Validation of an Alternative Extraction Method for FDA cleared 17-plex BioCode® Gastrointestinal Pathogen Panel

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Abstract

Introduction

Gastroenteritis is a leading cause of death worldwide across all age groups. It is estimated that 1.31 million people died from diarrheal disease in 2015. 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 high throughput.

To address this need, Applied BioCode has obtained FDA clearance for the BioCode® Gastrointestinal Pathogen Panel (GPP), which is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of 17 targets including bacteria (*Campylobacter*, *C. difficile* toxin A/B, *Salmonella*, Shigella/enteroinvasive *E. coli*, enteroaggregative *E. coli*, enterotoxigenic *E. coli*, Shiga toxin-producing *E. coli*, *E. coli* O157, Vibrio, *Yersinia enterocolitica*), viruses (norovirus GI/GII, adenovirus F, rotavirus A), and parasites (Cryptosporidium, *E. histolytica*, *G. lamblia*). The BioCode® GPP assay was cleared by FDA with the NucliSENS® easyMAG® (bioMérieux) and the BioCode® MDx-3000. This study is to validate the MagNA Pure 96 (MP96, Roche) as an alternative extraction system for the BioCode® GPP.

CDC. Defeating Diarrhea: CDC and Partners Tackle Causes and Consequences in Kenya and Beyond. http://www.cdc.gov/globalhealth/stories/diarrhea_kenya.html. January 2, 2015.

Methods

The MP96 was validated as an alternative extraction method by comparing the Limit of Detection (LoD) of this system with the LoD of the easyMAG® using 21 quantified bacteria, virus, or parasite and positive clinical specimens (for Norovirus GI/GII) tested in unpreserved stool (UNPs) and Cary-Blair stool (CBs). In addition, the comparison study of spiking single target versus multiple targets (for representative targets including *S. enterica*, *C. difficile*, *G. lamblia*, Rotavirus) in UNPs or CBs was assessed with MP96 and easyMAG®. Reproducibility of MP96 was performed by testing 7 Reproducibility Panels (RPs) assayed on 3 different MDx-3000, 2 runs per operator per day over 5 days, by 3 operators. Checkerboard test with high positive *Shigella sonnei* samples in every other well was conducted with MP96 to assess carry-over contamination.

Following extractions, the extracts were tested with the BioCode® MDx-3000 platform, which integrates and automates PCR, post-PCR sample handling and detection steps in a 96-well format. DNA and RNA targets were amplified by one-step RT-PCR. PCR products were captured by target-specific probes coupled to Barcoded Magnetic Beads (BMBs), and the presence of target sequence(s) was detected by a fluorescent conjugate. Qualitative results were based on median fluorescent index (MFI) compared to target-specific threshold values.

Conclusions

- The results of checkerboard test showed no carry-over contamination with GPP tested on MP96 and BioCode® MDx-3000 (Figure 2).
- The results of spiking single versus multiple organisms in unpreserved stool or CBs with both systems showed equivalent LoD (Table 1).
- Reproducibility performed by 3 operators was > 99% (Table 2).
- LoDs of targets were the same for both systems except the EIEC and Adenovirus 40/41 targets (unpreserved) and *Campylobacter spp.*, EIEC, O157, and Adenovirus 40 (Cary-Blair) for which LoDs for MP96 were 2-fold lower (Table 3a).
- For Norovirus, LoDs of unpreserved with easyMAG® was 2-fold and 8.3-fold lower than MP96 for Norovirus GI and GII, respectively. For CBs, LoD with easyMAG® was less than 2-fold lower than MP96 for Norovirus GI/GII (Table 3b).

The results of this validation demonstrated that BioCode® GPP produced equivalent results with MP96 or easyMAG®. This study supports using MP96, upon FDA clearance, as an alternative extraction method for the BioCode® GPP.

BioCode® GPP Workflow

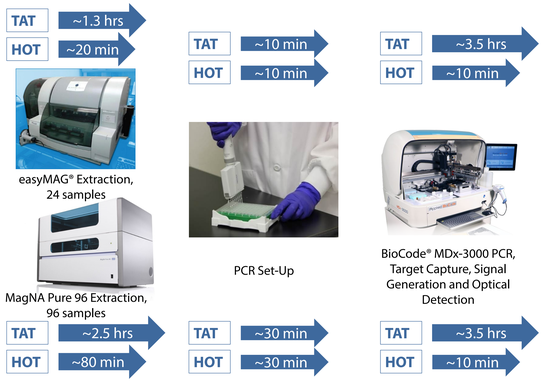

Figure 1. Workflow for BioCode® GPP Assay. 192 samples using easyMAG® extraction or 288 samples using MagNA Pure 96 extraction in an 8 hour shift with minimal hands on time for MDx-3000 system.

