DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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

September 28, 2018

Applied BioCode Inc.
Robert Di Tullio
Regulatory Consultant
10020 Pioneer Blvd. Suite 102
Santa Fe Springs, CA 90670

Re: K180041
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: January 08, 2018
Received: January 09, 2018

Dear Mr. Di 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. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

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

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

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

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

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

Sincerely,

Steven R. Gitterman -S for

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

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

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

510(k) Number *(if known)*
K180041

Device Name
BioCode Gastrointestinal Pathogen Panel (GPP)

Indications for Use *(Describe)*
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.

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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APPLIED BIOCODE, INC.
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SANTA FE SPRINGS, CA 90670, USA

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.
10020 Pioneer Blvd. Suite 102
Santa Fe Springs, CA 90670

Contact:
Robert Di Tullio
Regulatory Consultant
rditullio@apbiocode.com
Telephone: 310 801 1235
Fax: 323 372 3816

Date Submitted:
August 22, 2018

Trade Name:
BioCode Gastrointestinal Pathogen Panel

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

Predicate Device:
K121454 – Luminex xTAG® Gastrointestinal Pathogen Panel (GPP)

Intended Use:

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.

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.

A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.

Device Description:

The BioCode Gastrointestinal Pathogen Panel (GPP) is a multiplex nucleic acid 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 easyMAG, an automated system. Once the PCR plate is set up and sealed, all other operations are automated on MDx 3000. The BioCode Gastrointestinal Pathogen 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 test are available within less than 5 hours.

Bacteria, Viruses, and Parasites
Detected by the BioCode Gastrointestinal Pathogen Panel

Bacteria	Parasites
▪ Campylobacter spp. (*C. jejuni, C. coli*) ▪ *Clostridium difficile* toxin A/B ▪ 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.(*C. parvum/C. hominis)* ▪ *Entamoeba histolytica* ▪ *Giardia lamblia/intestinalis*
	Viruses
	▪ Adenovirus 40/41 ▪ Norovirus GI/GII ▪ Rotavirus A
	RNA Internal Control

Device Comparison:

Comparison of the BioCode Gastrointestinal Pathogen Panel with the Predicate Device

Characteristic	Proposed Device	Predicate
Name	Applied BioCode, Inc. BioCode Gastrointestinal Pathogen Panel	xTAG® 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	K121454
Regulation	21CFR 866.3990	21CFR 866.3990
Product Code	PCH, OOI	PCH, OOI
Device Class	II	II
Similarities		

Characteristic	Proposed Device	Predicate
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 (including several diarrheagenic *E. coli/Shigella* pathotypes), parasites, and viruses are identified using the BioCode GPP: *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. 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. 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. A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.	Equivalent

Characteristic	Proposed Device	Predicate
Instrument	Nucleic Acid Purification System BioCode MDx 3000	Nucleic Acid Purification System PCR Thermocycler Luminex® 100/200™ or MAGPIX instruments
Sample Type	Unpreserved stool and stool in Cary- Blair Media	Same
Controls	Externally Sourced -	Same
Differences		
Methodology	Multiplex RT-PCR and probe hybridization followed by fluorescence detection and decoding of barcoded magnetic beads (BMB) that are coupled to biotinylated products with streptavidin conjugate	Multiplex RT-PCR and multiplex TSPE followed by fluorescence-activated sorting of labeled beads coupled to streptavidin-conjugated biotinylated products.
Calibrators	Internal Calibration	External Calibration Kit

Summary of Performance Characteristics of the

BioCode GPP Clinical Performance

Testing of Prospectively collected Specimens (Categories I and II)

A clinical investigational study was performed in which a total of 1558 leftover, de-identified samples were prospectively collected from patients who underwent stool sample collection for clinical indications at four (4) investigational sites, with each collecting approximately 400 samples. The sites were located in the United States, with a broad geographic representation. The sites were located in the Northeast (Baltimore, MD), Southeast (Tampa, FL), Midwest (Memphis, TN), and West (Los Angeles, CA). 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 divided into aliquots and either tested freshly on the ABC IUO assay 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 ABC IUO assay 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, within which to enroll the specimens displaying due diligence in attempting to cover all panel targets with native specimens. The results of the clinical study follow.

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)

Clinical Sites collected both Category I and II see the following table for the breakdown.

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. Comparator (reference) methods for prospective clinical study.

Target Pathogen/Toxin	**Reference Method**
Adenovirus 40/41	Composite result of two PCR/sequencing assays
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 assays
Entamoeba histolytica	Composite result of two PCR/sequencing assays
Escherichia coli (*E. coli*) O157	Enrichment culture
Enterotoxigenic E. *coli* (ETEC) LT/ST	Composite result of two PCR/sequencing assays
Enteroaggregative E. *coli* (EAEC)	Composite result of two PCR/sequencing assays
Giardia lamblia /intestinalis	Composite result of two PCR/sequencing assays
Norovirus GI/GII	Composite result of two PCR/sequencing assays
Rotavirus A	Composite result of two PCR/sequencing assays
Salmonella spp.	Enrichment culture
Shiga-like Toxin producing *E. coli* (STEC) stx1/stx2	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

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

Table. 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)	(96.97, 99.9)
Clostridium difficile[b]	26/27 (96.3)	(81.0, 99.9)	208/210 (99.1)	(96.6, 99.9)
E.coli 0157	N/A	N/A	237/237 (100.0)	(98.46, 100.0)
EAEC	1/1 (100.0)	(2.5, 100.0)	234/234 (100.0)	(98.44, 100.0)
ETEC[c]	3/3 (100.0)	(29.2, 100.0)	229/232 (98.7)	(96.27, 99.7)
STEC[d]	N/A	N/A	235/237 (99.2)	(96.99, 99.9)
Salmonella spp[e]	3/3 (100.0)	(29.2, 100.0)	232/234 (99.2)	(96.95, 99.9)
Shigella/EIEC[f]	1/1 (100.0)	(2.5, 100.0)	233/236 (98.7)	(96.33, 99.7)
Vibrio parahaemolyticus[g]	N/A	N/A	236/237 (99.6)	(97.67, 100.0)
Vibrio spp	N/A	N/A	237/237 (100.0)	(98.46, 100.0)
Yersinia enterocolitica[h]	N/A	N/A	236/237 (99.6)	(97.67, 100.0)
Cryptosporidium spp	1/1 (100.0)	(2.5, 100.0)	234/234 (100.0)	(98.44, 100.0)
Entamoeba histolytica	N/A	N/A	235/235 (100.0)	(98.44, 100.0)
Giardia lamblia[i]	N/A	N/A	234/235 (99.6)	(97.65, 100.0)
Adenovirus 40/41[j]	N/A	N/A	233/235 (99.2)	(96.96, 100.0)
Norovirus GI/GII	1/1 (100.0)	(2.50, 100.0)	235/235 (100.0)	(98.44, 100.0)
Rotavirus A	1/1 (100.0)	(2.50, 100.0)	234/235 (99.6)	(97.65, 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. 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)
Clostridium difficile[b]	N/A	N/A	N/A	N/A
E.coli 0157[c]	1/2 (50.0)	(1.3, 98.7)	950/954 (99.6)	(98.9, 99.9)
EAEC[d]	25/29 (86.2)	(68.3, 96.1)	916/919 (99.7)	(99.1, 99.9)
ETEC[e]	7/10 (70.0)	(34.8, 93.3)	934/939 (99.5)	(98.8, 99.8)
STEC[f]	3/3 (100.0)	(29.2, 100.0)	918/919 (99.9)	(99.4, 100.0)
Salmonella spp[g]	18/22 (81.8)	(59.7, 94.8)	926/934 (99.1)	(98.3, 99.6)
Shigella/EIEC[h]	4/5 (80.00)	(28.4, 99.5)	940/951 (98.8)	(97.9, 99.4)
Vibrio parahaemolyticus[i]	N/A	N/A	955/957 (99.8)	(99.3, 100.0)
Vibrio spp	N/A	N/A	956/956 (100.0)	(99.6, 100.0)
Yersinia enterocolitica[j]	N/A	N/A	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	N/A	N/A	948/948 (100.0)	(99.6, 100.0)
Giardia lamblia[k]	2/2 (100.0)	(15.8, 100.0)	940/946 (99.4)	(98.6, 99.8)
Adenovirus 40/41[l]	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 - *Clostridium difficile*: *C. difficile* testing must be performed with fresh specimens only, not with previously frozen specimens.

c - *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.

d - 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.

e - 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.

f - STEC: The 1 false positive compared to the culture reference method was tested by bidirectional sequencing and confirmed as positive.

g – *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.

h - *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.

i - *Vibrio parahaemolyticus*: The 2 false positives compared to the culture reference method were tested by bidirectional sequencing

and, 1 of 2 confirmed as positive.
j - *Yersinia enterocolitica*: The 5 false positives compared to the culture reference method were tested by bidirectional sequencing and, 3 of 5 confirmed as positive.
k – *Giardia lamblia*: The 4 false positives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing: none were confirmed as positives.
l - 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. 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]	N/A	N/A	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]	N/A	N/A	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	N/A	N/A	361/361 (100.0)	(99.0, 100.0)
Vibrio spp	N/A	N/A	361/361 (100.0)	(99.0, 100.0)
Yersinia enterocolitica[i]	N/A	N/A	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	N/A	N/A	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	N/A	N/A	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.

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 reflex testing for the prospective study was approximately 0.2% (3/1558).

Analysis of mixed infections in the prospective study

The BioCode GPP detected a total of 49 samples with mixed infections in the prospective clinical study. This represents 3.1 % of the total number of specimens (49/1558). 40 were double infections, 8 were triple infections, and 1 was quadruple infection. The most common pathogens in co-infections were with EAEC (22/49, 44.9%) and ETEC (18/49, 36.7%). The most common co-infection combinations detected by the BioCode GPP in the prospective clinical study are summarized in the table below.

Most prevalent multiple detection combinations (5 or more instances) from clinical evaluation.

Multiple Detection Combination	Number of Specimens
EAEC + ETEC	8
Clostridium difficile + *Salmonella* spp	5

Clinical co-infection combinations detected by BioCode GPP (unpreserved stool).

