BioCode®

Gastrointestinal Pathogen Panel (GPP)

Package Insert

Applied BioCode

Barcoded Magnetic Beads | Flexible Syndromic Tests

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Barcoded Magnetic Beads | Flexible Syndromic Tests

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For *in vitro* Diagnostic Use.

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NAME AND INTENDED USE

BioCode® Gastrointestinal Pathogen Panel (GPP)

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

- Adenovirus F 40/41
- *Campylobacter (C. jejuni/C. coli)*
- *Clostridium difficile* (*C. difficile*) toxin A/B (from fresh specimens only)
- *Cryptosporidium* (*C. hominis/C. parvum)*
- *Entamoeba histolytica*
- *Escherichia coli (E. coli)* O157
- Enterotoxigenic *E. coli* (ETEC) LT/ST
- Enteroaggregative *E. coli* (EAEC)f
- *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*)/ Enteroinvasive *E. coli* (EIEC)
- *Vibrio* spp. (*V. cholerae/V. parahaemolyticus/V. vulnificus*), specific identification of *V. parahaemolyticus*
- *Yersinia enterocolitica*

The BioCode GPP is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness and results are meant to be used in conjunction with other clinical, laboratory, and epidemiological data.

Positive results do not rule out co-infection with organisms not included in the BioCode Gastrointestinal Pathogen Panel. 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.

SUMMARY AND EXPLANATION OF THE TEST

Gastroenteritis is a leading cause of death worldwide across all age groups.[1] It is estimated that 1.31 million people died from diarrheal disease in 2015, with nearly 500,000 of those fatalities occurring in children under five years old.[1,2] The burden is greatest in low-income countries with poor sanitation and limited access to clean water.[1] Outbreaks caused by many of these microbes are tracked by the Centers for Disease Control and Prevention (CDC) in the United States as well as the European Surveillance System (TESSy), the disease surveillance data bank for the European Centre for Disease Prevention and Control (ECDC). 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 GPP test are available within about 5 hours.

Table. Bacteria, Viruses, Diarrheagenic *E. coli/Shigella*, and Parasites Detected by the BioCode Gastrointestinal Pathogen Panel

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

Summary of Detected Organisms

Bacteria

Campylobacter (*C. coli* and *C. jejuni*)

Campylobacter spp. are gram-negative, S-shaped or spiral-shaped bacteria that are non-spore forming and often motile. Diarrheal illness is most commonly associated with *C. jejuni* and *C. coli*, and less frequently with *C. upsaliensis*. These bacteria are responsible for a large portion of food poisoning stemming from undercooked poultry, but can also be transmitted through unpasteurized dairy and contaminated water and produce. *Campylobacter* infections peak during the summer months.[3] Campylobacteriosis became a national notifiable disease in the United States in 2015, and causes an estimated 1.3 million illnesses and 120 deaths in the US annually.[3,4] In Europe *Campylobacter* is responsible for more foodborne illness cases than any other pathogen.[5,6,7] TESSy has noted an increase in the number of reported *Campylobacter* cases across Europe over the past several years, with over 220,000 cases reported in both 2014 and 2015.[6,7] *Campylobacter* is the leading bacterial cause of diarrhea in tourists visiting Southeast Asia.[8] Campylobacteriosis symptoms include watery or bloody diarrhea, fever, and abdominal cramps, although some patients are asymptomatic while a minority may acquire Guillain-Barré syndrome or arthritis.[9]

Clostridium difficile

Clostridium difficile is a gram-positive, anaerobic, rod-shaped bacterium. This organism sheds robust

spores that can maintain virulence even after exposure to antibiotics or disinfectants. Among the causative agents of acute gastroenteritis (AGE), *C. difficile* claims the highest mortality rate in high income countries.[1] Studies estimate that the US experiences nearly half a million *C. difficile* cases each year, with a majority of infections associated with health care and hospitalization.[10] The Infectious Diseases Society of America (IDSA) found that *C. difficile* is the bacterium most implicated in gastroenteritis-associated deaths in the US.[11] Tracking *C. difficile* cases and creating a standardized system for *C. difficile* diagnosis and reporting between European countries have been important issues for the ECDC in recent years.[12,13,14] *C. difficile* infections are most often acquired when a person touches their mouth or mucous membranes after touching a surface or item contaminated with feces.[15] Importantly, *C. difficile* infections may arise during prolonged antibiotic therapy when commensal bacteria in the intestines are eliminated by antibiotic treatment.[15,16] *C. difficile* acquired in this manner often results in the discontinuation of other antibiotics so as to focus on treating the patient's new *Clostridium* infection.[15] Furthermore, *C. difficile* is notoriously resistant to many types of antibiotics and is classified as an urgent threat by the CDC.[17] *C. difficile* infection symptoms include dysentery, fever, severe abdominal pain, and pseudomembranous colitis, although a small minority of people are asymptomatic carriers.[15,16]

Escherichia coli* and *Shigella

E. coli are gram-negative, rod-shaped bacteria found in the lower intestine of all individuals. *Shigella* are gram-negative, non-spore forming, rod-shaped bacteria closely related to *E. coli* genetically. Most types of *E. coli* are benign, but some strains are pathogenic and can be transmitted through contaminated food via the fecal-oral route. Pathogenic *E. coli* can be classified into several different pathotypes, each with their own distinct plasmids, toxins, and mechanisms of enteric colonization. Several *E. coli* pathotypes are prominent sources of traveler's diarrhea.[8,24] Recent studies have reported discoveries of *E. coli* pathotype recombinants that contain characteristics from multiple strains; these strains may acquire genes from other pathotypes via divergent plasmids.[18,19] The emergent prevalence of multi-drug resistant strains of *E. coli* is a growing concern for clinicians.[20,21]

The BioCode GPP tests for five different diarrheagenic *E. coli* pathotypes: Enteroaggregative *coli* (EAEC), Enterotoxigenic *E. coli* (ETEC), Shiga-like toxin-producing *E. coli* (STEC), *E. coli* 0157, and Shigella spp./Enteroinvasive *E. coli* (EIEC).

Enteroaggregative *E. coli* (EAEC)

Enteroaggregative *E. coli* is an *E. coli* pathotype that aggregates into a distinct "stacked brick" pattern. This type of *E. coli* is further classified as typical or atypical EAEC, with typical EAEC containing the gene *aggR* on the pAA plasmid and atypical EAEC lacking this marker. EAEC is the second leading cause of traveler's diarrhea.[8] While some EAEC infections are asymptomatic, the pathotype may cause watery or bloody diarrhea, fever, vomiting, and abdominal cramps.

Enterotoxigenic *E. coli* (ETEC)

Enterotoxigenic *E. coli* contain heat-labile (LT) or heat-stable (ST) enterotoxins, with some strains presenting both. These two toxins induce watery diarrhea after binding to the intestinal epithelium and triggering the intestinal epithelial cells to secrete fluids and electrolytes. According to the CDC, ETEC is the most common cause of traveler's diarrhea and is prominent in low-income countries.[24] It is estimated that ETEC caused 120,000 deaths worldwide in 2010.[23]

Shiga-like toxin-producing *E. coli* (STEC)

Shiga-like toxin-producing *E. coli* may contain either one or both of the Shiga-like toxins Stx1 and Stx2. STEC is sometimes referred to as verotoxigenic *E. coli* (VTEC) or enterohemorrhagic *E. coli* (EHEC). In 2015,

STEC was the third highest cause of foodborne illnesses and outbreaks reported in the US behind Norovirus and *Salmonella*.[25] STEC causes more than 265,000 illnesses, 3,600 hospitalizations, and 30 deaths annually in the US.[4] STEC cases are closely monitored in Europe, with improved testing and reporting following outbreaks in the early 2010s.[5,7,26,27] Like ETEC and EAEC, STEC is an important cause of traveler's diarrhea.[8] STEC infections may lead to hemolytic uremic syndrome (HUS), a potentially fatal disease where red blood cells damaged by Shiga-like toxins clog the kidneys, leading to renal failure. The CDC reports that children aged 1-4 years old typically make up the majority of HUS cases in the US.[3]

***E. coli* O157**

STEC O157:H7 is the most commonly identified STEC strain in North America.[28] This strain contains the O157 antigen and flagellar H7 antigen. Like other types of STEC, O157 can cause hemolytic uremic syndrome. Out of over 4,000 STEC cases reported to the CDC in 2014, 43.2% were due to O157.[29] This strain is also the most commonly reported type of STEC infection in Europe, accounting for 41.7% of STEC infections in 2015.[7] There are also reports of *E. coli* O157 diarrhea with no expression of stx1 and stx2.[30,31] Such cases are thought to occur at a low frequency. Because of its distinct antigens and prominence in reported STEC infections, the BioCode GPP probes for O157 independently from STEC strains.

***Shigella* spp./Enteroinvasive *E. coli* (EIEC)**

S*higella* and enteroinvasive *E. coli* both contain the plasmid-borne *ipaH* gene, which enables these bacteria to invade host cells. EIEC causes symptoms that are identical to the profuse diarrhea and high fever produced by *Shigella* infection. Each year, *Shigella* is responsible for an estimated 500,000 illnesses in the US with most shigellosis cases occurring in children under ten years old.[3,4] *S. sonnei* accounts for the vast majority of shigellosis in the US, whereas *S. dysenteriae* and *S. boydii* are rare.[32] Shigellosis does not have a predictable seasonality and the number of outbreaks per month varies from year to year.[3,32] Worldwide, *Shigella* is second only to rotavirus in the number of deaths it causes as an agent of AGE.[1] In addition to transmission via the fecal-oral route, shigellosis can also be acquired through certain types of sexual contact.[33]

***Salmonella* spp.**

Salmonella is a major source of foodborne outbreaks and is typically responsible for a number of meat, poultry, produce, and processed food recalls each year.[25] It is the second leading cause of AGE in the US after Norovirus, and the second leading bacterial cause of AGE in Europe after *Campylobacter*.[6,7,34] *Salmonella* includes the *S. enterica* and *S. bongori* species. These bacteria are gram- negative, motile, and rod-shaped, and are classified as either typhoidal or non-typhoidal. Typhoidal *Salmonella* causes a serious disease known as typhoid fever, whereas non-typhoidal *Salmonella* causes watery diarrhea coupled with fever and is often self-limiting. Every year around 1 million *Salmonella* cases occur in the US, and the CDC reports that in 2015 *Salmonella* accounted for 34% of US foodborne outbreaks and 39% of foodborne illnesses.[25,35] Data gathered by the ECDC shows that *Salmonella* has been the most common cause of foodborne outbreaks with confirmed origin in Europe for the past several years, and approximately 90,000 individual infections were noted in both 2014 and 2015.[5,6,7] Worldwide, *Salmonella* is one of the leading causes of diarrheal disease-related death.[1] Outbreaks for these bacteria occur most often in the summer or early fall.[3]

***Vibrio* spp.**

Vibrio spp. are gram-negative, comma-shaped, non-spore forming motile bacteria associated with aquatic environments. Vibriosis often arises after consuming contaminated seafood or after coming into contact with contaminated sea water or marine wildlife.[3,36] As such, the majority of vibriosis cases in the US

occur in the Gulf Coast and West Coast states.[3,36] From the mid-1990s to early 2000s, the US saw an increase in the rate of reported vibriosis cases.[37] Currently, *Vibrio* is responsible for 80,000 illnesses in the US each year.[38] Of the *Vibrio* cases reported to the CDC in 2014, one in four patients required hospitalization and 4% of all patients died.[36] *Vibrio* species are also tracked closely in Europe, where the ECDC has created an online real-time map monitoring *Vibrio* growth conditions in the Baltic Sea.[39]

The BioCode GPP detects different Vibrio species, including *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*, and specifically identifies *V. parahaemolyticus*.

Vibrio parahaemolyticus

V. parahaemolyticus is found in brackish saltwater and is notably associated with the consumption of raw and undercooked seafood, especially raw oysters. It is the most common cause of vibriosis in the US.[36] Symptoms of *V. parahaemolyticus* infections include watery diarrhea, nausea, vomiting, abdominal cramps, and sometimes fever.

Other *Vibrio* species

There are several non-*parahaemolyticus Vibrio* species, including *V. vulnificus* and *V. cholerae*. *V. cholerae* is the best known species and is responsible for endemic, epidemic, and pandemic cholera. The hallmark of cholera is profuse watery, white diarrhea that may lead to death from severe dehydration if the infected individual does not receive treatment. It has been estimated that between 1.3 million and 4 million cholera cases occur worldwide each year with between 21,000 and 143,000 fatalities.[40]

Yersinia enterocolitica

Yersinia enterocolitica is a gram-negative rod-shaped bacterium. This bacterium is frequently associated with the consumption of undercooked pork. Around 117,000 yersiniosis cases are reported annually in the US, with an average of 640 hospitalizations and 35 deaths.[41] TESSy closely monitors yersiniosis cases as *Y. enterocolitica* is the third leading cause of AGE outbreaks in Europe, with over 6,500 confirmed cases annually.[5,6,7] Children become infected with *Y. enterocolitica* more often than adults.[42] Yersiniosis symptoms vary between children and adults, young children experience bloody diarrhea accompanied by fever and abdominal pain, whereas older children and adults present fever and pain on the right side of the abdomen very similar to appendicitis.

Parasites

Cryptosporidium (C. hominis/C. parvum)

Cryptosporidium spp. are protozoan parasites that can cause both gastrointestinal and respiratory illness in humans. These parasites are most often transmitted via contaminated water and can survive under chlorinated conditions. Approximately 90% of cryptosporidiosis is caused by *C. parvum* and *C. hominis*.[3] In the US the highest rates of reported cryptosporidiosis come from the Midwestern states and peak in the summer when people are more likely to partake in recreational water activities.[3,43] More than 8,000 cryptosporidiosis cases were reported in the US in both 2011 and 2012, and over 7,300 cases were noted in Europe in 2014.[43,44] Worldwide, *Cryptosporidium* claims the second highest mortality rate for gastrointestinal pathogens in children under five years old.[1] The most common symptom of cryptosporidiosis is watery diarrhea, with other symptoms including fever, vomiting, and abdominal cramps.

Giardia lamblia

Giardia lamblia (a.k.a. *Giardia intestinalis*) is a flagellated protozoan that infects and reproduces inside

the intestinal lumen. In addition to sickening humans, this parasite also infects other animals such as cats, dogs, and livestock. *Giardia* is the most common enteric parasitic pathogen in the US, causing an estimated 1.2 million giardiasis cases each year.[4] Over 17,000 giardiasis cases were reported to the ECDC in Europe in 2014.[45] Like *Cryptosporidium, Giardia* is hardly enough to withstand chlorinated environments and is most commonly transmitted through the consumption of contaminated water.[46] Giardiasis cases peak in the early summer through fall in the US, with the highest incidence in the Northwestern states.[47] Although a small percentage of infected individuals are asymptomatic, giardiasis can cause diarrhea with greasy stools, abdominal cramps, excessive gas, and nausea.

Entamoeba histolytica

Entamoeba histolytica is a parasitic amoeba that infects humans and other primates. *E. histolytica* infections are encountered most often in tropical areas with poor sanitation.[48] Cysts can survive outside the body for a few months in contaminated food, water, and soil, but can be killed by heat or freezing.[49] Once cysts are ingested, the active parasite can bore into the intestinal wall and potentially reach the blood stream. Around 10% of individuals infected with *E. histolytica* experience symptoms such as dysentery, colitis, and in some cases amoebic liver abscess, but roughly 90% are asymptomatic.[50] *E. histolytica* has recently caused sporadic outbreaks in non-endemic countries as the organism can be transmitted through certain types of sexual contact.[51]

Viruses

Adenovirus 40/41

Adenoviruses are non-enveloped icosahedral viruses from the *Adenoviridae* family and contain double-stranded DNA. Adenoviruses are ubiquitous in the environment as they are resistant to chemical and physical damage. These viruses are classified into seven different species (A-G), with Adenoviruses 40 and 41 belonging to species F. While other Adenovirus species cause respiratory illness or conjunctivitis, serotypes 40 and 41 are implicated in AGE. Adenoviruses 40 and 41 are associated with hospital-acquired infections in children, with some studies indicating that the incidence of hospital- acquired Adenovirus surpasses that of community-acquired infections.[52] Although AGE symptoms of Adenoviruses 40 and 41 infections are generally mild, they may last 5-12 days with viral particles detectable in stool for weeks or even months after symptoms subside.

