DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

March 22, 2016

GREAT BASIN SCIENTIFIC, INC.
CHUCK OWEN
DIRECTOR, REGULATORY AFFAIRS & QUALITY ASSURANCE
2441 S. 3850 WEST
SALT LAKE CITY, UT 84120

Re: K152955
Trade/Device Name: Great Basin Shiga Toxin Direct Test
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay
Regulatory Class: II
Product Code: PCH
Dated: March 9, 2016
Received: March 10, 2016

Dear Mr. Owen:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

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

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

Sincerely yours,

Ribhi Shawar -S

For Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K152955

Device Name
Great Basin Shiga Toxin Direct Test

Indications for Use *(Describe)*
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 (STEC) 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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary – Shiga Toxin Direct Test

A. Submitted by:

Great Basin Scientific
2441 South 3850 West
Salt Lake City, Utah 84120
Phone: 801-990-1055
Fax: 801-990-1051

Summary Preparation Date

March 08, 2016

Contact Information

Chuck Owen, Director of Regulatory Affairs
Phone: 385-215-3313
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B. Name of Device

Proprietary Name:	Great Basin Shiga Toxin Direct Test
Common or Usual Names:	Shiga Toxin Direct Test Shiga Toxin Direct Shiga Tox STEC Test/Assay

C. Regulatory Information:

a.	Regulation Section:	21 CFR 866.3990, Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay
b.	Classification:	Class II
c.	Classification panel:	(83) Microbiology
d.	Product Code:	PCH Gastrointestinal Pathogen Panel Multiplex Nucleic Acid-Based Assay System OOI Real time nucleic acid amplification system

D. Intended use(s) / Indications 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* (STEC) 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.

E. Device Description:

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. The Portrait System was granted 510(k) clearance for the Portrait Toxigenic *C. difficile* Assay (DEN120013) and the Portrait GBS Assay (K143312).

Target genomic DNA is extracted from microbial cells 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 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.

Test Device:

The Portrait System is a fully 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 2 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.

F. Substantial Equivalence Information:

a. Predicate Device: *FilmArray® Gastrointestinal (GI) Panel* from BioFire Diagnostics, LLC
b. Predicate 510(k) number: K140407
c. Comparison with Predicate, see chart:

Predicate Device Comparison Chart		
Item	**Shiga Toxin Direct Test**	**Predicate (K140407)**
Manufacturer	Great Basin Scientific, Inc.	BioFire Diagnostics, LLC
Trade Name	Portrait™ Shiga Toxin Direct Test	FilmArray™ Gastrointestinal (GI) Panel
510(k) Number	K141658	K140407
Similarities		
Classification	Class II	same
Intended Use/ Indications for Use	Direct detection of nucleic acids from enteric pathogens and toxin genes directly from transport media preserved clinical stool specimens.	same
Target Sequence Detected	Shiga toxin 1 (*stx1*) and Shiga toxin 2 (*stx2*) gene virulence markers for the identification of Shiga toxin-producing *Escherichia coli* (STEC), including specific identification of the *E. coli* O157 serotype within STEC.	same
Qualitative/ Quantitative	Qualitative	same
Single-Use Test Cartridge	Disposable, single-use, self-contained fluidic test cartridge	same
Automated	Yes	same
Test Principle	Nucleic Acid Amplification Assay	same
Sample Types	Human stool sample preserved in transport media.	same
Assay Controls	Sample processing controls are incorporated directly into single-use test cartridges in freeze-dried form and are rehydrated into the test prior to sample lysis.	same
Calibration	Not required	same
Differences		
Organisms Detected	Shiga toxin-producing *Escherichia coli* (STEC), including specific identification of the *E. coli* O157 serotype within STEC.	· *Campylobacter (C. jejuni/C. coli/C. upsaliensis)* · *Clostridium difficile (C. difficile)* toxin A/B · *Plesiomonas shigelloides* · *Salmonella* · *Vibrio (V. parahaemolyticus/V. vulnificus/ V. cho*lerae), including specific identification of *Vibrio cholerae* · *Yersinia enterocolitica* · Enteroaggregative *Escherichia coli* (EAEC) · Enteropathogenic *Escherichia coli* (EPEC) · Enterotoxigenic *Escherichia coli* (ETEC) *lt/st* · Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1/stx2* (including specific identification of the *E. coli* O157 serogroup within STEC) · *Shigella/ Enteroinvasive Escherichia coli* (EIEC) · *Cryptosporidium* · *Cyclospora cayetanensis* · *Entamoeba histolytica* · *Giardia lamblia* · Adenovirus F 40/41 · Astrovirus · Norovirus GI/GII · Rotavirus A · Sapovirus (Genogroups I, II, IV, and V)
Compatible Media Types	Cary-Blair and C&S stool Preservation and Transport Medias	Cary-Blair Preservation and Transport Media
Analyte	DNA	DNA/RNA

Predicate Device Comparison Chart		
Item	**Shiga Toxin Direct Test**	**Predicate (K140407)**
Differences		
Amplification Technology	Multiplex polymerase chain reaction (PCR)	Nested multiplex RT-PCR
Instrument	PA500 Portrait™ Analyzer	FilmArray™ Instrument
Time to Result	Approximately 2 hours	Less than 1 hour
Detection Method	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.
Reagent Storage	Reagents stored at 4°C	Reagents stored at Room Temperature
Clinical Sensitivity Shiga Toxin	100% [95% CI: 39.8% - 100%]	Not Reported
Clinical Specificity Shiga Toxin	99.3% [95% CI: 98.5%- 99.7%]	Not Reported
Clinical Sensitivity O157 Serotype	Not Reported	Not Reported
Clinical Specificity O157 Serotype	83.3% [95% CI: 51.6%- 97.9%]	Not Reported
Positive Percent Agreement Shiga Toxin	92.7% [95% CI: 82.4%- 98.0%]	100% [95% CI: 89.4%- 100%]
Negative Percent Agreement Shiga Toxin	100% [95% CI: 89.4%- 100%]	99.7% [95% CI: 99.2%- 99.9%]
Positive Percent Agreement O157 Serotype	95.7% [95% CI: 78.1%- 99.9%]	100% [95% CI: 29.2%- 100%]
Positive Percent Agreement O157 Serotype	100% [95% CI: 85.8%- 100%]	97.1% [95% CI: 85.1%- 99.9%]

Performance Data – Analytical Studies

d. Analytical Sensitivity

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 1.

