510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K180041

B. Purpose for Submission:

To obtain a substantial equivalence determination for the BioCode Gastrointestinal Pathogen Panel (GPP) for the detection of microbial nucleic acids extracted from human stool specimens.

C. Measurands:

Target nucleic acid sequences of the following gastrointestinal microorganisms:

- *Campylobacter* (*C. jejuni/C. coli*)
- *Clostridium difficile* (*C. difficile*) toxin A/B
- *Salmonella* spp.
- *Vibrio* (*V. parahaemolyticus/V. vulnificus/ V. cholerae*), including specific identification of *Vibrio parahaemolyticus*
- *Yersinia enterocolitica*
- Enteroaggregative *Escherichia coli* (EAEC)
- Enterotoxigenic *Escherichia coli* (ETEC) lt/st
- *E. coli* O157 serogroup
- Shiga-like toxin-producing *Escherichia coli* (STEC) stx1/stx2
- *Shigella*/ Enteroinvasive *Escherichia coli* (EIEC)
- *Cryptosporidium* spp.
- *Entamoeba histolytica*
- *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*)
- Adenovirus F 40/41
- Norovirus GI/GII
- Rotavirus A

D. Type of Test:

Qualitative nucleic acid multiplex test

E. Applicant:

Applied BioCode

F. Proprietary and Established Names:

BioCode Gastrointestinal Pathogen Panel (GPP)

G. Regulatory Information:

1. Regulation section:

866.3990

Gastrointestinal microorganisms multiplex nucleic acid-based assay

2. Classification:

Class II

3. Product code:

PCH, OOI

4. Panel:

Microbiology (83)

H. Intended Use:

1. Intended use(s):

The BioCode Gastrointestinal Pathogen Panel (GPP) is a qualitative, gastrointestinal microorganism multiplexed nucleic acid-based assay capable of detecting of nucleic acids from the following organisms in unpreserved stool and Cary-Blair media:

- Adenovirus 40/41
- *Campylobacter* (*C. jejuni, C. coli*)
- *Clostridium difficile* (*C. difficile*) toxin A/B (from fresh specimens only)
- *Cryptosporidium* (*C. parvum, C. hominis*)
- *Entamoeba histolytica*
- *Escherichia coli* (*E. coli*) O157
- Enterotoxigenic *E. coli* (ETEC) LT/ST
- Enteroaggregative *E. coli* (EAEC)
- *Giardia lamblia (also known as G. intestinalis and G. duodenalis)*
- Norovirus GI/GII
- Rotavirus A
- *Salmonella* spp.
- Shiga-like Toxin producing *E. coli* (STEC) stx1/stx2
- *Shigella* (*S. boydii, S. sonnei, S. flexneri, S. dysenteriae*)/EIEC
- *Vibrio spp*. (*V. cholerae, V. parahaemolyticus, V. vulnificus*), specific identification of *V. parahaemolyticus*
- *Yersinia enterocolitica*

The BioCode Gastrointestinal Pathogen Panel (GPP) is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness and results are meant to be used in conjunction with other clinical, laboratory, and epidemiological data. Positive results do not rule out co-infection with organisms not included in the BioCode Gastrointestinal Pathogen Panel (GPP).

The agent detected may not be the 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.

Concomitant culture is necessary for organism recovery and further typing of bacterial agents. This device is not intended to monitor or guide treatment for *C. difficile* infection.

Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Campylobacter spp.*, *E. coli* O157, *Shigella/*EIEC, *Yersinia enterocolitica,* and Adenovirus 40/41 were established primarily with retrospective clinical specimens.

Performance characteristics for *Entamoeba histolytica,* and *Vibrio* spp. *(V. parahaemolyticus, V. vulnificus,* and *Vibrio cholerae)* were established primarily using contrived clinical specimens.

Indication(s) for use:

Same as intended use.

1. Special conditions for use statement(s):

   For prescription use only.

2. Special instrument requirements:

   For use with the BioCode MDx 3000 instrument.

I. Device Description:

The BioCode Gastrointestinal Pathogen Panel (GPP)is a multiplex nucleic acid test designed to be used with the BioCode MDx 3000 instrument. The BioCode Gastrointestinal Pathogen Panel (GPP) is an automated system that integrates PCR amplification, target capture, signal generation and optical detection for multiple gastrointestinal pathogens from a single stool specimen, either unpreserved or in Cary Blair medium. Stool specimens are processed and nucleic acids are extracted with the bioMérieux NucliSENSE easyMag. Once the PCR plate is set up and sealed, all other operations are automated. The BioCode Gastrointestinal Pathogen Panel (GPP) simultaneously tests for 17 pathogens from unpreserved stool specimens or stool preserved in

Cary-Blair medium. Results from the BioCode Gastrointestinal Pathogen Panel (GPP) test are available within 5 hours.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   xTAG Gastrointestinal Pathogen Panel

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

   K121454

3. Comparison with predicate:

   See table below.

Table 1. Comparison with Predicate Device

Name	Predicate: Luminex xTAG GPP K121454	Applied BioCode Gastrointestinal Pathogen Panel (GPP)
Intended Use	The xTAG Gastrointestinal Pathogen Panel (GPP) is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of multiple viral, parasitic, and bacterial nucleic acids in human stool specimens from individuals with signs and symptoms of infectious colitis or gastroenteritis. The following pathogen types, subtypes and toxin genes are identified using the xTAG GPP: Campylobacter spp. (C. *jejuni*, *C. coli* and *C. lari* only) *Clostridium difficile* (*C. difficile*) toxin A/B *Cryptosporidium* (*C. parvum* and *C. hominis* only) *Escherichia coli* (*E. coli*) O157 Enterotoxigenic *Escherichia coli* (ETEC) LT/ST *Giardia* (*G. lamblia* only - also known as *G. intestinalis* and *G. duodenalis*) Norovirus GI/GII Rotavirus A *Salmonella* Shiga-like Toxin producing *E. coli* (STEC) stx 1/stx 2 Shigella (*S. boydii*, *S. sonnei*, *S. flexneri* and *S. dysenteriae*) The detection and identification of specific gastrointestinal microbial nucleic acid from individuals exhibiting signs and symptoms of gastrointestinal infection aids in the diagnosis of gastrointestinal infection when used in conjunction with clinical evaluation, laboratory findings and epidemiological information. A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the detection and identification of acute gastroenteritis in the context of outbreaks. xTAG GPP positive results are presumptive and must be confirmed by FDA-cleared tests or other acceptable reference methods. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out coinfection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative xTAG Gastrointestinal Pathogen Panel 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. xTAG GPP is not intended to monitor or guide treatment for C. difficile infections. The xTAG GPP is indicated for	The BioCode Gastrointestinal Pathogen Panel (GPP) is a qualitative, gastrointestinal microorganism multiplexed nucleic acid-based assay capable of detecting of nucleic acids from the following organisms in unpreserved stool and Cary-Blair media: •Adenovirus 40/41 •*Campylobacter* (*C. jejuni, C. coli*) •*Clostridium difficile* (*C. difficile*) toxin A/B (from fresh specimens only) •*Cryptosporidium* (*C. parvum, C. hominis*) •*Entamoeba histolytica* •*Escherichia coli* (*E. coli*) O157 •Enterotoxigenic *E. coli* (ETEC) LT/ST •Enteroaggregative *E. coli* (EAEC) •*Giardia lamblia (also known as G. intestinalis and G. duodenalis)* •Norovirus GI/GII •Rotavirus A •*Salmonella* spp. •Shiga-like Toxin producing *E. coli* (STEC) stx1/stx2 •*Shigella* (*S. boydii, S. sonnei, S. flexneri, S. dysenteriae*)/EIEC •*Vibrio spp*. (*V. cholerae, V. parahaemolyticus, V. vulnificus*), specific identification of *V. parahaemolyticus* •*Yersinia enterocolitica* The BioCode Gastrointestinal Pathogen Panel (GPP) is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness and results are meant to be used in conjunction with other clinical, laboratory, and epidemiological data. Positive results do not rule out co-infection with organisms not included in the BioCode Gastrointestinal Pathogen Panel (GPP). The agent detected may not be the 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. Concomitant culture is necessary for organism recovery and further typing of bacterial agents.

	use with the Luminex 100/200 instrument.	This device is not intended to monitor or guide treatment for *C. difficile* infection. Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Campylobacter spp.*, *E. coli* O157, *Shigella/*EIEC, *Yersinia enterocolitica,* and Adenovirus 40/41 were established primarily with retrospective clinical specimens. Performance characteristics for *Entamoeba histolytica,* and *Vibrio* spp. *(V. parahaemolyticus, V. vulnificus,* and *Vibrio cholerae)* were established primarily using contrived clinical specimens.
Sample type	Unpreserved stool and stool in Cary-Blair medium	Same
Control	Externally sourced	Same
Instrument	Nucleic acid purification system PCR thermocycler Luminex 100/200 or MAGPIX	BioCode MDx 3000
Assay Methodology	Multiplex PCR and multiplex TSPE followed by fluorescence-activated sorting of labeled beads coupled to streptavidin-conjugated biotinylated products	Multiplex PCR and probe hybridization followed by fluorescence detection and decoding of barcoded magnetic beads (BMB) that are coupled to biotinylated products with streptavidin conjugate
Time to result	Approximately 5 hours	Approximately 5 hours
Test Interpretation	Automated test interpretation and report generation.	Automated test interpretation and report generation.

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

Not applicable.

L. Test Principle:

The BioCode Gastrointestinal Pathogen Panel (GPP) is an automated system that integrates PCR amplification, target capture, signal generation and optical detection for multiple gastrointestinal pathogens from a single stool specimen. Stool specimens are processed and nucleic acids are extracted with the bioMérieux NucliSENSE easyMAG automated system. Once the PCR plate is set up and sealed, all other operations are automated.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Reproducibility:

A study was performed to assess the Reproducibility of the BioCode Gastrointestinal Pathogen Panel (GPP) on the BioCode MDx 3000. This study was designed to assess intra-assay (within run), inter-assay (run-to-run), day-to-day and site-to-site reproducibility. One lot of reagents was assayed at 3 sites by 2 operators on 1 instrument per site for 5 days (total of 10 runs per site; 90 replicates total).

The reproducibility panel consisted of 7 contrived samples (sample 7 was a negative control) extracted in triplicate and each assayed in singlet. The samples consisted of combinations of 12 representative targets at 1.5x LoD (Low) and 3x LoD (Medium). Reproducibility for each analyte and panel member was > 99%. Results from the study are shown in Table 2.

