510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K163571

B. Purpose for Submission:

To obtain a substantial equivalence determination for a new device.

C. Measurand:
Nucleic acids from:

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

D. Type of Test:

Qualitative real-time polymerase chain reaction (PCR) coupled with chip based detection

E. Applicant:

Great Basin Scientific, Inc.

F. Proprietary and Established Names:

Assay: Great Basin Stool Bacteria Pathogens Panel
Instrument: Portrait Analyzer System, Model: PA500

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3990, Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay

2. Classification:

   Class II

3. Product code:

   PCH – Gastrointestinal Pathogen Panel Multiplex Nucleic Acid-Based Assay System
   PCI – Gastrointestinal Pathogen Panel Multiplex Nucleic Acid-Based Assay System
   OOI – Real time nucleic acid amplification system

4. Panel:

   83 - Microbiology

H. Intended 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.

2. Indication(s) for use:

   See Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   For use with the PA500 Portrait Analyzer System

I. Device Description:

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.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Nanosphere Verigene Enteric Pathogens Nucleic Acid Test

2. Predicate 510(k) number(s):

   K140083

3. Comparison with predicate:

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 (SBPP)	Verigene Enteric Pathogen Nucleic Acid Test
510(k) Number	K163571	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

K. Standard/Guidance Document Referenced (if applicable):

Class II Special Controls Guideline: Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assays for Detection and Identification of Microorganisms and Toxin Genes from Human Stool Specimens

L. Test Principle:

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).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/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 Stool Bacterial Pathogens Panel (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.

The overall results of the Reproducibility Study are summarized in the table below. 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). There was 100% agreement in negative results from the negative sample.

Summary of Reproducibility Results

Analyte	Concentration	% Agreement
Campylobacter coli/jejuni	1.5X LoD	100% (90/90)
	3X LoD	100% (90/90)
	Negative	99.8% (540/541)
Salmonella	1.5X LoD	96.7% (87/90)
	3X LoD	100% (90/90)
	Negative	99.8% (540/541)
Shiga toxin 1	1.5X LoD	97.8% (90/92)
	3X LoD	100% (91/91)
	Negative	100% (540/540)
Shiga toxin 2	1.5X LoD	95.7% (88/92)
	3X LoD	100% (91/91)
	Negative	100% (540/540)
E. coli Serotype O157	1.5X LoD	97.8% (90/92)
	3X LoD	100% (91/91)
	Negative	100% (540/540)
Shigella	1.5X LoD	100% (90/90)
	3X LoD	100% (90/90)
	Negative	99.8% (540/541)
Negative	Negative	100% (540/540)

b. *Linearity/assay reportable range:*

Not applicable (N/A)

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

External Controls:

External positive and negative controls are intended to monitor for correct procedural technique and reagent integrity. External controls are not provided by Great Basin.

- External Controls should be treated as if they are patient specimens.
- The External Negative Control is intended to detect reagent or environmental contamination by target nucleic acids.
- All External Controls should yield the expected results, with no internal control failures (INVALID results).
- The following External Control materials are recommended to allow the user to choose the most appropriate controls for the lab's Quality Control Plan:

  External Negative Control may be any of the following:
  - an aliquot of pure Cary Blair or C&S media
  - a fresh (never frozen), previously characterized sample known to be negative for all assay targets
  - a frozen, raw stool specimen that has been thawed and then preserved and known to be negative for all assay targets

  External Positive Control: commercially-available ATCC strains for all assay targets are available, and may be pooled and diluted in stool preservation media and processed as a patient specimen.

Specimen Processing Control (SPC):

The SPC controls for all analytical steps in the procedure, including DNA extraction, PCR amplification of target DNA sequences, hybridization, and detection on the chip surface. The SPC consists of *B. subtilis* cells lyophilized directly onto the frit of the SPD, and is processed along with the stool sample. The SPC detects inhibition of PCR reactions, ensuring the reaction conditions are appropriate and that the amplification reagents are functional. The SPC signal should be "Detected" in a sample in which target sequences were "Not Detected" for all Stool Bacterial Pathogens Panel targets, and can be "Detected" or "Not Detected" in samples for which at least one target sequence of the SBPP was "Detected".

Specimen Stability:

The recommended storage time and temperature conditions for Cary-Blair or C&S preserved stool specimens, prior to testing via the SBPP are as follows:

- Refrigerated storage (2°- 8° C) for up to 120 hours (5 days)
- Room temperature (RT) (20°-25°C) storage for up to 2 hours
- The combination of 2-hour storage at room temperature (20º-25ºC) followed by refrigerated storage for up to 120 hours.