Table 1. Limit of Detection (LoD) of the Shiga Toxin Direct Test.

Shiga toxin-producing *E. coli* (STEC) Strain	**Shiga Toxin(s) Present**	**Serotype**	**Expected Shiga Toxin Direct Test Result**	**Correct Results**	**LoD**
ATCC BAA-2191	*stx1+*	O45:H2	STEC POSITIVE/ Serotype O157 NEGATIVE	25/25	5.5 x 10^3 CFU/mL
ATCC 51434	*stx2+*	O91:H21	STEC POSITIVE/ Serotype O157 NEGATIVE	21/22	2.8 x 10^3 CFU/mL
ATCC BAA-2192	*stx1+, stx2+*	O145:NM	STEC POSITIVE/ Serotype O157 POSITIVE	26/26	5.2 x 10^3 CFU/mL
ATCC 43895	*stx1+, stx2+*	O157:H7	STEC POSITIVE/ Serotype O157 POSITIVE	20/20	5.0 x 10^3 CFU/mL

e. Specimen Stability

The recommended storage time and temperature conditions for Cary-Blair or C&S preserved stool specimens prior to testing via the Shiga Toxin Direct Test includes:

- Refrigerated storage (2°- 8° C) for up to 120 hours (5 days)
- Room temperature (RT) 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 each 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 (Tables 2-3).

Table 2. Specimen Stability Study Protocol Overview. A summary of the Specimen Stability panel and testing overview.

Total Strains Tested	4 Shiga toxin-producing *E.coli* (STEC) strains
Strains Tested	1. ATCC BAA-2192
	2. ATCC 51434
	3. ATCC 43889
Replicates	Each strain was formulated into 5 unique stool matrices* at each concentration and tested in a single replicate at each time point
Total Panel Size	30 samples
Panel Runs	6 time points
Time Points	T_0: 0 hr (freshly prepared)
	T_1: 4 hr Room Temp. storage
	T_2: 24 hr 2°-8° C storage
	T_3: 72 hr 2°-8° C storage
	T_4: 120 hr 2°-8° C storage
	T_5: 4 hr Room Temp. + 120 hr 2°-8° C storage
Total Runs	**30 samples x 6 time points = 180 runs**

* Stool matrices for this study were provided by a clinical test site and were previously characterized as 'Shiga toxin Negative' by Shiga Toxin Immunoassay.

Table 3. Specimen Stability Study Summary Results. Shiga toxin-producing *E. coli* samples stored at either RT and/or 2°-8°C and tested by the Shiga Toxin Direct Test at multiple time points.

	% Agreement		
Shiga toxin-producing *E. coli* (STEC) Strain Tested	**ATCC BAA-2191 (*stx1+*)**	**ATCC 51434 (*stx2+*)**	**ATCC 43889^ (*stx2+*/O157)**
Concentration (2X LoD)	**1.1×10^4 CFU/mL**	**6.0×10^3 CFU/mL**	**8.5×10^3 - 1.0×10^4 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 storage	100% (5/5)	100% (5/5)	100% (10/10)
T_3: 72 hr 2°- 8° C storage	100% (5/5)	100% (5/5)	100% (10/10)
T_4: 120 hr 2°- 8° C storage	100% (5/5)	100% (5/5)	100% (10/10)
T_5: 4 hr Room Temp. + 120 hr 2°- 8° C storage	100% (5/5)	100% (5/5)	100% (10/10)
Overall	100% (30/30)	100% (30/30)	100% (60/60)

^ Limit of Detection (LoD) was approximated for this strain.

f. Fresh vs. 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 (Table 4).

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. Demonstration of specimen stability after the second freeze/thaw cycle is important because generation of a Frozen Retrospective panel from archived clinical specimens requires a freeze/thaw to prepare panel prior to re-freezing for shipment to clinical sites. Clinical sites performed the second thawing of archived specimens prior to testing. Samples tested in the Reproducibility Study or for follow-up testing at the reference site were subjected to a single freeze/thaw cycle.

The Fresh vs. Frozen was conducted using contrived positive samples that were prepared using fresh (i.e. never frozen) enriched broth cultures. The panel for the Fresh vs. Frozen Study was comprised of 6 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 4 concentrations: ≤ 0.5X LoD, 1X LoD, 3X LoD, and 10X LoD. 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 1 week at which time the samples were tested for a second, and final, thaw (T_2).

This study demonstrates sufficient integrity of the Shiga Toxin Direct Test targets (nucleic acid from Shiga toxin 1, Shiga toxin 2 gene, and O157 serotype identification genes) in frozen stool specimens preserved in C&S media for up to 2 freeze/thaw cycles.

Table 4. Fresh vs. Frozen Study. Shiga Toxin Direct Test results of STEC samples evaluated prior to freezing at ≤-70°C and after multiple freeze/thaw cycles.

			% Agreement					
Shiga toxin-producing *E. coli* (STEC) Strain	**Expected Shiga Toxin Direct Test Result**	**Concentration**	**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.

g. Analytical Reactivity (Inclusivity)

The Analytical Reactivity of the Shiga Toxin Direct Test was tested against 30 well-characterized Shiga toxin-producing *E. coli* (STEC) strains from ATCC 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.

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'. Furthermore, all nine (9) O157 serotype STEC strains were identified as 'STEC POSITIVE/ Serotype O157 POSITIVE' (Table 5).

Table 5. Analytical Reactivity (Inclusivity) Panel. Shiga toxin-producing *E. coli* (STEC) and *Shigella dysenteriae* strains tested for inclusivity by the Shiga Toxin Direct Test.

