DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993-0002

July 12, 2017

GREAT BASIN SCIENTIFIC, INC.
SUZETTE CHANCE
SENIOR DIRECTOR OF CLINICAL AFFAIRS
2441 S. 3850 WEST
SALT LAKE CITY UT 84120

Re: K163571
Trade/Device Name: Great Basin Stool Bacterial Pathogens Panel
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay
Regulatory Class: II
Product Code: PCI, PCH
Dated: December 16, 2016
Received: December 19, 2016

Dear Dr. Chance:

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 (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. 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.

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 Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Steven R. Gitterman -S for

Uwe Scherf, M.S., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
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: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K163571

Device Name
Great Basin Stool Bacterial Pathogens Panel

Indications for Use *(Describe)*

The Great Basin Stool Bacterial Pathogens Panel is a multiplexed, qualitative test for the detection and identification of DNA targets of enteric bacterial pathogens. The Stool Bacterial Pathogens Panel detects nucleic acids from:

- *Campylobacter (C. coli/C. jejuni)*
- *Salmonella*
- Shiga toxin 1 *(stx1)*
- Shiga toxin 2 *(stx2)*
- *Escherichia coli* serotype O157
- *Shigella*

Shiga toxin genes are found in Shiga toxin-producing strains of *E. coli* (STEC/EHEC/VTEC) and *Shigella dysenteriae*. The *E. coli* O157 test result is only reported if a Shiga toxin gene (*stx1* and/or *stx2*) is also detected.

The Stool Bacterial Pathogens Panel is performed directly from Cary Blair or C&S Medium preserved stool specimens from symptomatic patients with suspected acute gastroenteritis, enteritis, or colitis and is performed on the Portrait™ Analyzer.

The test is intended for use as an aid in the diagnosis of specific agents of gastrointestinal illness in conjunction with clinical and epidemiological information; however, it is not to be used to monitor these infections. Positive results do not rule out co-infection with other organisms and may not be the definitive cause of patient illness. Negative test 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. Concomitant culture is necessary if organism recovery or further typing of bacterial agents is desired.

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.

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5.0 510(k) Summary – Stool Bacterial Pathogens Panel

A. Submitted by:

Great Basin Corporation
2441 South 3850 West
Salt Lake City, Utah 84120

Contact Information

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Senior Director of Clinical Affairs
Great Basin Scientific
2441 S. 3850 West
Salt Lake City, Utah 84120
Phone: 385-215-3369
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B. Name of Device

Proprietary Name: Great Basin Stool Bacterial Pathogens Panel
Common or Usual Names: Stool Bacteria Pathogens Panel
SBPP
GI Panel

C. Regulatory Information:

a.	Regulation Section:	21 CFR 866.3990, Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay 21 CFR 862.2570 – Instrumentation for clinical multiplex test systems
b.	Classification:	Class II (Stool Bacterial Pathogen Panel; non-exempt); Class II (PA500 Portrait Analyzer System)
c.	Classification panel: Product Code:	Microbiology Devices, OIVD (83) Microbiology PCI Gastrointestinal Bacterial Panel Multiplex Nucleic Acid-Based Assay System PCH Gastrointestinal Pathogen Panel Multiplex Nucleic Acid-Based Assay System OOI Real-Time Nucleic Amplification System

D. Intended use(s)/Indications for Use:

The Great Basin Stool Bacterial Pathogens Panel is a multiplexed, qualitative test for the detection and identification of DNA targets of enteric bacterial pathogens. The Stool Bacterial Pathogens Panel detects nucleic acids from:

- *Campylobacter (C. coli/C. jejuni)*
- *Salmonella*
- Shiga toxin 1 *(stx1)*
- Shiga toxin 2 *(stx2)*
- *Escherichia coli* serotype O157
- *Shigella*

Shiga toxin genes are found in Shiga toxin-producing strains of *E. coli* (STEC/EHEC/VTEC) and *Shigella dysenteriae*. The *E. coli* O157 test result is only reported if a Shiga toxin gene (*stx1* and/or *stx2*) is also detected.

The Stool Bacterial Pathogens Panel is performed directly from Cary Blair or C&S Medium preserved stool specimens from symptomatic patients with suspected acute gastroenteritis, enteritis, or colitis and is performed on the Portrait™ Analyzer.

The test is intended for use as an aid in the diagnosis of specific agents of gastrointestinal illness in conjunction with clinical and epidemiological information; however, it is not to be used to monitor these infections. Positive results do not rule out co-infection with other organisms and may not be the definitive cause of patient illness. Negative test 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. Concomitant culture is necessary if organism recovery or further typing of bacterial agents is desired.

Device Description

Test Principle:

The Great Basin Stool Bacterial Pathogens Panel on the PA500 Portrait™ System utilizes automated, hot-start PCR amplification technology to amplify specific nucleic acid sequences that are then detected using hybridization probes immobilized on a modified silicon chip surface, in a single-use, self-contained test cartridge.

An aliquot of the specimen (stool preserved in stool transport media) is first processed using the Sample Preparation Device (SPD). An aliquot of the eluate obtained from the SPD is loaded into the sample port of the SBPP Test Cartridge.

Genomic DNA is extracted from microbial cells and diluted to reduce potential inhibitors of the PCR. During the PCR process, biotin-labeled primers direct the amplification of specific nucleic acid sequences within a conserved region for identification of: a bacterial sample processing control (SPC), *Campylobacter coli/Campylobacter jejuni*, *Salmonella* spp.*, Shigella* spp., Shiga toxin 1, Shiga toxin 2, and *E. coli* serotype O157.

Following PCR, biotin-labeled, amplified target DNA sequences are hybridized to sequence specific probes immobilized on the silicon chip surface, and incubated with anti-biotin antibody conjugated to the horseradish peroxidase enzyme (HRP). The unbound conjugate is washed away, and tetramethylbenzidine (TMB) is added to produce a colored precipitate at the location of the probe/target sequence complex. The resulting signal is detected by the automated Portrait™ Optical Reader within the PA500 Portrait™ Analyzer System. The SPC undergoes the same extraction, amplification, and detection steps as the sample in order to monitor for inhibitory substances, as well as process inefficiency due to instrument or reagent failure. No operator intervention is required once the sample is loaded into the sample port, and the Stool Bacterial Pathogens Panel cartridge is loaded into the Portrait™ Analyzer.

Test Device:

The PA500 Portrait™ Analyzer System is a fully automated system that includes: the Portrait™ Analyzer, single-use Stool Bacterial Pathogen Panel Cartridges, and the Portrait™ Data Analysis Software Program. The Portrait™ System is designed to perform automated sample preparation, PCR, and optical chip-based detection with integrated data analysis in less than two hours. The Portrait System was granted 510(k) clearance for the Portrait Toxigenic *C. difficile* Assay (K113358), Portrait GBS Assay (K143312), Staph ID/R Blood Culture Panel (K152470) and the Shiga Toxin Direct Test (K152955).

E. Substantial Equivalence Information:

Predicate Device: Nanosphere Verigene® Enteric Pathogens Nucleic Acid Test (K140083)

The following table provides a comparison of the Stool Bacterial Pathogens Panel and the predicate device:

Features/Characteristics	Stool Bacterial Pathogens Panel (SBPP)	Predicate Device Verigene® EP (K140083)
Manufacturer	Great Basin Scientific, Inc.	Nanosphere
Trade Name	Great Basin Stool Bacterial Pathogen Panel	Verigene Enteric Pathogen Nucleic Acid Test
510(k) Number		K140083
Classification	II	II
Qualitative/Quantitative	Qualitative	Qualitative
Intended Use/Indications for Use	The Great Basin Stool Bacterial Pathogens Panel is a multiplexed, qualitative test for the detection and identification of DNA targets of enteric bacterial pathogens. The Stool Bacterial Pathogens Panel detects nucleic acids from: • *Campylobacter (C. coli* and *C. jejuni)* • *Salmonella* • Shiga toxin 1 *(stx1)* • Shiga toxin 2 *(stx2)* • *Escherichia coli* serotype O157 • *Shigella* Shiga toxin genes are found in Shiga toxin-producing strains of *E. coli* (STEC/EHEC/VTEC) and *Shigella dysenteriae*. The *E. coli* O157 test result is only reported if a Shiga toxin gene (*stx1* and/or *stx2*) is also detected. The Stool Bacterial Pathogens Panel is performed directly from Cary Blair or C&S Medium preserved stool specimens from symptomatic patients with suspected acute gastroenteritis, enteritis, or colitis and is performed on the Portrait™ Analyzer. The test is intended for use as an aid in the diagnosis of specific agents of gastrointestinal illness in conjunction with clinical and epidemiological information. Positive results do not rule out co-infection with other organisms and may not be the definitive cause of patient illness. Negative test 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. Concomitant culture is necessary if organism recovery or further typing of bacterial agents is desired.	The Verigene® Enteric Pathogens Nucleic Acid Test (**EP**) is a multiplexed, qualitative test for simultaneous detection and identification of common pathogenic enteric bacteria, viruses, and genetic virulence markers from liquid or soft stool preserved in Cary-Blair medium, collected from individuals with signs and symptoms of gastrointestinal infection. The test is performed on the automated Nanosphere Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific gastrointestinal microbial nucleic acid gene sequences associated with the following pathogenic bacteria and viruses: • *Campylobacter* Group (composed of *C. coli*, *C. jejuni*, and *C. lari*) • *Salmonella* species • *Shigella* species (including *S. dysenteriae*, *S. boydii*, *S. sonnei*, and *S. flexneri*) • *Vibrio* Group (composed of *V. cholerae* and *V. parahaemolyticus*) • *Yersinia enterocolitica* • Norovirus GI/GII • Rotavirus A In addition, **EP** detects the Shiga toxin 1 gene and Shiga toxin 2 gene virulence markers. Shiga toxin producing *E. coli* (STEC) typically harbor one or both genes that encode for Shiga toxins 1 and 2. **EP** is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness, in conjunction with other clinical, laboratory, and epidemiological information; however, is not to be used to monitor these infections. **EP** also aids in the detection and identification of acute

