June 5, 2019

Applied Biocode, Inc.
Robert Tullio
Regulatory Consultant
10020 Pioneer Blvd.
Suite 102
Santa Fe Springs, California 90067-0

Re: K190585
Trade/Device Name: Biocode Gastrointestinal Pathogen Panel (GPP)
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal microorganism multiplex nucleic acid-based assay
Regulatory Class: Class II
Product Code: PCH, OOI
Dated: March 4, 2019
Received: March 6, 2019

Dear Robert Tullio:

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,

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

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K190585

Device Name
BioCode Gastrointestinal Pathogen Panel (GPP)

Indications for Use *(Describe)*

The BioCode Gastrointestinal Pathogen Panel (GPP) is a qualitative multiplexed nucleic acid-based in vitro diagnostic test intended for use with the BioCode MDx 3000 Instrument. The BioCode GPP is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites extracted directly from unpreserved stool samples or stool preserved in Cary-Blair transport medium obtained from individuals with signs and/or symptoms of gastrointestinal infection. The following bacteria, parasites, and viruses are identified using the BioCode Gastrointestinal Pathogen Panel:

- Campylobacter (C. jejuni/C. coli)
- Clostridium difficile (C. difficile) toxin A/B (Fresh samples only)
- 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 (C. parvum/C. hominis)
- Entamoeba histolytica
- Giardia lamblia (also known as G. intestinalis and G. duodenalis)
- Adenovirus F 40/41
- Norovirus GI/GII
- Rotavirus A

The BioCode 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 GPP. The agent detected may not be the definite cause of the disease. 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 Adenovirus 40/41, Campylobacter, E. coli O157, Shigella/EIEC, Yersinia enterocolitica, and Giardia lamblia were established additionally with retrospective clinical specimens. Performance characteristics for Entamoeba histolytica, Giardia lamblia, Yersinia enterocolitica and Vibrio (V. parahaemolyticus, V. vulnificus, and V. cholerae) were established primarily using contrived clinical specimens.

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

Introduction: According to the requirements of 21 CFR 807.92, the following provides sufficient information to understand the basis for a determination of substantial equivalence.

Submitted by:
Applied BioCode, Inc.
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Santa Fe Springs, CA 90670

Contact:
Robert Di Tullio
Regulatory Consultant
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Telephone: 310 801 1235
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Date Submitted:
March 5, 2019

Trade Name:
BioCode Gastrointestinal Pathogen Panel (GPP)

Classification Name and Regulation Number:
Gastrointestinal microorganism multiplex nucleic acid-based assay (21 CFR 866.3990)

Predicate Device:
K180041 – BioCode Gastrointestinal Pathogen Panel (GPP)

Intended Use:

The BioCode Gastrointestinal Pathogen Panel (GPP) is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with the BioCode MDx 3000 Instrument. The BioCode GPP is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites extracted directly from unpreserved stool samples or stool preserved in Cary-Blair transport medium obtained from individuals with signs and/or symptoms of gastrointestinal infection. The following bacteria, parasites, and viruses are identified using the BioCode Gastrointestinal Pathogen Panel:

- *Campylobacter* (*C. jejuni/C. coli*)
- *Clostridium difficile* (*C. difficile*) toxin A/B (Fresh samples only)
- *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 (*C. parvum/C. hominis*)
- *Entamoeba histolytica*
- *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*)
- Adenovirus F 40/41
- Norovirus GI/GII
- Rotavirus A

The BioCode 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 GPP. The agent detected may not be the definite cause of the disease. 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 Adenovirus 40/41, *Campylobacter*, *E. coli* O157, *Shigella*/EIEC, *Yersinia enterocolitica,* and *Giardia lamblia* were established additionally with retrospective clinical specimens. Performance characteristics for *Entamoeba histolytica, Giardia lamblia, Yersinia enterocolitica* and *Vibrio* (*V. parahaemolyticus, V. vulnificus,* and *V. cholerae*) were established primarily using contrived clinical specimens.

Device Description:

The BioCode Gastrointestinal Pathogen Panel (GPP) is a multiplexed nucleic acid-based test designed to be used with the BioCode MDx 3000 system. The BioCode MDx 3000 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. Stool specimens are processed and nucleic acids extracted with the easyMAG and MagNa Pure. Once the PCR plate is set up and sealed, all other operations are automated on MDx 3000. The BioCode MDx 3000 Gastrointestinal Infection Panel simultaneously tests for 17 pathogens (see table below) from unpreserved stool specimens or stool collected in Cary-Blair transport medium. Results from the BioCode Gastrointestinal Pathogen Panel (GPP) test are available within less than 4 hours.

Bacteria, Viruses, Diarrheagenic *E. coli/Shigella*, and Parasites Detected by the BioCode MDx Gastrointestinal Pathogen Panel (GPP)

Bacteria	Parasites
▪ *Campylobacter* spp. (*C. jejuni, C. coli*) ▪ *Clostridium difficile* toxin A/B (Fresh samples only) ▪ Enteroaggregative *E. coli* (EAEC) ▪ Enterotoxigenic *E. coli* (ETEC): LT/ST ▪ Shiga-toxin producing *E. coli* (STEC): stx1/stx2 ▪ *E.coli* O157 ▪ *Shigella* spp. /Enteroinvasive *E.coli* (EIEC) ▪ *Salmonella* spp. ▪ *Vibrio parahaemolyticus* ▪ *Vibrio* spp *(*not *parahaemolyticus)* ▪ *Yersinia enterocolitica*	▪ *Cryptosporidium* spp. ▪ *Entamoeba histolytica* ▪ *Giardia lamblia*
	Viruses
	▪ Adenovirus 40/41 ▪ Norovirus GI/GII ▪ Rotavirus A
	RNA Internal Control

Device Comparison:

Comparison of the Applied BioCode GPP with the Predicate Device

Characteristic	Proposed Device	Predicate
Name	BioCode Gastrointestinal Pathogen Panel (GPP)	BioCode Gastrointestinal Pathogen Panel (GPP)
Common Name	Gastrointestinal Microorganism Multiplex Nucleic acid-based assay	Gastrointestinal Microorganism Multiplex Nucleic acid-based assay
510(k) No.	N/A	K180041
Regulation	21CFR 866.3990	21CFR 866.3990
Product Code	PCH, OOI	PCH, OOI
Device Class	II	II
Similarities		

Intended Use	The BioCode Gastrointestinal Pathogen Panel (GPP) is a qualitative, multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with the BioCode MDx 3000 Instrument. The BioCode GPP is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites extracted directly from unpreserved stool samples or stool preserved in Cary-Blair transport medium obtained from individuals with signs and/or symptoms of gastrointestinal infection. The following bacteria, parasites, and viruses are identified using the BioCode Gastrointestinal Pathogen Panel: ▪ *Campylobacter* (*C. jejuni/C. coli*) ▪ *Clostridium difficile* (*C. difficile*) toxin A/B (Fresh samples only) ▪ *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 (*C. parvum/C. hominis*) ▪ *Entamoeba histolytica* ▪ *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*) ▪ Adenovirus F 40/41 ▪ Norovirus GI/GII ▪ Rotavirus A The BioCode 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. For *In Vitro* Diagnostic Use Only. For Prescription Use Only. Positive results do not rule out co-infection with organisms not included in the BioCode GPP. The agent detected may not be the definite cause of the disease. 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.	Same

Characteristic	Proposed Device	Predicate
Instrument	Nucleic Acid Purification System BioCode MDx 3000	Same
Sample Type	Unpreserved stool and stool in Cary Blair Medium	Same
Controls	Externally Sourced - Zeptometrix	Same
Methodology	Multiplex RT-PCR and probe hybridization to biotinylated PCR product(s) followed by fluorescence detection and decoding of barcoded magnetic beads (BMB) that are coupled to target-specific probes	Same
Calibrators	Internal Calibration	Same
	Difference	
Sample Extraction	Roche MagNA Pure 96	easyMAG

Summary of Performance Characteristics of the Biocode GPP.

Clinical Performance

A clinical investigational study was performed in which a total of 466 leftover, de-identified samples (275 frozen unpreserved and 191 inoculated Cary-Blair) that were prospectively collected for the clinical study that resulted in the K180041 BioCode GPP clearance were extracted using the MagNA Pure 96 and the easyMAG, and tested on the MDx 3000 system. Fifty-three (53) freshly collected leftover samples were used for the *C. difficile* testing. In addition, a total of 120 samples were contrived and tested to determine the performance characteristics for *Entamoeba histolytica, Yersinia enterocolitica* and *Vibrio* (*V. parahaemolyticus, V. vulnificus,* and *V. cholerae*). The results of the clinical investigational study follow.

