June 19, 2019

Serosep, Ltd.
% Fran White
MDC Associates, LLC
180 Cabot Street
Beverly, Massachusetts 01915

Re: K182703
Trade/Device Name: EntericBio Dx Assay
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal microorganism multiplex nucleic acid-based assay
Regulatory Class: Class II
Product Code: PCH, OOI, NSU
Dated: September 26, 2018
Received: September 27, 2018

Dear Fran White:

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. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. 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) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; 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 https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). 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 (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

for

Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
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)*
k190121

Device Name
IDS SHBG

Indications for Use *(Describe)*
The IDS SHBG assay is an in vitro diagnostic device intended for the quantitative determination of SHBG in human serum or plasma on the IDS System. Results are to be used as an aid in the diagnosis of androgen disorders

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

Date of Summary: June 18, 2019

Sponsor
Serosep, Ltd.
Annacotty Business Park
Annacotty, Limerick, Ireland

Correspondent
MDC Associates, Inc
180 Cabot Street
Beverly, MA 01915
Contact : Fran White
Phone : (978) 705 5011
Fax : (978) 927 1308
Email : fran@mdcassoc.com

Device Trade or Proprietary Name
EntericBio® Dx Assay

Common Name
Gastrointestinal microorganism multiplex nucleic acid-based assay

Product Classification
866.3990

Classification
PCH, Class II

Substantial Equivalency
Serosep, Ltd. believes that the subject devices (new device) of this pre-submission document and subsequently a premarket notification submission (510k) is similar to other molecular devices currently marketed in the US. The device design, features and performance when compared to these devices is similar to BioFire, FilmArray Gastrointestinal (GI) Panel (K140407) in that the intended use, the targeted organism for detection, the analytes, technological principles, and specimen types are similar or the same.

There are differences between the subject new device and the predicate device. Because of these differences, the subject device features and suitability will be validated for its intended use using clinical specimens sourced from symptomatic patients or clinical library specimens and tested by the predicate device.

The similarities and differences between the subject device and the predicate device are summarized below.

Element	Subject (New) Device	Proposed Predicate Device	Discussion
Device Name	**EntericBio® Dx Assay**	**FilmArray Gastrointestinal (GI) Panel**	New vs. Predicate Device
FDA Device Premarket Notification	New device	K140407	New vs Predicate Device
FDA Device Classification	Class II, 21 CFR 866.3990 – Gastrointestinal microorganism multiplex nucleic acid-based assay, Microbiology (83 Panel)	Class II, 21 CFR 866.3990 – Gastrointestinal microorganism multiplex nucleic acid-based assay, Microbiology (83 Panel)	Same
Type of Test	Qualitative nucleic acid test	Qualitative nucleic acid test	Same
Users	CLIA certified clinical laboratories	CLIA certified clinical laboratories	Same
Assay Method	The EntericBio® Dx Assay provides PCR reagents to be used in conjunction with an automated pipetting system and the ABI 7500 Fast Dx instrument using standard filters. Results are interpreted using the EntericBio FastFinder plugin. The system provides automated, real-time amplification, detection and analysis and a user constructed template suitable for the EntericBio® Dx Assay.	The BioFire FilmArray Gastrointestinal Panel is designed to be used with the FilmArray® instrument. The FilmArray GI pouch contains freeze-dried reagents to perform nucleic acid purification and nested, multiplex PCR with DNA melt analysis.	Different: New device differs from the predicate device in that the realtime PCR assay kits are designed to be used with an automated pipetting station to accelerate sample preparation, and a commercially available, FDA-cleared PCR instrument to measure the fluorescent probe signal generated during amplification that are analyzed by the EntericBio automated analysis and interpretation software.
Targets for Detection	*Salmonella enterica* spp. *Shigella* spp./ Enteroinvasive *E. coli* (EIEC) *Campylobacter* spp. (*jejuni*, *coli* and *lari*)	*Clostridium difficile* (*C. difficile*) toxin A/B**,** *Campylobacter* spp. (*C. jejuni*, *C. coli* and *C. upsaliensis*), *Plesiomonas shigelloides*, *Salmonella* spp., *Vibrio* spp., *Yersinia enterocolitica*,	Similar, except the predicate device is additionally cleared for detection of a range of other nucleic acid targets from bacteria, parasites and viruses.

Element	Subject (New) Device	Proposed Predicate Device	Discussion
	STEC (Shiga toxin-producing *E. coli*), *stx1*/*stx2* genes *Vibrio* spp. (*cholerae* and *parahaemolyticus*) *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*) *Entamoeba histolytica*	Enteriaggregative *E. coli*, Enteropathogenic *E. coli*, Enterotoxigenic *E. coli* LT/ST toxins, Shiga-like toxin-producing *E. coli*, *Shigella*/ Enteroinvasive *E. coli*, *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia lamblia*, Adenovirus F40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, Sapovirus (GI, GII, GIV and GV).	
Intended Use	The EntericBio® Dx Assay performed on ABI 7500 Fast Dx real-time instrument, is an *in vitro* multiplexed nucleic acid test for the direct, simultaneous qualitative detection and identification of multiple enteric pathogens in Cary-Blair preserved stool specimens from individuals with signs and symptoms of infectious colitis or gastroenteritis. The test is based on detection of nucleic acids from the following organisms: (see organisms above).	The FilmArray Gastrointestinal Panel (GI) is intendent for use with the FilmArray® instrument for the qualitative *in vitro* detection and identification of multiple bacteria, viruses and parasites. The FilmArray GI Panel is performed directly from stool specimens in Cary-Blair transport media. The following pathogen types, subtypes and toxin genes are identified using the FilmArray GI Panel: (see organisms above).	Similar except for the additional organisms detected by the predicate device.
Analyte	DNA/RNA from Cary-Blair preserved fecal specimens	DNA/ RNA from Cary-Blair preserved fecal specimens	Same
Technological Principles	Multiplex nucleic acid PCR	Multiplex nucleic acid PCR	Same
Specimen Types	Human stool (Cary-Blair preserved)	Human stool (Cary-Blair preserved)	Same

Element	Subject (New) Device	Proposed Predicate Device	Discussion
Controls	Internal Amplification Control for each sample. Kit positive and negative controls are processed with each batch of samples.	Two controls are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis.	Similar: The new device IAC is lyophilized within the PCR mix whereas the predicate device has two controls included in each reagent pouch. Substantial equivalence will be demonstrated in clinical testing using human specimens and compared to the predicate device.
PCR Sample Preparation/ Extraction	Sample processed directly following heat treatment of specimen in a SPS tube.	Sample processing is automated in the FilmArray® instrument. The sample is lysed by a combination of mechanical (bead beating) and chemical means and the liberated nucleic acid is captured, washed and eluted using magnetic bead technology.	EntericBio® Dx kit provides reagents and procedure for DNA testing without extraction of stool specimens. Substantial equivalence will be demonstrated in clinical testing using human specimens and compared to the predicate device.
Technological Principles	Real-time multiplex RT-PCR based on the hydrolysis probe reagent chemistry.	Nested multiplex RT-PCR followed by high resolution melting analysis to confirm identity of the PCR product.	Different: Both devices use multiplex real-time PCR however the new device uses hydrolysis probe reagent chemistry compared to melting analysis in the predicate device. Substantial equivalence will be demonstrated in clinical testing using human specimens and compared to the predicate device.