		gastroenteritis in the context of outbreaks. Due to the limited number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Yersinia enterocolitica*, *Vibrio* Group and *Shigella* species were primarily established with contrived specimens. Concomitant culture is necessary for organism recovery and further typing of bacterial agents. **EP** results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed 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 **EP** results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or noninfectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.
Specimen Type	Human Stool sample preserved in Cary Blair or C&S Preservation and Transport Media	Human Stool sample preserved in Cary-Blair Medium
Sample Lysis and DNA Extraction	Automated sample lysis and DNA extraction in a self-contained cartridge	Same
Amplification Technology	Multiplex polymerase chain reaction (PCR)	Reserve transcription (RT) polymerase change reaction (PCR)
Detection Technology	Colorimetric target specific hybridization to probe on a chip surface, optical reader, automated software with built-in result interpretation.	Gold/Silver nanoparticle probe detection of bacterial-specific DNA on complementary oligo-microarray. Optical light scatter detection of gold-silver aggregates
Controls	One internal processing control (whole organism) – complete assay control	Two internal processing controls (hybridization control and extraction/assay control)
Instrument	PA500 Portrait™ Analyzer	Verigene Reader and Processor SP
Time to Result	<2 hours	~2 hours

The following summarizes the differences and similarities between the Stool Bacterial Pathogens Panel and the predicate device:

The Stool Bacterial Pathogens Panel (SBPP) is similar to the Verigene® Enteric Pathogens (EP) Nucleic Acid Test in the following ways:

- The SBPP and the EP have similar Intended Uses and both detect the following analytes: *Campylobacter coli, jejuni, Salmonella* spp., *Shigella* spp., Shiga toxin 1 and Shiga toxin 2
- The SBPP and the EP use the same sample type (preserved human stool)
- The SBPP and the EP are both qualitative, automated, multiplex nucleic acid based tests

The Stool Bacterial Pathogens Panel differs from the Verigene® Enteric Pathogens Nucleic Acid Test in the following:

- The EP detects additional analytes not detected in the SBPP (*Campylobater lari, Vibrio* Group, *Yersinia enterocolitica*, Norovirus GI/GII, Rotovirus A)
- The SBPP detects *E. coli* Serotype O157 whereas EP does not

F. Performance Summary – Analytical Studies

a. Analytical Sensitivity

The limit of detection (LoD) of the SBPP was assessed by testing 10 bacterial strains which included all the target organisms detected in the SBPP. Preparation of *E. coli*, *Salmonella* and *Shigella* included overnight incubations of each bacterial stock in Tryptic Soy Broth. The broths were serially diluted into a pool of preserved negative clinical stool and plated onto Tryptic Soy Agar plates to determine the cell concentrations.

Preparation of Campylobacter spp. included incubation in Campy-Thioglycollate Broth (using cells obtained from a Campy CVA agar plate). The broths were serially diluted into a pool of preserved negative clinical stool and plated onto blood agar plates to determine the cell concentrations.

The LoDs for each strain tested is shown in Table 1.

Table 1. SBPP Limit of Detection (LoD)

Strain	ATCC ID	SBPP Target Gene Present	LoD (CFU/mL)
Campylobacter coli	43486	*cadF*	1.8×10^3
Campylobacter jejuni	49943	*cadF*	1.3×10^3
Escherichia coli	BAA-2215	*stx1*	5.7×10^3
Escherichia coli	51435	*stx2*	4.4×10^3
Escherichia coli	BAA-2196	*stx1, stx2*	1.1×10^4
Escherichia coli	43895	*stx1, stx2,* O157	1.6×10^4
Salmonella bongori	43975	*invA*	2.5×10^3
Salmonella enterica	13311	*invA*	1.9×10^4
Shigella flexneri	25929	*ipaH*	5.2×10^3
Shigella sonnei	29930	*ipaH*	1.4×10^4

b. Analytical Reactivity (Inclusivity)

The analytical reactivity of the SBPP was assessed by testing an additional 91 well characterized bacterial strains representing the organisms detected in the SBPP. The strains were obtained from the ATCC and contained the following:

- 5 *Campylobacter coli*,
- 6 *Campylobacter jejuni*,
- 16 *Escherichia coli* (*stx1*+ and/or *stx2*+)
- 8 *Escherichia coli* (*stx1*+ and/or *stx2*+, with serotype O157+)
- 3 *Shigella dysenteriae* serotype 1 (*stx1*+) strains
- 33 *Salmonella* spp.
- 20 *Shigella* spp.

Organisms were grown as described in the LoD studies and were diluted to a concentration of 2X LoD into pooled negative preserved clinical stool matrix. At least 3 replicates per strain were tested. The results for are shown in Tables 2 through 5.

Table 2. Analytical Reactivity *Campylobacter* spp.

***Campylobacter* spp.**	**ATCC ID**	**Concentration 2X LoD (CFU/mL)**	**Correct SBPP Results**
Campylobacter coli	33559	3.6×10^3	9/9
Campylobacter coli	43135	3.6×10^3	3/3
Campylobacter coli	43486	3.6×10^3	6/6
Campylobacter coli	49941	3.6×10^3	3/3
Campylobacter coli	51729	3.6×10^3	3/3
Campylobacter jejuni subsp. jejuni	29428	2.6×10^3	3/3
Campylobacter jejuni subsp. jejuni	33560	2.6×10^3	3/3
Campylobacter jejuni subsp. jejuni	43434	2.6×10^3	3/3
Campylobacter jejuni subsp. doylei	49349	2.6×10^3	6/6
Campylobacter jejuni	49943	2.6×10^3	3/3
Campylobacter jejuni subsp. jejuni	33292	2.6×10^3	3/3
n=11			

Table 3. Analytical Reactivity *Salmonella* spp.

***Salmonella* spp.**	**ATCC ID**	**Concentration 2X LoD (CFU/mL)**	**Correct SBPP Results**
Salmonella enterica subsp. enterica Typhi	6539	3.8×10^4	3/3
Salmonella enterica subsp. enterica Newport	6962	3.8×10^4	3/3
Salmonella enterica subsp. enterica Choleraesuis	7001	3.8×10^4	3/3
Salmonella enterica subsp. enterica Stanley	7308	3.8×10^4	3/3
Salmonella enterica subsp. enterica Heidelberg	8326	3.8×10^4	6/6
Salmonella enterica subsp. enterica Muenchen	8388	3.8×10^4	6/6
Salmonella enterica subsp. enterica Paratyphi B	8759	3.8×10^4	6/6
Salmonella enterica subsp. enterica Bareilly	9115	3.8×10^4	6/6*
Salmonella enterica subsp. enterica Kentucky	9263	3.8×10^4	3/3
Salmonella enterica subsp. enterica Saint Paul	9712	3.8×10^4	3/3*
Salmonella enterica subsp. enterica Tennessee	10722	3.8×10^4	6/6
Salmonella enterica subsp. enterica Paratyphi A	12176	3.8×10^4	3/3
Salmonella enterica subsp. enterica Typhimurium	13311	3.8×10^4	3/3
Salmonella enterica subsp. enterica Choleraesuis	13312	3.8×10^4	6/6*
Salmonella enterica subsp. enterica arizonae	13314	3.8×10^4	3/3
Salmonella enterica subsp. enterica Typhimurium	14028	3.8×10^4	3/3
Salmonella enterica subsp. enterica Dublin	15480	3.8×10^4	3/3
Salmonella enterica subsp. enterica houtenae	15788	3.8×10^4	3/3
Salmonella enterica subsp. enterica Newport	27869	3.8×10^4	3/3
Salmonella enterica subsp. enterica diarizonae	29226	3.8×10^4	6/6
Salmonella enterica subsp. enterica Newington	29628	3.8×10^4	3/3
Salmonella enterica subsp. salamae	43972	3.8×10^4	6/6*
Salmonella enterica subsp. diarizonae	43973	3.8×10^4	3/3
Salmonella enterica subsp. houtenae	43974	3.8×10^4	6/6
Salmonella bongori	43975	3.8×10^4	3/3
Salmonella enterica subsp. indica	43976	3.8×10^4	3/3
Salmonella enterica subsp. enterica Virchow	51955	3.8×10^4	5/6
Salmonella enterica subsp. enterica Agona	51957	3.8×10^4	3/3
Salmonella enterica subsp. enterica Bristol	700138	3.8×10^4	3/3
Salmonella enterica subsp. enterica Montevideo	BAA-710	3.8×10^4	3/3
Salmonella enterica subsp. salamae	BAA-1576	3.8×10^4	3/3
Salmonella enterica subsp. enterica Infantis	BAA-1675	3.8×10^4	3/3
Salmonella enterica subsp. enterica Mississippi	BAA-2739	3.8×10^4	3/3
n=33			
* This set of test runs also contained 1 "Invalid" Run			