Distinct Co-Infection Combinations Detected by BioCode GPP						
Analyte_1	**Analyte_2**	**Analyte_3**	**Analyte_4**	**Total Co-infections**	**Number of Discrepant Co-infections**	**Discrepant Analyte(s)**
Adenovirus 40/41	Rotavirus A	N/A	N/A	1	1	Adenovirus 40/41 (x1)
Campylobacter spp	*Shigella*/EIEC	N/A	N/A	1	1	All
Campylobacter spp	*Giardia lamblia*	N/A	N/A	1	1	All
Campylobacter spp	Norovirus GI/GII	N/A	N/A	1	1	*Campylobacter* spp (x1)
Campylobacter spp	STEC	N/A	N/A	1	1	All
Campylobacter spp	*Y. enterocolitica*	N/A	N/A	1	1	All
C. difficile	ETEC	N/A	N/A	1	1	ETEC (x1)
Cryptosporidium spp	*Campylobacter* spp	N/A	N/A	1	1	*Campylobacter* spp (x1)
Cryptosporidium spp	*Giardia lamblia*	N/A	N/A	1	1	*Giardia lamblia* (x1)
E.coli O157	Norovirus GI/GII	N/A	N/A	1	1	*E.coli* O157 (x1)
E.coli O157	*Shigella*/EIEC	N/A	N/A	1	1	*E.coli* O157 (x1)
EAEC	*Shigella*/EIEC	N/A	N/A	1	1	*Shigella*/EIEC (x1)

Distinct Co-Infection Combinations Detected by BioCode GPP						
Analyte_1	**Analyte_2**	**Analyte_3**	**Analyte_4**	**Total Co-infections**	**Number of Discrepant Co-infections**	**Discrepant Analyte(s)**
EAEC	*Shigella*/EIEC	Norovirus GI/GII	N/A	1	1	*Shigella*/EIEC (x1)
EAEC	ETEC	N/A	N/A	4	1	ETEC (x1)
EAEC	ETEC	Norovirus GI/GII	N/A	1	1	EAEC (x1);ETEC (x1)
EAEC	*Giardia lamblia*	N/A	N/A	1	1	All
EAEC	Rotavirus A	N/A	N/A	1	1	Rotavirus A (x1)
Shigella/EIEC	ETEC	N/A	N/A	1	1	*Shigella*/EIEC (x1)
Norovirus GI/GII	Rotavirus A	N/A	N/A	1	1	Norovirus GI/GII (x1)
Norovirus GI/GII	Rotavirus A	STEC	N/A	1	1	Rotavirus A (x1);STEC (x1);
Norovirus GI/GII	*V. parahaemolyticus*	*Y. enterocolitica*	N/A	1	1	All
Total Co-infections				24	21	
Double Infections				20	17	
Triple Infections				4	4	

Clinical co-infection combinations detected by BioCode GPP (Cary-Blair).

Distinct Co-Infection Combinations Detected by BioCode GPP						
Analyte_1	**Analyte_2**	**Analyte_3**	**Analyte_4**	**Total Co-infections**	**Number of Discrepant Co-infections**	**Discrepant Analyte(s)**
C. difficile	*Salmonella* spp	N/A	N/A	3	1	All
E.coli O157	EAEC	ETEC	STEC	1	1	*E.coli* O157 (x1);STEC (x1)
EAEC	ETEC	N/A	N/A	4	1	EAEC (x1)
EAEC	ETEC	*Y. enterocolitica*	N/A	2	2	ETEC (x1);*Y. enterocolitica* (x2)
EAEC	Norovirus GI/GII	N/A	N/A	3	1	EAEC (x1)
EAEC	STEC	N/A	N/A	1	1	STEC (x1)
Total Co-infections				14	7	
Double Infections				11	4	
Triple Infections				2	2	

Distinct Co-Infection Combinations Detected by BioCode GPP						
Analyte_1	**Analyte_2**	**Analyte_3**	**Analyte_4**	**Total Co-infections**	**Number of Discrepant Co-infections**	**Discrepant Analyte(s)**
Quadruple Infections				1	1	

Clinical co-infection combinations detected by reference methods (unpreserved stool).

Distinct Co-Infection Combinations Detected by Reference Methods					
Analyte_1	**Analyte_2**	**Analyte_3**	**Total Co-infections**	**Number of Discrepant Co-infections**	**Discrepant Analyte(s)**
Adenovirus 40/41	EAEC	N/A	1	1	Adenovirus 40/41 (x1)
EAEC	ETEC	N/A	4	1	EAEC (x1)
EAEC	ETEC	Norovirus GI/GII	2	2	EAEC (x1);ETEC (x2)
Total Co-infections			7	4	
Double Infections			5	2	
Triple Infections			2	2	

Clinical co-infection combinations detected by reference methods (Cary-Blair).

Distinct Co-Infection Combinations Detected by Reference Methods					
Analyte_1	**Analyte_2**	**Analyte_3**	**Total Co-infections**	**Number of Discrepant Co-infections**	**Discrepant Analyte(s)**
EAEC	*Shigella*/EIEC	ETEC	1	1	*Shigella*/EIEC (x1)
Total Co-infections			1	1	
Double Infections			0	0	
Triple Infections			1	1	

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. 3 were removed from the study for improper storage prior to testing. 2 were invalid for RNA IC failure in the unpreserved stool. The following table summarizes the comparison of the results from the initial unpreserved testing and subsequent inoculated results.

Summary of Inoculated Cary-Blair or unpreserved samples Vs. reference method. Agreements were calculated compared to reference method testing of unpreserved stool. Reference testing was not repeated after samples were inoculated to Cary-Blair.

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Campylobacter spp.[a]	Unpreserved	394	2/2 (100.0)	(15.81, 100.0)	385/392 (98.21)	(96.36, 99.28)
	Cary-Blair (Inoculated)	396	2/2 (100.0)	(15.81, 100.0)	388/394 (98.48)	(96.72, 99.44)
E. coli O157[b]	Unpreserved	395	1/2 (50.00)	(1.26, 98.74)	389/393 (98.98)	(97.41, 99.72)
	Cary-Blair (Inoculated)	397	1/2 (50.00)	(1.26, 98.74)	391/395 (98.99)	(97.43, 99.72)
Enteroaggregative *E. coli* (EAEC)[c]	Unpreserved	394	12/14 (85.71)	(57.19, 98.22)	378/380 (99.47)	(98.11, 99.94)
	Cary-Blair (Inoculated)	396	12/14 (85.71)	(57.19, 98.22)	382/382 (100.0)	(99.04, 100.0)
Enterotoxigenic *E. coli* (ETEC)[d]	Unpreserved	394	4/6 (66.67)	(22.28, 95.67)	387/388 (99.74)	(98.57, 99.99)
	Cary-Blair (Inoculated)	396	4/6 (66.67)	(22.28, 95.67)	386/390 (98.97)	(97.39, 99.72)
Shiga toxin-producing *E. coli* (STEC)	Unpreserved	361	2/2 (100.0)	(15.81, 100.0)	359/359 (100.0)	(98.98, 100.0)
	Cary-Blair (Inoculated)	363	2/2 (100.0)	(15.81, 100.0)	361/361 (100.0)	(98.98, 100.0)
Salmonella spp.[e]	Unpreserved	395	5/6 (83.33)	(35.88, 99.58)	385/389 (98.97)	(97.39, 99.72)
	Cary-Blair (Inoculated)	397	6/6 (100.0)	(54.07, 100.0)	389/391 (99.49)	(98.16, 99.94)
Shigella/ EIEC[f]	Unpreserved	395	1/1 (100.0)	(2.50, 100.0)	389/394 (98.73)	(97.06, 99.59)
	Cary-Blair (Inoculated)	397	1/1 (100.0)	(2.50, 100.0)	391/396 (98.74)	(97.08, 99.59)
Vibrio parahaemolyticus	Unpreserved	395	N/A	N/A	395/395 (100.0)	(99.07, 100.0)
	Cary-Blair (Inoculated)	397	N/A	N/A	397/397 (100.0)	(99.08, 100.0)
Vibrio spp. (not parahaemolyticus)	Unpreserved	395	N/A	N/A	395/395 (100.0)	(99.07, 100.0)
	Cary-Blair (Inoculated)	397	N/A	N/A	397/397 (100.0)	(99.08, 100.0)
Yersinia enterocolitica[g]	Unpreserved	395	N/A	N/A	394/395 (99.75)	(98.60, 99.99)
	Cary-Blair (Inoculated)	397	N/A	N/A	396/397 (99.75)	(98.60, 99.99)
Cryptosporidium spp	Unpreserved	394	2/2 (100.0)	(15.81, 100.0)	392/392 (100.0)	(99.06, 100.0)
	Cary-Blair (Inoculated)	396	2/2 (100.0)	(15.81, 100.0)	394/394 (100.0)	(99.07, 100.0)
Entamoeba histolytica	Unpreserved	394	N/A	N/A	394/394 (100.0)	(99.07, 100.0)
	Cary-Blair (Inoculated)	396	N/A	N/A	396/396 (100.0)	(99.07, 100.0)

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Giardia lamblia[h]	Unpreserved	394	N/A	N/A	391/394 (99.24)	(97.79, 99.84)
	Cary-Blair (Inoculated)	396	N/A	N/A	394/396 (99.49)	(98.19, 99.94)
Adenovirus 40/41[i]	Unpreserved	394	3/6 (50.00)	(11.81, 88.19)	388/388 (100.0)	(99.05, 100.0)
	Cary-Blair (Inoculated)	396	2/6 (33.33)	(4.33, 77.72)	385/390 (98.72)	(97.03, 99.58)
Norovirus (GI/GII)	Unpreserved	395	28/28 (100.0)	(87.66, 100.0)	364/367 (99.18)	(97.63, 99.83)
	Cary-Blair (Inoculated)	397	28/28 (100.0)	(87.66, 100.0)	364/369 (98.64)	(96.87, 99.56)
Rotavirus A	Unpreserved	395	11/12 (91.67)	(61.52, 99.79)	380/383 (99.22)	(97.73, 99.84)
	Cary-Blair (Inoculated)	397	11/12 (91.67)	(61.52, 99.79)	380/385 (98.70)	(97.00, 99.58)

a – *Campylobacter* spp. Unpreserved: The 6 false positives compared to reference culture method were tested by bidirectional sequencing and 4 of 6 confirmed as positives. Cary-Blair: 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. Unpreserved: 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 the reference culture method were tested by bidirectional sequencing and 3 of 4 confirmed as positives. Cary-Blair: 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. Unpreserved: The 2 false negatives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing; 1 of the 2 confirmed as positive. Cary-Blair: 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. Unpreserved: The 2 false negatives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing; none were confirmed as positives. None of 4 false positives were confirmed as positive by 2 additional rounds of sequencing. Cary-Blair: 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. Unpreserved: The 2 false positives compared to the reference culture method were tested by bidirectional sequencing and 1 of 2 confirmed as positives. Cary-Blair: 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. Unpreserved: The 5 false positives compared to the reference culture method were tested by bidirectional sequencing and 4 of 5 confirmed as positives. Cary-Blair: 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*. Unpreserved: The 1 false positive compared to the reference culture method was tested by bidirectional sequencing and confirmed as positive. Cary-Blair: The 1 false positive compared to the reference culture method were tested by bidirectional sequencing and confirmed as positive.

h - *Giardia lamblia*. Unpreserved: The 2 false positives compared to bidirectional sequencing were not confirmed as positive by 2 additional rounds of sequencing. Cary-Blair: The 3 false positives compared to bidirectional sequencing were not confirmed as positive by 2 additional rounds of sequencing.

i – Adenovirus 40/41. Unpreserved: The 4 false negatives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing; 1 of 4 was confirmed as positive. The 5 false positives were not confirmed as positives by an additional round of sequencing. Cary-Blair: 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, 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 GPP were blinded to the expected test result. A summary of the demographic information of the tested samples and the results of the BioCode GPP testing are presented in Tables below.

