BETA SITE EVALUATION OF AN 18-PLEX GASTROINTESTINAL PATHOGEN PANEL WITH AN AUTOMATED, HIGH THROUGHPUT SYSTEM

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Introduction

Introduction: 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.[1] 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 for clinical labs with medium to high-throughput automation.

To address this need, Applied BioCode® has developed an automated high-throughput molecular diagnostic assay system in a 96-well format*. The BioCode® Gastrointestinal Pathogen Panel (GPP) is an 18-plex molecular assay for detection of gastrointestinal pathogens (Table 1).

Table 1. Organisms and toxins detected by the BioCode® GPP

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

Method

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, 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. For Reproducibility, pooled custom NATrol molecular controls (ZeptoMetix) were run in quadruplicate over 5 days.

BioCode® MDx 3000

Extraction (EasyMAG)

PCR Set-Up

PCR, Target Capture, Signal Generation and Optical Detection

	Extraction (EasyMAG)	PCR Set-Up	PCR, Target Capture, Signal Generation and Optical Detection
TAT	~75 min	~10 min	~3.5 hours
HOT	~20 min	~10 min	~10 min

Figure 1. Workflow for BioCode® GI Pathogen Panel. 192 samples can be processed in an 8-hour shift with minimal hands-on time.

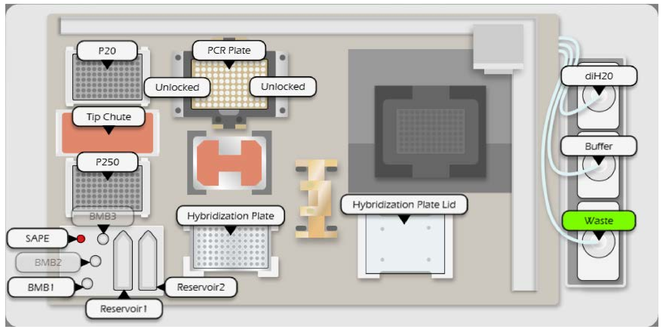

Figure 2. Schematic for BioCode® MDx 3000 deck layout. This system incorporates thermal-cycling, BMB-Capture and detection into one automated system. MDx 3000 is designed to run 3 different assays simultaneously in one plate.

Reproducibility

Table 2. Reproducibility of BioCode® GPP on BioCode® MDx 3000. Reproducibility was evaluated with contrived mixed analytes prepared with NATrol controls from Zeptometrix. Detection was highly reproducible across 5 days: 99.2% (119/120) overall agreement with inter-assay CVs between 5 - 25%.

Diarrheagenic *E.coli*							
		Day 1	Day 2	Day 3	Day 4	Day 5	Between Days
EAEC	MFI	26246	28542	26527	28645	21881	26368
	%CV	3%	4%	5%	4%	6%	10%
EPEC	MFI	28692	30532	30224	32918	26806	29834
	%CV	5%	4%	2%	4%	2%	8%
ETEC (LT)	MFI	5380	7904	5457	7449.3	4938	6225
	%CV	8%	19%	15%	11%	12%	24%
ETEC (ST-a)	MFI	40198	42641	38720	43861	34491	39982
	%CV	3%	2%	1%	2%	6%	9%
ETEC (ST-b)	MFI	23876	30139	25870	28176	20487	25710
	%CV	10%	3%	7%	8%	13%	15%
O157	MFI	29546	29776	29303	30614	22328	28313
	%CV	4%	4%	3%	1%	3%	11%
STEC (stx1)	MFI	7728	8865.3	8998.5	10812	4896	8260
	%CV	9%	5%	12%	3%	11%	25%
STEC (stx2)	MFI	35188	37363	37292	38643	28677	35433
	%CV	4%	5%	3%	5%	5%	11%

Bacteria							
		Day 1	Day 2	Day 3	Day 4	Day 5	Between Days
Campy	MFI	37462	36308	37909	36456	33557	36338
	%CV	1%	2%	2%	2%	3%	5%
C.diff (tcdA)	MFI	11776	14521	14931	16224	11085	13707
	%CV	19%	8%	5%	8%	16%	18%
C. diff (tcdB)	MFI	13417	15297	14929	17694	11679	14603
	%CV	10%	4%	7%	1%	13%	16%
Salmonella	MFI	19166	19800	19401	20588	16399	19071
	%CV	1%	3%	3%	4%	3%	8%
Shigella	MFI	17460	17010	15655	20849	14690	17133
	%CV	9%	27%	7%	3%	11%	18%
V.para	MFI	27404	28503	33279	24705	29440	28666
	%CV	4%	8%	2%	12%	5%	12%
Vibrio spp.	MFI	15008	15408	14494	15442	12612	14593
	%CV	3%	3%	2%	3%	2%	8%
Y.enterocolitica	MFI	27318	30909	25869	31718	21048	27372
	%CV	5%	3%	6%	2%	4%	15%