Norovirus GI/GII

Noroviruses are non-enveloped icosahedral viruses with positive-sense single-strand RNA. They belong to the *Caliciviridae* family and contain five subgroups, with GI and GII causing mild to severe AGE. Estimates indicate that Noroviruses GI and GII are responsible for 90% of epidemic non-bacterial AGE around the world.[53] In developing countries, Norovirus causes over 200,000 deaths annually.[54] The CDC reports that in 2015 Norovirus accounted for 37% of all reported foodborne outbreaks and 39% of individual foodborne illnesses in the US – higher than any other organism or virus.[25] The prominence of Norovirus as a causative agent of AGE has been magnified in recent years following the success of Rotavirus vaccine programs and the decline of Rotavirus diagnoses in certain countries.[55,56] As such, Norovirus has surpassed Rotavirus as the pathogen responsible for the most cases of pediatric gastroenteritis requiring medical attention in high income countries.[55] Symptoms of Norovirus infection include watery diarrhea, nausea, vomiting, abdominal cramps, lethargy, and in some patients loss of taste.

Rotavirus A

Rotaviruses are members of the *Reoviridae* family that contain double-stranded RNA. Eight species of Rotaviruses have been classified (A-H), with type A accounting for the majority of Rotavirus infections in

humans. Worldwide, Rotavirus infections cause more fatalities than any other diarrheal disease, especially for children under five years old.[1] The virus can cause severe watery diarrhea, fever, vomiting, and abdominal pain. Similar to Adenovirus, Rotavirus has been shown to be a significant source of hospital-acquired diarrhea in young children.[52]

PRINCIPLE OF PROCEDURE

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 are extracted with either the BioMérieux NucliSENS® easyMAG® or Roche MagNA Pure 96 automated system. Once the PCR plate is set up and sealed, all other operations are automated on the MDx-3000.

Nucleic Acid Extraction

A sample of unpreserved stool or stool in Cary-Blair transport medium is transferred to an SK38 tube containing glass and ceramic beads with S.T.A.R. buffer for mechanical lysis. After lysis, nucleic acids (both RNA and DNA) are captured by silica coated magnetic beads and eluted on the NucliSENS® easyMAG® or Roche MagNA Pure 96 automated system according to the manufacturer provided protocol.

Overview of a BioCode MDx-3000 Run

1. **Reverse Transcription and Multiplex PCR** – Since targets of the BioCode GPP include RNA viruses, a reverse transcription (RT) step is performed to convert the viral RNA into cDNA prior to amplification. The purified nucleic acid solution is combined with a freshly prepared reaction mix for the RT step and subsequent thermal cycling for multiplex PCR to enrich the target nucleic acids present in the sample. One of the target-specific primers for each pathogen is biotinylated at the 5'-end to generate labeled PCR product for subsequent detection.
2. **Dispensing BMB-Probe Mix** – Towards the end of PCR amplification, the robotic head dispenses BMB-Probe mix into the designated reaction wells of the capture plate using disposable pipette tips.
3. **PCR Product Transfer** – After PCR amplification is completed, the robotic head pierces the foil seal with disposable pipette tips and transfers PCR products into corresponding wells of the capture plate.
4. **Target Capture** – Amplified PCR products labeled with biotin are captured at a defined temperature by target-specific probes that are covalently coupled to designated Barcoded Magnetic Beads (BMBs). During this step, BMBs are kept in suspension by gentle agitation. Differentiation of captured targets is achieved by assigning a unique barcode pattern (BMB) for each pathogen and the internal control.
5. **Signal Generation** – After washing off unbound PCR products and unused primers, a streptavidin-phycoerythrin (SA-PE) conjugate is automatically added to the reaction by the robot. High affinity binding between biotin and streptavidin ensures that captured PCR products with the biotin moiety are labeled with phycoerythrin in close proximity to the BMBs.
6. **Optical Detection** – Optical detection is performed for each reaction well of the capture plate, an optically clear, flat-bottom microtiter plate. After washing off unbound SA-PE, excitation of the fluorophore at the designated wavelength emits fluorescence signal from BMBs tagged with SA-PE conjugates. Each reaction well is imaged at a specific emission wavelength for fluorescent signal and under bright field for identifying the barcode patterns (decoding).

The BioCode MDx-3000 Software controls the operation of the instrument, collects and analyzes data, and automatically generates interpretation for test reports at the end of the run. Fluorescent signals from BMBs with the same barcode are sorted and calculated to generate median fluorescence index (MFI) for each analyte. The presence or absence of a pathogen is determined relative to the validated assay cutoff by MFI. The software also analyzes the results of external and internal controls to validate the run and individual specimen results for reporting.

MATERIALS REQUIRED

Materials Provided With Each Kit

- BioCode Gastrointestinal Pathogen Kit (This kit is sufficient to perform up to 96 tests.) (Applied BioCode 63-G0002)
  - BioCode Master Mix A (store at -20°C, after thaw store at 4°C for up to 30 days)
  - BioCode GPP Primer Mix (store at -20°C, after thaw store at 4°C for up to 30 days)
  - BioCode RT Mix (store at -20°C)
  - BioCode GPP BMB-Probe Mix (store at -20°C, after thaw vortex for 30 sec, store at 4°C up to 90 days)
  - BioCode RNA-IC (store at -20°C, after thaw store at 4°C for up to 30 days)

Materials Required But Not Provided With Each Kit

- BioCode SA-PE Mix (single use; protect from light; store at 4°C. **Do Not Freeze**) (Applied BioCode 63-S0001)
- BioCode Buffer A (store at room temp) (Applied BioCode 44-B0003)
- BioCode MDx-3000
- BioCode MDx-3000 Consumables
  - Reagent Reservoirs (Integra 4332, 01-R0005)
  - Waste Bin and Lid (Applied BioCode 01-W0105 and 01-W0104)
  - 20 µL pipette tips (Beckman 717256, 01-P0006)
  - 250 µL pipette tips (Beckman 717252, 01-P0007)
  - Bio-Rad 96-well hard shell plate 0.1 mL (Bio-Rad HSL9601, 01-P0011)
  - PCR Adhesive Foil (Thermo Fisher Scientific AB-0626, 01-P0012)
  - Microtiter plate (Greiner Bio-One 655101, 01-P0009)
  - Microtiter plate lid (Nunc 5500, 01-P0010)
- S.T.A.R. Buffer (store at room temp; for extraction) (Roche 3335208001, 04-B0009)
- Bertin SK38 Soil Grinding Tubes (Bertin P000915-LYSK0-A.0, 01-B0010)
- NucliSENS easyMAG Extraction Systems (BioMérieux)
- easyMAG supplies for extraction
  - Lysis Buffer
  - Buffer 1
  - Buffer 2
  - Buffer 3
  - Magnetic silica
  - Nuclease free water
  - Consumables
  - ELISA strip plate
- MagNA Pure 96 Extraction System (Roche)
- MagNA Pure96 supplies for extraction
  - MagNA Pure 96 DNA and Viral nucleic acid kit
  - MagNA Pure 96 system fluid
  - Consumables
- Vortex – attachment for multiple specimens recommended
- Centrifuge
- Pipettes – single, multi-channel and/or repeater with accuracy range between 1-10 µL, 10-200 µL, and 100 – 1000 µL

- Sterile, RNase/DNase-free disposable aerosol-barrier micro pipettor tips
- 1.5 mL polypropylene micro centrifuge tubes and racks (RNase/DNase free recommended)
- Cooler racks for 1.5 mL tubes and 0.1 mL 96 well plate
- Biosafety cabinet (laminar flow hood) for extractions

WARNINGS AND PRECAUTIONS

General Precautions

1. For *In Vitro* Diagnostic Use only.
2. For Prescription Use Only.
3. Results should be interpreted in combination with the patient's signs and symptoms and results from other diagnostic tests by a trained healthcare professional.
4. The BioCode Gastrointestinal Pathogen Panel is for use with the BioCode MDx-3000 instrument.

Precaution Related to Public Health Reporting

Local, state, and federal rules and regulations for notification of reportable diseases are continually updated and include a number of organisms that are important for surveillance and outbreak investigations.[52] Laboratories are responsible for following their state and/or local rules pertaining to reportable pathogens and should consult their local and/or state public health laboratories for isolate and/or clinical sample submission guidelines.

Laboratory Precautions

1. Perform the protocol described in this insert. Deviations from this protocol may produce erroneous results.
2. The BioCode GPP should be performed in clearly defined work areas moving in one direction from pre-amplification areas to the amplification/detection area to reduce potential for contamination.
   a. Begin with specimen preparation and reagent preparation before moving to amplification/detection.
   b. Use dedicated equipment and supplies for each area (including personal protective equipment, such as lab coats and disposable gloves).
   c. Clean work areas with 10% bleach or similar disinfectant followed by water before and after assay preparation.
3. A negative control must be tested for each run. If multiple lots are assayed at the same time, a negative control must be assayed for each lot.
4. Do not use reagents past the expiration date. Do not mix reagents or interchange kit components from different kit lots. Kit configurations are identified on the Kit outer carton and Kit Card.
5. Assay setup should be performed at room temperature. Keep Reaction Mix cold using a cool block during formulation and loading of amplification plate.

Safety Precautions

1. Follow universal safety procedures. All patient specimens should be considered potentially infectious and handled accordingly.
2. Dispose of unused kit reagents and specimens according to local, state and federal regulations.
3. Wear appropriate personal protective equipment including, but not limited to, lab coats, gloves, and protective eyewear. Change gloves often.
4. Do not pipette by mouth.
5. Applied BioCode RT Mix is classified as an irritant. See SDS for details.

REAGENT STORAGE, HANDLING AND STABILITY

1. Store the GPP kit components frozen (-20°C) prior to use.
2. Store RT Mix frozen (-20°C) except during use.
3. Once thawed, store Master Mix, Primer Mix, and RNA IC refrigerated (2-8°C) for up to 30 days.
4. Once thawed, store BMB-Probe Mix refrigerated (2-8°C) for up to 90 days.
5. SA-PE mix is for single use only. Store refrigerated (2-8°C). Protect from light. **DO NOT FREEZE.**
6. Store the Buffer A at room temperature (15-25°C).
7. Avoid storage of any materials near heating or cooling vents or in direct sunlight.
8. Always check the expiration date and do not use reagents beyond the expiration date printed.
9. Once RT-PCR reaction mix is prepared, the test run should be started as soon as possible (within 60 minutes).
10. Remove BMB-Probe Mix from MDx-3000 once the run is completed and store refrigerated (2-8°C).

SAMPLE REQUIREMENTS

This section describes the requirements for specimen collection, preparation, and handling that will help ensure accurate test results.

Stool in Cary-Blair Transport Media

Specimen Collection - Stool specimens should be collected in Cary-Blair transport media according to manufacturer's instructions.

Minimum Sample Volume - 100 µL of sample is required for testing.

Transport and Storage - Specimens should be processed and tested as soon as possible, though stool in Cary-Blair may be stored at room temperature or under refrigeration for up to four days.

Stool without Preservatives

Specimen Collection - Unformed stool specimens without preservatives should be placed in a sterile cup/container and stored refrigerated as soon as possible.

Minimum Sample Volume - 100 µL of sample is required for testing.

Transport and Storage - Specimens should be processed and tested as soon as possible. Unpreserved stool specimens should be kept cold during transport and stored refrigerated within 24 hours. Freeze at-80°C or below if testing cannot be completed within 4 days.

Note: Samples for *Clostridium difficile* testing must not be frozen. Results for C. difficile will not be reported for frozen specimens.

PROCEDURE

Refer to the BioCode MDx-3000 Operator's Manual for more detail and pictorial representations of the BioCode MDx-3000 set up instructions.

Gloves and other Personal Protective Equipment (PPE) should be used when handling specimens and reagents. Once PCR reagents are prepared and sample is added to PCR plate, it should be promptly transferred to the instrument to start the run. After the run is complete, the PCR plate and capture plate should be sealed and discarded in a biohazard container.

Extraction Method for Unpreserved or Cary-Blair Stool Samples

Note: It is strongly recommended that sample preparation be performed in a biosafety cabinet with gloves and appropriate personal protective equipment (PPE).

1. Mix RNA IC and S.T.A.R. Buffer at a 1/100 ratio (v/v) to prepare 1 mL solution for each specimen or controls (see table below). Vortex for 5 to 10 seconds.

Table. RNA IC/ S.T.A.R. buffer preparation for extraction.

Reagent	1 specimen	8 specimens*	16 specimens*	24 specimens*	48 specimens*
RNA IC	10 µL	100 µL	180 µL	260 µL	500 µL
S.T.A.R. Buffer	990 µL	9,900 µL	17,820 µL	25,740 µL	49,500 µL

* - indicates that the mix is prepared with overage for 2 additional specimens.

2. Add 1000 µL of S.T.A.R. Buffer/RNA IC mix to each SK38 Bead tube.
3. Add 100 µL Cary-Blair or watery stool or one loopful (~100 mg) of formed stool to the SK38 tubes. **Do not add more stool than instructed. Doing so may lead to 'invalid results'.**
4. Add 100 µL S.T.A.R. buffer or well characterized negative sample for the Negative Control.
5. Vortex SK38 tubes for 5 minutes at high speed. A vortex attachment is recommended for multiple samples.
6. Spin for 2 minutes at 3500 - 5000 rpm.
7. **Extract specimens with easyMAG as follows:**

   7.1 Transfer 200 µL from the SK38 tube into an easyMAG cartridge and load onto the easyMAG.

   7.2 Perform Protocol: Specific A.1.0.2, Volume: 0.200 mL, Eluate: 70.0 µL, Sample Type: Primary, Matrix: Feces (stool)

   7.3 Start 10 minute on-board incubation.

   7.4 When prompted add magnetic silica.
   - 7.4.1 Mix 550 µL nuclease free water and 550 µL magnetic silica in a 1.5 mL tube per easyMAG cartridge.
   - 7.4.2 Mix well and dispense 125 µL into each well of an 8-well ELISA strip plate for each cartridge.
   - 7.4.3 Add 100 µL to each cartridge well and mix thoroughly.

   7.5 Proceed with the remainder of the run.

8 **Extract specimens with MagNA Pure 96 as follows:**

8.1 Transfer 200µL from the SK38 tube into the MagNA Pure 96 processing cartridge (be careful to pipet directly to the bottom and not produce bubbles. Liquid on the side of the well and bubbles will lead to incorrect volume sensing and the extraction will be aborted.)

8.2 Perform Protocol: Pathogen Universal 200 3.1 for MagNA Pure Kit: DNA/Viral NA SV 2.0. Volume: 200µL, Eluate: 50µL.

Nucleic Acid Storage Conditions

Transfer sample extracts from the cartridge into PCR grade micro-tubes, strips or plates and store samples in a 2-8°C refrigerator if testing within 24 hours. Store at -80°C or below if testing cannot be completed within 24 hours of extraction. Extracted nucleic acids may be stored at -80°C or below for 90 days. Leftover pretreated samples (in SK38 tubes) can be stored at -80°C or below for 90 days.

BioCode GPP Set Up

Note: Prepare the PCR Plate in a dedicated reaction mix prep area.

1. Thaw Primer Mix, Master Mix and BMB-Probe Mix at room temperature. Perform a quick vortex (2-3 seconds) and centrifuge to collect reagents at the bottom of the tube.
2. Prepare the reaction mix in a polypropylene microcentrifuge tube as described below:

Table. Reaction Mix Formulation

Component	Reaction Mix Volume (µL) per reaction	Reaction Mix Volume (µL) per 10 reactions
BioCode Master Mix A	10.0 µL	100 µL
BioCode GPP Primer Mix	9.5 µL	95 µL
BioCode RT Mix	0.5 µL	5 µL
Reaction Mix Volume (µL)	**20 µL**	**200 µL**

3. Mix reaction mix by pipetting up and down 8 to 10 times and centrifuge to collect contents at the bottom of the tube. Store at 2-8°C or on a cool block until ready to set up PCR (not to exceed one hour). Do NOT vortex reaction mix.
4. Pipette 20 µL of reaction mix into appropriate wells of a 96-well plate.
5. Pipette 5 µL of each extracted sample into the wells.
6. Pipette 5 µL extracted negative control into the NC well.
7. Seal plate with pierceable foil. Store at 2-8°C or on a cool block until ready to load onto the BioCode MDx-3000 (not to exceed one hour from the time the reaction mix is prepared).
8. Briefly centrifuge plate to collect samples at the bottom of the plate.
9. Load plate onto BioCode MDx-3000.Vortex thawed room temperature BMB-Probe Mix for 30 seconds at high speed and load onto the BioCode MDx-3000. **(Note: Precipitates may appear at cold temperatures. If precipitants are present, allow the BMB-Probe Mix to warm to room temperature and vortex additional 30 seconds.)**
10. Load reagents and consumables as prompted by graphic user interface and run BioCode Gastrointestinal Pathogen Panel Protocol.