Element	Subject (New) Device	Proposed Predicate Device	Discussion
Detection Methodology/ Platform	The Applied Biosystems 7500 Fast Dx Real-Time PCR instrument is a real-time nucleic acid amplification and five color fluorescence detection system for use with the EntericBio® Dx Assay. Results are analyzed and interpreted using the EntericBio® FastFinder plugin.	Detection and interpretation is automated on the FilmArray® instrument by analysis of the specific PCR product melts (melting temperature).	Different: The detection system and analysis differ between the two devices. Substantial equivalence will be demonstrated in clinical testing using human specimens and compared to the predicate device.
Device Format	The EntericBio® Dx Assay kits provide a PCR master mix with all the reagents required to perform each test which are lyophilized into individual reaction wells. Each reaction well contains an Internal Amplification Control (IAC) to monitor for PCR inhibition. A synthetic Positive Control (containing target sequences) is provided with each kit to monitor the thermal cycling steps and reagent integrity during amplification and detection process.	The FilmArray GI panel provides all the reagents lyophilized into a disposable pouch. Each pouch contains two controls which monitor sample processing and both stages of PCR and melt analysis.	Assay specific requirements

Intended Use

The EntericBio® Dx assay, performed on ABI 7500 Fast Dx real-time instrument, is an *in vitro* multiplexed nucleic acid test for the direct, simultaneous, qualitative detection and identification of multiple enteric pathogens in Cary-Blair preserved stool specimens from individuals with signs and symptoms of infectious colitis or gastroenteritis. The test is based on detection of nucleic acids from:

- *Salmonella enterica* spp.
- *Shigella* spp./ Enteroinvasive *E. coli* (EIEC)
- *Campylobacter* spp. (*jejuni*, *coli* and *lari*)
- *STEC* (Shiga-like toxin-producing *E. coli*), *stx*1/*stx*2 genes
- *Vibrio* spp. (*cholerae* and *parahaemolyticus*)
- *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*)
- *Entamoeba histolytica*

Testing is performed on Cary-Blair preserved diarrheal specimens from symptomatic patients with suspected acute gastroenteritis, enteritis or colitis of bacterial or parasitic origin. The test is performed directly on the specimen, utilizing real-time polymerase chain reaction (PCR) for the amplification of Salmonella-specific, Campylobacter-specific, Shigella/ EIEC-specific *ipaH*, *stx1*/*stx2*, Vibrio-specific, Entamoeba-specific and Giardia-specific gene sequences. The test utilizes fluorogenic sequence-specific hybridization probes for the detection of the amplified DNA.

This test is intended for use, in conjunction with clinical presentation, laboratory findings and epidemiological information, as an aid in the diagnosis of *Salmonella, Shigella* / EIEC*,* Shiga-like toxin-producing *E. coli, Campylobacter* spp*.*, *Vibrio* spp., *Entamoeba histolytica* and *Giardia* spp. infections in humans.

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 or definitive cause of patient illness. Negative EntericBio® Dx assay 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.

Methodology

The EntericBio® Dx assay is a molecular *in vitro* diagnostic test for direct, qualitative detection and identification of the following enteric organisms, associated with human gastroenteritis, directly, from Cary-Blair preserved fecal specimens:

- ***Salmonella enterica* spp.,**
- ***Shigella* spp./ Enteroinvasive *E. coli*,**
- ***Campylobacter spp*. (*jejuni*, *coli* and *lari*),**
- ***Vibrio spp.* (*cholerae* and *parahaemolyticus*),**

- **STEC(Shiga-like toxin-producing E. coli) *stx1*/*stx2* genes**,
- ***Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*),**
- ***Entamoeba histolytica***

The assay is composed of Stool Preparation Solution (SPS) tubes, PCR reagent strips containing lyophilized reagents, Resuspension Buffer (Negative Kit Control), Positive Kit Control containing DNA from all target analytes (with appropriate reconstitution buffer), and associated accessories,instruments and software for detection of bacterial and parasitic causes of gastroenteritis in humans.

The EntericBio® Dx assay detects target DNA from diarrheal Cary-Blair stool specimens from symptomatic individuals with suspected gastroenteritis or infectious colitis.

The assay works directly from a Cary-Blair preserved stool sample and does not require commercial nucleic acid extraction /purification. The PCR master mix with all the reagents required to perform each test is lyophilized into individual reaction wells on a strip. Each reaction well contains an Internal Amplification Control (IAC) to monitor for PCR inhibition.

Performance Data:
Analytical Performance

Reproducibility
A reproducibility study was performed to determine the inter-site and overall reproducibility of the EntericBio® Dx assay. Reproducibility testing was performed in-house, simulating a multi-site study by using three instruments (hereinafter 'in-house sites') and panels of contrived stool samples, each spiked with various combinations of *Vibrio parahaemolyticus, Shigella sonnei and Giardia lamblia.* These three target analytes were representative of each of the three component multiplex assays and each analyte was evaluated at three different concentrations (True Negative, Low Positive and Moderate Positive) in three independently manufactured batches of EntericBio® Dx Assay. Reproducibility panels were tested at each of the in-house sites by two different operators for five non-consecutive days.

The acceptance criteria for this study were that the moderate positive target concentration (3x LoD) must show 100% agreement with the expected result for each target analyte and the low target concentration (1.5x LoD) must show ≥95% agreement with the expected result for each target analyte.

All targets showed 100% agreement with the expected result for moderate positive concentrations tested across the in-house sites. *Shigella sonnei* and *Vibrio parahaemolyticus* samples showed 100% agreement with the expected result for low positive concentrations tested the in-house sites. *Giardia lamblia* at the low positive concentration was observed at 98% relative to the expected result. All acceptance criteria for each target analyte at each concentration tested across the in-house sites were met, as shown in Tables 1-2 below.

Table 1: Reproducibility study results

Organism Tested	Concentration Tested	FastFinder Expected Result	% Agreement with Expected Result			
			Serosep 1	Serosep 2	Serosep 3	All
Shigella sonnei DSM 5570	**Moderate Positive** 3x LoD	**Positive**	30/30 100%	30/30 100%	30/30 100%	**90/90 100%**
	Low Positive 1.5x LoD	**Positive**	30/30 100%	30/30 100%	30/30 100%	**90/90 100%**
	True Negative	**Negative**	60/60 **100%**	60/60 **100%**	59/59* **100%**	**179/179 100%**
Vibrio parahaemolyticus CCUG 14474	**Moderate Positive** 3x LoD	**Positive**	30/30 100%	30/30 100%	30/30 100%	**90/90 100%**
	Low Positive 1.5x LoD	**Positive**	30/30 100%	30/30 100%	30/30 100%	**90/90 100%**
	True Negative	**Negative**	60/60 **100%**	60/60 **100%**	59/59* **100%**	**179/179 100%**
Giardia lamblia P101	**Moderate Positive** 3x LoD	**Positive**	30/30 100%	30/30 100%	30/30 100%	**90/90 100%**
	Low Positive 1.5x LoD	**Positive**	30/30 100%	30/30 100%	29/30 95%	**89/90 98%**
	True Negative	**Negative**	60/60 **100%**	60/60 **100%**	59/59* **100%**	**179/179 100%**

* *One True Negative sample was invalid on the EntericBio FastFinder plugin and subsequently removed from study*

