510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

K152955

B. Purpose for Submission:

To obtain a substantial equivalence determination for a new device

C. Measurand:

Shiga toxin 1 and Shiga toxin 2 gene virulence markers for the identification of Shiga toxin-producing *Escherichia coli* (STEC), including gene markers of the *E. coli* O157 serotype within STEC

D. Type of Test:

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

E. Applicant:

Great Basin Corporation

F. Proprietary and Established Names:

Great Basin Shiga Toxin Direct Test

G. Regulatory Information:

1. Regulation section:

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

2. Classification:

   II

3. Product code:

   PCH, OOI

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Great Basin Shiga Toxin Direct Test performed on the Portrait Analyzer is an automated, in vitro diagnostic assay for the qualitative detection of Shiga Toxin 1 (*stx1*)/ Shiga Toxin 2 (*stx2*) genes and specific identification of a conserved genetic region of the *E. coli* O157 serogroup. Shiga Toxin genes are found in Shiga Toxin producing strains of *E. coli* and *Shigella dysenteriae.*

   The *E. coli* O157 test result is reported only if a Shiga Toxin gene is also detected. The test is performed directly from Cary Blair or C&S Medium preserved stool specimens from symptomatic patients with suspected acute gastroenteritis, enteritis, or colitis in hospital laboratories. The assay is intended for use in conjunction with clinical presentation as an aid in the diagnosis of STEC infections. Positive results do not rule out co-infection with other organisms, and may not be the definitive cause of patient illness.

   The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Shiga Toxin Direct Test negative results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

2. Indication(s) for use:

   The Great Basin Shiga Toxin Direct Test performed on the Portrait Analyzer is an automated, in vitro diagnostic assay for the qualitative detection of Shiga Toxin 1 (*stx1*) /Shiga Toxin 2 (*stx2*) genes and specific identification of a conserved genetic region of the *E. coli* O157 serogroup. Shiga Toxin genes are found in Shiga Toxin producing strains of *E. coli* and *Shigella dysenteriae.*

   The *E. coli* O157 test result is reported only if a Shiga Toxin gene is also detected. The test is performed directly from Cary Blair or C&S Medium preserved stool specimens from symptomatic patients with suspected acute gastroenteritis, enteritis, or colitis in hospital laboratories. The assay is intended for use in conjunction with clinical presentation as an aid in the diagnosis of STEC infections. Positive results do not rule out co-infection with other organisms, and may not be the definitive cause of patient illness.

   The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Shiga Toxin Direct Test negative results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative

colitis, irritable bowel syndrome, or Crohn's disease.

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 Portrait System is an automated system that includes the Portrait Analyzer, single-use Great Basin Shiga Toxin Direct Test cartridges, and the Portrait System data analysis software. The Portrait System is designed to perform automated sample preparation, PCR, and optical chip-based detection with integrated data analysis in approximately two hours.

The single-use Test Cartridge contains blister packs, fluidic channels, processing chambers, a waste chamber, and an assay chip coated with an array of sequence-specific detection probes. All reagents are contained within the integrated blister packs with the exception of the amplification reagents and SPC, which are dried into the Amplification Chamber and SPC Chambers of the Cartridge, respectively.

The appropriate specimen for use in the Test Cartridge is an aliquot of stool from symptomatic patients preserved in Cary-Blair or C&S transport media. A preserved stool specimen is placed into the sample port of the Test Cartridge for processing. Multiple fluidic channels move reagents from integrated blister packs to chambers where reagent mixing and sample processing occur. A waste chamber, self-contained and segregated within the Test Cartridge, collects and stores reagent waste.

Reagents and materials provided:

- Portrait™ STEC Assay Test Cartridge Kit - Each test cartridge pouch with integrated reaction buffers

Additional materials required but not provided:

- Portrait System Analyzer and Operator Manual
- Compatible Computer with Microsoft Windows® application, or stand-alone PC
- Compatible Printer
- Calibrated, fixed volume pipette

J. Substantial Equivalence Information:

1. Predicate device name(s):

   FilmArray® Gastrointestinal (GI) Panel

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

   K140407

3. Comparison with predicate:

Similarities		
Item	Device Portrait Shiga Toxin Direct Test	Predicate FilmArray GI Panel (K140407)
Intended use	Detection of nucleic acids and toxin gene sequences from enteric pathogens in transport media preserved stool specimens from patients with symptoms of gastrointestinal infection	Same (See below for target differences)
Target DNA detected	Shiga Toxin 1 (*stx1*) /Shiga Toxin 2 (*stx2*) genes found in Shiga Toxin producing strains of *E. coli* and *Shigella dysenteriae.* A genetic region of the *E. coli* O157 serogroup within STEC.	Similar (See below for noted differences)
Test Interpretation	Automated	Same
Qualitative/ Quantitative	Qualitative	Same
Technology	Nucleic acid amplification and detection	Same (See below for differences)
Specimen Types	Human stool specimens preserved in Cary Blair or C&S transport media	Similar (See below for differences)
Test cartridge	Disposable, single-use, self-contained fluidic test cartridge	Same

Differences		
Item	Device Portrait Shiga Toxin Direct Test	Predicate FilmArray GI Panel (K140407)
Target DNA detected	Shiga Toxin 1 (*stx1*)/Shiga Toxin 2 (*stx2*) genes found in Shiga Toxin producing strains of *E. coli* and *Shigella dysenteriae.* A genetic region of the *E. coli* O157 serogroup within STEC.	*Campylobacter* (*C. jejuni/C. coli/C. upsaliensis*), *Clostridium difficile* (*C. difficile*) toxin A/B, *Plesiomonas shigelloides, Salmonella, Vibrio* (*V. parahaemolyticus/V.*

Differences		
Item	Device Portrait Shiga Toxin Direct Test	Predicate FilmArray GI Panel (K140407)
		vulnificus/ V. cholerae), including specific identification of *Vibrio cholera, Yersinia enterocolitica,* Enteroaggregative *Escherichia coli* (EAEC), Enteropathogenic *Escherichia coli* (EPEC), Enterotoxigenic *Escherichia coli* (ETEC) *lt/st,*, *Shigella*/ Enteroinvasive *Escherichia coli* (EIEC), *Cyclospora cayetanensis,· Cryptosporidium* (genus claim), *Entamoeba histolytica, Giardia lamblia,* Adenovirus F 40/41, Astrovirus,·Norovirus GI/GII, Rotavirus A, Sapovirus (Genogroups I, II, IV, and V)
Instrument platform	PA500 Portrait Analyzer	FilmArray Instrument
Specimen Types	Stool specimens preserved in Cary Blair or C&S transport media	Stool specimens preserved in Cary Blair transport medium
Analyte	DNA	DNA/RNA
Amplification technology	Multiplex Polymerase Chain Reaction (PCR)	Nested multiplex RT-PCR
Detection technology	Colorimetric target specific hybridization to probe on a chip surface, optical reader, automated software with built-in result interpretation	High resolution melting analysis to confirm identity of amplified product with automated software with built-in result interpretation.
Time to result	Approximately two hours	Less than one hour

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

Not applicable

L. Test Principle:

The 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.