Table 4. Analytical Reactivity: Shiga toxin 1, Shiga toxin 2 and *E. coli* Serotype O157

ATCC ID	Serotype	Shiga Toxin Gene(s) Present	Expected Result	Concentration 2X LoD (CFU/mL)	Correct SBPP Results
Shiga-toxin producing *E. coli*					
700840	O111:H8	*stx1+/stx2+*	Shiga Toxin 1 DETECTED Shiga Toxin 2 DETECTED	2.2×10^4	3/3
BAA-2196	O26:H11	*stx1+/stx2+*		2.2×10^4	3/3*
BAA-2221	O21:H19	*stx1+/stx2+*		2.2×10^4	3/3
BAA-2440	O111	*stx1+/stx2+*		2.2×10^4	3/3
BAA-2181	O26:H11	*stx1+*	Shiga Toxin 1 DETECTED	1.4×10^4	3/3
BAA-2191	O45:H2	*stx1+*		1.4×10^4	3/3
BAA-2193	O45:H2	*stx1+*		1.4×10^4	3/3
BAA-2199	O123:H25	*stx1+*		1.4×10^4	3/3
BAA-2210	O103:H2	*stx1+*		1.4×10^4	3/3
BAA-2215	O103:H11	*stx1+*		1.4×10^4	3/3
51435	O91:H21	*stx2+*	Shiga Toxin 2 DETECTED	8.8×10^3	3/3
BAA-183	0113:H21	*stx2+*		8.8×10^3	3/3
BAA-2129	O145:H28	*stx2+*		8.8×10^3	3/3
BAA-2211	O145:H25	*stx2+*		8.8×10^3	3/3
BAA-2219	O121:H19	*stx2+*		8.8×10^3	3/3
BAA-2326	O104:H4	*stx2+*		8.8×10^3	5/6
35150	O157:H7	*stx1+/stx2+*	Shiga Toxin 1 DETECTED Shiga Toxin 2 DETECTED Serotype O157 DETECTED	3.2×10^4	3/3
43894	O157:H7	*stx1+/stx2+*		3.2×10^4	3/3
700378	O157:NM	*stx1+/stx2+*		3.2×10^4	5/5*
700927	O157:H7:K	*stx1+/stx2+*		3.2×10^4	3/3
43890	O157:H7	*stx1+*	Shiga Toxin 1 DETECTED Serotype O157 DETECTED	3.2×10^4	3/3
700376	O157:NM	*stx1+*		3.2×10^4	8/9
43889	O157:H7	*stx2+*	Shiga Toxin 2 DETECTED Serotype O157 DETECTED	3.2×10^4	3/3
700377	O157:NM	*stx2+*		3.2×10^4	3/3
n=24					
Shiga-toxin producing *Shigella dysenteriae*					
9361	Type 1	*stx1+*	Shiga Toxin 1 DETECTED	1.4×10^4	3/3
27345	Type 1	*stx1+*		1.4×10^4	6/7
27346	Type 1	*stx1+*		1.4×10^4	4/6
n=3					
* This set of test runs also contained 1 "Invalid" Run					

Table 5. Analytical Reactivity: *Shigella*

***Shigella* spp.**	**ATCC ID**	**Concentration 2X LoD (CFU/mL)**	**Correct SBPP Results**
Shigella boydii Serotype 2	8700	2.8×10^4	3/3
Shigella boydii Serotype 3	8702	2.8×10^4	3/3
Shigella boydii Serotype 1	9207	2.8×10^4	3/3
Shigella boydii Serotype 8	12028	2.8×10^4	3/3
Shigella boydii	29928	2.8×10^4	3/3
Shigella flexneri Serotype 5	9204	2.8×10^4	3/3
Shigella flexneri Serotype 2b	12022	2.8×10^4	3/3
Shigella flexneri Serotype 6	12025	2.8×10^4	3/3
Shigella flexneri Serotype 1a	25929	2.8×10^4	3/3
Shigella flexneri Serotype 2a	29903	2.8×10^4	3/3
Shigella sonnei	9290	2.8×10^4	3/3
Shigella sonnei	11060	2.8×10^4	3/3
Shigella sonnei	25931	2.8×10^4	3/3
Shigella sonnei	29029	2.8×10^4	3/3
Shigella sonnei	29930	2.8×10^4	3/3
Shigella dysenteriae Serotype 1	27345	2.8×10^4	6/6
Shigella dysenteriae Serotype 2	29027	2.8×10^4	3/3
Shigella dysenteriae Serotype 3	29028	2.8×10^4	5/5*
Shigella dysenteriae Serotype 12	49551	2.8×10^4	3/3
Shigella dysenteriae Serotype 13	49555	2.8×10^4	3/3
n= 20			
* This set of test runs also contained 1 "Invalid" Run			

Conclusion: The SBPP correctly identified all 91 organisms tested in the Inclusivity Study indicating that the SBPP can detect additional strains of *Campylobacter coli*, *Campylobacter jejuni, Shigella, Salmonella* and Shiga toxin producing *Escherichia coli*.

c. Analytical Specificity (Exclusivity)

The potential for cross-reactivity was evaluated in an Exclusivity Study, by testing non-target organisms commonly found in stool, in the SBPP. The study included 100 organisms phylogenetically related to targeted organisms as well as other bacteria, fungi/yeast, parasites, viruses, and human genomic DNA (84 bacterial strains, 3 yeast, 3 parasites, 9 viruses and human genomic DNA). For those isolates that were classified as Biosafety level III, or unable to be cultured via standard clinical microbiology techniques, genomic DNA was tested in place of whole organism.

Each non-target organism or nucleic acid was prepared in pooled, preserved, negative, clinical stool matrix. All bacterial and yeast strains were tested at concentrations ≥ 1.0×10^6 CFU/mL. Genomic DNA templates, viral strains, and parasites, were tested at ≥1 ug/mL, ≥1×10^6 copies/mL, or ≥1×10^5 $TCID_{50}$/mL, respectively. A minimum of 3 replicates were tested for each organism evaluated for cross-reactivity.

In addition, *in silico* analysis was performed on the SBPP primers and probes against the six (6) published complete Norovirus genomes in the NCBI data base (https://www.ncbi.nlm.nih.gov /assembly/?term=norovirus). Based on low % Match Scores, low sequence similarities, and sequence alignments, it is highly unlikely that any of the Noroviruses would be amplified or detected by the SBPP primer-probe set.

The results of the study, including the specific concentrations at which each organism was evaluated are provided in Table 6.

