



BioCode[®]


Gastrointestinal Pathogen Panel (GPP)

Package Insert



Applied BioCode

Barcoded Magnetic Beads | Flexible Syndromic Tests



Contact Info for Customers & Tech Services

Customers Services:

Telephone: 1-833-Bio-Code
(1-833-246-2633)
Email: Orders@ApBioCode.com

Technical Services:

Telephone: 1-833-BMB-Tech
(1-833-262-8324)
Email: TechSupport@ApBioCode.com

Website:

www.ApBioCode.com

Fax Number:

1-562-777-9805

Mailing Address:

12130 Mora Dr., Unit 2
Santa Fe Springs, CA 90670, USA



Applied BioCode, Inc.
12130 Mora Dr., Unit 2
Santa Fe Springs, CA 90670, USA



The information contained in this document is subject to change without notice. No part of this document may be reproduced or transmitted in any form or by any means, electronic or mechanical, for any purpose, without express written permission of Applied BioCode, Inc.

BioCode is a registered trademark of Applied BioCode, Inc.

All other names of products and brands appearing in this manual are trademarks or registered trademarks of their respective owners.

Table of Contents

| | |
|---|----|
| NAME AND INTENDED USE | 3 |
| SUMMARY AND EXPLANATION OF THE TEST | 4 |
| Summary of Detected Organisms | 4 |
| Bacteria | 4 |
| Parasites | 7 |
| Viruses | 8 |
| PRINCIPLE OF PROCEDURE | 10 |
| Nucleic Acid Extraction | 10 |
| Overview of a BioCode MDx-3000 Run | 10 |
| MATERIALS REQUIRED | 11 |
| Materials Provided With Each Kit | 11 |
| Materials Required But Not Provided With Each Kit | 11 |
| WARNINGS AND PRECAUTIONS | 13 |
| General Precautions | 13 |
| Precaution Related to Public Health Reporting | 13 |
| Laboratory Precautions | 13 |
| Safety Precautions | 13 |
| REAGENT STORAGE, HANDLING AND STABILITY | 14 |
| SAMPLE REQUIREMENTS | 14 |
| Stool in Cary-Blair Transport Media | 14 |
| Stool without Preservatives | 14 |
| PROCEDURE | 15 |
| Extraction Method for Unpreserved or Cary-Blair Stool Samples | 15 |
| BioCode GPP Set Up | 16 |
| INTERPRETATION OF RESULTS | 18 |
| Negative Controls | 18 |
| External Positive Controls | 18 |
| Internal Control | 19 |
| Target Pathogen Interpretation | 20 |
| BioCode GPP Test Report | 23 |
| LIMITATIONS OF THE PROCEDURE | 25 |

| | |
|---|-----------|
| EXPECTED VALUES | 27 |
| PERFORMANCE CHARACTERISTICS..... | 29 |
| Clinical Performance..... | 29 |
| Limit of Detection..... | 47 |
| Analytical Reactivity (Inclusivity) | 50 |
| Analytical Specificity (Cross-Reactivity and Exclusivity)..... | 59 |
| Cross-Contamination and Carryover | 66 |
| Reproducibility..... | 66 |
| Interference | 69 |
| Competitive Inhibition..... | 71 |
| REFERENCES | 73 |
| TABLE OF SYMBOLS..... | 76 |

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 |
|--|---|
| <ul style="list-style-type: none"> ▪ <i>Campylobacter</i> (<i>C. jejuni</i>, <i>C. coli</i>) ▪ <i>Clostridium difficile</i> toxin A/B (fresh specimens only) ▪ Enteroaggregative <i>E. coli</i> (EAEC) ▪ Enterotoxigenic <i>E. coli</i> (ETEC): LT/ST ▪ Shiga-toxin producing <i>E. coli</i> (STEC): stx1/stx2 ▪ <i>E.coli</i> O157 ▪ <i>Shigella</i> spp. /Enteroinvasive <i>E.coli</i> (EIEC) ▪ <i>Salmonella</i> spp. ▪ <i>Vibrio parahaemolyticus</i> ▪ <i>Vibrio</i> spp. (not <i>parahaemolyticus</i>) ▪ <i>Yersinia enterocolitica</i> | <ul style="list-style-type: none"> ▪ <i>Cryptosporidium</i> spp. (<i>C. hominis/C. parvum</i>) ▪ <i>Entamoeba histolytica</i> ▪ <i>Giardia lamblia</i> |
| | Viruses |
| | <ul style="list-style-type: none"> ▪ Adenovirus 40/41 ▪ Norovirus GI/GII ▪ Rotavirus A |
| | RNA Internal Control |
| | |

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)**

Shigella 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

| <i>Vibrio</i> (Vibrio Assay) | <i>V. parahaemolyticus</i> (V.para Assay) | Description |
|---|--|--|
| Not detected | Not detected | No <i>Vibrio</i> species detected |
| Detected | Not detected | <i>Vibrio</i> species DNA detected (not <i>V. parahaemolyticus</i>) |
| Any Results | Detected | <i>Vibrio parahaemolyticus</i> 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 10³ CFU/mL). *Y. rohdei* was also detected when present at high levels (>6.8 x 10⁴ 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 <i>stx1/2</i> (<i>stx1/ stx2</i>) Assays | <i>E. coli</i> O157 (O157) Assay | Description |
|---|----------------------------------|---|
| Not detected | Not detected | <i>stx1/stx2</i> DNA not detected. <i>E. coli</i> O157 DNA not detected. |
| Not detected | Detected | <i>stx1/stx2</i> DNA not detected. <i>E. coli</i> O157 DNA detected. |
| Detected ^a | Not detected | <i>stx1/stx2</i> DNA detected, <i>E. coli</i> O157 not detected. |
| Detected ^a | Detected ^b | <i>stx1/stx2</i> DNA detected, <i>E. coli</i> 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 asymptotically 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) |
|--|-----------------------|----------------------|-----------------------|------------------------|----------------------|
| <i>Campylobacter</i> spp | 35 (2.25%) | 5 (3.57%) | 4 (1.69%) | 20 (2.79%) | 6 (1.30%) |
| <i>Clostridium difficile</i> * | 69 (11.54%) | 3 (16.67%) | 4 (7.14%) | 30 (9.32%) | 32 (15.84%) |
| <i>E. coli</i> 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%) |
| <i>Salmonella</i> spp | 37 (2.37%) | 9 (6.43%) | 10 (4.22%) | 12 (1.67%) | 6 (1.30%) |
| <i>Shigella</i> /EIEC | 23 (1.48%) | 8 (5.71%) | 8 (3.38%) | 6 (0.84%) | 1 (0.22%) |
| <i>Vibrio parahaemolyticus</i> | 3 (0.19%) | 0 (0.00%) | 0 (0.00%) | 3 (0.42%) | 0 (0.00%) |
| <i>Vibrio</i> spp (not <i>parahaemolyticus</i>) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| <i>Yersinia enterocolitica</i> | 10 (0.64%) | 1 (0.71%) | 3 (1.27%) | 5 (0.70%) | 1 (0.22%) |
| <i>Entamoeba histolytica</i> | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| <i>Cryptosporidium</i> spp | 13 (0.83%) | 1 (0.71%) | 0 (0.00%) | 11 (1.53%) | 1 (0.22%) |
| <i>Giardia lamblia</i> | 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)

| Analyte | Prevalence in Mixed Infection N = 49 | |
|--------------------------------|---|-------|
| | n/N | % |
| <i>Campylobacter</i> spp | 8/49 | 16.3% |
| <i>Clostridium difficile</i> | 9/49 | 18.4% |
| <i>E.coli</i> 0157 | 4/49 | 8.2% |
| EAEC | 22/49 | 44.9% |
| <i>Shigella</i> /EIEC | 5/49 | 10.2% |
| ETEC | 18/49 | 36.7% |
| STEC | 5/49 | 10.2% |
| <i>Salmonella</i> spp. | 7/49 | 14.3% |
| <i>Vibrio parahaemolyticus</i> | 1/49 | 2.0% |
| <i>Yersinia enterocolitica</i> | 4/49 | 8.2% |
| <i>Cryptosporidium</i> spp | 4/49 | 8.2% |
| <i>Giardia lamblia</i> | 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 |
| <i>Campylobacter</i> (<i>C. jejuni</i> , <i>C. coli</i>) | Culture |
| <i>Clostridium difficile</i> (<i>C. difficile</i>) toxin A/B | FDA cleared NAAT |
| <i>Cryptosporidium</i> (<i>C. parvum</i> , <i>C. hominis</i>) | Composite result of two PCR/sequencing assays |
| <i>Entamoeba histolytica</i> | Composite result of two PCR/sequencing assays |
| <i>Escherichia coli</i> (<i>E. coli</i>) O157 | Enrichment culture |
| Enterotoxigenic <i>E. coli</i> (ETEC) LT/ST | Composite result of two PCR/sequencing assays |
| Enterobacteriaceae <i>E. coli</i> (EAEC) | Composite result of two PCR/sequencing assays |
| <i>Giardia lamblia</i> / <i>intestinalis</i> | 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 |
| <i>Salmonella</i> spp. | Enrichment culture |
| Shiga-like Toxin producing <i>E. coli</i> (STEC) stx1/stx2 | Enrichment culture/FDA cleared antigen test |
| <i>Shigella</i> (<i>S. boydii</i> , <i>S. sonnei</i> , <i>S. flexneri</i> , <i>S. dysenteriae</i>)/EIEC | Enrichment culture |
| <i>Vibrio</i> spp. (<i>V. cholerae</i> , <i>V. parahaemolyticus</i> , <i>V. vulnificus</i>) | Culture |
| <i>Yersinia enterocolitica</i> | 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).