Target genomic DNA is extracted from preserved stool specimens alongside sample processing control cells (SPC) and diluted to reduce potential inhibitors of the PCR reaction. During the PCR process, double-stranded DNA is separated and target nucleic acid sequences are amplified by thermal cycling. Biotin-labeled primers direct amplification of specific nucleic acid sequences within a conserved region of the *stx1*, *stx2*, and O157 antigen-specific genes for identification of Shiga toxin producing *E. coli*.

Following the PCR process, biotin-labeled, amplified target DNA sequences are hybridized to an array of probes immobilized on the silicon chip surface, then incubated with anti-biotin antibody conjugated to the horseradish peroxidase enzyme (HRP). These probes are specific for Shiga toxin 1 (*stx1*), Shiga toxin 2 (*stx2*), an O157 antigen marker gene, and the SPC. The unbound conjugate is removed by washing 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 Portrait Analyzer. While the Shiga Toxin Direct Test is designed to detect and distinguish between *stx1* and *stx2* toxin types, the assay does not report results to the individual toxin level.

M. Performance Characteristics:

1. Analytical performance:

   a. Reproducibility:

   Reproducibility testing of the Shiga Toxin Direct Test was conducted using a panel of four positive samples and one negative sample. The positive panel members consisted of two Shiga toxin-producing *E. coli* (STEC) strains: ATCC BAA- 2192 (O145:NM) and ATCC strain 43895 (O157:H7), each at a moderate positive concentration (~3X LoD) and a low positive concentration (~1.5X LoD). The positive samples were contrived by spiking enriched broth cultures of known concentration into negative clinical stool matrix consisting of clinical Shiga toxin negative stool preserved in ParaPak® C&S media. The negative samples consisted of clinical negative stool matrix.

   The reproducibility studies were performed at three external clinical sites using

randomized, blind-coded panels and two different Shiga Toxin Direct Test cartridge lots. The studies were performed over the course of five (5), nonconsecutive days. For each day of testing, two (2) panel runs were performed with three (3) replicates of each sample per run on each day. A minimum of two (2) operators performed the testing at each site.

For moderate and low positive ATCC BAA-2192 samples, % agreement = "STEC Positive; O157 Negative" calls per total sample runs. For moderate and low positive ATCC 43895 samples, % agreement = "STEC Positive; O157 Positive" calls per total sample runs. For clinical negative samples, % agreement = "STEC Negative; O157 Not Tested" calls per total samples runs.

The Shiga Toxin Direct Test results agreed with the expected results 100% across all three sites, with the exception of a single Low Positive replicate for ATCC BAA-2192 that produced a 'STEC POSITIVE/Serotype O157 POSITIVE' test result instead of the expected result of 'STEC POSITIVE/Serotype O157 NEGATIVE'.

The invalid and incomplete test rates for the reproducibility study were 1.1% (5 invalid runs/ 458 total runs) and 0.7% (3 test incomplete runs/ 458 total runs), respectively. In all eight (8) instances, the sample was re-tested on a new cartridge according to the package insert and each resolved to the expected result. The reproducibility study results were acceptable. The results of the reproducibility studies are summarized in Table 1.

Table 1. Overall Site-to-Site Reproducibility Results

Panel	**Expected result**	**% Agreement**							
		Site 1		**Site 2**		**Site 3**		**All Sites**	
Moderate Positive ATCC BAA-2192	STEC Positive/ Serotype O157 Negative	30/30	100%	30/30	100%	30/30	100%	90/90	100%
Low Positive ATCC BAA-2192	STEC Positive/ Serotype O157 Negative	30/30	100%	30/30	100%	29/30	97%	89/90	99%
Moderate Positive ATCC 43895	STEC Positive/ Serotype O157 Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%
Low Positive ATCC 43895	STEC Positive/ Serotype O157 Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%
Clinical Negative	STEC Negative/ Serotype O157 Not Tested	30/30	100%	30/30	100%	30/30	100%	90/90	100%

b. Linearity/assay reportable range:

Not applicable

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 and are not included in the package insert.

1. Commercially available ATCC Shiga toxin producing *E. coli* strains, for example ATCC 700376 (*E. coli* O157:NM, stx1+) and ATCC 51434 (*E. coli* O91:H21, stx2+), which have been diluted into Cary Blair or C&S media, are recommended for use as positive controls.

2. An aliquot of Cary Blair or C&S media may be used as a Negative Control. Alternatively, a known negative stool sample preserved in Cary Blair or C&S media may be used.

The external Positive Control is intended to monitor for substantial reagent failure. The external Negative Control is intended to confirm non-reactivity. An 'INVALID' result for any controls invalidates the Test result. Good laboratory practice recommends the use of control samples. The Shiga Toxin Direct Test should not be used in patient testing if the appropriate controls do not produce the expected results. If the external controls do not produce the expected results, the test should be repeated with a new Test Cartridge.

Daily Quality Control (QC) testing was conducted at each site during prospective and frozen retrospective clinical testing. The daily QC panel consisted of one negative and two positive samples to control for all assay outcomes. A total of 164 negative controls and 328 positive controls were tested across six test sites producing valid expected initial results for 163 (99.4%) and 317 (96.6%) negative controls and positive controls, respectively. One negative control and two positive controls produced initial 'Test Incomplete' results (0.6%). Seven positive controls produced initial 'Invalid' results (2.1%), and two positive controls produced valid but incorrect results (0.6%). All initially invalid and unexpected results (100%) resolved to the expected result upon re-test.

