PERFORMANCE EVALUATION OF AN 18-PLEX MOLECULAR GASTROINTESTINAL PATHOGEN PANEL WITH AN AUTOMATED, HIGH-THROUGHPUT SYSTEM

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Introduction

Gastroenteritis is the second most common cause of death among children under the age of 5, accounting for 1 in 9 child deaths worldwide; 2,195 children each day. High-throughput, highly multiplexed assays can aid in rapid identification of pathogens that can cause outbreaks of diarrhea and for infection control in healthcare settings. Despite recent introduction of molecular multiplex pathogen detection platforms, there is a limited choice of systems for clinical labs with high specimen throughput. To address this need, Applied BioCode has developed an automated high-throughput molecular diagnostic assay system in a 96-well format.

BioCode® Gastrointestinal Pathogen Panel

Table 1. Organisms and toxins detected by the BioCode® 18-Plex GI Pathogen Panel

Bacteria

- *Campylobacter* spp.
- *Clostridium difficile* toxin A/B
- Enteroaggregative *E. coli* (EAEC)
- Enteropathogenic *E. coli* (EPEC)
- Enterotoxigenic *E. coli* (ETEC)
- *Salmonella* spp.
- Shiga toxin producing *E. coli* (STEC)
- *E. coli* O157
- *Shigella*/ Enteroinvasive *E. coli* (EIEC)
- *Vibrio parahaemolyticus*
- *Vibrio* spp.
- *Yersinia enterocolitica*

Parasites

- *Cryptosporidium* spp.
- *Entamoeba histolytica*
- *Giardia lamblia*

Viruses

- Adenovirus 40/41
- Norovirus GI/GII
- Rotavirus A

Methods

The BioCode® MDx 3000 platform integrates and automates PCR, post-PCR sample handling and detection steps in a 96-well format. Following extraction of nucleic acids from either unpreserved stool or stool in Cary-Blair Transport medium with an automated system, DNA and RNA targets are amplified by one-step RT-PCR. PCR products are captured by target-specific probes coupled to Barcoded Magnetic Beads (BMBs), and the presence of target sequence(s) is detected by a fluorescent conjugate. Qualitative results are determined by a median fluorescent index (MFI) value relative to assay cutoff.

BioCode® MDx 3000

Extraction

~1.3 hours

PCR Set-Up

~10 min

PCR, Target Capture, Signal Generation and Optical Detection

~3.5 hours

Figure 1. Workflow for BioCode® GI Pathogen Panel. The system enables testing 192 samples in an 8 hour shift with minimal hands-on time. Three different panels can be performed simultaneously in one plate.

Preliminary Limit of Detection (LoD)

Table 2. Limit of Detection (LoD) for the BioCode® 18-Plex GI Pathogen Panel performed on the BioCode® MDx 3000