Carry-over Contamination Study

Shigella sonnei MFI												
	1	2	3	4	5	6	7	8	9	10	11	12
A	9217	5	8797	8	7782	2	8530	5	8200	4	8671	7
B	3	7753	6	7216	9	7185	4	6936	6	8874	2	9260
C	7297	7	7959	2	6418	6	8958	1	6868	1	11058	4
D	2	7327	3	7319	3	7087	1	6717	8	7333	4	7427
E	8584	2	7422	3	7173	2	8022	3	8734	3	7730	6
F	2	7359	2	7424	4	7424	3	6885	1	7722	1	7971
G	8633	5	7607	5	8619	7	7028	5	8068	8	8513	2
H	8	7282	1	7133	4	7559	7	7899	1	7627	8	8895

Figure 2. A Representative Plate of Carry-Over Contamination. Five 96-well plates tested with alternating negative samples and high positive samples of *Shigella sonnei* (ATCC 29930) at 5.42 x 10^6 CFU/mL. No Carry-over contamination was observed using MagNA Pure 96 as the extraction system.

Spiking Single vs. Multiple Organisms

Table 1. Samples spiked with single or multiple organism. Single and multi-spiked samples both achieved ≥95% detection of 20 replicates (≥19 out of 20) at same concentrations for the challenge organisms (concentrations indicated in the tables below).

Strain/ Source	Single Spike/ Multiple Spike	Unpreserved Stool				Cary-Blair Stool			
		easyMAG®		MagNA Pure 96		easyMAG®		MagNA Pure 96	
		LoD	n/N	LoD	n/N	LoD	n/N	LoD	n/N
Clostridium difficile (toxinotype 0) ATCC 9689	Single Spike	9.50 x 10^1 CFU/mL	20/20	9.50 x 10^1 CFU/mL	20/20	9.50 x 10^1 CFU/mL	20/20	9.50 x 10^1 CFU/mL	20/20
	Multi Spike		20/20		20/20		19/20		20/20
Salmonella enterica spp. enterica ATCC 14028	Single Spike	1.10 x 10^3 CFU/mL	20/20	1.10 x 10^3 CFU/mL	20/20	1.10 x 10^3 CFU/mL	20/20	1.10 x 10^3 CFU/mL	20/20
	Multi Spike		20/20		20/20		20/20		20/20
Giardia intestinalis Waterborne P101	Single Spike	9.00 x 10^2 cysts/mL	20/20	9.00 x 10^2 cysts/mL	20/20	9.00 x 10^2 cysts/mL	20/20	9.00 x 10^2 cysts/mL	20/20
	Multi Spike		20/20		20/20		20/20		20/20
Rotavirus A ATCC VR-2018	Single Spike	2.50 x 10^3 $TCID_{50}$/mL	20/20	1.25 x 10^3 $TCID_{50}$/mL	20/20	1.25 x 10^3 $TCID_{50}$/mL	20/20	1.25 x 10^3 $TCID_{50}$/mL	20/20
	Multi Spike		20/20		20/20		20/20		20/20

Reproducibility

Table 2. Reproducibility. Samples extracted with the MagNA Pure 96 and assayed on 3 BioCode® MDx-3000 instruments by 3 operators, 2 runs per day per operator for 5 days (total of 30 runs). The reproducibility panel consisted of 7 contrived samples: each was extracted in triplicate and assayed in singlet. The samples consisted of combinations of 12 representative targets at 1.5x LoD (Low Positive) and 3x LoD (Medium Positive). The overall reproducibility was > 99%.