Note: Samples for *Clostridium difficile* testing must not be frozen. Results for C. difficile will not be reported for frozen specimens. The graphic user interface will require the user to indicate fresh or frozen status for each specimen.

INTERPRETATION OF RESULTS

The BioCode MDx-3000 software will analyze data based on plate validity, sample validity and Median Fluorescent Intensity (MFI) compared to an MFI threshold. The software will suppress results if Internal or Negative controls are invalid. The software will indicate if external positive controls are valid or invalid, but will not suppress results if the positive control is not valid.

Negative Controls

External negative controls can be RNase-free water, S.T.A.R. Buffer, or well characterized negative specimens. The negative control should go through all processing steps (pretreatment, extractions, amplification, and detection). At least one negative control is required for each plate/kit lot. The BioCode MDx-3000 software will suppress results for all samples if the Negative Control(s) are not valid (see table below).

Table. Criteria for Valid Negative Control

Control	Targets	RNA IC	Description
Negative Control	Not Detected	Detected	Plate Status: Valid. Samples can be interpreted.
Negative Control	Detected	N/A	Plate Status: Invalid. Samples results cannot be interpreted. Results suppressed by software.
Negative Control	N/A	Not Detected	Plate Status: Invalid. Samples results cannot be interpreted. Results suppressed by software.

External Positive Controls

Each laboratory should establish its own Quality Control ranges and frequency of QC testing based on applicable local laws, regulations and good laboratory practices.

External positive controls can be well characterized clinical samples or positive strains. Controls can be single analytes or pooled specimens. The positive controls should go through all processing steps (pretreatment, extractions, amplification, and detection). It is recommended that at least one positive control be included for each plate/kit lot on a rotating schedule. Wells identified as Positive Controls will be trended by the BioCode MDx-3000 software and the report will indicate a valid or invalid result on the report header (see table below). The software will not suppress results based on positive control results. If a positive control does not perform as expected, the user should review all samples in that batch to determine if results should be reported.

Table. Criteria for Valid Positive Control

Control	Targets	RNA IC	Recommendations
Positive Control	Expected Target Detected	N/A	Report will indicate Positive control Valid. No user intervention required.
Positive Control	Expected Target Not Detected	N/A	Report will indicate Positive control Invalid. User should review results prior to release.
Positive Control	Unexpected Target Detected	N/A	Report will indicate Positive control Invalid. User should review results prior to release.

Internal Control

An RNA Internal Control (RNA IC: bacteriophage MS2) is added to each sample during pre-treatment. The internal control monitors the efficiency of the extraction, reverse transcription, amplification and detection stages of the assay. Positive results may be reported in the absence of RNA IC detection. However, the BioCode MDx-3000 software will suppress negative results for any wells with invalid RNA IC results (see table).

Table. Criteria for RNA Internal Control (RNA IC)

Targets	RNA IC	Recommendations
N/A	Detected	Well status: Valid. Report all results.
Detected	Not Detected	Well status: Invalid. Detected results may be reported. Repeat/reflex testing.
Not Detected	Not Detected	Well status: Invalid. Not Detected results suppressed by software. Repeat/reflex testing.

Lack of RNA IC signal may indicate sample-associated inhibition or reagent/instrumentation issues. If reagent or instrument issues are suspected specimens may be repeated for reserved nucleic acid extracts. If sample is suspected of being inhibitory, repeat from extraction.

EasyMAG repeat/reflex extraction

- Method 1:
  - Transfer 50 µL from the SK38 tube into an easyMAG cartridge and load onto the easyMAG.
  - Perform Protocol: Specific A.1.0.2, Volume: 0.050 mL, Eluate: 70.0 µL, Sample Type: Primary, Matrix: Feces (stool).
- Method 2:
  - Transfer 50 µL from the SK38 tube and 150 µL S.T.A.R. buffer into an easyMAG cartridge and load onto the easyMAG.
  - Perform Protocol: Specific A.1.0.2, Volume: 0.200 mL, Eluate: 70.0 µL, Sample Type: Primary, Matrix: Feces (stool).

MagNA Pure 96 repeat/reflex extraction

- Transfer 50 µL from the SK38 tube and 150 µL S.T.A.R. buffer into a MagNA Pure 96 processing cartridge.
- Perform Protocol: Pathogen Universal 200 for MagNA Pure Kit: DNA/Viral NA SV 2.0. Volume: 200µL, Eluate: 50µL.

Target Pathogen Interpretation

For many organisms detected by the BioCode GPP, the organism is considered to be detected if a single corresponding assay is positive. For example, *Campylobacter* spp. will have a result of "*Campylobacter* spp. Detected" if target specific MFI is at or above the threshold. The following organisms are detected using a single assay: *Campylobacter (C. jejuni/C. coli)*, *Salmonella,* EAEC, *Shigella/*EIEC, *E. coli* O157, *Y. enterocolitica*, Adenovirus F 40/41, Rotavirus A, *Cryptosporidium, E. histolytica,* and *G. lamblia*.

In contrast, the test results for several organisms rely on the combination of multiple assays. These include *C. difficile, Vibrio* (*V. parahaemolyticus/V. vulnificus/V. cholerae)*, and Norovirus GI/GII. The test results for several Diarrheagenic *E. coli*(s) include multiple assays for genetic markers to identify various classic pathotypes of *E. coli* including ETEC and STEC (as well as O157, EAEC and *Shigella*/EIEC included above). Interpretation rules for these assays are described below. Also included are summary descriptions of the assays' expected reactivity; for a full description of assay reactivity see Inclusivity.

Note: As polymicrobial results with four or more distinct organisms in a single sample are unusual based upon data from the prospective clinical study, confirmation of this result is recommended to rule out any unexpected error, either caused by the user's handling of the sample or the test system. Polymicrobial results of four or more organisms were detected in less than 0.06% (1/1558) of the BioCode GPP prospective study specimens.

Bacteria

Campylobacter (*C. jejuni/C. coli*): The BioCode GPP contains one assay (Campy) designed to detect, but not differentiate, the most common *Campylobacter* species associated with human gastrointestinal illness: *C. jejuni,* and *C. coli*. These are the same species that are identified using standard clinical laboratory practices. Other *Campylobacter* species will not be identified by the BioCode GPP. A positive result for the assay will give a *Campylobacter* Detected test result.

Clostridium difficile toxin A/B: The BioCode Gastrointestinal Pathogen Panel contains two assays (tcdA and tcdB) for the identification of toxigenic *C. difficile* which targets the toxin A gene (*tcdA*) and the toxin B gene (*tcdB*). Typical toxigenic strains produce both toxins, but the presence of either is indicative of a pathogenic strain. Empirical testing and *in silico* sequence analysis support that the assay will detect all toxinotypes and the epidemic BI/NAP1/027 hypervirulent strain, although these will not be specifically differentiated by the assay. Detection of either or both toxin genes by this assay gives a test result for *Clostridium difficile* toxin A/B Detected. As rates of asymptomatic carriage of *C. difficile* can be high in very young children and hospitalized patients, the detection of toxigenic *C. difficile* should be interpreted within the context of guidelines developed by the testing facility or other experts (e.g., guidelines/policy statements published by The American Academy of Pediatrics or the Society for Healthcare Epidemiology of America and the Infectious Disease Society of America).

Salmonella: The BioCode GPP contains a single assay (Salm) designed to detect both species of *Salmonella*: *S. enterica* and *S. bongori*. Empirical testing and *in silico* sequence analysis supports the ability to detect all subspecies and serovars of *Salmonella*.

Vibrio (V. parahaemolyticus/V. vulnificus/V. cholerae) and Vibrio parahaemolyticus: The BioCode GPP contains a single assay (Vib) for detection of Vibrio species most commonly implicated in gastroenteritis (V. parahaemolyticus, V. vulnificus, and V. cholerae). Empirical testing and in silico sequence analysis indicate that the assay may also react with some less common Vibrio species (i.e., V. alginolyticus, and V. mimicus). The Vibrio assay does not indicate which species has been detected and the Vibrio assay is not expected to detect the rarer V. hollisae and V. fluvialis. A second assay (V.para) is also included for the specific

detection of Vibrio parahaemolyticus. A Vibrio parahaemolyticus Detected result will be reported when the V. parahaemolyticus-specific assay is positive, while a positive result for Vib (not parahaemolyticus) will only be reported if the V. parahaemolyticus specific assay is Not detected (see table below).

Table. Possible Assay Results and Corresponding *Vibrio* Test Results

Vibrio **(Vibrio Assay)**	***V. parahaemolyticus*** **(V.para Assay)**	**Description**
Not detected	Not detected	No *Vibrio* species detected
Detected	Not detected	*Vibrio* species DNA detected (not *V. parahaemolyticus*)
Any Results	Detected	*Vibrio parahaemolyticus* DNA detected

Yersinia enterocolitica: The BioCode Gastrointestinal Pathogen Panel contains a single assay (Y.ent) designed to detect all known serotypes/biotypes of *Y. enterocolitica*. 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. enterocolitica* (~1.5 x 10^3 CFU/mL). *Y. rohdei* was also detected when present at high levels (>6.8 x 10^4 CFU/mL). These species are in the *Y. enterocolitica* group and are difficult to differentiate from *Y. enterocolitica* by culture methods. All have been isolated from humans; *Y. frederiksenii* and *Y. intermedia* are known human pathogens.

Diarrheagenic E. coli

The BioCode GPP contains multiple assays designed to detect genetic determinants associated with classic diarrheagenic *E. coli/ Shigella* pathotypes. Horizontal transfer of these genes between organisms has been documented; therefore, Detected results for multiple diarrheagenic *E. coli/ Shigella* may be due to the presence of multiple pathotypes or a single strain containing the characteristic determinants of multiple pathotypes. An example of this is the 2011 *E. coli* O104:H4 outbreak strain that contains determinants of both Shiga-like toxin-producing *E. coli* (STEC) and Enteroaggregative *E. coli* (EAEC).

Enteroaggregative E. coli (EAEC): The BioCode GPP contains a single assay (EAEC) for the identification of a gene typically associated with enteroaggregative *E. coli*: the *aggR* regulatory gene located on the partially-conserved pAA plasmid. pAA is not present in all strains phenotypically identified as EAEC, and not all pAA plasmids carry *aggR*; therefore the BioCode GPP will not detect all members of this diverse pathotype, but is likely to detect most pathogenic strains (including *E. coli* O104:H4, which was responsible for recent outbreaks in Europe).

Enterotoxigenic (ETEC), heat-labile (lt) and heat-stable (st) enterotoxins: The BioCode GPP contains three assays (LT, ST-a, ST-b) for the detection of enterotoxins found in Enterotoxigenic *E. coli* (ETEC). The assays are designed for the detection of heat-labile (LT) enterotoxin (*ltA*) and two heat- stable (ST) enterotoxin variants (*st1a*, also known as STp; and *st1b*, also known as STh). The reported results do not indicate which of these toxin(s) have been detected. A positive result for any combination of the three assays will give an Enterotoxigenic *E. coli* (ETEC) *lt/st* Detected test result. The variant LT-II toxin (structurally similar to LT) and the STb/ST2 toxin (structurally dissimilar to ST1) are not targeted by the ETEC assays and have not been established as important in human disease.

Shiga-like toxin-producing *E. coli* (STEC) Shiga-like toxin genes 1 and 2 (*stx1a&b/stx1c/stx2*): The BioCode GPP contains three assays (stx1, stx1c and stx2) for the detection of Shiga-like toxin 1 (*stx1a,b,c*) and Shiga-like toxin 2 (*stx2*) sequences. The reported results do not indicate which of these toxin(s) have been detected. A positive result for any of these assays will give a Shiga-like toxin- producing *E. coli* (STEC)

stx1/stx2 detected test result (see table below). 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) *stx1/stx2* and Shigella/Enteroinvasive *E. coli* (EIEC) in the same sample may indicate the presence of *S. dysenteriae*.

E. coli O157: To aid in the identification of *E. coli* O157 serotype, the BioCode GPP contains a single assay (O157) to detect a gene target that is specific to this serotype. Strains of *E. coli* O157 that do not carry the Shiga-like toxin genes have also been identified. Although the pathogenicity of these non-STEC strains remains to be defined, the *E. coli* O157 assay result is reported independent of Shiga-like toxin gene detection (STEC Detected or Not Detected). Detection of STEC *stx1/stx2* and the *E. coli* O157 target results in a reporting of positive STEC and *E. coli* O157 results. If STEC *stx1/stx2* is Not Detected and *E. coli* O157 is Detected, the result is reported as positive result for *E. coli* O157. The BioCode GPP cannot distinguish between infections with a single toxigenic STEC O157 or rare co-infections of STEC (non-O157) with a *stx1/stx2*-negative *E. coli* O157 (see table below).

Table. Possible Assay Results and Corresponding Test Results for *E. coli* O157 and Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx*

STEC *stx1/2* (stx1/ stx2) Assays	*E. coli* O157 (O157) Assay	Description
Not detected	Not detected	*stx1/stx2* DNA not detected. *E. coli* O157 DNA not detected.
Not detected	Detected	*stx1/stx2* DNA not detected. *E. coli* O157 DNA detected.
Detected[a]	Not detected	*stx1/stx2* DNA detected, *E. coli* O157 not detected.
Detected[a]	Detected[b]	*stx1/stx2* DNA detected, *E. coli* O157 DNA detected.

a - Positive results for the STEC assay(s) and the Shigella/Enteroinvasive E. coli (EIEC) assay may indicate the presence of Shigella dysenteriae.

b - O157 determinant may be from the STEC or may be due to the rare possibility of a shiga-like toxin-negative E. coli O157 being in the same specimen with a non-O157 STEC.

*Shigella/*Enteroinvasive *E. coli* (EIEC): The BioCode GPP contains a single assay (Shig) for the detection of *ipaH,* a gene specifically found in all *Shigella* species as well as Enteroinvasive *E. coli* (EIEC). It is not possible to differentiate *Shigella* from EIEC using this method, and detection of *ipaH* will result in a *Shigella*/Enteroinvasive *E. coli* (EIEC) Detected test result. 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) *stx1/stx2* with *Shigella*/ Enteroinvasive *E. coli* (EIEC) in the same sample may indicate the presence of *S. dysenteriae*.

Parasites

Cryptosporidium: The BioCode GPP contains a single assay (Crypto) for detection of *Cryptosporidium* species (*C. hominis* and *C. parvum*). Empirical testing indicates potential for cross reactivity with *C. cuniculus* and *C. meleagridis*.

Entamoeba histolytica: The BioCode GPP contains a single assay (E.his) for the detection of *E. histolytica*, the only *Entamoeba* species implicated in gastroenteritis. Empirical testing with a gene fragment and *in silico* sequence analysis do not predict cross reactivity with the closely related *E. dispar*.

Giardia lamblia: The BioCode GPP contains a single assay (G.lam) designed to detect *G. lamblia* (aka *G. intestinalis*, *G. duodenalis*), the only *Giardia* species infectious to humans.

Viruses

Adenovirus F40/41: The BioCode GPP contains a single multiplexed assay (AdenoF) for the specific detection of both Adenovirus F40 and F41 (i.e., the assay will not cross-react with respiratory non-40/41 Adenovirus species when shed in the stool). The reported results do not indicate which serotype (40 or 41) has been detected. The assay will not detect other adenovirus species, such as species B, C, and E, which are associated with respiratory infections.

Norovirus GI/GII: The BioCode GPP contains two assays (NoVG1 and NoVG2) that together target the Norovirus genogroups most commonly associated with human infections (GI and GII). Neither assay will detect genogroup GIV, non-human genogroups, or closely related Caliciviruses such as Sapovirus. The reported results do not indicate which genogroup(s) (GI and/or GII) have been detected. A positive result for either or both assays will produce test result of Norovirus GI/GII Detected.

Rotavirus A: The BioCode GPP contains a single assay (Rota) to be inclusive of all strains of Rotavirus A. *In silico* sequence analysis indicates potential for cross reactivity with Porcine Rotavirus C while no cross reactivity was predicted with the human strains for Rotavirus C in the NCBI data base. In addition, *in silico* analysis suggests that the assay should not cross react with Rotaviruses B, D, or F. Empirical testing has demonstrated that these assays will detect recombinant viruses included in Rotavirus vaccines.