To assess the stability of the SBPP nucleic acid targets under the recommended storage conditions, a Specimen Stability Study was conducted to evaluate each suggested time and temperature condition. The Specimen Stability Study tested 8 strains for which the LoD was experimentally measured:

- *Campylobacter coli* (ATCC 43486)
- *Campylobacter jejuni* (ATCC 49943)
- *Escherichia coli* (ATCC BAA-2196, *stx1+/stx2+*/non-O157)
- *Escherichia coli* (ATCC 43895, *stx1+/stx2+*/O157+)
- *Salmonella bongori* (ATCC 43975)
- *Salmonella enterica* (ATCC 13311)
- *Shigella flexneri* (ATCC 25929)
- *Shigella sonnei* (ATCC 29930)

The contrived positive samples were prepared by spiking fresh cultures of each organism into similar pooled, preserved, clinical negative stool matrix. The results of the specimen stability study support the recommended storage time and temperature conditions for Cary-Blair or C&S preserved stool specimens Results are summarized in the tables below:

Specimen Stability Study Results

			Correct SBPP Result					
Organism	**ATCC ID**	**Sample Input ≤3X LoD (CFU/mL)**	**Fresh (< 30 min)**	**2 hr RT (20º-25ºC)**	**24 hr 2º-8ºC**	**48 hr 2º-8ºC**	**72 hr 2º-8ºC**	**2 hr RT (20-25ºC) + 6 days 2º-8ºC**
Campylobacter coli	43486	5.1 x 10^3	3/3	3/3	3/3	3/3	3/3	3/3
Campylobacter jejuni	49943	7.8 x 10^2	3/3	3/3	3/3	3/3	3/3	3/3
Escherichia coli (stx1+/stx2+/ non-O157*)*	BAA-2196	3.4 x 10^4	3/3	3/3	3/3	3/3	3/3	3/3
Escherichia coli (stx1+/stx2+/O157+)	43895	3.9 x 10^4	3/3	3/3	3/3	3/3	3/3	1/3
Salmonella bongori	43975	6.5 x 10^3	3/3	3/3	3/3	3/3	3/3	–
Salmonella enterica	13311	5.9 x 10^4	3/3	3/3	3/3	3/3	3/3	1/3
Shigella flexneri	25929	1.2 x 104	3/3	3/3	3/3	3/3	3/3	–
Shigella sonnei	29930	4.4 x 104	3/3	3/3	3/3	3/3	3/3	3/3

Specimen Stability Study Results

			Correct SBPP Result			
Organism	**ATCC ID**	**Concentration ≤3X LoD (CFU/mL)**	**Fresh (< 30 min)**	**96 hr 2º-8ºC**	**120 hr 2º-8ºC**	**2 hr RT (20º-25ºC) + 5 days 2º-8ºC**
Campylobacter coli	43486	2.1 x 10^3	3/3	3/3	3/3	3/3
Campylobacter jejuni	49943	4.4 x 10^3	3/3	3/3	3/3	3/3
Escherichia coli (stx1+/stx2+/ non-O157*)*	BAA-2196	2.1 x 10^4	3/3	3/3	3/3	3/3
Escherichia coli (stx1+/stx2+/O157+)	43895	3.8 x 104	3/3	2/2	3/3	3/3
Salmonella bongori	43975	6.5 x 10^3	3/3	3/3	3/3	3/3a
Salmonella enterica	13311	5.4 x 10^4	3/3	3/3	3/3	3/3
Shigella flexneri	25929	1.2 x 10^4	3/3	3/3	3/3	3/3
Shigella sonnei	29930	2.3 x 10^4	3/3	3/3	3/3	3/3
a One replicate in this set correctly identified *Salmonella* , but additionally detected Shiga toxin 1						

Fresh versus Frozen:

A Fresh vs. Frozen Study was performed to support the use of frozen, transport media preserved stool specimens in SBPP for the Frozen Retrospective and Reproducibility Studies, as well as for discordant resolution of prospective samples.