Organism Tested	Concentration Tested	Expected Results	Agreement with Expected Result			
			Instrument 1 Operator 1	Instrument 2 Operator 2	Instrument 3 Operator 3	All Instruments/ Operators
Campylobacter jejuni spp. jejuni ATCC 33292	Medium Positive	Detected	30/30	30/30	30/30	90/90
	Low Positive	Detected	30/30	30/30	30/30	90/90
	None	Not Detected	150/150	150/150	150/150	450/450
Clostridium difficile (toxinotype III; Nap1) Zeptometrix 0801619cf	Medium Positive	Detected	30/30	30/30	30/30	90/90
	Low Positive	Detected	30/30	30/30	30/30	90/90
	None	Not Detected	150/150	150/150	150/150	450/450
Enteroaggregative *E. coli* O92:H33 (EAEC) STEC TW04440	Medium Positive	Detected	30/30	30/30	30/30	90/90
	Low Positive	Detected	30/30	30/30	30/30	90/90
	None	Not Detected	150/150	150/150	150/150	450/450
Enterotoxigenic *E. coli* O78:H11 H10407 (ETEC) ATCC 35401	Medium Positive	Detected	30/30	30/30	30/30	90/90
	Low Positive	Detected	30/30	30/30	30/30	90/90
	None	Not Detected	150/150	150/150	150/150	450/450
Salmonella enterica ssp. enterica ATCC 14028	Medium Positive	Detected	30/30	30/30	30/30	90/90
	Low Positive	Detected	30/30	30/30	30/30	90/90
	None	Not Detected	150/150	150/150	150/150	450/450
Shiga-like toxin producing *E. coli* (STEC) ATCC BAA-2217	Medium Positive	Detected	30/30	30/30	30/30	90/90
	Low Positive	Detected	30/30	30/30	30/30	90/90
	None	Not Detected	150/150	150/150	150/150	450/450
Shigella sonnei ATCC 29930	Medium Positive	Detected	30/30	30/30	30/30	90/90
	Low Positive	Detected	30/30	30/30	30/30	90/90
	None	Not Detected	150/150	150/150	150/150	450/450
Vibrio parahaemolyticus ATCC 17802	Medium Positive	Detected	30/30	30/30	30/30	90/90
	Low Positive	Detected	30/30	30/30	30/30	90/90
	None	Not Detected	150/150	150/150	150/150	450/450
Yersinia enterocolitica ATCC 23715	Medium Positive	Detected	30/30	30/30	30/30	90/90
	Low Positive	Detected	30/30	30/30	30/30	90/90
	None	Not Detected	150/150	150/150	150/150	450/450
Cryptosporidium parvum waterborne P102	Medium Positive	Detected	30/30	30/30	29/30 (97%)	89/90 (99%)
	Low Positive	Detected	30/30	30/30	29/30 (97%)	89/90 (99%)
	None	Not Detected	150/150	150/150	150/150	450/450
Giardia intestinalis (aka G. lamblia) waterborne P101	Medium Positive	Detected	30/30	30/30	30/30	90/90
	Low Positive	Detected	30/30	30/30	30/30	90/90
	None	Not Detected	150/150	150/150	150/150	450/450
Rotavirus ATCC VR-2018	Medium Positive	Detected	30/30	30/30	30/30	90/90
	Low Positive	Detected	30/30	30/30	30/30	90/90
	None	Not Detected	150/150	150/150	150/150	450/450

Limit of Detection

Table 3a. Limit of Detection. Comparison of the Limit of Detection (LoD) of the MagNA Pure 96 with the LoD of the easyMAG® using 21 quantified bacteria, virus, or parasites. The LoDs of targets were the same for both systems except EIEC and Adenovirus 40/41 targets (for Unpreserved Stool) and *Campylobacter spp.*, EIEC, O157, and Adenovirus 40 (for Cary-Blair) for which LoDs for MP96 were 2-fold lower.