Table. Demographic data for archived specimens (frozen unpreserved and inoculated Cary-Blair)

Archived Samples	
Total Specimen Count	466
Gender	
Male	248/466 (53.22%)
Female	211/466 (45.28%)
Unknown	7/466 (1.50%)
Age Category	
≤ 5 yrs	85/466 (18.24%)
6-21 yrs	91/466 (19.53%)
22-59 yrs	194/466 (41.63%)
60+yrs	89/466 (19.10%)
Unknown	7/466 (1.50%)

Table. Demographic data for fresh specimens (unpreserved)

Fresh Samples	
Total Specimen Count	53
Gender	
Male	9/53 (16.98%)
Female	44/53 (83.02%)
Age Category	
≤ 5 yrs	1/53 (1.89%)
6-21 yrs	5/53 (9.43%)
22-59 yrs	23/53 (43.40%)
60+yrs	24/53 (45.28%)

Clinical sensitivity (positive agreement) was calculated as TP/(TP + FN). TP = true positive or positive by both the EasyMag and the MagNA Pure 96; FN = false negative or negative by the MagNA Pure 96 only. Clinical specificity (negative agreement) was calculated as TN/(TN + FP). TN = true negative or negative by the EasyMag and the MagNA Pure 96; FP = false positive or positive by the MagNA Pure 96 only. The exact binomial two-sided 95% confidence interval was calculated. The results stratified by sample type and storage method are presented in the table below.

Table. Performance compared to historical Reference results. Stratified by sample type and extraction method. N=290 samples. 1 Invalid for EasyMAG (Cary-Blair sample); 3 Invalid for MP96 (1 Cary-Blair, 2 Unpreserved).

		EasyMAG		MP96	
Target	**Specimen Type**	**Positive Agreement**	**Negative Agreement**	**Positive Agreement**	**Negative Agreement**
		PPA (%)	**NPA (%)**	**PPA (%)**	**NPA (%)**
Campylobacter spp.	Inoculated Cary-Blair	3/3 (100%)	135/140 (96%)	3/3 (100%)	134/140 (96%)
	Unpreserved	1/1 (100%)	139/145 (96%)	1/1 (100%)	135/143 (94%)
	All Archived	4/4 (100%)	274/285 (96%)	4/4 (100%)	269/283 (95%)
Clostridium difficile	Inoculated Cary-Blair	9/12 (75%)	131/131 (100%)	9/12 (75%)	131/131 (100%)
	Unpreserved	12/13 (92%)	131/133 (98%)	12/13 (92%)	127/131 (97%)
	All Archived	21/25 (84%)	262/264 (99%)	21/25 (84%)	258/262 (98%)
E. coli O157	Inoculated Cary-Blair	1/1 (100%)	141/142 (99%)	0/1 (N/A)	141/142 (99%)
	Unpreserved	0/0 (N/A)	144/146 (99%)	0/0 (N/A)	142/144 (99%)
	All Archived	1/1 (100%)	285/288 (99%)	0/1 (N/A)	283/286 (99%)
Enteroaggregative *E. coli* (EAEC)	Inoculated Cary-Blair	14/14 (100%)	128/129 (99%)	13/14 (93%)	127/129 (98%)
	Unpreserved	13/14 (93%)	131/132 (99%)	13/14 (93%)	130/131 (99%)

		EasyMAG		MP96	
Target	**Specimen Type**	**Positive Agreement**	**Negative Agreement**	**Positive Agreement**	**Negative Agreement**
		PPA (%)	**NPA (%)**	**PPA (%)**	**NPA (%)**
	All Archived	27/28 (96%)	259/261 (99%)	26/28 (93%)	257/260 (99%)
Enterotoxigenic E. coli (ETEC)	Inoculated Cary-Blair	3/6 (50%)	137/137 (100%)	3/6 (50%)	137/137 (100%)
	Unpreserved	6/7 (86%)	139/139 (100%)	6/7 (86%)	138/138 (100%)
	All Archived	9/13 (69%)	276/276 (100%)	9/13 (69%)	275/275 (100%)
Shiga toxin-producing *E. coli* (STEC)	Inoculated Cary-Blair	1/3 (33%)	139/140 (99%)	1/3 (33%)	139/140 (99%)
	Unpreserved	0/0 (N/A)	144/146 (99%)	0/0 (N/A)	142/144 (99%)
	All Archived	1/3 (33%)	283/286 (99%)	1/3 (33%)	281/284 (99%)
Salmonella spp.	Inoculated Cary-Blair	8/10 (80%)	129/133 (97%)	9/10 (90%)	126/133 (95%)
	Unpreserved	10/10 (100%)	132/136 (97%)	10/10 (100%)	131/134 (98%)
	All Archived	18/20 (90%)	261/269 (97%)	19/20 (95%)	257/267 (96%)
Shigella/ EIEC	Inoculated Cary-Blair	3/3 (100%)	134/140 (96%)	3/3 (100%)	134/140 (96%)
	Unpreserved	2/2 (100%)	136/144 (94%)	2/2 (100%)	135/142 (95%)
	All Archived	5/5 (100%)	270/284 (95%)	5/5 (100%)	269/282 (95%)
Vibrio parahaemolyticus	Inoculated Cary-Blair	0/0 (N/A)	142/143 (99%)	0/0 (N/A)	142/143 (99%)
	Unpreserved	0/0 (N/A)	145/146 (99%)	0/0 (N/A)	143/144 (99%)
	All Archived	0/0 (N/A)	287/289 (99%)	0/0 (N/A)	285/287 (99%)
Vibrio spp. (not *parahaemolyticus*)	Inoculated Cary-Blair	0/0 (N/A)	143/143 (100%)	0/0 (N/A)	143/143 (100%)
	Unpreserved	0/0 (N/A)	146/146 (100%)	0/0 (N/A)	144/144 (100%)
	All Archived	0/0 (N/A)	289/289 (100%)	0/0 (N/A)	287/287 (100%)
Yersinia enterocolitica	Inoculated Cary-Blair	0/0 (N/A)	141/143 (99%)	0/0 (N/A)	141/143 (99%)
	Unpreserved	0/0 (N/A)	146/146 (100%)	0/0 (N/A)	144/144 (100%)
	All Archived	0/0 (N/A)	287/289 (99%)	0/0 (N/A)	285/287 (99%)
Cryptosporidium spp.	Inoculated Cary-Blair	3/4 (75%)	139/139 (100%)	2/4 (50%)	139/139 (100%)
	Unpreserved	3/4 (75%)	142/142 (100%)	3/4 (75%)	140/140 (100%)
	All Archived	6/8 (75%)	281/281 (100%)	5/8 (63%)	279/279 (100%)
Entamoeba histolytica	Inoculated Cary-Blair	0/0 (N/A)	143/143 (100%)	0/0 (N/A)	143/143 (100%)

Target	Specimen Type	EasyMAG		MP96	
		Positive Agreement	Negative Agreement	Positive Agreement	Negative Agreement
		PPA (%)	NPA (%)	PPA (%)	NPA (%)
	Unpreserved	0/0 (N/A)	146/146 (100%)	0/0 (N/A)	144/144 (100%)
	All Archived	0/0 (N/A)	289/289 (100%)	0/0 (N/A)	287/287 (100%)
Giardia lamblia	Inoculated Cary-Blair	1/1 (100%)	141/142 (99%)	1/1 (100%)	141/142 (99%)
	Unpreserved	1/1 (100%)	142/145 (98%)	1/1 (100%)	140/143 (98%)
	All Archived	2/2 (100%)	283/287 (99%)	2/2 (100%)	281/285 (99%)
Adenovirus 40/41	Inoculated Cary-Blair	1/2 (50%)	142/142 (100%)	1/2 (50%)	138/141 (98%)
	Unpreserved	5/6 (83%)	137/140 (98%)	5/6 (83%)	134/138 (97%)
	All Archived	6/8 (75%)	279/282 (99%)	6/8 (75%)	272/279 (97%)
Norovirus (GI/GII)	Inoculated Cary-Blair	19/19 (100%)	124/124 (100%)	19/19 (100%)	122/124 (98%)
	Unpreserved	19/19 (100%)	125/127 (98%)	19/19 (100%)	124/125 (99%)
	All Archived	38/38 (100%)	249/251 (99%)	38/38 (100%)	246/249 (99%)
Rotavirus A	Inoculated Cary-Blair	11/11 (100%)	130/132 (98%)	11/11 (100%)	132/133 (99%)
	Unpreserved	9/9 (100%)	135/137 (99%)	9/9 (100%)	130/135 (96%)
	All Archived	20/20 (100%)	264/269 (99%)	20/20 (100%)	262/268 (98%)

Footnotes (D = Detected, N = Not Detected)