ATCC Strain	Serotype	Shiga Toxin Gene(s) Present	Expected Shiga Toxin Direct Test Result	Concentration	Positive Results
Shiga toxin-producing *Escherichia coli* (STEC)					
BAA-2181	O26:H11	*stx1+*	STEC POSITIVE/ Serotype O157 NEGATIVE	1.0 x 10^4 CFU/mL	3/3
BAA-2215	O103:H11	*stx1+*		1.0 x 10^4 CFU/mL	3/3
BAA-2199	O123:H25	*stx1+*		1.0 x 10^4 CFU/mL	3/3
BAA-2210	O103:H2	*stx1+*		1.0 x 10^4 CFU/mL	3/3
BAA-2191	O45:H2	*stx1+*		1.0 x 10^4 CFU/mL	3/3
BAA-2201	O111:H8	*stx1+*		1.0 x 10^4 CFU/mL	3/3
51435	O91:H21	*stx2+*		1.0 x 10^4 CFU/mL	3/3
51434	O91:H21	*stx2+*		1.0 x 10^4 CFU/mL	3/3
BAA-182	O104:H21	*stx2+*		1.0 x 10^4 CFU/mL	3/3
BAA-2326	O104:H4	*stx2+*		1.0 x 10^4 CFU/mL	3/3
BAA-183	O113: H21	*stx2+*		1.0 x 10^4 CFU/mL	3/3
BAA-2220	O121:H19	*stx2+*		1.0 x 10^4 CFU/mL	3/3
BAA-2219	O121:H19	*stx2+*	STEC POSITIVE/ Serotype O157 NEGATIVE	1.0 x 10^4 CFU/mL	3/3
BAA-2211	O145: H25	*stx2+*		1.0 x 10^4 CFU/mL	3/3
BAA-2129	O145:H28	*stx2+*		1.0 x 10^4 CFU/mL	3/3
BAA-2221	O21:H19	*stx1+/stx2+*		1.0 x 10^4 CFU/mL	3/3
BAA-2196	O26:H11	*stx1+/stx2+*		1.0 x 10^4 CFU/mL	3/3
BAA-2193	O45:H2	*stx1+/stx2+*		1.0 x 10^4 CFU/mL	3/3
BAA-2440	O111	*stx1+/stx2+*		1.0 x 10^4 CFU/mL	3/3
700840	O111:H8	*stx1+/stx2+*		1.0 x 10^4 CFU/mL	3/3
BAA-2192	O145	*stx1+/stx2+*		1.0 x 10^4 CFU/mL	3/3
n = 21					
43890	O157:H7	*stx1+*	STEC POSITIVE/ Serotype O157 POSITIVE	1.0 x 10^4 CFU/mL	3/3
700376	O157:NM	*stx1+*		1.0 x 10^4 CFU/mL	3/3
43889	O157:H7	*stx2+*		1.0 x 10^4 CFU/mL	3/3
700377	O157:NM	*stx2+*		1.0 x 10^4 CFU/mL	3/4‡
700378	O157:NM	*stx1+/stx2+*		1.0 x 10^4 CFU/mL	3/3
700927	O157:H7:K	*stx1+/stx2+*		1.0 x 10^4 CFU/mL	3/3
43894	O157:H7	*stx1+/stx2+*		1.0 x 10^4 CFU/mL	3/3
43895	O157:H7	*stx1+/stx2+*		1.0 x 10^4 CFU/mL	3/3
35150	O157:H7	*stx1+/stx2+*		1.0 x 10^4 CFU/mL	3/3
n = 9					
Shigella dysenteriae					
9361	Type 1	^*stx+*	STEC POSITIVE/ Serotype O157 NEGATIVE	1.0 x 10^4 CFU/mL	3/3
27346†	Type 1	^*stx+*		≤1.0 x 10^4 CFU/mL	3/3
27345†	Type 1	^*stx+*		≤1.0 x 10^4 CFU/mL	3/3*
n = 3					
Total		**n = 33**			

* Represents each 'Test Incomplete' run in this dataset.
‡ Represents each replicate in this set that resulted in 'STEC NEGATIVE/Serotype O157 Not Tested'.
† 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.'

h. Analytical Specificity (Exclusivity)

Studies were conducted to assess the potential for cross-reactivity of non-target organisms, some of which are commonly found in stool specimens. Included in Analytical Specificity testing were well known enteric pathogens that present clinically with symptoms similar to STEC, such as diarrhea. The study evaluated a total of 118 microorganisms, including: bacteria, fungi/yeasts, parasites, viruses, and human genomic DNA. For organisms that where classified as Biosafety level III or unable to culture 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 6).

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. The Shiga Toxin Direct Test controls are built such that SPC amplification failure in the absence of Shiga Toxin signal results in an 'invalid' test. Therefore during exclusivity testing both non-STEC O157:H7 *E. coli* strains (ATCC 43888 and ATCC 700728) resulted in 'invalid' test results when tested at concentrations ≥ $1.0x10^8$ CFU/mL. The test concentration for both strains (ATCC 43888 and ATCC 700728) was lowered to approximately $1.0x10^6$ CFU/mL, each strain was re-retested, and resulted in the correct call of 'STEC NEGATIVE/Serotype O157 Not Tested'.

In total 104 unique bacterial strains, three (3) yeast, three (3) parasites, seven (7) viruses, and human genomic DNA were evaluated for cross-reactivity. With the exception of the 2 previously mentioned *E. coli* strains (43888 and 700728), none of the tested nucleic acids (genomic DNA or viruses) or cultured organisms (bacteria, yeasts, parasites) interfered with the internal controls and all of the calls were 'STEC NEGATIVE/Serotype O157 Not Tested,' indicating no cross-reactivity (Table 6).

Table 6. Analytical Specificity (Exclusivity) Panel. Non-Shiga toxin-producing enteric flora, including: bacteria, viruses, parasites, fungi and nucleic acids from various pathogens, tested for exclusivity by the Shiga Toxin Direct Test.