Specimen Processing Control (SPC):

The SPC controls for all analytical steps in the procedure, including: DNA extraction from organisms present in the stool specimen, PCR amplification of target DNA sequences, hybridization, and detection on the chip surface. The SPC contains *B. subtilis* cells in the form of a lyophilized cake that is incorporated directly into each Test Cartridge to verify adequate processing of each clinical stool specimen sample. If enteric microorganisms (such as bacteria including but not limited to *E. coli*) are found in the stool specimen, the SPC verifies that proper cell lysis has occurred and verifies that specimen processing is adequate. Additionally, SPC detects inhibition of

PCR reactions, ensuring the PCR reaction conditions are appropriate and that the amplification reagents are functional. The SPC signal should be positive in a sample that is negative for Shiga Toxin Direct Test analyte(s), and can be either negative or positive in a sample containing analyte(s).

Specimen stability:

The recommended storage time and temperature conditions for transport media preserved stool specimens prior to testing via the Shiga Toxin Direct Test include:

- Refrigerated storage (2°- 8° C) for up to 120 hours (5 days).
- Room temperature (25 °C ± 2 °C) storage for up to 4 hours.
- The combination of up to 4 hours storage at room temperature followed by refrigerated storage for up to 120 hours.

To assess the stability of the Shiga toxin and serotype O157 nucleic acid targets of the Shiga Toxin Direct Test under the recommended storage conditions, a specimen stability study was performed to evaluate the recommended time and temperature storage conditions. The study tested two non-O157 STEC strains (ATCC BAA-2191 and ATCC 51434) and one O157 STEC strain (ATCC 43889). Each sample was contrived from freshly cultured STEC cells spiked at 2X LoD into negative stool matrix and stored under the recommended storage conditions. The LoD was approximated for the O157 STEC strain (ATCC 43889). A second O157 STEC strain (ATCC 43890) was tested but was not included in the final analysis when baseline (T_0) testing results of 80% positivity indicated that the strain was spiked at concentrations below 2X LoD. At five pre-defined testing time points, an aliquot of each sample was removed and evaluated on the Shiga Toxin Direct Test. The results of the specimen stability study support the recommended storage time and temperature conditions for transport media preserved stool specimens. The results of Specimen Stability Testing are summarized in Table 2.

Table 2. Specimen Stability Study Results

	% Agreement		
Shiga toxin-producing *E. coli* (STEC) Strain Tested	**ATCC BAA-2191 (*stx1*+)**	**ATCC 51434 (*stx2*+)**	**ATCC 43889 (*stx2*+/O157)**
Concentration (2X LoD)	**1.1 x 10^4 CFU/mL**	**6.0 x 10^3 CFU/mL**	**1.0 x 10^4 CFU/mL**
Expected Shiga Toxin Direct Test Result	**STEC POSITIVE/ Serotype O157 NEGATIVE**		**STEC POSITIVE/ Serotype O157 POSITIVE**
T_0: 0 hr	100% (5/5)	100% (5/5)	100% (10/10)
T_1: 4 hr Room Temp.	100% (5/5)	100% (5/5)	100% (10/10)
T_2: 24 hr 2°- 8° C	100% (5/5)	100% (5/5)	100% (10/10)
T_3: 72 hr 2°- 8° C	100% (5/5)	100% (5/5)	100% (10/10)
T_4: 120 hr 2°- 8° C	100% (5/5)	100% (5/5)	100% (10/10)
T_5: 4 hr Room Temp. + 120 hr 2°- 8° C	100% (5/5)	100% (5/5)	100% (10/10)
Overall	100% (30/30)	100% (30/30)	100% (60/60)

Fresh versus Frozen:

A Fresh vs. Frozen Study was performed to support the use of frozen, transport media preserved stool specimens in the Shiga Toxin Direct Test for the Frozen Retrospective and Reproducibility Studies, as well as for follow-up testing of prospective samples.

The Fresh vs. Frozen Study tested the performance of the Shiga Toxin Direct Test on contrived positive samples that were subjected to two freeze/thaw cycles. The contrived positive samples were prepared using fresh (i.e. never frozen) enriched broth cultures. The panel for the Fresh vs. Frozen Study was comprised of six STEC strains: ATCC BAA-2191 (*stx1*+/O157-), ATCC 51434 (*stx2*+/O157-), ATCC BAA-2192 (*stx1*+/*stx2*+/O157-), ATCC 43890 (*stx1*+/O157+), ATCC 43889 (*stx2*+/O157+), and ATCC 43895 (*stx1*+/*stx2*+/O157+). Each strain was tested in replicate at four concentrations: ≤ 0.5X LoD, 1X LoD, 3X LoD, and 10X LoD. The LoD was approximated for strains ATCC 43890 and ATCC 43889

The panel was initially tested on the Shiga Toxin Direct Test within 30 minutes of construction to establish the 'fresh' activity prior to freezing (T_0). The entire panel was then placed at ≤-70°C for 1 week at which time it was thawed and re-tested (T_1). The entire panel was returned to ≤-70°C for a second freezing cycle for an additional one week at which time the samples were tested for a second, and final, thaw (T_2).

The study results support the testing of stool specimens preserved in C&S media for up to two freeze/thaw cycles. The results of the Fresh vs. Frozen study are summarized in Table 3.

Table 3. Fresh vs. Frozen Study Results

			% Agreement					
Shiga toxin-producing *E. coli* (STEC) Strain	**Expected Shiga Toxin Direct Test Result**	**Conc.**	**T_0 = pre-freeze**		**T_1 = 1X freeze/thaw**		**T_2 = 2X freeze/thaw**	
ATCC BAA-2191 (*stx1*+)	STEC POSITIVE/ Serotype O157 NEGATIVE	10X LoD	2/2	100%	2/2	100%	2/2	100%
		3X LoD	4/4	100%	4/4	100%	4/4	100%
		1X LoD	4/4	100%	4/4	100%	4/4	100%
		≤ 0.5X LoD	3/4	75%	4/4	100%	4/4	100%
ATCC 51434 (*stx2*+)		10X LoD	2/2	100%	2/2	100%	2/2	100%
		3X LoD	4/4	100%	4/4	100%	4/4	100%
		1X LoD	4/4	100%	4/4	100%	4/4	100%
		≤ 0.5X LoD	3/4	75%	3/4	75%	4/4	100%
ATCC BAA-2192 (*stx1*+/*stx2*+)		10X LoD	2/2	100%	2/2	100%	2/2	100%
		3X LoD	4/4	100%	4/4	100%	4/4	100%
		1X LoD	4/4	100%	4/4	100%	4/4	100%
		≤ 0.5X LoD	3/4	75%	4/4	100%	4/4	100%
ATCC 43890⁺ (*stx1*+/O157)	STEC POSITIVE/ Serotype O157 POSITIVE	10X LoD	2/2	100%	2/2	100%	2/2	100%
		3X LoD	4/4	100%	4/4	100%	4/4	100%
		1X LoD	4/4	100%	4/4	100%	3/4	75%
		≤ 0.5X LoD	3/4	75%	2/4	50%	3/4	75%
ATCC 43889⁺ (*stx2*+/O157)		10X LoD	2/2	100%	2/2	100%	2/2	100%
		3X LoD	4/4	100%	4/4	100%	4/4	100%
		1X LoD	4/4	100%	4/4	100%	4/4	100%
		≤ 0.5X LoD	3/4^	75%	2/4	50%	3/4	75%
ATCC 43895 (*stx1*+/*stx2*+/O157)		10X LoD	2/2	100%	2/2	100%	2/2	100%
		3X LoD	4/4	100%	4/4	100%	4/4	100%
		1X LoD	4/4	100%	4/4	100%	4/4	100%
		≤ 0.5X LoD	2/5*	40%	2/4*	50%	4/4	100%

