

Table 3: 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	327	6540
Salmonella enterica subsp. *enterica* (I)	13076	Enteritidis, CDC K--1891	144	2880
	14028[a]	Typhimurium, CDC 6516--60	144	2880
	15791	Sloterdijk	144	2880
	15611	Vellore, V1796	144	2880
	11646	Illinois, CDC	144	2880
	8391	Thompson, 2988	144	2880
	19430	Typhi, NCTC 8385	144	2880
	7378	Panama, Hochberg 2460	144	2880
	6962	Newport, NCTC 129	144	2880
	8388	Muenchen, 54	144	2880
	8326	Heidelberg, 16	144	2880
	9712	Saintpaul, 127	144	2880
	8387	Montevideo, 623	144	2880
	6539	Typhi, AMC	144	2880
	9150	Paratyphi A	144	2880
	10719	Paratyphi B, AMC 41-H-6	144	2880
	13428	Paratyphi C, CDC 3310-52	144	2880
	33062	Typhimurium, LJ211	144	2880
	13311	Typhimurium, NCTC 74	144	2880
	51956	Hadar, CDC 347	144	2880
	51741	DUP-103	144	2880
	10721	Javiana, ETS 146	144	2880
	9239	Oranienburg, E1093	144	2880
	51955	Virchow, CDC 41	144	2880
	51957	Agona, CDC 873	144	2880
	BAA-2739	Mississippi, CDC 2012K-0487	144	2880
	13312	Choleraesuis, NCTC 5735	144	2880
	700136	Braenderup, NCTC 5750	144	2880
	15480	Dublin, HWS 51	144	2880
	CCUG 21280	Schwarzengrund	144	2880

Table 3: 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	2880
	Univ of Calgary 2425	argC95	144	2880
	700148	NCTC 10252	144	2880
	43972	CIP 82.29	144	2880
Salmonella enterica subsp. *arizonae* (IIIa)	12323	CDC 3153--55	144	2880
	12324	CDC 1089-53	144	2880
	13314	NCTC 8297	144	2880
Salmonella enterica subsp. *diarizonae* (IIIb)	12325	CDC	144	2880
	29226	CDC 656/75	144	2880
	43973	CIP 82.31	144	2880
Salmonella enterica subsp. houtenae (IV)	29932	16:z4,z23: -	144	2880
Salmonella enterica subsp. indica (VI)	43976	CIP 102501	144	2880
	Univ of Calgary 2430	pyrE20	144	2880
Shigella dysenteriae (A)	13313	Type 1, NCTC 4837	204	4080
	49555	Type 13, CDC 8008-79	204	4080
	29028	Type 3, CDC 3596-74	204	4080
	49551	Type 12, CDC 2243-66	204	4080
	11835	AMC 43--A--1	204	4080
	9361	Type 1, AMC 43-A-14	204	4080
	12021	Type 8, CDC 2116-52	204	4080
	12037	Type 9, CDC A-58:1646	204	4080
	49547	Type 11, CDC 3883-66	204	4080
Shigella flexneri (B)	29903	Type 2a, 24570	204	4080
	12022	Type 2b, CDC 3591-52	204	4080
	9199	Type 1a, AMC 43-G-68	204	4080
	33948	612-003	204	4080
	11836	Type 3, AMC 43-G-100	204	4080
	12023	Type 4a, CDC 5380-52	204	4080
	12025	Type 6, CDC 64	204	4080
	700930	Type 2a, 2457T	204	4080