Organism	Strain ID	Input Tested	STEC NEGATIVE/ Serotype O157 Not Tested Result
Bacteria			
Abiotrophia defective	ATCC 49176	≥1.0×10^6 CFU/mL†	3/3
Acinetobacter baumannii	ATCC 19606	1.3×10^8 CFU/mL	3/3
Aeromonas hydrophila	ATCC 35654	4.6×10^8 CFU/mL	3/3
Anaerococcus tetradius	ATCC 35098	1.6×10^7 CFU/mL	3/3
Bacillus cereus	ATCC 14579	7.6×10^7 CFU/mL	3/3
Bacteroides fragilis	ATCC 23745	2.7×10^7 CFU/mL	3/3
Bacteroides vulgatus	ATCC 8482	≥1.0×10^6 CFU/mL†	3/3
Bifidobacterium adolescentis	ATCC 15703	≥1.0×10^6 CFU/mL†	3/3***
Bifidobacterium bifidum	ATCC 11863	≥1.0×10^6 CFU/mL†	3/3
Bifidobacterium longum	ATCC 15707	≥1.0×10^6 CFU/mL†	3/3
Camphylobacter coli	ATCC 33559	5.4×10^7 CFU/mL	3/3
Camphylobacter fetus	ATCC 15296	≥1.0×10^6 CFU/mL†	3/3^
Campylobacter jejuni	ATCC 49943	4.9×10^7 CFU/mL	3/3
Camphylobacter lari	ATCC 35221	4.0×10^6 CFU/mL	3/3
Citrobacter amalonaticus	ATCC 25406	1.3×10^8 CFU/mL	3/3
Citrobacter freundii	ATCC 8090	3.4×10^8 CFU/mL	3/3

Organism	Strain ID	Input Tested	STEC NEGATIVE/ Serotype O157 Not Tested Result
Clostridium difficile (A-, B-)	ATCC BAA-1801	≥1.0 x 10^6 CFU/mL†	3/3
Clostridium difficile (A+, B+) (gDNA)	ATCC BAA-1382D	5.0 x 10^6 copies/uL	3/3
Clostridium difficile (A+, B+)	ATCC 43255	≥1.0 x 10^6 CFU/mL†	3/3
Clostridium histolyticum	ATCC 19401	≥1.0 x 10^6 CFU/mL†	3/3
Clostridium perfringens	ATCC 12915	1.6 x 10^7 CFU/mL	3/3
Clostridium sordellii	ATCC 9715	≥1.0 x 10^6 CFU/mL†	3/3
Enterobacter aerogenes	ATCC 15038	2.7 x 10^8 CFU/mL	3/3
Enterobacter cloacae	ATCC 13047	4.6 x 10^8 CFU/mL	3/3
Enterococcus cecorum	ATCC 43198	8.2 x 10^5 CFU/mL	3/3
Enterococcus faecalis	ATCC 29212	1.2 x 10^8 CFU/mL	3/3
Enterococcus faecium	ATCC 19434	6.4 x 10^7 CFU/mL	3/3
Enteroaggregative *Escherichia coli* (EAEC)	ATCC 29552	5.6 x 10^7 CFU/mL	3/3
Enteroaggregative *Escherichia coli* (EAEC)	STEC Center JM221	4.0 x 10^7 CFU/mL	3/3
Enteroinvasive *Escherichia coli* (EIEC)	STEC Center 1885-77	2.2 x 10^7 CFU/mL	3/3
Enteroinvasive *Escherichia coli* (EIEC)	ATCC 43892	2.4 x 10^8 CFU/mL	3/3
Enteropathogenic *Escherichia coli* (EPEC)	STEC Center E2348/69	4.4 x 10^7 CFU/mL	3/3
Enteropathogenic *Escherichia coli* (EPEC)	STEC Center TW07897	5.1 x 10^7 CFU/mL	3/3^
Enteropathogenic *Escherichia coli* (EPEC)	STEC Center TW07886	5.3 x 10^7 CFU/mL	3/3
Enteropathogenic *Escherichia coli* (EPEC)	STEC Center E851/71	2.6 x 10^7 CFU/mL	3/3
Enterotoxigenic *Escherichia coli* (ETEC)	ATCC 35401	2.5 x 10^8 CFU/mL	3/3
Escherichia coli (non-STEC O157)	ATCC 700728	3.9 x 10^8 CFU/mL	0/3^^^
Escherichia coli (non-STEC O157)	ATCC 700728	1.0 x 10^6 CFU/mL	3/3
Escherichia coli (non-STEC O157)	ATCC 43888	4.9 x 10^7 CFU/mL	0/3^^^
Escherichia coli (non-STEC O157)	ATCC 43888	1.0 x 10^6 CFU/mL	3/3
Escherichia fergusonii	ATCC 35469	2.2 x 10^8 CFU/mL	3/3
Escherichia hermannii	ATCC 33650	3.7 x 10^8 CFU/mL	3/3^
Fusobacterium varium	ATCC 27725	1.1 x 10^8 CFU/mL	3/3
Gardnerella vaginalis	ATCC 14018	≥1.0 x 10^6 CFU/mL†	3/3
Helicobacter fennelliae	ATCC 35683	≥1.0 x 10^6 CFU/mL†	3/3
Helicobacter pylori	ATCC 49503	≥1.0 x 10^6 CFU/mL†	3/3
Klebsiella oxytoca	ATCC 13182	2.5 x 10^6 CFU/mL	3/3
Klebsiella pneumonia	ATCC 13883	3.2 x 10^8 CFU/mL	3/3*
Lactobacillus acidophilus	ATCC 4356	4.3 x 10^5 CFU/mL	3/3
Lactobacillus lactis	ATCC 49032	2.6 x 10^8 CFU/mL	3/3^^
Leminorella grimonti	ATCC 43007	1.2 x 10^8 CFU/mL	3/3
Listeria grayi	ATCC 19120	4.5 x 10^8 CFU/mL	3/3^
Listeria innocua	ATCC 33090	1.2 x 10^8 CFU/mL	3/3
Listeria monocytogenes	ATCC 19115	2.0 x 10^8 CFU/mL	3/3
Morganella morganii	ATCC 25829	7.7 x 10^7 CFU/mL	3/3
Peptostreptococcus anaerobius	ATCC 27337	1.0 x 10^8 CFU/mL	3/3
Plesiomonas shigelloides	ATCC 51903	3.5 x 10^8 CFU/mL	3/3^
Prevotella melaninogenica	ATCC 25845	1.6 x 10^7 CFU/mL	3/3
Proteus mirabilis	ATCC 25933	5.4 x 10^6 CFU/mL	3/3*
Proteus penneri	ATCC 33519	5.5 x 10^7 CFU/mL	3/3
Proteus vulgaris	ATCC 6896	1.3 x 10^8 CFU/mL	3/3
Providiencia alcalifaciens	ATCC 9886	4.4 x 10^7 CFU/mL	3/3
Providencia rettgeri	ATCC 9250	2.4 x 10^8 CFU/mL	3/3
Providencia stuartii	ATCC 49762	5.7 x 10^7 CFU/mL	3/3
Pseudomonas aeruginosa	ATCC 10145	2.4 x 10^8 CFU/mL	3/3
Pseudomonas mosselii	ATCC 49838	5.8 x 10^6 CFU/mL	3/3
Ruminococcus bromii	ATCC 27255	≥1.0 x 10^6 CFU/mL†	3/3
Salmonella enterica subp Arizonae	ATCC 13314	3.4 x 10^8 CFU/mL	3/3
Salmonella enterica subp Cholerasuis	ATCC 13312	5.3 x 10^8 CFU/mL	3/3^
Salmonella enterica subp Heidelberg	ATCC 8326	4.0 x 10^8 CFU/mL	3/3
Salmonella enterica subp Newington	ATCC 29628	1.6 x 10^8 CFU/mL	3/3
Salmonella enterica subp Newport	ATCC 6962	1.8 x 10^8 CFU/mL	3/3