⁺ Limit of Detection (LoD) was approximated for this strain.
^ Represents each 'INVALID' run in this dataset.
* Represents each 'Test Incomplete' run in this dataset.

Media Equivalency:

A Media Equivalency Study was conducted to demonstrate equivalent Shiga Toxin Direct Test performance in six (6) widely used stool preservation media types: Thermo Scientific™ Remel™ Cary Blair Transport Medium, Meridian™ Para-Pak® Enteric Plus Transport System, Thermo Scientific™ Protocol™ Cary Blair Media, Thermo Scientific™ Protocol™ Culture & Sensitivity (C&S) Medium, Meridian™ Para-Pak® 10% Formalin Stool Transport Vial, Meridian™ Para-Pak® Zn PVA Stool Transport Vial.

Analytical Sensitivity (LoD) was established for several strains in Meridian™ Para-Pak® C&S which served as the reference medium. The media equivalency study

evaluated three (3) of the previously characterized STEC strains at concentrations near LoD (2X LoD), above LoD (5X LoD) and below LoD (0.5X LoD) in different media. To generate the unique stool matrices for each media type, raw clinical stool specimens that previously tested negative for Shiga Toxin were preserved in each preservation medium per the manufacturer's instructions. The resulting six stool matrices were evaluated directly as clinical negative samples and as contrived positives.

For the Thermo Scientific™ Remel™ Cary Blair Transport Medium, Meridian™ Para-Pak® Enteric Plus Transport System, Thermo Scientific™ Protocol™ Cary Blair Media, and Thermo Scientific™ Protocol™ Culture & Sensitivity (C&S) Medium, results indicated that the media were equivalent to each other and equivalent to the reference medium (Meridian™ Para-Pak® C&S) for test performance. At 5X LoD, for all strains tested, there was 100% agreement with the expected results in all four (4) media types. Likewise, at 2X LoD there was ≥95% agreement with the expected results for all strains tested across all four (4) media types. Also as expected, the percent agreement for strains below LoD (0.5X LoD) varied from 50% to 100% across these four (4) media types.

The Meridian™ Para-Pak® 10% Formalin Stool Transport Vial media did not produce the expected results for positive or negative samples. At concentrations of STEC strains where a positive Shiga Toxin Direct Test result was expected in ≥ 95% of replicates, all of the testing produced either 'STEC Negative/Serotype O157 Not Tested' (12%) or 'Invalid' (88%) results. In negative stool matrix formulated with this media, only 20% (2/10 replicates) of the Shiga Toxin Direct Test results resolved as the expected 'STEC Negative/Serotype O157 Not Tested' result, and the remaining 80% of negative stool replicates (8/10 replicates) produced 'Invalid' results. The overall invalid rate for initial testing (35 samples in total) was abnormally high at 85.7%, suggesting that 10% Formalin transport media inhibits the Shiga Toxin Direct Test. Due to the evident inhibition and hindered performance of the Shiga Toxin Direct Test, no further testing was conducted on this media type.

The Meridian™ Para-Pak® Zn PVA Stool Transport Vial media also produced aberrant results with the Shiga Toxin Direct Test. At approximately 2X-5X LoD when ≥95% of the Shiga Toxin Direct Test replicates are expected to be 'STEC Positive/Serotype O157 Negative', 100% of the Shiga Toxin Direct Test replicates produced 'Invalid' test results. Similarly, 100% of the negative stool matrix samples formulated with this medium produced 'Invalid' Shiga Toxin Direct Test results, indicating that this transport medium completely inhibits the Shiga Toxin Direct Test. No further testing was conducted on this media type.

A summary of all media types tested and their resultant compatibility with the Shiga Toxin Direct Test is provided in Table 4.

Table 4. Media Equivalency.

Stool Preservation Media that are Compatible with the Shiga Toxin Direct Test
Meridian™ Para-Pak® C&S
Thermo Scientific™ Remel™ Cary Blair Transport Medium
Meridian™ Enteric Plus Transport System
Thermo Scientific™ Protocol™ Cary Blair Media
Thermo Scientific™ Protocol™ Culture & Sensitivity (C&S) Medium
Fixative-containing Media that are not Compatible with Shiga Toxin Direct Test (Interference Observed)
Meridian™ Para-Pak® 10% Formalin Stool Transport Vial
Meridian™ Para-Pak® Zn PVA Stool Transport Vial

d. Detection limit:

The limit of detection (LoD) for four (4) Shiga toxin-producing *E. coli* (STEC) strains was measured for the Shiga Toxin Direct Test. The LoD for each toxin gene, *stx1* and *stx2*, was assessed and measured independently by testing a non-O157 *stx1+ Escherichia coli* strain (ATCC BAA-2191) and a non-O157 *stx2+ Escherichia coli* strain (ATCC 51434), respectively. In addition the LoD for a non-O157 *Escherichia coli* strain containing both toxin genes (*stx1+*/*stx2+*/O157-) was measured (ATCC BAA-2192). Finally, the LoD for an O157 Serotype *Escherichia coli* strain containing both toxin genes (*stx1+*/*stx2+*/O157+) was also measured (ATCC 43895). The LoD for each strain is listed in Table 5.