| Target | PPA | | NPA | |
|---|------------------------|---------------|------------------------|----------------|
| | Agreement Rate n/N (%) | 95% CI | Agreement Rate n/N (%) | 95% CI |
| <i>Campylobacter</i> spp. ^a | 1/1 (100.0) | (2.5, 100.0) | 234/236 (99.2) | (96.97, 99.9) |
| <i>Clostridium difficile</i> ^b | 26/27 (96.3) | (81.0, 99.9) | 208/210 (99.1) | (96.6, 99.9) |
| <i>E.coli</i> O157 | 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) |
| <i>Salmonella</i> spp. ^e | 3/3 (100.0) | (29.2, 100.0) | 232/234 (99.2) | (96.95, 99.9) |
| <i>Shigella</i> /EIEC ^f | 1/1 (100.0) | (2.5, 100.0) | 233/236 (98.7) | (96.33, 99.7) |
| <i>Vibrio parahaemolyticus</i> ^g | N/A | N/A | 236/237 (99.6) | (97.67, 100.0) |
| <i>Vibrio</i> spp | N/A | N/A | 237/237 (100.0) | (98.46, 100.0) |

| Target | PPA | | NPA | |
|---|---------------------------|---------------|---------------------------|----------------|
| | Agreement Rate n/N (%) | 95% CI | Agreement Rate n/N (%) | 95% CI |
| <i>Yersinia enterocolitica</i> ^h | N/A | N/A | 236/237 (99.6) | (97.67, 100.0) |
| <i>Cryptosporidium</i> spp | 1/1 (100.0) | (2.5, 100.0) | 234/234 (100.0) | (98.44, 100.0) |
| <i>Entamoeba histolytica</i> | N/A | N/A | 235/235 (100.0) | (98.44, 100.0) |
| <i>Giardia lamblia</i> 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).

| Target | PPA | | NPA | |
|---|---------------------------|---------------|---------------------------|---------------|
| | Agreement Rate n/N (%) | 95% CI | Agreement Rate n/N (%) | 95% CI |
| <i>Campylobacter</i> spp. ^a | 3/3 (100.0) | (29.2, 100.0) | 936/952 (98.3) | (97.3, 99.0) |
| <i>Clostridium difficile</i> ^b | N/A | N/A | N/A | N/A |
| <i>E.coli</i> 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) |
| <i>Salmonella</i> spp ^g | 18/22 (81.8) | (59.7, 94.8) | 926/934 (99.1) | (98.3, 99.6) |
| <i>Shigella</i> /EIEC ^h | 4/5 (80.00) | (28.4, 99.5) | 940/951 (98.8) | (97.9, 99.4) |
| <i>Vibrio parahaemolyticus</i> ⁱ | N/A | N/A | 955/957 (99.8) | (99.3, 100.0) |

| Target | PPA | | NPA | |
|---|---------------------------|---------------|---------------------------|---------------|
| | Agreement Rate n/N (%) | 95% CI | Agreement Rate n/N (%) | 95% CI |
| <i>Vibrio</i> spp | N/A | N/A | 956/956 (100.0) | (99.6, 100.0) |
| <i>Yersinia enterocolitica</i> ^l | N/A | N/A | 951/956 (99.5) | (98.8, 99.8) |
| <i>Cryptosporidium</i> spp | 7/7 (100.0) | (59.0, 100.0) | 941/941 (100.0) | (99.6, 100.0) |
| <i>Entamoeba histolytica</i> | N/A | N/A | 948/948 (100.0) | (99.6, 100.0) |
| <i>Giardia lamblia</i> ^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 additional 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.

| Bacteria | Positive Agreement | | Negative Agreement | |
|---|---------------------------|----------------|---------------------------|----------------|
| | Agreement Rate n/N (%) | 95% CI | Agreement Rate n/N (%) | 95% CI |
| <i>Campylobacter</i> spp. ^a | 2/3 (66.7) | (9.4, 99.2) | 347/358 (96.9) | (94.6, 98.5) |
| <i>Clostridium difficile</i> ^b | 37/38 (97.4) | (86.2, 99.9) | 318/322 (98.8) | (96.9, 99.7) |
| <i>E.coli</i> O157 ^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) |
| <i>Salmonella</i> spp. ^g | 4/5 (80.0) | (28.36, 99.5) | 354/356 (99.4) | (98.0, 99.9) |
| <i>Shigella</i> /EIEC ^h | 1/2 (50.0) | (1.26, 98.7) | 356/359 (99.2) | (97.6, 99.8) |
| <i>Vibrio</i> <i>parahaemolyticus</i> | N/A | N/A | 361/361 (100.0) | (99.0, 100.0) |
| <i>Vibrio</i> spp | N/A | N/A | 361/361 (100.0) | (99.0, 100.0) |
| <i>Yersinia enterocolitica</i> ⁱ | N/A | N/A | 357/361 (98.9) | (97.2, 99.7) |
| <i>Cryptosporidium</i> spp. ^j | 3/3 (100.0) | (29.24, 100.0) | 354/356 (99.4) | (98.0, 99.9) |
| <i>Entamoeba histolytica</i> | N/A | N/A | 359/359 (100.0) | (99.0, 100.0) |
| <i>Giardia lamblia</i> ^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.

I - 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 |
| <i>Campylobacter</i> 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 |
| <i>Clostridium difficile</i> ^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 |
| <i>E. coli</i> 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 <i>E. coli</i> (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 <i>E. coli</i> (EPEC) ^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 <i>E. coli</i> (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 |
| <i>Salmonella</i> 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 |
| <i>Shigella</i> / 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 |
| <i>Vibrio parahaemolyticus</i> 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 |
| <i>Vibrio</i> spp. (not <i>parahaemolyticus</i>) | 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 |
| <i>Yersinia enterocolitica</i> ^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 |
| <i>Cryptosporidium</i> 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 |
| <i>Entamoeba histolytica</i> | 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 |
| <i>Giardia lamblia</i> ^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 |
| <i>Clostridium difficile</i> + <i>Salmonella</i> spp | 5 |

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

| Distinct Co-Infection Combinations Detected by BioCode GPP | | | | Total Co-infections | Number of Discrepant Co-infections | Discrepant Analyte(s) |
|--|----------------------------|--------------------------|-----------|---------------------|------------------------------------|-------------------------------|
| Analyte_1 | Analyte_2 | Analyte_3 | Analyte_4 | | | |
| Adenovirus 40/41 | Rotavirus A | N/A | N/A | 1 | 1 | Adenovirus 40/41 (x1) |
| <i>Campylobacter</i> spp | <i>Shigella</i> /EIEC | N/A | N/A | 1 | 1 | All |
| <i>Campylobacter</i> spp | <i>Giardia lamblia</i> | N/A | N/A | 1 | 1 | All |
| <i>Campylobacter</i> spp | Norovirus GI/GII | N/A | N/A | 1 | 1 | <i>Campylobacter</i> spp (x1) |
| <i>Campylobacter</i> spp | STEC | N/A | N/A | 1 | 1 | All |
| <i>Campylobacter</i> spp | <i>Y. enterocolitica</i> | N/A | N/A | 1 | 1 | All |
| <i>C. difficile</i> | ETEC | N/A | N/A | 1 | 1 | ETEC (x1) |
| <i>Cryptosporidium</i> spp | <i>Campylobacter</i> spp | N/A | N/A | 1 | 1 | <i>Campylobacter</i> spp (x1) |
| <i>Cryptosporidium</i> spp | <i>Giardia lamblia</i> | N/A | N/A | 1 | 1 | <i>Giardia lamblia</i> (x1) |
| <i>E.coli</i> O157 | Norovirus GI/GII | N/A | N/A | 1 | 1 | <i>E.coli</i> O157 (x1) |
| <i>E.coli</i> O157 | <i>Shigella</i> /EIEC | N/A | N/A | 1 | 1 | <i>E.coli</i> O157 (x1) |
| EAEC | <i>Shigella</i> /EIEC | N/A | N/A | 1 | 1 | <i>Shigella</i> /EIEC (x1) |
| EAEC | <i>Shigella</i> /EIEC | Norovirus GI/GII | N/A | 1 | 1 | <i>Shigella</i> /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 | <i>Giardia lamblia</i> | N/A | N/A | 1 | 1 | All |
| EAEC | Rotavirus A | N/A | N/A | 1 | 1 | Rotavirus A (x1) |
| <i>Shigella</i> /EIEC | ETEC | N/A | N/A | 1 | 1 | <i>Shigella</i> /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 | <i>V. parahaemolyticus</i> | <i>Y. enterocolitica</i> | 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) |
| <i>C. difficile</i> | <i>Salmonella</i> spp | N/A | N/A | 3 | 1 | All |
| <i>E.coli</i> O157 | EAEC | ETEC | STEC | 1 | 1 | <i>E.coli</i> O157 (x1);STEC (x1) |
| EAEC | ETEC | N/A | N/A | 4 | 1 | EAEC (x1) |
| EAEC | ETEC | <i>Y. enterocolitica</i> | N/A | 2 | 2 | ETEC (x1); <i>Y. enterocolitica</i> (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 | EPEC | N/A | 4 | 1 | EAEC (x1) |
| EAEC | EPEC | Norovirus GI/GII | 2 | 2 | EAEC (x1);EPEC (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 | <i>Shigella</i> /EIEC | EPEC | 1 | 1 | <i>Shigella</i> /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 |
| <i>Campylobacter</i> 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) |
| <i>E. coli</i> 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 <i>E. coli</i> (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 <i>E. coli</i> (EPEC) ^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 <i>E. coli</i> (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) |
| <i>Salmonella</i> 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) |
| <i>Shigella</i> / 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) |
| <i>Vibrio parahaemolyticus</i> | 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) |
| <i>Vibrio</i> 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) |
| <i>Yersinia enterocolitica</i> ^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) |
| <i>Cryptosporidium</i> 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) |
| <i>Entamoeba histolytica</i> | 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 |
| <i>Giardia lamblia</i> ^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)