Organism	Strain ID	Input Tested	STEC NEGATIVE/ Serotype O157 Not Tested Result
Salmonella paratyphi A	ATCC 9150	2.8×10^8 CFU/mL	3/3
Salmonella paratyphi B	ATCC 8759	7.6×10^8 CFU/mL	3/3*
Salmonella typhimurium	ATCC 13311	4.1×10^8 CFU/mL	3/3*
Selenomonas ruminantium	ATCC 35018	≥1.0×10^6 CFU/mL†	3/3
Serratia liquefaciens	ATCC 35551	6.4×10^8 CFU/mL	3/3^
Serratia marcescens	ATCC 13880	9.2×10^8 CFU/mL	3/3
Shigella boydii	ATCC 29928	2.5×10^8 CFU/mL	3/3
Shigella boydii	ATCC 12028	1.8×10^7 CFU/mL	3/3
Shigella dysenteriae (Type 2)	ATCC 29027	7.2×10^7 CFU/mL	3/3
Shigella dysenteriae (Type 3)	ATCC 29028	3.8×10^7 CFU/mL	3/3
Shigella dysenteriae (Type 12)	ATCC 49551	3.4×10^7 CFU/mL	3/3
Shigella dysenteriae (Type 13)	ATCC 49555	4.0×10^7 CFU/mL	3/3
Shigella flexneri	ATCC 25929	3.6×10^8 CFU/mL	3/3
Shigella sonnei	ATCC 25931	2.2×10^8 CFU/mL	3/3
Shigella sonnei	ATCC 29930	6.4×10^7 CFU/mL	3/3
Staphylococcus aureus	ATCC BK23738	4.5×10^8 CFU/mL	3/3
Staphylococcus epidermidis	ATCC 700567	3.5×10^8 CFU/mL	3/3
Stenotrophomonas maltophilia	ATCC 13637	2.4×10^7 CFU/mL	3/3
Streptococcus agalactiae	ATCC BAA-611	4.0×10^7 CFU/mL	3/3
Streptococcus dysgalactiae	ATCC 43078	7.0×10^6 CFU/mL	3/3
Streptococcus intermedius	ATCC 27335	8.0×10^5 CFU/mL	3/3
Streptococcus pyogenes	ATCC 49399	8.0×10^5 CFU/mL	3/3
Streptococus uberis	ATCC 9927	6.0×10^6 CFU/mL	3/3
Trabulsiella guamensis	ATCC 49492	5.5×10^7 CFU/mL	3/3
Veillonella parvula	ATCC 10790	6.6×10^7 CFU/mL	3/3
Vibrio cholera	ATCC 55188	3.8×10^8 CFU/mL	3/3
Vibrio parahaemolyticus	ATCC 17802	3.2×10^6 CFU/mL	3/3
Vibrio vulnificus	ATCC 27562	1.5×10^8 CFU/mL	4/4
Yersinia bercovieri	ATCC 43970	3.4×10^7 CFU/mL	3/3
Yersinia enterocolitica	ATCC 49397	1.6×10^8 CFU/mL	4/4
Yersinia pseudotuberculosis	ATCC 23207	1.3×10^7 CFU/mL	3/3
Yersinia rohdei	ATCC 43380	1.9×10^7 CFU/mL	3/3
Yeasts, Parasites, and Viruses			
Candida albicans	ATCC 18804	3.0×10^6 CFU/mL	3/3
Candida catenulata	ATCC 10565	5.0×10^5 CFU/mL	3/3
Cryptosporidium parvum	ATCC PRA-67D	1 ug/mL	3/3
Entamoeba histolytica	ATCC 30459DQ	1.0×10^8 CFU/mL	3/3
Giardia lamblia (G. intestinalis)	ATCC 50803D	1 ug/mL	3/3
Saccharomyces cerevisiae	ATCC MYA-796	≥1.0×10^6 CFU/mL†	3/3^
Human mastadenovirus F	ATCC VR-931D	1 ug/mL	3/3
Adenovirus type 41	ATCC VR-930D	1 ug/mL	3/3
Coxsackie B4	ATCC VR-184	1.0×10^6 $TCID_{50}$/mL	3/3
Enterovirus 71	ATCC VR-1775DQ	4.8×10^5 copies/uL	3/3
Norovirus G1	ATCC VR-3234SD	4.7×10^5 copies/uL	3/3
Norovirus G2	ATCC VR-3235SD	4.8×10^5 copies/uL	3/3*
Rotavirus	ATCC VR-1546	1.0×10^5 $TCID_{50}$/mL	3/3
Human genomic DNA (HT-29)	**ATCC HTB-38D**	**1 ug/mL**	**3/3**

† Actual concentration undetermined, estimate based on optical density measurement.
* Represents each 'Test Incomplete' run in this dataset.
^ Represents each 'INVALID' run in this dataset.

i. Microbial Interference

As a follow up to the previous Exclusivity studies, the Shiga Toxin Direct Test was further evaluated for interference from mixed microbial populations using a subset of 42 of the same microflora (bacterial, yeast, parasite, and viral stock strains). The potential for cross-reactivity in mixed infections was evaluated with a comprehensive panel created with a specific focus on common gastrointestinal pathogens encountered in stool that cause similar disease states to Shiga Toxins, including other common pathogenic, non-STEC *E. coli* species.