Table 5. Limit of Detection

Shiga toxin-producing *E. coli* (STEC) Strain	**Shiga Toxin Gene(s) Present**	**Serotype**	**Expected Shiga Toxin Direct Test Result**	**LoD**
ATCC BAA-2191	*stx1+*	O45:H2	STEC POSITIVE/ Serotype O157 NEGATIVE	5.5×10^3 CFU/mL
ATCC 51434	*stx2+*	O91:H21		2.8×10^3 CFU/mL
ATCC BAA-2192	*stx1+*, *stx2+*	O145:NM		5.2×10^3 CFU/mL
ATCC 43895	*stx1+*, *stx2+*	O157:H7	STEC POSITIVE/ Serotype O157 POSITIVE	5.0×10^3 CFU/mL

Inclusivity/reactivity

The inclusivity/reactivity of the Shiga Toxin Direct Test was tested against 30 well-characterized Shiga toxin-producing *E. coli* (STEC) strains representing the serotypes of *E. coli* that are most often associated with disease: serotypes O26, O45, O103, O111, O121, O145, and O157. The Shiga toxin gene (*stx)* which is identical in sequence to the STEC*stx1* gene is also commonly found in *Shigella dysenteriae* serotype 1 strains. Therefore in addition to STEC strains, three (3) serotype 1

Shigella dysenteriae strains were tested.

Cultured organism concentrations were verified by colony counting, spiked into negative clinical stool matrix in Parapak® C&S media at approximately 2XLoD (1.0 x10^4 CFU/mL), and tested in triplicate. The Shiga Toxin Direct Test correctly detected all 21 of the non-O157 Serotype STEC and three (3) Serotype 1 *Shigella dysenteriae* strains as 'STEC POSITIVE/Serotype O157 Negative.' All nine (9) O157 serotype STEC strains were identified as 'STEC POSITIVE/ Serotype O157 POSITIVE'. The inclusivity panel organisms are listed in Table 6.

Table 6. Inclusivity Panel.

Shiga toxin-producing *E. coli* (STEC)					
ATCC Strain	**Serotype**	**Shiga Toxin Gene(s) Present**	**ATCC Strain**	**Serotype**	**Shiga Toxin Gene(s) Present**
BAA-2181	O26:H11	*stx1+*	BAA-2193	O45:H2	*stx1+/stx2+*
BAA-2215	O103:H11	*stx1+*	BAA-2440	O111	*stx1+/stx2+*
BAA-2199	O123:H25	*stx1+*	700840	O111:H8	*stx1+/stx2+*
BAA-2210	O103:H2	*stx1+*	BAA-2192	O145	*stx1+/stx2+*
BAA-2191	O45:H2	*stx1+*	43890	O157:H7	*stx1+*
BAA-2201	O111:H8	*stx1+*	700376	O157:NM	*stx1+*
51435	O91:H21	*stx2+*	43889	O157:H7	*stx2+*
51434	O91:H21	*stx2+*	700377	O157:NM	*stx2+*
BAA-182	O104:H21	*stx2+*	700378	O157:NM	*stx1+/stx2+*
BAA-2326	O104:H4	*stx2+*	700927	O157:H7:K	*stx1+/stx2+*
BAA-183	O113: H21	*stx2+*	43894	O157:H7	*stx1+/stx2+*
BAA-2220	O121:H19	*stx2+*	43895	O157:H7	*stx1+/stx2+*
BAA-2219	O121:H19	*stx2+*	35150	O157:H7	*stx1+/stx2+*
BAA-2211	O145: H25	*stx2+*	***Shigella dysenteriae***		
BAA-2129	O145:H28	*stx2+*	9361	Type 1	*stx+* ^
BAA-2221	O21:H19	*stx1+/stx2+*	27346†	Type 1	*stx+* ^
BAA-2196	O26:H11	*stx1+/stx2+*	27345†	Type 1	*stx+* ^

† Concentration of broth culture estimated from optical density due to lack of growth on plates for exact colony counting.
^ This *Shigella dysenteriae* strain contains the Shiga toxin gene (*stx*) which is identical in sequence to *stx1*; therefore the Shiga Toxin Direct Test reports this strain as 'STEC POSITIVE/ Serotype O157 Negative.'

e. Analytical specificity:

Cross reactivity:

A study was conducted to assess the potential for cross-reactivity of non-target organisms found in stool specimens, including well-known enteric pathogens that present clinically with symptoms similar to STEC, such as diarrhea. In total 104 unique bacterial strains, three (3) yeast, three (3) parasites, seven (7) viruses, and human genomic DNA were evaluated for cross-reactivity. For some organisms that were classified as Biosafety level III or that could not be cultured via standard clinical microbiology techniques, genomic DNA was tested in lieu of whole organism. Each

non-target organism or nucleic acid was tested in the background of negative clinical stool matrix consisting of clinical Shiga toxin negative stool preserved in ParaPak® C&S media (Table 7).

Due to the design of the Sample Processing Control (SPC) in the Shiga Toxin Direct Test, very high concentrations of non-STEC O157 *E. coli* can compete with amplification of the SPC. An SPC amplification failure in the absence of a Shiga toxin target signal will produce an invalid test result. Accordingly, two non-STEC O157:H7 *E. coli* strains (ATCC 43888 and ATCC 700728) produced invalid test results when tested at concentrations greater than or equal to $1.0x10^7$ CFU/mL. When the test concentrations for these strains (ATCC 43888 and ATCC 700728) were lowered to approximately $1.0x10^6$ CFU/mL prior to re-testing, the correct expected result of 'STEC Negative/Serotype O157 Not Tested' was produced. None of the other cultured organisms or genomic DNA that were tested interfered with the internal controls or demonstrated cross reactivity.

Table 7. Cross Reactivity Panel.