		Historical Results (K180041)		MP96 Extraction (K190585)		
Target	Specimen ID	Reference	BioCode GPP	easy MAG	MP 96	Comment
Campylobacter spp	01-0027	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq C. jejuni, but contig too short; concordant with historical GPP result
	01-0048	N	D	D	D	Reference assay (culture) not as sensitive, PCR/Seq C. jejuni; concordant with historical GPP result
	01-0137	N	D	D	D	Reference assay (culture) not as sensitive, PCR/Seq C. jejuni; concordant with historical GPP result
	01-0237	N	D	D	D	Reference assay (culture) not as sensitive, PCR/Seq C. jejuni; concordant with historical GPP result
	01-0352	N	D	D	D	Reference assay (culture) not as sensitive, PCR/Seq C. jejuni; concordant with historical GPP result
	02-0159	N	D	D	D	Reference assay (culture) not as sensitive, PCR/Seq C. jejuni; concordant with historical GPP result
	02-0183	N	D	D	D	Reference assay (culture) not as sensitive, PCR/Seq C. jejuni; concordant with historical GPP result
	02-0367	N	D	D	D	Reference assay (culture) not as sensitive, PCR/Seq not detected, concordant with historical GPP result
	03-0153	N	D	D	D	Reference assay (culture) not as sensitive, PCR/Seq not detected, concordant with historical GPP result

		Historical Results (K180041)		MP96 Extraction (K190585)		
Target	Specimen ID	Reference	BioCode GPP	easy MAG	MP 96	Comment
	03-0308	N	D	D	D	Reference assay (culture) not as sensitive, PCR/Seq C. jejuni; concordant with historical GPP result
	03-0334	N	D	D	D	Reference assay (culture) not as sensitive, PCR/Seq C. jejuni; concordant with historical GPP result
	02-0069	N	D	N	D	Reference assay (culture) not as sensitive, PCR/Seq C. jejuni; MP96 concordant with historical GPP result
	02-0193	N	N	N	D	Possible Contamination; see repeat results for additional information
	03-0364	N	N	N	D	Possible Contamination; see repeat results for additional information
Clostridium difficile	01-0031	N	D	D	D	Reference Ct beyond cut-off (38.7); concordant with historical GPP Result
	01-0063	N	D	D	D	Reference Ct beyond cut-off with ref (38.3); concordant with historical GPP Result
	02-0201	D	N	D	N	Late Ct with ref (36.6) tested fresh, low positive; see repeat results for additional informations
	02-0350	N	N	N	D	Possible Contamination; see repeat results for additional information
	03-0053	D	D	N	D	Late Ct with ref (36.2) tested fresh; low positive; see repeat results for additional informations
	03-0351	N	N	N	D	Possible Contamination; see repeat results for additional information
	01-0002	D	N	N	N	Late Ct with ref (36.6) tested fresh; concordant with historical GPP Result (frozen)
	02-0378	D	N	N	N	Late Ct with ref (36.6) tested fresh; concordant with historical GPP Result (fresh)
	03-0353	D	D	N	N	Late Ct with ref (35.5) tested frozen; possible sample degradation
E. coli O157	02-0080	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq O157; concordant with historical GPP result
	02-0082	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq O157; concordant with historical GPP result
	02-0206	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq O157; concordant with historical GPP result
	02-0279	D	D	N	N	Possible sample degradation; current testing concordant
Enteroaggregative *E. coli* (EAEC)	03-0140	N	D	D	D	Late Ct with ref (37.1) on repeat testing; concordant with historical GPP Result
	02-0183	N	N	D	N	Possible low positive; see repeat results for additional information
	03-0004	D	D	D	N	Possible low positive; see repeat results for additional information
	02-0367	N	N	N	D	Possible Contamination; see repeat results for additional information
	03-0046	N	N	N	D	Possible Contamination; see repeat results for additional information
	02-0203	D	N	N	N	Reference result could not be confirmed by 2 additional rounds of PCR/Seq; concordant with historical GPP Result
Enterotoxigenic *E. coli* (ETEC)	02-0203	D	N	N	N	Reference result could not be confirmed by 2 additional rounds of PCR/Seq; concordant with historical GPP Result
	02-0207	D	N	N	N	Late Ct (35.2) Reference result could not be confirmed by 2 additional rounds of PCR/Seq; concordant with historical GPP Result
	03-0004	D	N	N	N	Reference result could not be confirmed by 2 additional rounds of PCR/Seq; concordant with historical GPP Result
	02-0391	D	D	N	N	Late Ct (37.8) with ref tested fresh; possible sample degradation
Salmonella spp.	01-0091	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq S.enterica; concordant with historical GPP result

		Historical Results (K180041)		MP96 Extraction (K190585)		
Target	Specimen ID	Reference	BioCode GPP	easy MAG	MP 96	Comment
	01-0137	N	N	D	N	Possible Contamination; see repeat results
	01-0278	D	D	N	D	See repeat results for additional information
	01-0346	D	D	N	N	Possible sample degradation; current testing concordant
	02-0195	N	N	N	D	Possible Contamination; see repeat results for additional information
	02-0213	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq S.enterica; concordant with historical GPP result
	02-0260	N	N	N	D	Possible Contamination; see repeat results for additional information
	02-0378	N	N	N	D	Possible Contamination; see repeat results for additional information
	03-0116	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq S.enterica; concordant with historical GPP result
	03-0147	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq S.enterica; concordant with historical GPP result
	03-0173	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq S.typhi; concordant with historical GPP result
	03-0189	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq S.enterica; concordant with historical GPP result
	03-0206	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq S.typhi; concordant with historical GPP result
Shiga toxin-producing *E. coli* (STEC)	01-0098	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq STEC; concordant with historical GPP result
	02-0367	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq STEC; concordant with historical GPP result
	02-0393	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq STEC; concordant with historical GPP result
	02-0279	D	D	D	N	See repeat results for additional information
	02-0081	D	D	N	N	Possible sample degradation; current testing concordant
Shigella/EIEC	01-0019	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq Shigella spp; concordant with historical GPP result
	01-0020	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq Shigella spp; concordant with historical GPP result
	01-0027	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq Shigella spp; concordant with historical GPP result
	01-0099	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq Shigella spp; concordant with historical GPP result
	01-0199	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq Shigella spp; concordant with historical GPP result
	01-0201	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq Shigella spp; concordant with historical GPP result
	01-0202	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq Shigella spp; concordant with historical GPP result
	01-0259	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq Shigella spp; concordant with historical GPP result
	02-0176	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq Shigella spp; concordant with historical GPP result
	03-0056	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq Shigella spp; concordant with historical GPP result
	03-0090	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq Shigella spp; concordant with historical GPP result
	03-0238	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq Shigella spp; concordant with historical GPP result

		Historical Results (K180041)		MP96 Extraction (K190585)		
Target	Specimen ID	Reference	BioCode GPP	easy MAG	MP 96	Comment
	03-0380	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq Shigella spp; concordant with historical GPP result
	01-0319	N	N	D	N	Possible low positive; see repeat results for additional information
Vibrio parahaemolyticus	03-0194	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq V.parahaemolyticus; concordant with historical GPP result
	03-0351	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq V.parahaemolyticus; concordant with historical GPP result
Yersinia enterocolitica	01-0024	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq V.parahaemolyticus; concordant with historical GPP result
	03-0307	N	D	D	D	Reference assay (culture) not as sensitive, PCR/seq not detected; concordant with historical GPP result
Cryptosporidium parvum	01-0212	D	D	D	N	See repeat results for additional information
	01-0265	D	D	N	N	Possible sample degradation; current testing concordant
	01-0285	D	D	N	N	Possible sample degradation; current testing concordant
Giardia intestinalis	01-0002	N	D	D	D	Reference assay (PCR/Seq) not as sensitive; concordant with historical GPP result
	01-0300	N	D	D	D	Reference assay (PCR/Seq) not as sensitive; concordant with historical GPP result
	02-0258	N	D	D	D	Reference assay (PCR/Seq) not as sensitive; concordant with historical GPP result
	03-0140	N	D	D	D	Reference assay (PCR/Seq) not as sensitive; concordant with historical GPP result
Human adenovirus 40/41	01-0128	N	N	D	D	concordant with historical GPP result
	01-0021	N	N	D	N	Possible Contamination; see repeat results for additional information
	02-0288	N	N	D	N	Possible Contamination; see repeat results for additional information
	01-0137	N	N	N	D	Possible Contamination; see repeat results for additional information
	02-0161	N	N	N	D	Possible Contamination; see repeat results for additional information
	02-0183	N	N	N	D	Possible Contamination; see repeat results for additional information
	02-0379	N	N	N	D	Possible Contamination; see repeat results for additional information
	02-0391	N	N	N	D	Possible Contamination; see repeat results for additional information
	03-0400	N	N	N	D	Possible Contamination; see repeat results for additional information
	02-0137	D	N	N	N	Reference result could not be confirmed by 2 additional rounds of PCR/Seq; concordant with historical GPP Result
	02-0147	D	D	N	N	Possible sample degradation; current testing concordant
Norovirus GI/GII	01-0004	N	D	D	D	concordant with historical GPP result
	01-0036	N	N	D	N	Low positive based on MFI (3150)
	02-0376	N	N	N	D	Low positive based on MFI (1265)
	02-0394	N	N	N	D	Low positive based on MFI (2249)
Human rotavirus A	01-0128	N	N	D	D	Current testing concordant

