Evaluation Of MagNA Pure 96 As Alternative Extraction Method for the BioCode® Gastrointestinal Pathogen Panel

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Abstract

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 [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 high throughput.

To address this need, Applied BioCode® has developed the BioCode® Gastrointestinal Pathogen Panel (GPP), which is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of 18 targets including 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 spp., *Yersinia enterocolitica*), viruses (norovirus I/II, adenovirus F, rotavirus A), and parasites (Cryptosporidium, *Entamoeba histolytica*, *Giardia lamblia*). The BioCode® GPP assay has been validated with the NucliSENS easyMag (bioMerieux) and the BioCode® MDx 3000. This study is to evaluate the MagNA Pure 96 (Roche) as an alternative extraction system for the BioCode® GPP.

References

1. 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 MagNA Pure 96 was evaluated as an alternative extraction method by comparing results between this system and the NucliSENS easyMag using pathogen pools (48 samples) prepared in prescreened negative unpreserved stools and Cary-Blair stools. Four Zeptometrix control pools were each tested in duplicates. In addition, the comparison study was also performed using 200 clinical samples and contrived samples. Checkerboard test with high positive specimens in every other well was conducted with the MagNA Pure 96 to assess carry-over contamination.

Following extractions, the extracts from both MagNA Pure 96 and the easyMag 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 based on median fluorescent index (MFI) threshold values were compared.

Conclusions

- For pathogen pools in Cary-Blair stool, except for *C. difficile* (5/6), 100% positive agreement was achieved for other targets. Overall positive agreement with the MagNA Pure 96 was 99%.
- For pathogen pools in unpreserved stool, except for *C. difficile* (5/6), 100% positive agreement was achieved with both MagNA Pure 96 and the easyMag systems.
- For commercial positive control pools, except for *Y. enterocolitica* (1/2), 100% positive agreement was obtained for other targets. Overall 97% and 100% positive agreement were obtained with the easyMag and the MagNA Pure 96, respectively.
- For clinical and contrived samples, 100% negative agreement was obtained for all targets except *Shigella spp.* (17/18) with the MagNA Pure 96. 100% negative agreement for all targets was achieved with the easyMag. 100% positive agreement was obtained with the MagNA Pure 96 and the easyMag.
- No clinical or contrived samples tested gave invalid results with the easyMag or the MagNA Pure 96.
- No Carry-over contamination was observed with the MagNA Pure 96.

The results of this evaluation indicated that BioCode GPP produced equivalent results with MagNA Pure 96 or NucliSENS easyMag, and MagNA Pure 96 may be utilized as an alternative extraction method for the BioCode® GPP.

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Barcoded Magnetic Bead (BMB) Technology

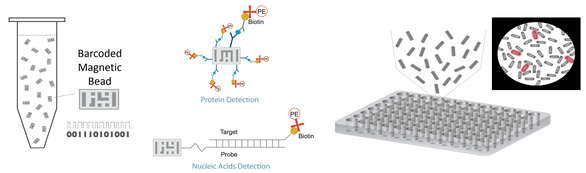


Figure 1. Barcoded Magnetic Beads (BMBs) are coupled to proteins or nucleic acids probes and used for target capture in microtiter plates. In the BioCode® GI Pathogen Panel, biotinylated PCR product is captured by target-specific nucleic acid probes coupled to BMBs then labeled by SA-PE for detection.

BioCode® GPP Workflow

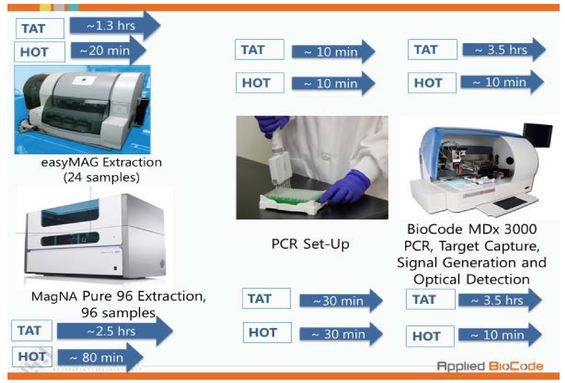


Figure 2. Workflow for BioCode® GPP Assay. 192 samples using easyMag extraction or 288 samples using MagNA Pure 96 extraction in an 8 hour shift with minimal hands on time.