Table 6. Analytical Specificity (Exclusivity) Study Results

Species	Strain ID	Input Tested	SBPP NEGATIVE Result
Bacteria			
Abiotrophia defectiva	ATCC 49176	≥ 1 x 10^6 CFU/mL#	3/3
Acinetobacter baumannii	ATCC19606	9.6 x 10^7 CFU/mL	3/3
Aeromonas hydrophila	ATCC 35654	8.7 x 10^8 CFU/mL	3/3
Anaerococcus tetradius	ATCC 35098	≥ 1 x 10^6 CFU/mL#	3/3
Bacillus cereus	ATCC 14579	1 x 10^8 CFU/mL	3/3
Bacteriodes fragilis	ATCC 23745	≥ 1 x 10^6 CFU/mL#	3/3*
Bacteriodes vulgatus	ATCC 8482	≥ 1 x 10^6 CFU/mL#	3/3
Bifidobacterium adolescentis	ATCC 15703	≥ 1 x 10^6 CFU/mL#	3/3
Bifidobacterium bifidum	ATCC 11863	≥ 1 x 10^6 CFU/mL#	3/3
Bifidobacterium longum	ATCC 15707	≥ 1 x 10^6 CFU/mL#	3/3
Campylobacter curvus (gDNA)	ATCC BAA-1459D-5	≥ 1 µg/mL	6/6
Campylobacter fetus fetus	ATCC 27374	2.65 x 10^7 CFU/mL	5/5*
Campylobacter fetus venerealis	ATCC 33561	≥ 1 x 10^6 CFU/mL#	3/3
Campylobacter hyointestinalis	ATCC 35217	≥ 1 x 10^6 CFU/mL#	3/3
Campylobacter lari	ATCC 35222	9.0 x 10^6 CFU/mL	3/3
Campylobacter lari	ATCC 35223	≥ 1 x 10^6 CFU/mL#	3/3
Campylobacter lari	ATCC 35221	≥ 1 x 10^6 CFU/mL#	3/3
Campylobacter lari	ATCC 43675	1.75 x 10^7 CFU/mL	3/3
Campylobacter lari	ATCC BAA-1060	5.95 x 10^7 CFU/mL	3/3
Campylobacter upsaliensis	ATCC 49816	≥ 1 x 10^6 CFU/mL#	3/3
Citrobacter amalonaticus	ATCC 25406	≥ 1 x 10^6 CFU/mL#	3/3
Citrobacter freundii	ATCC 8090	8.47 x 10^7 CFU/mL	3/3
Clostridium difficle	ATCC 43594	≥ 1 x 10^6 CFU/mL#	3/3
Clostridium histolyticum	ATCC 19401	≥ 1 x 10^6 CFU/mL#	3/3
Clostridium perfringens	ATCC 13124	≥ 1 x 10^6 CFU/mL#	3/3
Clostridium sordellii	ATCC 9714	≥ 1 x 10^6 CFU/mL#	3/3
Enterobacter aerogenes	ATCC 15038	8.43 x 10^7 CFU/mL	3/3
Enterobacter cloacae	ATCC 13047	8.33 x 10^7 CFU/mL	3/3
Enterococcus cecorum	ATCC 43918	≥ 1 x 10^6 CFU/mL#	3/3
Enterococcus faecalis	ATCC 29212	4.8 x 10^7 CFU/mL	3/3
Enterococcus faecium	ATCC 19434	5.85 x 10^7 CFU/mL	3/3
EAEC Escherichia coli	ATCC 29552	8.53 x 10^7 CFU/mL	3/3
Escherichia coli	ATCC 23544	≥ 1 x 10^6 CFU/mL#	3/3
EIEC Escherichia coli	ATCC 43892	2.8 x 10^4 CFU/mL	0/3
EIEC Escherichia coli	ATCC 43893	2.8 x 10^4 CFU/mL	0/3
EIEC Escherichia coli	ATCC 12806	2.8 x 10^4 CFU/mL	0/3
ETEC Escherichia coli	ATCC 31703	3.37 x 10^7 CFU/mL	3/3
Escherichia fergusonii	ATCC 35469	2.47 x 10^7 CFU/mL	3/3
Escherichia hermannii	ATCC 33650	5.57 x 10^7 CFU/mL	3/3
Fusobacterium varium	ATCC 27725	≥ 1 x 10^6 CFU/mL#	3/3
Gardnerella vaginalis	ATCC 14018	3.53 x 10^7 CFU/mL	3/3
Helicobacter fennelliae	ATCC 35683	≥ 1 x 10^6 CFU/mL#	3/3
Helicobacter pylori	ATCC 49503	≥ 1 x 10^6 CFU/mL#	3/3
Klebsiella pneurmoniae	ATCC 13883	5.7 x 10^7 CFU/mL	3/3
Klebsiella oxytoca	ATCC 49131	7.8 x 10^7 CFU/mL	3/3
Lactobacillus acidophilus	ATCC 4356	≥ 1 x 10^6 CFU/mL#	3/3
Lactobacillus casei	ATCC 393	≥ 1 x 10^6 CFU/mL#	3/3
Leminorella grimonti	ATCC 43007	≥ 1 x 10^6 CFU/mL#	3/3
Listeria grayi	ATCC 19120	≥ 1 x 10^6 CFU/mL#	3/3
Listeria innocua	ATCC 33090	6.6 x 10^7 CFU/mL	3/3
Listeria monocytogenes	ATCC 15313	≥ 1 x 10^6 CFU/mL#	3/3
Morganella morganii	ATCC 25829	1.72 x 10^8 CFU/mL	3/3
Peptostreptococcus anaerobius	ATCC 27337	≥ 1 x 10^6 CFU/mL#	3/3
Plesiomonas shigelloides	ATCC 51903	≥ 1 x 10^6 CFU/mL#	3/3
Porphyromonas asaccharolytica	ATCC 27908	≥ 1 x 10^6 CFU/mL#	3/3
Prevotella melaninogenicus	ATCC 25845	≥ 1 x 10^6 CFU/mL#	3/3
Proteus mirabilis	ATCC 25933	≥ 1 x 10^6 CFU/mL#	3/3

Species	Strain ID	Input Tested	SBPP NEGATIVE Result
Proteus penneri	ATCC 33519	≥ 1 x 10^6 CFU/mL[#]	3/3
Proteus vulgaris	ATCC 6896	≥ 1 x 10^6 CFU/mL[#]	3/3
Providencia alcalifaciens	ATCC 9886	≥ 1 x 10^6 CFU/mL[#]	3/3
Providencia rettgeri	ATCC 9250	≥ 1 x 10^6 CFU/mL[#]	3/3
Providencia stuartii	ATCC 49762	≥ 1 x 10^6 CFU/mL[#]	3/3
Pseudomonas aeruginosa	ATCC 10145	≥ 1 x 10^6 CFU/mL[#]	3/3
Pseudomonas putida	ATCC 49128	≥ 1 x 10^6 CFU/mL[#]	3/3
Ruminococcus bromii	ATCC 27255	≥ 1 x 10^6 CFU/mL[#]	3/3
Serratia liquefaciens	ATCC 27592	7.17 x 10^7CFU/mL	3/3
Serratia marcescens	ATCC 13880	4.27 x 10^7 CFU/mL	3/3
Staphylococcus aureus	ATCC 6538	3.37 x 10^7 CFU/mL	6/7[a]
Staphylococcus epidermidis	ATCC 12228	3.7 x 10^7 CFU/mL	3/3
Stenotrophomonas maltophilia	ATCC 13637	≥ 1 x 10^6 CFU/mL[#]	3/3
Streptococcus agalactiae	ATCC 13813	4.43 x 10^7 CFU/mL	5/5
Streptococcus dysgalactiae	ATCC 43078	≥ 1 x 10^6 CFU/mL[#]	3/3
Streptococcus intermedius	ATCC 27335	≥ 1 x 10^6 CFU/mL[#]	3/3
Streptococcus pyogenes	ATCC 4543	4.4 x 10^7 CFU/mL	5/6[b]
Streptococcus uberis	ATCC 9927	≥ 1 x 10^6 CFU/mL[#]	3/3
Trabulsiella guamensis	ATCC 49492	≥ 1 x 10^6 CFU/mL[#]	3/3
Veillonella parvula	ATCC 10790	≥ 1 x 10^6 CFU/mL[#]	3/3
Vibrio cholera	ATCC 55188	≥ 1 x 10^6 CFU/mL[#]	3/3
Vibrio parahaemolyticus	ATCC 17802	6.2 x 10^7 CFU/mL	3/3
Vibrio vulnificus	ATCC 27562	1.48 x 10^8 CFU/mL	3/3
Yersinia bercovieri	ATCC 43970	2.57 x 10^8 CFU/mL	3/3
Yersinia enterocolitica	ATCC 49397	1.81 x 10^8 CFU/mL	3/3
Yersinia pseudotuberculosis	ATCC 23207	4 x 10^7 CFU/mL	3/3
Yersinia rohdei	ATCC 43380	1.92 x 10^7 CFU/mL	3/3
Fungi			
Candida albicans	ATCC 18804	≥ 1 x 10^6 CFU/mL[#]	3/3
Candida catenulata	ATCC 10565	≥ 1 x 10^6 CFU/mL[#]	3/3
Saccharomyces boulardii	ATCC MYA-796	≥ 1 x 10^6 CFU/mL[#]	3/3
Viruses and Parasites			
Adenovirus Type 2 (gDNA)	ATCC VR-846D	≥ 1 µg/mL	3/3
Adenovirus type 40, strain Dugan (gDNA)	ATCC VR-931D	≥ 1 µg/mL	3/3
Adenovirus type 41, strain Tak (gDNA)	ATCC VR-930D	≥ 1 µg/mL	3/3
Coxsackie B4	ATCC VR-184	1 x 10^5 $TCID_{50}$/mL	3/3
Cryptosporidium parvum (gDNA)	ATCC PRA-67D	≥ 1 µg/mL	3/3
Entamoeba histolytica (gDNA)	ATCC 30459DQ	1 x 10^6 copies/mL	3/3
Enterovirus (RNA)	ATCC VR-1775DQ	1 x 10^5 $TCID_{50}$/mL	5/5*
Giardia intestinalis (gDNA)	ATCC 50803D	≥ 1 µg/mL	3/3
Norovirus GI (synthetic RNA)	ATCC VR-3234SD	1 x 10^6 copies/mL	3/3
Norovirus GII (synthetic RNA)	ATCC VR-3235SD	1 x 10^6 copies/mL	3/3
Rotavirus	ATCC VR-1546	1 x 10^5 $TCID_{50}$/mL	3/3
Rotavirus A (RNA)	ATCC VR-2018DQ	1 x 10^6 copies/mL	3/3
Human genomic DNA	Roche 11691112001	≥ 1 µg/mL[g]	3/3

[#] Concentration estimated based on OD_{600}

*This set of test runs also contained 1 'INVALID' run

[a] '*Salmonella* DETECTED' for 1/4 replicates. An additional 3 replicates were run and the expected NEGATIVE result obtained for all replicates.