		Historical Results (K180041)		MP96 Extraction (K190585)		
Target	Specimen ID	Reference	BioCode GPP	easy MAG	MP 96	Comment
	01-0337	N	D	D	D	concordant with historical GPP result
	02-0175	N	D	D	D	concordant with historical GPP result
	02-0246	N	D	D	N	Possible low positive; see repeat results for additional information
	01-0098	N	D	N	D	Possible low positive; see repeat results for additional information
	01-0202	N	N	N	D	Possible Contamination/low positive; see repeat results for additional information
	03-0400	N	N	N	D	Possible Contamination; see repeat results for additional information

Comparator (Reference) methods from BioCode GPP original submission

Target Pathogen/Toxin	**Reference Method**
Adenovirus 40/41	Composite result of PCR/sequencing
Campylobacter (C. *jejuni*, C. *coli*)	Culture
Clostridium difficile (C. *difficile*) toxin A/B	FDA cleared NAT
Cryptosporidium (C. *parvum*, C. *hominis*)	PCR/sequencing
Entamoeba histolytica	PCR/sequencing
Escherichia coli (E. *coli*) O157	Enrichment culture
Enteropathogenic E. *coli* (EPEC)	Composite result of PCR/sequencing
Enterotoxigenic E. *coli* (ETEC) LT/ST	Composite result of PCR/sequencing
Enteroaggregative E. *coli* (EAEC)	Composite result of PCR/sequencing
Giardia lamblia /*intestinalis*	Composite result of PCR/sequencing
Norovirus GI/GII	Composite result of PCR/sequencing
Rotavirus A	Composite result of PCR/sequencing
Salmonella	Enrichment culture
Shiga-like Toxin producing *E. coli* (STEC) stx1/stx2	Enrichment culture/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. Summary of Clinical Investigational Study Results (Archived Specimens) stratified by sample type and storage

Target	**Specimen Type**	**(n)**	**Positive Agreement**		**Negative Agreement**	
			PPA (%)	**95% CI**	**NPA (%)**	**95% CI**
Campylobacter spp.[a]	Inoculated Cary-Blair	190	22/23 (95.65)	79.0 – 99.2	164/167 (98.20)	94.9 – 99.4
	Unpreserved (Frozen)	274	27/27 (100)	87.5 - 100	244/247 (98.80)	96.5 – 99.6
	All Archived	464	49/50 (98.0)	89.5 – 99.6	408/414 (98.60)	96.9 – 99.3
Clostridium difficile[b]	Inoculated Cary-Blair	190	10/11 (90.91)	62.3 – 98.4	178/179 (99.44)	96.9 – 99.9
	Unpreserved (Frozen)	274	21/22 (95.45)	78.2 – 99.2	250/252 (99.20)	97.2 – 99.8

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
	All Archived	464	31/33 (93.94)	80.4 – 98.3	428/431 (99.30)	98.0 – 99.8
E. coli O157	Inoculated Cary-Blair	190	3/3 (100)	43.9 - 100	187/187 (100)	98.0 - 100
	Unpreserved (Frozen)	274	14/14 (100)	78.5 - 100	260/260 (100)	98.5 - 100
	All Archived	464	17/17 (100)	81.6 - 100	447/447 (100)	99.1 - 100
Enteroaggregative *E. coli* (EAEC) [c]	Inoculated Cary-Blair	190	15/17 (88.24)	65.7 – 96.7	171/173 (98.8)	95.9 – 99.7
	Unpreserved (Frozen)	274	29/29 (100)	88.3 - 100	244/245 (99.59)	97.7 – 99.9
	All Archived	464	44/46 (95.65)	85.5 – 98.8	416/418 (99.50)	98.3 -99.9
Enterotoxigenic *E. coli* (ETEC) [d]	Inoculated Cary-Blair	190	3/5 (60.00)	23.1 – 88.2	185/185 (100)	98.0 - 100
	Unpreserved (Frozen)	274	13/13 (100)	77.2 - 100	261/261 (100)	98.5 - 100
	All Archived	464	16/18 (88.89)	67.2 – 96.9	446/446 (100)	99.1 - 100
Shiga toxin-producing *E. coli* (STEC) [e]	Inoculated Cary-Blair	190	12/13 (92.31)	66.7 – 98.6	177/177 (100)	97.9 - 100
	Unpreserved (Frozen)	274	29/30 (96.67)	83.3 – 99.4	243/244 (99.60)	97.7 – 99.9
	All Archived	464	41/43 (95.35)	84.5 – 98.7	420/421 (99.80)	98.7 - 100
Salmonella spp.[f]	Inoculated Cary-Blair	190	17/17 (100)	81.6 - 100	169/173 (97.70)	94.2 – 99.1
	Unpreserved (Fresh)	274	25/27 (92.59)	76.6 – 97.9	246/247 (99.60)	97.7 – 99.9
	All Archived	464	42/44 (95.45)	84.9 – 98.7	415/420 (98.80)	97.2 – 99.5
Shigella/ EIEC [g]	Inoculated Cary-Blair	190	9/9 (100)	70.1 - 100	181/181 (100)	97.9 - 100
	Unpreserved (Frozen)	274	20/22 (90.91)	72.2 – 97.5	252/252 (100)	98.5 - 100
	All Archived	464	29/31 (93.55)	79.3 – 98.2	433/433 (100)	99.1 - 100
Vibrio parahaemolyticus [h]	Inoculated Cary-Blair	190	1/1 (100)	20.7 - 100	189/189 (100)	98.0 - 100
	Unpreserved (Frozen)	274	1/1 (100)	20.7 - 100	272/273 (99.6)	98.0 – 99.9
	All Archived	464	2/2 (100)	34.2 – 100	461/462 (99.8)	99.8 - 100
Vibrio spp. (not *parahaemolyticus*)[i]	Inoculated Cary-Blair	190	N/A	N/A	190/190 (100)	98.0 - 100
	Unpreserved (Frozen)	274	0/1 (0%)	N/A	273/274 (99.6)	98.0 – 99.6
	All Archived	464	0/1 (0%)	N/A	463/464 (99.8)	98.8 - 100
Yersinia enterocolitica [j]	Inoculated Cary-Blair	190	3/3 (100)	43.9 - 100	187/187 (100)	98.0 - 100
	Unpreserved (Frozen)	274	3/3 (100)	43.9 - 100	269/271 (99.26)	97.3 – 99.8
	All Archived	464	6/6 (100)	61.0 – 100	456/458 (99.6)	98.4- 99.9

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Cryptosporidium spp. [k]	Inoculated Cary-Blair	190	11/12 (91.67)	64.6 – 98.5	178/178 (100)	97.9 - 100
	Unpreserved (Frozen)	274	22/24 (91.67)	74.2 – 97.7	248/250 (99.20)	97.1 – 99.8
	All Archived	464	33/36 (91.67)	78.2 – 97.1	426/428 (99.5)	98.3 – 99.9
Entamoeba histolytica	Inoculated Cary-Blair	190	N/A	N/A	190/190 (100)	98.0 - 100
	Unpreserved (Frozen)	274	N/A	N/A	274/274 (100)	98.6 - 100
	All Archived	464	N/A	N/A	464/464 (100)	99.2 - 100
Giardia lamblia [l]	Inoculated Cary-Blair	190	3/3 (100)	43.90 - 100	187/187 (100)	98.0 - 100
	Unpreserved (Frozen)	274	14/14 (100)	78.5 - 100	255/260 (98.1)	95.6 – 99.2
	All Archived	464	17/17 (100)	81.6 - 100	442/447 (98.9)	97.4 – 99.5
Adenovirus 40/41 [m]	Inoculated Cary-Blair	190	7/10 (70.00)	39.7 – 89.2	177/180 (98.32)	95.2 – 99.4
	Unpreserved (Frozen)	274	11/14 (78.60)	52.4 – 92.4	252/260 (96.90)	94.0 – 98.4
	All Archived	464	21/24 (87.50)	69.0 – 95.7	429/440 (97.5)	95.6 – 98.6
Norovirus (GI/GII) [n]	Inoculated Cary-Blair	190	19/19 (100)	83.2 - 100	168/171 (98.20)	95.0 – 99.4
	Unpreserved (Frozen)	274	21/22 (95.45)	78.2 – 99.2	248/252 (98.40)	96.0 – 99.4
	All Archived	464	37/41 (90.24)	77.5 – 96.1	416/423 (98.3)	96.6 – 99.2
Rotavirus A [o]	Inoculated Cary-Blair	190	12/13 (92.31)	66.7 – 98.6	176/177 (99.44)	96.9 – 99.9
	Unpreserved (Frozen)	274	15/15 (100)	79.6 - 100	255/259 (98.5)	96.1 – 99.4
	All Archived	464	27/28 (96.43)	82.3 – 99.4	431/436 (98.9)	97.3 – 99.5