The Fresh vs. Frozen Study tested the performance of the SBPP on contrived positive samples that were subject to three (3) freeze/thaw cycles. Demonstration of specimen

stability after the third freeze/thaw is important because generation of a Frozen Retrospective panel from archived clinical specimens requires a two freeze thaws prior to shipment to clinical sites. Clinical sites will perform the third thawing of archived specimens prior to testing. Samples tested in the Reproducibility Study were subjected to two freeze/thaws.

Contrived positive samples for the Fresh vs. Frozen Study were prepared with fresh bacterial cells spiked into a pooled, preserved, negative, clinical stool. The panel for the Fresh vs. Frozen Study was comprised of eight (8) SBPP target strains that included:

- *Campylobacter coli* (ATCC 43486 *cadF*+)
- *Campylobacter jejuni* (ATCC 49943 *cadF*+)
- *Escherichia coli* (ATCC BAA-2196 *stx1*+/*stx2*+/non-O157)
- *Escherichia coli* (ATCC 43895 *stx1*+/*stx2*+/O157+)
- *Salmonella bongori* (ATCC 43975 *invA*+)
- *Salmonella enterica* (ATCC 13311 *invA*+)
- *Shigella flexneri* (ATCC 25929 *ipaH*+)
- *Shigella sonnei* (ATCC 29930 *ipaH*+)

The *E. coli* (ATCC BAA-2196), *E. coli* (ATCC 43895), *S. flexneri* (ATCC 25929) and *S. sonnei* (ATCC 29930) strains were stable following three (3) freeze thaw cycles at <1X LoD, 1X LoD, and 5X LoD.

Freeze/thaw had a negative impact on contrived samples spiked with *Salmonella* species*, S. enterica* (ATCC 13311) and *S. bongori* (ATCC 43975), and the *Campylobacter* species, *C. coli* (ATCC 43486) and *C. jejuni* (ATCC 49943) .

The results of the Fresh vs. Frozen study are summarized in the tables below.

Fresh vs. Frozen Study Summary (*E. coli* and *Shigella* sp)

Organism	ATCC ID	Input Conc. (X LOD)	CFU/mL	Correct SBPP Results			
				T_0= Fresh Pre-Freeze	T_1= 1X Freeze/Thaw	T_2 = 2X Freeze/Thaw	T_3 = 3X Freeze/Thaw
E. coli (stx1+/stx2+/non-O157)	BAA-2196	<1X LOD	2.0×10^3	2/5	2/5	4/5	1/5
		1X LOD	1.5×10^4	10/10	10/10	10/10	9/10
		5X LOD	5.0×10^4	5/5	5/5	5/5	5/5
E. coli (stx1+/stx2+/O157+)	43895	<1X LOD	2.0×10^3	2/5	3/5	1/5	0/5
		1X LOD	2.4×10^4	10/10	10/10	10/10	9/10
		5X LOD	8.0×10^4	5/5	5/5	5/5	5/5
Shigella flexneri	25929	<1X LOD	1.4×10^3	2/5	2/5	2/5	3/5
		1X LOD	2.3×10^4	10/10	10/10	10/10	10/10
		5X LOD	7.0×10^4	5/5	5/5	5/5	5/5
Shigella sonnei	29930	<1X LOD	1.4×10^3	1/5	0/5	2/5	2/5
		1X LOD	2.1×10^4	10/10	10/10	10/10	10/10
		5X LOD	7.0×10^4	5/5	5/5	5/5	5/5

Fresh vs. Frozen Study Summary (*Salmonella* spp.)

Organism	ATCC ID	Input Conc. (X LOD)	CFU/mL	Correct SBPP Results		
				T_0= Fresh Pre-Freeze	T_1= 1X Freeze/Thaw	T_2 = 2X Freeze/Thaw
Salmonella enterica	13311	<1X LOD	5.6×10^3	3/3	3/3	1/3
		1X LOD	2.1×10^4	4/4	4/4	0/4
		5X LOD	1.1×10^5	3/4	2/3	1/3
Salmonella enterica (repeated)	13311	<1X LOD	2.8×10^3	2/5	2/5	–
		1X LOD	2.9×10^4	10/10	8/10	–
		5X LOD	1.3×10^5	5/5	5/5[a,b]	–
Salmonella abongori	43975	<1X LOD	1.2×10^3	1/5	1/5	0/5
		1X LOD	2.6×10^3	4/4	2/4	0/4
		5X LOD	1.3×10^4	3/3	1/3	2/3
Salmonella abongori (repeated)	43975	1X LOD	2.3×10^4	10/10	10/10	3/10
		5X LOD	1.1×10^5	5/5	5/5	4/5

[a] One replicate in this set correctly identified *Salmonella,* but additionally detected *C. coli/jejuni.*
[b] One replicate in this set correctly identified *Salmonella* , but additionally detected *C. coli/jejuni* & Shiga toxin 1

Fresh vs. Frozen Study Summary (*Campylobacter* spp.)