| Target | Positive Agreement | | Negative Agreement | |
|--------------------------------|--------------------|----------------|--------------------|----------------|
| | Agreement n/N (%) | 95% CI | Agreement n/N (%) | 95% CI |
| <i>Campylobacter</i> spp | 38/40 (95.0) | (83.1, 99.4) | 152/152 (100.0) | (97.6, 100.0) |
| <i>E.coli</i> 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) |
| <i>Salmonella</i> spp. | 29/30 (96.7) | (82.8, 99.9) | 152/152 (100.0) | (97.6, 100.0) |
| <i>Shigella</i> / EIEC | 43/45 (95.6) | (84.9, 99.5) | 151/152 (99.3) | (96.4, 100.0) |
| <i>Yersinia enterocolitica</i> | 3/3 (100.0) | (29.24, 100.0) | 152/152 (100.0) | (97.6, 100.0) |
| <i>Cryptosporidium</i> spp. | 16/19 (84.2) | (60.4, 96.6) | 152/152 (100.0) | (97.6, 100.0) |
| <i>Giardia lamblia</i> | 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 |
|--------------------------------|-----------------|---------------------|--------------------------------|---------------------|----------------------|
| <i>Giardia lamblia</i> | Waterborne P101 | Assemblage B | <i>Entamoeba histolytica</i> | 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 |
| <i>Yersinia enterocolitica</i> | ATCC 29913 | O:8; biotype 2 | <i>Vibrio cholerae</i> | 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 | <i>Vibrio vulnificus</i> | ATCC 27562 | Strain 324 CDC B9629 |
| | BEI NR-213 | O:9 | | ATCC29306 | Strain CDC A1402 |
| <i>Vibrio parahaemolyticus</i> | ATCC 17802 | Strain EB101 | <i>Vibrio parahaemolyticus</i> | Zeptomatrix 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

| Target | PPA ^a | | NPA | |
|---|-------------------|--------------|-------------------|---------------|
| | Agreement n/N (%) | 95% CI | Agreement n/N (%) | 95% CI |
| <i>Vibrio parahaemolyticus</i> | 88/96 (91.7) | (84.2, 96.3) | 516/516 (100.0) | (99.3, 100.0) |
| <i>Vibrio</i> spp. (not <i>parahaemolyticus</i>) | 82/94 (87.2) | (78.8, 93.2) | 518/518 (100.0) | (99.3, 100.0) |
| <i>Vibrio cholerae</i> | 40/47 (85.1) | (72.3, 92.6) | 518/518 (100.0) | (99.3, 100.0) |
| <i>Vibrio vulnificus</i> | 42/47 (89.4) | (77.4, 95.4) | 518/518 (100.0) | (99.3, 100.0) |
| <i>Yersinia enterocolitica</i> | 95/98 (96.9) | (91.3, 99.4) | 514/514 (100.0) | (99.3, 100.0) |
| <i>Entamoeba histolytica</i> | 96/99 (97.1) | (91.4, 99.4) | 507/513 (98.8) | (97.5, 99.6) |
| <i>Giardia lamblia</i> | 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%) |
| <i>Clostridium difficile</i> | 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%) |
| EPEC | 0 (0.00%) | 0 (0.00%) | 2 (3.28%) | 0 (0.00%) |
| <i>Salmonella</i> spp | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 1 (1.67%) |
| <i>Giardia lamblia</i> | 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 $\geq 95\%$ 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 |
|--|-----------------------|--------------------------|-----------------------------|--------------------------|----------------------------|
| <i>Campylobacter coli</i> | ATCC 33559 | 5.6×10^1 CFU/mL | 20/20 | 5.6×10^1 CFU/mL | 20/20 |
| <i>Campylobacter jejuni</i> subsp. <i>jejuni</i> | ATCC 33292 | 7.0×10^2 CFU/mL | 20/20 | 7.0×10^2 CFU/mL | 20/20 |
| <i>Clostridium difficile</i> (toxintype 0) | ATCC 9689 | 1.9×10^2 CFU/mL | 20/20 | 1.9×10^2 CFU/mL | 20/20 |
| <i>Clostridium difficile</i> (toxintype III; Nap1) | Zeptomatrix 0801619cf | 8.3×10^2 CFU/mL | 20/20 | 3.3×10^3 CFU/mL | 20/20 |
| Enteroaggregative <i>E. coli</i> O92:H33 (EAEC) | STEC TW04440 | 1.4×10^3 CFU/mL | 20/20 | 1.4×10^3 CFU/mL | 20/20 |
| Enteroinvasive <i>E. coli</i> O29:NM (EIEC) | ATCC 43892 | 3.6×10^2 CFU/mL | 20/20 | 7.5×10^2 CFU/mL | 20/20 |
| Enterotoxigenic <i>E. coli</i> O78:H11 H10407 (ETEC) | ATCC 35401 | 5.6×10^2 CFU/mL | 20/20 | 5.6×10^2 CFU/mL | 20/20 |
| <i>Salmonella bongori</i> | SGSC 4900 | 1.4×10^3 CFU/mL | 20/20 | 5.5×10^3 CFU/mL | 20/20 |
| <i>Salmonella enterica</i> subsp. <i>enterica</i> | ATCC 14028 | 2.2×10^3 CFU/mL | 20/20 | 1.1×10^3 CFU/mL | 19/20 |
| Shiga-like toxin producing <i>E. coli</i> (STEC) | ATCC BAA-2217 | 2.5×10^3 CFU/mL | 20/20 | 2.5×10^3 CFU/mL | 20/20 |
| <i>E. coli</i> O157 | ATCC 700376 | 3.3×10^3 CFU/mL | 20/20 | 3.3×10^3 CFU/mL | 20/20 |
| <i>Shigella sonnei</i> | ATCC 29930 | 4.4×10^2 CFU/mL | 20/20 | 1.7×10^3 CFU/mL | 20/20 |
| <i>Vibrio cholerae</i> | ATCC 25870 | 4.9×10^2 CFU/mL | 20/20 | 4.9×10^2 CFU/mL | 20/20 |
| <i>Vibrio parahaemolyticus</i> | ATCC 17802 | 1.3×10^1 CFU/mL | 20/20 | 5.0×10^1 CFU/mL | 20/20 |
| <i>Yersinia enterocolitica</i> | 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 |
|--|---------------------|---|-----------------------------|---|----------------------------|
| <i>Cryptosporidium hominis</i> | UKRC | 1.3×10^4 oocysts/mL | 20/20 | 1.3×10^4 oocysts/mL | 19/20 |
| <i>Cryptosporidium parvum</i> | Waterborne P102C | 3.1×10^3 oocysts/mL | 20/20 | 3.1×10^3 oocysts/mL | 20/20 |
| <i>Entamoeba histolytica</i> | BEI NR-178 | 3.1×10^{-1} cysts/mL | 20/20 | 3.1×10^{-1} cysts/mL | 20/20 |
| <i>Giardia intestinalis</i> (aka <i>G. lamblia</i>) | Waterborne P101 | 1.8×10^3 cysts/mL | 20/20 | 1.8×10^3 cysts/mL | 20/20 |
| Adenovirus 40 (dugan) | Zeptomatrix 0810084 | 1.0×10^{-1} TCID ₅₀ /mL | 20/20 | 1.0×10^{-1} TCID ₅₀ /mL | 20/20 |
| Adenovirus 41 (TAK) | Zeptomatrix 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 |
|--|-----------------------|--------------------------|-----------------------------|--------------------------|----------------------------|
| <i>Campylobacter coli</i> | ATCC 33559 | 2.8×10^1 CFU/mL | 20/20 | 2.8×10^1 CFU/mL | 20/20 |
| <i>Campylobacter jejuni</i> spp. <i>jejuni</i> | ATCC 33292 | 3.5×10^2 CFU/mL | 19/20 | 7.0×10^2 CFU/mL | 20/20 |
| <i>Clostridium difficile</i> (toxintype 0) | ATCC 9689 | 9.5×10^1 CFU/mL | 20/20 | 9.5×10^1 CFU/mL | 20/20 |
| <i>Clostridium difficile</i> (toxintype III; Nap1) | Zeptomatrix 0801619cf | 4.2×10^2 CFU/mL | 20/20 | 4.1×10^2 CFU/mL | 20/20 |
| Enterotoxigenic <i>E. coli</i> O92:H33 (EAEC) | STEC TW04440 | 7.0×10^2 CFU/mL | 20/20 | 7.0×10^2 CFU/mL | 20/20 |
| Enteroinvasive <i>E. coli</i> O29:NM (EIEC) | ATCC 43892 | 1.8×10^2 CFU/mL | 20/20 | 1.8×10^2 CFU/mL | 20/20 |
| Enterotoxigenic <i>E. coli</i> O78:H11 H10407 (ETEC) | ATCC 35401 | 2.8×10^2 CFU/mL | 20/20 | 2.8×10^2 CFU/mL | 19/20 |
| <i>Salmonella bongori</i> | SGSC 4900 | 1.4×10^3 CFU/mL | 20/20 | 1.4×10^3 CFU/mL | 20/20 |
| <i>Salmonella enterica</i> ssp. <i>enterica</i> | 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 <i>E. coli</i> (STEC) | ATCC BAA-2217 | 1.3 x10 ³ CFU/mL | 20/20 | 1.3 x10 ³ CFU/mL | 20/20 |
| <i>E. coli</i> O157 | ATCC 700376 | 1.7 x10 ³ CFU/mL | 20/20 | 1.7 x10 ³ CFU/mL | 20/20 |
| <i>Shigella sonnei</i> | ATCC 29930 | 2.2 x10 ² CFU/mL | 20/20 | 2.2 x10 ² CFU/mL | 20/20 |
| <i>Vibrio cholerae</i> | ATCC 25870 | 2.5 x10 ² CFU/mL | 20/20 | 2.5 x10 ² CFU/mL | 20/20 |
| <i>Vibrio parahaemolyticus</i> | ATCC 17802 | 6.5 x10 ⁰ CFU/mL | 20/20 | 6.5 x10 ⁰ CFU/mL | 20/20 |
| <i>Yersinia enterocolitica</i> | ATCC 23715 | 7.5 x10 ² CFU/mL | 20/20 | 7.5 x10 ² CFU/mL | 20/20 |
| <i>Cryptosporidium parvum</i> | waterborne P102C | 3.1 x10 ³ oocysts/mL | 20/20 | 3.1 x10 ³ oocysts/mL | 20/20 |
| <i>Entamoeba histolytica</i> HB-301:NIH | BEI NR-178 | 1.6 x10 ⁻¹ cysts/mL | 20/20 | 1.6 x10 ⁻¹ cysts/mL | 20/20 |
| <i>Giardia intestinalis</i> (aka <i>G. lamblia</i>) | waterborne P101 | 9.0 x10 ² cysts/mL | 20/20 | 9.0 x10 ² cysts/mL | 20/20 |
| Adenovirus 40 (dugan) | Zeptomatrix 0810084 | 1.0 x10 ⁻¹ TCID ₅₀ /mL | 20/20 | 1.0 x10 ⁻¹ TCID ₅₀ /mL | 20/20 |
| Adenovirus 41 (TAK) | Zeptomatrix 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 titrated 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 titrated 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 |
|--|------------|-------------------------------|--------------------------|
| <i>Campylobacter jejuni</i> subsp. <i>jejuni</i> | BEI NR-399 | 2.10 x 10 ³ CFU/mL | 3x |
| | BEI NR-400 | 2.10 x 10 ³ CFU/mL | 3x |
| <i>Campylobacter jejuni</i> subsp. <i>doylei</i> | ATCC 49350 | 2.10 x 10 ³ CFU/mL | 3x |
| | ATCC 49349 | 2.10 x 10 ³ CFU/mL | 3x |
| <i>Campylobacter coli</i> | 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 |
|------------------------------|------------|------------------|-------------------------------|--------------------------|
| <i>Clostridium difficile</i> | 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 |
|---------------------|--------------------------------------|----------------------|------------------------------|--------------------------|--------------------|
| <i>E. coli</i> O157 | <i>E. coli</i> O157:H45 | STEC SC373/2 TW07922 | 9.9 x 10 ³ CFU/mL | 3x | non- STEC |
| | <i>E. coli</i> O157:HNM | STEC DA-26 TW07952 | 9.9 x 10 ³ CFU/mL | 3x | stx1/stx2 |
| | <i>E. coli</i> O157:H7 | STEC 93-111 TW04863 | 9.9 x 10 ³ CFU/mL | 3x | stx1/stx2 |
| | <i>E. coli</i> O157: H7 | STEC MI06-19 TW14301 | 9.9 x 10 ³ CFU/mL | 3x | stx2 |
| | <i>E. coli</i> O157:HNT | STEC DA-27 TW07953 | 9.9 x 10 ³ CFU/mL | 3x | stx1/stx2 |
| | <i>E. coli</i> 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 | <i>stx1/stx2</i> |
|---|------------------------|----------------------|------------------------------|--------------------------|------------------|
| Shiga toxin producing <i>E. coli</i> (STEC) | <i>E. coli</i> O26:H11 | STEC 2332/00 TW08998 | 7.5 x 10 ³ CFU/mL | 3x | <i>stx1</i> |
| | <i>E. coli</i> O45:H2 | STEC DEC11C DEC11c | 7.5 x 10 ³ CFU/mL | 3x | <i>stx1</i> |
| | <i>E. coli</i> O103:H2 | STEC 107-226 TW07881 | 7.5 x 10 ³ CFU/mL | 3x | <i>stx1</i> |
| | <i>E. coli</i> O26:NM | STEC DA-22 TW07948 | 7.5 x 10 ³ CFU/mL | 3x | <i>stx1</i> |