Table 2. Qualitative results from Reproducibility study.

	Concentration Level					
	Medium Positive		**Low Positive**		**Negative**	
Target	**Detection n/N (%)**	**95% CI**	**Detection n/N (%)**	**95% CI**	**Agreement n/N (%)**	**95% CI**
Salmonella bongori	90/90 (100.0)	(95.9 - 100.0)	90/90 (100.0)	(95.9 - 100.0)	450/450 (100.0)	(99.2 - 100.0)
Clostridium difficile	90/90 (100.0)	(95.9 - 100.0)	90/90 (100.0)	(95.9 - 100.0)	450/450 (100.0)	(99.2 - 100.0)
Giardia lamblia	90/90 (100.0)	(95.9 - 100.0)	90/90 (100.0)	(95.9 - 100.0)	449/450 (99.78)	(98.7 - 99.9)
Adenovirus 40/41	90/90 (100.0)	(95.9 - 100.0)	90/90 (100.0)	(95.9 - 100.0)	450/450 (100.0)	(99.2 - 100.0)
Shigella sonnei	90/90 (100.0)	(95.9 - 100.0)	90/90 (100.0)	(95.9 - 100.0)	450/450 (100.0)	(99.2 - 100.0)
Vibrio parahaemolyticus	90/90 (100.0)	(95.9 - 100.0)	90/90 (100.0)	(95.9 - 100.0)	450/450 (100.0)	(99.2 - 100.0)
ETEC	90/90 (100.0)	(95.9 - 100.0)	90/90 (100.0)	(95.9 - 100.0)	450/450 (100.0)	(99.2 - 100.0)
Yersinia enterocolitica	90/90 (100.0)	(95.9 - 100.0)	90/90 (100.0)	(95.9 - 100.0)	450/450 (100.0)	(99.2 - 100.0)
STEC	90/90 (100.0)	(95.9 - 100.0)	89/90 (98.89)	(93.96-99.97)	450/450 (100.0)	(99.2 - 100.0)
Campylobacter jejuni	90/90 (100.0)	(95.9 - 100.0)	90/90 (100.0)	(95.9 - 100.0)	450/450 (100.0)	(99.2 - 100.0)
Rotavirus A	90/90 (100.0)	(95.9 - 100.0)	90/90 (100.0)	(95.9 - 100.0)	450/450 (100.0)	(99.2 - 100.0)
Cryptosporidium parvum	90/90 (100.0)	(95.9 - 100.0)	90/90 (100.0)	(95.9 - 100.0)	450/450 (100.0)	(99.2 - 100.0)

b. Fresh versus Frozen Study:

Since many of the unpreserved samples underwent a freeze thaw prior to testing with the BioCode Gastrointestinal Pathogen Panel (GPP), a study was performed using 60 spiked

unpreserved stool specimens to assess fresh vs frozen specimen stability. Specimens were contrived and tested initially as fresh as well as after ≥ 2 freeze thaw cycles. Samples were tested at low positive (1X - 1.5X LoD) spiked into 7 negative stool samples. Cary-Blair specimens were not subjected to frozen storage during the clinical study, therefore they were not tested. Results for the study are presented in Table 3 with mean fluorescence intensities listed in Table 4 along with the normalized values. Typical raw signal for positive values are greater than 18,000 mean fluorescence intensity units. Frozen samples displayed stability throughout the length of the study as exemplified by changes in mean fluorescent intensity of less than 20%.

Table 3. Fresh Vs. Frozen Summary.

Acceptance Criteria	Results
95% replicates for all positive targets in the contrived sample should be Valid and Detected (≥60/63)	63/63
95% of negative targets should be Valid and not detected (≥60/63)	63/63[a]

a – Sample Target was detected but was invalid for RNA IC.

Table 4. Analysis of results from Fresh Vs Frozen study.

Analysis			Delta Fresh - Frozen	Percent Decrease
Campylobacter jejuni subsp .jejuni	ATCC 33292	FF1	3835	14%
Escherichia coli 10C-3114 (STEC)	ATCC BAA-2217	FF2	3019	12%
Human rotavirus A	ATCC VR-2018	FF3	4437	13%
Vibrio parahaemolyticus	ATCC 17802	FF4	1260	7%
O78:H11 Escherichia coli strain H10407 (ETEC)	ATCC 35401	FF5	2503	8%
Shigella sonnei	ATCC 29930	FF6	2365	20%
Salmonella enterica subsp. Enterica	ATCC 14028	FF7	-148	-2%
Human adenovirus 40 (dugan)	Zeptometrix	FF8	610	3%
Cryptosporidium parvum	waterborne P102M	FF9	3134	14%
Negative	Negative	FF10	1719	6%

c. Specimen stability:

A study was performed to establish the specimen stability limitations of the BioCode Gastrointestinal Pathogen Panel (GPP). This study employed the use of spiked specimens to assess the following storage conditions:

- Samples in Cary-Blair - 0, 2, 4 and 6 days at room temperature (20-25°C); 2, 4 and 6 days at 2-8°C
- Unpreserved Stool - 0, 2, 4 and 6 days at 2-8°C
- Fresh vs. Frozen (-90°C to -60°C) (2x freeze thaws) - 30, 60 and 90 days at -90°C to -60°C
- S.T.A.R. buffer after pretreatment (SK38 Tubes prior to extraction) - 24 hours at 2-8°C Fresh vs. Frozen (-90°C to -60°C) (2x freeze thaws) - 30, 60 and 90 days at -90°C to -60°C Extracted Nucleic Acid

- 24 hours at 2-8°C

- Fresh vs. Frozen (-90°C to -60°C) (2x freeze thaws) - 30, 60 and 90 days at -90°C to -60°C

One parasite (*C. parvum*), one virus (Rotavirus A), one gram-positive bacterium (*C. difficile*), and one gram-negative bacterium (STEC) were used as representative panel analyte constituents for this study. Spiked and clinical samples were tested at ~3X LoD with two organisms in each sample.

Concentrated organism stocks were serially diluted in Cary-Blair and combined with prescreened negative stool and each condition was assayed with 3 replicates at each time point. Samples displayed acceptable stability under the all conditions listed above.

d. Linearity/assay reportable range:

Not applicable because the BioCode Gastrointestinal Pathogen Panel (GPP) is qualitative.

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

External Control Summary:

Four pools of external controls were used on a rotating basis throughout testing. Each of these pools consisted of inactivated organisms sourced from Waterborne Inc. or Zeptometrix as shown in Table 5. Results from control testing performed in the clinical and reproducibility studies are shown in Table 6.

Table 5. Summary of Controls

Pool	Organism	Strain	Source	Dilution Factor
Pool 1	*Clostridium difficile*	NAP1	Natrol (ZeptoMetrix)	1/4
	Rotavirus A	WA	Natrol (ZeptoMetrix)	1/4
	Shigella sonnei	Z004	Natrol (ZeptoMetrix)	1/4
Pool 2	*Escherichia coli* (EAEC)	92.0147; EAEC	Natrol (ZeptoMetrix)	1/5
	Entamoeba histolytica	DS4-868	Natrol (ZeptoMetrix)	1/5
	Yersinia enterocolitica	Clinical isolate	Natrol (ZeptoMetrix)	1/5
	Norovirus GII	recombinant	Natrol (ZeptoMetrix)	1/5
	Vibrio parahaemolyticus	Clinical isolate	Natrol (ZeptoMetrix)	1/5
Pool 3	Adenovirus Type 41	TAK	Natrol (ZeptoMetrix)	1/4
	Escherichia coli O157/STEC	EDL933	Natrol (ZeptoMetrix)	1/4
	Giardia lamblia	H3	Waterborne Inc.	1/4
	Salmonella typhimurium	Z005	Natrol (ZeptoMetrix)	1/4
Pool 4	*Campylobacter jejuni*	Clinical isolate	Natrol (ZeptoMetrix)	1/4
	Cryptosporidium parvum	Iowa	Natrol (ZeptoMetrix)	1/4
	Escherichia coli (ETEC)	ETEC; ST+, LT+	Natrol (ZeptoMetrix)	1/4
	Norovirus GI	recombinant	Natrol (ZeptoMetrix)	1/4

Table 6. Performance of Controls during Clinical Trials (including Reproducibility testing)

	Site 001 (valid/total)	Site 002 (valid/total)	Site 003 (valid/total)	Site 006 (valid/total)	All sites (valid/total)
Pool 1	5/5	6/6	11/14	6/6	28/31
Pool 2	5/5	8/8	5/5	5/5	23/23
Pool 3	8/8	5/5	3/3	4/4	20/20
Pool 4	3/3	5/5	3/3	4/4	15/15
NC	24/27	34/35	38/39	15/15	111/116

f. Limit of detection:

A study was performed to assess the performance of the BioCode Gastrointestinal Pathogen Panel (GPP) at the Limit of Detection (LoD) for both unpreserved Stool and Cary- Blair specimens. In this study the GI Panel was tested with quantified stocks of bacteria, virus or parasites. For initial screening, four replicates of each concentration in negative stool and Cary-Blair were extracted on the easyMag System and tested in singlet with the BioCode Gastrointestinal Pathogen Panel (GPP) to estimate LoD. The LoD was confirmed by extracting 20 replicates of each sample type and testing each in singlet for a total of 20 replicates at or near the presumptive LoD. LoD for each stock was defined as the lowest concentration with ≥95% detection of 20 replicates (19 out of 20) and was determined separately for unpreserved stool and Cary-Blair preserved stool. Results for LoD testing are shown in Table 7 below.