BioCode GPP Test Report

The analyzed BioCode MDx-3000 results are displayed in two report formats: Run Report for the entire run including multiple specimens, or Sample Report for individual specimens. Both reports can be exported as a PDF or CSV file. Each report includes fully analyzed and interpreted results for specimens and/or controls but is formatted differently. Refer to operator manual for more details and examples of the BioCode MDx-3000 reports.

The Run Report displays analyzed results in a tabular format for all wells (specimens/controls) in a run from a specific Kit lot. If more than one lot is run together, separate Run Reports will be generated by the software for each lot. Possible results by target are: Detected, Not detected, Invalid, or N/A (not ordered; see interpretation of results for details).

The Sample report displays results for a single well (specimen/control). In addition to results for each target, the Sample Reports include a results summary section which allows positive results to be reviewed at a glance. The Sample Report results summary will also indicate well validity based on BMB counts, background MFI, and external and internal controls. Sample reports also include any samples specific comments entered during setup.

Both report headers provide traceability information for: Run name, Run start and finish time, User ID, Software version, Instrument ID, Kit Name, and Reagent lots and expiration dates. The headers also include sections for Run Status and External Controls status. The Run Status section will specify if the run is Incomplete, Valid or Invalid based on the Negative Control results for the specific run/kit lot. The External Controls section indicates the results for the negative controls (Valid or Invalid) and Positive Controls (Valid, Invalid, or N/A if not assayed). The Run Status and Controls sections should be reviewed prior to review of target results. In addition to these summaries, the software will also mask results in the detailed tabular sections based on plate and well validity requirements (see interpretation of results for details).

Completed reports can be electronically reviewed. Reviewer comments will be added to the report footer for traceability under the review section. MFI (Median Florescence Intensity) reports are available for information only for administrator level users.

LIMITATIONS OF THE PROCEDURE

- The BioCode Gastrointestinal Pathogen Panel is for use with the BioCode MDx-3000 instrument.
- The performance of the BioCode GPP has been validated with unpreserved human stool or stool collected in Cary-Blair transport medium, per manufacturer's instructions. It has not been validated for other specimen types, rectal swabs or samples stored in other transport media or fixatives.
- *C. difficile* testing must be performed with fresh specimens only, not with previously frozen specimens. Results for *C. difficile* will not be reported for frozen specimens.
- Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient. Due to high rates of asymptomatic carriage of *Clostridium difficile*, especially in very young children and hospitalized patients, the detection of toxigenic *C. difficile* should be interpreted within the context of guidelines developed by the testing facility or other experts (e.g., guidelines/policy statements published by The American Academy of Pediatrics or the Society for Healthcare Epidemiology of America and the Infectious Disease Society of America).
- A negative result for adenovirus may not rule out adenovirus infection. If an adenovirus infection is suspected an alternative test should be performed to confirm negative results.
- A negative result for ETEC may not rule out ETEC infection. If an ETEC infection is suspected an alternative test should be performed to confirm negative results.
- The performance of the BioCode GPP is dependent upon proper sample collection, handling, transportation, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive and false negative results caused by improperly collected, transported, or handled specimens. The internal control (RNA-IC) will not indicate whether or not nucleic acid has been lost due to inadequate collection, transport or storage of specimens. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.
- Negative results do not exclude the possibility of gastrointestinal infection. Negative test results may occur from sequence variants in the region targeted by the assay, the presence of inhibitors, technical error, sample mix-up, or an infection caused by an organism not detected by the panel. Test results may also be affected by concurrent antimicrobial therapy or levels of organism in the sample that are below the limit of detection for the test. Negative results should not be used as the sole basis for diagnosis, treatment, or other management decisions.
- The performance of the assay has not been established in individuals who received Rotavirus A vaccine. Recent oral administration of a Rotavirus A vaccine may cause positive results for Rotavirus A if the virus is passed in the stool.
- Results of the BioCode GPP should be interpreted by a trained clinician in conjunction with clinical history, epidemiological data and any other laboratory data.
- If four or more distinct organisms are detected in a specimen, retesting the specimen is recommended to confirm the polymicrobial result.
- This assay is qualitative and does not provide a quantitative value for the organism(s) present in the sample.
- Nucleic acid may persist independently of an organism's viability or organisms may be asymptomatically carried. Therefore, a positive result does not necessarily indicate the presence of

viable organisms or that the organism is the causative agent for the clinical symptoms.

- *Shigella dysenteriae* possess a shiga toxin gene (*stx*) that is identical to the *stx1* gene of STEC. The detection of both *Shigella*/ Enteroinvasive *E. coli* (EIEC) and STEC *stx1/stx2* analytes in the same specimen may indicate the presence of *S. dysenteriae*.
- This test only detects *Campylobacter jejuni* and *Campylobacter coli* and does not differentiate between these two species. Additional testing is required to differentiate between these species and to detect other Campylobacter species that may be present in stool specimens
- This test detects only the 'typical' EAEC which contain the gene aggR on the pAA plasmid and that the assay will not detect atypical EAEC strains lacking this marker (i.e., it does not detect all strains exhibiting an aggregative adherence pattern).
- Based on *in silico* analysis stx2 subtype f is predicted to be detected with reduced sensitivity or not detected by the BioCode GPP.
- Results for the BioCode GPP do not rule out disease caused by other pathogens.
- State and local public health authorities have published guidelines for notification of reportable diseases in their jurisdictions including *Salmonella*, *Shigella*, *V. cholerae*, *E. coli* O157, Enterotoxigenic *E. coli* (ETEC) *lt*/*st*, and Shiga-like toxin-producing *E. coli* (STEC) *stx1*/*stx2* to determine necessary measures for verification of results to identify and trace outbreaks. Laboratories are responsible for following their state or local regulations for submission of clinical material or isolates on positive specimens to their state public health laboratories.
- The effect of interfering substances has only been evaluated for those listed in the labeling. Interference by substances other than those described in the Interference section below could lead to erroneous results.
- The performance of this test has not been established for patients without signs and symptoms of gastrointestinal illness.
- The performance of this test has not been established for monitoring treatment of infection with any of the panel organisms.
- The performance of this test has not been evaluated for immunocompromised individuals.
- Positive and negative predictive values are highly dependent on prevalence. False negative results are more likely during peak activity when prevalence of disease is high. False positive results are more likely during periods when prevalence is moderate to low.
- Discrepancies between the BioCode GPP and other microbial identification methods may be caused by the inability to reliably differentiate species based on standard phenotypic microbial identification methods. Examples include differentiation of *Yersinia enterocolitica* from other *Y. enterocolitica* group members such as *Y. rhodei or Y. kristenssenii,* differentiation of *Entamoeba histolytica* from *E. dispar*, and differentiation of *Helicobacter pullorum* from *Campylobacter*.
- Several organisms were shown to have the potential to cross-react with BioCode GPP. These include *Vibrio mimicus*, *V. alginolyticus* (*Vibrio* assay), *Yersinia bercovieri, Y. frederiksenii, Y. kristensenii, Y. molaretii*, *Y. rohdei*, and *Y. intermedia,* which are members of the *Y. enterocolitica* group (*Y. enterocolitica* assay), as well as *Cryptosporidium meleagridis* and *C. cuniculus* (*Cryptosporidium* assay). Please refer to the Target Pathogen Interpretation section of this document for additional information.
- Cross-reactivity with organisms other than those listed above or in the Target Pathogen Interpretation section may lead to erroneous results.

EXPECTED VALUES

For this clinical investigational study, a total of 1558 leftover, de-identified samples were prospectively collected from patients who underwent stool sample collection for clinical evaluation at four (4) investigational sites, with each collecting approximately 400 samples. The sites were located in the United States, with a broad geographic representation, Midwest (site 001), Southeast (site 002), Northeast (site 003), and West (site 006). The collection began in January 2015 and was completed in August 2017. The table below presents the positive results via the BioCode GPP by stratified age group.

Table. Expected values (as determined by the BioCode GPP) by age group from the prospective clinical specimens (January 2015 – August 2017)

Analyte	Overall (N = 1558)	< 5 yrs (N = 140)	6-21 yrs (N = 237)	22-59 yrs (N = 720)	60+ yrs (N = 464)
Campylobacter spp	35 (2.25%)	5 (3.57%)	4 (1.69%)	20 (2.79%)	6 (1.30%)
*Clostridium difficile**	69 (11.54%)	3 (16.67%)	4 (7.14%)	30 (9.32%)	32 (15.84%)
E. coli O157	7 (0.45%)	3 (2.14%)	1 (0.42%)	3 (0.42%)	0 (0.00%)
EAEC	52 (3.34%)	9 (6.43%)	10 (4.22%)	28 (3.90%)	5 (1.08%)
ETEC	33 (2.12%)	2 (1.43%)	4 (1.69%)	16 (2.23%)	11 (2.38%)
STEC	8 (0.51%)	2 (1.43%)	2 (0.84%)	3 (0.42%)	1 (0.22%)
Salmonella spp	37 (2.37%)	9 (6.43%)	10 (4.22%)	12 (1.67%)	6 (1.30%)
Shigella/EIEC	23 (1.48%)	8 (5.71%)	8 (3.38%)	6 (0.84%)	1 (0.22%)
Vibrio parahaemolyticus	3 (0.19%)	0 (0.00%)	0 (0.00%)	3 (0.42%)	0 (0.00%)
Vibrio spp (not *parahaemlyticus*)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Yersinia enterocolitica	10 (0.64%)	1 (0.71%)	3 (1.27%)	5 (0.70%)	1 (0.22%)
Entamoeba histolytica	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Cryptosporidium spp	13 (0.83%)	1 (0.71%)	0 (0.00%)	11 (1.53%)	1 (0.22%)
Giardia lamblia	11 (0.71%)	1 (0.71%)	1 (0.42%)	8 (1.11%)	1 (0.22%)
Adenovirus 40/41	12 (0.77%)	6 (4.29%)	1 (0.42%)	3 (0.42%)	2 (0.43%)
Norovirus GI/GII	50 (3.21%)	10 (7.14%)	8 (3.38%)	25 (3.48%)	7 (1.51%)
Rotavirus A	30 (1.93%)	17 (12.14%)	7 (2.95%)	2 (0.28%)	4 (0.86%)

* - *C. difficile* was calculated for fresh specimens only (N=598): <5 yrs = 18, 6–21 yrs = 56, 22-59 yrs = 322, 60+ yrs = 202.

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).

Table. Expected values for mixed infections (as determined by the BioCode GPP) from the prospective clinical specimens (January 2015 – August 2017)

	Prevalence in Mixed Infection N = 49	
Analyte	**n/N**	**%**
Campylobacter spp	8/49	16.3%
Clostridium difficile	9/49	18.4%
E.coli 0157	4/49	8.2%
EAEC	22/49	44.9%
Shigella/EIEC	5/49	10.2%
ETEC	18/49	36.7%
STEC	5/49	10.2%
Salmonella spp.	7/49	14.3%
Vibrio parahaemolyticus	1/49	2.0%
Yersinia enterocolitica	4/49	8.2%
Cryptosporidium spp	4/49	8.2%
Giardia lamblia	4/49	8.2%
Adenovirus 40/41	1/49	2.0%
Norovirus GI/GII	12/49	24.5%
Rotavirus A	4/49	8.2%

PERFORMANCE CHARACTERISTICS

Clinical Performance

For this clinical investigational study, a total of 1558 leftover, de-identified samples were prospectively collected from patients who underwent stool sample collection for clinical evaluation at four (4) investigational sites, with each collecting approximately 400 samples. The sites were located in the United States, with a broad geographic representation, Midwest (site 001), Southeast (site 002), Northeast (site 003), and West (site 006). The collection began in January 2015 and was completed in August 2017. Demographic details are presented in the table below.

Table. 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 fresh (Category I) prospectively collected and tested and frozen (Category II) prospectively collected and retrospectively tested specimens.

Table. 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 (Prospective specimens) Results 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)

	PPA		NPA	
Target	**Agreement Rate n/N (%)**	**95% CI**	**Agreement Rate n/N (%)**	**95% CI**
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.
i – *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 (Prospective specimens) Results 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)

	PPA		NPA	
Target	**Agreement Rate n/N (%)**	**95% CI**	**Agreement Rate n/N (%)**	**95% CI**
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 and an FDA cleared NAAT; 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 (Prospective specimens) Results 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.

Table. Summary of Clinical Study Results (Prospective specimens) stratified by sample type and storage.

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Campylobacter spp.[a]	Cary-Blair (Fresh)	361	2/3 (66.7)	9.4 - 99.2	347/358 (96.9)	94.6 - 98.5
	Unpreserved (Frozen)	955	3/3 (100.0)	29.2 - 100.0	936/952 (98.3)	97.3 - 99.04
	Unpreserved (Fresh)	237	1/1 (100.0)	2.5 - 100.0	234/236 (99.2)	97.0 - 99.9
	All Prospective	1553	6/7 (85.7)	42.1 – 99.6	1517/1546 (98.1)	97.3 - 98.7
Clostridium difficile[b]	Cary-Blair (Fresh)	360	37/38 (97.4)	86.2 - 99.9	318/322 (98.8)	96.9 - 99.7
	Unpreserved (Frozen)	N/A	N/A	N/A	N/A	N/A
	Unpreserved (Fresh)	237	26/27 (96.3)	81.0 - 99.9	208/210 (99.1)	96.6 - 99.9
	All Prospective	597	63/65 (96.9)	89.5 – 99.2	526/532 (98.9)	97.6 – 99.5
E. coli O157 [c]	Cary-Blair (Fresh)	361	N/A	N/A	359/361 (99.5)	98.0 - 99.9
	Unpreserved (Frozen)	956	1/2 (50.0)	1.3 - 98.7	950/954 (99.6)	98.9 - 99.9
	Unpreserved (Fresh)	237	N/A	N/A	237/237 (100.0)	98.5 - 100.0
	All Prospective	1554	1/2 (50.0)	1.3 – 98.7	1546/1552 (99.6)	99.2 - 99.9
Enteroaggregative *E. coli* (EAEC)[d]	Cary-Blair (Fresh)	359	17/18 (94.4)	72.7 - 99.9	336/341 (98.5)	96.6 - 99.5
	Unpreserved (Frozen)	948	25/29 (86.2)	68.3 - 96.1	916/919 (99.7)	99.1 - 99.9
	Unpreserved (Fresh)	235	1/1 (100.0)	2.5 - 100.0	234/234 (100.0)	98.4 - 100.0
	All Prospective	1542	43/48 (89.6)	77.3 – 96.5	1486/1494 (99.5)	99.0 - 99.8
Enterotoxigenic *E. coli* (ETEC)[e]	Cary-Blair (Fresh)	359	13/14 (92.9)	66.1 - 99.8	343/345 (99.4)	97.9 - 99.9
	Unpreserved (Frozen)	949	7/10 (70.0)	34.8 - 93.3	934/939 (99.5)	98.8 - 99.8
	Unpreserved (Fresh)	235	3/3 (100.0)	29.2 - 100.0	229/232 (98.7)	96.3 - 99.7
	All Prospective	1543	23/27 (85.2)	66.3 – 95.8	1506/1516 (99.3)	98.8 - 99.7
Shiga toxin-producing *E. coli* (STEC)[f]	Cary-Blair (Fresh)	361	N/A	N/A	359/361 (99.5)	98.0 - 99.9
	Unpreserved (Frozen)	922	3/3 (100.0)	29.2 - 100.0	918/919 (99.9)	99.4 - 100.0
	Unpreserved (Fresh)	237	N/A	N/A	235/237 (99.2)	97.0 - 99.9
	All Prospective	1520	3/3 (100.0)	29.2 – 100.0	1512/1517 (99.7)	99.2 - 99.9

