June 1, 2018

Micronics, Inc
Karen Hedine
President
8464 154th Ave NE
Redmond, Washington 98052

Re: K173330
Trade/Device Name: PanNAT STEC Test
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal microorganism multiplex nucleic acid-based assay
Regulatory Class: Class II
Product Code: PCI, PCH, OOI
Dated: April 27, 2018
Received: May 1, 2018

Dear Karen Hedine:

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 Part 801 and Part 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.

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.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/) and CDRH Learn (http://www.fda.gov/Training/CDRHLearn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (http://www.fda.gov/DICE) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

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: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K173330

Device Name
PanNAT STEC Test

Indications for Use *(Describe)*
The Micronics PanNAT STEC Test is a qualitative, *in vitro* nucleic acid amplification-based test intended for the simultaneous detection and identification of the *stx*1 and *stx*2 Shiga toxin genes and the O-antigen gene cluster of *E.coli* O157 (*fcl*). Testing is performed in a unitized cartridge on the PanNAT System on soft to diarrheal unpreserved or Cary-Blair preserved stool specimens from individuals with signs and symptoms of gastrointestinal infection. The PanNAT STEC Test is intended for use, in conjunction with clinical presentation, laboratory findings and epidemiological risk factors, as an aid in detection of specific Shiga-toxin expressing strains of *E. coli* ("STEC") from patients with diarrhea.

The results of this test should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Positive PanNAT STEC Test results do not rule out the potential for coinfection with other pathogens that are not detected by this device and may not be indicative of the sole or definitive cause of patient illness. Negative PanNAT STEC Test results in the context 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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5. 510(k) Summary

5.1 Applicant

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Date of Preparation: May 22, 2018

5.2 Device

Trade Name: PanNAT® STEC Test
Common name: Gastrointestinal Bacterial Panel Multiplex Nucleic Acid-Based Assay System
Regulation number: 866.3990, Gastrointestinal microorganism multiplex nucleic acid-based assay
Product Code: PCI, PCH, OOI
Class: 2

5.3 Predicate Device

BD Max Enteric Bacterial Panel (K140111)

5.4 Intended Use

The Micronics PanNAT STEC Test is a qualitative, *in vitro* nucleic acid amplification-based test intended for the simultaneous detection and identification of the *stx1* and *stx2* Shiga toxin genes and the O-antigen gene cluster of *E.coli* O157 (*fcl*). Testing is performed in a unitized cartridge on the PanNAT System on soft to diarrheal unpreserved or Cary-Blair preserved stool specimens from individuals with signs and symptoms of

gastrointestinal infection. The PanNAT STEC Test is intended for use, in conjunction with clinical presentation, laboratory findings and epidemiological risk factors, as an aid in detection of specific Shiga-toxin expressing strains of *E. coli* ("STEC") from patients with diarrhea.

The results of this test should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Positive PanNAT STEC Test results do not rule out the potential for coinfection with other pathogens that are not detected by this device and may not be indicative of the sole or definitive cause of patient illness. Negative PanNAT STEC Test results in the context 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.

5.5 Device Description

The PanNAT System consists of the instrument with onboard software and 9-inch touchscreen used to process PanNAT STEC Test cartridges. The instrument automates all steps of the assay after sample addition to the test cartridge and insertion into the instrument; including DNA purification, nucleic acid amplification and detection of the target nucleic acid sequences using qualitative real-time PCR.

The system is a portable device that is powered by an external mains supply with a voltage range of 100-240VAC and a frequency range of 50-60 Hz. An onboard battery is included to provide power to the instrument for up to one hour in the event that mains power supply is interrupted. The instrument includes a pneumatic subsystem, a thermal control unit, fluorescent optical detectors, and software needed to process a test cartridge.

The PanNAT STEC Test cartridge is a unique, single use, disposable device in which all test reagents and controls are incorporated and all steps of the assay are performed. The PanNAT STEC Test cartridge uses a PanNAT Sample Transfer Pack accessory that contains a flocked swab, a prefilled Sample Buffer Tube and an Adaptor Cap.

There are three integrated controls for the PanNAT STEC Test: an endogenous human DNA internal process control that is coextracted and coamplified with the target nucleic acids, a negative control, and a positive control. These controls are performed automatically and do not require any action from the operator. External quality controls for the PanNAT STEC Test are also commercially available. Alternatively, laboratories may prepare their own controls.

5.6 Principle of Operation

Using the swab from the Sample Transfer Pack, the operator transfers a specimen (either soft to diarrheal unpreserved stool specimen or Cary-Blair preserved stool specimen) to the Sample Buffer Tube, which is then sealed using the Adaptor Cap. The Sample Buffer Tube is then attached to the sample inlet port of the cartridge by means of the attached Adaptor Cap; fully containing all fluids within the cartridge-tube assembly. Once the test cartridge with attached Sample Buffer Tube is inserted into the instrument, the cartridge barcode is scanned and the test starts automatically.

Utilizing proprietary microfluidic design, and under control of the instrument, sample and reagents are moved through the cartridge to complete all of the steps of the procedure.

DNA is extracted from lysed cells, captured on silica membranes, and eluted to accomplish purification. DNA sequences specific to *stx1*, *stx2* and O157, together with the internal process control, are amplified and then detected by fluorescent probe hybridization for qualitative real-time PCR detection.

When the test is completed, sample results are displayed on the screen and stored in the instrument database. Test results can be exported to a USB flash drive or printer.

5.7 Substantial Equivalence

A comparison of similarities and differences between the PanNAT STEC Test and the predicate device is provided in Table 5-1.