Organism	ATCC ID	Input Conc. (XLOD)	CFU/mL	Correct SBPP Results		
				T0= Fresh Pre-Freeze	T1= 1X Freeze/Thaw	T2 = 2X Freeze/Thaw
Campylobacter coli	43486	<1XLOD	4.2×10^2	3/5	1/5	1/5
		1XLOD	1.8×10^3	10/10	8/10	–
		5XLOD	1.1×10^4	9/9*	3/5	1/5
Campylobacter jejuni	49943	<1XLOD	2.0×10^1	2/3	0/3	0/3
		1XLOD	2.4×10^3	4/4	3/4	1/4
		5XLOD	1.2×10^4	3/3	3/3	1/3
Campylobacter jejuni (repeated)	49943	<1XLOD	1.0×10^2	5/8	0/5	–
		1XLOD	1.5×10^3	9/9	4/10	–
		5XLOD	1.3×10^4	5/5	5/5	1/5

* This set of test runs also contained 1 "Invalid" Run

Media Equivalency:

A Media Equivalency Study was conducted to demonstrate equivalent SBPP performance in five (5) prevalent stool preservation media types:

- Thermo Scientific™ Remel™ Cary Blair Transport Medium
- Meridian™ Para-Pak® Enteric Plus Transport Medium
- Meridian™ Para-Pak® Culture & Sensitivity (C&S) Medium
- Thermo Scientific™ Protocol™ Cary Blair Medium
- Thermo Scientific™ Protocol™ Culture & Sensitivity (C&S) Medium

To generate the unique stool matrices for each media type, raw clinical stool specimens, that previously tested negative for SBPP targets, were preserved in each preservation medium, listed above, per the Manufacturer's instructions. The subsequent stool matrices were used as the base for contrived positive samples, and the clinical negative sample, for each media type.

The Media Equivalency Study was designed to demonstrate equivalent SBPP test performance in the 5 media types, by evaluating eight (8) strains for which LoD was initially measured. The 8 strains included:

- *C. coli* (ATCC 43486)
- *C. jejuni* (ATCC 49443)
- *E. coli* (ATCC BAA-2196 *stx1+/stx2+*/non-O157)
- *E. coli* (ATCC 43895 *stx1+/stx2+*/O157+)
- *S. bongori* (ATCC 43975)
- *S. enterica* (ATCC 13311)
- *S. flexneri* (ATCC 25929)*S. sonnei* (ATCC 29930)

All stool transport media tested: Remel™ Cary Blair Transport Medium; Protocol™ Cary Blair Medium; Protocol™ Culture & Sensitivity (C&S) Medium; Para-Pak® Enteric Plus Transport Medium; and Para- Pak® Culture & Sensitivity (C&S) Medium, performed as expected, and each test medium type demonstrated equivalent performance to all other test media. At 5X LoD, for all strains tested, there was 100% agreement with the expected results in all five (5) media types. Likewise, at 2X LoD there was ≥95% agreement with the expected results for all strains tested across all five (5) media types. Also, as expected, the percent agreement for strains below LoD (<1X LoD) varied from 10% to 90% across these five (5) media types. All the media types tested, therefore, are compatible with the SBPP Test.