The same high concentrations of potentially interfering DNA and microorganisms were spiked into negative clinical preserved stool matrix containing a *stx1+*/*stx2+*/O157+ STEC strain at low positive concentration of 2X LoD. In total two (2) STEC strains (ATCC 43895 and ATCC 43894) containing all assay targets were evaluated.

This Microbial Interference Study assessed potential Shiga Toxin Direct Test interference due to mixed infections by evaluating detection of a STEC strain (ATCC 43895) consisting of all 3 analytes (*stx1+*/*stx2+*/O157) at near LoD concentrations in the background of high concentrations of non-Shiga toxin-producing enteric flora, including: bacteria, viruses, parasites, fungi and nucleic acids from various pathogens. A minimum of three replicate Shiga Toxin Direct Tests were performed for each potentially interfering organism or nucleic acid (Table 7).

In total 30 unique bacterial strains, two (2) yeast, four (4) parasites, five (5) viruses, and human genomic DNA were evaluated for microbial interference. All of the valid test runs resulted in the expected 'STEC POSITIVE/Serotype O157 POSITIVE' call indicating that none of the tested nucleic acids (genomic DNA or viruses) or cultured organisms (bacteria, yeasts, parasites) interfered with the detection of both Shiga Toxin Direct Test analytes (Shiga toxin and the O157 serotype) at 2X LoD (Table 7).

Table 7. Microbial Interference Panel. A panel of non-Shiga toxin-producing enteric flora, including: bacteria, viruses, parasites, fungi and nucleic acids from various pathogens, tested for microbial interference in detection of Shiga toxins and the O157 serotype via the Shiga Toxin Direct Test.

			STEC Strain 43895	**STEC Strain 43894**
Organism	**Strain ID**	**Input Tested**	**STEC POSITIVE/ Serotype O157 POSITIVE Test Result**	
Bacteria				
Aeromonas hydrophila	ATCC 35654	4.6×10^8 CFU/mL	4/4	3/3
Bacteroides fragilis	ATCC 23745	2.7×10^7 CFU/mL	3/3	3/3
Bacteroides vulgatus	ATCC 8482	$\geq 1.0 \times 10^6$ CFU/mL†	3/3	3/3
Bifidobacterium bifidum	ATCC 11863	$\geq 1.0 \times 10^6$ CFU/mL†	3/3	3/3
Campylobacter jejuni	ATCC 49943	4.9×10^7 CFU/mL	3/3	3/3
Clostridium difficile (A+, B+)	ATCC 43255	$\geq 1.0 \times 10^6$ CFU/mL†	3/3	3/3
Clostridium perfringens	ATCC 12915	1.6×10^7 CFU/mL	3/3	3/3
Enterobacter aerogenes	ATCC 15038	2.7×10^8 CFU/mL	3/3	3/3
Enterococcus faecalis	ATCC 29212	1.2×10^8 CFU/mL	3/3	3/3
Escherichia coli (non-STEC O157)	ATCC 700728	3.9×10^8 CFU/mL	3/3	3/3
Enteroaggregative *Escherichia coli* (EAEC)	ATCC 29552	5.6×10^7 CFU/mL	3/3	3/3

Organism	Strain ID	Input Tested	STEC Strain 43895	STEC Strain 43894
			STEC POSITIVE/ Serotype O157 POSITIVE Test Result	
Enteroaggregative *Escherichia coli* (EAEC)	STEC Center JM221	4.0 x 10^7 CFU/mL	3/3	3/3
Enteroinvasive *Escherichia coli* (EIEC)	ATCC 43892	2.4 x 10^8 CFU/mL	3/3	3/3
Enteroinvasive *Escherichia coli* (EIEC)	STEC Center 1885-77	2.2 x 10^7 CFU/mL	3/3	3/3
Enteropathogenic *Escherichia coli* (EPEC)	STEC Center E2348/69	4.4 x 10^7 CFU/mL	3/3	3/3
Enteropathogenic *Escherichia coli* (EPEC)	STEC Center TW07897	5.1 x 10^7 CFU/mL	3/3	3/3
Enteropathogenic *Escherichia coli* (EPEC)	STEC Center TW07886	5.3 x 10^7 CFU/mL	3/5‡§	3/3
Enteropathogenic *Escherichia coli* (EPEC)	STEC Center E851/71	2.6 x 10^7 CFU/mL	3/6‡‡‡	3/3
Enterotoxigenic *Escherichia coli* (ETEC)	ATCC 35401	2.5 x 10^8 CFU/mL	3/3	3/3
Helicobacter pylori	ATCC 49503	≥1.0 x 10^6 CFU/mL†	3/3	3/3
Klebsiella pneumonia	ATCC 13883	3.2 x 10^8 CFU/mL	3/3	3/4§
Lactobacillus acidophilus	ATCC 4356	4.3 x 10^5 CFU/mL	3/3	3/3
Listeria monocytogenes	ATCC 19115	2.0 x 10^8 CFU/mL	3/3	3/3
Prevotella melaninogenicus	ATCC 25845	1.6 x 10^7 CFU/mL	3/3	3/3
Prevotella oralis	ATCC 33322	≥1.0 x 10^6 CFU/mL†	3/3	3/3
Salmonella typhimurium	ATCC 13311	4.1 x 10^8 CFU/mL	3/3	3/4§
Shigella sonnei	ATCC 29930	6.4 x 10^7 CFU/mL	3/3	3/3
Staphylococcus aureus	ATCC BK-23738	4.5 x 10^8 CFU/mL	3/3*	3/3
Vibrio cholera	ATCC 55188	3.8 x 10^8 CFU/mL	3/3	3/3
Yersinia enterocolitica	ATCC 49397	1.6 x 10^8 CFU/mL	3/3	3/3
Yeasts, Parasites, and Viruses				
Blastocystis hominis (gDNA)	ATCC 50177D	1 ug/mL	3/3	6/6
Entamoeba histolytica	ATCC 30459DQ	1.0 x 10^8 copies/mL	3/3	3/3
Cryptosporidium parvum	ATCC PRA67D	1 ug/mL	3/3	3/3
Giardia lamblia (G. intestinalis)	ATCC 50803D	1 ug/mL	3/3	3/3
Candida albicans	ATCC 18804	3.0 x 10^6 CFU/mL	3/3	3/3
Saccharomyces cerevisiae	ATCC MYA-796	≥1.0 x 10^6 CFU/mL†	3/3^	3/4§
Adenovirus 40	ATCC VR-931D	1 ug/mL	3/3	3/3
Adenovirus 41	ATCC VR-930D	1 ug/mL	3/3	3/4§
Norovirus GI	ATCC VR-3234SD	1.0 x 10^8 copies/mL	3/3	3/4§
Norovirus GII	ATCC VR-3235SD	1.0 x 10^8 copies/mL	3/3	3/3
Rotavirus	ATCC VR-1546	1.0 x 10^5 TCID 50/mL	3/3	3/3
Human genomic DNA (HT-29)	**ATCC HTB-38D**	**1 ug/mL**	**3/3**	**3/3**