Demographic summary for 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)

Summary of Clinical specimen Results (Archived specimens)

	Positive Agreement		**Negative Agreement**	
Target	**Agreement n/N (%)**	**95% CI**	**Agreement n/N (%)**	**95% CI**
Campylobacter spp[a]	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)	(97.55, 100.0)
ETEC	20/20 (100.0)	(83.2, 100.0)	152/152 (100.0)	(97.6, 100.0)
STEC[b]	30/33 (90.9)	(75.7, 98.1)	152/152 (100.0)	(97.6, 100.0)
Salmonella spp.[c]	29/30 (96.7)	(82.8, 99.9)	152/152 (100.0)	(97.6, 100.0)
Shigella/ EIEC[d]	43/45 (95.6)	(84.9, 99.5)	151/152 (99.3)	(96.4, 100.0)
Yersinia enterocolitica	3/3 (100.0)	(29.24, 100.0)	152/152 (100.0)	(97.6, 100.0)
Cryptosporidium spp.[e]	16/19 (84.2)	(60.4, 96.6)	152/152 (100.0)	(97.6, 100.0)
Giardia lamblia[f]	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)

Distribution of Mixed Infections in Archived positives.

Analytes Detected Simultaneously	n/N (%)
Total Mixed Infections	**84**
2	71/84 (84.5)
3	11/84 (13.1)
4	2/84 (2.4)

Prevalence of Analytes in Mixed Infections in Archived positives.

Prevalence in Mixed Infection N = 84		
Analyte	**n/N**	**%**
Campylobacter spp	7/84	8.3
E.coli 0157	28/84	33.3
EAEC	30/84	35.7
Shigella/EIEC	18/84	21.4
ETEC	17/84	20.2
STEC	33/84	39.3
Salmonella spp	12/84	14.3
Yersinia enterocolitica	4/84	4.8
Cryptosporidium spp	6/84	7.1
Giardia lamblia	6/84	7.1
Adenovirus 40/41	11/84	13.1
Norovirus GI/GII	4/84	4.8
Rotavirus A	7/84	8.3

Most Prevalence Multiple Detection Combinations (5 or more instances) in Archived positives.

Multiple Detection Combination	Number of Specimens
E.coli 0157+STEC	21
EAEC+ETEC	10

Testing of Contrived Specimens (Category IV)

For some analytes both prospective and archived testing were insufficient to demonstrate system 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 specimens that had previously tested negative for all BioCode GPP 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 GPP testing are presented in the Table below.

Summary of contrived specimen results

	PPA[a]		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)

a - All false negative specimens were tested by PCR/bidirectional sequencing and none could be confirmed as positives (not positive via sequencing). It is likely that these were either prepared incorrectly or degraded during shipping and handling.

Detailed breakdown of contrived testing samples

Organism	Strain ID	Strain characterization	LoD (CFU/mL or oocysts/mL)	Concentration Prepared (CFU/mL or oocysts/mL)	Multiple of LOD	Replicates tested	False negative	False negative distribution
Vibrio parahaemolyticus	ATCC 17802	strain EB101	5.00E+01	1.50E+02	3x	7	2	2
				1.00E+03	20x	1	0	0
				1.50E+03	30x	4		0
				2.50E+03	50x	3		0
Vibrio parahaemolyticus	BEI NR-21991	O1:K56 strain 10295	7.50E+02	1.50E+02	0.2x	10	3	3
				1.50E+03	2x	3		0
				2.37E+03	3x	5		0
				2.50E+03	3.15	2	0	0
				5.00E+03	6.3x	3		0

Organism	Strain ID	Strain characterization	LoD (CFU/mL or oocysts/mL)	Concentration Prepared (CFU/mL or oocysts/mL)	Multiple of LOD	Replicates tested	False negative	False negative distribution
				7.90E+03	10x	1		0
Vibrio parahaemolyticus	BEI NR-21990	O4:K12 strain 48057	5.00E+01	1.50E+02	3x	8	0	0
				1.00E+03	20x	3	1	0
				2.30E+04	460x	3		1
Vibrio parahaemolyticus	BEI NR-22002	O3:K6 strain TX2103	1.71E+02	1.50E+02	0.88x	9	1	1
				5.13E+02	3x	4		0
				2.50E+03	14.6x	4	0	0
				5.00E+03	29x	4		0
				1.71E+05	1000x	4		0
Vibrio parahaemolyticus	Zepto 0801903	strain Z134	5.00E+01	1.50E+02	3x	6	1	1
				5.00E+03	100x	4	0	0
				1.50E+04	300x	4		0
				1.92E+05	3840x	4		0
Vibrio cholerae	ATCC 25870	O:1 Vibrio, vib+	4.90E+02	1.47E+03	3x	9	0	0
				2.45E+04	50x	4	3	1
				4.90E+04	100x	3		1
				9.80E+04	200x	1		1
				9.80E+05	2000x	1		0
Vibrio cholerae	BEI NR-146	O:1 strain 2125 same as ATTC 39050 EL Tor	1.47E+03	1.47E+03	1x	6	1	1
				4.44E+03	3x	3		0
				1.48E+04	10x	1	0	0
				4.90E+04	33x	3		0
				1.47E+05	100x	4		0
Vibrio cholerae	BEI NR-149	O:2 (non-O:1, non- O:139 strain) Nanking 32/123	9.20E+03	1.47E+03	0.16x	3	3	2
				9.80E+03	1.1x	3		1
				1.47E+04	1.6x	1		0
				2.45E+04	2.7x	1		0
				2.76E+04	3x	3		0
				9.20E+04	9x	1	0	0
Vibrio vulnificus	ATCC 27562	(vib+) strain 324 CDC B9629	4.90E+02	1.47E+03	3x	13	2	2
				1.96E+03	4x	14	0	0
Vibrio vulnificus	ATCC 29306	strain CDC A1402	2.45E+03	7.35E+03	3x	10	3	3
				7.35E+04	30x	3	0	0
				1.23E+05	50x	3		0
				2.45E+05	100	4		0
Yersinia	ATCC	Bilups-1803-68,	1.50E+03	4.50E+03	3x	9	0	0

Organism	Strain ID	Strain characterization	LoD (CFU/mL or oocysts/mL)	Concentration Prepared (CFU/mL or oocysts/mL)	Multiple of LOD	Replicates tested	False negative	False negative distribution
enterocolitica	23715	biotype2, serotype O:8		4.50E+04	30x	4	0	0
				1.50E+05	100x	4		0
				1.48E+07	9900x	4		0
Yersinia enterocolitica	ATCC 29913	serotype O:8 CDC 497-70, BEI NR-207	1.50E+03	4.50E+03	3x	8	0	0
				6.00E+03	4x	4	0	0
				7.50E+04	50x	3		0
Yersinia enterocolitica	ATCC 9610	O:8 biovar 1 strain NCTC 12982	1.50E+03	4.50E+03	3x	8	1	1
				7.50E+04	50x	7	0	0
				1.50E+05	100x	4		0
Yersinia enterocolitica	BEI NR-206	O:8 ATCC 27729 strain WA	1.50E+03	4.50E+03	3x	8	0	0
				3.00E+04	20x	4	1	1
				4.50E+04	30x	4		0
Yersinia enterocolitica	BEI NR-212	O:3 strain NCTC 11175	1.50E+03	4.50E+03	3x	7	0	0
				1.50E+05	100x	4	0	0
				4.50E+05	300x	4		0
Yersinia enterocolitica	BEI NR-213	O:9 Strain NCTC 11174	1.50E+03	4.50E+03	3x	9	1	1
				1.50E+05	100x	2	0	0
Entamoeba histolytica	BEI NR-176	HB-301:NIH	3.10E+01	9.30E-01	3x	13	1	1
				1.24E+00	4x	2	1	0
				9.30E+00	30x	4		0
				3.10E+01	100x	4		0
				9.30E+01	300x	4		1
Entamoeba histolytica	BEI NR-177	200:NIH	3.10E+01	9.30E-01	3x	12	0	0
				1.55E+01	50x	8	1	1
				3.10E+01	100x	4		0
Entamoeba histolytica	BEI NR-178	HM-1:IMSS	3.10E+01	9.30E-01	3x	11	0	0
				6.20E+00	20x	4	1	0
				3.10E+01	100x	4		1
				9.30E+01	300x	4		0
Entamoeba histolytica	BEI NR-179	Rahman	3.10E+01	9.30E-01	3x	13	1	1
				9.30E+00	30x	4	0	0
				1.55E+01	50x	3		0
				3.00E+01	100x	4		0
Giardia lamblia	BEI NR-9232	Mario strain	1.81E+03	3.62E+03	2x	2	0	0
				5.42E+03	3x	12	0	0

Organism	Strain ID	Strain characterization	LoD (CFU/mL or oocysts/mL)	Concentration Prepared (CFU/mL or oocysts/mL)	Multiple of LOD	Replicates tested	False negative	False negative distribution
				9.04E+03	5x	3		0
				1.28E+04	7x	3		0
				1.81E+04	10x	2		0
				6.92E+04	38x	2		0
Giardia lamblia	BEI NR-9234	D.Hall strain	1.81E+03	5.42E+03	3x	14	0	0
				9.94E+03	5x	2	3	2
				1.63E+04	9x	2		0
				1.81E+04	10x	5		0
				3.62E+04	20x	2		1
				4.52E+04	25x	2		0
Giardia lamblia	BEI NR-9235	DAN strain	1.81E+03	5.42E+03	3x	13	1	1
				9.04E+03	5x	2	0	0
				3.62E+04	20x	2		0
				5.42E+04	30x	2		0
				6.36E+04	35x	2		0
Giardia lamblia	Water borne P101	Lot# 160428	1.81E+03	5.42E+03	3x	12	0	0
				2.71E+04	15x	6	0	0
				3.62E+04	20x	6		0
				3.75E+04	21x	2		0

Performance of Controls During Clinical Trials

During clinical evaluation of the BioCode GPP, at least one negative control was included on each run. Negative controls were S.T.A.R. Buffer, or well characterized negative specimens. The negative controls completed all processing steps (pretreatment/extractions/amplification/detection). It was recommended that external controls consisting of 4 pools of inactivated organisms (see table) be assayed on a rotating basis with the exception of Giardia (Waterborne Inc.), all controls were prepared by ZeptoMetrix (cat no. NATGIP-ABC; Giardia is now also available with that catalog number).