Table 7. Limits of Detection in CFU/mL

Strain	Source	Unpreserved Stool LoD	Unpreserved Stool Detection	Cary-Blair Stool LoD	Cary-Blair Stool Detection
Campylobacter coli	ATCC 33559	5.6×10^1 CFU/mL	20/20	5.6×10^1 CFU/mL	20/20
Campylobacter jejuni subsp. jejuni	ATCC 33292	7.0×10^2 CFU/mL	20/20	7.0×10^2 CFU/mL	20/20
Clostridium difficile (toxinotype 0)	ATCC 9689	1.9×10^2 CFU/mL	20/20	1.9×10^2 CFU/mL	20/20
Clostridium difficile (toxinotype III; Nap1)	Zeptometrix 0801619cf	8.3×10^2 CFU/mL	20/20	3.3×10^3 CFU/mL	20/20
Enteroaggregative *E. coli* O92:H33 (EAEC)	STEC TW04440	1.4×10^3 CFU/mL	20/20	1.4×10^3 CFU/mL	20/20
Enteroinvasive *E. coli* O29:NM (EIEC)	ATCC 43892	3.6×10^2 CFU/mL	20/20	7.5×10^2 CFU/mL	20/20
Enterotoxigenic *E. coli* O78:H11 H10407 (ETEC)	ATCC 35401	5.6×10^2 CFU/mL	20/20	5.6×10^2 CFU/mL	20/20
Salmonella bongori	SGSC 4900	1.4×10^3 CFU/mL	20/20	5.5×10^3 CFU/mL	20/20
Salmonella enterica ssp. enterica	ATCC 14028	2.2×10^3 CFU/mL	20/20	1.1×10^3 CFU/mL	19/20
Shiga-like toxin producing *E. coli* (STEC)	ATCC BAA-2217	2.5×10^3 CFU/mL	20/20	2.5×10^3 CFU/mL	20/20
E. coli O157	ATCC 700376	3.3×10^3 CFU/mL	20/20	3.3×10^3 CFU/mL	20/20
Shigella sonnei	ATCC 29930	4.4×10^2 CFU/mL	20/20	1.7×10^3 CFU/mL	20/20
Vibrio cholerae	ATCC 25870	4.9×10^2 CFU/mL	20/20	4.9×10^2 CFU/mL	20/20
Vibrio parahaemolyticus	ATCC 17802	1.3×10^1 CFU/mL	20/20	5.0×10^1 CFU/mL	20/20
Yersinia enterocolitica	ATCC 23715	1.5×10^3 CFU/mL	20/20	1.5×10^3 CFU/mL	20/20
Cryptosporidium hominis	UKRC	1.3×10^4 oocysts/mL	20/20	1.3×10^4 oocysts/mL	19/20
Cryptosporidium parvum	waterborne P102C	3.1×10^3 oocysts/mL	20/20	3.1×10^3 oocysts/mL	20/20
Entamoeba histolytica HB-301:NIH	BEI NR-178	3.1×10^{-1} cysts/mL	20/20	3.1×10^{-1} cysts/mL	20/20
Giardia intestinalis (aka G. lamblia)	waterborne P101	1.8×10^3 cysts/mL	20/20	1.8×10^3 cysts/mL	20/20
Adenovirus 40 (dugan)	Zeptometrix 0810084	1.0×10^{-1} TCID50/mL	20/20	1.0×10^{-1} TCID50/mL	20/20
Adenovirus 41 (TAK)	Zeptometrix 0810085	9.4×10^{-2} TCID50/mL	20/20	7.5×10^{-1} TCID50/mL	20/20
Rotavirus A	ATCC VR-2018	2.5×10^3 TCID50/mL	20/20	6.2×10^2 TCID50/mL	20/20
Norovirus GIa	CDC	6.4×10^5 copies/gram	20/20	6.5×10^5 copies/gram	20/20
Norovirus GIIa	CDC	5.2×10^4 copies/gram	20/20	9.96×10^4 copies/gram	20/20

g. Analytical Reactivity/Inclusivity:

A study was performed to verify analytical reactivity of the BioCode Gastrointestinal Pathogen Panel (GPP). Different strains were selected that represent various temporal, geographic, and genetic diversity for each analyte. This study tested a panel of titered stocks for relevant organisms diluted in pre-screened negative stools at 3X LoD. Stocks not detected

at 3X LoD, if applicable, were tested at higher concentrations. Due to a lack of titered specimens, Adenovirus 40/41 clinical samples and Cryptosporidium DNA from the Cryptosporidium reference unit were used (Public Health England). Norovirus GI and GII genotypes and the Rotavirus vaccine strain were tested by the CDC. All of the organisms were detected at the concentrations indicated. The strains evaluated are shown in Tables 8-21 below.

Table 8. Shiga-like toxin producing *E. coli* (STEC) stx1/stx2 and *E. coli* O157 Inclusivity results

Organism	Serotype	Source
E. coli O157	*E. coli* O157:H45	STEC SC373/2 TW07922
	E. coli 0157:HNM	STEC DA-26 TW07952
	E. coli 0157:H7	STEC 93-111 TW04863
	E. coli O157: H7	STEC MI06-19 TW14301
	E. coli O157:HNT	STEC DA-27 TW07953
	E. coli O157:H7 Strain EDL933	*BEI NR-11*
Shiga toxin producing *E. coli* (STEC)	*E. coli* O26:H11	STEC 2332/00 TW08998
	E. coli O113:H21	STEC CL-15 TW02318
	E. coli O45:H2	STEC DEC11C DEC11c
	E. coli O103:H2	STEC 107-226 TW07881
	E. coli O104:H21	STEC G5506 TW04909
	E. coli O111:H2	STEC RD8 TW06296
	E. coli O111:H8	STEC DEC8B
	E. coli O26:NM	STEC DA-22 TW07948
	E. coli O145:NM	ATCC BAA-2192
	E. coli O146:21	STEC DEC16E TW01383
	E. coli O45:H2	STEC MI05-14 TW14003
	E. coli O121:19	STEC MDCH-4 TW07614
	E. coli O121:NM	STEC DA-37 TW07972
	E. coli *O104:H4	ATCC BAA-2326
	E. coli 045:H2	ATCC BAA-2193
	E. coli O26: H11	BAA-2196
	E. coli O121:H19	BAA-2219

Table 9. Enterotoxigenic *E. coli* (ETEC), Enteroaggregative *E. coli* (EAEC) Inclusivity results

Organism	Serotype	Source
Enteroaggregative *E. coli* (EAEC)	*E. coli* O44:H18	STEC 042 TW04393
	E. coli O111a, 111b:K58:H21	ATCC 29552
	E. coli O104:H4	ATCC BAA-2326
	E. coli O3:K2a	BEI NR-102

Table 10. *Shigella*/Enteroinvasive *E. coli* (EIEC) Inclusivity results

Organism	Serotype	Source
Enteroinvasive *E. coli* (EIEC)	*E. coli O121*	ATCC BAA-2190
	E. coli O124:HNM	STEC 929-78 TW16574
	E. coli O136:H	STEC LT-41 TW06139
	E. coli O285A:HNM	BEI NR-101
	E. coli O15	ATCC 49105
Shigella boydii	*Shigella boydii* (Type 1)	ATCC 9207
	Shigella boydii, (Type 2)	BEI NR-521
	Shigella boydii (Type 7)	ATCC 9905
	Shigella boydii (Type 20)	ATCC BAA-1247
	Shigella boydii (Type 3)	ATCC 8702
Shigella dysenteriae	*Shigella dysenteriae* (Type 1)	BEI NR-520
	Shigella dysenteriae (Type 3)	ATCC 9751
	Shigella dysenteriae (Type 2)	ATCC 9750
	Shigella dysenteriae (Type 5)	ATCC 9764
	Shigella dysenteriae (Type 12)	ATCC 49552
Shigella flexneri	*Shigella flexneri*, strain 24570 (Type 2a)	BEI NR-517
	Shigella flexneri, strain 2457T (Type 2a)	BEI NR-518
	Shigella flexneri (Type 2b)	ATCC 12022
	Shigella flexneri (Type 6)	ATCC 12025
	Shigella flexneri (Type 1b)	ATCC 9380
Shigella sonnei	*Shigella sonnei*	ATCC 25931
	Shigella sonnei	ATCC 11060
	Shigella sonnei	ATCC 9290
	Shigella sonnei	ATCC 29029

Table 11. *Salmonella* Inclusivity results

Organism	Serotype	Source
Salmonella enterica subsp. *arizonae*	*Salmonella enterica subsp. Enterica*	ATCC 13314
Salmonella enterica subsp. *salamae*	serovar Tranoroa	ATCC 700148
Salmonella enterica subsp. *enterica*	serovar Montevideo	ATCC 7001 BAA-710
	serovar Enteritidis	SGSC4901
	serovar Enteritidis	ATCC 4931
	serovar Oranienburg	SGSC4079
	serovar Paratyphi B var.L(+) tartrate+	SGSC4150
	serovar Typhimurium	SGSC1412
	serovar Saintpaul	SGSC2512
	serovar S. typhimurium LT2	SGSC2666
	serovar Newport	SGSC2493
	serovar Newport	ATCC 6962
	serovar Muenchen	SGSC2490
	serovar Agona	SGSC2458
	serovar Javiana	SGSC4917
	serovar Schwarzengrund	SGSC2514
	serovar Heidelberg	SGSC2480
	serovar Infantis	SGSC2484
	serovar Montevideo	SGSC2487
	serovar Thompson	SGSC 2519
	serovar Hadar	SGSC4965
	serovar Mississippi	SGSC4078
	serovar Paratyphi A	SGSC2499
	serovar Choleraesuis	SGSC4770
	serovar Choleraesuis	ATCC 13312
	serovar Dublin	SGSC4157
	serovar Braenderup	ATCC® 700136
	serovar Bareilly	ATCC® 9115
	serovar Typhi	Zeptometrix 0801933
Salmonella enterica subsp. II	*Salmonella enterica* subs. II	SGSC3039
Salmonella enterica subsp. IIIa	*Salmonella enterica* subs. IIIa	SGSC3061
Salmonella enterica subsp. IIIb	*Salmonella enterica* subs. IIIb	SGSC3068
Salmonella enterica subsp. IV	*Salmonella enterica* subs. IV	SGSC3074
Salmonella enterica subsp. VI	*Salmonella enterica* subs VI	SGSC3116

Table 12. *Campylobacter* inclusivity results.

Organism	Serotype	Source
Campylobacter spp.	*Campylobacter jejuni* subsp. *jejuni*	BEI NR-399
	Campylobacter jejuni subsp. *jejuni*	BEI NR-400
	Campylobacter jejuni subsp. *doylei*	ATCC 49350
	Campylobacter jejuni subsp. *doylei*	ATCC 49349
	Campylobacter coli	ATCC 43478
	Campylobacter coli	ATCC 43485
	Campylobacter coli	BEI HM-296
	Campylobacter coli	ATCC 43484

Table 13. *Vibrio* spp inclusivity results.