[b] One out of 3 replicates gave a '*Salmonella* DETECTED' result. An additional 3 replicates were run, and the expected NEGATIVE result obtained for all replicates.

Conclusion: All of the organisms shown in Table 6 gave the expected "Not Detected" result indicating that there was no cross-reactivity with the SBPP. The only exceptions were the three Enteroinvasive *Escherichia coli* (EIEC) strains which were "Detected" in the SBPP (0/3 SBPP Negative Results for each). This cross-reactivity was expected since the gene target used to detect *Shigella* spp. *(ipaH)* is also present EIEC (see Limitations in the Package Insert).

d. Competitive Inhibition

To evaluate potential for competitive interference in the SBPP, combinations of the 8 SBPP target organisms, representative of potential dual infections, were tested. The organisms included: *C. coli* (ATCC 43486), *C. jejuni* (ATCC 49943), *E. coli* (ATCC BAA-2196 *stx1+/stx2+*), *E. coli* (ATCC 43895 *stx1+/stx2+*/O157+), *S. bongori* (ATCC 43975), *S. enterica* (ATCC 13311), *S. flexneri* (ATCC 25929), and *S. sonnei* (ATCC 29930). The panels were designed such that one organism of each bacterial species was present at a low titer (2X LoD) with a second organism present at a high titer (≥10^6 CFU/mL). The samples were generated by spiking previously frozen and quantified enriched broth cultures of all bacterial species, into pooled, negative, preserved clinical stool at the required concentration. This resulted in 48 unique combinations, each of which were tested in triplicate in the SBPP. The combinations and concentrations tested along with the study results are shown in Table 7.

Table 7. Competitive Inhibition Study Results

Organism at Low Titer (2X) LoD	Organisms at High Titer: ≥10^6 CFU/mL							
	C. coli (ATCC 43486)	*C. jejuni* (ATCC 49943)	*E. coli* (stx1+/stx2+/ non-O157) (ATCC BAA-2196)	*E. coli* (stx1+/stx2+/ O157+) (ATCC 43895)	*S. bongori* (ATCC 43975)	*S. enterica* (ATCC 13311)	*S. flexneri* (ATCC 25929)	*S. sonnei* (ATCC 29930)
Campylobacter coli (ATCC 43486)	--	--	3/3	3/3	3/3	3/3	3/3	3/3
Campylobacter jejuni (ATCC 49943)	--	--	3/3	3/3	3/3	3/3	3/3	3/3
Escherichia coli (stx1+/stx2+/O157-) (ATCC BAA-2196)	3/3	3/3	--	--	3/3	3/3	3/3	3/3
Escherichia coli (stx1+/stx2+/O157+) (ATCC 43895)	3/3	3/3	--	--	3/3	3/3	3/3	3/3
Salmonella bongori (ATCC 43975)	3/3	3/3	3/3	3/3	--	--	3/3	5/6[a]
Salmonella enterica (ATCC 13311)	7/9[b]	3/3	3/3	14/19[c] 6/6[d]	--	--	3/3	3/3
Shigella flexneri (ATCC 25929)	3/3	3/3	3/3	3/3	3/3	3/3	--	--
Shigella sonnei (ATCC 29930)	3/3	3/3	5/6[e]	3/3	3/3	3/3	--	--

[a] In 1/3 replicates, 'high titer' *Shigella sonnei* was not detected and contamination with a *Campylobacter* sp. was noted. An additional 3 replicates were tested and the expected result was obtained for both analytes, in all replicates.

[b] For a 'low titer' *Salmonella enterica* and 'high titer' *Campylobacter coli* sample, the SBPP did not detect *Salmonella* in 2/3 replicates, although *Campylobacter* was correctly identified in all cases. An additional 6 replicates were tested, and the expected result was obtained for both analytes, in all replicates.

[c] For a 'low titer' *Salmonella enterica* and 'high titer' *Escherichia coli* (ATCC 43895, ≥10^6 CFU/mL) sample, the SBPP did not detect *Salmonella* in 1/3 replicates. An additional 16 replicates were tested and 12/16 detected 'low titer' *Salmonella.*

[d] We decreased the concentration of the 'high titer' *E. coli* to 1 x 10^5 CFU/mL in combination with 'low titer' *Salmonella* and tested 6 replicates. The expected result was obtained for both analytes, in all replicates.

[e] In 1/3 replicates, 'low titer' *Shigella sonnei* was not detected, although Shiga Toxin 1 & 2 was detected in all cases. An additional 3 replicates were tested, and the expected result obtained for both analytes, in all replicates.

Conclusion: Competitive inhibition was only observed for *Salmonella* when *E. coli* (*stx1+/stx2+*/O157+) was present at concentrations ≥ 1 x 10^6 CFU/mL. No other combinations of organisms showed competitive inhibition.

e. Interfering Substances

Potential interference in the SBPP from 19 different substances that are common stool contaminants, or likely to be present in patients with diarrhea were evaluated in an Interfering Substances Study. Each substance was added to a positive stool prepared by adding a single SBPP target organism to pooled, negative, preserved clinical stool at ≤ 3X LoD. The organisms tested represented each analyte detected by the SBPP and included: *C. coli* (ATCC 43486), *C. jejuni* (ATCC 49943), *E. coli* (ATCC BAA-2196 *stx1+/stx2+*), *E. coli* (ATCC 43895 *stx1+/stx2+*/O157+), *S. bongori* (ATCC 43975), *S. enterica* (ATCC 13311), *S. flexneri* (ATCC 25929), and *S. sonnei* (ATCC 29930).

A clinical, negative, un-spiked stool matrix was also tested as a control to evaluate potential interference with the internal assay control in the absence of analyte. A minimum of 3 replicates were tested for each substance. Samples for which 1 or more of the replicates gave unexpected results were re-tested. If 1 or more replicates still gave the unexpected result, the substance was considered to demonstrate interference in the SBPP at the concentration tested. The concentration at which each substance was tested along with the SBPP results are summarized in Table 8.

Table 8. Interfering Substances Study Results

Potentially Interfering Substances and Input Concentration		*Campylobacter coli* ATCC 43486 ≤3X LoD (3.6×10^3 CFU/mL)	*Campylobacter jejuni* ATCC 49943 ≤ 3X LoD (2.6×10^3 CFU/mL)	*Escherichia coli* (*stx1+/stx2+* O157+) ATCC 43895 ≤ 3X LoD (3.2×10^4 CFU/mL)	*Escherichia coli* (*stx1+/stx2+* non-O157) ATCC 2196 ≤ 3X LoD (2×10^4 CFU/mL)	*Salmonella bongori* ATCC 43975 ≤ 3X LoD (7.5×10^3 CFU/mL)	*Salmonella enterica* ATCC 13311 ≤ 3X LoD (3.8×10^4 CFU/mL)	*Shigella flexneri* ATCC 25929 ≤ 3X LoD (1.6×10^4 CFU/mL)	*Shigella sonnei* ATCC 29930 ≤ 3X LoD (2.8×10^4 CFU/mL)	Negative Stool Samples Assay Control DETECTED
Ampicillin	50 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Bacitracin Zinc ointment	50 mg/mL	3/3	3/3	3/3	3/3	3/3	6/6	3/3	3/3	3/3
Benzalkonium chloride, ethanol (moist towelettes)	9.5% v/v	3/3	3/3	3/3	3/3	3/3	3/3	3/3	4/6	3/3
Bovine Mucin	6.25 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	5/6	3/3
Calcium carbonate	200 mg/mL	3/3	3/3	3/3	5/6	3/3	3/3	3/3	3/3	3/3
Cholesterol	5% v/v	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Hemoglobin	10% w/v	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/4	3/3
Human Whole Blood	50% v/v	3/3	3/3	3/3	3/3	5/6	3/3	3/3	3/3	3/3
Hydrocortisone	75 mg/mL	3/3	3/3	5/6	3/3	3/3	3/3	3/3	3/3	3/3
Imodium	10% v/v	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Kaopectate	10% v/v	3/3	3/3	3/3	3/3	5/6	3/3	3/3	3/3	3/3
Milk of Magnesia	5% v/v	3/3	3/3	2/3	3/3	2/6[b] 3/3[c]	3/3	3/3	3/3	
Mineral Oil	50% v/v	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Naproxen Sodium	9.5% w/v	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Nystatin	5% v/v	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Pepto-Bismol	10% v/v	3/3	3/3	3/3	5/5*[a]	3/3	3/3	3/3	3/3	3/3
Pork Mucin	6.25 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Sennosides	9.7 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Triglycerides	10% v/v	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3

* This set of test runs also contained 1 "Invalid" run.