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

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[a]	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	20/20
NC	24/27[b]	34/35[c]	38/39[d]	15/15	111/116

a – The same pool 1 extract was being repeated freeze-thawed. It failed 3 times in a row for low signals, while the RNA IC signals were normal in the other samples and NC for the plate. When the group performed fresh extractions the PC signals returned to normal.

b – 2 failed for False positive signals and 1 for RNA IC not being detected

c – 1 RNA IC not detected

d – 1 RNA IC not detected

General Performance of Assay During Clinical Trials

Accounting of valid, partially invalid, and invalid runs.

Run Description	Number	% of Total
Valid runs with complete results	53	49.5
Valid runs with RNA-IC failures for one or more samples[a]	36	33.6
Partially or completely invalid runs	18	16.8
Total	107	100

a – All invalid results were reflex tested according to the IFU, and all but 3 were resolved as valid results.

Summary of issues causing partially or completely invalid runs.

Reason for failure	Number	% of Total
User error	4	3.7
Instrument/Alignment [a]	4	3.7
Negative Control contamination	2	1.9
Software installation error [b]	3	2.8
Reagent storage/ handling [c]	2	1.9
Unknown reason	3	2.8
Total Invalid runs	18	16.8

a MDx 3000 Alignment error at one clinical site accounted for 3 consecutive failed runs before it was corrected.
b Unapproved Software for remote access was installed that resulted in a software error for 2 runs software was removed and issue did not repeat.
c Reagent storage/handling error at one site accounted for 2 consecutive failed runs.

Clinical Specificity – Microbial Detection in Asymptomatic Volunteers

In order to determine baseline levels for each analyte included in the BioCode Gastrointestinal Pathogen Panel 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, Tampa General Hospital (clinical site 2) and University of Maryland (clinical site 3) 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 two samples (1.6%). After re-running this sample in accordance with the package insert instructions for use, inhibition was still observed in, so no result was reported. A total of 26 samples were positive. The results are summarized in the Table below.

Table. Demographic information for Asymptomatic Volunteers.

Gender	Number of Subjects
Male	67
Female	58
Total	125
Age	
<1-5	1
6-21	3
22-59	61
>60	60

Table. 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%)	49 (80.33%)	46 (76.67%)
Clostridium difficile	0 (0.00%)	0 (0.00%)	9 (14.75%)	11 (18.33%)
EAEC	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (1.67%)
ETEC	0 (0.00%)	0 (0.00%)	2 (3.28%)	0 (0.00%)
Salmonella spp	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (1.67%)
Giardia lamblia	0 (0.00%)	0 (0.00%)	1 (1.64%)	0 (0.00%)
Norovirus GI/GII	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (1.67%)

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 Gastrointestinal Pathogen Panel assay.

REPRODUCIBILITY STUDY

A study was performed to assess the Reproducibility of the BioCode Gastrointestinal Pathogen Panel 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 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%.

Qualitative results from Reproducibility study. All results are as expected with the exception of one false positive for *Giardia lamblia* and one false negative for STEC.

	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)	(95.98, 100.0)	90/90 (100.0)	(95.98, 100.0)	450/450 (100.0)	(99.18, 100.0)
Clostridium difficile	90/90 (100.0)	(95.98, 100.0)	90/90 (100.0)	(95.98, 100.0)	450/450 (100.0)	(99.18, 100.0)
Giardia lambia	90/90 (100.0)	(95.98, 100.0)	90/90 (100.0)	(95.98, 100.0)	449/450 (99.78)	(98.77, 99.99)
Adenovirus 40/41	90/90 (100.0)	(95.98, 100.0)	90/90 (100.0)	(95.98, 100.0)	450/450 (100.0)	(99.18, 100.0)
Shigella sonnei	90/90 (100.0)	(95.98, 100.0)	90/90 (100.0)	(95.98, 100.0)	450/450 (100.0)	(99.18, 100.0)
Vibrio parahaemolyticus	90/90 (100.0)	(95.98, 100.0)	90/90 (100.0)	(95.98, 100.0)	450/450 (100.0)	(99.18, 100.0)
ETEC	90/90 (100.0)	(95.98, 100.0)	90/90 (100.0)	(95.98, 100.0)	450/450 (100.0)	(99.18, 100.0)
Yersinia enterocolitica	90/90 (100.0)	(95.98, 100.0)	90/90 (100.0)	(95.98, 100.0)	450/450 (100.0)	(99.18, 100.0)
STEC	90/90 (100.0)	(95.98, 100.0)	89/90 (98.89)	(93.96, 99.97)	450/450 (100.0)	(99.18, 100.0)
Campylobacter jejuni	90/90 (100.0)	(95.98, 100.0)	90/90 (100.0)	(95.98, 100.0)	450/450 (100.0)	(99.18, 100.0)
Rotavirus A	90/90 (100.0)	(95.98, 100.0)	90/90 (100.0)	(95.98, 100.0)	450/450 (100.0)	(99.18, 100.0)
Cryptosporidium parvum	90/90 (100.0)	(95.98, 100.0)	90/90 (100.0)	(95.98, 100.0)	450/450 (100.0)	(99.18, 100.0)

Quantitative results from Reproducibility study. Fluorescent signals from BMBs with the same barcode are sorted and calculated to generate median fluorescence index (MFI) for each analyte (shown below). The presence or absence of a pathogen is determined relative to the validated assay cutoff by MFI.

Target	Analyte	Analyte	Repeatability		Between Runs		Between Days		Between Sites		Total	
Analyte/ Probe	Concentration	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Salmonella	Low	9174	1853	20.2	622	6.78	1464	15.95	0	0	2442	26.62
Salmonella	Med	11572	1204	10.41	469	4.05	1980	17.11	1190	10.28	2647	22.87
tcdA (C. difficile)	Low	5144	1407	27.35	1306	25.4	689	13.39	882	17.15	2222	43.2
tcdA (C. difficile)	Med	8802	2381	27.05	1444	16.4	1645	18.69	1337	15.19	3499	39.76
tcdB (C. difficile)	Low	11595	2004	17.29	847	7.31	2293	19.78	0	0	3161	27.27
tcdB (C. difficile)	Med	16441	1878	11.42	2219	13.49	2804	17.05	682	4.15	4096	24.91
Giardia lamblia	Low	30206	2436	8.06	2164	7.16	2516	8.33	3342	11.07	5302	17.55
Giardia lamblia	Med	20485	1024	5	3824	18.67	0	0	2839	13.86	4871	23.78
Adenovirus 40	Low	17155	2043	11.91	2850	16.62	1987	11.58	1030	6.01	4160	24.25
Adenovirus 40	Med	20850	1699	8.15	2666	12.79	1949	9.35	2447	11.74	4448	21.33
Shigella sonnei	Low	6115	1654	27.05	1487	24.31	1337	21.87	1043	17.06	2797	45.74
Shigella sonnei	Med	9707	2492	25.68	1968	20.28	0	0	1669	17.19	3588	36.96
Vibrio parahaemolyticus	Low	9927	2690	27.1	792	7.98	2956	29.78	966	9.73	4188	42.18
Vibrio parahaemolyticus	Med	12421	1540	12.39	2018	16.25	3126	25.16	0	0	4026	32.42
ST-a (ETEC)	Low	14272	3469	24.31	3562	24.96	3910	27.4	0	0	6326	44.32
ST-a (ETEC)	Med	21453	4151	19.35	2943	13.72	4869	22.7	2322	10.83	7416	34.57
ST-b (ETEC)	Low	13193	3246	24.6	1992	15.1	3719	28.19	733	5.56	5373	40.73
ST-b (ETEC)	Med	19807	3205	16.18	2141	10.81	4756	24.01	1829	9.23	6389	32.26
Yersinia enterocolitica	Low	19049	1970	10.34	1729	9.08	1647	8.65	2326	12.21	3872	20.33
Yersinia enterocolitica	Med	20020	1246	6.22	1474	7.36	2036	10.17	2156	10.77	3538	17.67
stx2	Low	16291	3347	20.54	5757	35.34	2353	14.44	5161	31.68	8747	53.69
stx2	Med	21117	2977	14.1	6931	32.82	0	0	5593	26.49	9390	44.47
Campylobacter jejuni	Low	21865	2384	10.9	3282	15.01	2727	12.47	2302	10.53	5402	24.71
Campylobacter jejuni	Med	26561	2992	11.26	3871	14.58	2549	9.6	2903	10.93	6234	23.47

Rotavirus A	Low	45792	4269	9.32	3274	7.15	2960	6.46	5063	11.06	7959	17.38
Rotavirus A	Med	45552	4673	10.26	0	0	4124	9.05	7622	16.73	9846	21.61
Cryptosporidium parvum	Low	25298	3754	14.84	2212	8.74	3314	13.1	3639	14.38	6573	25.98
Cryptosporidium parvum	Med	27966	2649	9.47	3123	11.17	2672	9.56	3226	11.54	5859	20.95

LIMIT OF DETECTION (LoD)

A study was performed to assess the performance of the BioCode Gastrointestinal Pathogen Panel on the BioCode MDx 3000 at the Limit of Detection (LoD) for both unpreserved Stool and Cary- Blair specimens. In this study the BioCode GPP was tested with quantified bacteria, virus or parasite stocks (note Norovirus GI and Norovirus GII were tested by CDC). 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 GPP on the BioCode MDx 3000 system 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 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.

Limit of Detection (LoD) for BioCode GPP.

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

Strain	Source	Unpreserved Stool LoD	Unpreserved Stool Detection	Cary-Blair Stool LoD	Cary-Blair Stool Detection
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} $TCID_{50}$/mL	20/20	1.0×10^{-1} $TCID_{50}$/mL	20/20
Adenovirus 41 (TAK)	Zeptometrix 0810085	9.4×10^{-2} $TCID_{50}$/mL	20/20	7.5×10^{-1} $TCID_{50}$/mL	20/20
Rotavirus A	ATCC VR-2018	2.5×10^3 $TCID_{50}$/mL	20/20	6.2×10^2 $TCID_{50}$/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

INTERFERING SUBSTANCES

A study was performed to demonstrate the accuracy of the BioCode GPP on the BioCode MDx 3000 in the presence of potentially inhibiting substances or microorganisms. Each member of the interfering substance panel (ISP) was added to prescreened negative stool sample spiked with representative members of the BioCode GPP at 3X LoD and a negative matrix only pre-screened negative stool. One parasite, one virus, one gram-positive bacterium, and one gram-negative bacterium were used as representatives for this study. Each sample was tested with and without potentially interfering substances. Each sample was extracted in triplicate on the easyMag System and tested in singlet with the BioCode GPP on the BioCode MDx 3000 system. Concentrations were determined by reviewing 510k summary results of previous GI panel clinical trials and FDA Guidance. No interfering substances or microorganisms were identified.

