REPRODUCIBILITY AND FRONT-END EXTRACTION COMPATIBILITY FOR AN 18-PLEX GASTROINTESTINAL PATHOGEN PANEL WITH AN AUTOMATED, HIGH-THROUGHPUT SYSTEM

Jakob Kirchner, Colleen Knoth, Melissa Henrie, Taihra Ul-Hasan, Derrek Mantzke, Anh Pham, Jesse Granados, Kassturi Jeevaprakash, Michael Aye*

Applied BioCode, Santa Fe Springs, CA 90670, USA

Abstract

Background

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 multiplex 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 that provide high specimen throughput for clinical labs.

To address this need, Applied BioCode has developed the BioCode® MDx 3000, an automated, high-throughput molecular diagnostic assay system in a 96-well format. The BioCode® GI Pathogen Panel is an 18-plex molecular assay for detection of gastrointestinal pathogens which include bacteria (*Campylobacter*, *C. difficile* toxin A/B, *Salmonella*, *Shigella*/enteroinvasive *E. coli*, enteroaggregative *E. coli*, enteropathogenic *E. coli*, enterotoxigenic *E. coli*, shiga toxin-producing *E. coli*, *E. coli* O157, *Vibrio*, *Yersinia enterocolitica*), viruses (norovirus group I/II, adenovirus F, rotavirus A), and parasites (*Cryptosporidium*, *Entamoeba histolytica*, *Giardia lamblia*).

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.

Barcoded Magnetic Bead Technology

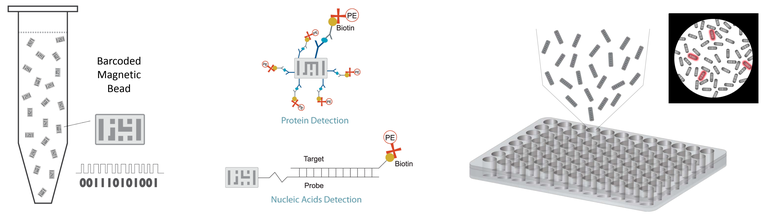

Figure 1. Barcoded Magnetic Beads (BMBs) are coupled to proteins or nucleic acids probes and used for target capture in microtiter plates. In the BioCode® GI Pathogen Panel, biotinylated PCR product is captured by target-specific nucleic acid probes coupled to BMBs then labeled by SA-PE for detection.

BioCode® MDx 3000

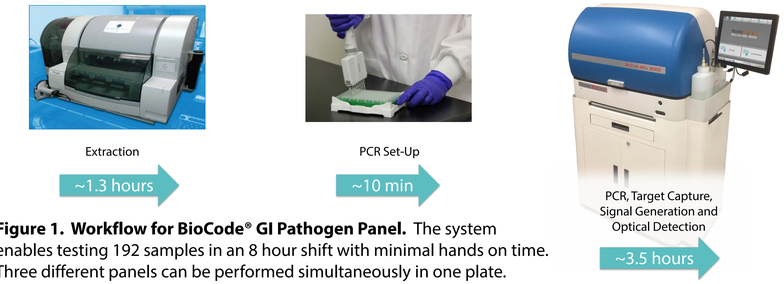

Figure 1. Workflow for BioCode® GI Pathogen Panel. The system enables testing 192 samples in an 8 hour shift with minimal hands on time. Three different panels can be performed simultaneously in one plate.

BioCode® Gastrointestinal Pathogen Panel

Table 1. Organisms and toxins detected by the BioCode® 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.
- ♦ 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

Reproducibility

Table 2. Reproducibility of BioCode® GI Pathogen Panel on BioCode® MDx 3000. Detection of representative targets for the BioCode® GI Pathogen Panel was highly reproducible across 12 runs (2 instruments, multiple operators over 3 days). The positive agreement was 100% for all targets. The CVs for the study ranged from a low of 6% for *G. lamblia* at 3X LoD to a high of 44% for STEC *stx2* at 1.5 X LoD.

Organism	Concentration	Agreement with expected result	Mean MFI	%CV
Salmonella enterica	3 X LoD (4.14 x10^3 CFU/mL)	(72/72) 100%	9960	21%
	1.5X LoD (2.07 x 10^3 CFU/mL)	(72/72) 100%	7560	17%
Clostridium difficile (*tcd B*)	3 X LoD (9.9 x 10^3 CFU/mL)	(72/72) 100%	27322	8%
	1.5X LoD (4.95 x 10^3 CFU/mL)	(72/72) 100%	23158	9%
Giardia lamblia	3 X LoD (1.35 x 10^3 cyst/mL)	(72/72) 100%	38297	8%
	1.5X LoD (6.75 x 10^2 cysts/mL)	(72/72) 100%	41062	6%
Adenovirus 40	3 X LoD (1.2 U/mL)	(72/72) 100%	15771	16%
	1.5X LoD (0.6 U/mL)	(72/72) 100%	19453	12%
Shigella sonnei	3 X LoD (1.04 x 10^4 CFU/mL)	(72/72) 100%	25934	8%
	1.5X LoD (5.22 x 10^3 CFU/mL)	(72/72) 100%	21924	12%
ETEC	3 X LoD (2.7 x 10^4 CFU/mL)	(72/72) 100%	31803	12%
	1.5X LoD (1.4 e4 cfu/mL)	(72/72) 100%	22959	20%
V. paraheamlyticus	3 X LoD (7.50 x 10^1 CFU/mL)	(72/72) 100%	13987	22%
	1.5X LoD (3.75 x 10^1 CFU/mL)	(72/72) 100%	17681	18%
STEC	3 X LoD (1.5 x 10^4 CFU/mL)	(72/72) 100%	25047	25%
	1.5 X LoD (7.5 x 10^3 CFU/mL)	(72/72) 100%	16655	44%
Campylobacter jejuni	3 X LoD (8.4 x 10^3 CFU/mL)	(72/72) 100%	16526	14%
	1.5X LoD (4.20 x 10^3 CFU/mL)	(72/72) 100%	10841	21%
Cryptosporidium parvum	3 X LoD (1.88 x 10^4 cysts/mL)	(72/72) 100%	16012	16%
	1.5X LoD (9.38 x 10^3 cysts/mL)	(72/72) 100%	19638	17%
Rotavirus A	3 X LoD (1.86 x 10^3 U/mL)	(72/72) 100%	26169	18%
	1.5X LoD (9.3 x 10^2 U/mL)	(72/72) 100%	24044	22%
Negative	N/A	(71/72) 98.6%	33942	10%