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Salmonella spp.[g]	Cary-Blair (Fresh)	361	4/5 (80.0)	28.4 - 99.5	354/356 (99.4)	98.0 - 99.9
	Unpreserved (Frozen)	956	18/22 (81.8)	59.7 - 94.8	926/934 (99.1)	98.3 - 99.6
	Unpreserved (Fresh)	237	3/3 (100.0)	29.2 - 100.0	232/234 (99.2)	96.9 - 99.9
	All Prospective	1554	25/30 (83.3)	65.3 – 94.4	1512/1524 (99.2)	98.6 - 99.6
Shigella/ EIEC[h]	Cary-Blair (Fresh)	361	1/2 (50.0)	1.3 - 98.7	356/359 (99.2)	97.6 - 99.8
	Unpreserved (Frozen)	956	4/5 (80.0)	28.4 - 99.5	940/951 (98.8)	97.9 - 99.4
	Unpreserved (Fresh)	237	1/1 (100.0)	2.5 - 100.0	233/236 (98.7)	96.3 -99.7
	All Prospective	1554	6/8 (75.0)	34.9 – 96.8	1529/1546 98.9	98.3 - 99.4
Vibrio parahaemolyticus[i]	Cary-Blair (Fresh)	361	N/A	N/A	361/361 (100.0)	99.0 - 100.0
	Unpreserved (Frozen)	957	N/A	N/A	955/957 (99.8)	99.3 - 99.97
	Unpreserved (Fresh)	237	N/A	N/A	236/237 (99.6)	97.7 - 99.99
	All Prospective	1555	N/A	N/A	1552/1555 (99.8)	99.4 - 100.0
Vibrio spp. (not *parahaemolyticus*)	Cary-Blair (Fresh)	361	N/A	N/A	361/361 (100.0)	99.0 - 100.0
	Unpreserved (Frozen)	956	N/A	N/A	956/956 (100.0)	99.6 - 100.0
	Unpreserved (Fresh)	237	N/A	N/A	237/237 (100.0)	98.5 - 100.0
	All Prospective	1554	N/A	N/A	1554/1554 (100)	99.8 - 100.0
Yersinia enterocolitica[j]	Cary-Blair (Fresh)	361	N/A	N/A	357/361 (98.9)	97.2 - 99.7
	Unpreserved (Frozen)	956	N/A	N/A	951/956 (99.5)	98.8 - 99.8
	Unpreserved (Fresh)	237	N/A	N/A	236/237 (99.6)	97.7 - 99.99
	All Prospective	1554	N/A	N/A	1544/1554 (99.4)	98.8 - 99.7
Cryptosporidium spp.[k]	Cary-Blair (Fresh)	359	3/3 (100.0)	29.2 - 100.0	354/356 (99.4)	98.0 - 99.9
	Unpreserved (Frozen)	948	7/7 (100.0)	59.0 - 100.0	941/941 (100.0)	99.6 - 100.0
	Unpreserved (Fresh)	235	1/1 (100.0)	2.5 - 100.0	234/234 (100.0)	98.4 - 100.0
	All Prospective	1542	11/11 (100.0)	71.5 – 100.0	1529/1531 (99.9)	99.5 - 100.0
Entamoeba histolytica	Cary-Blair (Fresh)	361	N/A	N/A	359/359 (100.0)	99.0 – 100.0
	Unpreserved (Frozen)	948	N/A	N/A	948/948 (100.0)	99.6 - 100.0
	Unpreserved (Fresh)	235	N/A	N/A	235/235 (100.0)	98.4 - 100.0
	All Prospective	1542	N/A	N/A	1542/1542 (100)	99.8 - 100.0

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Giardia lamblia [l]	Cary-Blair (Fresh)	359	1/1 (100.0)	2.5 – 100.0	357/358 (99.7)	98.5 - 100.0
	Unpreserved (Frozen)	948	2/2 (100.0)	15.8 - 100.0	940/946 (99.4)	98.6 - 99.8
	Unpreserved (Fresh)	235	N/A	N/A	234/235 (99.6)	97.7 - 99.99
	All Prospective	1542	3/3 (100.0)	29.2 – 100.0	1531/1539 (99.5)	99.0 - 99.8
Adenovirus 40/41[m]	Cary-Blair (Fresh)	359	N/A	N/A	359/359 (100.0)	99.0 - 100.0
	Unpreserved (Frozen)	948	7/10 (70.0)	34.8 - 93.3	935/938 (99.7)	99.1 - 99.9
	Unpreserved (Fresh)	235	N/A	N/A	233/235 (99.2)	97.0 - 99.9
	All Prospective	1542	7/10 (70.0)	34.8 – 93.3	1527/1532 (99.7)	99.2 - 100.0
Norovirus (GI/GII)[n]	Cary-Blair (Fresh)	354	6/7 (85.7)	42.1 - 99.6	354/354 (100.0)	99.0 - 100.0
	Unpreserved (Frozen)	956	39/39 (100.0)	91.0 - 100.0	913/917 (99.6)	98.9 - 99.9
	Unpreserved (Fresh)	236	1/1 (100.0)	2.5 - 100.0	235/235 (100.0)	98.4 - 100.0
	All Prospective	1553	46/47 (97.9)	88.7 – 100.0	1502/1506 (99.7)	99.3 - 99.9
Rotavirus A	Cary-Blair (Fresh)	361	1/1 (100.0)	2.5 - 100.0	360/360 (100.0)	99.0 - 100.0
	Unpreserved (Frozen)	956	19/20 (95.0)	75.1 - 99.9	928/936 (99.2)	98.3 - 99.6
	Unpreserved (Fresh)	236	1/1 (100.0)	2.5 - 100.0	234/235 (99.6)	97.7 - 99.99
	All Prospective	1553	21/22 (95.5)	77.2 – 99.9	1522 /1531 (99.4)	98.9 - 99.7

a - *Campylobacter* spp. The 1 false negative compared to reference culture method was tested by bidirectional sequencing and confirmed as positive. The 29 false positives compared to the reference culture method were tested by bidirectional sequencing, and 20 of 29 confirmed as positives.

b - *Clostridium difficile*: The 2 false negatives compared to a FDA cleared NAAT produced high Ct values (Ct ≥ 35), and the 6 false positives had low MFIs which indicate that these samples are low positives. *C. difficile* must be tested fresh.

c - *E. coli* O157. The one false negative compared to the reference culture method was tested by bidirectional sequencing and could not be confirmed as positive. The 6 false positive samples compared to the reference culture method were tested by bidirectional sequencing, and 5 of 6 confirmed as positives.

d - EAEC. The 5 false negatives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing; 4 of the 5 confirmed as positive. 2 of the 8 false positives could not be repeated due to low sample volume. For the remaining samples, 5 of 6 were not detected by addition rounds of sequencing.

e - ETEC. The 4 false negatives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing; none were confirmed as positives. 1 of the 10 false positives could not be repeated due to low sample volume. Of the remaining 9 false positives, 8 were not confirmed as positives by an additional round of sequencing.

f - STEC. The 5 false positive samples compared to the reference culture method were tested by bidirectional sequencing, and all 5 were confirmed as positives.

g - *Salmonella* spp. The 5 false negative samples compared to the reference culture method were tested by bidirectional sequencing and 4 of 5 confirmed as positives. The 12 false positives compared to reference culture method were tested by bidirectional sequencing and 9 of 12 confirmed as positives. *Salmonella* species observed in the clinical study were: 8 *S.* enterica groups B-D, 9 S. enterica (untyped), and 17 Salmonella species (untyped).

h - *Shigella*/EIEC. The 2 false negatives compared to the reference culture method were tested by bidirectional sequencing and were not confirmed as positives. The 17 false positive samples compared to reference culture were tested by bidirectional sequencing and 16 of 17 confirmed as positives.

i - *Vibrio parahaemolyticus*. The 3 false positive samples compared to the reference culture method were tested by bidirectional sequencing, and 2 of 3 confirmed as positives.
j - *Yersinia enterocolitica.* The 10 false positive samples compared to the reference culture method were tested by bidirectional sequencing, and 3 of 10 confirmed positives.
k - *Cryptosporidium* spp. The 2 false positive samples compared to bidirectional sequencing were confirmed as positive by 2 additional rounds of sequencing.
l - *Giardia lamblia*. The 8 false positive samples compared to bidirectional sequencing were not confirmed as positive by 2 additional rounds of sequencing.
m - Adenovirus 40/41. The 3 false negatives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing and a FDA-cleared NAAT; none were confirmed as positives by either method. On initial testing these were detected by only one sequencing assay and all had high Ct values, which indicate that they are low positives. The 5 false positives were not confirmed as positives by an additional round of sequencing.
n - Norovirus GI/GII. The 1 false negative compared to bidirectional sequencing produced a high Ct (37).

Mixed Infections

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.

Table. 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

Table. 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)
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	

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)**
Double Infections				20	17	
Triple Infections				4	4	

Table. 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	
Quadruple Infections				1	1	

Table. 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	

Table. 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.

Table. Summary of unpreserved samples with their inoculated Cary-Blair samples Vs. reference method. Agreements were calculated compared to reference method results 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)	(15.8, 100.0)	385/392 (98.2)	(96.4, 99.3)
	Cary-Blair (Inoculated)	396	2/2 (100.0)	(15.8, 100.0)	388/394 (98.5)	(96.7, 99.4)
E. coli O157[b]	Unpreserved	395	1/2 (50.0)	(1.3, 98.7)	389/393 (99.0)	(97.4, 99.7)
	Cary-Blair (Inoculated)	397	1/2 (50.0)	(1.3, 98.7)	391/395 (99.0)	(97.4, 99.7)
Enteroaggregative *E. coli* (EAEC)[c]	Unpreserved	394	12/14 (85.7)	(57.2, 98.2)	378/380 (99.5)	(98.1, 99.9)
	Cary-Blair (Inoculated)	396	12/14 (85.7)	(57.2, 98.2)	382/382 (100.0)	(99.0, 100.0)
Enterotoxigenic *E. coli* (ETEC)[d]	Unpreserved	394	4/6 (66.7)	(22.3, 95.7)	387/388 (99.7)	(98.6, 100.0)
	Cary-Blair (Inoculated)	396	4/6 (66.7)	(22.3, 95.7)	386/390 (99.0)	(97.4, 99.7)
Shiga toxin-producing *E. coli* (STEC)	Unpreserved	361	2/2 (100.0)	(15.8, 100.0)	359/359 (100.0)	(99.0, 100.0)
	Cary-Blair (Inoculated)	363	2/2 (100.0)	(15.8, 100.0)	361/361 (100.0)	(99.0, 100.0)
Salmonella spp.[e]	Unpreserved	395	5/6 (83.3)	(35.9, 99.6)	385/389 (99.0)	(97.4, 99.7)
	Cary-Blair (Inoculated)	397	6/6 (100.0)	(54.1, 100.0)	389/391 (99.5)	(98.2, 99.9)
Shigella/ EIEC[f]	Unpreserved	395	1/1 (100.0)	(2.5, 100.0)	389/394 (98.7)	(97.1, 99.56)
	Cary-Blair (Inoculated)	397	1/1 (100.0)	(2.5, 100.0)	391/396 (98.7)	(97.1, 99.6)
Vibrio parahaemolyticus	Unpreserved	395	N/A	N/A	395/395 (100.0)	(99.1, 100.0)
	Cary-Blair (Inoculated)	397	N/A	N/A	397/397 (100.0)	(99.1, 100.0)
Vibrio spp. (not parahaemolyticus)	Unpreserved	395	N/A	N/A	395/395 (100.0)	(99.1, 100.0)
	Cary-Blair (Inoculated)	397	N/A	N/A	397/397 (100.0)	(99.1, 100.0)
Yersinia enterocolitica[g]	Unpreserved	395	N/A	N/A	394/395 (99.8)	(98.6, 100.0)
	Cary-Blair (Inoculated)	397	N/A	N/A	396/397 (99.8)	(98.6, 100.0)
Cryptosporidium spp	Unpreserved	394	2/2 (100.0)	(15.8, 100.0)	392/392 (100.0)	(99.1, 100.0)
	Cary-Blair (Inoculated)	396	2/2 (100.0)	(15.8, 100.0)	394/394 (100.0)	(99.1, 100.0)
Entamoeba histolytica	Unpreserved	394	N/A	N/A	394/394 (100.0)	(99.1, 100.0)
	Cary-Blair (Inoculated)	396	N/A	N/A	396/396 (100.0)	(99.1, 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 (99.2)	(97.8, 99.8)
	Cary-Blair (Inoculated)	396	N/A	N/A	394/396 (99.5)	(98.2, 99.9)
Adenovirus 40/41[i]	Unpreserved	394	3/6 (50.0)	(11.8, 88.2)	388/388 (100.0)	(99.1, 100.0)
	Cary-Blair (Inoculated)	396	2/6 (33.3)	(4.3, 77.7)	385/390 (98.7)	(97.0, 99.6)
Norovirus (GI/GII)	Unpreserved	395	28/28 (100.0)	(87.7, 100.0)	364/367 (99.2)	(97.6, 99.8)
	Cary-Blair (Inoculated)	397	28/28 (100.0)	(87.7, 100.0)	364/369 (98.6)	(96.9, 99.6)
Rotavirus A	Unpreserved	395	11/12 (91.7)	(61.5, 99.8)	380/383 (99.2)	(97.7, 99.8)
	Cary-Blair (Inoculated)	397	11/12 (91.7)	(61.5, 99.8)	380/385 (98.7)	(97.0, 99.6)

a - *Campylobacter* spp. Cary-Blair: The 6 false positives compared to reference culture method were tested by bidirectional sequencing and 4 of 6 confirmed as positives. Unpreserved: 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. 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 the reference culture method were tested by bidirectional sequencing and 3 of 4 confirmed as positives. 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 reference culture method were tested by bidirectional sequencing and 3 of 4 confirmed as positives.

c - EAEC. 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. Unpreserved: 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. Cary-Blair: 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. Unpreserved: 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. Cary-Blair: The 2 false positives compared to the reference culture method were tested by bidirectional sequencing and 1 of 2 confirmed as positives. Unpreserved: 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. Cary-Blair: The 5 false positives compared to the reference culture method were tested by bidirectional sequencing and 4 of 5 confirmed as positives. Unpreserved: 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*. Cary-Blair: The 1 false positive compared to the reference culture method was tested by bidirectional sequencing and confirmed as positive. Unpreserved: The 1 false positive compared to the reference culture method were tested by bidirectional sequencing and confirmed as positive.

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

i - Adenovirus 40/41. Cary-Blair: The 4 false negatives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing and a FDA-cleared NAAT; 1 of 4 was confirmed as positive by both methods. The 5 false positives were not confirmed as positives by an additional round of sequencing. Unpreserved: The 3 false negatives compared to bidirectional sequencing were tested by 2 additional rounds of sequencing and a FDA-cleared NAAT; none confirmed as positive.

Testing of Pre-selected Archived Specimens (Category III)

Several analytes were either 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 specimens were archived clinical specimens that had previously tested positive. Prior to

testing with the 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 assay 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.

Table. Demographic summary for archived specimens

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

Table. Summary of Clinical specimen Results (Archived specimens)

	Positive Agreement		**Negative Agreement**	
Target	**Agreement n/N (%)**	**95% CI**	**Agreement n/N (%)**	**95% CI**
Campylobacter spp	38/40 (95.0)	(83.1, 99.4)	152/152 (100.0)	(97.6, 100.0)
E.coli 0157	19/19 (100.0)	(82.4, 100.0)	152/152 (100.0)	(97.55, 100.0)
ETEC	20/20 (100.0)	(83.2, 100.0)	152/152 (100.0)	(97.6, 100.0)
STEC	30/33 (90.9)	(75.7, 98.1)	152/152 (100.0)	(97.6, 100.0)
Salmonella spp.	29/30 (96.7)	(82.8, 99.9)	152/152 (100.0)	(97.6, 100.0)
Shigella/ EIEC	43/45 (95.6)	(84.9, 99.5)	151/152 (99.3)	(96.4, 100.0)
Yersinia enterocolitica	3/3 (100.0)	(29.24, 100.0)	152/152 (100.0)	(97.6, 100.0)
Cryptosporidium spp.	16/19 (84.2)	(60.4, 96.6)	152/152 (100.0)	(97.6, 100.0)
Giardia lamblia	25/26 (96.2)	(83.2, 99.9)	152/152 (100.0)	(97.6, 100.0)
Adenovirus 40/41	26/26 (100.0)	(86.8, 100.0)	151/152 (99.3)	(96.4, 100.0)

Testing of Contrived Specimens (Category IV)

For some analytes, both prospective and archived testing were insufficient to 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 unique stool 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 for each microorganism) or greater using multiple strains for each organism (see Table below).

Table. Summary of contrived specimens.

Organism	Source	Characterization	Organism	Source	Characterization
Giardia lamblia	Waterborne P101	Assemblage B	*Entamoeba histolytica*	BEI NR-176	HB-301:NH
	BEI NR-9232	Mario		BEI NR-177	200:NH
	BEI NR-9234	D. Hall		BEI NR-178	HM-1:IMSS
	BEI NR-9235	Dan		BEI NR-179	Rahman
Yersinia enterocolitica	ATCC 29913	O:8; biotype 2	*Vibrio cholerae*	ATCC 25870	O:1
	ATCC 9610	O:8; biotype 1		BEI NR-146	O:1; El Tor
	BEI NR-206	O:8		BEI NR-149	O:2; Nanking 32/123
	BEI NR-212	O:3	*Vibrio vulnificus*	ATCC 27562	Strain 324 CDC B9629
	BEI NR-213	O:9		ATCC29306	Strain CDC A1402
Vibrio parahaemolyticus	ATCC 17802	Strain EB101	*Vibrio parahaemolyticus*	Zeptometrix 0801903	Strain Z134
	BEI NR-21991	Strain 10295 O1:K56		BEI NR-2202	Strain TX2103 O3:K6
	BEI NR-21990	Strain 48057 O4:K12			

Positive contrived samples of each of the above strains 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.