| Organism | Serotype | Source | Concentration Detected | Multiple of LoD Detected | stx1/stx2 |
|----------|-------------------------------------|----------------------|------------------------------|--------------------------|-----------|
| | <i>E. coli</i> O45:H2 | STEC MI05-14 TW14003 | 7.5 x 10 ³ CFU/mL | 3x | stx1 |
| | <i>E. coli</i> O45:H2 | ATCC BAA-2193 | 7.5 x 10 ³ CFU/mL | 3x | stx1 |
| | <i>E. coli</i> O111:H8 | STEC DEC8B | 7.5 x 10 ³ CFU/mL | 3x | stx1/stx2 |
| | <i>E. coli</i> O145:NM | ATCC BAA-2192 | 7.5 x 10 ³ CFU/mL | 3x | stx1/stx2 |
| | <i>E. coli</i> O26:H11 | BAA-2196 | 7.5 x 10 ³ CFU/mL | 3x | stx1/stx2 |
| | <i>E. coli</i> O146:21 | STEC DEC16E TW01383 | 7.5 x 10 ³ CFU/mL | 3x | stx1c |
| | <i>E. coli</i> O104:H4 ^a | ATCC BAA-2326 | 7.5 x 10 ³ CFU/mL | 3x | stx2 |
| | <i>E. coli</i> O113:H21 | STEC CL-15 TW02318 | 7.5 x 10 ³ CFU/mL | 3x | stx2 |
| | <i>E. coli</i> O104:H21 | STEC G5506 TW04909 | 7.5 x 10 ³ CFU/mL | 3x | stx2 |
| | <i>E. coli</i> O111:H2 | STEC RD8 TW06296 | 7.5 x 10 ³ CFU/mL | 3x | stx2 |
| | <i>E. coli</i> O121:19 | STEC MDCH-4 TW07614 | 7.5 x 10 ³ CFU/mL | 3x | stx2 |
| | <i>E. coli</i> O121:NM | STEC DA-37 TW07972 | 7.5 x 10 ³ CFU/mL | 3x | stx2 |
| | <i>E. coli</i> 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 <i>E. coli</i> (EAEC) | <i>E. coli</i> O44:H18 | STEC 042 TW04393 | 4.08 x 10 ³ CFU/mL | 3x |
| | <i>E. coli</i> O111a, 111b:K58:H21 | ATCC 29552 | 4.08 x 10 ³ CFU/mL | 3x |
| | <i>E. coli</i> O104:H4 ^a | ATCC BAA-2326 | 4.08 x 10 ³ CFU/mL | 3x |
| | <i>E. coli</i> 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 <i>E. coli</i> (ETEC) | <i>E. coli</i> O25:K98 | ATCC 43886 | LT | 1.68 x 10 ³ CFU/mL | 3x |
| | <i>E. coli</i> O78:K80:H12 | ATCC 43896 | ST-b | 1.68 x 10 ³ CFU/mL | 3x |
| | <i>E. coli</i> O8:K85:K99 | ATCC 31618 | ST-a | 1.68 x 10 ³ CFU/mL | 3x |
| | <i>E. coli</i> | Zeptomatrix 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 |
|---|---|--------------------|-------------------------------|--------------------------|
| <i>Salmonella bongori</i> ^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 |
| <i>Salmonella enterica</i> subsp. II | | SGSC3039 | 6.45 x 10 ³ CFU/mL | 3x |
| <i>Salmonella enterica</i> subsp. IIIa | | SGSC3061 | 6.45 x 10 ³ CFU/mL | 3x |
| <i>Salmonella enterica</i> subsp. IIIb | | sGSC3068 | 6.45 x 10 ³ CFU/mL | 3x |
| <i>Salmonella enterica</i> subsp. IV | | SGSC3074 | 6.45 x 10 ³ CFU/mL | 3x |
| <i>Salmonella enterica</i> subsp. VI | | SGSC3116 | 6.45 x 10 ³ CFU/mL | 3x |
| <i>Salmonella enterica</i> subsp. <i>arizonae</i> | Unknown | ATCC 13314 | 6.45 x 10 ³ CFU/mL | 3x |
| <i>Salmonella enterica</i> subsp. <i>salamae</i> | serovar Tranoroa | ATCC 700148 | 6.45 x 10 ³ CFU/mL | 3x |
| <i>Salmonella enterica</i> subsp. <i>enterica</i> | 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 | Zeptomatrix 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 <i>E. coli</i> (EIEC) | <i>E. coli</i> O121 | ATCC BAA-2190 | 1.08 x 10 ³ CFU/mL | 3x |
| | <i>E. coli</i> O124:HNM | STEC 929-78 TW16574 | 1.08 x 10 ³ CFU/mL | 3x |
| | <i>E. coli</i> O136:H | STEC LT-41 TW06139 | 1.08 x 10 ³ CFU/mL | 3x |
| | <i>E. coli</i> O285A:HNM | BEI NR-101 | 1.08 x 10 ³ CFU/mL | 3x |
| | <i>E. coli</i> O15 | ATCC 49105 | 1.08 x 10 ³ CFU/mL | 3x |
| <i>Shigella boydii</i> | 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 |
| <i>Shigella dysenteriae</i> | 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 |
| <i>Shigella flexneri</i> (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 |
| <i>Shigella sonnei</i> | 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 | |
|--------------------------------|---------------------------|---------------------------|-------------------------------|-------------------------------|------------------|
| <i>Vibrio</i> spp. | <i>Vibrio cholerae</i> | 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 |
| | <i>Vibrio vulnificus</i> | | 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 |
| <i>Vibrio parahaemolyticus</i> | 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 | Zeptomatrix 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 |
|--------------------------------|----------|------------|-------------------------------|--------------------------|
| <i>Yersinia enterocolitica</i> | 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 |
|--------------------------------|--------------------------|------------|-------------------------|-------------------|
| <i>Cryptosporidium parvum</i> | DNA subtype / IlaA17G1R1 | UKCR UK28 | Clinical Sample unknown | Ct = 24.4 |
| | DNA subtype / IlaA15G2R1 | UKCR UK29 | Clinical Sample unknown | Ct = 23.5 |
| | DNA subtype / IlaA19G1R1 | 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 |
| <i>Cryptosporidium hominis</i> | 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 |
|------------------------------|------------|------------|--------------------------------|--------------------------|
| <i>Entamoeba histolytica</i> | 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 |
|--------------------------------------|----------|-------------|-----------------------------|--------------------------|
| <i>Giardia lamblia/ intestinalis</i> | 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 | 7.44×10^3 IU/mL | 3x |
| | HRV 89-12C2 | ATCC VR-2272 | 7.44×10^3 IU/mL | 3x |
| | WISC2 | ATCC VR-2417 | 7.44×10^3 IU/mL | 3x |
| | HRV 408 | ATCC VR-2273 | 7.44×10^3 IU/mL | 3x |
| | HRV 248 | ATCC VR-2274 | 7.44×10^3 IU/mL | 3x |
| | HU | ATCC VR-1546 | 7.44×10^3 IU/mL | 3x |
| | HRV CJN | ATCC VR-2275 | 7.44×10^3 IU/mL | 3x |
| | W161 | ATCC VR-2551 | 7.44×10^3 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 titrated 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 (TCID₅₀/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. enterocolitica* ($\sim 1.5 \times 10^3$ CFU/mL). *Y. rohdei* was also detected when present at high levels ($>6.8 \times 10^4$ 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 |
|---|-----------------------------|----------------------------------|--|---|
| <i>Vibrio alginolyticus</i> | ATCC 17749 | 6.40 x 10 ⁶ CFU/mL | 3/3 (<i>Vibrio</i> spp ^a) | 6.40 x 10 ³ CFU/mL |
| <i>Vibrio mimicus</i> | ATCC 700326 | 8.30 x 10 ⁶ CFU/mL | 3/3 (<i>Vibrio</i> spp ^a) | 8.30 x 10 ³ CFU/mL |
| <i>Yersinia bercovieri</i> | ATCC 43970 | 1.24 x 10 ⁶ CFU/mL | 3/3 (<i>Yersinia enterocolitica</i> ^b) | < 1.24 x 10 ² CFU/mL |
| <i>Yersinia frederiksenii</i> | ATCC 33642 | 1.29 x 10 ⁶ CFU/mL | 3/3 (<i>Yersinia enterocolitica</i> ^b) | 1.29 x 10 ³ CFU/mL |
| <i>Yersinia intermedia</i> | DD-750 (DNA) | 1.80 x 10 ⁶ copies/mL | 3/3 (<i>Yersinia enterocolitica</i> ^b) | N/A |
| <i>Yersinia mollaretii</i> | ATCC 43969 | 3.40 x 10 ⁶ CFU/mL | 3/3 (<i>Yersinia enterocolitica</i> ^b) | < 3.40 x 10 ² CFU/mL |
| <i>Yersinia rohdei</i> | ATCC 43380 | 6.80 x 10 ⁶ CFU/mL | 3/3 (<i>Yersinia enterocolitica</i> ^b) | 6.80 x 10 ⁴ CFU/mL |
| <i>Cryptosporidium cuniculus</i> (DNA) | UKCU12 - DNA | Unknown | 1/3 (<i>Cryptosporidium</i> spp.) | N/A |
| <i>Cryptosporidium meleagridis</i> (DNA) | UKMEL10 - DNA | Unknown | 1/3 (<i>Cryptosporidium</i> spp.) | N/A |
| <i>Cryptosporidium meleagridis</i> | BEI NR-2521 | 1.01 x 10 ⁵ Cysts/mL | 3/3 (<i>Cryptosporidium</i> spp.) | N/A |
| <i>Cryptosporidium meleagridis</i> ^c | UKMEL10 | Clinical Sample (Ct = 32.5) | 3/3 (<i>Cryptosporidium</i> spp. ^c) | N/A |