Table 3: 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	4080
	29928	Type10, C-10	204	4080
	9207	Type 1, AMC 43-G-58	204	4080
	BAA-1247	Type 20, SH-108	204	4080
	12030	Type 10, CDC 6336-52	204	4080
	12028	Type 8	204	4080
	12031	Type 11, CDC 1624-54	204	4080
	9905	Type 7, AMC 4006	204	4080
Shigella sonnei (D)	9290	AMC 43-GG9	204	4080
	29930[a]	WRAIR I virulent	204	4080
	11060	4628	204	4080
	29031	CDC 45-75	204	4080
	25931	NCDC 1120-66	204	4080
Enteroinvasive *E. coli* (EIEC)	43893	Type O124:NM, CDC EDL 1284	69	1380
	BAA-2190	Type O121, 98-3306	69	1380
	49105	Type O15, 1/1/7482	69	1380
	12806	Type O124:K72 (B17):H, CDC	69	1380
	43892[a]	Type O29:NM, CDC EDL 1282	69	1380
Campylobacter jejuni subsp. *jejuni*	33560	CIP 702	75	1500
	43432	Type O:4, MK7	75	1500
	35920	BG 22	75	1500
	43459	Type O:40, MPD570102	75	1500
	29428	VPI H840	75	1500
	33252	C3692	75	1500
	33291[a]	AS-83-79	75	1500
	700819	NCTC 11168	75	1500
	BAA-1062	RM 1221	75	1500
	BAA-1234	RM3193	75	1500
	33292	AS-84-79	75	1500
	35918	BG 177	75	1500
	43434	Type O:6, C6	75	1500
	43435	Type O:7, DPH-1	75	1500
	43449	Type O:23, MK 198	75	1500
	43503	UA466	75	1500
	43472	Type O:5, CFJ29	75	1500
	43430	Type O:2, CJC-25	75	1500

Table 3: 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[a]	CIP 7080	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)	700377	O157:NM (*stx2*), CDC 92-3099	60	1200
	700927	O157:H7:K- (*stx1/stx2*), EDL 933	60	1200
	35150[a]	O157:H7 (*stx1/stx2*), EDL 931	60	1200
	43894	O157:H7 (*stx1/stx2*), CDC EDL 932	60	1200
	700378	O157:NM (*stx1/stx2*), CDC 92-3073	60	1200
	43890	O157:H7 (*stx1*) CDC C984	60	1200
	43895	O157:H7 (*stx1/stx2*), CDC EDL 933	60	1200

Table 3: 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* (non-O157)	51435	O91:H21 (*stx2*), B2F1	318	6360
	700840	O111:H8 (*stx1/stx2*), B99BE001161	318	6360
	51434	O91:H21 (*stx2*), H414-36/89	318	6360
	BAA-181	O111:H8 (*stx1/stx2*), CDC 1999-3249	318	6360
	BAA-180	O111:H8 (*stx1*), CDC 1999-3302	318	6360
	BAA-176	O113:H21 (*stx2*), CDC 2001-3004	318	6360
	BAA-177	O113:H21 (*stx1/stx2*), CDC 2000-3159	318	6360
	BAA-182	O104:H21 (*stx2*), CDC 1994-3023	318	6360
	BAA-1653[a]	O26:H11 (*stx1/stx2*), EH1534	318	6360
	BAA-2193	O45:H2 (*stx1*), 2000-3039	318	6360
	BAA-2210	O103:H2 (*stx1*), 2003-3112	318	6360
	BAA-2211	O145:H25 (*stx2*), 2003-3375	318	6360
	BAA-2219	O121:H19 (*stx2*), 2002-3211	318	6360
	BAA-2222	O145:Nonmotile (*stx1/stx2*), 2006-3142	318	6360
	BAA-2326	O104:H4 (*stx2*), TY-2482	318	6360
	BAA-2196	O26:H11 (*stx1/stx2*), 2003--3014	318	6360
	BAA-2215	O103:H11 (*stx1*), 2006-3008	318	6360
	BAA-2213	O103:H25 (stx1), 2005-3546	318	6360
	BAA-178	O104:H21(*stx2*), CDC 1994-3024	318	6360
	BAA-184	O111:H8 (*stx1*), CDC 2000-3025	318	6360
	BAA-2217	O146 (*stx2*), 10C-3114	318	6360
	BAA-179	O111:H8 (*stx1/stx2*), CDC 1997-3215	318	6360
	BAA-2129	O145:H28 (*stx2*), TW07865	318	6360
	BAA-1652	O145:H48 (*stx2*), EH1533	318	6360
	BAA-2192	O145:Nonmotile (*stx1/stx2*), 99-3311	318	6360