Sixty-four (64) archived samples with discordant results were retested twice with both easyMag and/or MagNA Pure 96 systems.

a - *Campylobacter* spp. One (1) false negative retested became true positive. Of the 5 false positives retested, 2 were true negative, one became true positive, two remained false positive. One false positive not retested due to insufficient volume to retest.

b - *Clostridium difficile*: Of the 2 false negatives retested, 1 became true positive, the other one was true negative. Of the 3 false positives retested, 2 were true negative and 1 was true positive.

c - EAEC: Of the 2 false negatives retested, 1 was false positive and 1 remained false negative. Of the 3 false positives retested, 2 were true negative and 1 was true positive.

d - ETEC: Two (2) false negatives tested were true negative.

e - STEC: Of 3 false positives retested, 2 were true positive and 1 was true negative.

f - *Salmonella* spp. Of the 2 false negatives retested, one was false positive and the other one was true negative. Of the 5 false positives retested, 4 were true negative and 1 was true positive.

g - *Shigella*/EIEC: Of the 2 false negatives retested, 1 was true positive and the other remained false negative.

h - *Vibrio parahaemolyticus*: The 1 false positive retested was true negative.

i - *Vibrio spp*. The 1 false negative retested remained false negative. Detected as *Vibrio parahaemolyticus* by MP96.

j - *Yersinia enterocolitica*: Two (2) false positives retested were true negative.

k - *Cryptosporidium spp*: Of the 3 false negatives retested, 1 was true negative and 2 were false negative. The 2 false positives retested remained false positive.

l - *Giardia lamblia*: Of the 5 false positives retested, 1 became true positive, 2 were true negative. The remaining 2 were not retested due insufficient volume to retest.
m- Adenovirus 40/41: All 6 false negatives retested were true negatives. Of the 11 false positives, 9 were retested and became true negative, and the remaining 2 were not retested due insufficient volume to retest.
n - Norovirus G1/G2: The 1 false negative retested was true negative. Of the 7 false positives retested, 6 were true negative and one became true positive.
o - Rotavirus: The 1 false negative retested was true negative. Of the 5 false positives retested, 1 was true positive, 3 were true negative, and 1 remained false positive.

Table. Results of Reflex Testing of Invalid Samples (Archived Samples)

Target	Sample Name	Sample Type	Extraction	Original Run	Reflex Testing Result	Final Result
Internal Control (MS2)	01-0079	Inoculated Cary-Blair	EasyMag	IC Invalid	IC Valid	Valid
	01-0073	Inoculated Cary-Blair	MagNA Pure	IC Invalid	IC Invalid	Invalid
	02-0211	Unpreserved	MagNa Pure	IC Invalid	IC Invalid	Invalid
	02-0228	Unpreserved	EasyMag	IC Invalid	IC Valid	Valid
	Mayo-G1_124	Unpreserved	MagNA Pure	IC Invalid	IC Valid	Valid

Note. Five (5) archived samples were invalid. After relflex testing, two samples (1 inoculated Cary-Blair and 1 unpreserved) were still invalid.

Table. Summary of Clinical Investigational Study Results of Fresh Specimens

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Campylobacter spp.	Unpreserved (Fresh)	47	N/A	N/A	47/47(100)	92.4– 100
Clostridium difficile	Unpreserved (Fresh)	52	30/30 (100)	88.6 -100	22/22 (100)	85.1 – 100
E. coli O157	Unpreserved (Fresh)	47	N/A	N/A	47/47(100)	92.4– 100
Enteroaggregative *E. coli* (EAEC)	Unpreserved (Fresh)	47	N/A	N/A	47/47(100)	92.4– 100
Enterotoxigenic *E. coli* (ETEC)	Unpreserved (Fresh)	47	N/A	N/A	47/47(100)	92.4– 100
Shiga toxin-producing *E. coli* (STEC)	Unpreserved (Fresh)	47	N/A	N/A	47/47(100)	92.4– 100
Salmonella spp.	Unpreserved (Fresh)	47	1/1 (100)	20.7 -100	46/46 (100)	92.3– 100
Shigella/ EIEC	Unpreserved (Fresh)	47	1/1 (100)	20.7 -100	46/46 (100)	92.3– 100
Vibrio parahaemolyticus	Unpreserved (Fresh)	47	N/A	N/A	47/47(100)	92.4– 100
Vibrio spp. (not *parahaemolyticus*)	Unpreserved (Fresh)	47	N/A	N/A	47/47(100)	92.4– 100
Yersinia enterocolitica	Unpreserved (Fresh)	47	N/A	N/A	47/47(100)	92.4– 100

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Cryptosporidium spp.	Unpreserved (Fresh)	47	N/A	N/A	47/47(100)	92.4– 100
Entamoeba histolytica	Unpreserved (Fresh)	47	N/A	N/A	47/47(100)	92.4– 100
Giardia lamblia[l]	Unpreserved (Fresh)	47	N/A	N/A	47/47(100)	92.4– 100
Adenovirus 40/41	Unpreserved (Fresh)	47	N/A	N/A	47/47(100)	92.4– 100
Norovirus (GI/GII)	Unpreserved (Fresh)	47	1/1 (100)	20.7 -100	46/46 (100)	92.3– 100
Rotavirus A	Unpreserved (Fresh)	47	N/A	N/A	47/47(100)	92.4– 100

Note. Fifty-three (53) fresh unpreserved samples were tested. The Internal Control (MS2) result of the 5 *C. difficile* positives and 1 *C. difficile* negative were invalid. Therefore, one sample for *C. difficile* target was invalid. For the remaining targets, 6 samples were invalid.

Table. Summary of Contrived Specimen Results

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Vibrio parahaemolyticus [a]	Unpreserved (Frozen)	119	28/30 (93.3)	78.7 – 98.2	89/89 (100)	95.9 – 100
Vibrio spp. (not *parahaemolyticus*) [b]	Unpreserved (Frozen)	118	29/29 (100)	88.3 - 100	89/89 (100)	95.9 – 100
Yersinia enterocolitica	Unpreserved (Frozen)	118	30/30 (100)	88.6 – 100	88/88 (100)	95.8 – 100
Entamoeba histolytica	Unpreserved (Frozen)	118	30/30 (100)	88.6 – 100	88/88 (100)	96.8 – 100
All other targets	Unpreserved (Frozen)	118	N/A	N/A	118/118 (100)	96.8 – 100

Note. One hundred twenty (120) contrived samples were tested. [a] The Internal control (MS2) result of one of the *Vibrio parahaemolyticus* positives was invalid. [b] One (1) of the *Vibrio spp.* positives was negative and invalid (IC negative) with both the easyMag and the MagNA Pure 96.

Table. Clinical Co-infection Summary

Co-infection summary	
+1 Target	246/466 (52.8%)
+2 Targets	78/466 (16.7%)
+3 Targets	13/466 (2.8%)

Analytical Performance

The results of the analytical studies summarized in the following paragraphs met the acceptance criteria and successfully demonstrated the analytical performance characteristics of the proposed BioCode GPP using the MagNA Pure 96 extraction system.

REPRODUCIBILITY STUDY

A study was performed to assess the Reproducibility of the BioCode GPP using samples extracted with the MagNA Pure 96. This study was designed to assess intra-assay (within run), Inter-assay (run-to-run), day-to-day and instrument-to-instrument (operator-to-operator) reproducibility. One lot of reagents was assayed at Applied BioCode on 3 instruments by 3 operators, 2 runs per day per operator for 5 days (total of 30 runs). The reproducibility panel consisted of 7 contrived samples (sample 7 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 was > 99%.

All results are as expected with the exception of two false negative results for *Cryptosporidium parvum* (one low positive and one medium positive).

Table. Reproducibility of BioCode GPP with MP96 extractions- Qualitative results.