Organism	Serotype	Source
Vibrio spp. (not *parahaemolyticus*)	*Vibrio cholerae* (O:1 Inaba, Biotype E1 Tor)	BEI NR-147
	Vibrio cholerae (O:1 Inaba, Biotype E1 Tor)	BEI NR-146
	Vibrio cholerae(O:2)	BEI NR-149
	Vibrio cholerae (O:4)	BEI NR-151
	Vibrio cholerae (O:139)	BEI NR-144
	Vibrio cholerae (O:1 Ogawa)	ATCC 14035
	Vibrio vulnificus	ATCC 27562
	Vibrio vulnificus	ATCC BAA-88
	Vibrio vulnificus	ATCC 43382
	Vibrio vulnificus	ATCC 29306
	Vibrio vulnificus	ATCC 29307
Vibrio parahaemolyticus	*Vibrio parahaemolyticus*	BEI NR-21990
	Vibrio parahaemolyticus	BEI NR- 21991
	Vibrio parahaemolyticus	BEI NR-22002
	Vibrio parahaemolyticus	Zepto 0801903
	Vibrio parahaemolyticus	BEI NR-22013

Table 14. *Yersinia enterocolitica* inclusivity results.

Organism	Serotype	Source
Yersinia enterocolitica	*Yersinia enterocolitica* (O:8)	ATCC 9610
	Yersinia enterocolitica (O:3)	BEI NR-212
	Yersinia enterocolitica (O:8)	BEI NR-206
	Yersinia enterocolitica	ATCC 29913
	Yersinia enterocolitica	BEI NR-213

Table 15. *Clostridium difficile* inclusivity results.

Organism	TOXINOTYPE	Source
Clostridium difficile	0 A+B+	ATCC 43255-FZ
	0 A+B+	ATCC 700792-FZ
	0 A+B+	ATCC BAA-1382-fz
	0 A+B+	ATCC 51695-fz
	0 A+B+	ATCC 43599-fz
	0 A+B+	ATCC 43596-fz
	0 A+B+	ATCC 17858-fz
	0 A+B+	ATCC 43594
	0 A+B+	ATCC 43600
	0 A+B+	ATCC 17857
	0 A+B+	ATCC BAA-1871
	0 A+B+	ATCC BAA-1872
	VIII A-B+	ATCC 43598
	III A+B+ (Nap1)	ATCC BAA-1805
	XXII A+B+	ATCC BAA-1814
	III A+B+	ATCC BAA-1870
	V A+B+	ATCC BAA-1875

Table 16. Adenovirus 40/41 inclusivity results.

Organism	Serotype	Source
Adenovirus 40	*Human adenovirus 40 (dugan)*	lot #K1220A
	n/a	*sample ID # SP143*
	n/a	*sample ID # SP258*
Adenovirus 41	*n/a*	*sample ID # SP170*
	n/a	*sample ID # SP174*
	n/a	*sample ID # SP 276*
	n/a	*sample ID # SP288*
	n/a	*sample ID # SP309*
	n/a	*sample ID # SP329*
	n/a	*sample ID # SP446*

Table 17. Norovirus GI/GII inclusivity results.

Organism	Genotype	Source
*Norovirus GI	GI.1	Clinical Specimen
	GI.2	Clinical Specimen
	GI.3	Clinical Specimen
	GI.4	Clinical Specimen
	GI.5	Clinical Specimen
	GI.6	Clinical Specimen
	GI.7	Clinical Specimen
	GI.8	Clinical Specimen
[a]Norovirus GII	GII.1	Clinical Specimen
	GII.2	Clinical Specimen
	GII.3	Clinical Specimen
	GII.4 New Orleans	Clinical Specimen
	GII.4 Sydney	Clinical Specimen
	GII.5	Clinical Specimen
	GII.6	Clinical Specimen
	GII.7	Clinical Specimen
	GII.8	Clinical Specimen
	GII.12	Clinical Specimen
	GII.13	Clinical Specimen
	GII.14	Clinical Specimen
	GII.16	Clinical Specimen
	GII.17	Clinical Specimen

Table 18. Rotavirus inclusivity results.

Organism	Serotype	Source
Rotavirus A	Rotavirus A (DS-1)	ATCC VR-2550
	Rotavirus A (HRV 89-12C2)	ATCC VR-2272
	Rotavirus A (WISC2)	ATCC VR-2417
	Rotavirus A (HRV 408)	ATCC VR-2273
	Rotavirus A (HRV 248)	ATCC VR-2274
	Rotavirus A (HU)	ATCC VR-1546
	Rotavirus A (HRV CJN)	ATCC VR-2275
	Rotavirus A G1	Clinical Sample
	Rotavirus A G2	Clinical Sample
	Rotavirus A G3	Clinical Sample
	Rotavirus A G4	Clinical Sample
	Rotavirus A G9	Clinical Sample
	Rota vaccine strain	RotaTeq vaccine
	Rota vaccine strain	Rotarix vaccine

Table 19. *Cryptosporidium* spp inclusivity results.

Organism	Serotype	Source
C. parvum	*DNA subtype /IIaA17G1R1*	UKCR UK28
	DNA subtype /IIaA15G2R1	UKCR UK29
	DNA subtype /IIaA19G1R1	UKCR UK30
	DNA subtype /IIdA22G1	UKCR UK31
	DNA subtype /IIdA15G1	UKCR UK32
C. hominis	*DNA subtype IaA14R3*	UKCR UKH14
	DNA subtype IdA18	UKH12
	DNA subtype IbA10G2	UKH13
	DNA E. coli O103:H2	NR2520

Table 20. *Entamoeba histolytica* inclusivity results.

Organism	Serotype	Source
Entamoeba histolytica	200:NIH	BEI NR-177
	HB-301:NIH	BEI NR-176
	Rahman	BEI NR-179
	H-303:NIH	BEI NR-180

Table 21. *Giardia lamblia* inclusivity results.

Organism	Serotype	Source
Giardia lamblia/ intestinalis	Egypt-4	BEI NR-9231
	Mario	BEI NR-9232
	D.Hall	BEI NR-9234
	DAN	BEI NR-9235

h. Analytical Cross-reactivity:

A study was performed to verify that the BioCode Gastrointestinal Pathogen Panel (GPP) does not detect DNA or RNA from organisms commonly found in stool specimens or from organisms that can cause similar clinical symptoms. In addition, on-panel organisms were tested at high concentrations to insure there is no cross-reactivity with other panel targets. This study tested a panel of titered stocks and genomic DNA extracts for relevant organisms. Organisms that were not available for wet testing were analyzed *in silico* comparing the whole organism sequence against all primers to assess potential for cross reactivity. Analysis was conducted using BlastN and Primer Blast programs. Results from evaluation of cross-reactivity are shown in Tables 21-25.

Cross-reactivity was not observed with microorganisms tested in this study except for the

following:

Y. enterocolitica: Wet testing and *in silico* sequence analysis indicate a potential for cross-reactivity with *Y. bercovieri, Y. frederiksenii, Y. intermedia* and *Y. mollaretii* near the established LoD for *Y. enterocolitica* (~1.5 x 10^3 CFU/mL). *Y. rohdei* was also detected when present at high levels (>6.8 x 10^4 CFU/mL). These species are in the *Y. enterocolitica* group and are suspected human pathogens.

Shiga toxin: Shiga toxin (*stx*), that is identical to *stx*1 of STEC is found in *Shigella dysenteriae*; therefore, a BioCode Gastrointestinal Pathogen Panel (GPP) GI Panel report with positive test results for Shiga-like toxin-producing *E. coli* (STEC) and Shigella/Enteroinvasive *E. coli* (EIEC) in the same sample may indicate the presence of *Shigella dysenteriae* since the genetic relatedness of these two pathogens indicate they could constitute a single species but they are maintained as separately named entities for the purposes of epidemiology and clinical medicine.

Rotavirus: Wet testing has demonstrated that the GPP assay will detect recombinant viruses included in Rotavirus vaccines.

Cryptosporidium: Wet testing demonstrated cross reactivity with *C. cuniculus* and *C. meleagridis* .

E. histolytica: Wet testing with a gene fragment construct and *in silico* sequence analysis do not predict cross reactivity with the closely related *E. dispar*.

The following tables (Table 22, Table 23, and table 24) present a listing of organisms for which there was no observed cross-reactivity:

Table 22. Bacteria tested for Cross-reactivity.

Bacteria		
Aeromonas jandaei	*Egglerthella lenta*	*Proteus penneri*
Aeromonas media	Enterobacter aerogenes	*Proteus vulgaris*
Aeromonas trota	*Enterobacter cloacae*	*Providencia alcalifaciens*
Aeromonas caviae	*Enterococcus faecalis*	*Providencia stuartii*
Aeromonas hydrophila	*Enterococcus faecium*	*Pseudomonas aeruginosa*
Acinetobacter baumannii	Enteropathogenic *Escherichia coli*	*Pseudomonas fluorescens*
Acinetobacter lwoffii	*Escherichia coli* Non pathogenic	*Pseudomonas putida*
Alcaligenes faecalis	*Escherichia hermannii*	*Saccharomyces boulardii*
Bacillus cereus	*Escherichia vulneris*	*Serratia liquefaciens*
Bacteroides fragilis	*Fusobacterium varium*	*Serratia marcescens*
Bacteroides thetaiotaomicron	*Gardnerella vaginalis*	*Shewanella algae*
Bifidobacterium breve	*Gemella morbillorum*	*Staphylococcus aureus*
Campylobacter fetus	*Grimontia hollisae (formerly vibrio)*	*Staphylococcus epidermidis*
Campylobacter hyointestinalis	*Haemophilus influenzae*	*Stenotrophomonas maltophilia*
Campylobacter lari	*Hafnia alvei*	*Streptococcus agalactiae*
Campylobacter upsaliensis	*Helicobacter pylori*	*Streptococcus intermedius*
Candida albicans	*Klebsiella oxytoca*	*Streptococcus pyogenes*
Cedecea davisae	*Klebsiella pneumoniae*	*Streptococcus salivarius*
Chlamydia trachomatis	*Lactobacillus acidophilus*	*Streptococcus suis*
Citrobacter amalonaticus	*Lactobacillus reuteri*	*Trabulsiella guamensis*
Citrobacter freundii	*Lactococcus lactis*	*Veillonella parvula*
Clostridium difficile non-toxigenic	*Leminorella grimontii*	*Vibrio alginolyticus* [a]
Clostridium histolyticum	*Listeria monocytogenes*	*Vibrio fluvialis*
Clostridium perfringens	*Morganella morganii*	*Vibrio mimicusa*
Clostridium septicum	*Peptoniphilus asaccharolyticus*	*Yersinia bercovieri* [b]
Clostridium sordellii	*Plesiomonas shigelloides*	*Yersinia frederiksenii* [b]
Clostridium sporogenes	*Porphyromonas asaccharolytica*	*Yersinia intermedia* [b]
Clostridium tetani	*Prevotella melaninogenica*	*Yersinia mollaretii* [b]
Edwardsiella tarda	*Proteus mirabilis*	*Yersinia pseudotuberculosis*
		Yersinia rohdei [b]

[a] Detected as *Vibrio spp.* at high titers, see full submission for details.
[b] Detected as *Yersinia enterocolitica* at high titers.