Table 2: Summary of the Reproducibility of the Cq Values

Organism Tested	Assay	Concentration Tested	Test Site	Cq Reproducibility		
				Mean (Cq)	STDEV	%CV
Shigella sonnei DSM 5570	**Well A**	**Moderate Positive** 3x LoD	**Serosep 1**	27.10	±0.68	2.51
			Serosep 2	26.84	±0.47	1.74
			Serosep 3	27.01	±0.79	2.91
			All Sites	26.98	±0.66	2.44
		Low Positive 1.5x LoD	**Serosep 1**	29.07	±0.57	1.97
			Serosep 2	28.88	±0.53	1.85
			Serosep 3	28.95	±0.57	1.98
			All Sites	28.97	±0.57	1.97
Vibrio parahaemolyticus CCUG 14474	**Well B**	**Moderate Positive** 3x LoD	**Serosep 1**	31.73	±0.42	1.34
			Serosep 2	31.87	±0.24	0.74
			Serosep 3	31.74	±0.44	1.40
			All Sites	31.79	±0.38	1.21
		Low Positive 1.5x LoD	**Serosep 1**	33.50	±0.48	1.44
			Serosep 2	33.34	±0.50	1.51
			Serosep 3	33.30	±0.79	2.37
			All Sites	33.38	±0.61	1.82
Giardia lamblia P101	**Well C**	**Moderate Positive** 3x LoD	**Serosep 1**	32.96	±0.85	2.58
			Serosep 2	33.04	±1.36	4.12
			Serosep 3	33.30	±1.27	3.81
			All Sites	33.10	±1.17	3.53
		Low Positive 1.5x LoD	**Serosep 1**	33.98	±1.18	3.49
			Serosep 2	34.40	±1.38	4.01
			Serosep 3	33.11	±1.26	3.80
			All Sites	33.85	±1.37	4.05

Limit of Detection (LoD)

The analytical sensitivity (limit of detection or LoD) of the EntericBio® Dx Assay was determined using contrived samples with target analytes spiked into a negative stool matrix (Cary-Blair preserved stool) at three concentrations: greater than estimated LoD (High), estimated LoD (Medium) and less than LoD (Low). A total of 20 replicate EntericBio® Stool Preparation Solution (SPS) were tested from each sample using three independently manufactured lots of EntericBio® Dx assay. The LoD is defined as the lowest concentration of analyte that can be consistently detected ≥95% of the time. A minimum of two strains were tested for each EntericBio® Dx target organism and toxin gene. Table 3 lists the LoD determined for each target organism.

Table 3: LoD determined using the EntericBio® Dx Assay

Organism	Strain	LoD
Salmonella spp.	*Salmonella enterica* serovar Enteritidis DSM 17420	8 x 10^4 CFU/mL
	Salmonella enterica serovar Typhi NCTC 10787	8 x 10^4 CFU/mL
Shigella spp./EIEC	*Shigella sonnei* DSM 5570	1.25 x 10^4 CFU/mL
	E. coli (ipaH) DSM 9029	1 x 10^4 CFU/mL
Campylobacter spp.	*Campylobacter jejuni* ATCC 33560	4 x 10^4 CFU/mL
	Campylobacter coli DSM 4689	4 x 10^4 CFU/mL
	Campylobacter lari DSM 11375	1 x 10^4 CFU/mL
Escherichia coli (STEC)	*E. coli* (stx1) NVRL 15x23 RE-008 (O111:H-)	5 x 10^5 CFU/mL
	E. coli (stx2) NVRL 15x24 RE-006 (O26:H11)	1 x 10^6 CFU/mL
Vibrio spp.	*Vibrio parahaemolyticus* CCUG 14474	1 x 10^4 CFU/mL
	Vibrio cholerae NCTC 3661	1 x 10^4 CFU/mL
Giardia lamblia	*Giardia intestinalis* (WB) ATCC 30957	25 cells/mL
	Giardia intestinalis (New Orleans) ATCC 50137	100 cells/mL
Entamoeba histolytica	*Entamoeba histolytica* (HM-1: IMSS) ATCC 30459	25 cells/mL
	Entamoeba histolytica (HK-9) ATCC 30015	100 cells/mL

Fresh vs. Frozen

The Fresh versus Frozen Specimen Stability study was performed to support the inclusion of frozen, retrospective specimens and contrived samples in the clinical and analytical studies of the EntericBio® Dx assay; the test is not intended for use on frozen specimens.

Sixty contrived samples were prepared for each EntericBio® Dx target analyte and tested at Time 0 (T0). These samples were subsequently frozen at -20°C for 3 months before re-testing. Contrived samples were prepared in a negative stool matrix (Cary-Blair preserved stool) with target analytes spiked at three concentrations (5X, 2X and 1X LoD).

Agreement between detection of fresh and frozen samples was 100% for Salmonella, Shigella and STEC and <80% for four analytes (Campylobacter, Vibrio, Giardia and Entamoeba). Additional testing was performed using frozen clinical specimens from the prospective study to further support the inclusion of these four analytes in clinical studies.

Analytical Reactivity/Inclusivity

The analytical reactivity (Inclusivity) of the EntericBio® Dx Assay was determined using a panel of 101 target organisms (Table 4 below) which represents the diversity of the EntericBio® Dx target analytes. Fifteen of these organisms were also evaluated in the LoD determination study.

Table 4: EntericBio® target analytes used in this study

Organism	Supplier	Catalogue Number
Salmonella enterica serovar Enteritidis	DSMZ[1]	DSM 17420
Salmonella enterica serovar Typhi	NCTC[2]	NCTC 10787
Salmonella enterica subsp. enterica I serovar Cholerasuis	ATCC[3]	ATCC 7001
Salmonella enterica subsp. enterica I serovar Paratyphi B	ATCC	ATCC 8759
Salmonella enterica subsp. enterica I serovar Paratyphi A	ATCC	ATCC 9281
Salmonella enterica subsp. enterica I serovar Paratyphi C	ATCC	ATCC 13428
Salmonella enterica subsp. enterica I serovar Typhimurium	DSMZ	DSM 101475
Salmonella enterica subsp. enterica I serovar Dublin	DSMZ	DSM 102345
Salmonella enterica subsp. enterica I serovar Agona	DSMZ	DSM 102864
Salmonella enterica subsp. enterica I serovar Heidelberg	DSMZ	DSM 9379
Salmonella enterica subsp. enterica I serovar Infantis	NCTC	NCTC 10679
Salmonella enterica subsp. enterica I serovar Thompson	NCTC	NCTC 2252
Salmonella enterica subsp. enterica I serovar Oranienburg	NCTC	NCTC 5743
Salmonella enterica subsp. enterica I serovar Bareilly	NCTC	NCTC 5745
Salmonella enterica subsp. enterica I serovar Montevideo	NCTC	NCTC 5747
Salmonella enterica subsp. enterica I serovar Braenderup	NCTC	NCTC 5750
Salmonella enterica subsp. enterica I serovar Muenchen	NCTC	NCTC 5755
Salmonella enterica subsp. enterica I serovar Saintpaul	NCTC	NCTC 6022
Salmonella enterica subsp. enterica I serovar Mississippi	NCTC	NCTC 6487
Salmonella enterica subsp. enterica I serovar Javiana	NCTC	NCTC 6495
Salmonella enterica subsp. enterica I serovar Newport	NCTC	NCTC 6704
Salmonella enterica subsp. enterica I serovar Schwarzengrund	NCTC	NCTC 6756
Salmonella enterica subsp. enterica I serovar Hadar	NCTC	NCTC 9877
Salmonella enterica subsp. enterica I serovar 4, [5] 12:i:-	NSSLRL[5]	NSSLRL Ms170397
Salmonella enterica subsp. II (salame)	DSMZ	DSM 9220