Organism Tested	Target Probe	Concentration Tested	Expected Results	% Agreement with Expected Result			
				Instrument1 - Operator 1	Instrument2 - Operator 2	Instrument3 - Operator 3	All Instruments/ Operators
Campylobacter jejuni spp. jejuni ATCC 33292	Campy	Medium Positive 3xLoD 1.05×10^3 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		Low Positive 1.5xLoD 5.25×10^2 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
Clostridium difficile (toxinotype III; Nap1) Zeptometrix 0801619cf	tcdB	Medium Positive 3xLoD 1.25×10^3 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		Low Positive 1.5xLoD 6.23×10^2 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
Enteroaggregative E. coli O92:H33 (EAEC) STEC TW04440	EAEC	Medium Positive 3xLoD 2.10×10^3 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		Low Positive 1.5xLoD 1.05×10^3 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)

Organism Tested	Target Probe	Concentration Tested	Expected Results	% Agreement with Expected Result			
				Instrument1 - Operator 1	Instrument2 - Operator 2	Instrument3 - Operator 3	All Instruments/ Operators
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
Enterotoxigenic E. coli O78:H11 H10407 (ETEC) ATCC 35401	ST-1a	Medium Positive 3xLoD 8.40 x 10^2 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		Low Positive 1.5xLoD 4.20 x 10^2 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
Salmonella enterica ssp. enterica ATCC 14028	Salm	Medium Positive 3xLoD 3.30 x 10^3 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		Low Positive 1.5xLoD 1.65 x 10^3 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
Shiga-like toxin producing E. coli (STEC) ATCC BAA-2217	stx2	Medium Positive 3xLoD 3.75 x 10^3 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		Low Positive 1.5xLoD 1.88 x10^3 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
Shigella sonnei ATCC 29930	Shig	Medium Positive 3xLoD 6.60 x 10^2 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		Low Positive 1.5xLoD 3.30 x 10^2 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
Vibrio parahaemolyticus ATCC 17802	V.para	Medium Positive 3xLoD 1.95 x10^1 CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)

Organism Tested	Target Probe	Concentration Tested	Expected Results	% Agreement with Expected Result			
				Instrument1 - Operator 1	Instrument2 - Operator 2	Instrument3 - Operator 3	All Instruments/ Operators
		Low Positive 1.5xLoD $9.75x10^0$ CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
Yersinia enterocolitica ATCC 23715	Y.ent	Medium Positive 3xLoD $2.25 x10^3$ CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		Low Positive 1.5xLoD $1.13 x10^3$ CFU/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
Cryptosporidium parvum waterborne P102	Crypto	Medium Positive 3xLoD $9.30 x 10^3$ oocysts/mL	Detected	30/30 (100%)	30/30 (100%)	29/30 (97%)	89/90 (99%)
		Low Positive 1.5xLoD $4.65 x 10^3$ oocysts/mL	Detected	30/30 (100%)	30/30 (100%)	29/30 (97%)	89/90 (99%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
Giardia intestinalis (aka G. lamblia) waterborne P101	G.lam	Medium Positive 3xLoD $2.70 x 10^3$ cysts/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		Low Positive 1.5xLoD $1.35 x 10^3$ cysts/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
Rotavirus ATCC VR-2018	Rota	Medium Positive 3xLoD $3.75 x 10^3$ $TCID_{50}$/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		Low Positive 1.5xLoD $1.88 x 10^2$ $TCID_{50}$/mL	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)

Organism Tested	Target Probe	Concentration Tested	Expected Results	% Agreement with Expected Result			
				Instrument1 - Operator 1	Instrument2 - Operator 2	Instrument3 - Operator 3	All Instruments/ Operators
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)

Table. Reproducibility of BioCode GPP with MP96 extractions- Quantitative results

Organism	Target Probe	Test Level	Instrument/Operator	MFI Reproducibility				
				Mean	StDev	%CV	Min	Max
Campylobacter jejuni spp. jejuni ATCC 33292	Campy	Moderate Positive 3xLoD 1.05 x 10^3 CFU/mL	C2- Operator 1	26128	4013	15	8265	29801
			C3- Operator 2	28562	2652	9	22187	33021
			C4- Operator 3	24154	2714	11	18890	28822
			All Instruments/Operators	26281	3637	14	8265	33021
		Low Positive 1.5xLoD 5.25 x 10^2 CFU/mL	C2- Operator 1	24831	3563	14	14326	30367
			C3- Operator 2	25831	4285	17	14406	31649
			C4- Operator 3	21655	4326	20	10566	30156
			All Instruments/Operators	24106	4407	14	10566	31649
Clostridium difficile (toxinotype III; Nap1) Zeptometrix 0801619cf	tcdB	Moderate Positive 3xLoD 1.25 x 10^3 CFU/mL	C2- Operator 1	27589	2276	8	22487	31505
			C3- Operator 2	22672	3726	16	17112	32047
			C4- Operator 3	22780	2838	11	16926	27598
			All Instruments/Operators	24347	3762	15	16926	32047
		Low Positive 1.5xLoD 6.23 x 10^2 CFU/mL	C2- Operator 1	25007	1761	7	20436	27552
			C3- Operator 2	20116	3513	17	13752	27461
			C4- Operator 3	20902	2561	11	15608	27662
			All Instruments/Operators	22008	3438	16	13752	27662
Enteroaggregative E. coli O92:H33 (EAEC) STEC TW04440	EAEC	Moderate Positive 3xLoD 2.10 x 10^3 CFU/mL	C2- Operator 1	30474	1672	5	25616	33694
			C3- Operator 2	28424	3220	14	20108	34967
			C4- Operator 3	25129	2198	9	18787	28489
			All Instruments/Operators	28009	3281	12	18787	34967
		Low Positive 1.5xLoD 1.05 x 10^3 CFU/mL	C2- Operator 1	29161	1492	5	26343	31716
			C3- Operator 2	25402	3630	14	18723	32890
			C4- Operator 3	22836	2538	11	15300	27002
			All Instruments/Operators	25800	3734	15	15300	32890
Enterotoxigenic E. coli O78:H11 H10407 (ETEC) ATCC 35401	ST-1a	Moderate Positive 3xLoD 8.40 x 10^2 CFU/mL	C2- Operator 1	35769	1009	3	33677	38306
			C3- Operator 2	34358	4831	14	25398	42656
			C4- Operator 3	28730	3638	13	22254	35493
			All Instruments/Operators	32952	4648	14	22254	42656
		Low Positive 1.5xLoD 4.20 x 10^2 CFU/mL	C2- Operator 1	32795	2240	7	28536	36716
			C3- Operator 2	30726	3040	10	24219	35644
			C4- Operator 3	24907	2765	11	20236	30170
			All Instruments/Operators	29476	4291	15	20236	36716
	Salm		C2- Operator 1	15291	1496	10	12588	18918

Organism	Target Probe	Test Level	Instrument/Operator	MFI Reproducibility				
				Mean	StDev	%CV	Min	Max
Salmonella enterica ssp. enterica ATCC 14028		Moderate Positive 3xLoD 3.30×10^3 CFU/mL	C3- Operator 2	13931	2645	19	6284	18489
			C4- Operator 3	15701	1456	9	13306	19184
			All Instruments/Operators	14974	2068	14	6284	19184
		Low Positive 1.5xLoD 1.65×10^3 CFU/mL	C2- Operator 1	17247	1108	6	15153	19682
			C3- Operator 2	14880	1682	11	10847	19127
			C4- Operator 3	16234	1455	7	13301	19674
			All Instruments/Operators	16120	1721	10	10847	19682
Shiga-like toxin producing E. coli (STEC) ATCC BAA-2217	stx2	Moderate Positive 3xLoD 3.75×10^3 CFU/mL	C2- Operator 1	27217	1112	4	23606	30023
			C3- Operator 2	31888	1316	4	29183	34448
			C4- Operator 3	26310	1515	6	22970	30028
			All Instruments/Operators	28472	2785	10	22970	34448
		Low Positive 1.5xLoD 1.88×10^3 CFU/mL	C2- Operator 1	27467	1547	6	23914	30339
			C3- Operator 2	31460	1501	5	28002	34121
			C4- Operator 3	25293	2097	8	20340	29403
			All Instruments/Operators	28073	3089	11	20340	34121
Shigella sonnei ATCC 29930	Shig	Moderate Positive 3xLoD 6.60×10^2 CFU/mL	C2- Operator 1	17531	1173	7	15327	19469
			C3- Operator 2	15369	3781	25	7573	21561
			C4- Operator 3	16454	2056	13	11910	21114
			All Instruments/Operators	16451	2697	16	7573	21561
		Low Positive 1.5xLoD 3.30×10^2 CFU/mL	C2- Operator 1	13919	1862	13	9233	17022
			C3- Operator 2	12242	2503	20	6435	16434
			C4- Operator 3	12832	2184	17	8352	17489
			All Instruments/Operators	12998	2283	18	6435	17489
Vibrio parahaemolyticus ATCC 17802	V.para	Moderate Positive 3xLoD 1.95×10^1 CFU/mL	C2- Operator 1	23605	2305	10	17750	29798
			C3- Operator 2	21458	3112	15	13138	27526
			C4- Operator 3	17486	2821	16	8543	22789
			All Instruments/Operators	20850	3739	18	8543	29798
		Low Positive 1.5xLoD 9.75×10^0 CFU/mL	C2- Operator 1	22628	2402	11	17145	26762
			C3- Operator 2	18000	6067	34	6043	27526
			C4- Operator 3	16263	3060	19	6989	23597
			All Instruments/Operators	18964	4922	26	6043	27526
Yersinia enterocolitica ATCC 23715	Y.ent	Moderate Positive 3xLoD 2.25×10^3 CFU/mL	C2- Operator 1	14365	1847	13	10209	17892
			C3- Operator 2	15303	1786	12	10813	17893
			C4- Operator 3	14256	2325	16	9523	19982
			All Instruments/Operators	14641	2033	14	9523	19982
		Low Positive 1.5xLoD 1.13×10^3 CFU/mL	C2- Operator 1	14741	1694	11	11853	19628
			C3- Operator 2	15592	2014	13	12978	20765
			C4- Operator 3	14732	2253	16	10750	19994
			All Instruments/Operators	15022	2019	13	10750	20765