Strain	Unpreserved Stool LoD	Cary-Blair Stool LoD
Camplyobacter coli	5.60 x10^1 CFU/mL	5.60 x10^1 CFU/mL
Campylobacter jejuni sub sp .jejuni	7.00 x10^2 CFU/mL	7.00 x10^2 CFU/mL
Clostridium difficile (toxinotype 0)	1.93 x 10^2 CFU/mL	1.93 x 10^2 CFU/mL
Clostridium difficile (toxinotype III) Nap1	3.30 x 10^3 CFU/mL	3.30 x 10^3 CFU/mL
Enteroaggregative *E.coli (EAEC)*	5.50 x 10^3 CFU/mL	5.50 x 10^3 CFU/mL
Enteroinvasive *E. coli* (EIEC)	7.53 x 10^2 CFU/mL	7.53 x 10^2 CFU/mL
Enteropathogenic *E. coli (EPEC)*	3.50 x 10^3 CFU/mL	3.50 x 10^3 CFU/mL
Enterotoxigenic *E. coli* (ETEC)	2.25 x10^3 CFU/mL	2.25 x10^3 CFU/mL
Salmonella bongori	1.38 x 10^3 CFU/mL	1.38 x 10^3 CFU/mL
Salmonella enterica	2.15 x10^3 CFU/mL	2.15 x10^3 CFU/mL
Shiga toxin producing *E. coli* (STEC)	5.00 x 10^3 CFU/mL	5.00 x 10^3 CFU/mL
E. coli O157	6.00 x10^4 CFU/mL	1.20 x10^4 CFU/mL
Shigella sonnei	3.48 x 10^3 CFU/mL	3.48 x 10^3 CFU/mL
Vibrio cholerae	9.80 x10^2 CFU/mL	9.80 x10^2 CFU/mL
Vibrio parahaemolyticus	2.5 x10^1 CFU/mL	2.5 x10^1 CFU/mL
Yersinia enterocolitica	5.90 x10^3 CFU/mL	5.90 x10^3 CFU/mL
Cryptosporidium parvum	6.25 x 10^3 oocysts/mL	6.25 x 10^3 oocysts/mL
Entamoeba histolytica	1.56 x10^{-1} cysts/mL	1.56 x10^{-1} cysts/mL
Giardia intestinalis (aka G. lamblia)	4.50 x 10^2 oocysts/mL	4.50 x 10^2 oocysts/mL
Human adenovirus 40 (dugan)	4.00 x10^{-1} $TCID_{50}$/mL	4.00 x10^{-1} $TCID_{50}$/mL
Human adenovirus 41	3.75 x10^{-1} $TCID_{50}$/mL	3.75 x10^{-1} $TCID_{50}$/mL
Norovirus GI	1.12 x10^2 $TCID_{50}$/mL	1.12 x10^2 $TCID_{50}$/mL
Norovirus GII	4.75 $TCID_{50}$/mL	4.75 $TCID_{50}$/mL
Human rotavirus A	6.20 x10^2 $TCID_{50}$/mL	6.20 x10^2 $TCID_{50}$/mL

- At least 2 additional strains were assayed for each target organism which were detected between 3–10 fold of LoD.

Method Comparison

Table 4. Assay performance was tested with a set of 398 unpreserved stool and 100 stool in Cary-Blair transport medium, and compared to the results of respective FDA-cleared assay or validated sequencing assay.

Unpreserved Stool

comparison to reference methods	*Campylobacter* spp	*Clostridium difficile*	ETEC	*Salmonella* spp	*Shigella* spp/ EIEC	STEC, Non O157	STEC O157	Non STEC, O157	*Vibrio* spp	*Cryptosporidium spp.*	*Entamoeba histolytica*	*Giardia lamblia*	Adenovirus 40/41	Norovirus G1/G2	Rotavirus A
True Negative	387	380	387	382	389	391	393	389	393	391	393	392	386	380	392
False Positive	0	2	1	0	0	0	0	0	0	0	0	0	2	1	0
False Negative	2	0	0	0	0	1	0	0	0	0	0	0	0	2	0
True Positive	4	11	5	11	4	1	0	4	0	2	0	1	5	9	0
% Negative Agreement	100% (387/387)	99.5% (380/382)	99.7% (387/388)	100% (382/382)	100% (389/389)	100% (391/391)	100% (393/393)	100% (389/389)	100% (393/393)	100% (391/391)	100% (393/393)	100% (392/392)	99.5% (386/388)	100% (380/381)	100% (392/392)
% Positive Agreement	66.7% (4/6)	100% (11/11)	100% (5/5)	100% (11/11)	100% (4/4)	50% (1/2)	N/A	100% (4/4)	N/A	100% (2/2)	N/A	100% (1/1)	100% (5/5)	81.8% (9/11)	N/A