[a] One replicate in this set correctly identified Shiga toxin 1/Shiga toxin 2, but additionally detected *Shigella*.

[b] Four *Salmonella bongori* (ATCC 43975, 7.5×10^3 CFU/mL) in stool with Milk of Magnesia (5% v/v) the SBPP did not detect *Salmonella* in 2/3 replicates. An additional 3 replicates were tested and similarly, 2/3 replicates did not detect *Salmonella*.

[C] The concentration of Milk of Magnesia was decreased to 2.5% v/v. The SBPP detected *Salmonella* in all replicates tested

Conclusion: No interference in the SBPP was observed for the substances tested at the concentrations shown in Table 8, with the exception of *S. bongori* in the presence of 5% Milk of Magnesia. However, no interference was observed at 2.5% Milk of Magnesia.

f. Microbial Interference

The potential for cross-reactivity in a mixed infection was evaluated in a Microbial Interference Study. A panel of non-target gastrointestinal pathogens commonly encountered in stool were tested in the presence of each of the analytes detected in the SBPP. The panel of non-target organisms tested was a subset of 29 of the organisms used in the Exclusivity Study and were commercially purchased at a given concentration, or previously frozen broth cultures, for which concentration was measured at the time of growth.

Similar to the Exclusivity Study, the non-target bacterial and yeast strains were prepared using previously frozen and enumerated aliquots of liquid cultures. Potentially interfering bacteria/fungi, viruses, and DNA were added at ≥10^6 CFU/mL, ≥1 x 10^6 copies/mL, and ≥ 1 µg/mL, respectively, to pooled, negative, preserved, clinical stool with a single SBPP target analyte added at ≤ 3X LoD. The following 8 strains representing all SBPP targets were tested in the presence of the 29 interfering organisms: *C. coli* (ATCC 43486), *C. jejuni* (ATCC 49943), *E. coli* (ATCC BAA-2196 *stx1+/stx2+*), *E. coli* (ATCC 43895 *stx1+/stx2+*/O157+), *S. bongori* (ATCC 43975), *S. enterica* (ATCC 13311), *S. flexneri* (ATCC 25929), and *S. sonnei* (ATCC 29930). In total, 21 unique bacterial strains, 2 yeast, 2 parasites, 3 viruses and human genomic DNA were tested for microbial interference with the 8 SBPP target strains.

A minimum of 3 replicates of each sample were tested. The specific concentrations at which each organism was evaluated along with the results are shown in Table 9.

Table 9. Microbial Interference Study Results

Species	Strain ID	Input Tested	*C. coli* ATCC 43486 ≤ 3X LoD (3.6×10^3 CFU/mL)	*C. jejuni* ATCC 49943 ≤3X LoD (2.6×10^3 CFU/mL)	*E. coli* (*stx1+/stx2+* non-O157) ATCC 2196 ≤3X LoD (2×10^4 CFU/mL)	*E. coli* (*stx1+/stx2+* O157+) ATCC 43895 ≤ 3X LoD (3.2×10^4 CFU/mL)	*Salmonella bongori* ATCC 43975 ≤ 3X LoD (7.5×10^3 CFU/mL)	*Salmonella enterica* ATCC 13311 ≤ 3X LoD (3.8×10^4 CFU/mL)	*Shigella flexneri* ATCC 25929 ≤ 3X LoD (1.6×10^4 CFU/mL)	*Shigella sonnei* ATCC 29930 ≤ 3X LoD (2.8×10^4 CFU/mL)
Bacteria										
Aeromonas hydrophilia	ATCC 35654	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Bacteroides fragilis	ATCC 23745	≥10^6 CFU/mL	3/3	3/3	3/3	5/5*	3/3	3/3	3/3	3/3
Bacteroides vulgatus	ATCC 8482	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	5/6	3/3	3/3	5/5*
Bifidobacterium bifidum	ATCC 11863	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3	5/6	3/3
Clostridium difficile (toxinA/B)	ATCC 43594	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3[a]	3/3	3/3	3/3
Clostridium perfringens	ATCC 13124	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3*	3/3	3/3
Enterobacter aerogenes	ATCC 15038	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Enterococcus faecalis	ATCC 29212	≥10^6 CFU/mL	3/3	3/3*	3/3	3/3	5/6	3/3	3/3	3/3
Escherichia coli (non-STEC O157)	ATCC 700728	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	8/9	3/3	3/3
Enteroaggregative E. coli (EAEC)	ATCC 29552	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Enterotoxigenic E. coli (ETEC)	ATCC 31703	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Enteropathogenic E. coli (EPEC)	ATCC 49106	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Helicobacter pylori	ATCC 49503	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Klebsiella pneumonia	ATCC 13883	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Lactobacillus acidophilus	ATCC 4356	≥10^6 CFU/mL	3/3	3/3	3/3	5/5*	5/6	3/3	3/3	3/3
Listeria monocytogenes	ATCC 15313	≥10^6 CFU/mL	3/3	3/3	5/5*	3/3	3/3	3/3	3/3	3/3
Prevotella melaninogenica	ATCC 25845	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3*
Pseudomonas aeruginosa	ATCC 10145	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	5/5*	3/3	3/3
Staphylococcus aureus	ATCC 6538	≥10^6 CFU/mL	3/3	3/3	3/3	5/5*	3/3	3/3	3/3	3/3
Vibrio cholera	ATCC 55188	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Yersinia enterocolitica	ATCC 49397	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
n=21										
Yeasts, Parasites, Viruses and DNA										
Candida albicans	ATCC 18804	>10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Saccharomyces boulardii	ATCC MYA-796	≥10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Entamoeba histolytica (gDNA)	ATCC 30459DQ	≥1 x10^6 copies/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Giardia intestinalis (gDNA)	ATCC 50803D	≥1 µg/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Enterovirus 71 (RNA)	ATCC VR-1775DQ	≥1 x10^6 copies/mL	3/3	3/3	3/3	3/3	3/3	3/3	5/5	3/3
Norovirus G1 (synthetic RNA)	ATCC VR-3234SD	≥1 x10^6 copies/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Rotavirus (RNA)	ATCC VR-2018DQ	≥1 x10^6 copies/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Human genomic DNA	Roche Cat No. 11691112001	≥1 µg/mL	3/3	3/3	5/6	5/6	3/3	3/3	3/3	3/3
n=8										

*This set of tests also contained 1 "Invalid" run

[a] One replicate in this set correctly identified *Salmonella*, but additionally detected *Shigella*.

Conclusion: No interference from non-target organisms was observed at the concentrations indicated in Table 9 in the mixed Microbial Interference Study.

g. Carry-over/Cross Contamination

To evaluate potential carry-over/cross-contamination of the SBPP a Carry-over Study was conducted. Briefly a contrived stool sample containing a high concentration of an analyte was alternated with a clinical negative stool sample in 10 consecutive testing rounds. The high positive sample was generated by adding previously frozen and quantified enriched broth cultures into pooled negative, preserved, clinical stool at a final concentration ≥ 1 x 10^6 CFU/mL. The organisms used in this study were *C. coli* (ATCC 43486), *E. coli* (ATCC 43895 s*tx1+/stx2+*/O157+), *S. bongori* (ATCC 43975), and *S. flexneri* (ATCC 25929). The negative sample was stool from symptomatic patients that previously tested negative for all SBPP targets.

The alternating pattern of 10 test rounds of high positive and negative samples were performed in direct succession on 4 different analyzers. In total, 80 SBPP tests were performed: 40 tests of a high positive sample and 40 tests of a negative sample. The concentrations tested along with the study results are shown in Table 10.