Table 23. Parasites tested for Cross-reactivity.

Viruses		Parasites
Adenovirus 3	Enterovirus 68	*Cryptosporidium cuniculus* (DNA)[a]
Adenovirus 4	Enterovirus	*Cryptosporidium felis* (DNA)
Adenovirus 7	Echovirus 11	*Cryptosporidium meleagridis*[1]
Adenovirus 8	HSV Type 2	*Cryptosporidium meleagridis* (DNA)[a]
Adenovirus 14	Norovirus GIV	*Cryptosporidium muris*
Adenovirus 37	Rhinovirus 1A	*Cryptosporidium ubiquitum* (DNA)
Astrovirus type 1	Sapovirus GI	*Encephalitozoon cuniculi*
Astrovirus type 4	Sapovirus GIV	*Encephalitozoon hellem*
Coronavirus 229E	Sapovirus GV	*Encephalitozoon intestinalis*
Coronavirus NL63	**Parasites**	*Giardia muris*
Coxsackie virus A16	*Blastocystis hominis*	*Pentatrichomonas hominis*
Coxsackievirus B3	*Blastocystis hominis*	*Toxoplasma gondii*
Cytomegalovirus (CMV)	*Cryptosporidium canis* (DNA)	

[a] Detected as Cryptosporidium spp.

Table 24. Microorganisms tested for Cross-reactivity.

GI Panel Targets		
Campylobacter coli	Enterotoxigenic *E. coli* O78:H11 H10407 (ETEC)	*Vibrio parahaemolyticus*
Campylobacter jejuni spp. jejuni	Shiga toxin producing *E. coli* (STEC)	*Yersinia enterocolitica*
Clostridium difficile (toxinotype 0)	*E. coli* O157	*Cryptosporidium parvum*
Clostridium difficile (toxinotype III; Nap1)	*Salmonella bongori*	*Entamoeba histolytica* HB-301:NIH
gregative *E. coli* O92:H33 (EAEC)	*Salmonella enterica ssp. enterica*	*Giardia intestinalis (aka G. lamblia)*
Enteroinvasive *E. coli* O29:NM (EIEC)	*Shigella sonnei*	Adenovirus 40 (dugan)
		Rotavirus A

Table 25. Analysis of microorganisms *in silico* for Cross-reactivity.

Bacteria	Bacteria	Parasites
Anaerococcus tetradius	*Eubacterium cylindroides*	*Ancylostoma duodenale*
Bifidobacterium adolescentis	*Eubacterium rectale*	*Ascaris lumbricoides*
Bifidobacterium longum	*Megamonas hypermegale*	*Balantidium coli*
Campylobacter concisus	*Methanobrevibacter smithii*	*Chilomastix mesnili*
Campylobacter curvus	*Peptoniphilus asaccharolyticus*	*Cryptosporidium bovis*
Campylobacter gracilis	*Ruminococcus bromii*	*Cryptosporidium canis*
Campylobacter helveticus	*Ruminococcus flavefaciens*	*Cryptosporidium cuniculus* [b]
Campylobacter hominis	*Ruminococcus obeum*	*Cryptosporidium felis*
Campylobacter lari	*Selenomonas ruminantium*	*Cryptosporidium fetus*
Campylobacter mucosalis	*Vibrio cincinnatiensis*	*Cryptosporidium meleagridis* [b]
Campylobacter rectus	*Vibrio furnissii*	*Cryptosporidium muris*
Campylobacter showae	*Vibrio metschnikovii*	*Cryptosporidium ryanae*
Campylobacter sputorum	*Yersinia kristensenii*	*Cryptosporidium xiaoi*
Campylobacter upsaliensis	**Viruses**	*Dientamoeba fragilis*
Campylobacter ureolyticus	Norovirus GIV	*Endolimax nana*
Clostridium acetobutylicum	Rotavirus B	*Entamoeba coli*
Clostridium methylpentosum	Rotavirus C [a]	*Entamoeba dispar*
Clostridium novyi	Rotavirus D	*Entamoeba hartmanni*
Clostridium ramosum	Rotavirus E	*Entamoeba moshkovskii*
Collinsella aerofaciens	Rotavirus F	*Entamoeba polecki*
Desulfovibrio piger	Sapovirus	

[a] Cross-reactivity predicted with Porcine Rotavirus C strains only, no cross-reactivity with human Rotavirus C.
[b] *C. cuniculus* and *C. meleagridis* cross reactivity for Cryptosporidium spp assay observed in lab testing was not predicted by *in silico* analysis (Primer Blast).

i. Interference:

Interfering substances:

A study was performed to demonstrate the accuracy of the BioCode Gastrointestinal

Pathogen Panel (GPP) in the presence of potentially inhibiting substances. The substances tested were:

EDTA blood (40% w/v)	benzalkonium chloride (50% w/v)
Ampicillin (152 µmol/L)	Supleco (5% w/v), mucin (3 mg/mL)
10% bleach (50% w/v)	naproxen sodium (14 mg/mL)
cholesterol (5% w/v)	Neosporin (50% w/v)
mineral oil (50% w/v)	nystatin (1000 U/mL)
hydrocortisone (50% w/v)	Pepto-Bismol (5% w/v)
Imodium (5% w/v)	Preparation H (5% w/v)
Senokot (5% w/v)	Tums (5% w/v)
Maalox (5% w/v)	vancomycin (12.5 mg/mL)
metronidazole (14 mg/mL)	

Each member of the interfering substance panel was added to prescreened negative stool sample spiked with representative members of the BioCode Gastrointestinal Pathogen Panel (GPP) at 3X LoD and a negative matrix only pre- screened negative stool. One parasite (*C. parvum*), one virus (Rotavirus A), one gram positive bacterium (*C. difficile*), and one gram negative bacterium (STEC) was used as representative analytes for this study. Each sample was tested with and without potentially interfering substances. Each sample was extracted in triplicate tested in singlet with the GI Panel on the BioCode Gastrointestinal Pathogen Panel (GPP).

Concentrations were determined by reviewing 510(k) summary results of previous GI panel clinical trials and CLSl-EP1-A2. None of the substances listed above returned interfering results at the concentrations tested.

Microbial Inhibition:

The accuracy of the BioCode Gastrointestinal Pathogen Panel (GPP) in the presence of potentially inhibiting microorganisms was assessed in the following study. The interfering organisms tested were *Bacteroides fragilis* (1 x 10^6 CFU/mL), *Blastocystis hominis* (1 x 10^5 CFU/mL), *Candida albicans* (1 x 10^5 CFU/mL), *Clostridium difficile* non-toxigenic (1 x 10*6* CFU/mL), *Enterococcus faecalis* (1 x 10^6 CFU/mL), *Escherichia coli* non-pathogenic (1 x 10^6 CFU/mL), *Pseudomonas aeruginosa* (1 x 10^6 CFU/mL), and *Saccharomyces boulardi* (1 x 10^5 CFU/mL).Each member of the interfering microorganism panel was added to prescreened negative stool sample spiked with representative members of the BioCode Gastrointestinal Pathogen Panel (GPP) GI Pathogen Panel at 3X LoD and a negative matrix only pre- screened negative stool. One parasite (*C. parvum*), one virus (Rotavirus A), one gram positive bacterium (*C. difficile*), and one gram negative bacterium (STEC) was used as representatives for this study. Each sample was tested with and without potentially interfering microorganisms. Each sample was extracted in triplicate and tested in singlet with the GI Panel on the BioCode Gastrointestinal Pathogen Panel (GPP).

Concentrations were determined by reviewing 510k summary results of previous GI panel clinical trials and CLSl-EP1-A2. None of the microorganisms listed above returned interfering results at the concentrations tested.

Competitive Interference:

A study was performed to evaluate the potential for inhibition in samples with mixed infectious agents. Prescreen negative stool was spiked with one target at high concentration ($\geq 10^6$ CFU/mL for bacteria and $\geq 10^5$ units/mL for viruses or parasites) and two targets at low concentration ($\leq$ 3 LoD). Common co-infections were determined by reviewing results of previous GI Panel clinical trials from 510k summaries, publications/posters and internal clinical sample testing. Each sample was extracted in triplicate on the easyMAG and each extraction tested in singlet with the BioCode GPP on the BioCode MDx 3000 system. No inhibition was observed.

Table 26. Competitive inhibition testing results.

Panel Designation	Viral/Bacteria Strain	Source	Level	Titer Tested	Target Probe	Result (n of 3 Detected)
Competitive Inhibition Sample 1	*Clostridium difficile*	Zeptometrix 801619	High	$3.0x10^6$ CFU/mL	tcdB	3/3
	Rotavirus A	ATCC VR-2018	Medium	$7.44X10^3$ $TCID_{50}$/mL	Rota	3/3
	Escherichia coli E2348/69 (EPEC)	STEC TW06375	Medium	$7.02x10^3$ CFU/mL	EPEC	3/3
Competitive Inhibition Sample 2	O92:H33 *Escherichia coli* (EAEC)	STEC JM221 TW04440	High	$3.0x10^6$ CFU/mL	EAEC	3/3
	Escherichia coli E2348/69 (EPEC)	STEC TW06375	Medium	$7.02X10^3$ CFU/mL	EPEC	3/3
	Clostridium difficile	Zeptometrix 801619	Medium	$5.7x10^2$ CFU/mL	tcdB	3/3
Competitive Inhibition Sample 3	*Escherichia coli* E2348/69 (EPEC)	STEC TW06375	High	$3.0x10^6$ CFU/mL	EPEC	3/3
	Clostridium difficile	Zeptometrix 801619	Medium	$5.7x10^2$ CFU/mL	tcdB	3/3
	Rotavirus A	ATCC VR-2018	Medium	$7.44X10^3$ $TCID_{50}$/mL	Rota	3/3
Competitive Inhibition Sample 4	*Escherichia coli* E2348/69 (EPEC)	STEC TW06375	High	$3x10^6$ CFU/mL	EPEC	3/3
	O92:H33 *Escherichia coli* (EAEC)	STEC JM221 TW04440	Medium	$4.08X10^3$ CFU/mL	EAEC	3/3
	Campylobacter jejuni subsp. jejuni	ATCC 33292	Medium	$2.1X10^3$ CFU/mL	Campy	3/3
Competitive Inhibition Sample 5	*Campylobacter jejuni subsp. Doylei*	ATCC 49349	High	$3.0x10^6$ CFU/mL	Campy	3/3
	Escherichia coli E2348/69 (EPEC)	STEC TW06375	Medium	$7.02X10^3$ CFU/mL	EPEC	3/3
	O92:H33 *Escherichia coli* (EAEC)	STEC JM221 TW04440	Medium	$4.08X10^3$ CFU/mL	EAEC	3/3