† Actual concentration undetermined, estimate based on optical density measurement.
^ Represents each 'INVALID' run in this dataset.
* Represents each 'Test Incomplete' run in this dataset.
‡ Represents each replicate in this set that resulted in 'STEC NEGATIVE/ Serotype O157 Not Tested'.
§ Represents each replicate in this set that resulted in 'STEC POSITIVE/ Serotype O157 NEGATIVE'.

j. Interfering Substances

The Shiga Toxin Direct Test was evaluated for chemical interference by the following panel of 26 different substances that are common stool contaminants or likely present in patients with diarrhea (Table 8). Each substance was tested in the background of a contrived, low positive that was generated by spiking a Shiga toxin-producing *E. coli* strain (ATCC 43895) containing all 3 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 the potential for chemical substances to interfere with assay controls without analyte present.

Table 8. Interfering Substances Panel. Shiga Toxin Direct Test performance evaluation for chemical interference in detecting a Shiga toxin-producing *E. coli* strain.

Interfering Substance	Concentration Tested	% Agreement			
		STEC Strain 43895		Clinical Negative	
		Expected Result			
		STEC POSITIVE/ Serotype O157 POSITIVE		STEC NEGATIVE/ Serotype O157 Not Tested	
Endogenous Substances					
Human Bile	25% v/v	100%	3/3	100%	3/3^^
Human Urine	50% v/v	100%	3/3	100%	3/3
Human Whole Blood	50% v/v	100%	3/3	100%	3/3
Cholesterol	5% w/v (50 mg/mL)	100%	3/3	100%	3/3
Fatty Acids	3.33% w/v (33.3 mg/mL)	100%	3/3	100%	3/3
Mucin	6.25% w/v (6.25 mg/mL)	100%	3/3	100%	3/3
Triglycerides	10% v/v	100%	3/3	100%	3/3
Exogenous Substances					
Amoxicillin	5% w/v (50 mg/mL)	100%	3/3	100%	3/3
Baby Wipes	5% v/v	100%	3/3	100%	3/3
Barium Sulfate	9.9% w/v (99 mg/mL)	100%	3/3	100%	3/3
Ciprofloxacin	1.25% w/v (12.5 mg/mL)	100%	3/3	100%	3/3
Fleet Enema	50% v/v	100%	3/3	100%	3/3
Gaviscon Liquid Anacid	10% v/v	100%	3/3	100%	3/3
Glycerin Laxative	50% v/v	100%	3/3	100%	3/3
Hydrocortisone Cream	7.5% w/v (75 mg/mL)	100%	3/3	100%	3/3
Imodium	10% v/v	100%	3/3	100%	3/3
Personal Lubricant (K-Y Jelly)	50% v/v	100%	3/3^	100%	3/3
Laxative Tablet	0.97% w/v (9.7 mg/mL)	100%	3/3	100%	3/3
Metronidazole	5% w/v (50 mg/mL)	100%	3/3	100%	3/3
Milk of Magnesia	10% v/v	100%	3/3	100%	3/3
Mineral Oil	50% v/v	100%	3/3	100%	3/3
Pepto Bismal	10% v/v	100%	3/3	100%	3/3*
Preparation H Cream	9.5% w/v (95 mg/mL)	100%	3/3	100%	3/3
Stool Softener	0.7% w/v (7 mg/mL)	100%	3/3*	100%	3/3**^
Tums	20% (200 mg/mL)	100%	3/3	100%	3/3^
Vaginal Contraceptive Gel	50% v/v	100%	3/3	100%	3/3

* Represents each 'Test Incomplete' run in this dataset.

^ Represents each 'INVALID' run in this dataset.

As summarized in Table 8, none of the chemical substances tested interfered with detection of either Shiga toxin or O157 Serotype gene targets, and each test resulted in 'STEC POSITIVE/Serotype O157 POSITIVE' calls as expected. Additionally, none of the chemical substances interfered with assays controls when negative stool was tested. During evaluation of chemical interference, four (4) runs yielded incomplete testing results and five (5) 'invalid' tests were observed. In each instance the specimen was re-retested on a new Shiga Toxin Direct Test cartridge and resulted in the correct call.

k. Carry-Over/Cross-Over 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. By running a series of alternating runs of high positive and negative samples on multiple Portrait Analyzers, potential carry-over/ cross-contamination was evaluated. 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 were in concordance with 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 3 samples were re-tested on new cartridges and all resolved to the expected result.

l. Media Equivalency Study (Poolability)

A Media Equivalency (Poolability) Study was conducted to demonstrate equivalent Shiga Toxin Direct Test performance in six (6) widely used stool preservation media types, including: 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 in Meridian™ Para-Pak® C&S. The Media Equivalency Study was designed to demonstrate equivalent Shiga Toxin Direct Test performance in each test media type by re-evaluating three (3) of the STEC strains for which LoD was initially measured at concentration near LoD (2X LoD), above LoD (5X LoD) and below LoD (0.5X LoD). 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 stool matrices (6 in total) were evaluated directly as clinical negative samples and as the base for contrived positives of each media type.