Organism	Target Probe	Test Level	Instrument/Operator	MFI Reproducibility				
				Mean	StDev	%CV	Min	Max
Cryptosporidium parvum waterborne P102	Crypto	Moderate Positive 3xLoD 9.30×10^3 oocysts/mL	C2- Operator 1	18731	4323	23	9904	28443
			C3- Operator 2	20843	3978	19	11526	28678
			C4- Operator 3*	14867	4457	31	5669	26611
			All Instruments/Operators	18222	4873	26	5669	28678
		Low Positive 1.5xLoD 4.65×10^3 oocysts/mL	C2- Operator 1	13816	5170	37	2572	24148
			C3- Operator 2	15402	3996	26	8166	22933
			C4- Operator 3*	10785	3409	33	4413	18053
			All Instruments/Operators	13363	4631	35	2572	24148
Giardia intestinalis (aka G. lamblia) waterborne P101	G.lam	Moderate Positive 3xLoD 2.70×10^3 cysts/mL	C2- Operator 1	14327	4487	31	7696	30050
			C3- Operator 2	21743	5006	23	8932	31538
			C4- Operator 3	16963	3780	22	11527	25036
			All Instrument/Operators	17677	5377	30	7696	31538
		Low Positive 1.5xLoD 1.35×10^3 cysts/mL	C2- Operator 1	17340	3668	21	11338	25626
			C3- Operator 2	24751	4701	19	12478	31282
			C4- Operator 3	19821	3772	20	12677	25949
			All Instruments/Operators	20637	5081	25	11338	31282
Rotavirus ATCC VR-2018	Rota	Moderate Positive 3xLoD 3.75×10^3 $TCID_{50}$/mL	C2- Operator 1	39523	2086	5	35835	44023
			C3- Operator 2	39162	3400	9	28863	44274
			C4- Operator 3	32899	2956	8	25107	38361
			All Instruments/Operators	37195	4170	13	25107	44274
		Low Positive 1.5xLoD 1.88×10^2 $TCID_{50}$/mL	C2- Operator 1	36515	5114	14	16127	40941
			C3- Operator 2	36312	3863	11	26491	41547
			C4- Operator 3	31925	3280	10	21114	36158
			All Instruments/Operators	34917	4628	11	16127	41547

*The one replicate with 'Not detected' result was not included in the calculations.

SINGLE VS. MULTI-SPIKE

To demonstrate that the LoD is equivalent when spiking with single versus multiple organisms, LoD for representative organisms (*S. enterica*, *C. difficile*, *G. lamblia*, and Rotavirus) were performed as single targets and with all representative organisms combined, using both extraction systems. The results of spiking single versus multiple organisms showed equivalent LoD (see Table below). LoD for the remaining organisms were performed in pairs, except for norovirus which was tested from positive clinical specimens.

Single and multi-spiked samples both achieved ≥95% detection of 20 replicates (≥19 out of 20) at same concentrations for the challenge organisms (concentrations indicated in the tables below).

Table. Comparison of results for limit of detection testing with single or multiple spiked samples for easyMAG extractions assayed with BioCode GPP.

Strain	Single Spike/ Multiple Spike	Source	Target Probe	Unpreserved Stool LoD	Unpreserved Stool Detection	Cary-Blair Stool LoD	Cary-Blair Stool Detection
Clostridium difficile (toxinotype 0)	Single Spike	ATCC 9689	tcdB	9.50 x 10^1 CFU/mL	20/20	9.50 x 10^1 CFU/mL	20/20
	Multi Spike				20/20		19/20
Salmonella enterica ssp. enterica	Single Spike	ATCC 14028	Salm	1.10 x 10^3 CFU/mL	20/20	1.10 x 10^3 CFU/mL	20/20
	Multi Spike				20/20		20/20
Giardia intestinalis (aka G. lamblia)	Single Spike	waterborne P101	G.lam	9.00 x 10^2 cysts/mL	20/20	9.00 x 10^2 cysts/mL	20/20
	Multi Spike				20/20		20/20
Rotavirus A	Single Spike	ATCC VR-2018	Rota	2.50 x 10^3 $TCID_{50}$/mL	20/20	1.25 x 10^3 $TCID_{50}$/mL	20/20
	Multi Spike				20/20		20/20

Table. Comparison of results for limit of detection testing with single or multiple spiked samples for MP96 extractions assayed with BioCode GPP.

Strain	Single Spike/ Multiple Spike	Source	Target Probe	Unpreserved Stool LoD	Unpreserved Stool Detection	Cary-Blair Stool LoD	Cary-Blair Stool Detection
Clostridium difficile (toxinotype 0)	Single Spike	ATCC 9689	tcdB	9.50 x 10^1 CFU/mL	20/20	9.50 x 10^1 CFU/mL	20/20
	Multi Spike				20/20		20/20
Salmonella enterica ssp. enterica	Single Spike	ATCC 14028	Salm	1.10 x 10^3 CFU/mL	20/20	1.10 x 10^3 CFU/mL	20/20
	Multi Spike				20/20		20/20
Giardia intestinalis (aka G. lamblia)	Single Spike	waterborne P101	G.lam	9.00 x 10^2 cysts/mL	20/20	9.00 x 10^2 cysts/mL	20/20
	Multi Spike				20/20		20/20
Rotavirus A	Single Spike	ATCC VR-2018	Rota	1.25 x 10^3 $TCID_{50}$/mL	20/20	1.25 x 10^3 $TCID_{50}$/mL	20/20
	Multi Spike				20/20		20/20

LIMIT OF DETECTION (LoD)

A study was performed to assess the performance of the BioCode GPP on the BioCode MDx 3000 at the Limit of Detection (LoD) for both unpreserved Stool and Cary-Blair specimens. In this study the GI Panel was tested with quantified bacteria, virus or parasite stocks (except norovirus which used clinical samples). For initial screening, four replicates of each concentration (near LoD for the predicate) in negative stool and Cary-Blair were extracted on the easyMAG and MagNA Pure 96 Systems and tested in singlet with the BioCode GPP on the BioCode MDx 3000 system. The LoD was confirmed by extracting 20 replicates of each sample type/extraction method and testing each in singlet for a total of 20 replicates at or near 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 unpreserved stool and Cary-Blair preserved stool. The LoDs determined from the quantitated stocks or clinical samples are presented below and in the labeling. The LoDs were the same or within 2-fold for each extraction system.

Table. Comparison of results for limit of detection testing for Unpreserved Stool extracted with the easyMAG or MP96 and assayed with BioCode GPP.