Table 10. Carry-over/Cross Contamination Study Results

Sample Type (Alternating Positive/Negative)		Stool Bacterial Pathogens Panel Results			
		Portrait Analyzer 5.315	**Portrait Analyzer 5.106**	**Portrait Analyzer 5.382**	**Portrait Analyzer 5.072**
High Positive	Runs 1 - 4	C. coli/jejuni DETECTED	Shiga Toxin 1 DETECTED Shiga Toxin 2 DETECTED Serotype O157 DETECTED	Salmonella DETECTED	Shigella DETECTED
Negative	Runs 5 - 8	Negative	Negative	Negative	Negative
High Positive	Runs 9 - 12	C. coli/jejuni DETECTED	Shiga Toxin 1 DETECTED Shiga Toxin 2 DETECTED Serotype O157 DETECTED	Salmonella DETECTED	Shigella DETECTED
Negative	Runs 13 - 16	Negative	Negative	Negative	Negative
High Positive	Runs 17 - 20	C. coli/jejuni DETECTED	Shiga Toxin 1 DETECTED Shiga Toxin 2 DETECTED Serotype O157 DETECTED	Salmonella DETECTED	Shigella DETECTED
Negative	Runs 21 - 24	Negative	Shiga Toxin 1 DETECTED	Negative	Negative
High Positive	Runs 25 - 28	C. coli/jejuni DETECTED	Shiga Toxin 1 DETECTED Shiga Toxin 2 DETECTED Serotype O157 DETECTED	Salmonella DETECTED	Shigella DETECTED
Negative	Runs 29 - 32	Negative	Negative	Negative	Negative
High Positive	Runs 33 - 36	C. coli/jejuni DETECTED	Shiga Toxin 1 DETECTED Shiga Toxin 2 DETECTED Serotype O157 DETECTED	Salmonella DETECTED	Shigella DETECTED
Negative	Runs 37 - 40	Negative	Negative	Negative	Negative

Conclusion: No carry-over or cross contamination was observed in the SBPP.

h. Reproducibility

A Reproducibility Study was conducted at 3 clinical study sites; 2 external and 1 internal. The study incorporated several variables including 6 different operators (2 per site), 70 different Portrait Analyzers and 10 different cartridge lots.

A panel consisting of 7 different samples was tested in triplicate over 5 non-consecutive days by each operator. Each analyte detected in the SBPP was included as a low positive (1.5X LoD) and a moderate positive (3X LoD) in the reproducibility panel. A single negative was also included.

Analysis of Positive Results

For each analyte, there were 90 possible positive results. However, due to circumstances which required additional runs, some samples have greater than 90 results which is explained below.

For Sample RP-03 two (2) of the three (3) replicates obtained by Operator 4 on Day 3 gave unexpected negative results for all 3 analytes that were supposed to be present in RP-03 (Shiga toxin 1, Shiga toxin 2, and *E. coli* O157 at 1.5X LoD). It was suspected that the negative results were due to a mix up with the sample ID. The third replicate was an aborted run ("Test Incomplete") and didn't produce any results. All 3 replicates were repeated. The original results along with the repeat test results were included in the analysis for a total "n" of 92 for each analyte in sample RP-03.

For Sample RP-04 one (1) of the replicates obtained by operator 5 gave positive results for all analytes detected in the SBPP (Sample RP-04 should only contain Shiga toxin 1, Shiga toxin 2, and *E. coli* O157 at 3X LoD). It was suspected that this sample was contaminated by the operator. Another aliquot of this sample was tested. The original result was included in the analysis along with the repeat test results giving a total "n" of 91 for the analytes in sample RP-04. The total number of replicates for all other samples was 90.

The percent agreement of the positive results is presented separately for each analyte and concentration, by operator, by site and in total, and is shown in Tables 11 through 16.

Table 11. ***Campylobacter* Reproducibility Results**

Analyte	Conc.	Operator	Correct Positive Results	Agreement	Site	Correct Positive Results	Agreement	Total Correct Positive Results
Campylobacter spp.	1. 5 X LOD	1	15/15	100%	1	30/30	100%	90/90 100%
		2	15/15	100%				
		3	15/15	100%	2	30/30	100%	
		4	15/15	100%				
		5	15/15	100%	5	30/30	100%	
		6	15/15	100%				
	3 X LOD	1	15/15	100%	1	30/30	100%	90/90 100%
		2	15/15	100%				
		3	15/15	100%	2	30/30	100%	
		4	15/15	100%				
		5	15/15	100%	5	30/30	100%	
		6	15/15	100%				

Table 12. ***Salmonella*** **Reproducibility Results**

Analyte	Conc.	Operator	Correct Positive Results	Agreement	Site	Correct Positive Results	Agreement	Total Correct Positive Results
Salmonella	1. 5 X LOD	1	15/15	100%	1	30/30	100%	87/90 96.7%
		2	15/15	100%				
		3	14/15	93.3%	2	28/30	93.3%	
		4	14/15	93.3%				
		5	14/15	93.3%	5	29/30	96.7%	
		6	15/15	100%				
	3 X LOD	1	15/15	100%	1	30/30	100%	90/90 100.0%
		2	15/15	100%				
		3	15/15	100%	2	30/30	100%	
		4	15/15	100%				
		5	15/15	100%	5	30/30	100%	
		6	15/15	100%				

Table 13. **Shiga toxin 1 Reproducibility Results**

Analyte	Conc.	Operator	Correct Positive Results	Agreement	Site	Correct Positive Results	Agreement	Total Correct Positive Results
Shia toxin 1	1. 5 X LOD	1	15/15	100%	1	30/30	100%	90/92 97.8%
		2	15/15	100%				
		3	15/15	100%	2	30/32	93.8%	
		4	15/17	88.2%				
		5	15/15	100%	5	30/30	100%	
		6	15/15	100%				
	3 X LOD	1	15/15	100%	1	30/30	100%	91/91 100.0%
		2	15/15	100%				
		3	15/15	100%	2	30/30	100%	
		4	15/15	100%				
		5	16/16	100%	5	31/31	100%	
		6	15/15	100%				

Table 14. **Shiga toxin 2 Reproducibility Results**

Analyte	Conc.	Operator	Correct Positive Results	Agreement	Site	Correct Positive Results	Agreement	Total Correct Positive Results
Shiga toxin 2	1. 5 X LOD	1	14/15	93.3%	1	29/30	96.7%	88/92 95.7%
		2	15/15	100%				
		3	15/15	100%	2	29/32	90.6%	
		4	14/17	82.4%				
		5	15/15	100%	5	30/30	100%	
		6	15/15	100%				
	3 X LOD	1	15/15	100%	1	30/30	100%	91/91 100.0%
		2	15/15	100%				
		3	15/15	100%	2	30/30	100%	
		4	15/15	100%				
		5	16/16	100%	5	31/31	100%	
		6	15/15	100%				

Table 15. ***E. coli* Serotype O157 Reproducibility Results**

Analyte	Conc.	Operator	Correct Positive Results	Agreement	Site	Correct Positive Results	Agreement	Total Correct Positive Results
E. coli serotype O157	1. 5 X LOD	1	15/15	100%	1	30/30	100%	90/92 97.8%
		2	15/15	100%				
		3	15/15	100%	2	30/32	93.8%	
		4	15/17	88.2%				
		5	15/15	100%	5	30/30	100%	
		6	15/15	100%				
	3 X LOD	1	15/15	100%	1	30/30	100%	91/91 100%
		2	15/15	100%				
		3	15/15	100%	2	30/30	100%	
		4	15/15	100%				
		5	16/16	100%	5	31/31	100%	
		6	15/15	100%				

Table 16. ***Shigella*** **Reproducibility Results**

Analyte	Conc.	Operator	Correct Positive Results	Agreement	Site	Correct Positive Results	Agreement	Total Correct Positive Results
Shigella	1. 5 X LOD	1	15/15	100%	1	30/30	100%	90/90 100.0%
		2	15/15	100%				
		3	15/15	100%	2	30/30	100%	
		4	15/15	100%				
		5	15/15	100%	5	30/30	100%	
		6	15/15	100%				
	3 X LOD	1	15/15	100%	1	30/30	100%	90/90 100.0%
		2	15/15	100%				
		3	15/15	100%	2	30/30	100%	
		4	15/15	100%				
		5	15/15	100%	5	30/30	100%	
		6	15/15	100%				

Analysis of Negative Results

To assess negative results a single negative stool sample (RP-05) was tested. All analytes in the SBPP should give negative "Not Detected" results for this sample except for *E. coli* O157. Since *E. coli* O157 is not evaluated when Shiga toxin 1 and or 2 are "Not Detected" the reported result for O157 is "Not Tested". Therefore, the SBPP will generate 5 negative results per replicate tested. Each operator tested 15 replicates of RP-05 for a total of 75 negative results per operator. The percent agreement of the negative results is presented by operator, by site and in total, and is shown in Table 17.

Table 17. **Negative Sample Reproducibility Results**

Analyte	Conc.	Operator	Correct Negative Results	Agreement	Site	Correct Negative Results	Agreement	Total Correct Negative Results
Negative	N/A	1	75/75	100%	1	150/150	100%	450/450 100%
		2	75/75	100%				
		3	75/75	100%	2	150/150	100%	
		4	75/75	100%				
		5	75/75	100%	5	150/150	100%	
		6	75/75	100%				

The overall results of the Reproducibility Study are summarized in Table 18. There was ≥ 95% agreement of positive results for analytes that were present in low concentrations in the samples (1.5X LoD) and 100% agreement of positive results for analytes present at moderate concentration (3X LoD). The was 100% agreement of negative results from the negative sample.