Table 5-1. Substantial Equivalence Table Comparing PanNAT STEC Test to Predicate

Features	PanNAT STEC Test	Predicate: BD Max™ Enteric Bacterial Panel (K140111)
Similarities		
Intended Use	The Micronics PanNAT STEC Test is a qualitative, *in vitro* nucleic acid amplification-based test intended for the simultaneous detection and identification of the *stx1* and *stx2* Shiga toxin genes and the O-antigen gene cluster of *E.coli* O157 (*fcl*). Testing is performed in a unitized cartridge on the PanNAT System on soft to diarrheal unpreserved or Cary-Blair preserved stool specimens from individuals with signs and symptoms of gastrointestinal infection. The PanNAT STEC Test is intended for use in conjunction with clinical presentation, laboratory findings and epidemiological risk factors, as an aid in detection of specific Shiga-toxin expressing strains of *E. coli* ("STEC") from patients with diarrhea. The results of this test should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Positive PanNAT STEC Test results do not rule out the potential for coinfection with other pathogens that are not detected by this device and may not be indicative of the sole or definitive cause of patient illness Negative PanNAT STEC Test results in the context 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	The BD MAX™ Enteric Bacterial Panel performed on the BD MAX™ System is an automated in vitro diagnostic test for the direct qualitative detection and differentiation of enteric bacterial pathogens. The BD MAX™ Enteric Bacterial Panel detects nucleic acids from: • *Salmonella* spp. • *Campylobacter* ssp. (*jejuni* and *coli*) • *Shigella* spp./Enteroinvasive *E. coli* (EIEC) • Shiga Toxin 1 (*stx1*)/Shiga toxin 2 (*stx2*) genes (found in Siga toxin-producing *E. coli* as well as *Shigella dysenteriae*, which can possess a Shiga toxin gene (*stx*) that is identical to the *stx1* gene of STEC. Testing is performed on unpreserved soft to diarrheal unpreserved stool specimens or Cary Blair preserved stool symptomatic patients with suspected acute gastroenteritis, enteritis or colitis. The test is performed directly on the specimen, utilizing real-time polymerase chain reaction (PCR) for the amplification of *SpaO*, a *Campylobacter* specific *tuf* gene sequence, *ipaH* and *stx1/stx2*. The test utilizes fluorogenic sequence-specific hybridization probes for detection of the amplified DNA. This test is intended for use, in conjunction with clinical presentation, laboratory findings and epidemiological

	colitis, irritable bowel syndrome, or Crohn's disease.	information, as an aid in the differential diagnosis of *Salmonella*, *Shigella*/EIEC, *Campylobacter* and Shiga toxin-producing *E. coli* (STEC) infections. Results of this test should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Positive results do not rule out co-infection with other organisms that are not detected by this test and may not be the sole definitive cause of patient illness. Negative results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.
Specimen Type	Unpreserved and Cary-Blair preserved stool	Same
Assay Format	Real-time PCR amplification: Fluorogenic target-specific hybridization	Same
Detection of stx1/stx2	Presence of *stx1* and *stx2* genes specific to Shiga toxin-producing organisms	Same
Sample Preparation	Automated by the PanNAT System	Automated by the BD MAX™ System
Detection probes	Fluorescent Probes MGB Pleiades™ Probe	Fluorescent Probes TaqMan® Probe
Assay controls	Internal Process Control and Integrated positive and negative controls	Sample Processing Control (SPC)
Differences		
Detection of *E. coli*	*fcl* gene for *E. coli* O157	*ipaH* gene for *E coli* (EIEC)
Analysis platform	PanNAT® System	BD MAX™ System

5.8 Analytical Performance Studies

Precision/ Reproducibility

Reproducibility of the PanNAT STEC Test was evaluated at three sites (2 external and 1 internal) utilizing 3 test cartridge lots, 6 operators (2 per site), and 13 PanNAT instruments. A total of 90 samples, 10 for each of 9 panel members were evaluated in triplicate over a minimum of 6 days at each site.

The coded 9-member panel consisted of *E. coli* strains containing a single analyte at low or moderate concentrations of *stx1, stx2* or O157, a strain positive for all analytes at low or moderate concentrations and a strain negative for all 3 analytes.

Agreement results by panel member by site are shown in Table 5-2. Each site used a different lot of cartridges; therefore lot-to-lot variability is demonstrated by site. In Table 5-3, agreement for each analyte is shown by concentration and by site/lot.

Table 5-2. Agreement by Panel Member by Site

Analyte Panel	Panel 1 All Neg	Panel 2 Low *stx1*	Panel 3 Med *stx1*	Panel 4 Low *stx2*	Panel 5 Med *stx2*	Panel 6 Low O157	Panel 7 Med O157	Panel 8 All Low	Panel 9 All Med	Total
Overall										
%Agmt (95% CI)	100.00% (95.6,100)	97.60% (91.8,99.4)	98.8% (93.4,99.8)	97.70% (91.9, 99.4)	100.00% (95.8, 100)	98.90% (93.8, 99.8)	100.00% (95.8, 100)	88.60% (80.3, 93.7)	98.90% (93.8, 99.8)	**97.8%**
Total (n)	83	85	82	86	87	88	87	88	87	773
Agmt (n)	83	83	81	84	87	87	87	78	86	756
Site A										
% Agmt (95% CI)	100.0% (87.9,100)	100.0% (87.9,100)	100.0% (87.5,100)	100.0% (87.5,100)	100.0% (87.9,100)	100.0% (88.6,100)	100.0% (88.6,100)	93.3% (78.7,98.2)	96.3% (81.7,99.3)	**98.8%**
Total (n)	28	28	27	27	28	30	30	30	27	255
Agmt (n)	28	28	27	27	28	30	30	28	26	252
Site B										
% Agmt (95% CI)	100.0% (88.3,100)	100.0% (88.3,100)	96.7% (83.3,99.4)	100.0% (88.3,100)	100.0% (88.3,100)	100.0% (88.3,100)	100.0% (87.9,100)	96.4% (82.3,99.4)	100.0% (88.6,100)	**99.2%**
Total (n)	29	29	30	29	29	29	28	28	30	261
Agmt (n)	29	29	29	29	29	29	28	27	30	259
Site C										
% Agmt (95% CI)	100.0% (87.1, 100)	92.90% (77.3, 98.0)	100.0% (87.1, 100)	93.30% (78.7, 98.2)	100.0% (88.6, 100)	96.60% (82.8, 99.4)	100.0% (88.3, 100)	76.70%* (59.1, 88.2)	100.0% (88.6, 100)	**95.3%**
Total (n)	26	28	25	30	30	29	29	30	30	257
Agmt (n)	26	26	25	28	30	28	29	23	30	245