Pathogen Pools

Table 1. Pathogen Pools prepared in prescreened negative Cary-Blair stool. Except the *C. difficile* (5/6 with MagNA Pure 96), 100% positive agreement was achieved for other targets. Overall positive agreement with the MagNA Pure 96 was 99%.

Pathogens	easyMag Cary-Blair Stool Positive Agreement		MagNA Pure 96 Cary-Blair Stool Positive Agreement	
	TP/(TP+FN)	%	TP/(TP+FN)	%
Escherichia coli (ETEC)	6/6	100%	6/6	100%
Escherichia coli (EPEC)	6/6	100%	6/6	100%
Escherichia coli (EAEC)	6/6	100%	6/6	100%
Clostridium difficile	6/6	100%	5/6	83%
Campylobacter jejuni	6/6	100%	6/6	100%
Shigella sonnei	6/6	100%	6/6	100%
Yersinia enterocolitica	6/6	100%	6/6	100%
Salmonella enterica	6/6	100%	6/6	100%
Escherichia coli O157/STEC	6/6	100%	6/6	100%
Vibrio parahaemolyticus	6/6	100%	6/6	100%
Adenovirus Type 41	6/6	100%	6/6	100%
Norovirus GI	6/6	100%	6/6	100%
Norovirus GII	6/6	100%	6/6	100%
Rotavirus A	6/6	100%	6/6	100%
Entamoeba histolytica	6/6	100%	6/6	100%
Cryptosporidium parvum	6/6	100%	6/6	100%
Giardia lamblia	6/6	100%	6/6	100%
Totals	122/122	100%	121/122	99%

Pathogen Pools (Continued)

Table 2. Pathogen Pools prepared in prescreened negative unpreserved stool. Except for *C. difficile* (5/6 with the MagNA Pure 96 and the easyMag), 100% positive agreement was achieved for all targets with both systems.

Pathogens	easyMag Unpreserved Stool Positive Agreement		MagNA Pure 96 Unpreserved Stool Positive Agreement	
	TP/(TP+FN)	%	TP/(TP+FN)	%
Escherichia coli (ETEC)	6/6	100%	6/6	100%
Escherichia coli (EPEC)	6/6	100%	6/6	100%
Escherichia coli (EAEC)	6/6	100%	6/6	100%
Clostridium difficile	5/6	83%	5/6	83%
Campylobacter jejuni	6/6	100%	6/6	100%
Shigella sonnei	6/6	100%	6/6	100%
Yersinia enterocolitica	6/6	100%	6/6	100%
Salmonella enterica	6/6	100%	6/6	100%
Escherichia coli O157/STEC	6/6	100%	6/6	100%
Vibrio parahaemolyticus	6/6	100%	6/6	100%
Adenovirus Type 41	6/6	100%	6/6	100%
Norovirus GI	6/6	100%	6/6	100%
Norovirus GII	6/6	100%	6/6	100%
Rotavirus A	6/6	100%	6/6	100%
Entamoeba histolytica	6/6	100%	6/6	100%
Cryptosporidium parvum	6/6	100%	6/6	100%
Giardia lamblia	6/6	100%	6/6	100%
Total	121/122	99%	121/122	99%

Commercial Positive Control Pools

Table 3. Commercial Positive Control Pools. Except for *Yersinia enterocolitica* (1/2 with easyMag), 100% positive agreement was obtained for other targets. Overall, 97% and 100% positive agreement were obtained with the easyMag and the MagNA Pure 96, respectively.

Pathogens	easyMag Positive Agreement		MagNA Pure 96 Positive Agreement	
	TP/(TP+FN)	%	TP/(TP+FN)	%
Escherichia coli (ETEC), ST+, LT+	2/2	100%	2/2	100%
Escherichia coli (EPEC)/O84:H28	2/2	100%	2/2	100%
Escherichia coli (EAEC)/92.0147	2/2	100%	2/2	100%
Clostridium difficile (NAP1)	2/2	100%	2/2	83%
Campylobacter jejuni Clinical isolate	2/2	100%	2/2	100%
Shigella sonnei /Z004	2/2	100%	2/2	100%
Yersinia enterocolitica Clinical Isolate	1/2	50%	2/2	100%
Salmonella typhimurium /Z005	2/2	100%	2/2	100%
Escherichia coli /O157/STEC /EDL933	2/2	100%	2/2	100%
Vibrio parahaemolyticus Clinical isolate	2/2	100%	2/2	100%
Adenovirus Type 41 (TAK)	2/2	100%	2/2	100%
Norovirus GI/ Recombinant	2/2	100%	2/2	100%
Norovirus GII/ Recombinant	2/2	100%	2/2	100%
Rotavirus A/ WA	2/2	100%	2/2	100%
Entamoeba histolytical DS4-868	2/2	100%	2/2	100%
Cryptosporidium parvuml Iowa	2/2	100%	2/2	100%
Giardia lamblia /Waterborne	2/2	100%	2/2	100%
Total	33/34	97%	34/34	100%