Media Equivalency Study

Results			Correct SBPP Results for Different Media Types				
Species	ATCC ID	Sample Input Conc. (X LoD)	Remel Cary Blair	Protocol Cary Blair	Protocol C&S	Para-Pak C&S	Para-Pak Enteric Plus
Campylobacter coli	43486	<1X	7/10	4/10	5/10	7/10	7/10
		2X	30/30	30/30	30/30	30/30*	30/30
		5X	10/10	10/10	10/10	10/10	10/10
Campylobacter jejuni	49443	<1X	7/10	5/10	7/10	6/10	3/10
		2X	30/30	30/30	30/30	30/30	30/30
		5X	10/10	10/10[a]	10/10	10/10	10/10
E. coli (stx1+/stx2+/ non-O157*)*	BAA-2196	<1X	8/10*	9/10	6/10	4/10	2/10
		2X	29/30*	29/30*	29/30	29/30	30/30
		5X	10/10	10/10	9/9*	10/10	19/20
E. coli (stx1+/stx2+/O157+)	43895	<1X	26/30	1/10	9/10	8/10	3/10
		2X	30/30	30/30	29/30	30/30	29/30
		5X	10/10	10/10	9/9	10/10	10/10
Salmonella bongori	43975	<1X	4/11	2/10	1/10	5/10	2/10
		2X	29/29	30/30	30/30	27/27	30/30
		5X	10/10	10/10	10/10	10/10	10/10
Salmonella enterica	13311	<1X	7/10	6/9	9/10	6/10	9/10
		2X	46/50	40/40	38/40	30/30	30/30
		5X	19/20*	10/10	9/9	10/10	10/10
Shigella flexneri	25929	<1X	1/10	4/10	7/10	1/10	7/10
		2X	30/30	30/30	30/30[c]	30/30	30/30[d]
		5X	10/10	10/10	10/10	10/10	10/10
Shigella sonnei	29930	<1X	1/10	10/11[b]	7/10	6/10	8/10
		2X	28/29	30/30	30/30	30/30	30/30
		5X	10/10	10/10	10/10	10/10	10/10
Clinical Negative		Negative	0/10	0/10	0/10	0/10	0/10

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

[a] One replicate detected *C. jejuni* correctly, but additionally detected Shiga toxin 2 (Protocol Cary Blair Media)

[b] One replicate detected *S. sonnei* correctly, but additionally detected Shiga toxin 1, Shiga toxin2, and Serotype C (Protocol Cary Blair Media)

[c] One replicate detected *S. flexneri* correctly, but additionally detected *C. coli/jejuni* (Protocol C&S)

[d] One replicate in this set correctly identified *S. flexneri* , but additionally detected *C. coli/jejuni* (Para-Pak Enteri

d. Detection limit:

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. The LoDs for each strain tested is shown in the table below

Limit of Detection by Analyte

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

Inclusivity/ Reactivity:

The analytical reactivity of the SBPP was assessed by testing an additional 91 well characterized bacterial strains representing the organisms detected in the SBPP. Organisms were diluted to a concentration of 2X LoD into pooled negative preserved clinical stool matrix. The additional organisms shown in the table below were detected in the SBBP.

Organisms Tested for Inclusivity

Organism/Serotype	Total Number	Concentration 2X LoD (CFU/mL)
Campylobacter coli	5	3.6×10^3
*Campylobacter jejuni (*subsp. *jejuni,* subsp. *doylei)*	6	2.6×10^3
*Salmonella enterica (*subsp. *enterica: Typhi, Newport, Choleraesuis, Stanley, Heidelber, Muenchen, Paratyphi B, Bareilly, Kentucky, Saint Paul, Tennessee, Paratyphi A, Typhimurium, Choleraesuis, arizonae, Typhimurium, Dublin, houtenae, Virchow, Agona, Bristol, Montevideo, Infantis, Mississippi,* subsp. *indica,* subsp. *salame,* subsp. *diarizonae,* subsp. *houtanae), Salmonella bongori*	33	3.8×10^4
*Shigella boydij (*Serotypes 1, 1a, 2, 2a, 3, 5, 6, 8), *Shigella sonnei, Shegella dysenteriae* (Serotypes 1, 2, 3, 12, 13)	20	2.8×10^4
Shiga-toxin producing *E. coli*		
O11:H8, O26:H11, O21:H19, O111	4	2.2×10^4
O111, O26:H11, O45:H2, O45:H2, O123:H25, O103:H2, O103:H11	6	1.4×10^4
O91:H21, 0113:H21, O145:H28, O145:H25, O121:H19, O104:H4	6	8.8×10^3
O157:H7, O157:H7, O157:NM, O157:H7:K, O157:H7, O157:NM, O157:H7, O157:NM	8	3.2×10^4
Shiga-toxin producing *Shigella dysentariae*		
Type 1	3	1.4×10^4

e. Analytical specificity:

The potential for cross-reactivity was evaluated in an Exclusivity Study, by testing non-target organisms commonly found in stool. 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 $\geq 1.0 \times 10^6$ CFU/mL. Genomic DNA (gDNA) templates, viral strains, and parasites, were tested at ≥1 ug/mL, $\geq 1 \times 10^6$ copies/mL, or $\geq 1 \times 10^5$ $TCID_{50}$/mL, respectively.