*83/90 possible results for Panel Member #8 were concordant (92% [80.0, 100]). The individual test results for this panel member at Site C were: *stx* 1: 93.3% (80, 100), 28/30 concordant results; *stx* 2: 86.6% (70, 90), 26/30 concordant results; *fcl:* 96.7% (80, 100), 29/30 concordant results. For all of the seven Panel Member #8 test results that did not demonstrate full concordance with the expected result, only one target was missed at each test point. None of these missed 2 targets and none missed 3 targets. Further, all three target concentrations in Panel Member #8 were at the LoD of the assay (1.00E+06 CFU/mL) and as such are not expected to be detected 100% of the time. When looking at the individual targets, the detection rate ranged from 86.6%-96.7%.

Table 5-3. Agreement by Concentration, Analyte and Site/Lot for Positive Panel Members

	Analyte	*stx1*			*stx2*			O157		
		Neg	Low	Med	Neg	Low	Med	Neg	Low	Med
Total^	%Agmt (95% CI)	100.0% (98.9,100)	97.1% (93.4,98.8)	98.8% (95.8,99.7)	100.0% (98.9,100)	96.0% (91.9,98.0)	100.0% (97.8,100)	100.0% (98.9,100)	97.2% (93.5,98.8)	99.4% (96.8,99.9)
	Total (n)	348	173	169	342	174	174	340	176	174
	Agmt (n)	348	168	167	342	167	174	340	171	173
Site A Lot 1	%Agmt (95% CI)	100.0% (96.8,100)	100.0% (93.8,100)	98.1% (90.2,99.7)	100.0% (96.8,100)	100.0% (93.7,100)	100.0% (93.5,100)	100.0% (96.6,100)	96.7% (88.6,99.1)	98.2% (90.7,99.7)
	Total (n)	115	58	54	115	57	55	110	60	57
	Agmt (n)	115	58	53	115	57	55	110	58	56
Site B Lot 2	%Agmt (95% CI)	100.0% (96.8,100)	98.2% (90.7,99.7)	98.3% (91.1,99.7)	100.0% (96.8,100)	98.2% (90.7,99.7)	100.0% (93.9,100)	100.0% (96.8,100)	98.2% (90.7,99.7)	100.0% (93.8,100)
	Total (n)	115	57	60	116	57	59	117	57	58
	Agmt (n)	115	56	59	116	56	59	117	56	58
Site C Lot 3	%Agmt (95% CI)	100.0% (96.9,100)	93.1% (83.6,97.3)	100.0% (93.5,100)	100.0% (96.7,100)	90.0% (79.8,95.3)	100.0% (94.0,100)	100.0% (96.7,100)	96.6% (88.5,99.1)	100.0% (93.9,100)
	Total (n)	118	58	55	111	60	60	113	59	59
	Agmt (n)	118	54	55	111	54	60	113	57	59

Total^: total combined across sites. *stx1* neg: panel members 4, 5, 6, 7, *stx1* low: panel 2 ,8, *stx1* medium: panel members 3, 9, *stx2* neg: panels 2, 3, 6, 7, *stx2* low: panel members 4, 8, *stx2* medium: panel members 5,9, O157 neg: panels 2, 3, 4, 5. O157 low: panels 6, 8, O157 medium: panel members 7, 9 *95% CI: 95% confidence intervals calculated utilizing Wilson's Method.

For evaluation of the quantitative results, all negative samples in panel member 1 reported "0" and were therefore in 100% agreement. For all positive samples with a valid result, the mean, standard deviation and coefficient of variation (%CV) are reported.

To further evaluate performance by Site/Lot the mean, standard deviation, and coefficient of variation of cycle number were calculated. These parameters are calculated excluding samples that were expected to yield a positive result but where the PanNAT STEC Test cycle number was 0 (Table 5-4).

Table 5-4. Reproducibility Cycle Numbers by Site, Analyte and Concentration

Site	Analyte:	*stx 1*		*stx 2*		O157		Process Control [1]
		Low	Med	Low	Med	Low	Med	
Site A Lot 1	n	58	53	57	55	58	56	84
	Mean (std)	36.2 (0.8)	34.8 (0.9)	35.5 (1.0)	33.6 (1.1)	35.4 (0.8)	34.0 (1.0)	28.6 (1.0)
	CV	2.2%	2.6%	2.7%	3.2%	2.1%	3.0%	3.6%
Site B Lot 2	n	56	59	56	59	56	58	87
	Mean (std)	36.0 (0.8)	34.9 (0.7)	35.4 (1.0)	33.9 (0.8)	35.6 (0.8)	34.2 (0.8)	28.7 (1.0)
	CV	2.2%	1.9%	2.7%	2.3%	2.1%	2.4%	3.4%
Site C Lot 3	n	54	55	54	60	57	59	78
	Mean (std)	35.3 (0.7)	34.0 (0.7)	35.1 (0.9)	33.5 (0.8)	35.4 (1.0)	33.9 (0.7)	28.9 (0.9)
	CV	2.0%	2.2%	2.7%	2.5%	2.7%	2.0%	3.0%

[1]In negative panel members
n=number of observations, mean = mean cycle number, std = standard deviation for mean cycle number, CV=percent coefficient of variation.