Panel Designation	Viral/Bacteria Strain	Source	Level	Titer Tested	Target Probe	Result
Competitive Inhibition Sample 6	O92:H33 *Escherichia coli* (EAEC)	STEC JM221 TW04440	High	$3x10^6$ CFU/mL	EAEC	3/3
	Campylobacter jejuni subsp. jejuni	ATCC 33292	Medium	$2.1X10^3$ CFU/mL	Campy	3/3
	Escherichia coli E2348/69 (EPEC)	STEC TW06375	Medium	$7.02X10^3$ CFU/mL	EPEC	3/3
Competitive Inhibition Sample 7	Shiga-toxin producing *E. coli* (STEC)	ATCC BAA-2217	High	$3.0x10^6$ CFU/mL	stx2	3/3
	Giardia intestinalis	waterborne P101	Medium	$5.42X10^3$ cysts/mL	G.lam	3/3
	Shigella sonnei	ATCC 29930	Medium	$1.31X10^3$ CFU/mL	Shig	3/3
Competitive Inhibition Sample 8	*Giardia intestinalis*	waterborne P101	High	$3.0x10^5$ cysts/mL	G.lam	3/3
	Shigella sonnei	ATCC 29930	Medium	$1.31X10^3$ CFU/mL	Shig	3/3
	Shiga-toxin producing *E. coli* (STEC)	ATCC BAA-2217	Medium	$7.5X10^3$ CFU/mL	stx2	3/3
Competitive Inhibition Sample 9	*Shigella sonnei*	ATCC 29930	High	$3.0x10^6$ CFU/mL	Shig	3/3
	Shiga-toxin producing *E. coli* (STEC)	ATCC BAA-2217	Medium	$7.5X10^3$ CFU/mL	stx2	3/3
	Giardia intestinalis	waterborne P101	Medium	$5.42X10^3$ cysts/mL	G.lam	3/3

j. Carry-Over/Cross-Contamination study:

The BioCode Gastrointestinal Pathogen Panel (GPP) assay was evaluated for the presence of contamination due to carry over in known negative specimens by alternating high positive samples with negative samples. No evidence of carry-over contamination was observed.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Not applicable

3. Clinical studies:

A total of 1558 leftover, de-identified samples were prospectively collected from patients who underwent stool sample collection for clinical indications of diarrhea caused by gastrointestinal infection at four (4) investigational sites, with each collecting approximately 400 samples. In addition testing was performed on archived known positives and contrived specimens to further augment the sample numbers. Each raw stool specimen was de-identified at the site and the de-identified leftover sample was divided into aliquots and either tested freshly on the BioCode Gastrointestinal Pathogen Panel (GPP) at the site, or frozen and tested at a later date at the site. Each stool specimen in Cary-Blair was similarly de-identified at the site and the de-identified leftover sample aliquoted into 4 aliquots and tested freshly on the BioCode Gastrointestinal Pathogen Panel (GPP) at the site. All prospectively collected samples had age, sex, and therapy status collected by the site. Enrollment of samples covered multiple calendar seasons beginning January 2015 and ending in August 2017. During the prospective clinical study 2.6% (41/1558) of samples were invalid for lack of RNA-IC signal on initial testing. The invalid rate after repeat testing was approximately 0.2% (3/1558). The results of the clinical study are presented in the tables below.

The performance for *C. difficile* for frozen specimens was not equivalent to the predicate device. Therefore, samples suspected of *C. difficile* presence should only be tested fresh. The assay software requires selection of sample type (fresh or frozen) and results for *C. difficile* are reported only for fresh specimens.

Table 27. Demographic data for prospective specimens (fresh and frozen).

Prospective Study Specimens	
Total Specimens	1558
Gender (n/N(%))	
Female	778/1558 (49.9)
Male	780/1558 (51.1)
Age Category (n/N(%))	
< 5 year	140/1558 (9.0)
6-21 yrs	237/1558 (15.2)
22-59 yrs	718/1558 (46.1)
60+ yrs	463/1558 (29.7)
Status (n/N(%))	
Inpatient	1212/1558 (77.8)
Outpatient	346/1558 (22.2)

Table 28. Breakdown of prospective specimen collection by site.

	Unpreserved (Fresh)	Unpreserved (Frozen)	Cary-Blair (Fresh)
Site 001	50	350	0
Site 002	50	347	0
Site 003	137	263	0
Site 006	0	0	361
Total	237	960	361

Table 29. Comparator (reference) methods for prospective clinical study.

Target Pathogen/Toxin	**Reference Method**
Adenovirus 40/41	Composite result of two PCR/sequencing
Campylobacter (*C. jejuni*, *C. coli*)	Culture
Clostridium difficile (*C. difficile*) toxin A/B	FDA cleared NAAT
Cryptosporidium (*C. parvum*, *C. hominis*)	Composite result of two PCR/sequencing
Entamoeba histolytica	Composite result of two PCR/sequencing
Escherichia coli (*E. coli*) O157	Enrichment culture
Enterotoxigenic *E. coli* (ETEC) LT/ST	Composite result of two PCR/sequencing
Enteroaggregative *E. coli* (EAEC)	Composite result of two PCR/sequencing
Giardia lamblia /*intestinalis*	Composite result of two PCR/sequencing
Norovirus GI/GII	Composite result of two PCR/sequencing
Rotavirus A	Composite result of two PCR/sequencing
Salmonella spp.	Enrichment culture
Shiga-like Toxin producing *E. coli* (STEC) *stx*1/*stx*2	Enrichment culture/FDA cleared antigen test
Shigella (*S. boydii*, *S. sonnei, S. flexneri*, *S. dysenteriae*)/EIEC	Enrichment culture
Vibrio spp. (*V. cholerae, V. parahaemolyticus, V. vulnificus*)	Culture
Yersinia enterocolitica	Culture

Table 30. Summary of Clinical Study Results (Prospective specimens) for Unpreserved Stool (Fresh).

	PPA		NPA	
Target	**Agreement Rate n/N (%)**	**95% CI**	**Agreement Rate n/N (%)**	**95% CI**
Campylobacter spp. [a]	1/1 (100.0)	(2.5 - 100.0)	234/236 (99.2)	(97.0 - 99.9)
Clostridium difficile [b]	26/27 (96.3)	(81.0 - 99.9)	208/210 (99.1)	(96.6 - 99.9)
E.coli 0157	0/0	0/0	237/237 (100.0)	(98.5 - 100.0)
EAEC	1/1 (100.0)	(2.5 - 100.0)	234/234 (100.0)	(98.4 - 100.0)
ETEC [c]	3/3 (100.0)	(29.2 - 100.0)	229/232 (98.7)	(96.4 - 99.7)
STEC [d]	0/0	0/0	235/237 (99.2)	(97.0 - 99.9)
Salmonella spp [e]	3/3 (100.0)	(29.2 - 100.0)	232/234 (99.2)	(97.0 - 99.9)
Shigella/EIEC [f]	1/1 (100.0)	(2.5 - 100.0)	233/236 (98.7)	(96.3 - 99.7)
Vibrio parahaemolyticus [g]	0/0	0/0	236/237 (99.6)	(97.7 - 100.0)
Vibrio spp	0/0	0/0	237/237 (100.0)	(98.5 - 100.0)
Yersinia enterocolitica [h]	0/0	0/0	236/237 (99.6)	(97.7 - 100.0)
Cryptosporidium spp	1/1 (100.0)	(2.5 - 100.0)	234/234 (100.0)	(98.4 - 100.0)
Entamoeba histolytica	0/0	0/0	235/235 (100.0)	(98.4 - 100.0)
Giardia lamblia [i]	0/0	0/0	234/235 (99.6)	(97.6 - 100.0)
Adenovirus 40/41 [j]	0/0	0/0	233/235 (99.2)	(97.0 - 100.0)
Norovirus GI/GII	1/1 (100.0)	(2.5 - 100.0)	235/235 (100.0)	(98.4 - 100.0)
Rotavirus A	1/1 (100.0)	(2.5 - 100.0)	234/235 (99.6)	(97.7 - 100.0)

a - *Campylobacter* spp: The 2 false positives compared to the culture reference method were tested by bidirectional sequencing, and 1 of 2 confirmed as positive.
b - *Clostridium difficile*: The 1 false negative compared to the FDA Cleared NAAT reference test produced high Ct (Ct 35.0).
c - ETEC: The 3 false positives compared to bidirectional sequencing were not confirmed as positives by an additional round of sequencing.
d - STEC: The 2 false positives compared to the culture reference method were tested by bidirectional sequencing, and both confirmed as positive.
e– *Salmonella* spp: The 2 false positives compared to the culture reference method were tested by bidirectional sequencing, and both confirmed as positives.
f - *Shigella*/EIEC: The 3 false positives compared to the culture reference method were tested by bidirectional sequencing, and all 3 confirmed as positives.
g - *Vibrio parahaemolyticus*: The 1 false positive sample compared to the culture reference method was tested by bidirectional sequencing and confirmed as positive.
h - *Yersinia enterocolitica*: The 1 false positive sample compared to the culture reference method was tested by bidirectional sequencing and could not be confirmed as positive.
ij – *Giardia lamblia*: The 1 false positive to bidirectional sequencing was not confirmed as positive by 2 additional rounds of sequencing.
j - Adenovirus 40/41: The 2 false positives to bidirectional sequencing were not confirmed as positives by an additional round of sequencing.

Table 31. Summary of Clinical Study Results (Prospective specimens) for Unpreserved Stool (Frozen).