Strain	Source	Unpreserved Stool		Cary-Blair Stool LoD	
		easyMAG® LoD	MP96 LoD	easyMAG® LoD	MP96 LoD
Campylobacter coli	ATCC 33559	2.81 x 10^1 CFU/mL	2.81 x 10^1 CFU/mL	5.61 x 10^1 CFU/mL	2.81 x 10^1 CFU/mL
Campylobacter jejuni spp. jejuni	ATCC 33292	3.50 x 10^2 CFU/mL	3.50 x 10^2 CFU/mL	3.50 x10^2 CFU/mL	7.00 x 10^2 CFU/mL
Clostridium difficile (toxinotype 0)	ATCC 9689	9.50 x 10^1 CFU/mL	9.50 x 10^1 CFU/mL	9.50 x 10^1 CFU/mL	9.50 x 10^1 CFU/mL
Clostridium difficile (toxinotype III; Nap1)	Zeptometrix 0801619cf	4.15 x 10^2 CFU/mL	4.15 x10^2 CFU/mL	4.15 x10^2 CFU/mL	4.15 x 10^2 CFU/mL
Enteroaggregative *E. coli* O92:H33 (EAEC)	STEC TW04440	7.00 x 10^2 CFU/mL	7.00 x10^2 CFU/mL	7.00 x10^2 CFU/mL	7.00 x 10^2 CFU/mL
Enteroinvasive *E. coli* O29:NM (EIEC)	ATCC 43892	3.60 x 10^2 CFU/mL	1.80 x10^2 CFU/mL	3.60 x10^2 CFU/mL	1.80 x 10^2 CFU/mL
Enterotoxigenic *E. coli* O78:H11 H10407 (ETEC)	ATCC 35401	2.80 x 10^2 CFU/mL	2.80 x 10^2 CFU/mL	2.80 x 10^2 CFU/mL	2.80 x 10^2 CFU/mL
Salmonella bongori	SGSC 4900	1.40 x 10^3 CFU/mL	1.40 x 10^3 CFU/mL	1.40 x 10^3 CFU/mL	1.40 x 10^3 CFU/mL
Salmonella enterica spp. enterica	ATCC 14028	1.10 x 10^3 CFU/mL	1.10 x 10^3 CFU/mL	1.10 x 10^3 CFU/mL	1.10 x 10^3 CFU/mL
Shiga-like toxin producing E. coli (STEC)	ATCC BAA-2217	1.25 x 10^3 CFU/mL	1.25 x 10^3 CFU/mL	1.25 x 10^3 CFU/mL	1.25 x 10^3 CFU/mL
E. coli O157	ATCC 700376	1.65 x 10^3 CFU/mL	1.65 x 10^3 CFU/mL	3.30 x 10^3 CFU/mL	1.65 x 10^3 CFU/mL
Shigella sonnei	ATCC 29930	2.20 x 10^2 CFU/mL	2.20 x 10^2 CFU/mL	2.20 x 10^2 CFU/mL	2.20 x 10^2 CFU/mL
Vibrio cholerae	ATCC 25870	2.45 x 10^2 CFU/mL	2.45 x 10^2 CFU/mL	2.45 x 10^2 CFU/mL	2.45 x 10^2 CFU/mL
Vibrio parahaemolyticus	ATCC 17802	6.50 x 10^0 CFU/mL	6.50 x 10^0 CFU/mL	6.50 x 10^0 CFU/mL	6.50 x 10^0 CFU/mL
Yersinia enterocolitica	ATCC 23715	7.50 x 10^2 CFU/mL	7.50 x 10^2 CFU/mL	7.50 x 10^2 CFU/mL	7.50 x 10^2 CFU/mL
Cryptosporidium parvum	waterborne P102C	3.10 x 10^3 oocysts/mL	3.10 x10^3 oocysts/mL	3.10 x 10^3 oocysts/mL	3.10 x 10^3 oocysts/mL
Entamoeba histolytica HB-301:NIH	BEI NR-178	1.55 x 10^{-1} cysts/mL	1.55 x 10^{-1} cysts/mL	1.55 x 10^{-1} cysts/mL	1.55 x 10^{-1} cysts/mL
Giardia intestinalis (aka G. lamblia)	waterborne P101	9.00 x 10^2 cysts/mL	9.00 x 10^2 cysts/mL	9.00 x 10^2 cysts/mL	9.00 x 10^2 cysts/mL
Adenovirus 40 (dugan)	Zeptometrix 0810084	2.00 x 10^{-1} $TCID_{50}$/mL	1.00 x 10^{-1} $TCID_{50}$/mL	2.00 x 10^{-1} $TCID_{50}$/mL	1.00 x 10^{-1} $TCID_{50}$/mL
Adenovirus 41 (TAK)	Zeptometrix 0810085	9.4 x 10^{-2} $TCID_{50}$/mL	4.70 x 10^{-2} $TCID_{50}$/mL	4.70 x 10^{-2} $TCID_{50}$/mL	4.70 x 10^{-2} $TCID_{50}$/mL
Rotavirus A	ATCC VR-2018	2.5 x 10^3 $TCID_{50}$/mL	1.25 x 10^3 $TCID_{50}$/mL	1.25 x 10^3 $TCID_{50}$/mL	1.25 x 10^3 $TCID_{50}$/mL

Table 3b. Limit of Detection. Comparison of the Limit of Detection (LoD) for MagNA Pure 96 with easyMAG® using serial dilutions of Norovirus GI- and GII-positive clinical samples. The LoDs in Unpreserved Stool with easyMAG® was 2-fold and 8.3-fold lower than MagNA Pure 96, for Norovirus GI and GII, respectively. For Cary-Blair Stool, the LoD with easyMAG® was less than 2-fold lower than MagNA Pure 96 for Norovirus GI/GII.

Target	Source	Unpreserved Stool		Cary-Blair Stool	
		easyMAG® Dilution	MP96 Dilution	easyMAG® Dilution	MP96 Dilution
Norovirus GI	Clinical Sample ID#60	1:10,000	1:5,000	1:50,000	1:80,000
Norovirus GII	Clinical Sample ID#54	1:250,000	1:30,000	1:100,000	1:80,000

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Validation data for MagNA Pure 96 as an alternative extraction for BioCode® GPP has been submitted to FDA and is currently under review.