Interference Test Panel.

Sample Name	Organism	Source
Sample A	*Clostridium difficile* (NAP1) *Cryptosporidium parvum*	Zeptometrix 0801619cf Waterborne P102C
Sample B	Human Rotavirus A *Escherichia coli* 10C-3114 (STEC)	ATCC VR-2018 ATCC BAA-2217
Sample C	Negative Matrix Only	N/A

Potential Microbial Interferents. No inhibition or unexpected results were observed in the presence of high titer for the organisms in the table below.

Microbial Interferent	Source	Concentration (CFU/mL)	Interference Yes (Y) or No (N)
No Interferent (Control)	N/A	N/A	N
Bacteroides fragilis[a]	Zeptometrix 0801583	1×10^6	N
Blastocystis hominis[b]	ATCC 50752	1×10^5	N
Candida albicans	ATCC 14053	1×10^5	N
Clostridium difficile non-toxigenic	ATCC 700057	1×10^6	N
Enterococcus faecalis	ATCC 51299	1×10^6	N
Escherichia coli nonpathogenic	ATCC BAA-97	1×10^6	N
Pseudomonas aeruginosa	ATCC 39324	1×10^6	N
Saccharomyces boulardii	ATCC MYA-796	1×10^5	N

a – 1/3 wells of *B. fragilis* in the negative stool only sample produced a false positive result for Adenovirus 40/41. An additional 5 extractions were then repeated with no false positives.
b – *Blastocystis hominis* is titered in cells/mL.

Potential Interfering Substances.

Substance Interferent	Brand/Source	Concentration	Interference Yes (Y) or No (N)
No Interferent	N/A	N/A	N
Blood (EDTA)	Clinical Sample	40% w/v	N
Ampicillin	Ampicillin	152 µmol/L	N
Sodium hypochlorite	Bleach (10%)	50% w/v	N
Cholesterol	Cholesterol	5% w/v	N
Mineral Oil	Mineral oil, USP	50% w/v	N
Hydrocortisone	Hydrocortisone cream	50% w/v	N
Loperamide hydrochloride	Imodium	5% v/v	N
Sennosides	Senokot	5% w/v	N
Magnesium Hydroxide, Aluminum Hydroxide	Maalox	5% w/v	N
Metronidazole	Metronidazole	14 mg/mL	N
Benzalkonium chloride, Ethanol	Moist towelettes	50% w/v	N
Mono, di, triglycerides mix	Supelco	5% w/v	N
Mucin	Mucin	3 mg/ml	N
Naproxen sodium	Naproxen sodium	14mg/ml	N
Polymyxin B sulfate, bacitracin zinc, Neomycin	Neosporin	50% w/v	N
Nystatin	Nystatin	1000 U/mL	N
Bismuth subsalicylate	Pepto-Bismol	5% v/v	N
Petrolatum	Preparation H	5% v/v	N
Calcium carbonate	Tums	5% w/v	N
Vancomycin	Vancomycin	12.5 mg/mL	N

ANALYTICAL REACTIVITY/INCLUSIVITY

A study was performed to verify Analytical Reactivity/Inclusivity of the BioCode Gastrointestinal Pathogen Panel. 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.

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

Organism	Serotype	Source	Concentration Detected	Multiples of LoD Detected
E. coli O157	*E. coli* O157:H45	STEC SC373/2 TW07922	9.9 x 10^3 CFU/mL	3x
	E. coli 0157:HNM	STEC DA-26 TW07952	9.9 x 10^3 CFU/mL	3x
	E. coli 0157:H7	STEC 93-111 TW04863	9.9 x 10^3 CFU/mL	3x
	E. coli O157: H7	STEC MI06-19 TW14301	9.9 x 10^3 CFU/mL	3x
	E. coli O157:HNT	STEC DA-27 TW07953	9.9 x 10^3 CFU/mL	3x

Organism	Serotype	Source	Concentration Detected	Multiples of LoD Detected
	E. coli O157:H7 Strain EDL933	BEI NR-11	9.9 x 10^3 CFU/mL	3x
Shiga toxin producing *E. coli* (STEC)	*E. coli* O26:H11	STEC 2332/00 TW08998	7.5 x 10^3 CFU/mL	3x
	E. coli O113:H21	STEC CL-15 TW02318	7.5 x 10^3 CFU/mL	3x
	E. coli O45:H2	STEC DEC11C DEC11c	7.5 x 10^3 CFU/mL	3x
	E. coli O103:H2	STEC 107-226 TW07881	7.5 x 10^3 CFU/mL	3x
	E. coli O104:H21	STEC G5506 TW04909	7.5 x 10^3 CFU/mL	3x
	E. coli O111:H2	STEC RD8 TW06296	7.5 x 10^3 CFU/mL	3x
	E. coli O111:H8	STEC DEC8B	7.5 x 10^3 CFU/mL	3x
	E. coli O26:NM	STEC DA-22 TW07948	7.5 x 10^3 CFU/mL	3x
	E. coli O145:NM	ATCC BAA-2192	7.5 x 10^3 CFU/mL	3x
	E. coli O146:21	STEC DEC16E TW01383	7.5 x 10^3 CFU/mL	3x
	E. coli O45:H2	STEC MI05-14 TW14003	7.5 x 10^3 CFU/mL	3x
	E. coli O121:19	STEC MDCH-4 TW07614	7.5 x 10^3 CFU/mL	3x
	E. coli O121:NM	STEC DA-37 TW07972	7.5 x 10^3 CFU/mL	3x
	E. coli *O104:H4	ATCC BAA-2326	7.5 x 10^3 CFU/mL	3x
	E. coli 045:H2	ATCC BAA-2193	7.5 x 10^3 CFU/mL	3x
	E. coli O26: H11	BAA-2196	7.5 x 10^3 CFU/mL	3x
	E. coli O121:H19	BAA-2219	7.5 x 10^3 CFU/mL	3x

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

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
Enteroaggregative *E. coli* (EAEC)	*E. coli* O44:H18	STEC 042 TW04393	4.08 x 10^3 CFU/mL	3x
	E. coli O111a, 111b:K58:H21	ATCC 29552	4.08 x 10^3 CFU/mL	3x
	E. coli O104:H4	ATCC BAA-2326	4.08 x 10^3 CFU/mL	3x
	E. coli O3:K2a	BEI NR-102	4.08 x 10^3 CFU/mL	3x

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

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
Enteroinvasive *E. coli* (EIEC)	*E. coli O121*	ATCC BAA-2190	1.08 x 10^3 CFU/mL	3x
	E. coli O124:HNM	STEC 929-78 TW16574	1.08 x 10^3 CFU/mL	3x
	E. coli O136:H	STEC LT-41 TW06139	1.08 x 10^3 CFU/mL	3x
	E. coli O285A:HNM	BEI NR-101	1.08 x 10^3 CFU/mL	3x
	E. coli O15	ATCC 49105	1.08 x 10^3 CFU/mL	3x
Shigella boydii	*Shigella boydii* (Type 1)	ATCC 9207	1.31 x 10^3 CFU/mL	3x
	Shigella boydii, (Type 2)	BEI NR-521	1.31 x 10^3 CFU/mL	3x
	Shigella boydii (Type 7)	ATCC 9905	1.31 x 10^3 CFU/mL	3x
	Shigella boydii (Type 20)	ATCC BAA-1247	1.31 x 10^3 CFU/mL	3x
	Shigella boydii (Type 3)	ATCC 8702	1.31 x 10^3 CFU/mL	3x
	Shigella dysenteriae (Type 1)	BEI NR-520	1.31 x 10^3 CFU/mL	3x

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
Shigella dysenteriae	*Shigella dysenteriae (Type 3)*	ATCC 9751	1.31 x 10^3 CFU/mL	3x
	Shigella dysenteriae (Type 2)	ATCC 9750	1.31 x 10^3 CFU/mL	3x
	Shigella dysenteriae (Type 5)	ATCC 9764	1.31 x 10^3 CFU/mL	3x
	Shigella dysenteriae (Type 12)	ATCC 49552	1.31 x 10^3 CFU/mL	3x
Shigella flexneri	*Shigella flexneri*, strain 24570 (Type 2a)	BEI NR-517	1.31 x 10^3 CFU/mL	3x
	Shigella flexneri, strain 2457T (Type 2a)	BEI NR-518	1.31 x 10^3 CFU/mL	3x
	Shigella flexneri (Type 2b)	ATCC 12022	1.31 x 10^3 CFU/mL	3x
	Shigella flexneri (Type 6)	ATCC 12025	1.31 x 10^3 CFU/mL	3x
	Shigella flexneri (Type 1b)	ATCC 9380	1.31 x 10^3 CFU/mL	3x
Shigella sonnei	*Shigella sonnei*	ATCC 25931	1.31 x 10^3 CFU/mL	3x
	Shigella sonnei	ATCC 11060	1.31 x 10^3 CFU/mL	3x
	Shigella sonnei	ATCC 9290	1.31 x 10^3 CFU/mL	3x
	Shigella sonnei	ATCC 29029	1.31 x 10^3 CFU/mL	3x

***Salmonella* Inclusivity results**

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
Salmonella enterica subsp. *arizonae*	*Salmonella enterica subsp. Enterica*	ATCC 13314	6.45 x 10^3 CFU/mL	3x
Salmonella enterica subsp. *salamae*	serovar Tranoroa	ATCC 700148	6.45 x 10^3 CFU/mL	3x
Salmonella enterica subsp. *enterica*	serovar Montevideo	ATCC 7001 BAA-710	6.45 x 10^3 CFU/mL	3x
	serovar Enteritidis	SGSC4901	6.45 x 10^3 CFU/mL	3x
	serovar Enteritidis	ATCC 4931	6.45 x 10^3 CFU/mL	3x
	serovar Oranienburg	SGSC4079	6.45 x 10^3 CFU/mL	3x
	serovar Paratyphi B var.L(+) tartrate+	SGSC4150	6.45 x 10^3 CFU/mL	3x
	serovar Typhimurium	SGSC1412	6.45 x 10^3 CFU/mL	3x
	serovar Saintpaul	SGSC2512	6.45 x 10^3 CFU/mL	3x
	serovar S. typhimurium LT2	SGSC2666	6.45 x 10^3 CFU/mL	3x
	serovar Newport	SGSC2493	6.45 x 10^3 CFU/mL	3x
	serovar Newport	ATCC 6962	6.45 x 10^3 CFU/mL	3x
	serovar Muenchen	SGSC2490	6.45 x 10^3 CFU/mL	3x
	serovar Agona	SGSC2458	6.45 x 10^3 CFU/mL	3x
	serovar Javiana	SGSC4917	6.45 x 10^3 CFU/mL	3x
	serovar Schwarzengrund	SGSC2514	6.45 x 10^3 CFU/mL	3x
	serovar Heidelberg	SGSC2480	6.45 x 10^3 CFU/mL	3x
	serovar Infantis	SGSC2484	6.45 x 10^3 CFU/mL	3x
	serovar Montevideo	SGSC2487	6.45 x 10^3 CFU/mL	3x
	serovar Thompson	SGSC 2519	6.45 x 10^3 CFU/mL	3x
	serovar Hadar	SGSC4965	6.45 x 10^3 CFU/mL	3x
	serovar Mississippi	SGSC4078	6.45 x 10^3 CFU/mL	3x
	serovar Paratyphi A	SGSC2499	6.45 x 10^3 CFU/mL	3x
	serovar Choleraesuis	SGSC4770	6.45 x 10^3 CFU/mL	3x
	serovar Choleraesuis	ATCC 13312	6.45 x 10^3 CFU/mL	3x