Organism	Supplier	Catalogue Number
Salmonella enterica subsp. IIIa (arizonae)	DSMZ	DSM 9386
Salmonella enterica subsp. IIIb (diarizonae)	DSMZ	DSM 14847
Salmonella enterica subsp. IV (houtenae)	DSMZ	DSM 9221
Salmonella enterica subsp. VI (indica)	DSMZ	DSM 14848
Shigella sonnei	DSMZ	DSM 5570
Shigella sonnei	DSMZ	DSM 25715
Shigella sonnei	ATCC	ATCC 11060
Shigella sonnei	ATCC	ATCC 25931
Shigella sonnei	ATCC	ATCC 9290
Shigella flexneri (serotype 2a)	DSMZ	DSM 4782
Shigella flexneri (serotype 2a)	ATCC	ATCC 700930
Shigella flexneri (serotype 1a)	ATCC	ATCC 9199
Shigella flexneri (serotype 2b)	ATCC	ATCC 12022
Shigella flexneri (serotype 6)	ATCC	ATCC 12025
Shigella boydii (serotype 2)	DSMZ	DSM 7532
Shigella boydii (serotype 1)	ATCC	ATCC 9207
Shigella boydii (serotype 20)	ATCC	ATCC BAA-1247
Shigella boydii (serotype 10)	ATCC	ATCC 12030
Shigella boydii (serotype 4)	ATCC	ATCC 9210
Shigella dysenteriae (serotype 1)	NCTC	NCTC 4837
Shigella dysenteriae (serotype 2)	NCTC	NCTC 5109
Shigella dysenteriae (serotype 7)	NCTC	NCTC 9763
Shigella dysenteriae (serotype 3)	NCTC	NCTC 6340
Shigella dysenteriae (serotype 9)	NCTC	NCTC 9347
Escherichia coli EIEC (serotype O28ac:H-)	DSMZ	DSM 9025
Escherichia coli EIEC (serotype O29:H10)	DSMZ	DSM 9026
Escherichia coli EIEC (serotype O136:H-)	DSMZ	DSM 9032
Escherichia coli EIEC (serotype O124:H30)	DSMZ	DSM 9031
Escherichia coli EIEC (serotype O144:H-) *(ipaH)*[8]	DSMZ	DSM 9029
Campylobacter jejuni	ATCC	ATCC 33560
Campylobacter jejuni subsp. jejuni	DSMZ	DSM 104743
Campylobacter jejuni subsp. jejuni	DSMZ	DSM 27585
Campylobacter jejuni subsp. doylei	DSMZ	DSM 104768
Campylobacter jejuni subsp. doylei	NCTC	NCTC 12208
Campylobacter coli	DSMZ	DSM 110395
Campylobacter coli	DSMZ	DSM 24155
Campylobacter coli	DSMZ	DSM 24106
Campylobacter coli	DSMZ	DSM 24206
Campylobacter coli	DSMZ	DSM 4689
Campylobacter lari	DSMZ	DSM 11375
Campylobacter lari	NCTC	NCTC 12892
Campylobacter lari	NCTC	NCTC 12893
Campylobacter lari	NCTC	NCTC 12894
Campylobacter lari	NCTC	NCTC 12895
Vibrio parahaemolyticus	DSM	DSM 101031

Organism	Supplier	Catalogue Number
Vibrio parahaemolyticus	DSM	DSM 11058
Vibrio parahaemolyticus	DSM	DSM 15477
Vibrio parahaemolyticus	DSM	DSM 27657
Vibrio parahaemolyticus	CCUG[6]	CCUG 14474
Vibrio cholerae (0:1 Ogawa classical)	NCTC	NCTC 3661
Vibrio cholerae O:1 Biotype El Tor	NCTC	NCTC 8457
Vibrio cholerae O:1 Ogawa	NCTC	NCTC 8021
Vibrio cholerae non-O:1, non-O139 (O:3)	NCTC	NCTC 11502
Escherichia coli O157 (stx2)	NVRL[4]	NVRL 17X01 RE-001
Escherichia coli O157 (stx1 & stx2)	NVRL	NVRL17X04 RE-002
Escherichia coli O157 (stx2)	NVRL	NVRL 17X09 RE-003
Escherichia coli O157 (stx1 & stx2)	NVRL	NVRL 17X15 RE-004
Escherichia coli O157 (stx2)	NVRL	NVRL 17S110 RE-005
Escherichia coli O103:H2 (stx1)	NVRL	NVRL 17X128 RE-010
Escherichia coli O111:H8 (stx1)	NVRL	NVRL 13S5371 RE-009
Escherichia coli O121:H19 (stx2)	NVRL	NVRL 15X18 RE-007
Escherichia coli O157:NM	NVRL	NVRL O6-CC3 RE-011
Escherichia coli O157:H7 (stx1 & stx2)	NCTC	NCTC 12079 NVRL RE-012
Escherichia coli O157:H- (stx2)	NCTC	NCTC 12080
Escherichia coli O111:H- (stx1)	NVRL	NVRL 15x23 RE-008
Escherichia coli O26:H11 (stx2)	NVRL	NVRL 15x24 RE-006
Escherichia coli O113 [7]	N/A	N/A
Escherichia coli O45 [7]	N/A	N/A
Escherichia coli O104 [7]	N/A	N/A
Escherichia coli O145 [7]	N/A	N/A
Giardia intestinalis (WB)	ATCC	ATCC 30957
Giardia intestinalis (New-Orleans-1)	ATCC	ATCC 50137
Giardia intestinalis GS Assemblage B	ATCC	ATCC 50581
Giardia intestinalis Portland 1	ATCC	ATCC 30888
Giardia intestinalis Mario	ATCC	ATCC PRA-244
Entamoeba histolytica (HM-1: IMSS)	ATCC	ATCC 30459
Entamoeba histolytica (HK-9)	ATCC	ATCC 30015
Entamoeba histolytica HB-301:NIH	ATCC	ATCC 30190
Entamoeba histolytica HU-21:AMC	ATCC	ATCC 30457
Entamoeba histolytica IP:1182:2	ATCC	ATCC PRA-357

1 DSMZ - Deutsche Sammlung von Mikroorganismen und Zenllkulturen
2 NCTC - National Collection of Type Cultures, a Culture Collection of Public Health England
3 ATCC – American Type Culture Collection
4 NVRL - National VTEC Reference Laboratory, Ireland
5 NSSLRL- National Salmonella, Shigella and Listeria Reference Lab, Galway, Ireland
6 CCUG – Culture Collection University of Gothenburg
7 Inclusivity predicted based on *in-silico* analysis
8 EIEC strain which also generates positive result for Shigella/EIEC (ipaH)

Testing was performed using contrived samples with target analytes spiked into a negative stool matrix (Cary-Blair preserved stool) at a concentration of approximately three times the respective LoD, where possible.

All organisms tested were detectable over three replicates demonstrating the inclusivity of the EntericBio® Dx assay.

Analytical Exclusivity

The analytical reactivity (Exclusivity) of the EntericBio® Dx Assay was determined using a comprehensive panel of 133 bacteria, viruses and parasites (Table 5 below). The panel of organisms tested consisted of organisms closely related to the EntericBio® Dx assay targets and organisms likely to be found in human feces.

Table 5: Microorganisms used in this study

Organism	Organism	Organism	Organism
Yersinia aldovae	*Cryptosporidium meleagridis*	*Citrobacter koseri*	*Pseudomonas putida*[1]
Yersinia bercovieri	*Cryptosporidium Skunk genotype*[1]	*Clostridium difficile*	*Rahnella aquatilis*
Yersinia entomophaga	*Cryptosporidium ubiquitum*[1]	*Clostridium perfringens*	Rotavirus A[1]
Yersinia frederiksenii	*Cryptosporidium viatorum*[1, 2]	*Clostridium sordelli*	*Ruminococcus gauvreauii*
Yersinia intermedia	*Escherichia vulneris*	*Cronobacter sakazakii*	*Saccharomyces cerevisiae*
Yersinia kristensenii	*Cryptosporidium cuniculus*[1,3]	*Dientamoeba fragilis*	Sapovirus[1]
Yersinia massiliensis	*Cryptosporidium Horse genotype*[1]	*Edwardsiella tarda*	*Serratia liquefaciens*
Yersinia mollaretti	*Cryptosporidium felis*[1]	*Encephalitozoon cuniculi*[1]	*Serratia marcescens*
Yersinia nurmii	*Cryptosporidium canis*[1]	*Enterobacter aerogenes*	*Serratia odoriferae*
Yersinia pekkaneneii	*Cryptosporidium xiaoi*[1, 4]	*Enterobacter cloacae*	*Serratia rubidaea*
Yersinia pseudotuberculosis	*Cryptosporidium andersoni*[1]	*Enterococcus faecalis*	*Staphylococcus aureus*
Yersinia rodhei	*Cryptosporidium baileyi*[1]	*Enterococcus faecium*	*Staphylococcus epidermidis*
Yersinia ruckeri	*Entamoeba dispar*[5]	*Eubacterium rectale*[1, 6]	*Stenotrophomonas maltophilia*
Yersinia similis	*Entamoeba moshkovoskii*	*Ewingella americana*	*Streptococcus agalactiae*
Vibrio alginolyticus	*Entamoeba invadens*	*Fusobacterium gonidiaformans*	*Streptococcus bovis*
Vibrio fluvialis	*Aeromonas hydrophilia*	*Fusobacterium nucleatum*	*Streptococcus equinus*