Strain	Source	EasyMag		MagNA Pure 96	
		Unpreserved Stool LoD	Detection	Unpreserved Stool LoD	Detection
Campylobacter coli	ATCC 33559	2.81 x 10^1 CFU/mL	19/20	2.81 x 10^1 CFU/mL	20/20
Campylobacter jejuni spp. jejuni	ATCC 33292	3.50 x 10^2 CFU/mL	19/20	3.50 x10^2 CFU/mL	19/20
Clostridium difficile (toxinotype 0)	ATCC 9689	9.50 x 10^1 CFU/mL	20/20	9.50 x 10^1 CFU/mL	20/20
Clostridium difficile (toxinotype III; Nap1)	Zeptometrix 0801619cf	4.15 x 10^2 CFU/mL	20/20	4.15 x10^2 CFU/mL	20/20
Enteroaggregative *E. coli* O92:H33 (EAEC)	STEC TW04440	7.00 x 10^2 CFU/mL	20/20	7.00 x10^2 CFU/mL	20/20
Enteroinvasive *E. coli* O29:NM (EIEC)	ATCC 43892	3.60 x 10^2 CFU/mL	20/20	1.80 x10^2 CFU/mL	20/20
Enterotoxigenic *E. coli* O78:H11 H10407 (ETEC)	ATCC 35401	2.80 x 10^2 CFU/mL	20/20	2.80 x10^2 CFU/mL	20/20
Salmonella bongori	SGSC 4900	1.40 x 10^3 CFU/mL	20/20	1.40 x10^3 CFU/mL	20/20
Salmonella enterica ssp. enterica	ATCC 14028	1.10 x 10^3 CFU/mL	20/20	1.10 x10^3 CFU/mL	20/20
Shiga-like toxin producing *E. coli* (STEC)	ATCC BAA-2217	1.25 x 10^3 CFU/mL	20/20	1.25 x10^3 CFU/mL	20/20
E. coli O157	ATCC 700376	1.65 x 10^3 CFU/mL	20/20	1.65 x10^3 CFU/mL	20/20
Shigella sonnei	ATCC 29930	2.20 x 10^2 CFU/mL	20/20	2.20 x10^2 CFU/mL	20/20
Vibrio cholerae	ATCC 25870	2.45 x 10^2 CFU/mL	20/20	2.45 x10^2 CFU/mL	20/20
Vibrio parahaemolyticus	ATCC 17802	6.50 x 10^0 CFU/mL	20/20	6.50 x10^0 CFU/mL	20/20

Strain	Source	EasyMag		MagNA Pure 96	
		Unpreserved Stool LoD	Detection	Unpreserved Stool LoD	Detection
Yersinia enterocolitica	ATCC 23715	7.50 x 10^2 CFU/mL	20/20	7.50 x10^2 CFU/mL	20/20
Cryptosporidium parvum	waterborne P102C	3.10 x 10^3 oocysts/mL	20/20	3.10 x10^3 oocysts/mL	20/20
Entamoeba histolytica HB-301:NIH	BEI NR-178	1.55 x 10^{-1} cysts/mL	20/20	1.55 x10^{-1} cysts/mL	20/20
Giardia intestinalis (aka G. lamblia)	waterborne P101	9.00 x 10^2 cysts/mL	20/20	9.00 x10^2 cysts/mL	20/20
Adenovirus 40 (dugan)	Zeptometrix 0810084	2.00 x 10^{-1} $TCID_{50}$/mL	20/20	1.00 x10^{-1} $TCID_{50}$/mL	20/20
Adenovirus 41 (TAK)	Zeptometrix 0810085	9.4 x 10^{-2} $TCID_{50}$/mL	20/20	4.70 x10^{-2} $TCID_{50}$/mL	20/20
Rotavirus A	ATCC VR-2018	2.5 x 10^3 $TCID_{50}$/mL	20/20	1.25 x10^3 $TCID_{50}$/mL	20/20

Table. Comparison of results for limit of detection testing for Cary-Blair Stool extracted with the easyMAG or MP96 and assayed with BioCode GPP.

Strain	Source	EasyMag		MagNA Pure 96	
		Cary-Blair Stool LoD	Detection	Cary-Blair Stool LoD	Detection
Campylobacter coli	ATCC 33559	5.61 x 10^1 CFU/mL	20/20	2.81 x 10^1 CFU/mL	20/20
Campylobacter jejuni spp. jejuni	ATCC 33292	3.50 x10^2 CFU/mL	20/20	7.00 x10^2 CFU/mL	20/20
Clostridium difficile (toxinotype 0)	ATCC 9689	9.50 x 10^1 CFU/mL	20/20	9.50 x 10^1 CFU/mL	20/20
Clostridium difficile (toxinotype III; Nap1)	Zeptometrix 0801619cf	4.15 x10^2 CFU/mL	20/20	4.15 x10^2 CFU/mL	20/20
Enteroaggregative *E. coli* O92:H33 (EAEC)	STEC TW04440	7.00 x10^2 CFU/mL	20/20	7.00 x10^2 CFU/mL	20/20
Enteroinvasive *E. coli* O29:NM (EIEC)	ATCC 43892	3.60 x10^2 CFU/mL	20/20	1.80 x10^2 CFU/mL	20/20
Enterotoxigenic *E. coli* O78:H11 H10407 (ETEC)	ATCC 35401	2.80 x10^2 CFU/mL	20/20	2.80 x10^2 CFU/mL	19/20
Salmonella bongori	SGSC 4900	1.40 x10^3 CFU/mL	19/20	1.40 x10^3 CFU/mL	20/20
Salmonella enterica ssp. enterica	ATCC 14028	1.10 x10^3 CFU/mL	20/20	1.10 x10^3 CFU/mL	20/20
Shiga-like toxin producing *E. coli* (STEC)	ATCC BAA-2217	1.25 x10^3 CFU/mL	20/20	1.25 x10^3 CFU/mL	20/20
E. coli O157	ATCC 700376	3.30 x10^3 CFU/mL	20/20	1.65 x10^3 CFU/mL	20/20

Strain	Source	EasyMag		MagNA Pure 96	
		Cary-Blair Stool LoD	Detection	Cary-Blair Stool LoD	Detection
Shigella sonnei	ATCC 29930	2.20 x10^2 CFU/mL	20/20	2.20 x10^2 CFU/mL	20/20
Vibrio cholerae	ATCC 25870	2.45 x10^2 CFU/mL	20/20	2.45 x10^2 CFU/mL	20/20
Vibrio parahaemolyticus	ATCC 17802	6.50 x10^0 CFU/mL	20/20	6.50 x10^0 CFU/mL	20/20
Yersinia enterocolitica	ATCC 23715	7.50 x10^2 CFU/mL	20/20	7.50 x10^2 CFU/mL	20/20
Cryptosporidium parvum	waterborne P102C	3.10 x10^3 oocysts/mL	20/20	3.10 x10^3 oocysts/mL	20/20
Entamoeba histolytica HB-301:NIH	BEI NR-178	1.55 x10^{-1} cysts/mL	20/20	1.55 x10^{-1} cysts/mL	20/20
Giardia intestinalis (aka G. lamblia)	waterborne P101	9.00 x10^2 cysts/mL	20/20	9.00 x10^2 cysts/mL	20/20
Adenovirus 40 (dugan)	Zeptometrix 0810084	2.00 x10^{-1} $TCID_{50}$/mL	20/20	1.00 x10^{-1} $TCID_{50}$/mL	20/20
Adenovirus 41 (TAK)	Zeptometrix 0810085	4.70 x10^{-2} $TCID_{50}$/mL	20/20	4.70 x10^{-2} $TCID_{50}$/mL	20/20
Rotavirus A	ATCC VR-2018	1.25 x10^3 $TCID_{50}$/mL	20/20	1.25 x10^3 $TCID_{50}$/mL	20/20

For Norovirus GI and GII targets, positive clinical specimens were used, and serial dilutions (initial 10-fold dilution series followed by finer dilutions) were performed. Four replicates of each dilution in negative unpreserved stool and Cary-Blair stool were extracted with the easyMAG and MagNA Pure 96 Systems and tested with the BioCode GPP on the BioCode MDx 3000 system. The LoD was confirmed by extracting 20 replicates of each sample type with each extraction method and testing at or near presumptive LoD. For unpreserved stool, LoD with the easyMag extraction was 2-fold and 8.3-fold lower than the MagNA Pure 96 extraction for Norovirus GI and Norovirus GII, respectively. For Cary-Blair stool, LoD with the easyMag extraction was less than 2-fold lower than the MagNA Pure 96 extraction for both Norovirus GI and GII.

Table. Norovirus - Comparison of results for limit of detection testing for Unpreserved Stool extracted with the easyMAG or MP96 and assayed with BioCode GPP.

Target	Source	Target Probe	EasyMag		MagNA Pure 96	
			Unpreserved Stool Dilution	Detection	Unpreserved Stool Dilution	Detection
Norovirus GI	Clinical Sample ID#60	NoVG1	1:10,000	20/20	1:5,000	19/20
Norovirus GII	Clinical Sample ID#54	NoVG2	1:250,000	20/20	1:30,000	20/20

Table. Norovirus - Comparison of results for limit of detection testing for Cary-Blair Stool extracted with the easyMAG or MP96 and assayed with BioCode GPP.

Target	Source	Target Probe	EasyMag		MagNA Pure 96	
			Cary-Blair Stool Dilution	Detection	Cary-Blair Stool Dilution	Detection
Norovirus GI	Clinical Sample ID#60	NoVG1	1:50,000	20/20	1:80,000	19/20
Norovirus GII	Clinical Sample ID#54	NoVG2	1:100,000	20/20	1:80,000	20/20

Conclusion:

The intended use and fundamental scientific technology of the BioCode GPP using the MagNA Pure 96 is substantially equivalent to the predicate device. Clinical and non-clinical studies have established that the BioCode GPP with the MagNA Pure 96 is substantially equivalent to the predicate device.