Organism	**Strain ID**	**Organism**	**Strain ID**
Bacteria			
Abiotrophia defective	ATCC 49176	*Listeria innocua*	ATCC 33090
Acinetobacter baumannii	ATCC 19606	*Listeria monocytogenes*	ATCC 19115
Aeromonas hydrophila	ATCC 35654	*Morganella morganii*	ATCC 25829
Anaerococcus tetradius	ATCC 35098	*Peptostreptococcus anaerobius*	ATCC 27337
Bacillus cereus	ATCC 14579	*Plesiomonas shigelloides*	ATCC 51903
Bacteroides fragilis	ATCC 23745	*Prevotella melaninogenica*	ATCC 25845
Bacteroides vulgatus	ATCC 8482	*Proteus mirabilis*	ATCC 25933
Bifidobacterium adolescentis	ATCC 15703	*Proteus penneri*	ATCC 33519
Bifidobacterium bifidum	ATCC 11863	*Proteus vulgaris*	ATCC 6896
Bifidobacterium longum	ATCC 15707	*Providiencia alcalifaciens*	ATCC 9886
Camphylobacter coli	ATCC 33559	*Providencia rettgeri*	ATCC 9250
Camphylobacter fetus	ATCC 15296	*Providencia stuartii*	ATCC 49762
Campylobacter jejuni	ATCC 49943	*Pseudomonas aeruginosa*	ATCC 10145
Camphylobacter lari	ATCC 35221	*Pseudomonas mosselii*	ATCC 49838
Citrobacter amalonaticus	ATCC 25406	*Ruminococcus bromii*	ATCC 27255
Citrobacter freundii	ATCC 8090	*Salmonella enterica* subp Arizonae	ATCC 13314
Clostridium difficile (A-, B-)	ATCC BAA-1801	*Salmonella enterica* subp Cholerasuis	ATCC 13312
Clostridium difficile (A+, B+) (gDNA)	ATCC BAA-1382D	*Salmonella enterica* subp Heidelberg	ATCC 8326
Clostridium difficile (A+, B+)	ATCC 43255	*Salmonella enterica* subp Newington	ATCC 29628
Clostridium histolyticum	ATCC 19401	*Salmonella enterica* subp Newport	ATCC 6962
Clostridium perfringens	ATCC 12915	*Salmonella paratyphi* A	ATCC 9150
Clostridium sordellii	ATCC 9715	*Salmonella paratyphi* B	ATCC 8759
Enterobacter aerogenes	ATCC 15038	*Salmonella typhimurium*	ATCC 13311
Enterobacter cloacae	ATCC 13047	*Selenomonas ruminantium*	ATCC 35018
Enterococcus cecorum	ATCC 43198	*Serratia liquefaciens*	ATCC 35551
Enterococcus faecalis	ATCC 29212	*Serratia marcescens*	ATCC 13880
Enterococcus faecium	ATCC 19434	*Shigella boydii*	ATCC 29928
Enteroaggregative *Escherichia coli*	ATCC 29552	*Shigella boydii*	ATCC 12028

Organism	Strain ID	Organism	Strain ID
(EAEC)			
Enteroaggregative *Escherichia coli* (EAEC)	STEC Center JM221	*Shigella dysenteriae* (Type 2)	ATCC 29027
Enteroinvasive *Escherichia coli* (EIEC)	STEC Center 1885-77	*Shigella dysenteriae* (Type 3)	ATCC 29028
Enteroinvasive *Escherichia coli* (EIEC)	ATCC 43892	*Shigella dysenteriae* (Type 12)	ATCC 49551
Enteropathogenic *Escherichia coli* (EPEC)	STEC Center E2348/69	*Shigella dysenteriae* (Type 13)	ATCC 49555
Enteropathogenic *Escherichia coli* (EPEC)	STEC Center TW07897	*Shigella flexneri*	ATCC 25929
Enteropathogenic *Escherichia coli* (EPEC)	STEC Center TW07886	*Shigella sonnei*	ATCC 25931
Enteropathogenic *Escherichia coli* (EPEC)	STEC Center E851/71	*Shigella sonnei*	ATCC 29930
Enterotoxigenic *Escherichia coli* (ETEC)	ATCC 35401	*Staphylococcus aureus*	ATCC BK23738
Escherichia coli (non-STEC O157)	ATCC 700728	*Staphylococcus epidermidis*	ATCC 700567
Escherichia coli (non-STEC O157)	ATCC 700728	*Stenotrophomonas maltophilia*	ATCC 13637
Escherichia coli (non-STEC O157)	ATCC 43888	*Streptococcus agalactiae*	ATCC BAA-611
Escherichia coli (non-STEC O157)	ATCC 43888	*Streptococcus dysgalactiae*	ATCC 43078
Escherichia fergusonii	ATCC 35469	*Streptococcus intermedius*	ATCC 27335
Escherichia hermannii	ATCC 33650	*Streptococcus pyogenes*	ATCC 49399
Fusobacterium varium	ATCC 27725	*Streptococus uberis*	ATCC 9927
Gardnerella vaginalis	ATCC 14018	*Trabulsiella guamensis*	ATCC 49492
Helicobacter fennelliae	ATCC 35683	*Veillonella parvula*	ATCC 10790
Helicobacter pylori	ATCC 49503	*Vibrio cholera*	ATCC 55188
Klebsiella oxytoca	ATCC 13182	*Vibrio parahaemolyticus*	ATCC 17802
Klebsiella pneumonia	ATCC 13883	*Vibrio vulnificus*	ATCC 27562
Lactobacillus acidophilus	ATCC 4356	*Yersinia bercovieri*	ATCC 43970
Lactobacillus lactis	ATCC 49032	*Yersinia enterocolitica*	ATCC 49397
Leminorella grimonti	ATCC 43007	*Yersinia pseudotuberculosis*	ATCC 23207
Listeria grayi	ATCC 19120	*Yersinia rohdei*	ATCC 43380
Yeasts, Parasites, Viruses, and Human gDNA			
Candida albicans	ATCC 18804	Adenovirus type 41	ATCC VR-930D
Candida catenulata	ATCC 10565	Coxsackie B4	ATCC VR-184
Cryptosporidium parvum	ATCC PRA-67D	Enterovirus 71	ATCC VR-1775DQ
Entamoeba histolytica	ATCC 30459DQ	Norovirus G1	ATCC VR-3234SD
Giardia lamblia (G. intestinalis)	ATCC 50803D	Norovirus G2	ATCC VR-3235SD
Saccharomyces cerevisiae	ATCC MYA-796	Rotavirus	ATCC VR-1546
Human mastadenovirus F	ATCC VR-931D	Human genomic DNA (HT-29)	ATCC HTB-38D

† Actual concentration undetermined, estimate based on optical density measurement.

Interference:

Twenty-six (26) endogenous and exogenous substances that are common stool contaminants or likely present in patients with diarrhea were evaluated for potential interference with the Shiga Toxin Direct Test (Table 8). Each substance was tested in

the background of a contrived, low positive sample that was generated by spiking a Shiga toxin-producing *E. coli* strain (ATCC 43895) containing all three test analytes (*stx1+*, *stx2+*, and serotype O157) into ParaPak® C&S preserved clinical negative stool matrix at 2X LoD ($1x10^4$ CFU/mL). Clinical negative stool matrix was also tested (i.e. negative stool specimen, non-STEC) to evaluate potential interference with assay controls in the absence of analyte.