All but one of the organisms shown gave the expected "Not Detected" result indicating that there was no cross-reactivity in the SBPP. The one exception was Enteroinvasive *Escherichia coli* (EIEC) which was "Detected" in the SBPP. This cross-reactivity was expected since the gene target used to detect *Shigella* spp. *(ipaH)* is also present EIEC.

Organisms Tested for Exclusivity

Bacteria			
Genus	**Species**	**Genus**	**Species**
Abiotrophia	*defectiva*	*Proteus*	*penneri*
Acinetobacter	*baumannii*	*Proteus*	*vulgaris*
Aeromonas	*hydrophila*	*Providencia*	*alcalifaciens*
Anaerococcus	*tetradius*	*Providencia*	*rettgeri*
Bacillus	*cereus*	*Providencia*	*stuartii*
Bacteriodes	*fragilis*	*Pseudomonas*	*aeruginosa*
Bacteriodes	*vulgatus*	*Pseudomonas*	*putida*
Bifidobacterium	*adolescentis*	*Ruminococcus*	*bromii*
Bifidobacterium	*bifidum*	*Serratia*	*liquefaciens*
Bifidobacterium	*longum*	*Serratia*	*marcescens*
Campylobacter	*curvus (gDNA)*	*Staphylococcus*	*aureus*
Campylobacter	*fetus*	*Staphylococcus*	*epidermidis*
Campylobacter	*fetus* subsp. *venerealis*	*Stenotrophomonas*	*maltophilia*
Campylobacter	*hyointestinalis*	*Streptococcus*	*agalactiae*
Campylobacter	*lari* (5 strains)	*Streptococcus*	*dysgalactiae*
Campylobacter	*upsaliensis*	*Streptococcus*	*intermedius*
Citrobacter	*amalonaticus*	*Streptococcus*	*pyogenes*
Citrobacter	*freundii*	*Streptococcus*	*uberis*
Clostridium	*difficile*	*Trabulsiella*	*guamensis*
Clostridium	*histolyticum*	*Veillonella*	*parvula*
Clostridium	*perfringens*	*Vibrio*	*cholera*
Clostridium	*sordellii*	*Vibrio*	*parahaemolyticus*
Enterobacter	*aerogenes*	*Vibrio*	*vulnificus*
Enterobacter	*cloacae*	*Yersinia*	*bercovieri*
Enterococcus	*cecorum*	*Yersinia*	*enterocolitica*
Enterococcus	*faecalis*	*Yersinia*	*pseudotuberculosis*
Enterococcus	*faecium*	*Yersinia*	*rohdei*
EAEC Escherichia	*coli*	**Fungi**	
Escherichia	*coli*	*Candida albicans*	
EIEC Escherichia	*coli* (3 strains)	*Candida catenulata*	
ETEC Escherichia	*coli*	*Saccharomyces boulardii*	
Escherichia	*fergusonii*	**Viruses and Parasites**	
Escherichia	*hermannii*	Adenovirus Type 2 (gDNA)	
Fusobacterium	*varium*	Adenovirus type 40, strain Dugan (gDNA)	
Gardnerella	*vaginalis*	Adenovirus type 41, strain Tak	
Helicobacter	*fennelliae*	Coxsackie B4	
Helicobacter	*pylori*	*Cryptosporidium parvum* (gDNA)	
Klebsiella	*pneumoniae*	*Entamoeba histolytica* (gDNA)	
Klebsiella	*oxytoca*	Enterovirus (RNA)	
Lactobacillus	*acidophilus*	*Giardia intestinalis* (gDNA)	
Lactobacillus	*casei*	Norovirus GI (synthetic RNA)	
Leminorella	*grimonti*	Norovirus GII (synthetic RNA)	
Listeria	*grayi*	Rotavirus	
Listeria	*innocua*	Rotavirus A (RNA)	
Listeria	*monocytogenes*	Human genomic DNA	
Morganella	*morganii*		
Peptostreptococcus	*anaerobius*		

Bacteria			
Genus	**Species**	**Genus**	**Species**
Plesiomonas	*shigelloides*		
Porphyromonas	*asaccharolytica*		
Prevotella	*melaninogenicus*		
Proteus	*mirabilis*		

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 combinations and concentrations tested along with the study results are shown in the table below.

Competitive inhibition was observed for Salmonella when *E. coli* (*stx1+/stx2+*/O157+) was present at concentrations ≥ 1 x 10^6 CFU/mL.