Table. Summary of contrived specimen results

	PPA[a]		NPA	
Target	**Agreement n/N (%)**	**95% CI**	**Agreement 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 - 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.

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 (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 for both, so no result was reported. A total of 26 samples were positive for at least one target. 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	Number of Subjects
<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 (100.00%)	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%)

General Performance of Assay during Clinical Trials

During the prospective clinical study 2.6% (41/1558) of samples were invalid for lack of RNA-IC signal on initial testing. After repeat or reflex testing, according to the IFU, the final invalid rate was 0.2% (3/1558).

Table. Summary of valid, partially invalid, and invalid runs.

Run Description	Number	Percentage 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.

Table. 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.

Limit of Detection

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 the presumptive LoD. LoD for each stock was defined as the lowest concentration with ≥95% detection of 20 replicates (19 out of 20), and was determined separately for unpreserved stool and Cary-Blair preserved stool.

Table. Limit of Detection for BioCode GPP, unpreserved and Cary-Blair specimens with easyMAG system.

Organism	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 subsp. *enterica*	ATCC 14028	2.2×10^3 CFU/mL	20/20	1.1×10^3 CFU/mL	19/20
Shiga-like toxin producing *E. coli* (STEC)	ATCC BAA-2217	2.5×10^3 CFU/mL	20/20	2.5×10^3 CFU/mL	20/20
E. coli O157	ATCC 700376	3.3×10^3 CFU/mL	20/20	3.3×10^3 CFU/mL	20/20
Shigella sonnei	ATCC 29930	4.4×10^2 CFU/mL	20/20	1.7×10^3 CFU/mL	20/20
Vibrio cholerae	ATCC 25870	4.9×10^2 CFU/mL	20/20	4.9×10^2 CFU/mL	20/20
Vibrio parahaemolyticus	ATCC 17802	1.3×10^1 CFU/mL	20/20	5.0×10^1 CFU/mL	20/20
Yersinia enterocolitica	ATCC 23715	1.5×10^3 CFU/mL	20/20	1.5×10^3 CFU/mL	20/20

Organism	Source	Unpreserved Stool LoD	Unpreserved Stool Detection	Cary-Blair Stool LoD	Cary-Blair Stool Detection
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	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 GI[a]	CDC	6.4×10^5 copies/gram	20/20	6.5×10^5 copies/gram	20/20
Norovirus GII[a]	CDC	5.2×10^4 copies/gram	20/20	9.96×10^4 copies/gram	20/20

a - Assayed at CDC.

Table. Limit of Detection for BioCode GPP, unpreserved and Cary-Blair specimens with MagNA Pure 96 system.

Organism	Source	Unpreserved Stool LoD	Unpreserved Stool Detection	Cary-Blair Stool LoD	Cary-Blair Stool Detection
Campylobacter coli	ATCC 33559	2.8×10^1 CFU/mL	20/20	2.8×10^1 CFU/mL	20/20
Campylobacter jejuni spp. jejuni	ATCC 33292	3.5×10^2 CFU/mL	19/20	7.0×10^2 CFU/mL	20/20
Clostridium difficile (toxinotype 0)	ATCC 9689	9.5×10^1 CFU/mL	20/20	9.5×10^1 CFU/mL	20/20
Clostridium difficile (toxinotype III; Nap1)	Zeptometrix 0801619cf	4.2×10^2 CFU/mL	20/20	4.1×10^2 CFU/mL	20/20
Enteroaggregative *E. coli* O92:H33 (EAEC)	STEC TW04440	7.0×10^2 CFU/mL	20/20	7.0×10^2 CFU/mL	20/20
Enteroinvasive *E. coli* O29:NM (EIEC)	ATCC 43892	1.8×10^2 CFU/mL	20/20	1.8×10^2 CFU/mL	20/20
Enterotoxigenic *E. coli* O78:H11 H10407 (ETEC)	ATCC 35401	2.8×10^2 CFU/mL	20/20	2.8×10^2 CFU/mL	19/20
Salmonella bongori	SGSC 4900	1.4×10^3 CFU/mL	20/20	1.4×10^3 CFU/mL	20/20
Salmonella enterica ssp. enterica	ATCC 14028	1.1×10^3 CFU/mL	20/20	1.1×10^3 CFU/mL	20/20

Organism	Source	Unpreserved Stool LoD	Unpreserved Stool Detection	Cary-Blair Stool LoD	Cary-Blair Stool Detection
Shiga-like toxin producing *E. coli* (STEC)	ATCC BAA-2217	1.3 x10^3 CFU/mL	20/20	1.3 x10^3 CFU/mL	20/20
E. coli O157	ATCC 700376	1.7 x10^3 CFU/mL	20/20	1.7 x10^3 CFU/mL	20/20
Shigella sonnei	ATCC 29930	2.2 x10^2 CFU/mL	20/20	2.2 x10^2 CFU/mL	20/20
Vibrio cholerae	ATCC 25870	2.5 x10^2 CFU/mL	20/20	2.5 x10^2 CFU/mL	20/20
Vibrio parahaemolyticus	ATCC 17802	6.5 x10^0 CFU/mL	20/20	6.5 x10^0 CFU/mL	20/20
Yersinia enterocolitica	ATCC 23715	7.5 x10^2 CFU/mL	20/20	7.5 x10^2 CFU/mL	20/20
Cryptosporidium parvum	waterborne P102C	3.1 x10^3 oocysts/mL	20/20	3.1 x10^3 oocysts/mL	20/20
Entamoeba histolytica HB-301:NIH	BEI NR-178	1.6 x10^{-1} cysts/mL	20/20	1.6 x10^{-1} cysts/mL	20/20
Giardia intestinalis (aka G. lamblia)	waterborne P101	9.0 x10^2 cysts/mL	20/20	9.0 x10^2 cysts/mL	20/20
Adenovirus 40 (dugan)	Zeptometrix 0810084	1.0 x10^{-1} $TCID_{50}$/mL	20/20	1.0 x10^{-1} $TCID_{50}$/mL	20/20
Adenovirus 41 (TAK)	Zeptometrix 0810085	4.7 x10^{-2} $TCID_{50}$/mL	20/20	4.7 x10^{-2} $TCID_{50}$/mL	20/20
Rotavirus A	ATCC VR-2018	1.3 x10^3 $TCID_{50}$/mL	20/20	1.3 x10^3 $TCID_{50}$/mL	20/20

Titered Norovirus GI and GII specimens were not available when determining the LoD for the MagNA Pure 96. Therefore, limit of detection was determined for positive clinical specimens in parallel for the easyMAG and MagNA Pure 96 systems and reported by dilution factor. For unpreserved stool, LoD with the easyMag extraction was 2-fold and 8.3-fold lower than the MagNA Pure 96 extraction for Norovirus GI and Norovirus GII, respectively. For Cary-Blair stool, LoD with the easyMag extraction was less than 2-fold lower than the MagNA Pure 96 extraction for both Norovirus GI and GII.

Table. Norovirus - Comparison of results for limit of detection testing for Unpreserved Stool extracted with the easyMAG and MagNA Pure 96 systems and assayed with BioCode GPP.

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

Table. Norovirus - Comparison of results for limit of detection testing for Cary-Blair Stool extracted with the easyMAG or MagNA Pure 96 systems and assayed with BioCode GPP.

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

Analytical Reactivity (Inclusivity)

A study was performed to verify Analytical Reactivity/Inclusivity of the BioCode GPP. Different strains were selected that represent various temporal, geographic, and genetic diversity for each analyte. This study tested a panel of titered stocks for relevant organisms diluted in Pre-Screened Negative Stools at 3X LoD. Stocks not detected at 3X LoD, if applicable, were tested at higher concentrations. Due to a lack of titered specimens, Adenovirus 40/41 clinical samples and *Cryptosporidium* DNA from the Cryptosporidium reference unit were used (Public Health England). Norovirus GI and GII genotypes and the Rotavirus vaccine strain were tested according to the same approach by the CDC. In addition, wet testing was supplemented with *in silico* analysis to assess reactivity for strains or serotypes that were not tested in this study.

Table. Campylobacter inclusivity results.

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

Table. *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
		ATCC 700792-FZ	2.48 x 10^3 CFU/mL	3x
		ATCC BAA-1382-FZ	2.48 x 10^3 CFU/mL	3x
		ATCC 51695-FZ	2.48 x 10^3 CFU/mL	3x
		ATCC 43599-FZ	2.48 x 10^3 CFU/mL	3x

Organism	Toxinotype	Source	Concentration Detected	Multiple of LoD Detected
		ATCC 43596-FZ	2.48 x 10^3 CFU/mL	3x
		ATCC 17858-FZ	2.48 x 10^3 CFU/mL	3x
		ATCC 43594	2.48 x 10^3 CFU/mL	3x
		ATCC 43600	2.48 x 10^3 CFU/mL	3x
		ATCC 17857	2.48 x 10^3 CFU/mL	3x
		ATCC BAA-1871	2.48 x 10^3 CFU/mL	3x
		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

Table. *E. coli* O157 inclusivity results.

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected	STEC Assay Results
E. coli O157	*E. coli* O157:H45	STEC SC373/2 TW07922	9.9 x 10^3 CFU/mL	3x	non- STEC
	E. coli 0157:HNM	STEC DA-26 TW07952	9.9 x 10^3 CFU/mL	3x	stx1/stx2
	E. coli 0157:H7	STEC 93-111 TW04863	9.9 x 10^3 CFU/mL	3x	stx1/stx2
	E. coli O157: H7	STEC MI06-19 TW14301	9.9 x 10^3 CFU/mL	3x	stx2
	E. coli O157:HNT	STEC DA-27 TW07953	9.9 x 10^3 CFU/mL	3x	stx1/stx2
	E. coli O157:H7 Strain EDL933	BEI NR-11	9.9 x 10^3 CFU/mL	3x	stx1/stx2

Table. Shiga-like toxin producing *E. coli* (STEC) *stx1/stx2* inclusivity results.

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected	*stx1/stx2*
Shiga toxin producing *E. coli* (STEC)	*E. coli* O26:H11	STEC 2332/00 TW08998	7.5 x 10^3 CFU/mL	3x	*stx1*
	E. coli O45:H2	STEC DEC11C DEC11c	7.5 x 10^3 CFU/mL	3x	*stx1*
	E. coli O103:H2	STEC 107-226 TW07881	7.5 x 10^3 CFU/mL	3x	*stx1*
	E. coli O26:NM	STEC DA-22 TW07948	7.5 x 10^3 CFU/mL	3x	*stx1*

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected	*stx1/stx2*
	E. coli O45:H2	STEC MI05-14 TW14003	7.5 x 10^3 CFU/mL	3x	*stx1*
	E. coli 045:H2	ATCC BAA-2193	7.5 x 10^3 CFU/mL	3x	*stx1*
	E. coli O111:H8	STEC DEC8B	7.5 x 10^3 CFU/mL	3x	*stx1/stx2*
	E. coli O145:NM	ATCC BAA-2192	7.5 x 10^3 CFU/mL	3x	*stx1/stx2*
	E. coli O26: H11	BAA-2196	7.5 x 10^3 CFU/mL	3x	*stx1/stx2*
	E. coli O146:21	STEC DEC16E TW01383	7.5 x 10^3 CFU/mL	3x	*stx1c*
	E. coli O104:H4[a]	ATCC BAA-2326	7.5 x 10^3 CFU/mL	3x	*stx2*
	E. coli O113:H21	STEC CL-15 TW02318	7.5 x 10^3 CFU/mL	3x	*stx2*
	E. coli O104:H21	STEC G5506 TW04909	7.5 x 10^3 CFU/mL	3x	*stx2*
	E. coli O111:H2	STEC RD8 TW06296	7.5 x 10^3 CFU/mL	3x	*stx2*
	E. coli O121:19	STEC MDCH-4 TW07614	7.5 x 10^3 CFU/mL	3x	*stx2*
	E. coli O121:NM	STEC DA-37 TW07972	7.5 x 10^3 CFU/mL	3x	*stx2*
	E. coli O121:H19	BAA-2219	7.5 x 10^3 CFU/mL	3x	*stx2*

a - 2011 German Outbreak Strain. Isolate has characteristics of STEC and EAEC.

Note: Based on *in silico* analysis stx2 subtypes a, b, c, d, e will be detected while subtype f is predicted to be detected with reduced sensitivity or not detected by the BioCode GPP.

Table. 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[a]	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

a - 2011 German Outbreak Strain. Isolate has characteristics of STEC and EAEC.

Table. Enterotoxigenic *E. coli* (ETEC) LT/ST inclusivity results.

Organism	Serotype	Source	ST/LT	Concentration Detected	Multiple of LoD Detected
Enterotoxigenic *E. coli* (ETEC)	*E.coli* O25:K98	ATCC 43886	LT	1.68 x 10^3 CFU/mL	3x
	E.coli O78:K80:H12	ATCC 43896	ST-b	1.68 x 10^3 CFU/mL	3x
	E.coli O8:K85:K99	ATCC 31618	ST-a	1.68 x 10^3 CFU/mL	3x
	E.coli	Zeptometrix 0801624	ST-a/ ST-b/ LT	1.68 x 10^3 CFU/mL	3x

Table. *Salmonella* inclusivity results.

Organism	serovar	Source	Concentration Detected	Multiple of LoD Detected
Salmonella bongori[a]	subsp V serotype 66:Z41	UC Irvine (MZ0091)	1.65 x 10^4 CFU/mL	3x
	subsp V serotype 48:Z41	UC Irvine (MZ0092)	1.65 x 10^4 CFU/mL	3x
	subsp V serotype 66:Z41	UC Irvine (MZ0162)	1.65 x 10^4 CFU/mL	3x
Salmonella enterica subsp. II		SGSC3039	6.45 x 10^3 CFU/mL	3x
Salmonella enterica subsp. IIIa		SGSC3061	6.45 x 10^3 CFU/mL	3x
Salmonella enterica subsp. IIIb		sGSC3068	6.45 x 10^3 CFU/mL	3x
Salmonella enterica subsp. IV		SGSC3074	6.45 x 10^3 CFU/mL	3x
Salmonella enterica subsp. VI		SGSC3116	6.45 x 10^3 CFU/mL	3x
Salmonella enterica subsp. *arizonae*	Unknown	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 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

Organism	serovar	Source	Concentration Detected	Multiple of LoD Detected
	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
	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

a - *Salmonella bongori* was examined during LoD and confirmed with a similar LoD concentration to *Salmonella enterica*. *In silico* analysis predict reactivity with the *Salmonella bongori* species in NCBI.

Note: *Salmonella* species observed in the clinical study were: 8 *S. enterica* groups B-D, 9 *S. enterica* (untyped), and 17 *Salmonella* species (untyped).

Table. Enteroinvasive *E. coli* (EIEC) and Shigella spp. 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	Type 1	ATCC 9207	1.31 x 10^3 CFU/mL	3x
	Type 2	BEI NR-521	1.31 x 10^3 CFU/mL	3x
	Type 7	ATCC 9905	1.31 x 10^3 CFU/mL	3x
	Type 20	ATCC BAA-1247	1.31 x 10^3 CFU/mL	3x
	Type 3	ATCC 8702	1.31 x 10^3 CFU/mL	3x
Shigella dysenteriae	Type 1	BEI NR-520[a]	1.31 x 10^3 CFU/mL	3x
	Type 3	ATCC 9751	1.31 x 10^3 CFU/mL	3x
	Type 2	ATCC 9750	1.31 x 10^3 CFU/mL	3x
	Type 5	ATCC 9764	1.31 x 10^3 CFU/mL	3x
	Type 12	ATCC 49552	1.31 x 10^3 CFU/mL	3x
Shigella flexneri (strain 2457T)	Type 2a	BEI NR-517	1.31 x 10^3 CFU/mL	3x
	Type 2a	BEI NR-518	1.31 x 10^3 CFU/mL	3x
	Type 2b	ATCC 12022	1.31 x 10^3 CFU/mL	3x
	Type 6	ATCC 12025	1.31 x 10^3 CFU/mL	3x
	Type 1b	ATCC 9380	1.31 x 10^3 CFU/mL	3x
Shigella sonnei	N/A	ATCC 25931	1.31 x 10^3 CFU/mL	3x
	N/A	ATCC 11060	1.31 x 10^3 CFU/mL	3x
	N/A	ATCC 9290	1.31 x 10^3 CFU/mL	3x
	N/A	ATCC 29029	1.31 x 10^3 CFU/mL	3x

a - *Shigella dysenteriae* (BEI NR-520) STEC detected as expected due to presence of stx1.