Table 18. Summary of Reproducibility Results

Analyte	Concentration	% Agreement
Campylobacter coli/jejuni	1.5X LoD	100% (90/90)
	3X LoD	100% (90/90)
Salmonella	1.5X LoD	96.7% (87/90)
	3X LoD	100% (90/90)
Shiga toxin 1	1.5X LoD	97.8% (90/92)
	3X LoD	100% (91/91)
Shiga toxin 2	1.5X LoD	95.7% (88/92)
	3X LoD	100% (91/91)
E. coli Serotype O157	1.5X LoD	97.8% (90/92)
	3X LoD	100% 91/91)
Shigella	1.5X LoD	100% (90/90)
	3X LoD	100% (90/90)
Negative	N/A	100% (450/450)

Conclusion: The study demonstrated acceptable reproducibility of the SBPP.

i. Specimen Stability and Storage

A sample stability study was conducted to determine the allowable storage conditions (time and temperature) for clinical specimens. The study included the following organisms prepared at a concentration of 2X LoD: *C. coli* (ATCC 43486), *C. jejuni* (ATCC 49943), *E. coli* (BAA-2196), *E. coli* (ATCC 43895), *S. bongori* (ATCC 43975), *S. enterica* (ATCC 13311), and *S. sonnei* (ATCC 29930), at 2X LoD.

Each sample was tested in triplicate after storage at the times and temperatures shown in Table 19.

Table 19. Specimen Stability Study Time/Temperature Storage Conditions

Time Point	Time tested and Storage Condition
T0	0 hr: Freshly prepared
T1	2 hr: Room temperature storage (20°- 25°C)
T2	24 hr: 2-8°C storage
T3	48 hr: 2-8°C storage
T4	72 hr: 2-8°C storage
T5	96 hr: 2-8°C storage
T6	120 hr: 2-8°C storage
T7	2 hr Room temperature (20°- 25°C) + 120 hr 2-8°C storage
T8	2 hr Room temperature (20°- 25°C) + 144 hr 2-8°C storage

The results demonstrated 100% agreement with the expected results supporting the specimen storage claims in the Product Insert.

G. Performance Summary – Clinical Studies

The clinical evaluation of the SBPP consisted of both a Prospective Sample and a Frozen Retrospective Sample Study. An additional study using selected fresh positive *Salmonella* samples was also performed (Selected Sample Study).

A prospective method comparison study was conducted to compare the performance of the SBPP to standard stool culture-based methods for identification of *Campylobacter jejuni /Campylobacter coli*, *Escherichia coli* serotype O157 *Salmonella* spp., Shiga toxin 1 Shiga toxin 2, and *Shigella* spp. The study was conducted at four external, geographically-diverse U.S. clinical study sites (Midwest, Northeast, Southwest and West) from July, 2016 through November, 2016. The specimens enrolled in the study were excess remnants of preserved stool samples collected from symptomatic individuals suspected of gastrointestinal infection that were processed according to routine standard care. A total of 1506 samples were collected for all four sites combined. Subsequent to enrollment, 24 samples were excluded from the data set leaving 1479 samples included in the analysis.

In addition, frozen archived de-identified specimens that were previously characterized as positive or negative by the standard of care method used at the institution (historical result) were obtained. The historical result for each sample was first confirmed by an FDA cleared Nucleic Acid Amplification Test (NAAT) prior to enrolling the sample in the study. A total of 150 frozen samples were included in the panel. The SBPP results were compared to the historical result.

To further increase the number of positive *Salmonella* samples evaluated, additional fresh samples selected as positive by the standard of care method used by the clinical study site were collected and tested. Intermountain Healthcare (IMC) in Salt Lake City, UT was also added as a sample collection site and testing samples from IMC was performed internally at Great Basin Scientific.

The positive (PPA) and negative (NPA) percent agreement for each of the three studies (Prospective Study; All sites Combined, Frozen Retrospective Sample study and Selected Sample Study) along with the 95% Confidence Intervals are shown in Table 20.

Table 20. Summary of Clinical Study Results

Specimen		n	% Agreement (95% CI)	
			Positive	Negative
Campylobacter	Fresh	1479	96.4% (82.3-99.4) 27/28	99.2% (98.6-99.5) 1439/1451
	Frozen	0	N/A	N/A
Salmonella	Fresh	1479	83.3% (55.2-95.3) 10/12	99.6% (99.1-99.8) 1461/1467
	Fresh Selected	28	92.9% (77.4-98.0) 26/28	N/A
	Frozen	206	94.4% (81.9-98.5) 34/36	100.0% (97.8-100.0) 170/170
Shiga Toxin 1	Fresh	1479	100.0% (20.7-100.0) 1/1	99.5% (99.0-99.8) 1471/1478
	Frozen	206	100.0% (88.3-100.0) 29/29	100.0% (97.9-100.0) 177/177
Shiga Toxin 2	Fresh	1479	100.0% (20.7-100.0) 1/1	99.4% (98.8-99.7 1469/1478)
	Frozen	206	100.0% (89.0-100.0) 31/31	100.0% (97.9-100.0) 175/175
***E. coli* Serotype O157**	Fresh	16	100% (51.0-100.0) 4/4	75.0% (46.8-91.1) 9/12
	Frozen	48	100.0% (81.6 -100.0) 17/17	100.0% (89.0-100.0) 31/31
Shigella	Fresh	1479	100% (56.6-100.0) 5/5	99.1% (98.4-99.4) 1460/1474
	Frozen	206	94.7% (75.4-99.1) 18/19	100.0% (98.0-100.0) 187/187

As shown in Table 20, in the SBPP Prospective Study the point estimate achieved for PPA was ≥ 95% (96.4% -100%) for all analytes, except for *Salmonella* which was 83.3%. Due to the low number of co-positive samples, the lower bound of the 95% CI was < 80% for all analytes, except for *Campylobacter* which was 82.3%. The point estimate for NPA was still ≥ 95% (99.1% - 99.6%) for all analytes except *E. coli* O157 which was 75.0%.

Samples with discrepant results for the *Campylobacter*, *Salmonella*, *Shigella* and *E. coli* Serotype O157 analytes were investigated by further testing in the BioFire Film Array GI Panel (K140407). Samples with discrepant results for Shiga toxin 1 and/or 2 were investigated by further testing in the Nanosphere Verigene® EP test (K140083). The results are shown in Table 21.

Table 21. Summary Table – Prospective Study Discrepant Results Resolution

Analyte	False Negatives Resolved by NAAT Reference Method	False Positives Resolved by NAAT Reference Method
Campylobacter	1/1	10/12
Salmonella	0/2	6/6
stx1	N/A	6/7[a]
stx2	N/A	8/9[b]
E. coli O157	N/A	3/3
Shigella	N/A	14/14

[a] The one false positive not concordant with Verigene® EP (negative for stx1 in the Verigene® EP) was positive for stx1/2 in the BioFire GI Panel.

[b] The one false positive not concordant with Verigene® EP (negative for stx2 in the Verigene® EP) was positive for stx1/2 in the BioFire GI Panel.

As shown in Table 21, the majority of the false positive results were found to be concordant (resolved) with the FDA cleared NAAT that was used in the investigation of the discrepant results (BioFire GI Panel or Verigene® EP).

The results from the Frozen Retrospective Study which utilized frozen archived samples further support the acceptable performance of the SBPP. As shown in Table 20, in the SBPP Frozen Retrospective Study the point estimate achieved for PPA was ≥ 90% (94.4% - 100.0%) for all analytes detected in the SBPP. The lower bound 95% CI for the PPA was ≥ 80% for all analytes except *Shigella* which was 75.4%. The point estimate for NPA was 100.0% for all analytes evaluated. The lower bound of the 95% CI was ≥ 95% for all analytes except for *E. coli* O157 which was 89.0% (due to the low sample size of the negatives). In the Frozen Retrospective study, there was one (1) false negative result for *Shigella*. This sample was further investigated and was also negative in the Verigene® EP test. There were two (2) false negative results for *Salmonella* which were further investigated. One (1) sample was positive and one (1) sample was negative in the BioFire GI Panel.

The results from the Selected Sample Study are also summarized in Table 20 and provide further support of the performance for the detection of *Salmonella* using fresh samples. The point estimate was obtained for PPA was 92.9% (77.4% - 98.0%). There were two (2) false negative results which were further investigated by testing in the Verigene® EP. One (1) of these was also negative in the Verigene® EP and the other was positive for *Salmonella* in the Verigene® EP.

Conclusion: The method comparison studies conducted as part of the clinical studies demonstrated acceptable performance of the SBPP and support the Intended Use of this product.

H. Overall Conclusion

The submitted information in this 510(k) pre-market notification is complete and supports substantial equivalence.