Competitive Inhibition

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.

Non-microbial Interference:

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. The list of potentially interfering substances along with the concentrations tested is shown below. No interference in the SBPP was observed for the substances tested shown in the table below at the concentrations listed.

Potential Intefereins Substances Tested

Substance	Concentration Tested
Ampicillin	50 mg/mL
Bacitracin Zinc ointment	50 mg/mL
Benzalkonium chloride, ethanol (moist towelettes)	9.5% v/v
Bovine Mucin	6.25 mg/mL
Calcium carbonate	200 mg/mL
Cholesterol	5% v/v
Hemoglobin	10% w/v
Human Whole Blood	50% v/v
Hydrocortisone	75 mg/mL
Imodium	10% v/v
Kaopectate	10% v/v
Milk of Magnesia	5% v/v
Mineral Oil	50% v/v
Naproxen Sodium	9.5% w/v
Nystatin	5% v/v
Pepto-Bismol	10% v/v
Pork Mucin	6.25 mg/mL
Sennosides	9.7 mg/mL
Triglycerides	10% v/v

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 was tested in the presence of each of the analytes detected in the SBPP.

Potentially interfering bacteria/fungi, viruses, and DNA were added at $\geq 10^6$ CFU/mL, $\geq 1 \times 10^6$ copies/mL, and ≥ 1 µg/mL, respectively, to pooled, negative, preserved, clinical stool with a single SBPP target analyte added at ≤ 3X LoD. 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.

No interference from non-target organisms was observed in the SBPP for any of the samples tested.

Organisms Tested for Microbial Interference

Bacteria		Yeasts, Parasites, Viruses and DNA
Aeromonas hydrophilia	*Enteropathogenic E. coli* (EPEC)	*Candida albicans*
Bacteroides fragilis	*Helicobacter pylori*	*Saccharomyces boulardii*
Bacteroides vulgatus	*Klebsiella pneumonia*	*Entamoeba histolytica* (gDNA)
Bifidobacterium bifidum	*Lactobacillus acidophilus*	*Giardia intestinalis* (gDNA)
Clostridium difficile (toxinA/B)	*Listeria monocytogenes*	Enterovirus 71 (RNA)
Clostridium perfringens	*Prevotella melaninogenica*	Norovirus G1 (synthetic RNA)
Enterobacter aerogenes	*Pseudomonas aeruginosa*	Rotavirus (RNA)
Enterococcus faecalis	*Staphylococcus aureus*	Human genomic DNA
Escherichia coli (non-STEC O157)	*Vibrio cholera*	
Enteroaggregative E. coli (EAEC)	*Yersinia enterocolitica*	
Enterotoxigenic E. coli (ETEC)		

Carryover/Cross-Contamination:

To evaluate potential carry-over/cross-contamination of the SBPP a Carry-over Study was conducted. Contrived stool samples containing a high concentration of an analyte were alternated with clinical negative stool samples 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 $\geq 1 \times 10^6$ 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. No carry-over or cross contamination was observed in the SBPP.

f. Assay cut-off: N/A

2. Comparison studies:

a. Method comparison with predicate device: **N/A**

b. Matrix comparison: See M1c.3.

3. Clinical studies:

a. Clinical Sensitivity:

The clinical evaluation of the SBPP consisted of a prospective and a frozen retrospective method comparison study. An additional study using selected fresh positive *Salmonella* samples was also performed.

The 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 that site were collected and tested.

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 *Salmonella* study) along with the 95% Confidence Intervals are shown in table below.

Summary of Clinical Study Results

Specimen		n	% Agreement (95% CI)	
			Positive	Negative
Campylobacter	Fresh	1479	96.4% (82.3-99.4) 27/28[1]	99.2% (98.6-99.5) 1439/1451[2]
	Frozen	0	N/A	N/A
Salmonella	Fresh	1479	83.3% (55.2-95.3) 10/12[3]	99.6% (99.1-99.8) 1461/1467[4]
	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[5]
	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[6]
	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[7]
	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[8]
	Frozen	206	94.7% (75.4-99.1) 18/19	100.0% (98.0-100.0) 187/187

[1] *Campylobacter* DNA sequence was detected in 0/1false negative specimens when tested with an alternate, FDA-cleared NAAT

[2] *Campylobacter* DNA sequence was detected in 10/12 false positives specimens when tested with an alternate, FDA-cleared NAAT