None of the tested substances interfered with detection of either Shiga toxin or the O157 Serotype gene targets, and each test resulted in 'STEC POSITIVE/Serotype O157 POSITIVE' calls as expected. Additionally, none of the substances interfered with assays controls when negative stool was tested. During testing, two (2) runs yielded incomplete testing results, and a single 'Invalid' test was observed. In each instance the specimen was re-retested using a new Shiga Toxin Direct Test cartridge, and the testing resolved to the correct result.

Table 8. Endogenous and Exogenous Substances Tested for Interference.

Potentially Interfering Substance	Concentration Tested
Endogenous Substances	
Human Bile	25% v/v
Human Urine	50% v/v
Human Whole Blood	50% v/v
Cholesterol	5% w/v (50 mg/mL)
Fatty Acids	3.33% w/v (33.3 mg/mL)
Mucin	6.25% w/v (6.25 mg/mL)
Triglycerides	10% v/v
Exogenous Substances	
Amoxicillin	5% w/v (50 mg/mL)
Baby Wipes	5% v/v
Barium Sulfate	9.9% w/v (99 mg/mL)
Ciprofloxacin	1.25% w/v (12.5 mg/mL)
Fleet Enema	50% v/v
Gaviscon Liquid Antacid	10% v/v
Glycerin Laxative	50% v/v
Hydrocortisone Cream	7.5% w/v (75 mg/mL)
Imodium	10% v/v
Personal Lubricant (K-Y Jelly)	50% v/v
Laxative Tablet	0.97% w/v (9.7 mg/mL)
Metronidazole	5% w/v (50 mg/mL)
Milk of Magnesia	10% v/v
Mineral Oil	50% v/v
Pepto Bismol	10% v/v
Preparation H Cream	9.5% w/v (95 mg/mL)
Stool Softener	0.7% w/v (7 mg/mL)
Tums	20% (200 mg/mL)
Vaginal Contraceptive Gel	50% v/v

Microbial Interference:

As a follow up to cross reactivity studies, the Shiga Toxin Direct Test was further evaluated for interference from mixed microbial populations using a subset of 42 organisms and genomic DNA from the cross reactivity panel with a specific focus on common gastrointestinal pathogens encountered in stool that cause similar disease states to Shiga toxins. In total, 30 bacterial strains, two (2) yeast, four (4) parasites, five (5) viruses, and human genomic DNA were tested (Table 9). Potential interference from mixed infections was evaluated by testing two (2) STEC strains (ATCC 43895 and ATCC 43894) that represent all three target analytes (*stx1*+/*stx2*+/O157) at 2X LoD in the background of high concentrations of the non-Shiga toxin-producing enteric organisms. A minimum of three replicate Shiga Toxin Direct Tests were performed for each potentially interfering organism or nucleic acid.

All of the valid test runs produced the expected 'STEC POSITIVE/Serotype O157 POSITIVE' result, indicating that none of the tested organisms or nucleic acids interfered with the detection of Shiga Toxin Direct Test gene targets at 2X LoD.

Table 9. Microbial Interference Panel.

Organism	**Strain ID**	**Organism**	**Strain ID**
Bacteria			
Aeromonas hydrophila	ATCC 35654	*E. coli* (non-STEC O157)	ATCC 700728
Bacteroides fragilis	ATCC 23745	Enteroaggregative *E coli* (EAEC)	ATCC 29552
Bacteroides vulgatus	ATCC 8482	Enteroaggregative *E coli* (EAEC)	STEC Center JM221
Bifidobacterium bifidum	ATCC 11863	Enteroinvasive *E coli* (EIEC)	ATCC 43892
Campylobacter jejuni	ATCC 49943	Enteroinvasive *E coli* (EIEC)	STEC Center 1885-77
Clostridium difficle (A+, B+)	ATCC 43255	Enteropathogenic *E coli* (EPEC)	STEC Center E2348/69
Clostridium perfringens	ATCC 12915	Enteropathogenic *E coli* (EPEC)	STEC Center TW07897
Enterobacter aerogenes	ATCC 15038	Enteropathogenic *E coli* (EPEC)	STEC Center TW07886
Enterococcus faecalis	ATCC 29212	Enteropathogenic *E coli* (EPEC)	STEC Center E851/71
Enterotoxigenic *E coli* (ETEC)	ATCC 35401	*Prevotella oralis*	ATCC 33322
Helicobacter pylori	ATCC 49503	*Salmonella typhimurium*	ATCC 13311
Klebsiella pneumonia	ATCC 13883	*Shigella sonnei*	ATCC 29930
Lactobacillus acidophilus	ATCC 4356	*Staphylococcus aureus*	ATCC BK-23738
Listeria monocytogenes	ATCC 19115	*Vibrio cholera*	ATCC 55188

Organism	Strain ID	Organism	Strain ID
Bacteria			
Prevotella melaninogenicus	ATCC 25845	*Yersinia enterocolitica*	ATCC 49397
Yeasts, Parasites, Viruses, and Human gDNA			
Blastocystis hominis (gDNA)	ATCC 50177D	Adenovirus 40	ATCC VR-931D
Entamoeba histolytica	ATCC 30459DQ	Adenovirus 41	ATCC VR-930D
Cryptosporidium parvum	ATCC PRA67D	Norovirus GI	ATCC VR-3234SD
Giardia lamblia (G. intestinalis)	ATCC 50803D	Norovirus GII	ATCC VR-3235SD
Candida albicans	ATCC 18804	Rotavirus	ATCC VR-1546
Saccharomyces cerevisiae	ATCC MYA-796	Human genomic DNA (HT-29)	ATCC HTB-38D

Carryover/Cross-Contamination:

A study was performed to assess the potential of carry-over or cross-contamination of the Shiga Toxin Direct Test by alternatively testing high positive contrived stool samples and clinical negative stool samples in direct succession for six (6) rounds on five (5) Portrait Analyzers. The high positive sample was formulated by spiking previously frozen and quantified enriched broth culture of STEC strain ATCC 43895 (*stx1+*/*stx2+*/O157) into negative clinical stool matrix consisting of clinical Shiga toxin negative stool preserved in ParaPak® C&S media to obtain a final concentration of $1x10^8$ CFU/mL. In total, 60 Shiga Toxin Direct Test runs were performed: 30 high positive runs and 30 negative runs.

All of the Shiga Toxin Direct Test results agreed with the expected test results. Therefore, there was no evidence of carry-over or cross-contamination in any of the tests. During carry-over/cross-contamination assessment, two (2) tests gave 'Test Incomplete' results and a single 'Invalid' test results was observed. All three samples were re-tested on new cartridges and all resolved to the expected result.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

See M1c.