Controls

The PanNAT STEC Test has an internal process control, a negative control, and a positive control, integrated within the cartridge. External controls are not provided, but are commercially available. Alternatively, a strain of *E. coli* that is positive for *stx1*, *stx2* and *fcl,* such as strain EDL933 (ATCC® #43895™) may be used as an external positive control. A known negative sample such as a suspension of human A549 cells (ATCC® CCL-185™) may be used as an external negative control.

Specimen Stability

The recommended storage time and temperature for Cary-Blair preserved and unpreserved stool specimens is:

- Refrigerated storage (2-8^{o}C) for up to 7 days

To assess the stability of PanNAT STEC Test targets, testing was performed on both Cary-Blair preserved and unpreserved specimens at 3X LoD. The study tested the O157 STEC reference strain (ATCC #43895), which contains all three PanNAT STEC Test targets. Specimens were stored at 2-8°C and tested at intervals over the course of the study. Five replicates per specimen type per time point were tested. Results demonstrated that both specimen types were stable for 7 days under these conditions for all PanNAT STEC Test analytes.

Analytical Sensitivity/ Limit of Detection (LoD)

An estimation of the LoD for seven *E. coli* strains with different combinations of the *stx1*, *stx2* and O157 was performed by testing dilutions of each strain with the PanNAT STEC Test. Unpreserved and preserved specimens were tested at different concentrations. The estimated LoD was the lowest concentration at which all replicates were positive. The estimated LoD was then confirmed with strains EDL933 (*stx1+*, *stx2+*, O157+) and 3007-85 (*stx1+*, *stx2+*, O157-) tested in replicates of 28 at the estimated LoD in both preserved and unpreserved specimen types. To be considered confirmed, ≥95% of replicates had to produce positive results at the LoD target level.

Results:

- In unpreserved stool specimens, the LoD for EDL933 and 3007-85 (*stx1*, *stx2*, and O157) is 1.00E+06 CFU/mL.
- In stool specimens preserved in Cary-Blair media, the LoD for EDL933 and 3007-85 (*stx1, stx2* and O157) is 1.75E+06 CFU/mL.

Analytical Reactivity

A collection of well-characterized O157 and non-O157 strains was tested using the PanNAT STEC Test. The presence of the two Shiga toxin genes, *stx1* and *stx2*, was assessed and measured in seven (7) O157 strains, twelve (12) non-O157 strains, six (6) un-typeable (O) strains, and in a single O-antigen negative laboratory strain as a control (K12).

A summary of results is listed below:

- The PanNAT STEC Test detected *stx1* and *stx2* in O157, non-O157 serotype strains and un-typeable O strains.
- The PanNAT STEC Test detected at least 2 subtypes of each toxin gene.
- For *stx1*, the PanNAT STEC Test generated the expected results for 24/26 strains (92%), including strains with subtypes *stx1a* and *stx1c*. The 2 strains that gave false negative results (TW07740, TW07754) were determined to be *stx1d* variants. The PanNAT STEC Test only weakly detects *stx1d* due to nucleotide mismatches in the probe-binding region.
- For *stx2*, the PanNAT STEC Test generated the expected results for 25/26 strains (96%), including strains with subtypes *stx2a* and *stx2c/d*. The strain that gave false negative results (TW07731) was determined to be an *stx2* variant that closely resembles *stx2b*. The PanNAT STEC Test cannot detect this variant due to extensive nucleotide mismatches in the primer and probe binding regions.
- For the O157 O-antigen (*fcl*) gene cluster, the PanNAT STEC Test generated the expected results in 26/26 strains (100%).

Analytical Specificity – Cross Reactivity

Thirty-seven (37) unique bacterial strains, eight (8) viruses, one (1) yeast organism, and three (3) parasites were spiked into a STEC negative pool matrix and evaluated for cross reactivity (Table 5-5).