a - *Vibrio* spp LoD established as 4.9x 10² CFU/mL

b - *Yersinia enterocolitica* LoD established as 1.5 x 10³ 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 |
|--|-----------------------------|-------------------------------|
| <i>Campylobacter coli</i> | ATCC 33559 | 2.40 x 10 ⁵ CFU/mL |
| <i>Campylobacter jejuni</i> spp. <i>jejuni</i> | ATCC 33292 | 7.10 x 10 ⁵ CFU/mL |
| <i>Clostridium difficile</i> (toxintype 0) | ATCC 9689 | 1.93 x 10 ⁶ CFU/mL |
| <i>Clostridium difficile</i> (toxintype III; Nap1) | Zeptomatrix 0801619CF | 7.43 x 10 ⁶ CFU/mL |
| Enteroggregative <i>E. coli</i> O92:H33 (EAEC) | STEC TW04440 | 6.18 x 10 ⁶ CFU/mL |
| Enteroinvasive <i>E. coli</i> O29:NM (EIEC) | ATCC 43892 | 4.70 x 10 ⁶ CFU/mL |
| Enterotoxigenic <i>E. coli</i> O78:H11 H10407 (ETEC) | ATCC 35401 | 4.07 x 10 ⁶ CFU/mL |
| Shiga-like toxin producing <i>E. coli</i> (STEC) | ATCC BAA-2217 | 5.90 x 10 ⁶ CFU/mL |
| <i>E. coli</i> O157 | ATCC 700376 | 2.36 x 10 ⁶ CFU/mL |
| <i>Salmonella bongori</i> | SGSC 4900 | 2.16 x 10 ⁷ CFU/mL |
| <i>Salmonella enterica</i> subsp. <i>enterica</i> | ATCC 14028 | 5.52 x 10 ⁷ CFU/mL |
| <i>Shigella sonnei</i> | ATCC 29930 | 5.42 x 10 ⁶ CFU/mL |
| <i>Vibrio cholerae</i> | ATCC 25870 | 9.80 x 10 ⁵ CFU/mL |
| <i>Vibrio parahaemolyticus</i> | ATCC 17802 | 6.20 x 10 ⁴ CFU/mL |
| <i>Yersinia enterocolitica</i> | ATCC 23715 | 1.48 x 10 ⁶ CFU/mL |
| <i>Cryptosporidium parvum</i> | Waterborne P102C | 1.20 x 10 ⁵ CFU/mL |
| <i>Entamoeba histolytica</i> HB-301:NIH | BEI NR-178 | 7.44 x 10 ³ CFU/mL |
| <i>Giardia intestinalis</i> (aka <i>G. lamblia</i>) | Waterborne P101 | 6.25 x 10 ⁵ CFU/mL |
| Adenovirus 40 (dugan) | Zeptomatrix 0810084CF | 1.69 x 10 ⁴ CFU/mL |
| Adenovirus 41 (TAK) | Zeptomatrix 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 |
|--------------------------------------|-----------------------------|-------------------------------|
| <i>Aeromonas jandaei</i> | ATCC 49568 | 1.21 x 10 ⁵ CFU/mL |
| <i>Aeromonas media</i> | ATCC BAA-229 | 5.10 x 10 ⁵ CFU/mL |
| <i>Aeromonas trota</i> | ATCC 49658 | 1.10 x 10 ⁶ CFU/mL |
| <i>Aeromonas caviae</i> | ATCC 14486 | 1.14 x 10 ⁶ CFU/mL |
| <i>Aeromonas hydrophila</i> | ATCC 7966 | 8.70 x 10 ⁶ CFU/mL |
| <i>Acinetobacter baumannii</i> | ATCC 19606 | 5.00 x 10 ⁶ CFU/mL |
| <i>Acinetobacter lwoffii</i> | ATCC 15309 | 1.80 x 10 ⁶ CFU/mL |
| <i>Alcaligenes faecalis</i> | ATCC 15554 | 1.09 x 10 ⁶ CFU/mL |
| <i>Bacillus cereus</i> | ATCC 13472 | 1.20 x 10 ⁶ CFU/mL |
| <i>Bacteroides fragilis</i> | Zeptomatrix 0801583 | 4.77 x 10 ⁶ CFU/mL |
| <i>Bacteroides thetaiotaomicron</i> | Zeptomatrix Z037 | 5.67 x 10 ⁶ CFU/mL |
| <i>Bifidobacterium breve</i> | Zeptomatrix 0801829 | 1.53 x 10 ⁶ CFU/mL |
| <i>Campylobacter fetus</i> | ATCC 25936 | 2.60 x 10 ⁶ CFU/mL |
| <i>Campylobacter hyointestinalis</i> | ATCC 35217 | 1.31 x 10 ⁶ CFU/mL |
| <i>Campylobacter lari</i> | ATCC 43675 | 1.60 x 10 ⁶ CFU/mL |