Interference

Table 3. Results of Inhibition Study. No inhibition was observed with 16 substances and 8 microbes that may be present in stool or Cary-Blair samples.

Substances		Microbes
Mucin (3 mg/mL)	Ampicillin (152 µmol/L)	*Bacteroides fragilis*
Cholesterol (5% w/v)	Vancomycin (12.5 mg/mL)	*Blastocystis hominis*
Pepto-Bismol (5% v/v)	Metronidazole (14 mg/mL)	*Candida albicans*
Imodium (5% v/v)	Nystatin (1000 U/mL)	*C. difficile* non-toxigenic
Laxative (5% v/v)	Mineral Oil (50% w/v)	*Enterococcus faecalis*
Antacid (Tums; 5% w/v)	Hydrocortisone cream (50% w/v)	*E. coli* (non-pathogenic)
Maalox (5% w/v)	Neosporin (50% w/v)	*Pseudomonas aeruginosa*
Naproxen Sodium (14 mg/mL)	Bleach (10%)	*Saccharomyces boulardii*

Organisms tested at >10^6 CFU/mL for bacteria and >10^5 units/mL for parasite or fungus.

NucliSENS easyMAG® vs. MagNA Pure 96

Table 4. Front-end nucleic acids extraction was evaluated with NucliSENS easyMAG® (bioMérieux) and MagNA Pure 96 (Roche). Both automated extraction systems gave nucleic acids with good purity and yield from contrived and limited clinical specimens in unpreserved stool and Cary-Blair medium (47 total samples).

Target Pathogens	Positive Results reported for NucliSENS easyMAG®	Positive Results reported for MagNA Pure 96
Clostridium difficile	3	3
***Campylobacter* spp.**	4	4
***Salmonella* spp.**	1	2
***Shigella* spp./ EIEC**	0	0
EAEC	2	2
***E. coli* O157**	1	1
EPEC	7	7
ETEC	1	3
STEC	0	0
Vibrio parahaemolyticus	0	0
***Vibrio* spp.**	0	0
Yersinia enterocolitica	2	2
Giardia lamblia	1	1
Cryptosporidium spp.	3	3
Entameoba histolytica	0	0
Norovirus (GI & GII)	2	2
Adenovirus F (40/41)	0	0
Rotavirus A	0	0

Overall Agreement		NucliSENS EasyMAG® Pos	Neg	Total
MagNA Pure 96	Pos	15	3	18
	Neg	0	29	29
	Total	15	32	47

Positive Agreement	100.0%
Negative Agreement	90.6%
Overall Agreement	93.6%

One sample was invalid with easyMAG® extraction, and no samples were invalid with MP96.

Carryover Contamination Study

Table 5. Results of carryover study using BioCode® MDx 3000. No carryover contamination was observed within run or between runs across 3 runs with a high positive *Salmonella enterica* sample (1.0 x 10^6 CFU/mL) assayed in a "checkerboard" pattern. MFI values are shown in 96-well format for a representative run. Within run CVs for positive wells were 17%, 21% and 22%.

	1	2	3	4	5	6	7	8	9	10	11	12
A	9345	8	11787	7	10146	4	10554	7	8599	17	9343	1
B	1	10946	9	9005	66	11215	34	11599	4	7792	23	10531
C	14843	4	13137	6	12796	4	14724	2	10891	4	13585	39
D	5	9432	7	9918	4	11509	3	10297	28	10844	144	11276
E	12261	8	12747	52	8133	3	12206	5	12933	1	10807	7
F	7	13599	7	10781	4	10694	23	11448	7	11065	5	11715
G	11976	5	12127	4	7683	8	8746	8	10949	6	13617	7
H	2	11927	6	9302	28	13442	5	13530	8	12531	25	6781

Conclusions

- ❖ Using the BioCode® MDx 3000 system, the BioCode® GI Pathogen Panel specifically and reproducibly detects bacteria/toxins, viruses and parasites.
- ❖ The BioCode® GI Pathogen Panel is compatible with commonly used automated extraction systems.
- ❖ No inhibition was observed with the substances or microbes tested.
- ❖ No carry-over contamination was observed, and signals were uniform within an assay run with "checkerboard pattern".
- ❖ The BioCode® MDx 3000 system combined with the BioCode® GI Pathogen Panel allows users to perform highly multiplexed molecular detection in a high-throughput, automated format with a simple workflow and minimal hands-on time.

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*Corresponding author: maye@apbiocode.com

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