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, the Shiga Toxin Direct Test performance was as expected and each test media type demonstrated equivalent performance to all other test mediums, as well as equivalent performance to the reference media, Meridian™ Para-Pak® C&S. 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 was initially tested at the highest concentrations, approximately 5X LoD for strain ATCC 51434 and approximately 5X and 2X LoD for ATCC BAA-2191 and ATCC 43895 strains. At these concentrations each strain was expected to return a 'positive' Shiga Toxin Direct Test result in ≥95% of replicates, however all of the test results were either 'negative' (12%) or 'invalid' (88%). Clinical negative stool matrix, formulated with 10% Formalin media, was also evaluated via Shiga Toxin Direct Test and expected to yield 100% 'negative' results, however only 20% (2/10 replicates) resolved as 'negative'. The remaining 80% of negative stool replicates tested (8/10 replicates) also yielded 'invalid' results. At 85.7%, the overall invalid rate for initial testing (35 samples in total) was abnormally high, 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 was initially tested at all three test concentrations for strain ATCC BAA-2191 (approximately 5X LoD, 2XLoD, and 0.5XLoD). At approximately 2X-5X LoD, ≥95% of the Shiga Toxin Direct Test replicates are expected to be 'STEC POSITIVE/Serotype O157 NEGATIVE'. However in Zn PVA transport media, 100% of the Shiga Toxin Direct Test replicates resulted in 'INVALID' test results. Clinical negative stool matrix, formulated with Zn PVA transport media, was also evaluated and expected to yield 100% 'negative' results, however 100% of the clinical negative replicated resulted in 'INVALID' Shiga Toxin Direct Test results. Therefore, the invalid rate for this initial testing (39 samples in total) was 100%, suggesting that Zn PVA transport media 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 9.

Table 9. Summary of Media Equivalency for Shiga Toxin Direct Test.

Stool Preservation Medias 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 Medias 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

m. Reproducibility

Reproducibility testing of the Shiga Toxin Direct Test was conducted using a panel of five (5) prepared samples consisting of four 'positive' samples and one 'negative'. The 'positive' panel constituents comprised 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 contrived positive samples were made by spiking the respective 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 only clinical negative stool matrix.

The Reproducibility studies were performed at three external clinical sites using randomized, blind-coded panels and two (2) different Shiga Toxin Direct Test cartridge lots. At each site, these studies were performed over the course of five (5), non-consecutive days. For each day of testing, two (2) panel runs were performed with three (3) replicates of each sample per run (Table 10) on each day. A minimum of two (2) operators was required to perform Reproducibility testing at each site. Results of the Reproducibility studies are summarized in Table 11.

Table 10. Reproducibility Study Testing Protocol Overview.

Test Sites	3 external sites
Panel Size	5 samples
Panel Constituents	1. Moderate Positive ATCC BAA-2192
	2. Low Positive ATCC BAA-2192
	3. Moderate Positive ATCC 43895
	4. Low Positive ATCC 43895
	5. Clinical Negative
Runs per Sample	3 replicates
Runs per Day (2 operators)	5 samples x 3 replicates x 2 operators = 30
Total Runs per Site (5 days)	30 runs/day x 5 days = 150
Total Runs	150 runs/site x 3 sites = 450

Table 11. Overall Results of the Reproducibility Studies.

Sample Type (Panel Constituents)	Expected Result	% Agreement							
		Site 1		Site 2		Site 5		All Sites	
1. Moderate Positive ATCC BAA-2192	STEC POSITIVE/ Serotype O157 NEGATIVE	30/30	100%	30/30	100%	30/30	100%	90/90	100%
2. Low Positive ATCC BAA-2192	STEC POSITIVE/ Serotype O157 NEGATIVE	30/30	100%	30/30	100%	29/30	97%	89/90	99%
3. Moderate Positive ATCC 43895	STEC POSITIVE/ Serotype O157 POSITIVE	30/30	100%	30/30	100%	30/30	100%	90/90	100%
4. Low Positive ATCC 43895	STEC POSITIVE/ Serotype O157 POSITIVE	30/30	100%	30/30	100%	30/30	100%	90/90	100%
5. Clinical Negative	STEC NEGATIVE/ Serotype O157 Not Tested	30/30	100%	30/30	100%	30/30	100%	90/90	100%

The cumulative data for Reproducibility testing of the Shiga Toxin Direct Test across all three sites is summarized in Table 11. 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. All panel members produced acceptable performance results.

G. Performance Data – Prospective Clinical Studies

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 stool samples were enrolled and evaluated. Of these, 1,082 clinical specimens met the inclusion criteria and were used in the prospective study to evaluate the performance of the Shiga Toxin Direct Test. Of these 1,082 specimens, 1,047 used C&S preservation medium, 34 used Cary-Blair preservation medium, and the preservation medium type was not specified for one specimen. Prospective evaluation was conducted by comparing the performance of the Portrait Shiga Toxin Direct Test to the reference clinical microbiology protocols for the detection of both Shiga Toxin and the *E. coli* O157 Serotype. Results from these studies from all five sites are combined and summarized in Table 12.

Table 12. Overall Shiga Toxin Direct Test Performance from Prospective Testing at all Clinical Evaluation Sites.

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	100.0%	39.8%	100.0%
Specificity	99.3%	98.5%	99.7%
PPV	33.3%	9.9%	65.1%
NPV	100.0%	99.7%	100.0%

‡ Shiga toxin 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	100.0%	69.2%	100.0%

‡ O157 serogroup 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), especially of the O157 serotype, a Frozen Retrospective panel was constructed and tested at three (3) clinical test sites in order to enrich the sample set for positives. This panel consisted of 92 unique clinical specimens previously characterized as positive or negative for STEC and the O157 serotype. Of these, 88 frozen clinical specimens met the inclusion criteria and were used in the Frozen Retrospective study to evaluate the performance of the Shiga Toxin Direct Test. Of these 88 specimens, 44 used C&S preservation medium, 40 used Cary-Blair preservation medium, and 4 used Enteric Transport medium. Results from this study is summarized in Table 13. The performance of the Shiga Toxin Direct Test, both in prospective and frozen retrospective specimen testing, is summarized in comparison in Table 14.

Table 13. Overall Shiga Toxin Direct Test Performance Results from Frozen Retrospective Specimen Testing.

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	100.0%	89.4%	100.0%

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	100.0%	85.8%	100.0%

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

Table 14. Combined Shiga Toxin Direct Test Clinical Performance Results including Prospective and Frozen Retrospective Studies.

Specimen Type			***n***	***% Agreement (95% CI)***	
				Positive	***Negative***
Shiga toxin (*sx1/stx2*)	Clinical Specimens	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**	Clinical Specimens	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)

H. Conclusion

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