Organism	Organism	Organism	Organism
Vibrio furnissii	Adenovirus F40[1]	*Fusobacterium varium*	*Toxoplasma gondii*
Vibrio mimicus	*Alcaligenes faecalis*	*Hafnia alvei*	*Campylobacter concisus*[1]
Vibrio harvei	*Anaerococcus hydrogenalis*	*Klebsiella oxytoca*	*Campylobacter curvus*[1]
Vibrio fischeri[1]	*Anaerostipes hadrus*[7]	*Klebsiella pneumoniae*	*Campylobacter fetus subsp. Fetus*
Vibrio damsela	*Arcobacter butzleri*	*Lactobacillus acidophilus*	*Campylobacter fetus subsp. Venerealis*
Griomontia hollisae	Astrovirus[1]	*Lactobacillus lactis*	*Campylobacter gracilis*
Vibrio diazotrophicus	*Bacillus cereus*	*Listeria monocytogenes*	*Campylobacter helveticus*[1]
Vibrio proteolyticus	*Bacillus subtilis subsp subtilis*	*Morganella morganii*	*Campylobacter hominis*
Vibrio natrigens	*Bacillus subtilis subsp spizizenii*	*Neisseria gonorrhoeae*	*Campylobacter hyointestinalis*
Vibrio pelagius[1]	*Bacteroides fragilis*	Norovirus GGI[1]	*Campylobacter mucosalis*[1]
Vibrio campbellii	*Bacteroides thetaiotaomicron*	Norovirus GGII[1]	*Campylobacter rectus*[1]
Vibrio vulnificus[8]	*Bifidobacterium breve*	*Plesiomonas shigelloides*	*Campylobacter showae*
Escherichia coli non toxigenic	*Bifidobacterium longum*	*Prevotella melaninogenica*	*Campylobacter sputorum*[1,9]
Escherichia coli (Enteropathogenic)	*Blastocystis hominis*	*Proteus mirabilis*	*Campylobacter ureolyticus*
Escherichia coli (Enterotoxigenic)	*Candida albicans*	*Proteus vulgaris*	*Salmonella bongori*
Escherichia hermannii	*Citrobacter amalonaticus*[1]	*Providencia stuartii*	*Salmonella subterranea*
Escherichia blattae	*Citrobacter freundii*	*Pseudomonas aeruginosa*	*Campylobacter upsaliensis*
Escherichia fergusonii			

[1] Organisms for which testing was performed using genomic DNA

[2] *Cryptosporidium viatorum*: *Shigella* detected in 3/3 replicates. Bi-directional sequencing demonstrated the presence of *Shigella* DNA suggesting possible sample contamination with *Shigella*. *In silico* analysis indicated no cross-reactivity should occur.

[3] *Cryptosporidium cuniculus*: *Giardia* detected in 2/3 replicates. Bi-directional sequencing of amplicon failed and thus it could not be confirmed empirically if the sample was contaminated with *Giardia* or if cross reactivity-occurred. *In silico* analysis indicated no cross-reactivity should occur.

[4] *Cryptosporidium xiaoi*: *Campylobacter* detected in 3/3 replicates. Bi-directional sequencing demonstrated the presence of *Campylobacter* DNA suggesting possible sample contamination with *Campylobacter*. *In silico* analysis indicated no cross-reactivity should occur.

[5] *Entamoeba dispar:* IAC failures were observed for 5/6 replicates after repeat testing. *In silico* analysis demonstrated homology for the primers and predicts amplification to occur and competitively inhibit the IAC, but the probe has several mismatches and is not expected to produce a detected signal.

[6] *Eubacterium rectale: Campylobacter* detected in 3/3 replicates and *Vibrio* in 1/3 replicates. Bi-directional sequencing demonstrated the presence of *Campylobacter* and *Vibrio* DNA suggesting possible sample contamination with *Vibrio* and *Campylobacter*. *In silico* analysis indicated no cross-reactivity should occur.

[7] *Anaerostipes hadrus*: *Campylobacter* detected in 2/3 replicates. Bi-directional sequencing demonstrated the presence of *Campylobacter* DNA suggesting possible sample contamination with *Campylobacter*. *In silico* analysis indicated no cross-reactivity should occur.

[8] *Vibrio vulnificus:* Evaluated by *in silico* analysis only. *In silico* analysis indicated no cross-reactivity should occur.

[9] *Campylobacter sputorum*: *Vibrio* detected in 1/3 replicates. Bi-directional sequencing demonstrated the presence of *Vibrio* DNA suggesting possible sample contamination with *Vibrio*. *In silico* analysis indicated no cross-reactivity should occur.

Testing was performed using contrived samples with target analytes spiked into a negative stool matrix (Cary-Blair preserved stool) at a concentration of 10^6 CFU or cells/mL. Genomic DNA/RNA was tested at a concentration of 10^6 genomic equivalents per reaction where possible.

The non-target organisms which were shown to cross react were all closely related to their respective target organism and shared significant sequence similarity and are presented below (Table 6).

Table 6: Non-target organisms for which cross reactivity was observed with the EntericBio® Dx assay

Cross Reacting Organisms	Target Analyte
Vibrio campbellii	Vibrio
Vibrio fluvialis	Vibrio
Vibrio furnissii	Vibrio
Vibrio mimicus	Vibrio
Vibrio fischeri	Vibrio
Vibrio natriegens	Vibrio

Microbial Interference

A study was performed to evaluate the performance of the EntericBio® Dx Assay in the presence of high concentrations of eleven microorganisms that are commonly found in fecal specimens (Table 7). Each potentially interfering microorganism was tested in the presence of 3X LoD of each target analyte in the EntericBio® Dx assay. No interference was observed between the potentially interfering organisms and the IAC or any of the EntericBio® Dx target analytes tested.

Table 7: Potentially interfering microorganisms tested in this study

Substance	Source/ID	Concentration tested
Aeromonas hydrophila	DSM 17695	10^6 CFU/mL
Bacteroides fragilis	DSM 2151	10^6 CFU/mL
Staphylococcus aureus	DSM 20231	10^6 CFU/mL
Escherichia coli	DSM 30083	10^6 CFU/mL
Enterococcus faecalis	DSM 20478	10^6 CFU/mL
Clostridium perfringens	DSM 798	10^6 CFU/mL
Saccharomyces cerevisiae	DSM 1848	10^6 CFU/mL
Blastocystis hominis	Clinical specimen	Not quantifiable
Pseudomonas aeruginosa	DSM 50071	10^6 CFU/mL
Klebsiella oxytoca	DSM 5175	10^6 CFU/mL
Candida albicans	DSM 1577	10^6 CFU/mL

DSMZ - Deutsche Sammlung von Mikroorganismen und Zenllkulturen

Potentially Interfering Substances

A study was performed to evaluate the performance of the EntericBio® Dx Assay in the presence of 23 potentially interfering/cross-reactive substances, at high, but clinically relevant levels, that might be present in fecal specimens. Each substance was tested in the presence of each target analyte detected by the assay at low positive concentrations. Each substance was also evaluated in negative stool samples (without target organisms) where results demonstrated IAC failure in the presence of Benzalkonium chloride at a concentration of >1% v/v and Hemorrhoidal cream at a concentration of >1% w/v.