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 the NCBI database and in 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 Bacterial Assay is predicted to detect 100% of 121 *Salmonella bongori*,

99.03% of 2365 *Salmonella enterica*, 96.43% of 392 *Campylobacter jejuni*, 99.09% of 1104 *Campylobacter coli*, 100% of 1080 *Shigella sonnei*, 100% of 1164 *Shigella flexneri*, 100% of 192 *Shigella dysenteriae*, 100% of 364 *Shigella boydii*, 98.71% of 387 STEC-expressing *stx1* and 97.35% of 1019 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 4 were tested in processed negative CBS matrix in the absence and in the presence of Panther Fusion 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 $TCID_{50}$/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 4: 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	*Eggerthella 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 hermannii*	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
Coxsackievirus 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

Table 4: Microorganisms Tested for Cross-Reactivity and Microbial Interference (continued)

Microorganism	Test Concentration	Microorganism	Test Concentration
Vibrio parahaemolyticus	10^6 CFU/mL	*Peptostreptococcus micros*	10^6 rRNA copies /mL
Abiotrophia defectiva	10^6 CFU/mL	*Photobacterium damselae*	10^6 CFU/mL
Acinetobacter baumannii	10^6 CFU/mL	*Plesiomonas shigelloides*	10^6 CFU/mL
Acinetobacter lwoffii	10^6 CFU/mL	*Prevotella bivia*	10^6 CFU/mL
Aeromonas hydrophila	10^6 CFU/mL	*Prevotella melaninogenica*	10^6 CFU/mL
Alcaligenes faecalis	10^6 CFU/mL	*Proteus mirabilis*	10^6 rRNA copies /mL
Campylobacter upsaliensis	10^6 CFU/mL	*Proteus penneri*	10^6 CFU/mL
Anaerococcus vaginalis	10^6 CFU/mL	*Proteus vulgaris*	10^6 CFU/mL
Arcobacter butzleri	10^6 CFU/mL	*Providencia alcalifaciens*	10^6 CFU/mL
Bacillus cereus	10^6 CFU/mL	*Providencia rettgeri*	10^6 CFU/mL
Bacteroides 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	*Megasphaera elsdenii*	10^6 CFU/mL
Clostridium tertium[b]	10^4 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 10^6 CFU/mL and 100% positivity was recovered for *Campylobacter* at ≤ 10^4 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 10^6 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 5 shows a summary of results observed in the competitive interference testing.

Table 5: 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, Conc = concentration, 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 6.

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

Table 6: 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
Antimicrobial and antifungal	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

Table 6: Substances Tested for Interference (continued)

Substance Type	Generic Name	Active Ingredient(s)	Test Concentration[a,b,c]
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
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[d]	Purified mucin protein	0.05% w/v

[a] Substance concentration in Aptima Multitest tube.
[b] v/v: volume by volume.
[c] w/v: weight by volume.
[d] Interference was observed in higher concentrations of Mucin.

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 7. 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 7: 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 9714 X LoD) were tested across 5 runs on 2 Panther Fusion Systems. 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 8, 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 8: 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	0.12	0.33	0.00	0.00	0.07	0.19	0.18	0.50	0.22	0.61	0.51	1.41	0.60	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	0.20	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	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	0.40	0.24	0.70	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	0.30	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 for *Salmonella, Campylobacter, Shigella,* and STEC (Table 9).

Table 9: Agreement of Panther Fusion GI Bacterial Assay Results with Expected Results

		Agreement with Expected Results	
Description	**Analyte**	**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 10). For the sources of variation except the 'within-run' 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 11).

Table 10: 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 11: 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.

Clinical Performance

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 GI Bacterial 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 specimens (1521 prospective and 219 retrospective specimens) is provided in Table 12.

Table 12: 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 13 through Table 16.

Table 13: 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 14: 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)	98.0 (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 15: 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 16: 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 were 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 17. Nine (9) coinfections were also detected by the comparator NAAT.

Table 17: 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

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