Table. Vibrio spp (V. cholerae and V. vulnificus) and Vibrio parahaemolyticus inclusivity results.

Organism	Species, Serotype, Strain		Source	Concentration Detected	Multiple of LoD Detected
Vibrio spp.	*Vibrio cholerae*	O:1 Inaba, Biotype El Tor	BEI NR-147	1.47 x 10^3 CFU/mL	3x
		O:1 Inaba, Biotype El Tor	BEI NR-146	1.47 x 10^3 CFU/mL	3x
		O:2	BEI NR-149	1.47 x 10^3 CFU/mL	3x
		O:4	BEI NR-151	1.47 x 10^3 CFU/mL	3x
		O:139	BEI NR-144	1.47 x 10^3 CFU/mL	3X
		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
			ATCC BAA-88	1.47 x 10^3 CFU/mL	3x
			ATCC 43382	4.9 x 10^3 CFU/mL	10X[a]
			ATCC 29306	1.47 x 10^3 CFU/mL	3x
			ATCC 29307	1.47 x 10^3 CFU/mL	3x
Vibrio parahaemolyticus	O4:K12 strain 48057		BEI NR-21990	3.75 x 10^1 CFU/mL	3x
	O1:K56 strain 10295		BEI NR-21991	3.75 x 10^1 CFU/mL	3x
	O3:K6 strain TX2103		BEI NR-22002	3.75 x 10^1 CFU/mL	3x
	strain Z134		Zeptometrix 0801903	3.75 x 10^1 CFU/mL	3x
	O4:K86 strain AN218		BEI NR-22013	3.75 x 10^1 CFU/mL	3x

a - *Vibrio vulnificus* ATCC 43382 was detected 2 of 3 replicates at 3x LoD, while all 3 replicates were detected at 10x LoD.

Table. *Yersinia enterocolitica* inclusivity results.

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
Yersinia enterocolitica	O:8	ATCC 9610	4.43 x 10^3 CFU/mL	3x
		BEI NR-206	4.43 x 10^3 CFU/mL	3x
		ATCC 29913	4.43 x 10^3 CFU/mL	3x
	O:3	BEI NR-212	4.43 x 10^3 CFU/mL	3x
	O:9	BEI NR-213	4.43 x 10^3 CFU/mL	3x

Table. Cryptosporidium spp inclusivity results.

Organism	Serotype	Source	Concentration Detected	Ct from ref assay
Cryptosporidium parvum	*DNA subtype / IIaA17G1R1*	UKCR UK28	Clinical Sample unknown	Ct = 24.4
	DNA subtype / IIaA15G2R1	UKCR UK29	Clinical Sample unknown	Ct = 23.5
	DNA subtype / IIaA19G1R1	UKCR UK30	Clinical Sample unknown	Ct = 24.9
	DNA subtype / IIdA22G1	UKCR UK31	Clinical Sample unknown	Ct = 31.0
	DNA subtype / IIdA15G1	UKCR UK32	Clinical Sample unknown	Ct = 27.0
Cryptosporidium hominis	*DNA subtype IaA14R3*	UKCR UKH14	Clinical Sample unknown	Ct = 28.0
	DNA subtype IdA18	UKH12	Clinical Sample unknown	Ct = 31.1
	DNA subtype IbA10G2	UKH13	Clinical Sample unknown	Ct = 35.2
	Unknown	NR2520	Clinical Sample unknown	Ct = 16.9

Ct values determined by Applied BioCode validated real-time PCR (SYBR) method.

Table. *Entamoeba histolytica* inclusivity results.

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

Table. *Giardia lamblia* (aka intestinalis) inclusivity results.

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

Table. Adenovirus 40/41 inclusivity results.

Organism	Serotype	Source	Concentration Detected	Ct from ref assays
Adenovirus 40	Dugan	Virapur	3.00×10^{-1} $TCID_{50}$/mL	N/A
Adenovirus 40	N/A	Sample ID # SP143	Clinical Sample unknown	12.4
Adenovirus 40	N/A	Sample ID # SP258	Clinical Sample unknown	10.6
Adenovirus 41	N/A	Sample ID # SP170	Clinical Sample unknown	15.0
Adenovirus 41	N/A	Sample ID # SP174	Clinical Sample unknown	13.8
Adenovirus 41	N/A	Sample ID # SP 276	Clinical Sample unknown	30.1
Adenovirus 41	N/A	Sample ID # SP288	Clinical Sample unknown	12.1
Adenovirus 41	N/A	Sample ID # SP309	Clinical Sample unknown	13.8
Adenovirus 41	N/A	Sample ID # SP326	Clinical Sample unknown	11.8
Adenovirus 41	N/A	Sample ID # SP446	Clinical Sample unknown	17.0

Ct values determined by Applied BioCode validated real-time PCR (SYBR) method.

Table. Norovirus GI/GII inclusivity results.

Organism	Genotype	Source	Concentration Detected	Multiple of LoD Detected
Norovirus GI[a]	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
Norovirus GII[a]	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 - Tested at CDC at 3x, 30x and 300x LoD only.

Table. Rotavirus A inclusivity results.

Organism	Serotype	Source	Concentration Detected	Multiple of LoD Detected
Rotavirus A	DS-1	ATCC VR-2550	7.44 x 10^3 IU/mL	3x
	HRV 89-12C2	ATCC VR-2272	7.44 x 10^3 IU/mL	3x
	WISC2	ATCC VR-2417	7.44 x 10^3 IU/mL	3x
	HRV 408	ATCC VR-2273	7.44 x 10^3 IU/mL	3x
	HRV 248	ATCC VR-2274	7.44 x 10^3 IU/mL	3x
	HU	ATCC VR-1546	7.44 x 10^3 IU/mL	3x
	HRV CJN	ATCC VR-2275	7.44 x 10^3 IU/mL	3x
	W161	ATCC VR-2551	7.44 x 10^3 IU/mL	3x
	G1b	Clinical Sample	Ct= 20[a]	N/A
	G2b	Clinical Sample	Ct= 22[a]	N/A
	G3b	Clinical Sample	Ct= 26[a]	N/A
	G4b	Clinical Sample	Ct= 21[a]	N/A
	G9b	Clinical Sample	Ct= 20[a]	N/A

a - Ct values provided by CDC from CDC validated Real-time PCR assay; considered to be moderate positives.

Analytical Specificity (Cross-Reactivity and Exclusivity)

- 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. Microorganisms were tested at 10^6 CFU/mL or higher for bacteria and 10^5 units/mL or higher for viruses ($TCID_{50}$/mL), protozoa (Cysts/mL), and fungi (CFU/mL) when possible. Each organism was extracted in triplicate on the easyMAG and assayed in singlet with the BioCode GPP on the BioCode MDx-3000 system per instructions for use. 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. Unless otherwise specified by asterisk * all testing was performed at Applied BioCode, Inc. Empirical testing with a gene fragment construct and *in silico* sequence analysis do not predict cross reactivity with the closely related *E. dispar*.

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 has demonstrated that the BioCode GPP will detect recombinant viruses included in Rotavirus vaccines.
- Empirical testing indicates cross reactivity with *C. cuniculus* and *C. meleagridis* with the Cryptosporidium assay.

Table. Reactivity was observed with the following organisms. 10-fold serial dilutions were performed to determine lower end of detection.

Organism	ATCC/Other Reference number	Titer Tested	N of 3 (target detected)	Titer detected 3 of 3 after 10- fold serial dilutions
Vibrio alginolyticus	ATCC 17749	6.40×10^6 CFU/mL	3/3 (*Vibrio* spp[a])	6.40×10^3 CFU/mL
Vibrio mimicus	ATCC 700326	8.30×10^6 CFU/mL	3/3 (*Vibrio* spp[a])	8.30×10^3 CFU/mL
Yersinia bercovieri	ATCC 43970	1.24×10^6 CFU/mL	3/3 (*Yersinia enterocolitica*[b])	$< 1.24 \times 10^2$ CFU/mL
Yersinia frederiksenii	ATCC 33642	1.29×10^6 CFU/mL	3/3 (*Yersinia enterocolitica*[b])	1.29×10^3 CFU/mL
Yersinia intermedia	DD-750 (DNA)	1.80×10^6 copies/mL	3/3 (*Yersinia enterocolitica*[b])	N/A
Yersinia mollaretii	ATCC 43969	3.40×10^6 CFU/mL	3/3 (*Yersinia enterocolitica*[b])	$< 3.40 \times 10^2$ CFU/mL
Yersinia rohdei	ATCC 43380	6.80×10^6 CFU/mL	3/3 (*Yersinia enterocolitica*[b])	6.80×10^4 CFU/mL
Cryptosporidium cuniculus(DNA)	UKCU12 - DNA	Unknown	1/3 (*Cryptosporidium* spp.)	N/A
Cryptosporidium meleagridis(DNA)	UKMEL10 - DNA	Unknown	1/3 (*Cryptosporidium* spp.)	N/A
Cryptosporidium meleagridis	BEI NR-2521	1.01×10^5 Cysts/mL	3/3 (Cryptosporidium spp.)	N/A
Cryptosporidium meleagridis[c]	UKMEL10	Clinical Sample (Ct = 32.5)	3/3 (Cryptosporidium spp.[c])	N/A

a - Vibrio spp LoD established as 4.9×10^2 CFU/mL
b - Yersinia enterocolitica LoD established as 1.5×10^3 CFU/mL
c - Ct values determined by Applied BioCode validated real-time PCR (SYBR) method.

Table. No cross-reactivity was observed for the on-panel GI Pathogens tested with the BioCode GPP.

BioCode GPP targets	ATCC/Other Reference number	Titer Tested
Campylobactercoli	ATCC 33559	2.40 x 10^5 CFU/mL
Campylobacter jejuni spp. jejuni	ATCC 33292	7.10 x 10^5 CFU/mL
Clostridium difficile (toxinotype 0)	ATCC 9689	1.93 x 10^6 CFU/mL
Clostridium difficile (toxinotype III; Nap1)	Zeptometrix 0801619CF	7.43 x 10^6 CFU/mL
Enteroaggregative *E. coli* O92:H33 (EAEC)	STEC TW04440	6.18 x 10^6 CFU/mL
Enteroinvasive *E. coli* O29:NM (EIEC)	ATCC 43892	4.70 x 10^6 CFU/mL
Enterotoxigenic *E. coli* O78:H11 H10407 (ETEC)	ATCC 35401	4.07 x 10^6 CFU/mL
Shiga-like toxin producing *E. coli* (STEC)	ATCC BAA-2217	5.90 x 10^6 CFU/mL
E. coli O157	ATCC 700376	2.36 x 10^6 CFU/mL
Salmonella bongori	SGSC 4900	2.16 x 10^7 CFU/mL
Salmonella enterica subsp. *enterica*	ATCC 14028	5.52 x 10^7 CFU/mL
Shigella sonnei	ATCC 29930	5.42 x 10^6 CFU/mL
Vibrio cholerae	ATCC 25870	9.80 x 10^5 CFU/mL
Vibrio parahaemolyticus	ATCC 17802	6.20 x 10^4 CFU/mL
Yersinia enterocolitica	ATCC 23715	1.48 x 10^6 CFU/mL
Cryptosporidium parvum	Waterborne P102C	1.20 x 10^5 CFU/mL
Entamoeba histolytica HB-301:NIH	BEI NR-178	7.44 x 10^3 CFU/mL
Giardia intestinalis (aka G. lamblia)	Waterborne P101	6.25 x 10^5 CFU/mL
Adenovirus 40 (dugan)	Zeptometrix 0810084CF	1.69 x 10^4 CFU/mL
Adenovirus 41 (TAK)	Zeptometrix 0810085CF	1.25 x 10^5 CFU/mL
Rotavirus A	ATCC VR-2018	3.00 x 10^5 CFU/mL

Table. No cross-reactivity was observed for the following bacteria.

Bacteria	ATCC/Other Reference number	Titer Tested
Aeromonas jandaei	ATCC 49568	1.21 x 10^5 CFU/mL
Aeromonas media	ATCC BAA-229	5.10 x 10^5 CFU/mL
Aeromonas trota	ATCC 49658	1.10 x 10^6 CFU/mL
Aeromonascaviae	ATCC 14486	1.14 x 10^6 CFU/mL
Aeromonashydrophila	ATCC 7966	8.70 x 10^6 CFU/mL
Acinetobacterbaumannii	ATCC 19606	5.00 x 10^6 CFU/mL
Acinetobacterlwoffii	ATCC 15309	1.80 x 10^6 CFU/mL
Alcaligenes faecalis	ATCC 15554	1.09 x 10^6 CFU/mL
Bacillus cereus	ATCC 13472	1.20 x 10^6 CFU/mL
Bacteroides fragilis	Zeptometrix 0801583	4.77 x 10^6 CFU/mL
Bacteroidesthetaiotaomicron	Zeptometrix Z037	5.67 x 10^6 CFU/mL
Bifidobacterium breve	Zeptometrix 0801829	1.53 x 10^6 CFU/mL
Campylobacterfetus	ATCC 25936	2.60 x 10^6 CFU/mL
Campylobacterhyointestinalis	ATCC 35217	1.31 x 10^6 CFU/mL
Campylobacterlari	ATCC 43675	1.60 x 10^6 CFU/mL

Bacteria	ATCC/Other Reference number	Titer Tested
Campylobacter upsaliensis	ATCC 49816	1.31 x 10^6 CFU/mL
Candida albicans	ATCC 14053	1.87 x 10^6 CFU/mL
Cedecea davisae	ATCC 43025	6.40 x 10^6 CFU/mL
Chlamydiatrachomatis	Zeptometrix 0801775	2.85 x 10^6 CFU/mL
Citrobacteramalonaticus	ATCC BAA-2562	1.74 x 10^6 CFU/mL
Citrobacter freundii	ATCC 8090	2.49 x 10^6 CFU/mL
Clostridium difficile non-toxigenic	ATCC 43593	1.25 x 10^6 CFU/mL
Clostridium difficile non-toxigenic	ATCC 43601	3.10 x 10^6 CFU/mL
Clostridium difficile non-toxigenic	ATCC 700057	1.01 x 10^6 CFU/mL
Clostridiumhistolyticum	ATCC 19401	1.09 x 10^6 CFU/mL
Clostridiumperfringens	Zeptometrix 0801585	1.06 x 10^6 CFU/mL
Clostridium septicum	Zeptometrix 0801885	1.62 x 10^6 CFU/mL
Clostridium sordellii	Zeptometrix 0801587	3.27 x 10^6 CFU/mL
Clostridium sporogenes	ATCC 3584	1.40 x 10^6 CFU/mL
Clostridium tetani	ATCC 19406	1.18 x 10^6 CFU/mL
Edwardsiella tarda	ATCC 15947	9.80 x 10^6 CFU/mL
Egglerthella lenta	ATCC 25559 N9	1.40 x 10^6 CFU/mL
Enterobacter aerogenes	Zeptometrix Z035	1.75 x 10^6 CFU/mL
Enterobactercloacae	ATCC 13047	2.62 x 10^6 CFU/mL
Enterococcusfaecalis	ATCC 51299	1.55 x 10^6 CFU/mL
Enterococcusfaecium	ATCC 700221	4.90 x 10^6 CFU/mL
Enteropathogenic *E. coli* O127:H6 (EPEC)	STEC TW06375	4.32 x 10^6 CFU/mL
Escherichia coli Non pathogenic	ATCC BAA-1431	4.50 x 10^6 CFU/mL
Escherichia coli Non pathogenic	ATCC 35328	3.40 x 10^6 CFU/mL
Escherichia coli Non pathogenic	ATCC BAA-97	2.40 x 10^6 CFU/mL
Escherichia hermannii	ATCC 55236	1.09 x 10^6 CFU/mL
Escherichia vulneris	ATCC 39368	2.00 x 10^6 CFU/mL
Fusobacterium varium	ATCC 8501	4.10 x 10^6 CFU/mL
Gardnerella vaginalis	Zeptometrix Z125	6.12 x 10^6 CFU/mL
Gemella morbillorum	HM-240	6.30 x 10^6 CFU/mL
Grimontia hollisae (formerly vibrio)	ATCC 33564	6.50 x 10^6 CFU/mL
Haemophilus influenzae	Zeptometrix type b; Eagan	2.40 x 10^6 CFU/mL
Hafnia alvei	ATCC 29926	4.20 x 10^6 CFU/mL
Helicobacter pylori	Zeptometrix Z040	1.96 x 10^6 CFU/mL
Klebsiella oxytoca	ATCC 33496	4.60 x 10^6 CFU/mL
Klebsiella pneumoniae	ATCC 13883	4.80 x 10^6 CFU/mL
Lactobacillus acidophilus	ATCC 4356	1.40 x 10^6 CFU/mL
Lactobacillus reuteri	HM-102	1.00 x 10^6 CFU/mL
Lactococcus lactis	ATCC 11454	1.00 x 10^6 CFU/mL
Leminorella grimontii	ATCC 43008	1.20 x 10^6 CFU/mL