Overall, the presence of 21/23 potentially interfering substances had no effect on the detection of the target analytes or the EntericBio® Dx assay internal control at the concentrations listed in Table 8 below. A false negative result for *V. parahaemolyticus* was observed for 1/3 sample replicates containing hemorrhoidal cream (1% w/v) and false negative results for *C. jejuni* were observed for 2/3 sample replicates containing tetracycline (1.6% w/v).

Table 8: Highest concentration of each substance for which most EntericBio® Dx target analytes were detected (Substances for which Interference was observed are bolded)

Substance	Passing Concentration
Amoxicillin	1% w/v[3]
Benzalkonium Chloride	0.15% v/v[3]
Ceftriaxone	1 % w/v
Cholesterol	7 % w/v
Ciprofloxacin	5.4% w/v
Erythromycin	1.5% w/v
Hemorrhoidal cream	**1% w/v[1,3]**
Human DNA	0.1% v/v
Hydrocortisone	50% w/v
Laxative	5% v/v
Loperamide Hydrochloride	0.5% w/v[3]
Lubricant	50% w/v
Magnesium Hydroxide	0.5% w/v[3]
Metronidazole	6% w/v
Mucin	10% w/v
Naproxen sodium (NSAID)	10% w/v
Nystatin Cream	50% w/v
Sudocrem	50% w/v
Sulfamethoxazole	4% w/v[3]
Tetracycline	**0.8% w/v[2,3]**
Trimethoprim	1.6% w/v
Vagisil	50% w/v
Whole Human Blood	5% v/v[3]

[1] *V. parahaemolyticus* failed in 1/3 replicates at this concentration

[2] *C. jejuni* was only tested at 1.6% w/v and 2/3 replicates failed at this concentration

[3] Interference for detection of one or more targeted organisms was observed for samples with higher than the listed concentration

Competitive Inhibition

This study was performed to evaluate the performance of the EntericBio® Dx Assay when challenged with combinations of target analytes in order to determine the potential for

competitive interference in patient specimens with mixed infections. The combinations of analytes tested were selected based on the frequency of co-infections reported in the literature. This study was performed using Cary Blair preserved negative stool specimens spiked with dual EntericBio® Dx target analyte combinations. Binary combinations of target analytes were spiked at both high and low concentrations and tested in triplicate (Table 9).

The potential for competitive inhibition was not evaluated for analytes detected in Well B (*Vibrio cholerae/parahaemolyticus* and STEC)
Based on the results data, competitive inhibition was observed for *Giardia lamblia* in the presence of both low or high concentrations of *Entamoeba histolytica*.

Table 9: Combinations of Entericbio® Dx Target Analytes Evaluated For Competitive Inhibition

EntericBio® Dx Target Analyte Combinations
Salmonella High (300X LoD) & Campylobacter Low (3X LoD)
Salmonella Low (18X LoD) & Campylobacter High (50X LoD)
Shigella High (200X LoD) & Giardia Low (3X LoD)
Shigella Low (12X LoD) & Giardia High (50X LoD)
Entamoeba High (50X LoD) & Cryptosporidium Low (10X LoD)
Entamoeba Low (3X LoD) & Cryptosporidium High (50X LoD)
Cryptosporidium High (50X LoD) & Giardia Low (3X LoD)
Cryptosporidium Low (10X LoD) & Giardia High (50X LoD)
Campylobacter High (50X LoD) & Giardia Low (3X LoD)
Campylobacter Low (3X LoD) & Giardia High (50X LoD)
Campylobacter High (50X LoD) & Cryptosporidium Low (10X LoD)
Campylobacter Low (3X LoD) & Cryptosporidium High (50X LoD)
Salmonella High (300X LoD) & Cryptosporidium Low (10X LoD)
Salmonella Low (12X LoD) & Cryptosporidium High (50X LoD)
Entamoeba High (50X LoD) & Giardia Low (3X LoD)
Entamoeba Low (3X LoD) & Giardia High (50X LoD)

Carry-Over and Cross Contamination

The Cross-Contamination (Carryover) study was performed to investigate the potential for carryover and cross-contamination of the EntericBio® Dx assay on the EntericBio® Workstation between and within experiments. Samples with a high concentration of target organism(s) were processed in alternating sequence with negative samples. Contrived samples were prepared in a negative stool matrix (Cary-Blair preserved stool) with two representative target analytes (one bacterial (*Shigella sonnei*) and one parasitic target (*Giardia lamblia*)) spiked at a high concentration (800x and 1000x LoD respectively). Two representative negative samples were prepared from an uninoculated negative stool matrix. The study consisted of three separate experiments for each target analyte and each experiment contained 30 samples (15 positive and 15 negative).

No carryover or cross-contamination occurred with the EntericBio® Dx assay on the EntericBio® Workstation between or within each of the assay experiments.

Specimen Stability

The specimen stability study was performed to determine the recommended specimen storage conditions for use with the EntericBio® Dx assay.

The recommended storage conditions for Cary-Blair preserved stool is 5 days at 2-8°C.

Real Time Stability

The objective of this study is to determine the shelf life in real-time of the EntericBio® Dx assay and its constituent components.

The shelf life was determined to be six (6) months at 2-8°C.

Clinical Performance

Performance characteristics of the EntericBio® Dx assay were established in a multi-site clinical study, including three (3) distinct study sites - two (2) US clinical sites and one (1) non-US clinical site.

The performance of the EntericBio® Dx assay was assessed by testing 4 specimen types:

1) Fresh, prospectively-collected, Cary-Blair preserved fecal specimens from patients presenting with symptoms of gastrointestinal infection;
2) Fresh, selected Cary-Blair preserved fecal specimens from patients presenting with symptoms of gastrointestinal infection. Specimens were selected based on the results obtained with Standard of Care methods in use at the study sites;
3) Frozen, well characterized Cary-Blair preserved fecal specimens from patients presenting with symptoms of gastrointestinal infection, known to be positive for selected target analytes;
4) Spiked/Contrived Cary-Blair preserved fecal samples for lower prevalence targets

The performance of the EntericBio® Dx assay in fresh and archived specimens was evaluated by comparing the test result for each target analyte with the appropriate comparator method(s).

For STEC and Campylobacter target analytes, a composite comparator method of three FDA-cleared assays was used for fresh specimens. A specimen was characterised as positive if 2 out of 3 comparator assays were positive and a specimen was characterized as negative if 2 out of 3 comparator assays were negative. For the remaining target analytes and archived specimens, the comparator method consisted of one, FDA-cleared assay.

A total of 1523 fresh samples (1491 prospective, 32 select) were enrolled during the clinical trial. Of the 1491 prospective samples, 19 were excluded from the study, giving 1472 evaluable samples. The reasons for exclusion included invalid test result for the EntericBio® Dx assay (n=9) or by the comparator method (n=6), Indeterminate result by the EntericBio® Dx assay (n=1), specimen not tested with comparator method within sample stability (n=2), duplicate specimen from previously enrolled patient (n=1).
Table 10 provides a summary of demographic information for the fresh specimens enrolled into the clinical study.