Cary-Blair Stool

comparison to reference methods	*Campylobacter* spp	*Clostridium difficile*	EAEC	EPEC	ETEC	*Salmonella* spp	*Shigella* spp/ EIEC	STEC, Non-O157	STEC, O157	*Vibrio parahaemolyticus*	*Vibrio* spp	*Yersinia enterocolitica*	*Cryptosporidium spp.*	*Entamoeba histolytica*	*Giardia lamblia*	Adenovirus 40/41	Norovirus G1/G2	Rotavirus A
True Negative	96	85	92	72	99	100	100	100	100	100	99	100	95	100	98	98	97	100
False Positive	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
False Negative	2	1	0	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0
True Positive	2	14	7	25	1	0	0	0	0	0	0	0	5	0	2	2	3	0
% Negative Agreement	100% (96/96)	100% (85/85)	98.9% (92/93)	100% (72/72)	100% (99/99)	100% (100/100)	100% (100/100)	100% (100/100)	100% (100/100)	100% (100/100)	100% (99/99)	100% (100/100)	100% (95/95)	100% (100/100)	100% (98/98)	100% (98/98)	100% (97/97)	100% (100/100)
% Positive Agreement	50% (2/4)	93.3% (14/15)	100% (7/7)	89.3% (25/28)	100% (1/1)	N/A	N/A	N/A	N/A	N/A	0% (0/1)	N/A	100% (5/5)	N/A	100% (2/2)	100% (2/2)	100% (3/3)	N/A

- Overall agreement was 96.7% for unpreserved stool compared to Luminex GPP and 93.0% for Cary-Blair specimens compared to BioFire GI panel as reference methods.
- 5 unpreserved specimens were invalid for lack of RNA IC during initial screening (1.3%). After reflex testing all 5 were valid.
- All Cary-Blair specimens gave valid results in this study.

Cross Reactivity Study

Table 3. Microorganisms tested for cross reactivity.

Bacteria				Viruses
Aeromonas hydrophila	*Citrobacter freundii*	*Klebsiella pneumoniae*	*Streptococcus pyogenes*	Adenovirus Type 3
Acinetobacter lwoffii	*C. difficile (non-toxigenic)*	*Lactobacillus acidophilus*	*Streptococcus suis*	Adenovirus Type 4
Alcaligenes faecalis	*Clostridium perfringens*	*Lactococcus lactis*	*Veillonella parvula*	Adenovirus Type 7a
Bacillus cereus	*Clostridium septicum*	*Leminorella grimontii*	[a]*Vibrio mimicus*	Adenovirus Type 8
Bifidobacterium breve	*Clostridium sordellii*	*Morganella morganii*	[a]*Vibrio alginolyticus*	Adenovirus Type 14
Campylobacter fetus	*Clostridium tetani*	*Plesiomonas shigelloides*	*Vibrio fluvialis*	Adenovirus Type 37
Campylobacter lari	*Enterobacter cloacae*	*Porphyromonas asaccharolytica*	[b]*Yersinia bercovieri*	Coronavirus NL63
Campylobacter upsaliensis	*Enterococcus faecalis*	*Proteus vulgaris*	[b]*Yersinia frederiksenii*	Cytomegalovirus (CMV)
Candida albicans	*E. coli (non- pathogenic)*	*Providencia alcalifaciens*	[b]*Yersinia mollaretii*	Enterovirus 68
Chlamydia trachomatis	*Escherichia hermannii*	*Pseudomonas aeruginosa*	[b]*Yersinia pseudotuberculosis*	**Parasites**
Citrobacter freundii	*Gemella morbillorum*	*Serratia liquefaciens*	*Yersinia rohdei*	*Cryptosporidium muris*
	Klebsiella oxytoca	*Staphylococcus aureus*		*Giardia muris*

No cross reactivity was observed with bacteria (≥10^6 CFU/mL), viruses or parasites (≥10^5 Units/mL)
[a,b] Detected for [a]Vibrio spp. or [b]Yersinia spp. respectively at high titer.

Conclusions

Using the BioCode® MDx 3000 system, the BioCode® 18-Plex GI Pathogen Panel specifically detects bacteria/toxins, viruses and parasites known to cause gastroenteritis or colitis. Combined, the automated system and molecular panel allows users to perform highly multiplexed molecular detection in a high-throughput, automated format with a simple workflow and minimal hands-on time.

- LoD of the BioCode® GI Pathogen Panel was comparable to current commercially available assays (Table 2)
- No cross reactivity was observed with the organisms tested (Table 3)
- Inclusivity was tested with several relevant pathogens for each target organism which were detected between 3 – 10 fold LoD
- Clinical performance with 398 unpreserved stool and 100 stool in Cary-Blair transport medium showed and overall agreement of 96.7% and 93.0% respectively (Table 4)0

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The BioCode® 18-Plex GI Pathogen Panel and BioCode® MDx 3000 are currently in development.