Bacteria	ATCC/Other Reference number	Titer Tested
Listeria monocytogenes	ATCC 19115	3.80 x 10^6 CFU/mL
Morganella morganii	ATCC 25830	1.90 x 10^6 CFU/mL
Peptoniphilus asaccharolyticus	ATCC 29743	1.21 x 10^6 CFU/mL
Plesiomonas shigelloides	ATCC 14029	8.60 x 10^6 CFU/mL
Porphyromonas asaccharolytica	ATCC 25260	1.90 x 10^6 CFU/mL
Prevotella melaninogenica[a]	ATCC 25845	1.00 x 10^6 CFU/mL
Proteus mirabilis	ATCC 25933	2.15 x 10^6 CFU/mL
Proteus penneri	ATCC 33519	1.90 x 10^6 CFU/mL
Proteus vulgaris	ATCC 6380	2.41 x 10^6 CFU/mL
Providencia alcalifaciens	ATCC 51902	3.70 x 10^6 CFU/mL
Providencia stuartii	ATCC 33672	2.31 x 10^6 CFU/mL
Pseudomonas aeruginosa	ATCC 39324	7.40 x 10^6 CFU/mL
Pseudomonas fluorescens	ATCC 13525	1.27 x 10^6 CFU/mL
Pseudomonas putida	Zeptometrix 0801722	1.00 x 10^6 CFU/mL
Saccharomyces boulardii	ATCC MYA-796	8.40 x 10^6 CFU/mL
Serratia liquefaciens	ATCC 27592	3.20 x 10^6 CFU/mL
Serratia marcescens	ATCC 13880	2.80 x 10^6 CFU/mL
Shewanella algae	ATCC 51181	1.20 x 10^6 CFU/mL
Staphylococcus aureus	ATCC 43300	2.40 x 10^6 CFU/mL
Staphylococcus epidermidis	ATCC 14990	8.20 x 10^6 CFU/mL
Stenotrophomonas maltophilia	DD-468 (DNA)	1.85 x 10^6 copies/mL
Streptococcus agalactiae	ATCC 55193	1.64 x 10^6 CFU/mL
Streptococcus intermedius	HM-368D (DNA)	Unknown
Streptococcus pyogenes	NR-15274	2.90 x 10^6 CFU/mL
Streptococcussalivarius	HM-121	1.12 x 10^6 CFU/mL
Streptococcus suis	ATCC 43765	1.64 x 10^6 CFU/mL
Trabulsiella guamensis	ATCC 49490	2.40 x 10^6 CFU/mL
Veillonella parvula	ATCC 10790	5.90 x 10^6 CFU/mL
Vibrio fluvialis	ATCC 33809	4.70 x 10^6 CFU/mL
Yersinia pseudotuberculosis	NR-4371	1.85 x 10^6 CFU/mL

a – *Prevotella melaninogenica*: On initial testing 1 of 3 replicates detected stx1. *In silico* analysis does not predict interaction with this primer set and 0 of 5 replicates detected stx1 upon repeat.

Table. No cross-reactivity was observed for the following viruses.

Viruses	ATCC/Other Reference number	Titer Tested
Adenovirus 3	Zeptometrix 0810062CF	4.17 x 10^4 $TCID_{50}$/mL
Adenovirus 4	Zeptometrix 0810070CF	2.45 x 10^4 $TCID_{50}$/mL
Adenovirus 7a	Zeptometrix 0810021CF	1.95 x 10^5 $TCID_{50}$/mL
Adenovirus 8	Zeptometrix 0810069CF	1.41 x 10^4 $TCID_{50}$/mL
Adenovirus 14	Zeptometrix 0810108CF	3.39 x 10^5 $TCID_{50}$/mL

Viruses	ATCC/Other Reference number	Titer Tested
Adenovirus 37	Zeptometrix 0810119CF	1.91 x 10^5 $TCID_{50}$/mL
*Astrovirus type 1	Clinical Sample - CDC	CT= 31
*Astrovirus type 4	Clinical Sample - CDC	CT= 25
Coronavirus 229E	Zeptometrix 0810229CF	1.51 x 10^5 $TCID_{50}$/mL
Coronavirus NL63[a]	Zeptometrix 0810228CF	1.41 x 10^4 $TCID_{50}$/mL
*Coxsackie virus A16	Cell culture - CDC	1.0 x 10^5 pfu/mL
Coxsackievirus B3	Zeptometrix 0810074CF	1.78 x 10^5 $TCID_{50}$/mL
Cytomegalovirus (CMV)	Zeptometrix 0810003CF	1.15 x 10^5 $TCID_{50}$/mL
Enterovirus 68	Zeptometrix 0810237CF	6.60 x 10^5 $TCID_{50}$/mL
*Enterovirus	Cell culture - CDC	1 x 10^9 pfu/mL
*Echovirus 11	Cell culture - CDC	1.0 x 10^6 pfu/mL
HSV Type 2	Zeptometrix 0810213CF	2.19 x 10^5 $TCID_{50}$/mL
*Norovirus GIV	Clinical Sample - CDC	CT= 24
*Norovirus GIV	Clinical Sample - CDC	CT = 32
Rhinovirus 1A	Zeptometrix 0810012CF	1.41 x 10^4 $TCID_{50}$/mL
*Sapovirus GI	Clinical Sample - CDC	CT=14
*Sapovirus GIV	Clinical Sample - CDC	CT=26
*Sapovirus GV	Clinical Sample - CDC	CT=25
*Rotavirus vaccine	RotaTeq vaccine	1.00 x 10^8 pfu/mL
	Rotarix vaccine	1.00 x 10^6 $CCID_{50}$/mL

* - Assayed at CDC.
a - Coronavirus NL63: On initial testing 1 of 3 replicates detected stx1. *In silico* analysis does not predict interaction with this primer set and 0 of 5 replicates detected stx1 upon repeat.

Table. No cross-reactivity was observed for the following parasites.

Parasites	ATCC/Other Reference number	Titer Tested
**Blastocystis hominis*	Clinical Sample - CDC	1.0 x 10^7 infectious units
Blastocystis hominis	ATCC 50613	3.40 x 10^5 cells/mL
Cryptosporidium canis (DNA)	UKCAN1 - DNA	Unknown
Cryptosporidium felis (DNA)	UKFEL3 - DNA	Unknown
Cryptosporidium muris	Waterborne P104	2.50 x 10^4 Cysts/mL
Cryptosporidium ubiquitum (DNA)	UKUB7 - DNA	Unknown
Encephalitozoon cuniculi	BEI NR-9073	1.73 x 10^4 Cysts/mL
Encephalitozoon hellem	BEI NR-9701	8.24 x 10^3 Cysts/mL
Encephalitozoon intestinalis	BEI NR-9702	1.52 x 10^3 Cysts/mL
Giardia muris	Waterborne P105	6.25 x 10^4 Cysts/mL
Pentatrichomonashominis	ATCC 30098	2.50 x 10^5 cells/mL
Toxoplasma gondii	NR-33509	3.17 x 10^5 Cysts/mL

* - Assayed at CDC.

Table. *In silico* evaluation of organisms for potential cross-reactivity. No cross-reactivity was predicted via *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*
Campylobacter hominis	*Ruminococcus obeum*	*Cryptosporidium felis*
Campylobacter lari	*Selenomonas ruminantium*	*Cryptosporidium fetus*
Campylobacter mucosalis	*Vibrio cincinnatiensis*	*Cryptosporidium meleagridis*
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 strains only, not with human Rotavirus C.

Cross-Contamination and Carryover

A study was performed to demonstrate the absence of carryover or cross-contamination when using the BioCode GPP on the BioCode MDx-3000 in conjunction with the NucliSENS easyMAG or the Roche MagNA Pure 96. High-positive samples were tested alternating with no-template control samples in a "checkerboard" pattern.

Samples were extracted checkerboard (singlet) and assayed in singlet. Each study consisted of five complete runs from extraction to BioCode MDx-3000 on one instrument. No cross-contamination was observed across all plates tested.

Reproducibility

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). The reproducibility panel consisted of 7 contrived mixed-analyte 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). All positive samples were contrived using pooled negative stool matrix.

All results are as expected with the exception of one false negative for STEC (low positive) and one false positive for *Giardia lamblia* (repro sample 4).

Table. Reproducibility of BioCode GPP- Qualitative results

	Concentration Level					
	Medium Positive		Low Positive		Negative	
Target	Detection Rate n/N (%)	95% CI	Detection Rate n/N (%)	95% CI	Agreement Rate n/N (%)	95% CI
Salmonella enterica	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 lamblia	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	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)

Table. Reproducibility of BioCode GPP- Quantitative results. The MDx-3000 software analyzes the raw data automatically. 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/ Probe	Analyte Concentration	Analyte Mean MFI	Repeatability		Between Run		Between Day		Between Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Salmonella enterica	Low	9174	1853	20.2	622	6.78	1464	15.95	0	0	2442	26.62
Salmonella enterica	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
V.parahaemolyticus	Low	9927	2690	27.1	792	7.98	2956	29.78	966	9.73	4188	42.18
V.parahaemolyticus	Med	12421	1540	12.39	2018	16.25	3126	25.16	0	0	4026	32.42
ST-a	Low	14272	3469	24.31	3562	24.96	3910	27.4	0	0	6326	44.32
ST-a	Med	21453	4151	19.35	2943	13.72	4869	22.7	2322	10.83	7416	34.57
ST-b	Low	13193	3246	24.6	1992	15.1	3719	28.19	733	5.56	5373	40.73
ST-b	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

Target Analyte/ Probe	Analyte Concentration	Analyte Mean MFI	Repeatability		Between Run		Between Day		Between Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
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

Interference

A study was performed to demonstrate the accuracy of the BioCode Gastrointestinal Pathogen Panel on the BioCode MDx-3000 in the presence of potentially inhibiting substances or microorganisms. Each member of the interfering substance panel was added to prescreened negative stool sample matrix spiked with representative members of the BioCode GPP at 3X LoD and a negative matrix comprised of only pre-screened negative stool. One parasite, one virus, one gram positive bacterium, and one gram negative bacterium were used as representative analytes for this study as shown in the Table below. Each sample was tested with and without potentially interfering substances or microbes. Each sample was prepared and extracted in triplicate on the easyMAG System and tested with the BioCode GPP on the BioCode MDx-3000 system.

Table. Interference test Panel.

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

Table. 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	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
Enterococcusfaecalis	ATCC 51299	1×10^6	N
Escherichia coli nonpathogenic	ATCC BAA-97	1×10^6	N
Pseudomonasaeruginosa	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.

Table. Potential interference 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
Loperamidehydrochloride	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

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 low concentration (≤3x LoD).

Common co-infections were determined by reviewing results of previous GI Panel clinical trials from 510k summaries, publications/posters and internal clinical sample testing. Each sample was extracted in triplicate on the easyMAG and each extraction tested in singlet with the BioCode GPP on the BioCode MDx-3000 system. No inhibition was observed.

Table. Competitive inhibition testing results.

Panel Designation	Viral/Bacteria Strain	Source	Level	Titer Tested	Target Probe	Result (n of 3 Detected)
Competitive Inhibition Sample 1	*Clostridium difficile*	Zeptometrix 801619	High	3.0 x 10^6 CFU/mL	tcdB	3/3
	Rotavirus A	ATCC VR-2018	Medium	7.44 x 10^3 $TCID_{50}$/mL	Rota	3/3
	Escherichia coli E2348/69 (EPEC)	STEC TW06375	Medium	7.02 x 10^3 CFU/mL	EPEC	3/3
Competitive Inhibition Sample 2	O92:H33 *Escherichia coli* (EAEC)	STEC JM221 TW04440	High	3.0 x 10^6 CFU/mL	EAEC	3/3
	Escherichia coli E2348/69 (EPEC)	STEC TW06375	Medium	7.02 x 10^3 CFU/mL	EPEC	3/3
	Clostridium difficile	Zeptometrix 801619	Medium	5.7 x 10^2 CFU/mL	tcdB	3/3
Competitive Inhibition Sample 3	*Escherichia coli* E2348/69 (EPEC)	STEC TW06375	High	3.0 x 10^6 CFU/mL	EPEC	3/3
	Clostridium difficile	Zeptometrix 801619	Medium	5.7 x 10^2 CFU/mL	tcdB	3/3
	Rotavirus A	ATCC VR-2018	Medium	7.44 x 10^3 $TCID_{50}$/mL	Rota	3/3
Competitive Inhibition Sample 4	*Escherichia coli* E2348/69 (EPEC)	STEC TW06375	High	3.0 x 10^6 CFU/mL	EPEC	3/3
	O92:H33 *Escherichia coli* (EAEC)	STEC JM221 TW04440	Medium	4.08 X 10^3 CFU/mL	EAEC	3/3
	Campylobacter jejuni subsp. *jejuni*	ATCC 33292	Medium	2.1 x 10^3 CFU/mL	Campy	3/3
Competitive Inhibition Sample 5	*Campylobacter jejuni* subsp. *doylei*	ATCC 49349	High	3.0 x 10^6 CFU/mL	Campy	3/3
	Escherichia coli E2348/69 (EPEC)	STEC TW06375	Medium	7.02 x 10^3 CFU/mL	EPEC	3/3
	O92:H33 *Escherichia coli* (EAEC)	STEC JM221 TW04440	Medium	4.08 x 10^3 CFU/mL	EAEC	3/3

Panel Designation	Viral/Bacteria Strain	Source	Level	Titer Tested	Target Probe	Result
Competitive Inhibition Sample 6	O92:H33 *Escherichia coli* (EAEC)	STEC JM221 TW04440	High	3.0 x 10^6 CFU/mL	EAEC	3/3
	Campylobacter jejuni subsp. *jejuni*	ATCC 33292	Medium	2.1 x 10^3 CFU/mL	Campy	3/3
	Escherichia coli E2348/69 (EPEC)	STEC TW06375	Medium	7.02 x 10^3 CFU/mL	EPEC	3/3
Competitive Inhibition Sample 7	Shiga-toxin producing *E. coli* (STEC)	ATCC BAA-2217	High	3.0 x 10^6 CFU/mL	stx2	3/3
	Giardia intestinalis	Waterborne P101	Medium	5.42 x 10^3 cysts/mL	G.lam	3/3
	Shigella sonnei	ATCC 29930	Medium	1.31 x 10^3 CFU/mL	Shig	3/3
Competitive Inhibition Sample 8	*Giardia intestinalis*	Waterborne P101	High	3.0 x 10^5 cysts/mL	G.lam	3/3
	Shigella sonnei	ATCC 29930	Medium	1.31 x 10^3 CFU/mL	Shig	3/3
	Shiga-toxin producing *E. coli* (STEC)	ATCC BAA-2217	Medium	7.5 x 10^3 CFU/mL	stx2	3/3
Competitive Inhibition Sample 9	*Shigella sonnei*	ATCC 29930	High	3.0 x 10^6 CFU/mL	Shig	3/3
	Shiga-toxin producing *E. coli* (STEC)	ATCC BAA-2217	Medium	7.5 x 10^3 CFU/mL	stx2	3/3
	Giardia intestinalis	Waterborne P101	Medium	5.42 x 10^3cysts/mL	G.lam	3/3

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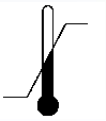
TABLE OF SYMBOLS

The following symbols are used on the BioCode Gastrointestinal Pathogen Panel kit components and/or in this package insert.

LOT	Batch code		Keep away from sunlight		Temperature limitations
REF	Catalog number		Contains sufficient for <n> tests		Consult instructions for use
	Use by YYYY-MM-DD		Do Not Reuse		Manufacturer
IVD	*In vitro* diagnostic device		For Prescription Use Only		

Applied BioCode

Barcoded Magnetic Beads | Flexible Syndromic Tests