	PPA		NPA	
Target	**Agreement Rate n/N (%)**	**95% CI**	**Agreement Rate n/N (%)**	**95% CI**
Campylobacter spp. [a]	3/3 (100.0)	(29.2 - 100.0)	936/952 (98.3)	(97.3 - 99.0)
E.coli 0157 [b]	1/2 (50.0)	(1.3 - 98.7)	950/954 (99.6)	(98.9 - 99.9)
EAEC [c]	25/29 (86.2)	(68.3 - 96.1)	916/919 (99.7)	(99.1 99.9)
ETEC [d]	7/10 (70.0)	(34.8 - 93.3)	934/939 (99.5)	(98.8 - 99.8)
STEC [e]	3/3 (100.0)	(29.2 - 100.0)	918/919 (99.9)	(99.4 - 100.0)
Salmonella spp [f]	18/22 (81.8)	(59.7 - 94.8)	926/934 (99.1)	(98.3 - 99.6)
Shigella/EIEC [g]	4/5 (80.00)	(28.4 - 99.5)	940/951 (98.8)	(97.9 - 99.4)
Vibrio parahaemolyticus [h]	0/0	0/0	955/957 (99.8)	(99.3 - 100.0)
Vibrio spp	0/0	0/0	956/956 (100.0)	(99.6 - 100.0)
Yersinia enterocolitica[i]	0/0	0/0	951/956 (99.5)	(98.8 - 99.8)
Cryptosporidium spp	7/7 (100.0)	(59.0 - 100.0)	941/941 (100.0)	(99.6 - 100.0)
Entamoeba histolytica	0/0	0/0	948/948 (100.0)	(99.6 - 100.0)
Giardia lamblia [j]	2/2 (100.0)	(15.8 - 100.0)	940/946 (99.4)	(98.6 - 99.8)
Adenovirus 40/41 [k]	7/10 (70.0)	(34.8 - 93.3)	935/938 (99.7)	(99.1 - 99.9)
Norovirus GI/GII	39/39 (100.0)	(91.0 - 100.0)	913/917 (99.6)	(98.9 - 99.9)
Rotavirus A	19/20 (95.0)	(75.1 - 99.9)	928/936 (99.2)	(98.3 - 99.6)

a - *Campylobacter* spp: The 16 false positives compared to the culture reference method were tested by bidirectional sequencing, and 8 of 16 confirmed as positives.
b - *E. coli* O157: The one false negative compared to the culture reference method was tested by bidirectional sequencing and could not be confirmed as positive. The 4 false positive samples compared to the culture reference method were tested by bidirectional sequencing, and 3 of 4 confirmed as positives.
c - EAEC: The 4 false negatives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing; 3 of the 4 confirmed as positives. 2 of the 3 false positives could not be repeated due to low sample volume. The remaining 1 was not detected by addition rounds of sequencing.
d - ETEC: The 3 false negatives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing; none were confirmed as positives. 1 of the 5 false positives could not be repeated due to low sample volume. The remaining 4 false positives were not confirmed as positives by an additional round of sequencing.
e - STEC: The 1 false positive compared to the culture reference method was tested by bidirectional sequencing and confirmed as positive.
f – *Salmonella* spp: The 4 false negatives compared to the culture reference method were tested by bidirectional sequencing, and 1 of 4 could not be confirmed as positives. The 8 false positive samples compared to the culture reference method were tested by bidirectional sequencing and 6 of 8 confirmed as positives.
g - *Shigella*/EIEC: The 1 false negative compared to the culture reference method was tested by bidirectional sequencing and could not be confirmed as positive. The 11 false positive samples compared to the culture reference method were tested by bidirectional sequencing, and 10 of 11 confirmed as positives.
h - *Vibrio parahaemolyticus*: The 2 false positives compared to the culture reference method were tested by bidirectional sequencing and, 1 of 2 confirmed as positive.
i - *Yersinia enterocolitica*: The 5 false positives compared to the culture reference method were tested by bidirectional sequencing and, 3 of 5 confirmed as positive.
j – *Giardia lamblia*: The 4 false positives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing: none were confirmed as positives.
k - Adenovirus 40/41: The 3 false negatives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing; none were confirmed as positives. The 3 false positives were not confirmed as positives by an additional round of sequencing.

Table 32. Summary of Clinical Study Results (Prospective specimens) for Native Cary-Blair Samples.

	Positive Agreement		Negative Agreement	
Bacteria	**Agreement Rate n/N (%)**	**95% CI**	**Agreement Rate n/N (%)**	**95% CI**
Campylobacter spp. [a]	2/3 (66.7)	(9.4 - 99.2)	347/358 (96.9)	(94.6 - 98.5)
Clostridium difficile [b]	37/38 (97.4)	(86.2 - 99.9)	318/322 (98.8)	(96.9 - 99.7)
E.coli 0157 [c]	0/0	0/0	359/361 (99.5)	(98.0 - 99.9)
EAEC [d]	17/18 (94.4)	(72.71 - 99.9)	336/341 (98.5)	(96.6 - 99.5)
ETEC [e]	13/14 (92.9)	(66.13 - 99.8)	343/345 (99.4)	(97.9 - 99.9)
STEC [f]	0/0	0/0	359/361 (99.5)	(98.0 - 99.9)
Salmonella spp [g]	4/5 (80.0)	(28.36 - 99.5)	354/356 (99.4)	(98.0 - 99.9)
Shigella/EIEC [h]	1/2 (50.0)	(1.26 - 98.7)	356/359 (99.2)	(97.6 - 99.8)
Vibrio parahaemolyticus	0/0	0/0	361/361 (100.0)	(99.0 - 100.0)
Vibrio spp	0/0	0/0	361/361 (100.0)	(99.0 - 100.0)
Yersinia enterocolitica [i]	0/0	0/0	357/361 (98.9)	(97.2 - 99.7)
Cryptosporidium spp [j]	3/3 (100.0)	(29.24 - 100.0)	354/356 (99.4)	(98.0 - 99.9)
Entamoeba histolytica	0/0	0/0	359/359 (100.0)	(99.0 - 100.0)
Giardia lamblia [k]	1/1 (100.0)	(2.50 - 100.0)	357/358 (99.7)	(98.5 - 100.0)
Adenovirus 40/41	0/0	0/0	359/359 (100.0)	(99.0 - 100.0)
Norovirus GI/GII [l]	6/7 (85.7)	(42.13 - 99.6)	354/354 (100.0)	(99.0 - 100.0)
Rotavirus A	1/1 (100.0)	(2.50 - 100.0)	360/360 (100.0)	(98.98 - 100.0)

a – *Campylobacter* spp. The 1 false negative compared to reference culture method was tested by bidirectional sequencing and confirmed positive. The 11 false positives compared to reference culture method were tested by bidirectional sequencing, and 11 of 11 confirmed as positives.
b – *Clostridium difficile*. The 1 false negative compared to the FDA cleared NAAT reference method produced high Ct (35).
c - *E. coli* O157. The 2 false positives compared to reference culture method were tested by bidirectional sequencing, and 2 of 2 confirmed as positives.
d – EAEC. The 1 false negative compared to bidirectional sequencing was tested by 2 additional rounds of sequencing and confirmed as positive. The 4 of 5 false positives were not detected by an addition round of sequencing.
e – ETEC. The 1 false negative compared to bidirectional sequencing was tested by 2 additional rounds of sequencing and was not confirmed as positive. 1 of 2 false positives was confirmed as positive by 2 additional rounds of sequencing.
f – STEC. The 2 false positives compared to reference culture method were tested by bidirectional sequencing, and 2 of 2 confirmed as positives.
g – *Salmonella* spp. The 1 false negative compared to the reference culture method was tested by bidirectional sequencing and confirmed as positive. The 2 false positives compared to reference culture method were tested by bidirectional sequencing and 1 of 2 confirmed as positive.
h – *Shigella*/EIEC. The 1 false negative compared to the reference culture method was tested by bidirectional sequencing and could not be confirmed as positive. The 3 false positives compared to reference culture method were tested by bidirectional sequencing, and all 3 confirmed as positives.
i - *Yersinia enterocolitica.* The 4 false positives compared to the reference culture method were tested by bidirectional sequencing, and none were confirmed as positive.
j – *Cryptosporidium* spp. The 2 false positives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing and both confirmed as positives.
k – Giardia lamblia. The 1 false positive compared to bidirectional sequencing was not confirmed as positive by 2 additional rounds of sequencing.
l - Norovirus GI/GII. The 1 false negative compared to bidirectional sequencing produced a high Ct (37) which indicates that this sample is low positive.

Testing of inoculated Cary-Blair specimens from previously frozen prospective specimens

To supplement the number of prospective Cary-Blair specimens, 400 unpreserved stool samples from sites 1 and 2 were thawed and inoculated into Cary-Blair. Three were removed from the study for improper storage prior to testing. Two were invalid for RNA IC failure in the unpreserved stool. The following table summarizes the performance of BioCode Gastrointestinal Pathogen Panel (GPP) for these inoculated Cary-Blair specimens with performance calculated based on a comparison to reference/comparator methods.

Table 33. Summary of Inoculated Cary-Blair specimens versus reference/comparator methods.

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Campylobacter spp. [a]	Cary-Blair (Inoculated)	396	2/2 (100.0)	(15.8 - 100.0)	388/394 (98.5)	(96.7 - 99.4)
E. coli O157 [b]	Cary-Blair (Inoculated)	397	1/2 (50.0)	(1.3 - 98.7)	391/395 (99.0)	(97.4 - 99.7)
Enteroaggregative *E. coli* (EAEC) [c]	Cary-Blair (Inoculated)	396	12/14 (85.7)	(57.2 - 98.2)	382/382 (100.0)	(99.0 - 100.0)
Enterotoxigenic *E. coli* (ETEC) [d]	Cary-Blair (Inoculated)	396	4/6 (66.7)	(22.3 - 95.7)	386/390 (99.0)	(97.4 - 99.7)
Shiga toxin-producing *E. coli* (STEC)	Cary-Blair (Inoculated)	363	2/2 (100.0)	(15.8 - 100.0)	361/361 (100.0)	(99.0 - 100.0)
Salmonella spp [e]	Cary-Blair (Inoculated)	397	6/6 (100.0)	(54.1 - 100.0)	389/391 (99.5)	(98.2 - 99.9)
Shigella/ EIEC [f]	Cary-Blair (Inoculated)	397	1/1 (100.0)	(2.5 - 100.0)	391/396 (98.7)	(97.1 - 99.6)
Vibrio parahaemolyticus	Cary-Blair (Inoculated)	397	N/A	N/A	397/397 (100.0)	(99.1 - 100.0)
Vibrio spp. (not parahaemolyticus)	Cary-Blair (Inoculated)	397	N/A	N/A	397/397 (100.0)	(99.1 - 100.0)
Yersinia enterocolitica [g]	Cary-Blair (Inoculated)	397	N/A	N/A	396/397 (99.8)	(98.6 - 100.0)
Cryptosporidium spp	Cary-Blair (Inoculated)	396	2/2 (100.0)	(15.8 - 100.0)	394/394 (100.0)	(99.1 - 100.0)
Entamoeba histolytica	Cary-Blair (Inoculated)	396	N/A	N/A	396/396 (100.0)	(99.1 - 100.0)
Giardia lamblia [h]	Cary-Blair (Inoculated)	396	N/A	N/A	394/396 (99.5)	(98.2- 99.9)
Adenovirus 40/41 [i]	Cary-Blair (Inoculated)	396	2/6 (33.3)	(4.3 - 77.7)	385/390 (98.7)	(97.0 - 99.6)
Norovirus (GI/GII)	Cary-Blair (Inoculated)	397	28/28 (100.0)	(87.7 - 100.0)	364/369 (98.6)	(96.9 - 99.6)
Rotavirus A	Cary-Blair (Inoculated)	397	11/12 (91.7)	(61.5 - 99.8)	380/385 (98.7)	(97.0 - 99.6)