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
	serovar Dublin	SGSC4157	6.45 x 10^3 CFU/mL	3x
	serovar Braenderup	ATCC® 700136	6.45 x 10^3 CFU/mL	3x
	serovar Bareilly	ATCC® 9115	6.45 x 10^3 CFU/mL	3x
	serovar Typhi	Zeptometrix 0801933	6.45 x 10^3 CFU/mL	3x
Salmonella enterica subsp. II	Salmonella enterica subs. II	SGSC3039	6.45 x 10^3 CFU/mL	3x
Salmonella enterica subsp. IIIa	Salmonella enterica subs. IIIa	SGSC3061	6.45 x 10^3 CFU/mL	3x
Salmonella enterica subsp. IIIb	Salmonella enterica subs. IIIb	SGSC3068	6.45 x 10^3 CFU/mL	3x
Salmonella enterica subsp. IV	Salmonella enterica subs. IV	SGSC3074	6.45 x 10^3 CFU/mL	3x
Salmonella enterica subsp. VI	Salmonella enterica subs VI	SGSC3116	6.45 x 10^3 CFU/mL	3x

Campylobacter **inclusivity results.**

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
Campylobacter spp.	*Campylobacter jejuni* subsp. *jejuni*	BEI NR-399	2.10 x 10^3 CFU/mL	3x
	Campylobacter jejuni subsp. *jejuni*	BEI NR-400	2.10 x 10^3 CFU/mL	3x
	Campylobacter jejuni subsp. *doylei*	ATCC 49350	2.10 x 10^3 CFU/mL	3x
	Campylobacter jejuni subsp. *doylei*	ATCC 49349	2.10 x 10^3 CFU/mL	3x
	Campylobacter coli	ATCC 43478	1.68 x 10^2 CFU/mL	3x
	Campylobacter coli	ATCC 43485	1.68 x 10^2 CFU/mL	3x
	Campylobacter coli	BEI HM-296	1.68 x 10^2 CFU/mL	3x
	Campylobacter coli	ATCC 43484	1.68 x 10^2 CFU/mL	3x

Vibrio **spp inclusivity results.**

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
Vibrio spp. (not *parahaemolyticus*)	*Vibrio cholerae* (O:1 Inaba, Biotype E1 Tor)	BEI NR-147	1.47 x 10^3 CFU/mL	3x
	Vibrio cholerae (O:1 Inaba, Biotype E1 Tor)	BEI NR-146	1.47 x 10^3 CFU/mL	3x
	Vibrio cholerae(O:2)	BEI NR-149	1.47 x 10^3 CFU/mL	3x
	Vibrio cholerae (O:4)	BEI NR-151	1.47 x 10^3 CFU/mL	3x
	Vibrio cholerae (O:139)	BEI NR-144	1.47 x 10^3 CFU/mL	3X
	Vibrio cholerae (O:1 Ogawa)	ATCC 14035	1.47 x 10^3 CFU/mL	3x
	Vibrio vulnificus	ATCC 27562	1.47 x 10^3 CFU/mL	3x
	Vibrio vulnificus	ATCC BAA-88	1.47 x 10^3 CFU/mL	3x
	Vibrio vulnificus	ATCC 43382	4.9 x 10^3 CFU/mL	10X
	Vibrio vulnificus	ATCC 29306	1.47 x 10^3 CFU/mL	3x
	Vibrio vulnificus	ATCC 29307	1.47 x 10^3 CFU/mL	3x
Vibrio parahaemolyticus	*Vibrio parahaemolyticus*	BEI NR-21990	3.75 x 10^1 CFU/mL	3x
	Vibrio parahaemolyticus	BEI NR- 21991	3.75 x 10^1 CFU/mL	3x

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
	Vibrio parahaemolyticus	BEI NR-22002	3.75 x 10^1 CFU/mL	3x
	Vibrio parahaemolyticus	Zepto 0801903	3.75 x 10^1 CFU/mL	3x
	Vibrio parahaemolyticus	BEI NR-22013	3.75 x 10^1 CFU/mL	3x

Yersinia enterocolitica **inclusivity results.**

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
Yersinia enterocolitica	*Yersinia enterocolitica* (O:8)	ATCC 9610	4.43 x 10^3 CFU/mL	3x
	Yersinia enterocolitica (O:3)	BEI NR-212	4.43 x 10^3 CFU/mL	3x
	Yersinia enterocolitica (O:8)	BEI NR-206	4.43 x 10^3 CFU/mL	3x
	Yersinia enterocolitica	ATCC 29913	4.43 x 10^3 CFU/mL	3x
	Yersinia enterocolitica	BEI NR-213	4.43 x 10^3 CFU/mL	3x

Clostridium difficile **inclusivity results.**

Organism	TOXINOTYPE	Source	Concentration Detected	Multiple of LoD Detected
Clostridium difficile	0 A+B+	ATCC 43255-FZ	2.48 x 10^3 CFU/mL	3x
	0 A+B+	ATCC 700792-FZ	2.48 x 10^3 CFU/mL	3x
	0 A+B+	ATCC BAA-1382-fz	2.48 x 10^3 CFU/mL	3x
	0 A+B+	ATCC 51695-fz	2.48 x 10^3 CFU/mL	3x
	0 A+B+	ATCC 43599-fz	2.48 x 10^3 CFU/mL	3x
	0 A+B+	ATCC 43596-fz	2.48 x 10^3 CFU/mL	3x
	0 A+B+	ATCC 17858-fz	2.48 x 10^3 CFU/mL	3x
	0 A+B+	ATCC 43594	2.48 x 10^3 CFU/mL	3x
	0 A+B+	ATCC 43600	2.48 x 10^3 CFU/mL	3x
	0 A+B+	ATCC 17857	2.48 x 10^3 CFU/mL	3x
	0 A+B+	ATCC BAA-1871	2.48 x 10^3 CFU/mL	3x
	0 A+B+	ATCC BAA-1872	2.48 x 10^3 CFU/mL	3x
	VIII A-B+	ATCC 43598	2.48 x 10^3 CFU/mL	3x
	III A+B+ (Nap1)	ATCC BAA-1805	2.48 x 10^3 CFU/mL	3x
	XXII A+B+	ATCC BAA-1814	2.48 x 10^3 CFU/mL	3x
	III A+B+	ATCC BAA-1870	2.48 x 10^3 CFU/mL	3x
	V A+B+	ATCC BAA-1875	2.48 x 10^3 CFU/mL	3x

Adenovirus 40/41 inclusivity results.

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
Adenovirus 40	*Human adenovirus 40 (dugan)*	lot #K1220A	3.0E-01 $TCID_{50}$/mL	3
	n/a	*sample ID # SP143*	Clinical Sample unknown	3
	n/a	*sample ID # SP258*	Clinical Sample unknown	3
Adenovirus 41	*n/a*	*sample ID # SP170*	Clinical Sample unknown	3
	n/a	*sample ID # SP174*	Clinical Sample unknown	3
	n/a	*sample ID # SP 276*	Clinical Sample unknown	3
	n/a	*sample ID # SP288*	Clinical Sample unknown	3
	n/a	*sample ID # SP309*	Clinical Sample unknown	3
	n/a	*sample ID # SP329*	Clinical Sample unknown	3
	n/a	*sample ID # SP446*	Clinical Sample unknown	3

Norovirus GI/GII inclusivity results.

Organism	Genotype	Source	Concentration Detected	Multiple of LoD Detected
*Norovirus GI	GI.1	Clinical Specimen	1.93×10^6 copies/gram	3x
	GI.2	Clinical Specimen	1.93×10^6 copies/gram	3x
	GI.3	Clinical Specimen	1.93×10^8 copies/gram	300x
	GI.4	Clinical Specimen	1.93×10^6 copies/gram	3x
	GI.5	Clinical Specimen	1.93×10^6 copies/gram	3x
	GI.6	Clinical Specimen	1.93×10^6 copies/gram	3x
	GI.7	Clinical Specimen	1.93×10^6 copies/gram	3x
	GI.8	Clinical Specimen	1.93×10^6 copies/gram	3x
[a]Norovirus GII	GII.1	Clinical Specimen	1.57×10^5 copies/gram	3x
	GII.2	Clinical Specimen	1.57×10^6 copies/gram	30x
	GII.3	Clinical Specimen	1.57×10^6 copies/gram	30x
	GII.4 New Orleans	Clinical Specimen	1.57×10^6 copies/gram	30x
	GII.4 Sydney	Clinical Specimen	1.57×10^5 copies/gram	3x
	GII.5	Clinical Specimen	1.57×10^5 copies/gram	3x
	GII.6	Clinical Specimen	1.57×10^5 copies/gram	3x
	GII.7	Clinical Specimen	1.57×10^5 copies/gram	3x
	GII.8	Clinical Specimen	1.57×10^6 copies/gram	30x
	GII.12	Clinical Specimen	1.57×10^5 copies/gram	3x
	GII.13	Clinical Specimen	1.57×10^5 copies/gram	3x
	GII.14	Clinical Specimen	1.57×10^6 copies/gram	30x
	GII.16	Clinical Specimen	1.57×10^6 copies/gram	30x
	GII.17	Clinical Specimen	1.57×10^5 copies/gram	3x

a – Assayed at CDC.

Norovirus GI/GII inclusivity results.