Table 5-5. Summary of Cross Reactivity Data

Organism	Strain ID/Catalog #	Input Tested	% Agreement with Expected Result (*stx1*- / *stx2*- / O157-)
Bacteria			
Acinetobacter baumannii	ZeptoMetrix 307-0294 0801597	1.00E+06 CFU/mL	3/3 (100%)
Aeromonas hydrophila	ZeptoMetrix Z161 0804098 and ATCC 35654	1.00E+06 CFU/mL	3/3 (100%)
Bacillus cereus	ZeptoMetrix ZO91 0801823	1.00E+06 CFU/mL	3/3 (100%)
Bacteroides fragilis	ZeptoMetrix Z029 0801583	1.00E+06 CFU/mL	3/3 (100%)
Campylobacter jejuni	ZeptoMetrix Z086 0801650	1.00E+06 CFU/mL	3/3 (100%)
Citrobacter koseri	ZeptoMetrix Z039 0801745	1.00E+06 CFU/mL	3/3 (100%)
Clostridium difficile	ZeptoMetrix NAP1 0801619	1.00E+06 CFU/mL	3/3 (100%)
Clostridium parabotulinum	ATCC 17863	1.00E+06 CFU/mL	3/3 (100%)
Clostridium perfringens	ZeptoMetrix TypeA 0801585	1.00E+06 CFU/mL	3/3 (100%)
Enterobacter cloacae	ZeptoMetrix Z101 0801830	1.00E+06 CFU/mL	3/3 (100%)
Enterococcus faecalis	ZeptoMetrix VRE 0801693	1.00E+06 CFU/mL	3/3 (100%)
Enterococcus faecium	ZeptoMetrix VRE, vanA 0801892	1.00E+06 CFU/mL	3/3 (100%)
Escherichia coli K12	ATCC 29425	1.00E+06 CFU/mL	3/3 (100%)
Escherichia coli O111a (EAEC)	ATCC 29552	1.00E+06 CFU/mL	3/3 (100%)
Escherichia coli O29:NM (EIEC)	STEC Center O29:NM TW01095	1.00E+06 CFU/mL	3/3 (100%)
Escherichia coli O111a:2 (EPEC)	STEC Center O111a:2 TW07886	1.00E+06 CFU/mL	3/3 (100%)
Escherichia coli O8:H19 (ETEC)	STEC Center O8:H19 TW09068	1.00E+06 CFU/mL	3/3 (100%)
Escherichia hermannii	ATCC 33650	1.00E+06 CFU/mL	3/3 (100%)
Hafnia alvei	ATCC 13337	1.00E+06 CFU/mL	3/3 (100%)
Klebsiella pneumoniae	ZeptoMetrix Z026 0801506	1.00E+06 CFU/mL	2/2^ (100%)
Listeria monocytogenes	ZeptoMetrix serotype 1/2b 0801534	1.00E+06 CFU/mL	3/3 (100%)
Morganella morganii	ATCC 25830	1.00E+06 CFU/mL	3/3 (100%)
Plesiomonas shigelloides	ZeptoMetrix Z130 0801899	1.00E+06 CFU/mL	3/3 (100%)
Proteus mirabilis	ZeptoMetrix Z050 0801544	1.00E+06 CFU/mL	3/3 (100%)
Pseudomonas aeruginosa	ZeptoMetrix clinical isolate 0801519	1.00E+06 CFU/mL	3/3 (100%)
Salmonella enterica	ATCC 35664	1.00E+06 CFU/mL	3/3 (100%)
Serratia marcescens	ZeptoMetrix Z053 0801723	1.00E+06 CFU/mL	3/3 (100%)
Shigella dysenteriae	**ATCC 13313**	**1.00E+06 CFU/mL**	**3/3‡ (100%)**
Shigella flexneri	ZeptoMetrix Z046 0801757	1.00E+06 CFU/mL	3/3 (100%)
Shigella sonnei	ZeptoMetrix clinical isolate 0801627	1.00E+06 CFU/mL	3/3 (100%)
Staphylococcus aureus	ZeptoMetrix Z020 0801531	1.00E+06 CFU/mL	2/2^ (100%)
Staphylococcus epidermidis	MSSE:HER1292 0801689	1.00E+06 CFU/mL	3/3 (100%)
Streptococcus agalactiae	ZeptoMetrix Z019 0801545	1.00E+06 CFU/mL	3/3 (100%)
Streptococcus dysgalactiae	ZeptoMetrix Z068 0801516	1.00E+06 CFU/mL	3/3 (100%)
Vibrio spp	ATCC 11985	1.00E+06 CFU/mL	3/3 (100%)
Yersinia enterocolitica	ZeptoMetrix Z036 0801734	1.00E+06 CFU/mL	3/3 (100%)
Yersinia pseudotuberculosis	ATCC 4284	1.00E+06 CFU/mL	3/3 (100%)
Viruses			
Adenovirus 18	ZeptoMetrix D.C.(VR-19) VPL-030	1.00E+05 PFU/mL	3/3 (100%)
Adenovirus 40	ZeptoMetrix Dugan HEK293A 0810084CF	1.00E+05 PFU/mL	3/3 (100%)
Coxsackie B4	ZeptoMetrix LLC-mk2 0810075CF	1.00E+05 PFU/mL	3/3 (100%)
Cytomegalovirus	ZeptoMetrix AD-169 MRC-5 0810003CF	1.00E+05 PFU/mL	3/3 (100%)
Echovirus 11	ZeptoMetrix LLC-MK2 0810023CF	1.00E+05 PFU/mL	3/3 (100%)
Enterovirus 68	ZeptoMetrix FO2-3607 corn (VR-1197) Hela VPL-030	1.00E+05 PFU/mL	3/3 (100%)
Norovirus (recombinant)	ZeptoMetrix Vero Group 10810086CF	1.00E+05 PFU/mL	3/3 (100%)
Rotavirus	ZeptoMetrix WA MA-104 MRC-5 0810041CF	1.00E+05 PFU/mL	3/3 (100%)
Yeast and Parasites			
Candida albicans	ZeptoMetrix Z006 0801504	1.00E+06 CFU/mL	3/3 (100%)

Cryptosporidium parvum	Waterborne Iowa Isolate	1.00E+06 Oocysts/mL	3/3 (100%)
*Entamoeba histolytica (*lysate*)*	ZeptoMetrix DS4-868 BacT-035R05	1.00E+06 Cysts/mL	3/3 (100%)
Giardia lamblia	Waterborne H3 isolate	1.00E+06 Cysts/mL	3/3 (100%)

^ Indicates that one of the replicates was "INCOMPLETE" and was removed from analysis.
‡ *S. dysenteriae* detection is expected.

Analytical Specificity – Microbial Interference

For microbial interference, the same 49 non-target organisms were spiked into Shiga toxin negative pooled, unpreserved matrix containing 1X LoD of the reference strain *E. coli* O157:H7 (EDL933 ATCC #43895), which is positive for target analytes *stx1*, *stx2* and O157 (Table 5-6).