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

| Bacteria | ATCC/Other Reference number | Titer Tested |
|---|-----------------------------|----------------------------------|
| <i>Listeria monocytogenes</i> | ATCC 19115 | 3.80 x 10 ⁶ CFU/mL |
| <i>Morganella morganii</i> | ATCC 25830 | 1.90 x 10 ⁶ CFU/mL |
| <i>Peptoniphilus asaccharolyticus</i> | ATCC 29743 | 1.21 x 10 ⁶ CFU/mL |
| <i>Plesiomonas shigelloides</i> | ATCC 14029 | 8.60 x 10 ⁶ CFU/mL |
| <i>Porphyromonas asaccharolytica</i> | ATCC 25260 | 1.90 x 10 ⁶ CFU/mL |
| <i>Prevotella melaninogenica</i> ^a | ATCC 25845 | 1.00 x 10 ⁶ CFU/mL |
| <i>Proteus mirabilis</i> | ATCC 25933 | 2.15 x 10 ⁶ CFU/mL |
| <i>Proteus penneri</i> | ATCC 33519 | 1.90 x 10 ⁶ CFU/mL |
| <i>Proteus vulgaris</i> | ATCC 6380 | 2.41 x 10 ⁶ CFU/mL |
| <i>Providencia alcalifaciens</i> | ATCC 51902 | 3.70 x 10 ⁶ CFU/mL |
| <i>Providencia stuartii</i> | ATCC 33672 | 2.31 x 10 ⁶ CFU/mL |
| <i>Pseudomonas aeruginosa</i> | ATCC 39324 | 7.40 x 10 ⁶ CFU/mL |
| <i>Pseudomonas fluorescens</i> | ATCC 13525 | 1.27 x 10 ⁶ CFU/mL |
| <i>Pseudomonas putida</i> | Zeptomatrix 0801722 | 1.00 x 10 ⁶ CFU/mL |
| <i>Saccharomyces boulardii</i> | ATCC MYA-796 | 8.40 x 10 ⁶ CFU/mL |
| <i>Serratia liquefaciens</i> | ATCC 27592 | 3.20 x 10 ⁶ CFU/mL |
| <i>Serratia marcescens</i> | ATCC 13880 | 2.80 x 10 ⁶ CFU/mL |
| <i>Shewanella algae</i> | ATCC 51181 | 1.20 x 10 ⁶ CFU/mL |
| <i>Staphylococcus aureus</i> | ATCC 43300 | 2.40 x 10 ⁶ CFU/mL |
| <i>Staphylococcus epidermidis</i> | ATCC 14990 | 8.20 x 10 ⁶ CFU/mL |
| <i>Stenotrophomonas maltophilia</i> | DD-468 (DNA) | 1.85 x 10 ⁶ copies/mL |
| <i>Streptococcus agalactiae</i> | ATCC 55193 | 1.64 x 10 ⁶ CFU/mL |
| <i>Streptococcus intermedius</i> | HM-368D (DNA) | Unknown |
| <i>Streptococcus pyogenes</i> | NR-15274 | 2.90 x 10 ⁶ CFU/mL |
| <i>Streptococcus salivarius</i> | HM-121 | 1.12 x 10 ⁶ CFU/mL |
| <i>Streptococcus suis</i> | ATCC 43765 | 1.64 x 10 ⁶ CFU/mL |
| <i>Trabulsiella guamensis</i> | ATCC 49490 | 2.40 x 10 ⁶ CFU/mL |
| <i>Veillonella parvula</i> | ATCC 10790 | 5.90 x 10 ⁶ CFU/mL |
| <i>Vibrio fluvialis</i> | ATCC 33809 | 4.70 x 10 ⁶ CFU/mL |
| <i>Yersinia pseudotuberculosis</i> | 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 | Zeptomatrix 0810062CF | 4.17 x 10 ⁴ TCID ₅₀ /mL |
| Adenovirus 4 | Zeptomatrix 0810070CF | 2.45 x 10 ⁴ TCID ₅₀ /mL |
| Adenovirus 7a | Zeptomatrix 0810021CF | 1.95 x 10 ⁵ TCID ₅₀ /mL |
| Adenovirus 8 | Zeptomatrix 0810069CF | 1.41 x 10 ⁴ TCID ₅₀ /mL |
| Adenovirus 14 | Zeptomatrix 0810108CF | 3.39 x 10 ⁵ TCID ₅₀ /mL |

| Viruses | ATCC/Other Reference number | Titer Tested |
|-------------------------------|-----------------------------|---|
| Adenovirus 37 | Zeptomatrix 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 | Zeptomatrix 0810229CF | 1.51 x 10 ⁵ TCID ₅₀ /mL |
| Coronavirus NL63 ^a | Zeptomatrix 0810228CF | 1.41 x 10 ⁴ TCID ₅₀ /mL |
| *Coxsackie virus A16 | Cell culture - CDC | 1.0 x 10 ⁵ pfu/mL |
| Coxsackievirus B3 | Zeptomatrix 0810074CF | 1.78 x 10 ⁵ TCID ₅₀ /mL |
| Cytomegalovirus (CMV) | Zeptomatrix 0810003CF | 1.15 x 10 ⁵ TCID ₅₀ /mL |
| Enterovirus 68 | Zeptomatrix 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 | Zeptomatrix 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 | Zeptomatrix 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 |
|--|-----------------------------|--|
| * <i>Blastocystis hominis</i> | Clinical Sample - CDC | 1.0 x 10 ⁷ infectious units |
| <i>Blastocystis hominis</i> | ATCC 50613 | 3.40 x 10 ⁵ cells/mL |
| <i>Cryptosporidium canis</i> (DNA) | UKCAN1 - DNA | Unknown |
| <i>Cryptosporidium felis</i> (DNA) | UKFEL3 - DNA | Unknown |
| <i>Cryptosporidium muris</i> | Waterborne P104 | 2.50 x 10 ⁴ Cysts/mL |
| <i>Cryptosporidium ubiquitum</i> (DNA) | UKUB7 - DNA | Unknown |
| <i>Encephalitozoon cuniculi</i> | BEI NR-9073 | 1.73 x 10 ⁴ Cysts/mL |
| <i>Encephalitozoon hellem</i> | BEI NR-9701 | 8.24 x 10 ³ Cysts/mL |
| <i>Encephalitozoon intestinalis</i> | BEI NR-9702 | 1.52 x 10 ³ Cysts/mL |
| <i>Giardia muris</i> | Waterborne P105 | 6.25 x 10 ⁴ Cysts/mL |
| <i>Pentatrichomonas hominis</i> | ATCC 30098 | 2.50 x 10 ⁵ cells/mL |
| <i>Toxoplasma gondii</i> | 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 |
|-------------------------------------|---------------------------------------|------------------------------------|
| <i>Anaerococcus tetradius</i> | <i>Eubacterium cylindroides</i> | <i>Ancylostoma duodenale</i> |
| <i>Bifidobacterium adolescentis</i> | <i>Eubacterium rectale</i> | <i>Ascaris lumbricoides</i> |
| <i>Bifidobacterium longum</i> | <i>Megamonas hypermegale</i> | <i>Balantidium coli</i> |
| <i>Campylobacter concisus</i> | <i>Methanobrevibacter smithii</i> | <i>Chilomastix mesnili</i> |
| <i>Campylobacter curvus</i> | <i>Peptoniphilus asaccharolyticus</i> | <i>Cryptosporidium bovis</i> |
| <i>Campylobacter gracilis</i> | <i>Ruminococcus bromii</i> | <i>Cryptosporidium canis</i> |
| <i>Campylobacter helveticus</i> | <i>Ruminococcus flavefaciens</i> | <i>Cryptosporidium cuniculus</i> |
| <i>Campylobacter hominis</i> | <i>Ruminococcus obeum</i> | <i>Cryptosporidium felis</i> |
| <i>Campylobacter lari</i> | <i>Selenomonas ruminantium</i> | <i>Cryptosporidium fetus</i> |
| <i>Campylobacter mucosalis</i> | <i>Vibrio cincinnatiensis</i> | <i>Cryptosporidium meleagridis</i> |
| <i>Campylobacter rectus</i> | <i>Vibrio furnissii</i> | <i>Cryptosporidium muris</i> |
| <i>Campylobacter showae</i> | <i>Vibrio metschnikovii</i> | <i>Cryptosporidium ryanae</i> |
| <i>Campylobacter sputorum</i> | <i>Yersinia kristensenii</i> | <i>Cryptosporidium xiaoi</i> |
| <i>Campylobacter upsaliensis</i> | Viruses | <i>Dientamoeba fragilis</i> |
| <i>Campylobacter ureolyticus</i> | Norovirus GIV | <i>Endolimax nana</i> |
| <i>Clostridium acetobutylicum</i> | Rotavirus B | <i>Entamoeba coli</i> |
| <i>Clostridium methylpentosum</i> | Rotavirus C ^a | <i>Entamoeba dispar</i> |
| <i>Clostridium novyi</i> | Rotavirus D | <i>Entamoeba hartmanni</i> |
| <i>Clostridium ramosum</i> | Rotavirus E | <i>Entamoeba moshkovskii</i> |
| <i>Collinsella aerofaciens</i> | Rotavirus F | <i>Entamoeba polecki</i> |
| <i>Desulfovibrio piger</i> | 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