Organism	Serotype	Source	Concentration Detected	Multiples of LoD Detected
Rotavirus A	Rotavirus A (DS-1)	ATCC VR-2550	7.44 x 10^3 IU/mL	3x
	Rotavirus A (HRV 89-12C2)	ATCC VR-2272	7.44 x 10^3 IU/mL	3x
	Rotavirus A (WISC2)	ATCC VR-2417	7.44 x 10^3 IU/mL	3x
	Rotavirus A (HRV 408)	ATCC VR-2273	7.44 x 10^3 IU/mL	3x
	Rotavirus A (HRV 248)	ATCC VR-2274	7.44 x 10^3 IU/mL	3x
	Rotavirus A (HU)	ATCC VR-1546	7.44 x 10^3 IU/mL	3x
	Rotavirus A (HRV CJN)	ATCC VR-2275	7.44 x 10^3 IU/mL	3x
	Rotavirus A G1	Clinical Sample	Ct= 20	N/A[a]
	Rotavirus A G2	Clinical Sample	Ct= 22	
	Rotavirus A G3	Clinical Sample	Ct= 26	
	Rotavirus A G4	Clinical Sample	Ct= 21	
	Rotavirus A G9	Clinical Sample	Ct= 20	
	Rota vaccine strain	RotaTeq vaccine	1.00 x 10^8 pfu/mL	
	Rota vaccine strain	Rotarix vaccine	1.00 x 10^6 $CCID_{50}$/mL	

a – Tested at CDC at indicated concentration at CDC, concentration cannot be related to LoD.

***Cryptosporidium* spp inclusivity results.**

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
C. parvum	*DNA subtype /IIaA17G1R1*	UKCR UK28	Clinical Sample unknown	3
	DNA subtype /IIaA15G2R1	UKCR UK29	Clinical Sample unknown	3
	DNA subtype /IIaA19G1R1	UKCR UK30	Clinical Sample unknown	3
	DNA subtype /IIdA22G1	UKCR UK31	Clinical Sample unknown	3
	DNA subtype /IIdA15G1	UKCR UK32	Clinical Sample unknown	3
C. hominis	*DNA subtype IaA14R3*	UKCR UKH14	Clinical Sample unknown	3
	DNA subtype IdA18	UKH12	Clinical Sample unknown	3
	DNA subtype IbA10G2	UKH13	Clinical Sample unknown	3
	DNA E. coli O103:H2	NR2520	Clinical Sample unknown	3

***Entamoeba histolytica* inclusivity results.**

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
Entamoeba histolytica	200:NIH	BEI NR-177	9.36 x 10^{-1} cysts/mL	3x
	HB-301:NIH	BEI NR-176	9.36 x 10^{-1} cysts/mL	3x
	Rahman	BEI NR-179	9.36 x 10^{-1} cysts/mL	3x
	H-303:NIH	BEI NR-180	9.36 x 10^{-1} cysts/mL	3x

***Giardia lamblia* inclusivity results.**

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
Giardia lamblia/ intestinalis	Egypt-4	BEI NR-9231	5.42 x 10^3 cysts/mL	3x
	Mario	BEI NR-9232	5.42 x 10^3 cysts/mL	3x
	D.Hall	BEI NR-9234	5.42 x 10^3 cysts/mL	3x
	DAN	BEI NR-9235	5.42 x 10^3 cysts/mL	3x

ANALYTICAL SPECIFICITY/CROSS REACTIVITY

A study was performed to verify that the BioCode 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.

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

Empirical testing and *in silico* sequence analysis indicate that the *Vibrio* spp assay may also react with some less common *Vibrio* species (i.e., *V. alginolyticus*, and *V. mimicus*).

Empirical 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. entericolitica* (~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 (stx; identical to stx1 of STEC) is found in *Shigella dysenteriae*; therefore, a BioCode GPP 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 *S. dysenteriae*.

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

Empirical testing has demonstrated that these assays will detect recombinant viruses included in Rotavirus vaccines.

Empirical testing indicates potential for cross reactivity with *C. cuniculus* and *C. meleagridis* with the Cryptosporidium assay.

No Cross reactivity was observed for the organisms tested below.

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 coli* Non pathogenic	*Saccharomyces boulardii*
Bacillus cereus	*Escherichia coli* Non pathogenic	*Serratia liquefaciens*
Bacteroides fragilis	*Escherichia hermannii*	*Serratia marcescens*
Bacteroides thetaiotaomicron	*Escherichia vulneris*	*Shewanella algae*
Bifidobacterium breve	*Fusobacterium varium*	*Staphylococcus aureus*
Campylobacter fetus	*Gardnerella vaginalis*	*Staphylococcus epidermidis*
Campylobacter hyointestinalis	*Gemella morbillorum*	*Stenotrophomonas maltophilia*
Campylobacter lari	*Grimontia hollisae (formerly vibrio)*	*Streptococcus agalactiae*
Campylobacter upsaliensis	*Haemophilus influenzae*	*Streptococcus intermedius*
Candida albicans	*Hafnia alvei*	*Streptococcus pyogenes*
Cedecea davisae	*Helicobacter pylori*	*Streptococcus salivarius*
Chlamydia trachomatis	*Klebsiella oxytoca*	*Streptococcus suis*
Citrobacter amalonaticus	*Klebsiella pneumoniae*	*Trabulsiella guamensis*
Citrobacter freundii	*Lactobacillus acidophilus*	*Veillonella parvula*
Clostridium difficile non-toxigenic	*Lactobacillus reuteri*	*Vibrio alginolyticusa*
Clostridium difficile non-toxigenic	*Lactococcus lactis*	*Vibrio fluvialis*
Clostridium difficile non-toxigenic	*Leminorella grimontii*	*Vibrio mimicusa*
Clostridium histolyticum	*Listeria monocytogenes*	*Yersinia bercovierib*
Clostridium perfringens	*Morganella morganii*	*Yersinia frederikseniib*
Clostridium septicum	*Peptoniphilus asaccharolyticus*	*Yersinia intermediab*
Clostridium sordellii	*Plesiomonas shigelloides*	*Yersinia mollaretiib*
Clostridium sporogenes	*Porphyromonas asaccharolytica*	*Yersinia pseudotuberculosis*
Clostridium tetani	*Prevotella melaninogenica*	*Yersinia rohdei*[b]
Edwardsiella tarda	*Proteus mirabilis*	

a – Detected as Vibrio spp at high titers, see full submission for details.

b – Detected as Yersinia enterocolitica at high titers, see full submission for details.

No Cross reactivity was observed for the viruses and parasites tested below.

Viruses		Parasites
Adenovirus 3	Enterovirus 68	*Cryptosporidium cuniculus* (DNA)b
Adenovirus 4	Enterovirusa	*Cryptosporidium felis* (DNA)
Adenovirus 7a	Echovirus 11a	*Cryptosporidium meleagridis* [b]
Adenovirus 8	HSV Type 2	*Cryptosporidium meleagridis* (DNA)[b]
Adenovirus 14	Norovirus GIV	*Cryptosporidium muris*
Adenovirus 37	Rhinovirus 1A	*Cryptosporidium ubiquitum* (DNA)
Astrovirus type 1a	Sapovirus GIa	*Encephalitozoon cuniculi*
Astrovirus type 4a	Sapovirus GIVa	*Encephalitozoon hellem*
Coronavirus 229E	Sapovirus GVa	*Encephalitozoon intestinalis*
Coronavirus NL63	**Parasites**	*Giardia muris*
Coxsackie virus A16a	*Blastocystis hominis*[a]	*Pentatrichomonas hominis*
Coxsackievirus B3	*Blastocystis hominis*	*Toxoplasma gondii*
Cytomegalovirus (CMV)	*Cryptosporidium canis* (DNA)	

a – tested at CDC

b – Detected as Cryptosporidium spp.

No Cross reactivity was observed for BioCode GPP targets.

BioCode GPP 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
Enteroaggregative *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

No Cross reactivity was predicted by *in silico* analysis.

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).

COMPETITIVE INHIBITION

A study was performed to evaluate the potential for inhibition in samples with mixed infections. Prescreened negative stool was spiked with one target at high concentration (≥10^6 CFU/mL for bacteria and ≥10^5 units/mL for viruses or parasites) and two targets at medium concentration (≤3x LoD). Common co-infections were determined by reviewing results of previous GI Panel clinical trials from 510(k) 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 Gastrointestinal Pathogen Panel on the BioCode MDx 3000 system. No competitive inhibition was observed.

Competitive inhibition testing results.

Panel Designation	Viral/Bacteria Strain	Source	Level	Screening Titer	Target Probe	Average MFI	Screening Result (n of 3)
CI-1	*Clostridium difficile*	Zepto 801619	High	3.0x10^6 CFU/mL	tcdB	23969	3/3
	Rotavirus A	ATCC VR-2018	Medium	7.44X10^3 $TCID_{50}$/mL	Rota	33899	3/3
	Escherichia coli E2348/69 (EPEC)	STEC TW06375	Medium	7.02x10^3 CFU/mL	EPEC	11585	3/3
CI-2	O92:H33 *Escherichia coli* (EAEC)	STEC JM221 TW04440	High	3.0x10^6 CFU/mL	EAEC	26578	3/3
	Escherichia coli E2348/69 (EPEC)	STEC TW06375	Medium	7.02X10^3 CFU/mL	EPEC	10680	3/3
	Clostridium difficile	Zepto 801619	Medium	5.7x10^2 CFU/mL	tcdB	8775	3/3
CI-3	*Escherichia coli* E2348/69 (EPEC)	STEC TW06375	High	3.0x10^6 CFU/mL	EPEC	27671	3/3
	Clostridium difficile	Zepto 801619	Medium	5.7x10^2 CFU/mL	tcdB	8876	3/3

Panel Designation	Viral/Bacteria Strain	Source	Level	Screening Titer	Target Probe	Average MFI	Screening Result (n of 3)
	Rotavirus A	ATCC VR-2018	Medium	$7.44X10^3$ $TCID_{50}$/mL	Rota	31617	3/3
CI-4	*Escherichia coli* E2348/69 (EPEC)	STEC TW06375	High	$3x10^6$ CFU/mL	EPEC	26059	3/3
	O92:H33 *Escherichia coli* (EAEC)	STEC JM221 TW04440	Medium	$4.08X10^3$ CFU/mL	EAEC	22175	3/3
	Campylobacter jejuni subsp .jejuni	ATCC 33292	Medium	$2.1X10^3$ CFU/mL	Campy	32286	3/3
CI-5	*Campylobacter jejuni subsp. Doylei*	ATCC 49349	High	$3.0x10^6$ CFU/mL	Campy	24738	3/3
	Escherichia coli E2348/69 (EPEC)	STEC TW06375	Medium	$7.02X10^3$ CFU/mL	EPEC	7382	3/3
	O92:H33 *Escherichia coli* (EAEC)	STEC JM221 TW04440	Medium	$4.08X10^3$ CFU/mL	EAEC	22488	3/3
CI-6	O92:H33 *Escherichia coli* (EAEC)	STEC JM221 TW04440	High	$3x10^6$ CFU/mL	EAEC	24968	3/3
	Campylobacter jejuni sub sp .jejuni	ATCC 33292	Medium	$2.1X10^3$ CFU/mL	Campy	33202	3/3
	Escherichia coli E2348/69 (EPEC)	STEC TW06375	Medium	$7.02X10^3$ CFU/mL	EPEC	10342	3/3
CI-7	Shiga-toxin producing *E. coli* (STEC)	ATCC BAA-2217	High	$3.0x10^6$ CFU/mL	stx2	34913	3/3
	Giardia intestinalis	waterborne P101	Medium	$5.42X10^3$ cysts/mL	G.lam	37282	3/3
	Shigella sonnei	ATCC 29930	Medium	$1.31X10^3$ CFU/mL	Shig	10518	3/3
CI-8	*Giardia intestinalis*	waterborne P101	High	$3.0x10^5$ cysts/mL	G.lam	12832	3/3
	Shigella sonnei	ATCC 29930	Medium	$1.31X10^3$ CFU/mL	Shig	12530	3/3
	Shiga-toxin producing *E. coli* (STEC)	ATCC BAA-2217	Medium	$7.5X10^3$ CFU/mL	stx2	29785	3/3
CI-9	*Shigella sonnei*	ATCC 29930	High	$3.0x10^6$ CFU/mL	Shig	12483	3/3
	Shiga-toxin producing *E. coli* (STEC)	ATCC BAA-2217	Medium	$7.5X10^3$ CFU/mL	stx2	31890	3/3
	Giardia intestinalis	waterborne P101	Medium	$5.42X10^3$ cysts/mL	G.lam	31227	3/3