Table 5-6. Summary of Microbial Interference Data

Organism	Strain ID/Catalog #	Input Tested CFU/mL	% Agreement with Expected Result (*stx1*+ / *stx2*+ / O157+)
Bacteria			
Acinetobacter baumannii	ZeptoMetrix 307-0294 0801597	1.00E+06 CFU/mL	3/3 (100%)
Aeromonas hydrophilia	ZeptoMetrix Z161 0804098 and ATCC 35654	1.00E+06 CFU/mL	3/3 (100%)
Bacillus cereus	ZeptoMetrix ZO91 0801823	1.00E+06 CFU/mL	3/3 (100%)
Bacteroides fragilis	ZeptoMetrix Z029 0801583	1.00E+06 CFU/mL	3/3 (100%)
Campylobacter jejuni	ZeptoMetrix Z086 0801650	1.00E+06 CFU/mL	3/3 (100%)
Citrobacter koceri	ZeptoMetrix Z039 0801745	1.00E+06 CFU/mL	3/3 (100%)
Clostridium difficile	ZeptoMetrix NAP1 0801619	1.00E+06 CFU/mL	3/3 (100%)
Clostridium parabotulinum	ATCC 17863	1.00E+06 CFU/mL	3/3 (100%)
Clostridium perfringens	ZeptoMetrix TypeA 0801585	1.00E+06 CFU/mL	3/3 (100%)
Enterobacter cloacae	ZeptoMetrix Z101 0801830	1.00E+06 CFU/mL	3/3 (100%)
Enterococcus faecalis	ZeptoMetrix VRE 0801693	1.00E+06 CFU/mL	3/3 (100%)
Enterococcus faecium	ZeptoMetrix VRE, vanA 0801892	1.00E+06 CFU/mL	3/3 (100%)
Escherichia coli K12	ATCC 29425	1.00E+06 CFU/mL	3/3 (100%)
Escherichia coli O111a (EAEC)	ATCC 29552	1.00E+06 CFU/mL	3/3 (100%)
Escherichia coli O29:NM (EIEC)	STEC Center O29:NM TW01095	1.00E+06 CFU/mL	3/3 (100%)
Escherichia coli O111a:2 (EPEC)	STEC Center O111a:2 TW07886	1.00E+06 CFU/mL	3/3 (100%)
Escherichia coli O8:H19 (ETEC)	STEC Center O8:H19 TW09068	1.00E+06 CFU/mL	3/3 (100%)
Escherichia hermannii	ATCC 33650	1.00E+06 CFU/mL	3/3 (100%)
Hafnia alvei	ATCC 13337	1.00E+06 CFU/mL	3/3 (100%)
Klebsiella pneumoniae	ZeptoMetrix Z026 0801506	1.00E+06 CFU/mL	3/3 (100%)
Listeria monocytogenes	ZeptoMetrix serotype 1/2b 0801534	1.00E+06 CFU/mL	3/3 (100%)
Morganella morganii	ATCC 25830	1.00E+06 CFU/mL	3/3 (100%)
Plesiomonas shigelloides	ZeptoMetrix Z130 0801899	1.00E+06 CFU/mL	3/3 (100%)
Proteus mirabilis	ZeptoMetrix Z050 0801544	1.00E+06 CFU/mL	3/3 (100%)
Pseudomonas aeruginosa	ZeptoMetrix clinical isolate 0801519	1.00E+06 CFU/mL	3/3 (100%)
Salmonella enterica	ATCC 35664	1.00E+06 CFU/mL	3/3 (100%)
Serratia marcescens	ZeptoMetrix Z053 0801723	1.00E+06 CFU/mL	3/3 (100%)
Shigella dysenteriae	ATCC 13313	1.00E+06 CFU/mL	3/3 (100%)
Shigella flexneri	ZeptoMetrix Z046 0801757	1.00E+06 CFU/mL	3/3 (100%)
Shigella sonnei	ZeptoMetrix clinical isolate 0801627	1.00E+06 CFU/mL	3/3 (100%)
Staphylococcus aureus	ZeptoMetrix Z020 0801531	1.00E+06 CFU/mL	3/3 (100%)
Staphylococcus epidermidis	ZeptoMetrix MSSE:HER1292 0801689	1.00E+06 CFU/mL	3/3 (100%)
Streptococcus agalactiae	ZeptoMetrix Z019 0801545	1.00E+06 CFU/mL	3/3 (100%)
Streptococcus dysgalactiae	ZeptoMetrix Z068 0801516	1.00E+06 CFU/mL	3/3 (100%)
Vibrio spp	ATCC 11985	1.00E+06 CFU/mL	3/3 (100%)

Yersinia enterocolitica	ZeptoMetrix Z036 0801734	1.00E+06 CFU/mL	3/3 (100%)
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Coxsackie B4	ZeptoMetrix LLC-mk2 0810075CF	1.00E+05 PFU/mL	3/3 (100%)
Cytomegalovirus	ZeptoMetrix AD-169 MRC-5 0810003CF	1.00E+05 PFU/mL	3/3 (100%)
Echovirus 11	ZeptoMetrix LLC-MK2 0810023CF	1.00E+05 PFU/mL	3/3 (100%)
Enterovirus 68	ZeptoMetrix FO2-3607 corn (VR-1197) Hela VPL-030	1.00E+05 PFU/mL	3/3 (100%)
Norovirus (recombinant)	ZeptoMetrix Vero Group 10810086CF	1.00E+05 PFU/mL	3/3 (100%)
Rotavirus	ZeptoMetrix WA MA-104 MRC-5 0810041CF	1.00E+05 PFU/mL	3/3 (100%)
Yeast and Parasites			
Candida albicans	ZeptoMetrix Z006 0801504	1.00E+06 CFU/mL	2/2^ (100%)
Cryptosporidium parvum	Waterborne Iowa Isolate	1.00E+06 Oocysts/mL	3/3 (100%)
*Entamoeba histolytica (*lysate*)*	ZeptoMetrix DS4-868 BacT-035R05	1.00E+06 Cysts/mL	3/3 (100%)
Giardia lamblia	Waterborne H3 isolate	1.00E+06 Cysts/mL	3/3 (100%)

^ Indicates that one of the replicates was "INCOMPLETE" and was removed from analysis.