| Target | Concentration Level | | | | | |
|--------------------------------|---------------------------|----------------|---------------------------|----------------|---------------------------|----------------|
| | Medium Positive | | Low Positive | | Negative | |
| | Detection Rate n/N (%) | 95% CI | Detection Rate n/N (%) | 95% CI | Agreement Rate n/N (%) | 95% CI |
| <i>Salmonella enterica</i> | 90/90 (100.0) | (95.98, 100.0) | 90/90 (100.0) | (95.98, 100.0) | 450/450 (100.0) | (99.18, 100.0) |
| <i>Clostridium difficile</i> | 90/90 (100.0) | (95.98, 100.0) | 90/90 (100.0) | (95.98, 100.0) | 450/450 (100.0) | (99.18, 100.0) |
| <i>Giardia lamblia</i> | 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) |
| <i>Shigella sonnei</i> | 90/90 (100.0) | (95.98, 100.0) | 90/90 (100.0) | (95.98, 100.0) | 450/450 (100.0) | (99.18, 100.0) |
| <i>Vibrio parahaemolyticus</i> | 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) |
| <i>Yersinia enterocolitica</i> | 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) |
| <i>Campylobacter jejuni</i> | 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) |
| <i>Cryptosporidium parvum</i> | 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 |
| <i>Salmonella enterica</i> | Low | 9174 | 1853 | 20.2 | 622 | 6.78 | 1464 | 15.95 | 0 | 0 | 2442 | 26.62 |
| <i>Salmonella enterica</i> | Med | 11572 | 1204 | 10.41 | 469 | 4.05 | 1980 | 17.11 | 1190 | 10.28 | 2647 | 22.87 |
| <i>tcdA (C. difficile)</i> | Low | 5144 | 1407 | 27.35 | 1306 | 25.4 | 689 | 13.39 | 882 | 17.15 | 2222 | 43.2 |
| <i>tcdA (C. difficile)</i> | Med | 8802 | 2381 | 27.05 | 1444 | 16.4 | 1645 | 18.69 | 1337 | 15.19 | 3499 | 39.76 |
| <i>tcdB (C. difficile)</i> | Low | 11595 | 2004 | 17.29 | 847 | 7.31 | 2293 | 19.78 | 0 | 0 | 3161 | 27.27 |
| <i>tcdB (C. difficile)</i> | Med | 16441 | 1878 | 11.42 | 2219 | 13.49 | 2804 | 17.05 | 682 | 4.15 | 4096 | 24.91 |
| <i>Giardia lamblia</i> | Low | 30206 | 2436 | 8.06 | 2164 | 7.16 | 2516 | 8.33 | 3342 | 11.07 | 5302 | 17.55 |
| <i>Giardia lamblia</i> | 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 |
| <i>Shigella sonnei</i> | Low | 6115 | 1654 | 27.05 | 1487 | 24.31 | 1337 | 21.87 | 1043 | 17.06 | 2797 | 45.74 |
| <i>Shigella sonnei</i> | Med | 9707 | 2492 | 25.68 | 1968 | 20.28 | 0 | 0 | 1669 | 17.19 | 3588 | 36.96 |
| <i>V.parahaemolyticus</i> | Low | 9927 | 2690 | 27.1 | 792 | 7.98 | 2956 | 29.78 | 966 | 9.73 | 4188 | 42.18 |
| <i>V.parahaemolyticus</i> | 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 |
| <i>Yersinia enterocolitica</i> | Low | 19049 | 1970 | 10.34 | 1729 | 9.08 | 1647 | 8.65 | 2326 | 12.21 | 3872 | 20.33 |
| <i>Yersinia enterocolitica</i> | 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 |
| <i>Campylobacter jejuni</i> | Low | 21865 | 2384 | 10.9 | 3282 | 15.01 | 2727 | 12.47 | 2302 | 10.53 | 5402 | 24.71 |
| <i>Campylobacter jejuni</i> | 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 prescreened 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 | <i>Clostridium difficile</i> (NAP1) | Zeptomatrix 0801619cf |
| | <i>Cryptosporidium parvum</i> | Waterborne P102C |
| Sample B | Human Rotavirus A | ATCC VR-2018 |
| | <i>Escherichia coli</i> 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 |
| <i>Bacteroides fragilis</i> ^a | Zeptomatrix 0801583 | 1 x 10 ⁶ | N |
| <i>Blastocystis hominis</i> ^b | ATCC 50752 | 1 x 10 ⁵ | N |
| <i>Candida albicans</i> | ATCC 14053 | 1 x 10 ⁵ | N |
| <i>Clostridium difficile</i> non-toxigenic | ATCC 700057 | 1 x 10 ⁶ | N |
| <i>Enterococcus faecalis</i> | ATCC 51299 | 1 x 10 ⁶ | N |
| <i>Escherichia coli</i> nonpathogenic | ATCC BAA-97 | 1 x 10 ⁶ | N |
| <i>Pseudomonas aeruginosa</i> | ATCC 39324 | 1 x 10 ⁶ | N |
| <i>Saccharomyces boulardii</i> | ATCC MYA-796 | 1 x 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 |
| Loperamide hydrochloride | Imodium | 5% v/v | N |
| Sennosides | Senokot | 5% w/v | N |
| Magnesium Hydroxide, Aluminum Hydroxide | Maalox | 5% w/v | N |
| Metronidazole | Metronidazole | 14 mg/mL | N |
| Benzalkonium chloride, Ethanol | Moist towelettes | 50% w/v | N |
| Mono, di, triglycerides mix | Supelco | 5% w/v | N |
| Mucin | Mucin | 3 mg/ml | N |
| Naproxen sodium | Naproxen sodium | 14mg/ml | N |
| Polymyxin B sulfate, bacitracin zinc, Neomycin | Neosporin | 50% w/v | N |
| Nystatin | Nystatin | 1000 U/mL | N |
| Bismuth subsalicylate | Pepto-Bismol | 5% v/v | N |
| Petrolatum | Preparation H | 5% v/v | N |
| Calcium carbonate | Tums | 5% w/v | N |
| Vancomycin | Vancomycin | 12.5 mg/mL | N |

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 ($\geq 10^6$ CFU/mL for bacteria and $\geq 10^5$ units/mL for viruses or parasites) and two targets at low concentration ($\leq 3 \times$ 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 | <i>Clostridium difficile</i> | Zeptomatrix 801619 | High | 3.0×10^6 CFU/mL | tcdB | 3/3 |
| | Rotavirus A | ATCC VR-2018 | Medium | 7.44×10^3 TCID ₅₀ /mL | Rota | 3/3 |
| | <i>Escherichia coli</i> E2348/69 (EPEC) | STEC TW06375 | Medium | 7.02×10^3 CFU/mL | EPEC | 3/3 |
| Competitive Inhibition Sample 2 | O92:H33 <i>Escherichia coli</i> (EAEC) | STEC JM221 TW04440 | High | 3.0×10^6 CFU/mL | EAEC | 3/3 |
| | <i>Escherichia coli</i> E2348/69 (EPEC) | STEC TW06375 | Medium | 7.02×10^3 CFU/mL | EPEC | 3/3 |
| | <i>Clostridium difficile</i> | Zeptomatrix 801619 | Medium | 5.7×10^2 CFU/mL | tcdB | 3/3 |
| Competitive Inhibition Sample 3 | <i>Escherichia coli</i> E2348/69 (EPEC) | STEC TW06375 | High | 3.0×10^6 CFU/mL | EPEC | 3/3 |
| | <i>Clostridium difficile</i> | Zeptomatrix 801619 | Medium | 5.7×10^2 CFU/mL | tcdB | 3/3 |
| | Rotavirus A | ATCC VR-2018 | Medium | 7.44×10^3 TCID ₅₀ /mL | Rota | 3/3 |
| Competitive Inhibition Sample 4 | <i>Escherichia coli</i> E2348/69 (EPEC) | STEC TW06375 | High | 3.0×10^6 CFU/mL | EPEC | 3/3 |
| | O92:H33 <i>Escherichia coli</i> (EAEC) | STEC JM221 TW04440 | Medium | 4.08×10^3 CFU/mL | EAEC | 3/3 |
| | <i>Campylobacter jejuni</i> subsp. <i>jejuni</i> | ATCC 33292 | Medium | 2.1×10^3 CFU/mL | Campy | 3/3 |
| Competitive Inhibition Sample 5 | <i>Campylobacter jejuni</i> subsp. <i>doylei</i> | ATCC 49349 | High | 3.0×10^6 CFU/mL | Campy | 3/3 |
| | <i>Escherichia coli</i> E2348/69 (EPEC) | STEC TW06375 | Medium | 7.02×10^3 CFU/mL | EPEC | 3/3 |
| | O92:H33 <i>Escherichia coli</i> (EAEC) | STEC JM221 TW04440 | Medium | 4.08×10^3 CFU/mL | EAEC | 3/3 |