3. Clinical studies:

a. Clinical Sensitivity:

Performance characteristics of the Shiga Toxin Direct Test were determined in a multi-site clinical study. Specimens for the clinical study were collected prospectively (fresh) at five sites during a three-month period from June to September 2015. A combined total of 1,116 clinical stool samples were enrolled and evaluated. Of these, 1,082 clinical specimens met the inclusion criteria. Of these 1,082 specimens, 1047 used C&S preservation medium, 34 used Cary Blair preservation medium, and the preservation medium type was not specified for one specimen. Performance was evaluated by comparing the performance of the Shiga Toxin Direct Test to reference clinical microbiology protocols for the independent detection of Shiga toxin and the *E. coli* O157 serotype. Combined results from the multi-site investigational study are summarized in Tables 10 for Shiga toxin and the *E. coli* O157 serotype.

Table 10. Prospective Clinical Study Results.

Shiga toxin (*stx1*/*stx2*)

Shiga Toxin Direct Test	Reference Clinical Microbiology - Shiga Toxin EIA: Positive	Negative	Total
Positive	4	8‡	12
Negative	0	1,070	1,070
Total	4	1,078	1,082

		Lower CI_{95}	Upper CI_{95}
Sensitivity	100%	39.8%	100%
Specificity	99.3%	98.5%	99.7%
PPV	33.3%	9.9%	65.1%
NPV	100%	99.7%	100%

‡ Shiga toxin DNA sequence was detected in 8/8 false positive specimens by both bi-directional sequencing and alternate, FDA-cleared comparator NAAT.

O157

Shiga Toxin Direct Test	Reference Clinical Microbiology - O157 Culture: Positive	Negative	Total
Positive	0	2‡	2
Negative	0	10	10
Total	0	12	12

		Lower CI_{95}	Upper CI_{95}
Sensitivity	N/A	N/A	N/A
Specificity	83.3%	51.6%	97.9%
PPV	0.0%	0.0%	84.2%
NPV	100%	69.2%	100%

‡ O157 serogroup DNA sequence was detected in 2/2 false positive specimens by alternate, FDA-cleared comparator NAAT.

Due to the low clinical prevalence of Shiga toxin-producing *E. coli* (STEC) and the O157 serotype in the prospective clinical study, a frozen retrospective panel of 88 unique, archived clinical stool specimens was tested with the Shiga Toxin Direct Test at multiple sites. Of these 88 specimens, 44 used C&S preservation medium, 40 used Cary Blair preservation medium, and 4 used Enteric Transport medium. The frozen retrospective panel consisted of 55 STEC positive and 33 STEC negative clinical specimens. Twenty three (23) of the STEC positive retrospective specimens were

serotype O157 positive. The initial clinical characterization (i.e. presence of Shiga toxins and O-antigen serotype) was confirmed for each frozen retrospective sample, and the samples were blinded and randomized before testing at multiple clinical sites with the Shiga Toxin Direct Test. Results of retrospective specimen testing are shown in Table 11. Summary results of all prospective and retrospective clinical study testing are shown in Table 12.

Table 11. Retrospective Clinical Study Results

Shiga toxin (stx1/stx2)

Shiga Toxin Direct Test	Clinical Characterization - Molecular and/or Shiga Toxin EIA		
	Positive	Negative	Total
Positive	51	0	51
Negative	4	33	37
Total	55	33	88

		Lower CI_{95}	Upper CI_{95}
PPA	92.7%	82.4%	98.0%
NPA	100%	89.4%	100%

O157

Shiga Toxin Direct Test	Clinical Characterization - Molecular and/or O157 Culture		
	Positive	Negative	Total
Positive	22	0	22
Negative	1‡	24‡	25
Total	23	24	47

		Lower CI_{95}	Upper CI_{95}
PPA	95.7%	78.1%	99.9%
NPA	100%	85.8%	100%

‡ The Shiga Toxin Direct Test result was 'Shiga Toxin NEGATIVE/Serotype O157 Not Tested' in 1/1 false negative and 2/24 true negative specimens.

Table 12. Summary of Prospective and Retrospective Clinical Study Results

Specimen Type		**n**	**% Agreement, n/N (95% CI)**	
			Positive	**Negative**
Shiga toxin (sx1/stx2)	Fresh	1,082	100% 4/4 (39.8-100)	99.3% 1,070/1,078 (98.5-99.7)
	Frozen	88	92.7% 51/55 (82.4-98.0)	100% 33/33 (89.4-100)
***E.coli* O157**	Fresh	12	-	83.3% 10/12 (51.6-97.9)
	Frozen	47	95.7% 22/23 (78.1-99.9)	100% 24/24 (85.8-100)

b. Clinical specificity: See M3a.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:
Clinical performance of the Shiga Toxin Direct Test was evaluated in a multi-site prospective clinical study at five sites during a three-month period from June to September 2015. A combined total of 1,116 stool samples were enrolled and evaluated. Of these, 1,082 clinical specimens met the inclusion criteria and were used to evaluate the performance of the Shiga Toxin Direct Test. The specimens were collected from 471 males and 611 females with ages ranging from less than one month old to greater than 85 years old. A total of 12 Shiga toxin positive samples were observed across all five test sites (1.1%). Two (2) of the 12 Shiga toxin positive specimens were also positive for O157 serotype (16.7%).

N. Instrument Name:

PA500 Portrait Analyzer System

O. System Descriptions:

1. Modes of Operation:

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes ___X_____ or No _____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _____ or No ___X_____

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ___X_____ or No _____

3. Specimen Identification:

   To perform a test, the user prepares and loads the cartridge according to instructions in the Package Insert. The required specimen and test information is entered into the computer (barcode reader optional) before starting the test. The specimen ID is associated with the test results and is shown in the Results Report.

4. Specimen Sampling and Handling:

   Specimens for use with the Portrait STEC Test Cartridges are stool specimens preserved in either Cary Blair or C&S media. Stool specimens should be collected, processed, and stored following standard laboratory procedures.

5. Calibration:

   All adjustments and calibration requirements are completed at the factory prior to shipment of each analyzer. There are no calibration requirements for instruments in the field; the user is not responsible for calibration activity.

6. Quality Control:

   The integrity of the system is verified and controlled by specific hardware/software checks during the cartridge load process and during the assay run. These checks, along with assay internal controls, are employed to monitor the performance of the system during operation and to alert the user of any out of specification conditions.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable

Q. Proposed Labeling:

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

R. Conclusion:

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