The analytical reactivity studies demonstrated no cross reactivity for any analytes tested except for *Shigella dysenteriae*, which is expected due to sequence homology between the *S. dysenteriae stxA* gene and the *stx1* region targeted by the PanNAT STEC Test. No competitive inhibition of PanNAT STEC Test analytes was demonstrated in any of the samples tested.

Analytical Specificity – Interfering Substances

The PanNAT STEC Test was evaluated for potential chemical interference from 25 endogenous and exogenous substances that are common stool contaminants, or likely to be present in patients with diarrhea (Table 5-7). All substances were tested in unpreserved stool specimens in the presence and absence of two *stx1+/stx2+*/O157+ strains of *E. coli*, with three replicates per condition. In the presence of an *stx 1+/stx2+*/O157+ strain, Gaviscon® and Imodium AD® each gave a *stx1+/stx2+*/O157- result on one of three replicates, while stearic acid gave an *stx1-/stx2-*/O157- result on one of three replicates. No interference was demonstrated for any of the remaining substances tested.

Table 5-7. List of Interferents Tested and Concentrations

Interfering Substance	Concentration tested
Whole Blood (EDTA)	40% v/v
Mucin	3.5% w/v (35mg/mL)
Cholesterol	4.80% w/v (48mg/mL)
Human genomic DNA	10 ug/mL
Bile	25% w/v (250mg/mL)
Kaopectate®	3.5% v/v
Pepto-Bismol®	5% v/v
Imodium AD®	5% v/v

Petroleum Jelly (Vaseline®)	50% w/v (500mg/mL)
Baby Wipes	50% v/v
Hydrocortisone Cream	50% w/v (500mg/mL)
Diaper Rash Cream	50% w/v (500mg/mL)
Stearic Acid	4.80% w/v (48mg/mL)
Palmitic Acid	4.80% w/v (48mg/mL)
Gaviscon®	10% w/v (100mg/mL)
Milk of Magnesia®	10% w/v (100mg/mL)
Aleve®	0.5 mg/mL
Preparation H®	9.5% w/v (95mg/mL)
Mineral oil	50% w/v (500mg/mL)
Amoxicillin	5%w/v (50mg/mL)
Nystatin	0.43%w/v (4.28mg/mL)
Metronidazole	5%w/v (50mg/mL)
Ciprofloxacin	1.25% w/v (12.5mg/mL)
Sennica laxatives	1%w/v (10mg/mL)
Diaper Eluent	25% v/v

Carry Over / Cross Contamination

The potential for false positive results with the PanNAT STEC Test due to run-to-run contamination was evaluated by testing an alternating series of contrived high positive stool samples and clinically negative stool samples in direct succession five (5) times on three (3) PanNAT instruments.

All positive samples reported *stx1*+/*stx2*+/O157+ results, and 14 of the 15 negative samples reported *stx1*-/*stx2*-/O157- results. One of the negative samples yielded an invalid result (negative sample, instrument 3, round 5). No carryover or cross contamination was observed.

Assay Cut-Off

The PanNAT STEC Test detects Shiga toxin 1 (*stx1*), Shiga toxin 2 (*stx2*), and *E. coli* O157. For each target, the valid cycle number range is 16 to 39. Cycle numbers are automatically calculated by the PanNAT system software. Each of the target analytes (*stx1, stx2*, O157), as well as the Process Control, must have a cycle number within the valid range in order to be reported as "Positive," otherwise it is reported as "Negative." Cycle number cutoffs were verified on a panel of seven (7) STEC strains of known genotypes, and subsequently validated in the multi-site clinical study.

5.9 Clinical Performance Studies

Prospective and Retrospective Specimen Testing

Performance characteristics of the PanNAT STEC Test were determined on stool samples prospectively collected at six (6) clinical sites and on frozen, retrospective samples. Prospective data were collected from pediatric and adult patients as part of routine patient

care. Results for standard of care testing (comparator method) were obtained using multiple methods, including a test for Shiga toxins 1 and 2 and a test for *E.coli* O157. A presumptive result for O157 on specimens that tested positive with sorbitol on MacConkey agar was confirmed by latex agglutination and biochemical analysis.

For prospective specimens (both unpreserved and Cary-Blair preserved), the leftover, de-identified specimens were tested by two comparator methods: 1) an immunoassay for detection of Shiga toxins 1 and 2 following enrichment culture, and 2) a SMAC plate for presumptive detection of O157. Discordant analyses were performed by an independent molecular reference laboratory on discrepant samples using alternative PCR and bi-directional (BDS) sequencing methods.

A total of 1331 prospective specimens (1231 Cary-Blair preserved and 100 unpreserved) from a range of age groups (Table 5-8) were enrolled in the clinical evaluation. Of these, 30 were removed from the analysis due to pre- or post-analytical errors. Of the remaining 1301 specimens, 7.6% (99/1301) were initially invalid. Specimens with initial invalid results were retested. On retest, 78 were valid resulting in a repeat invalidity rate of 1.6% (21/1301). The number of prospective specimens that qualified for the agreement analysis was 1280 (1184 Cary-Blair preserved and 96 unpreserved) (Table 5-9).

Table 5-8. Samples with Valid and Invalid Results by Age Group

	Total Results	**Valid Results**		**Invalid Results**	
Age Group	**n**	**n**	**%**	**n**	**%**
<=5	154	150	97.4%	4	2.6%
6-21	196	191	97.4%	5	2.6%
22-59	501	495	98.8%	6	1.2%
60+	450	444	98.7%	6	1.3%
Overall	1301	1280	98.4%	21	1.6%

Table 5-9. Study Samples with Valid and Invalid Results by Stool Preservation Status

	Total Results	**Valid Results**		**Invalid Results**	
Type	**n**	**n**	**%**	**n**	**%**
Preserved	1201	1184	98.6%	17	1.4%
Unpreserved	100	96	96.0%	4	4.0%
Overall	1301	1280	98.4%	21	1.6%

In the prospective clinical study with preserved specimens, although the number of positive specimens was small, positive percent agreement (PPA) for each analyte (*stx1*, *stx2* and O157) was 100% while negative percent agreement (NPA) ranged from 99.3% to 99.8% (Table 5-10). With unpreserved specimens, no *stx1* positive specimens were obtained, although PPA for *stx2* and O157 was 100% and NPA ranged from 98.6 to 100%.