a – *Campylobacter* spp. The 7 false positives compared to the reference culture method were tested by bidirectional sequencing and 4 of 7 confirmed as positives.
b - *E. coli* O157. The one false negative compared to the reference culture method was tested by bidirectional sequencing and could not be confirmed as positive. The 4 false positives compared to reference culture method were tested by bidirectional sequencing and 3 of 4 confirmed as positives.
c - EAEC. The 2 false negatives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing; 1 of the 2 confirmed as positive. The 2 false positives compared to bidirectional sequencing were not confirmed as positive by 2 additional rounds of sequencing.
d - ETEC. The 2 false negatives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing; none were confirmed as positives. The 1 false positive compared to bidirectional sequencing was not available for confirmation testing.
e – *Salmonella* spp. The one false negative compared to the reference culture method was tested by bidirectional sequencing and confirmed as positive. The 4 false positives compared to the reference culture method were tested by bidirectional sequencing and 2 of 4 confirmed as positives.
f - *Shigella*/EIEC. The 5 false positives compared to the reference culture method were tested by bidirectional sequencing and 4 of 5 confirmed as positives.
g - *Yersinia enterocolitica*. The 1 false positive compared to the reference culture method were tested by bidirectional sequencing and confirmed as positive.
h - *Giardia lamblia*. The 3 false positives compared to bidirectional sequencing were not confirmed as positive by 2 additional rounds of sequencing.

i – Adenovirus 40/41. The 3 false negatives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing; none confirmed as positive.

Testing of Pre-selected Archived Specimens (Category III)

Several analytes were not encountered or had low prevalence in the clinical study. To supplement the results of the prospective clinical study, 260 preselected archived specimens were assayed. These were archived clinical specimens that were previously tested positive by different methods. Prior to testing with the Applied BioCode Gastrointestinal Pathogen Panel (GPP), the presence of the expected analyte was verified in each specimen using analyte-specific PCR followed by bi-directional sequencing performed at Applied BioCode, Inc. The specimens were randomized with negative specimens, such that the users performing the BioCode Gastrointestinal Pathogen Panel (GPP) were blinded to the expected test result. A summary of results of the BioCode Gastrointestinal Pathogen Panel (GPP) testing are presented in the tables below.

Table 34. Demographics of Pre-selected Archived Specimens

Prospective Study Specimens	
Total Specimens	260
Gender (n/N(%))	
Female	123/260 (47.3)
Male	137/260 (52.7)
Age Category (n/N(%))	
< 5 year	54/260 (20.8)
6-21 yrs	46/260 (17.7)
22-59 yrs	123/260 (47.3)
60+ yrs	37/260 (14.2)

Table 35. Summary of Clinical Specimen Results (Archived specimens)

	Positive Agreement		Negative Agreement	
Target	**Agreement n/N (%)**	**95% CI**	**Agreement n/N (%)**	**95% CI**
Campylobacter spp.	38/40 (95.0)	(83.1 - 99.4)	152/152 (100.0)	(97.6 - 100.0)
E.coli 0157	19/19 (100.0)	(82.4 - 100.0)	152/152 (100.0)	(97.5 - 100.0)
ETEC	20/20 (100.0)	(83.2 - 100.0)	152/152 (100.0)	(97.6 - 100.0)
STEC	30/33 (90.9)	(75.7 - 98.1)	152/152 (100.0)	(97.6 - 100.0)
Salmonella spp.	29/30 (96.7)	(82.8 - 99.9)	152/152 (100.0)	(97.6 - 100.0)
Shigella/ EIEC	43/45 (95.6)	(84.9 - 99.5)	151/152 (99.3)	(96.4 - 100.0)
Yersinia enterocolitica	3/3 (100.0)	(29.2 - 100.0)	152/152 (100.0)	(97.6 - 100.0)
Cryptosporidium spp.	16/19 (84.2)	(60.4 - 96.6)	152/152 (100.0)	(97.6 - 100.0)
Giardia lamblia	25/26 (96.2)	(83.2 - 99.9)	152/152 (100.0)	(97.6 - 100.0)
Adenovirus 40/41	26/26 (100.0)	(86.8 - 100.0)	151/152 (99.3)	(96.4 - 100.0)

Testing of Contrived Specimens (Category IV)

For some analytes both prospective and archived testing were insufficient to establish performance. To supplement the prospective and archived data, contrived specimens were assayed. The contrived specimens were positive for *Giardia, E. histolytica, Yersinia enterocolitica, Vibrio parahaemolyticus*, and *Vibrio* spp. These contrived clinical specimens were prepared using individual clinical specimens that had

previously tested negative for all GI panel analytes. Specimens were spiked at levels of up to 3X LOD (~50% of contrived specimens) or greater using multiple strains for each organism. Positive samples of each were prepared and randomized by mixing with negative samples before testing. A total of 612 samples, 485 positives, were tested. The results of the BioCode Gastrointestinal Pathogen Panel (GPP) testing are presented in the table below.

Table 36. Summary of contrived specimen results

	PPA		NPA	
Target	**Agreement Rate n/N (%)**	**95% CI**	**Agreement Rate n/N (%)**	**95% CI**
Vibrio parahaemolyticus	88/96 (91.7)	(84.2, 96.3)	516/516 (100.0)	(99.3, 100.0)
Vibrio spp. (not *parahaemolyticus*)	82/94 (87.2)	(78.8, 93.2)	518/518 (100.0)	(99.3, 100.0)
Vibrio cholerae	40/47 (85.1)	(72.3, 92.6)	518/518 (100.0)	(99.3, 100.0)
Vibrio vulnificus	42/47 (89.4)	(77.4, 95.4)	518/518 (100.0)	(99.3, 100.0)
Yersinia enterocolitica	95/98 (96.9)	(91.3, 99.4)	514/514 (100.0)	(99.3, 100.0)
Entamoeba histolytica	96/99 (97.1)	(91.4, 99.4)	507/513 (98.8)	(97.5, 99.6)
Giardia lamblia	94/98 (95.9)	(89.9, 98.9)	513/514 (99.8)	(98.9, 100.0)

Clinical Specificity – Microbial Detection in Asymptomatic Volunteers

In order to investigate baseline levels for each analyte included in the BioCode Gastrointestinal Pathogen Panel (GPP) in individuals who are not exhibiting signs and symptoms of infectious gastroenteritis, 125 clinical stool samples were collected from healthy asymptomatic donors. These are defined as donors not exhibiting signs and symptoms, or on antibiotics (for symptoms) during the previous 30 days. Asymptomatic donors from two sites and various age groups were included in this study and the demographic information for the donors is shown in the table below. PCR inhibition, as determined by results of the assay internal control (bacteriophage MS2), was observed for one sample (0.8%). After re-running this sample in accordance with the package insert instructions for use, inhibition was still observed, so no result was reported. Therefore, the final data analysis was conducted on 124 of 125 samples collected for this study. A total of 26 samples were positive. The results are summarized in the tables below.

Table 37. Demographic information for Asymptomatic Volunteers.

Gender	Number of Subjects
Male	67
Female	58
Total	125
Age	
<1-5	1
6-21	3
22-65	86
>65	35

Table 38. Detections in Asymptomatic Volunteers-Stratified by Age

Analyte	< 5 yrs	6-21 yrs	22-59 yrs	60+ yrs
All Negative	1 (100.0%)	3 (100.0%)	47 (77.1%)	46 (76.7%)
Single Infection	0 (0%)	0 (0%)	13 (21.3%)	14 (23.3%)
Co-Infections	0 (0%)	0 (0%)	1 (1.6%)	0 (0%)
Clostridium difficile	0 (0%)	0 (0%)	9 (14.8%)	11 (18.3%)
EAEC	0 (0%)	0 (0%)	0 (0.0%)	1 (1.7%)
ETEC	0 (0%)	0 (0%)	2 (3.3%)	0 (0%)
Salmonella spp.	0 (0%)	0 (0%)	0 (0%)	1 (1.7%)
Giardia lamblia	0 (0%)	0 (0%)	1 (1.6%)	0 (0%)
Norovirus GI/GII	0 (0%)	0 (0%)	0 (0%)	1 (1.7%)

N. Instrument Name:

BioCode Gastrointestinal Pathogen Panel (GPP)

O. System Descriptions:

1. Modes of Operation:

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

   Yes

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

   No

2. Software:

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

   Yes

3. Specimen Identification:

   Specimen identity is provided by barcode magnetic beads.

4. Specimen Sampling and Handling:

   After extraction with the easyMag system and loading samples into a 96well formatted plate, the BioCode MDx 3000 processes all RT-PCR steps automatically.

5. Calibration: Optical calibration of the MDx 3000 is performed twice a year by Applied BioCode. No calibration kit is available.

6. Quality Control:

   Each laboratory should establish its own Quality Control ranges and frequency of QC testing based on applicable local laws, regulations and good laboratory practices. The BioCode Gastrointestinal Pathogen Panel (GPP) uses an internal control (bacteriophage MS2) which is added to each sample during pre- treatment. The internal control monitors the efficiency of the extraction, reverse transcription, amplification and detection stages of the assay. Positive results may be reported in the absence of RNA IC detection. The BioCode Gastrointestinal Pathogen Panel (GPP) software will suppress negative results for any wells with invalid RNA IC results.

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

Not applicable.

Q. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

R. Conclusion:

Samples suspected of *C. difficile* presence should only be tested fresh. The instrument software has been updated to select for fresh or frozen samples and omit results of *C. difficile*.

The submitted information in this premarket notification is sufficient to support a substantial equivalence decision for the BioCode Gastrointestinal Pathogen Panel (GPP).