[3] Salmonella DNA sequence was detected in 2/2 false negative specimens when tested with an alternate, FDA-cleared NAAT

[4] Salmonella DNA sequence was detected in 6/6 false positives specimens tested with an alternate, FDA-cleared NAAT

[5] Stx1 DNA sequence was detected in 6/7 false positive specimens when tested with an alternate, FDA-cleared NAAT

[6] Stx2 DNA sequence was detected in 8/9 false positive specimens when tested with an alternate, FDA-cleared NAAT

[7] *E. coli* O157 DNA sequence was detected in 3/3 false positive specimens when tested with an alternate, FDA-cleared NAAT

[8] *Shigella* DNA sequence was detected in 14/14 false positive specimens when tested with an alternate, FDA-cleared NAAT

Detection of Multiple Target Analytes

The tables below identify samples from the Prospective Study for which multiple target analytes were detected either by SBPP or the Reference Test. Each table also shows the analytes that were discrepant for a given sample.

Samples with Multiple Analytes as Determined by SBPP

Sample ID	Multiple Target Analytes as Determined by SBPP		Discrepant Results (Analytes not Detected by Reference Test)
	SBPP Detected Analytes	Reference Test Detected Analytes	
MCW-113	Stx2 *E. coli* Serotype O157	*E. coli* Serotype O157	Stx 2
LACNY-272	Stx2 *E. coli* Serotype O157	*E. coli* Serotype O157	Stx 2
LACNY-349	Stx2 *E. coli* Serotype O157	*E. coli* Serotype O157	Stx 2
LACNY-467	Stx2 *E. coli* Serotype O157	No Analytes Detected	Stx2 *E. coli* Serotype O157
TRCL-416	Stx2 *E. coli* Serotype O157	Stx2 *E. coli* Serotype O157	No Discrepant Results
TRCL-177	Stx2 *Salmonella*	No Analytes Detected	Stx2 *Salmonella*
CHLA-122	Stx1 *E. coli* Serotype O157	No Analytes Detected	Stx1 *E. coli* Serotype O157
CHLA-43	Stx1 *Shigella*	*Shigella*	Stx1
MCW-142	Stx1 *E. coli* Serotype O157 *Shigella*	No Analytes Detected	Stx1 *E. coli* Serotype O157 *Shigella*
CHLA-4	Stx1 Stx2	No Analytes Detected	Stx1 Stx2
CHLA-143	Stx1 Stx2	Stx1	Stx2

Samples with Multiple Analytes as Determined by Reference Test

Sample ID	Multiple Target Analytes as Determined by the Reference Test		Discrepant Results (Analytes not Detected by SBPP)
	Reference Test Detected Analytes	SBPP Detected Analytes	
TRCL-416	Stx2 *E. coli* Serotype O157	Stx2 *E. coli* Serotype O157	No Discrepant Results

b. Clinical specificity: See M3a.

4. Clinical cut-off**:** N/A

5. Expected values/Reference range:

In the SBPP clinical study, 1479 prospectively collected fresh specimens which were obtained from four geographically distributed U.S. healthcare institutions (Midwest, Northeast, Southwest, West) were included in the method comparison study. The number and percentage of positive SBPP results for each analyte (overall prevalence rate) are shown in below. The prevalence rate obtained in routine use may vary depending on the institution, geographic location and patient population.

Prevalence Rates of Analytes Detected by SBPP Observed in Clinical Studies

Site	*Campylobacter*	*Salmonella*	Shiga Toxin 1	Shiga Toxin 2	*E. coli* O157	*Shigella*
1	2.3% (8/348)	0.3% (1/348)	0.6% (2/348)	0.9% (3/348)	0.6% (2/348)	2.0% (7/348)
2	3.8% (19/505)	0.1% (5/505)	0.4% (2/505)	0.6% (3/505)	0.6% (3/505)	0.4% (2/505)
3	1.7% (8/465)	1.5% (7/465)	0.0% (0/465)	0.4% (2/465)	0.2% (1/465)	0.9% (4/465)
4	2.5% (4/161)	1.9% (3/161)	2.5% (4/161)	1.2% (2/161)	0.6% (1/161)	3.7% (6/161)
Total	2.6% (39/1479)	1.1% (16/1479)	0.5% (8/1479)	0.7% (10/1479)	0.5% (7/1479)	1.3% (19/1479)

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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