Due to the low prevalence of positive specimens in the prospective clinical study, additional testing was also performed using unpreserved, retrospective frozen specimens. The comparator method for the retrospective study was alternative PCR (nested)/bi-

directional sequencing. Of the 114 specimens initially enrolled in the study, eight (8) were excluded from the analysis of performance because they did not yield sufficient DNA for comparator testing (7) or previous enrollment (1). On initial testing 8/106 (7.5%) retrospective specimens produced invalid results, all of which were retested and three (3) of which remained invalid for a final invalid rate of 2.8% (3/106). The retrospective study data set therefore contained 103 evaluable results (Table 5-10). PPA for retrospective specimens ranged from 88.5% for *stx1* to 90.0% for *stx2* and NPA ranged from 97.4% to 98.6%.

To supplement the analysis of prospective and retrospective clinical specimens, additional testing was also performed with 16 contrived unpreserved specimens, each of which was spiked with an enumerated stock of a different strain of Shiga-toxin producing *E. coli* or *E. coli* O157:H7 to a final concentration of 5 x 10^6 CFU/mL (Table 5-10). The contrived specimens were frozen and tested at the clinical sites interspersed with the retrospective specimens.

Table 5-10. Prospective and Retrospective study results

Analyte	Study	Sample Type	PPA (95% CI)	NPA (95% CI)
stx1	Prospective	Preserved	100.0% (51.0,100.0) (4/4)	99.3% (98.7,99.7) (1172/1180)
		Unpreserved		100.0% (96.2,100.0) (96/96)
	Retrospective^*****	Unpreserved	88.5% (71, 96.0) (23/26)	97.4% (91.0, 99.3) (75/77)
	Contrived^	Unpreserved	100.0% (75.8, 100.0) (12/12)	100.0% (51.0, 100.0) (4/4)
stx2	Prospective	Preserved	100.0% (56.6,100.0) (5/5)	99.4% (98.8,99.7) (1172/1179)
		Unpreserved	100.0% (34.2,100.0) (2/2)	98.9% (94.2,99.8) (93/94)
	Retrospective^$^{\Phi}$	Unpreserved	90.0% (74.4, 96.5) (27/30)	98.6% (92.6, 99.8) (72/73)
	Contrived^	Unpreserved	100.0% (67.6, 100.0) (8/8)	100.0% (67.6, 100.0) (8/8)
O157	Prospective	Preserved	100.0% (43.9,100.0) (3/3)	99.8% (99.4,100.0) (1179/1181)
		Unpreserved	100.0% (34.2,100.0) (2/2)	100.0% (96.1,100.0) (94/94)
	Retrospective^ # $^{\delta}$	Unpreserved	89.7% (73.6, 96.4) (26/29)	98.6% (92.6, 99.8) (72/73)
	Contrived^	Unpreserved	100.0% (34.2, 100.0) (2/2)	100.0% (78.5, 100.0) (14/14)

PPA=positive percent agreement, NPA=negative percent agreement. 95% CI = 95% confidence interval using Wilson's method

^The alternative PCR/BDS comparator assay detects all known variants of O157, although it may demonstrate reduced sensitivity and/or inability to detect *stx1d*, variants of *stx2b*, *stx2e* and *stx2f*.

One specimen produced insufficient PCR product for sequencing of this target only.

***** 3/3 specimens that were negative by the PanNAT STEC Test but positive by PCR/BDS were negative by standard of care culture

$^{\Phi}$ 2/3 specimens that were negative by the PanNAT STEC Test but positive by PCR/BDS were negative by standard of care culture

$^{\delta}$ 1/3 specimens that was negative by the PanNAT STEC Test but positive by PCR/BDS was negative by standard of care culture

A summary of PanNAT STEC Test performance stratified by analyte is shown in Table 5-11.

Table 5-11. Summary of PanNAT STEC Test performance by study and analyte/analyte combination

Analyte(s) [1]	Prospective		Retrospective Unpreserved	Contrived Unpreserved
	Preserved	Unpreserved		
*stx*1 Only	100% (4/4)	Not applicable	25.0% (1/4)	100% (7/7)
*stx*2 Only	100% (2/2)	Not applicable	0.0% (0/2)	100% (3/3)
O157 Only	Not applicable	Not applicable	Not applicable	100% (1/1)
*stx*1 + *stx*2	Not applicable	Not applicable	Not applicable	100% (4/4)
*stx*1 + O157	Not applicable	Not applicable	0.0% (0/1)	Not applicable
*stx*2 + O157	100% (3/3)	100% (2/2)	85.7% (6/7)	Not applicable
*stx*1 + *stx*2 + O157	Not applicable	Not applicable	95.2% (20/21)	100% (1/1)
Negative	98.7% (1160/1175) [2]	98.9% (93/94) [3]	98.5% (67/68) [4]	Not applicable

[1] Analyte(s) present as determined by the applicable comparator method for the study

[2] Of the 15 specimens with false positive results by the PanNAT STEC Test, 8 were positive for *stx*1, 5 for *stx*2 and 2 for both *stx*2 and O157

[3] 1/94 negative specimens was positive by the PanNAT STEC Test for *stx*2

[4] 1/68 negative specimens was positive by the PanNAT STEC Test for *stx*1, *stx*2 and O157

5.10 Conclusion

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