Table 10: Demographic Summary for the fresh specimens (n=1523) enrolled into the clinical study

Gender	**Number of specimens (%)**
Male	636 (41.8%)
Female	880 (57.8%)
N/A	7 (0.4%)
Age Group	**Number of specimens (%)**
<1 year	11 (0.7%)
1-5 years	65 (4.3%)
6-12 years	27 (1.8%)
13-21 years	62 (4.1%)
22-65 years	863 (56.7%)
+ 65 years	489 (32.1%)
N/A	6 (0.4%)
Patient Status	**Number of specimens (%)**
Outpatient	692 (45.4%)
In-patient	700 (46.0%)
Emergency Care	94 (6.2%)
Long term care	22 (1.4%)
N/A	15 (1.0%)
Total	**1523**

Several target analytes had a low prevalence in the fresh clinical samples. To supplement the results of the fresh testing, 212 frozen, retrospective positive specimens were included in the

study. These specimens were selected and archived based on previously testing positive for the desired target analytes by routine diagnostic methods used by the collection sites.

Of the 212 archived specimens enrolled into the frozen clinical study, 3 samples were excluded from the study, giving 209 evaluable samples. The reason for exclusion was invalid test result for the EntericBio® Dx assay (n=1) and Indeterminate result by the EntericBio® Dx assay (n=2).
The archived specimens were distributed across the three testing sites and were randomized such that the users performing the testing were blinded as to the expected test result.
Table 11 provides a summary of demographic information for the archived specimens enrolled into the clinical study.

Table 11: Demographic Summary for the archived specimens (n=212) enrolled into the clinical study

Gender	**Number of specimens (%)**
Male	105 (49.5%)
Female	103 (48.6%)
N/A	4 (1.9%)
Age Group	**Number of specimens (%)**
<1 year	5 (2.4%)
1-5 years	51 (24.1%)
6-12 years	21 (9.9%)
13-21 years	14 (6.6%)
22-65 years	96 (45.3%)
+ 65 years	21 (9.9%)
N/A	4 (1.9%)
Patient Status	**Number of specimens (%)**
Outpatient	136 (64.2%)
In-patient	42 (19.8%)
Emergency Care	27 (12.7%)
Long term care	1 (0.5%)
N/A	6 (2.8%)
Total	**212**

Since prevalence of some target analytes (*Vibrio* and *Entamoeba histolytica*) was very low, both prospective and retrospective specimens did not yield adequate specimen numbers to demonstrate sufficient performance with the EntericBio® Dx assay. To supplement the fresh and frozen specimen data, contrived samples (n=310) were prepared and tested with the EntericBio® Dx assay. Contrived samples were prepared for each target analyte using unique fecal matrix from residual fresh specimens which had previously tested negative for all target analytes. Positive specimens were spiked at various levels, using multiple strains for each organism. Contrived samples were randomized with negative specimens so that the users were blinded to the expected result.

Of the 310 contrived samples prepared for the contrived sample study, there were no samples that were excluded from the study, giving 310 evaluable samples.
The performance of the EntericBio® Dx assay in contrived samples was evaluated by comparing the test result for each target analyte with the expected sample result, based on the organism/ strain used for spiking.

The results of the EntericBio® Dx assay testing are presented in Tables 12a-12g below. Clinical study results have been stratified by the specimen type.

Table 12a: Summary of the Clinical Performance for *Salmonella*

	Specimen Type			n=	% Agreement (95% CI)	
					Positive	Negative
Salmonella	**Clinical Specimens**	**Fresh**	**All-Comers**	1472	92.3% 24/26[1] (75.9-97.9)	100% 1446/1446 (99.7-100)
			Select	32	90.0% 9/10[2] (59.6-98.2)	100% 22/22 (85.1-100)
		Frozen	**Archived**	209	85.7% 12/14[3] (60.1-96.0)	100% 195/195 (98.1-100)
	Simulated			310	NA	99.7% 309/310 (98.2-99.9)

[1]2/2 Salmonella FN observed were negative for Salmonella when tested with an alternative FDA cleared PCR assay
[2]1/1 Salmonella FN observed was positive for Salmonella when tested with an alternative FDA cleared PCR assay
[3]1/2 Salmonella FN observed was negative for Salmonella when tested with an alternative FDA cleared PCR assay. 1/2 Salmonella FN observed was positive for Salmonella when tested with an alternative FDA cleared PCR assay.

Table 12b: Summary of the Clinical Performance for *Campylobacter*

	Specimen Type			n=	% Agreement (95% CI)	
					Positive	Negative
Campylobacter	Clinical Specimens	Fresh	All-Comers	1472	98.0% 50/51 (89.7-99.7)	99.8% 1418/1421 (99.4-99.9)
			Select	32	100% 9/9 (70.1-100)	100% 23/23 (85.7-100)
		Frozen	Archived	209	93.8% 15/16[4] (71.7-98.9)	100% 193/193 (98.0-100)
	Simulated			310	NA	100% 310/310 (98.8-100)

[4]1/1 Campylobacter FN observed were negative for Campylobacter when tested with an alternative FDA cleared PCR assay

Table 12c: Summary of the Clinical Performance for *Shigella/EIEC*

	Specimen Type			n=	% Agreement (95% CI)	
					Positive	Negative
Shigella/EIEC	Clinical Specimens	Fresh	All-Comers	1472	100% 14/14 (78.5-100)	100% 1458/1458 (99.7-100)
			Select	32	100% 3/3 (43.9-100)	100% 29/29 (88.3-100)
		Frozen	Archived	209	90.9% 10/11[5] (62.3-98.4)	100% 198/198 (98.1-100)
	Simulated			310	NA	100% 310/310 (98.8-100)

[5]1/1 Shigella/EIEC FN observed was negative for Shigella/EIEC when tested with an alternative FDA cleared PCR assay

Table 12d: Summary of the Clinical Performance for *STEC*						
	Specimen Type			n=	% Agreement (95% CI)	
					Positive	Negative
STEC	Clinical Specimens	Fresh	All-Comers	1472	100% 8/8 (67.6-100)	99.9% 1462/1464 (99.5-99.9)
			Select	32	100% 3/3 (43.9-100)	100% 29/29 (88.3-100)
		Frozen	Archived	209	94.6% 70-74[6] (86.9-97.9)	100% 135/135 (92.7-100)
	Simulated			310	NA	100% 310/310 (98.8-100)

[6]4/4 STEC FN observed were negative for STEC when tested with an alternative FDA cleared PCR assay

Table 12e: Summary of the Clinical Performance for *Vibrio*						
	Specimen Type			n=	% Agreement (95% CI)	
					Positive	Negative
Vibrio	Clinical Specimens	Fresh	All-Comers	1472	0.0% 0/3[7] (0.0-56.1)	100% 1469/1469 (99.7-100)
			Select	32	NA	100% 32/32 (89.3-100)
		Frozen	Archived	209	NA	100% 209/209 (98.2-100)
	Simulated			310	100% 100/100 (96.3-100)	100% 210/210 (98.2-100)

[7]3/3 Vibrio FN observed were negative for Vibrio when tested with an alternative FDA cleared PCR assay

Table 12f: Summary of the Clinical Performance for *Giardia lamblia*						
	Specimen Type			n=	% Agreement (95% CI)	
					Positive	Negative
Giardia lamblia	Clinical Specimens	Fresh	All-Comers	1472	85.7% 12/14[8] (60.1-96.0)	99.9% 1457/1458 (99.6-99.9)
			Select	32	100% 3/3 (43.9-100)	100% 29/29 (88.3-100)
		Frozen	Archived	209	100% 29/29 (88.3-100)	100% 180/180 (97.9-100)
	Simulated			310	NA	100% 310/310 (98.8-100)

[8]2/2 Giardia FN observed were negative for Giardia when tested with an alternative FDA cleared PCR assay

Table 12g: Summary of the Clinical Performance for *Entamoeba histolytica*						
	Specimen Type			n=	% Agreement (95% CI)	
					Positive	Negative
Entamoeba histolytica	Clinical Specimens	Fresh	All-Comers	1472	NA	100% 1472/1472 (99.7-100)
			Select	32	NA	100% 32/32 (89.3-100)
		Frozen	Archived	209	0.0% 0/2[9] (0.0-65.8)	100% 207/207 (98.2-100)
	Simulated			310	98.6% 74/75 (92.3-99.8)	100% 235/235 (98.4-100)

[9]2/2 Entamoeba FN observed were positive for Entamoeba when tested with an alternative FDA cleared PCR

The EntericBio® Dx assay reported multiple organism detections (co-infections) for a total of 3 specimens. This represents 0.20% of all fresh specimens tested (3/1504). All multiple detections contained two target analytes and all were concordant with the comparator method(s) used for the respective target analytes. The summary of the multi-detections reported by the EntericBio® Dx assay is presented in Table 13.