CROSS CONTAMINATION/SAMPLE CARRYOVER

A study was performed to demonstrate the absence of carryover or cross-contamination when using the BioCode Gastrointestinal Pathogen Panel in conjunction with the easyMag. High-positive samples (EAEC at 10^6 CFU/mL) were tested alternating with no-template control samples in a

"checkerboard" pattern. Samples were extracted checkerboard and assayed in singlet. The study consisted of five complete runs from extraction to BioCode MDx 3000 results on one instrument. No cross contamination was observed.

FRESH VS. FROZEN STABILITY

Since many of the unpreserved samples in three of the four sites using 60 spiked unpreserved stool specimens to assess fresh vs frozen specimen stability. Specimens were contrived and tested at time 0 and 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 there were not tested. Frozen samples displayed stability throughout the length of the study.

Fresh Vs. Frozen results.

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[a]/63

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

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) STa	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%

SPECIMEN STABILITY

A study was performed to assess the specimen stability limitations for the optimal performance of the BioCode Gastrointestinal Pathogen Panel on the BioCode MDx 3000. 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, one virus, one-gram positive bacterium, and one-gram negative bacterium was used for testing. 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 stability under the various conditions throughout the length of the study.

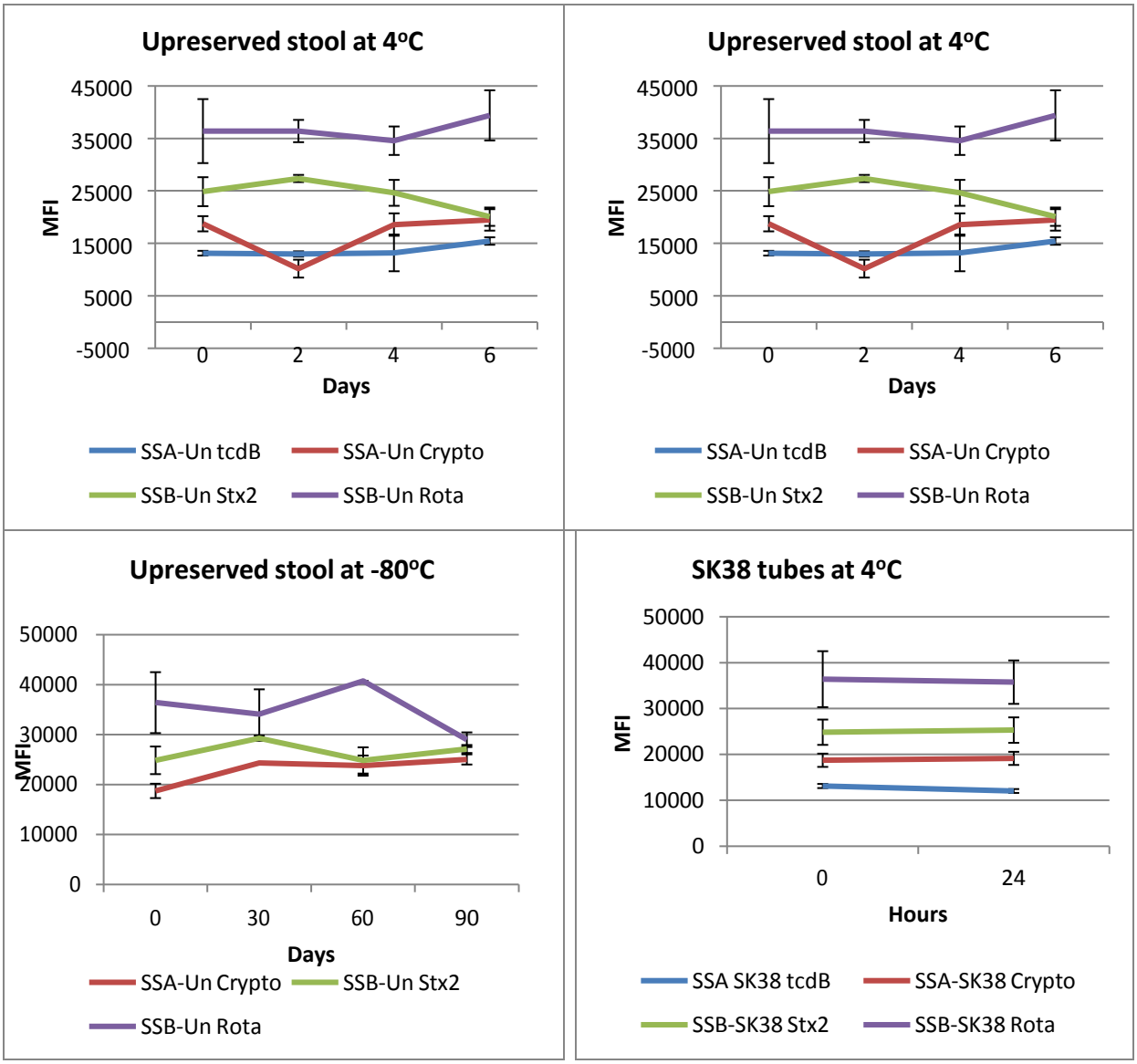

Figure. Graphic display of MFI results at indicated storage temperatures and timepoints.

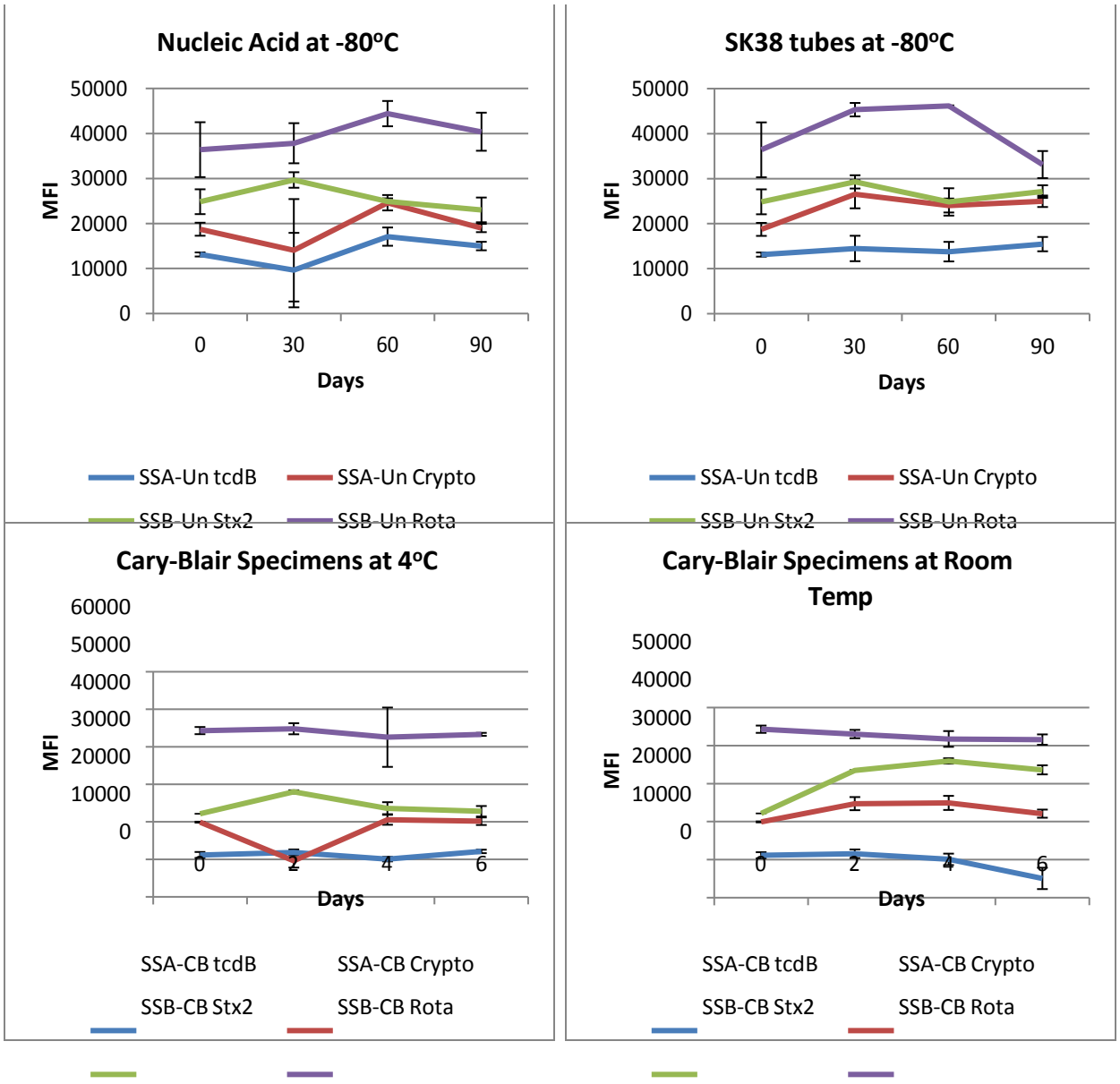

Figure. Graphic display of MFI results at indicated storage temperatures and timepoints.

CONCLUSION

The intended use and fundamental scientific technology of the BioCode Gastrointestinal Pathogen Panel Assay is substantially equivalent to the predicate device. Clinical and non-clinical studies have established that the BioCode Gastrointestinal Pathogen Panel is substantially equivalent to the predicate device.