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

REFERENCES



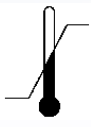








1. GBD Diarrhoeal Diseases Collaborators. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect Dis.* **17**, 909-948 (2017).
2. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet.* **388**, 1459-544 (2016).
3. Centers for Disease Control and Prevention. Summary of Notifiable Infectious Diseases and Conditions, 2015. *MMWR Morb Mortal Wkly Rep.* **64**(53), 1-148 (2017).
4. Scallan, E. et al. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis.* **17**, 7–15 (2011).
5. Eurosurveillance editorial team. The 2013 joint ECDC/EFSA report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks published. *Euro Surveill.* **20**(4), (2015).
6. European Food Safety Authority and European Centre for Disease Prevention and Control, 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. *EFSA Journal.* **13**(12):4329, (2015).
7. European Food Safety Authority and European Centre for Disease Prevention and Control. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA Journal.* **14**(12):4634, (2016).
8. Shah, N., DuPont, H. L., Ramsey D. J. Global etiology of travelers' diarrhea: systematic review from 1973 to the present. *Am J Trop Med Hyg.* **80**(4), 609–614 (2009).
9. Nachamkin, I., Szymanski, C. M. & Blaser, M. J. *Campylobacter*. (ASM Press, 2008).
10. Lessa, F. C. et al. Burden of *Clostridium difficile* Infection in the United States. *N Engl J Med.* **372**, 825-834 (2015).
11. Hall et al. The roles of *Clostridium difficile* and norovirus among gastroenteritis-associated deaths in the United States, 1999-2007. *Clin Infect Dis.* (2012).
12. Kola, A. et al., on behalf of members of ECDIS-Net. Survey of *Clostridium difficile* infection surveillance systems in Europe, 2011. *Euro Surveill.* **21**(29), 5-12 (2016).
13. European Centre for Disease Prevention and Control. European Surveillance of *Clostridium difficile* infections. Surveillance protocol version 2.2. [Internet] Stockholm: ECDC, 2015. Retrieved from http://ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=1402
14. van Dorp, S. M. et al., for the European *Clostridium difficile* Infection Surveillance Network (ECDIS-Net) project on behalf of all participants. Survey of diagnostic and typing capacity for *Clostridium difficile* infection in Europe, 2011 and 2014. *Euro Surveill.* **21**(29), 13-23 (2016).
15. Centers for Disease Control and Prevention. Healthcare-associated Infections. *Clostridium difficile* Infection Information for Patients. Retrieved from <https://www.cdc.gov/hai/organisms/cdiff/cdiff-patient.html>
16. Bien, J., Palagani, V., Bozko, P. The intestinal microbiota dysbiosis and *Clostridium difficile* infection: is there a relationship with inflammatory bowel disease? *Ther Adv Gastroenterol.* **6**(1), 53–68 (2013).
17. Centers for Disease Control and Prevention. Antibiotic/Antimicrobial Resistance. Biggest Threats. Retrieved from https://www.cdc.gov/drugresistance/biggest_threats.html
18. Bielaszewska, M. et al. Characterisation of the *Escherichia coli* strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. *Lancet Infect Dis.* **11**, 671–676 (2011).

19. Hazen, H. et al. Comparative genomics and transcriptomics of Escherichia coli isolates carrying virulence factors of both enteropathogenic and enterotoxigenic E. coli. *Sci Rep.* **7**, 3513 (2017).
20. Bou-Antoun, S. et al. Descriptive epidemiology of Escherichia coli bacteraemia in England, April 2012 to March 2014. *Euro Surveill.* **21**(35), (2016).
21. Chellapandi, K. et al. Prevalence of multi drug resistant enteropathogenic and enteroinvasive Escherichia coli isolated from children with and without diarrhea in Northeast Indian population. *Ann Clin Microbiol Antimicrob.* **16**(49), (2017).
22. Ochoa, T. J., Contreras, C. A. Enteropathogenic E. coli (EPEC) infection in children. *Curr Opin Infect Dis.* **24**(5): 478–483 (2011).
23. Lozano, R. et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet.* **380**, 2095–2128 (2012).
24. Centers for Disease Control and Prevention. E.coli (Escherichia coli). Enterotoxigenic E. coli (ETEC). Retrieved from <https://www.cdc.gov/ecoli/etec.html>
25. Centers for Disease Control and Prevention (CDC). Surveillance for Foodborne Disease Outbreaks, United States, 2015, Annual Report. Atlanta, Georgia: US Department of Health and Human Services, CDC, (2017).
26. McFarland, N. et al. Recurrent seasonal outbreak of an emerging serotype of Shiga toxin-producing Escherichia coli (STEC O55:H7 Stx2a) in the south west of England, July 2014 to September 2015. *Euro Surveill.* **22**(36), (2017).
27. Vygen-Bonnet, S. et al. Ongoing haemolytic uraemic syndrome (HUS) outbreak caused by sorbitol-fermenting (SF) Shiga toxin-producing Escherichia coli (STEC) O157, Germany, December 2016 to May 2017. *Euro Surveill.* **22**(21), (2017).
28. Centers for Disease Control and Prevention. E.coli (Escherichia coli). General Information. Retrieved from <https://www.cdc.gov/ecoli/general/index.html>
29. Centers for Disease Control and Prevention. National Enteric Disease Surveillance: Shiga toxin-producing Escherichia coli (STEC) Annual Report, 2014. Atlanta, Georgia, CDC, (2017).
30. Schmidt, H., Scheef, J., Huppertz, H. I., Frosch, M., Karch, H. Escherichia coli O157:H7 and O157:H(-) strains that do not produce Shiga toxin: phenotypic and genetic characterization of isolates associated with diarrhea and hemolytic-uremic syndrome. *J Clin Microbiol.* **37**(11), 3491- 3496 (1999).
31. Blank, T. E. et al. Enteropathogenic Escherichia coli O157 strains from Brazil. *Emerg Infect Dis.* **9**(1):113-155 (2003).
32. Centers for Disease Control and Prevention (CDC). National Shigella Surveillance Annual Report, 2013. Atlanta, Georgia: US Department of Health and Human Services, CDC, (2016).
33. Simms, I. et al. Intensified shigellosis epidemic associated with sexual transmission in men who have sex with men - Shigella flexneri and S. sonnei in England, 2004 to end of February 2015. *Euro Surveill.* **20**(15), (2015).
34. Hall, A. J. et al. Acute Gastroenteritis Surveillance through the National Outbreak Reporting System, United States. *Emerg Infect Dis.* **19**(8), 1305-1309 (2013).
35. Centers for Disease Control and Prevention. Salmonella. Retrieved from <https://www.cdc.gov/salmonella/index.html>
36. Centers for Disease Control and Prevention. National Disease Surveillance: COVIS Annual Summary, 2014. Atlanta, Georgia, CDC, (2016).
37. Newton, A. et al. Increasing rates of vibriosis in the United States, 1996–2010: review of surveillance data from 2 systems. *Clin Infect Dis.* **54**, (2012).
38. Center for Disease Control and Prevention. Vibrio Species Causing Vibriosis. Retrieved from <https://www.cdc.gov/vibrio/index.html>

39. European Centre for Disease Prevention and Control. E3 Geoportal. Vibrio Map Viewer. Retrieved from <https://e3geoportal.ecdc.europa.eu/SitsszePages/Vibrio%20Map%20Viewer.aspx>
40. Ali, M., Nelson, A. R., Lopez, A. L., Sack, D. A. Updated global burden of cholera in endemic countries. *PLOS Negl Trop Dis.* **9**(6), (2015).
41. Centers for Disease Control and Prevention. Yersinia enterocolitica (Yersiniosis). Retrieved from <https://www.cdc.gov/yersinia/>
42. Chakraborty, A. et al. The descriptive epidemiology of yersiniosis: a multistate study, 2005 - 2011. *Public Health Rep.* **130**,269-77 (2015).
43. Painter, J. E., Hlavsa, M. C., Collier, S. A., Xiao, L., Yoder, J. S. Cryptosporidiosis Surveillance - United States, 2011–2012. *MMWR Surveill Summ.* **64**(3), 1-13 (2015).
44. European Centre for Disease Prevention and Control. Annual Epidemiological Report 2016 - Cryptosporidiosis. Stockholm, ECDC, (2016).
45. European Centre for Disease Prevention and Control. Annual Epidemiological Report 2016 - Giardiasis. Stockholm, ECDC, (2016).
46. Centers for Disease Control and Prevention. Parasites - Giardia. Retrieved from <https://www.cdc.gov/parasites/giardia/index.html>
47. Painter, J. E., Gargano, J. W., Collier, S. A., Yoder, J. S. Giardiasis Surveillance - United States, 2011–2012. *MMWR Surveill Summ.* **64**(3), 15-25 (2015).
48. Centers for Disease Control and Prevention. Parasites - Amebiasis - Entamoeba histolytica Infection. Retrieved from <https://www.cdc.gov/parasites/amebiasis/>
49. *American Water Works Association (June 2006). Waterborne Pathogens.* American Water Works Association. ISBN 978-1-58321-403-9.
50. Lohia, A. The cell cycle of Entamoeba histolytica. *Mol Cell Biochem.* **253**, 217-222 (2003).
51. Escolà-Vergé, L. et al. Outbreak of intestinal amoebiasis among men who have sex with men, Barcelona (Spain), October 2016 and January 2017. *Euro Surveill.* **22**(30), (2017).
52. Liu et al. Epidemiological aspects of rotavirus and adenovirus in hospitalized children with diarrhea: a 5-year survey in Beijing. *BMC Infect Dis.* **16**(508), (2016).
53. Lindesmith, L. et al. Human susceptibility and resistance to Norwalk virus infection. *Nat. Med.* **9**(5), 548–53 (2003).
54. Pires, S. M. et al. Aetiology-Specific Estimates of the Global and Regional Incidence and Mortality of Diarrhoeal Diseases Commonly Transmitted through Food. *PLoS One.* **10**(12), (2015).
55. Payne, D. C. et al. Norovirus and medically attended gastroenteritis in U.S. children. *N Engl J Med.* **368**(12), 1121–1130 (2013).
56. Fletcher, S. et al. Gastrointestinal pathogen distribution in symptomatic children in Sydney, Australia. *J Epidemiol Glob Health.* **3**, 11-21 (2013).
57. *Summary of Notifiable Diseases.* available at <http://www.cdc.gov>
58. *CIFOR Analysis of State Legal Authorities.* available at <http://www.cifor.us>

TABLE OF SYMBOLS

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

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



Applied BioCode

Barcoded Magnetic Beads | Flexible Syndromic Tests