The comparator assay(s) reported multiple organism detections (co-infections) for a total of 5 specimens. This represents 0.33% of all fresh specimens tested (5/1504). All multiple detections contained two target analytes. From the 5 samples with reported co-detections, the EntericBio® Dx assay did not detect a second target analyte in 2 specimens and none of the discordant specimens reported as co-infections were confirmed with an alternative FDA-

cleared assay. The summary of the multi-detections reported by the comparator assay is presented in Table 14.

Table 13: Distinct Multi-detections detected in fresh clinical specimens (n=1,504) by the EntericBio® Dx assay

Analyte_1	Analyte_2	Prevalence		Number of Discrepant specimens (FP by EntericBio)	Discrepant Analyte(s)
		No.	%		
Campylobacter	Giardia	1	0.07	0	N/A
Shigella	Giardia	1	0.07	0	N/A
Campylobacter	STEC	1	0.07	0	N/A
Total		**3**	**0.20**		

Table 14: Distinct Multi-detections detected in fresh clinical specimens (n=1,504) by the comparator methods

Analyte_1	Analyte_2	Prevalence		Number of Discrepant specimens (FN by EntericBio)	Discrepant Analyte(s)
		No.	%		
Campylobacter	Giardia	2	0.13	1[a]	Giardia[a]
Shigella	Giardia	2	0.13	1[b]	Giardia[b]
Campylobacter	STEC	1	0.07	0	N/A
Total		**5**	**0.33**		

[a] Sample was negative with EntericBio® Dx and an alternative FDA-cleared assay

[b] Sample was negative with EntericBio® Dx and an alternative FDA-cleared assay

Of the 1482 prospective (fresh) specimens initially evaluated with EntericBio® Dx assay, 28 specimens (1.9%) were initially reported as Invalid. Following a repeat test, 19 out of 28 invalid specimens generated valid results. Repeat testing was not performed for 3 of the 28 invalid specimens and these specimens remained as Invalid. None of the 32 select (fresh) specimens were initially reported as invalid. Of the 212 retrospective specimens initially evaluated with EntericBio® Dx assay, 2 specimens (0.9%) were initially reported as Invalid. Following a repeat test, 1 out of 2 invalid specimens was resolved. Repeat testing was not performed for the other specimen which remained invalid. The total numbers provided in Table 15 are based on compliant specimens and EntericBio® Dx assay results.

Table 15: Summary of Invalid results observed during clinical trial with the EntericBio® Dx assay

	Initial Invalid			Final Invalid		
	Count	Percent	95% CI	Count	Percent	95% CI
Prospective (Fresh)	28/1482	1.9%	1.3-2.7%	9*/1482	0.6%	0.3-1.2%
Select (Fresh)	0/32	0.0%	0.0-10.7%	0/32	0.0%	0.0-10.7%
Total (Fresh)	28/1514	1.9%	1.3-2.7%	9*/1514	0.6%	0.3-1.1%
Retrospective (Frozen)	2/212	0.9%	0.3-3.4%	1**/212	0.5%	0.1-2.6%
Total (All)	**30/1726**	**1.7%**	**1.2-2.5%**	**10/1726**	**0.6%**	**0.3-1.2%**

*19/28 initial invalids for fresh specimens were resolved upon repeat; 6/28 were invalid upon repeat and 3/28 were not repeated
**1/2 initial Invalid results was resolved upon repeat and 1/2 was not repeated

Of the 1482 prospective (fresh) specimens initially evaluated with EntericBio® Dx assay, 1 specimen (0.1%) was initially reported as Indeterminate. Repeat testing was not performed and the specimen remained as Indeterminate.
None of the 32 select (fresh) specimens initially reported as Indeterminate. Of the 212 retrospective specimens initially evaluated with EntericBio® Dx assay, 2 specimens (0.9%) were initially reported as Indeterminate. Repeat testing for these 2 specimens was not performed and the specimens remained as Indeterminate. The total numbers provided in Table 16 are based on compliant specimens and EntericBio® Dx assay results.

Table 16: Summary of Indeterminate results observed during clinical trial with the EntericBio® Dx assay

	Initial Indeterminate			Final Indeterminate		
	Count	Percent	95% CI	Count	Percent	95% CI
Prospective (Fresh)	1/1482	0.1%	0.0-0.4%	1*/1482	0.1%	0.0-0.4%
Select (Fresh)	0/32	0.0%	0.0-10.7%	0/32	0.0%	0.0-10.7%
Total (Fresh)	1/1514	0.1%	0.0-0.4%	1*/1514	0.1%	0.0-0.4%
Retrospective (Frozen)	2/212	0.9%	0.3-3.4%	2**/212	0.9%	0.3-3.4%
Total (All)	**3/1726**	**0.2%**	**0.1-0.5%**	**3/1726**	**0.2%**	**0.1-0.5%**

*1/1 initial indeterminate result was not repeated
**2/2 initial indeterminate results were not repeated

Of the 1482 prospective (fresh) specimens initially evaluated with EntericBio® Dx assay, 29 specimens (2.0%) were initially Non-reportable (Invalid and Indeterminate combined). Following a repeat test, 19 out of 29 Non-reportable results were resolved. Repeat testing was not performed for 4 of the 29 non-reportable results and the specimens remained as Non-reportable. None of the 32 select (fresh) specimens were Non-reportable.

Of the 212 retrospective specimens initially evaluated with EntericBio® Dx assay, 4 specimens (1.9%) were initially Non-reportable. Following a repeat test, 1 out of 4 Non-reportable results was resolved. Repeat testing was not performed for 3 of the 4 specimens with non-reportable

results, and the specimens remained as Non-reportable.

The total numbers provided in Table 17 are based on compliant specimens and EntericBio® Dx assay results.

Table 17: Summary of all Non-reportable results observed during clinical trial with the EntericBio® Dx assay

	Initial All Non-reportable			**Final All Non-reportable**		
	Count	Percent	95% CI	Count	Percent	95% CI
Prospective (Fresh)	29/1482	2.0%	1.4-2.8%	10/1482	0.7%	0.4-1.2%
Select (Fresh)	0/32	0.0%	0.0-10.7%	0/32	0.0%	0.0-10.7%
Total (Fresh)	29/1514	1.9%	1.3-2.7%	10*/1514	0.7%	0.4-1.2%
Retrospective (Frozen)	4/212	1.9%	0.7-4.8%	3**/212	1.4%	0.5-4.1%
Total (All)	**33/1726**	**1.9%**	**1.4-2.7%**	**13/1726**	**0.8%**	**0.4-1.3%**

*19/29 initial non-reportable results for fresh specimens were resolved upon repeat; 6/29 were non reportable upon repeat and 4/29 were not repeated

**1/4 initial non-reportable results was resolved upon repeat and 3/4 were not repeated

Statement of Safety and Effectiveness

The data presented clearly demonstrates the safety and efficacy of the EntericBio® Dx assay as compared to the reference method when the product's Instructions for Use are followed.