Parasites							
		Day 1	Day 2	Day 3	Day 4	Day 5	Between Days
Crypto	MFI	25984	28992	26636	28865	22568	26609
	%CV	4%	6%	4%	6%	5%	10%
E.his	MFI	5400	5526	5428.3	6114	4126	5319
	%CV	10%	6%	4%	3%	8%	14%
G.lam	MFI	30980	30784	31541	33911	26500	30743
	%CV	4%	3%	3%	8%	6%	9%
Viruses							
		Day 1	Day 2	Day 3	Day 4	Day 5	Between Days
AdenoF	MFI	21232	20972	23652	22257	16302	20883
	%CV	11%	11%	12%	14%	16%	17%
NoVG1	MFI	18355	14244	17800	19390	12125	16383
	%CV	4%	3%	3%	2%	16%	18%
NoVG2	MFI	15477	15423	14946	16600	11585	14806
	%CV	4%	1%	4%	2%	3%	12%
Rota	MFI	36913	45359	46688	47929	32476	41873
	%CV	25%	2%	5%	2%	40%	21%
RNA Internal Control							
		Day 1	Day 2	Day 3	Day 4	Day 5	Between Days
RNA-IC	MFI	39503	40179	39650	40453	35519	39216
	%CV	5%	4%	4%	4%	4%	6%
	Detected	24/24	24/24	24/24	24/24	24/24	120/120

Clinical Performance / Method Comparison

Table 3. Clinical performance. 300 unpreserved stool samples were compared to composite results for Luminex xTAG GPP and Sequencing. Overall positive agreement was 96.1% (98/102); negative agreement was 93.4% (183/196). Invalid rate was <1% (2/300).

Target Pathogens	Positives without or with *discordant testing			Resolved agreement (%)	
	BioCode® GPP	Luminex xTAG GPP	Verified Luminex positives*	Negative	Positive
Campylobacter spp.	24	23	22*	99.3	100
Clostridium difficile Toxin A/B	20	17	17	98.9	100
EAEC	10	N/A	N/A	N/A	N/A
EPEC	9	N/A	N/A	N/A	N/A
ETEC	8	4	4	98.6	100
Salmonella spp.	8	15	8*	100	100
Shigella spp./ EIEC	7	7	7	100	100
STEC	11	12	11*	100	100
E. coli O157	1	1	1	100	100
Vibrio parahaemolyticus	1	N/A	N/A	N/A	N/A
Vibrio spp. (not *parahaemolyticus*)	0	N/A	N/A	N/A	N/A
Yersinia enterocolitica	1	N/A	N/A	N/A	N/A
Cryptosporidium spp.	4	4	4	100	100
Entameoba histolytica	1	6	1*	100	100
Giardia lamblia	5	26	5*	100	100
Adenovirus (Type 40 & 41)	9	5	5	98.6	100
Norovirus GI/GII	27	26	26	99.6	100
Rotavirus A	9	8	8	99.7	100

Table 4. Clinical performance. 100 Cary-Blair samples were compared to composite results for BioFire Filmarray GI Panel and Sequencing. Overall positive agreement was 94.1% (64/68), and negative agreement was 87.5% (28/32). There were no invalid samples.

Target Pathogens	Positive Results without or with *Discordant Testing			Resolved agreement (%)	
	BioCode® GPP	BioFire Filmarray GI Panel	Verified BioFire positives*	Negative	Positive
Campylobacter spp.	8	9	8*	100	100
Clostridium difficile Toxin A/B	13	15	15	100	87
EAEC	7	7	7	100	100
EPEC	11	14	12*	100	92
ETEC	9	9	9	100	100
Salmonella spp.	13	15	14*	100	93
Shigella spp./ EIEC	10	11	10*	100	100
STEC	4	4	4	100	100
E. coli O157	2	N/A	N/A	N/A	N/A
Vibrio parahaemolyticus	0	0	0	100	N/A
Vibrio spp.	0	0	0	100	N/A
Yersinia enterocolitica	1	1	1	100	100
Cryptosporidium spp.	3	3	3	100	100
Entameoba histolytica	0	0	0	100	N/A
Giardia lamblia	2	2	2	100	100
Adenovirus (Type 40 & 41)	3	2	2	99	100
Norovirus (GI & GII)	8	8	8	99	88
Rotavirus A	8	5	5	97	100

*Discordant samples tested and resolved by sequencing or repeat testing

Conclusions

- The BioCode® Gastrointestinal Pathogen Panel, performed with high clinical accuracy and precision for patient samples and controls.
- The invalid rate was <1%, resulting in minimal repeats or unresolved samples.
- Combined with the automated system, the 18-plex molecular panel was intuitive and easy to use.
- The software guided set up and clear results reports allowed quick implementation with minimal training time.

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).

The BioCode® GPP and BioCode® MDx 3000 have been submitted to the FDA and are currently under review.

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