Clinical and Contrived Samples

Table 4. Negative Clinical Samples. Except for *Shigella spp.* (17/18), 100% negative agreement for other targets was obtained with the MagNA Pure 96. 100% negative agreement for all targets was achieved with the easyMag.

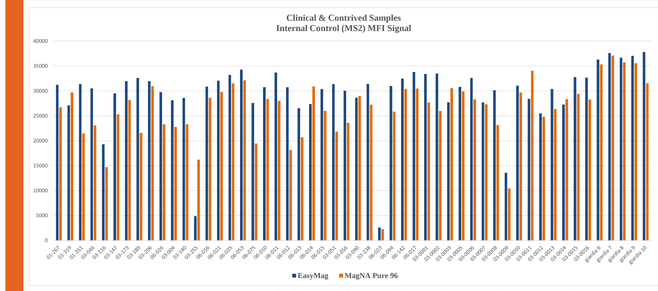
Pathogens	easyMag Negative Agreement		MagNA Pure 96 Negative Agreement	
	TN/(TN+FP)	%	TN/(TN+FP)	%
Shigella spp.	18/18	100%	17/18	94%
Other targets	18/18	100%	18/18	100%

Table 5. Positive Clinical and Contrived Samples. 100% positive agreement was obtained for all targets with MagNA Pure 96 and easyMag.

Pathogens	easyMag Positive Agreement		MagNA Pure 96 Positive Agreement	
	TP/(TP+FN)	%	TP/(TP+FN)	%
Escherichia coli (ETEC)- LT, ST1a, ST1b	9/9	100%	9/9	100%
Escherichia coli (EPEC)	28/28	100%	28/28	100%
Escherichia coli (EAEC)	11/11	100%	11/11	100%
Clostridium difficile	20/20	100%	20/20	100%
Campylobacter spp.	5/5	100%	5/5	100%
Shigella spp.	11/11	100%	11/11	100%
Yersinia enterocolitica	4/4	100%	4/4	100%
Salmonella spp.	11/11	100%	11/11	100%
Shiga-toxin producing *E. coli* (STEC) stx1/stx2	4/4	100%	4/4	100%
*Vibrio parahaemolyticus**	10/10	100%	10/10	100%
*Vibrio spp. (not V. parahaemolyticus)**	10/10	100%	10/10	100%
Adenovirus Type 40/41	6/6	100%	6/6	100%
Norovirus GI	4/4	100%	4/4	100%
Norovirus GII	16/16	100%	16/16	100%
Rotavirus A	8/8	100%	8/8	100%
*Entamoeba histolytica**	10/10	100%	10/10	100%
Cryptosporidium spp.	5/5	100%	5/5	100%
*Giardia intestinalis**	10/10	100%	10/10	100%
Totals	182/182	100%	182/182	100%

*** indicates contrived samples**

Internal Control (MS2) Detection



Figures 3: Internal Control (MS2) MFI Signal. No clinical and contrived samples tested with the easyMag and the MagNA Pure 96 had invalid results.

Carry-over Contamination study

MagNA Pure 96 CheckerBoard-ETEC target

	1	2	3	4	5	6	7	8	9	10	11	12
A	25894	8	31680	1	29272	1	27665	1	30988	4	32646	1
B	7	28271	6	31960	2	32641	8	28764	1	35007	22	35379
C	30418	7	32478	4	30433	6	32211	7	32222	22	34036	28
D	7	34763	12	33209	6	33041	3	31675	6	34394	1	33564
E	30932	4	32466	2	31399	12	30983	3	34136	2	32547	8
F	1	27234	22	28097	4	25281	1	33049	7	32140	35	29793
G	25747	2	19989	5	30190	7	29527	6	30734	5	32448	2
H	57	28625	14	29396	1	29737	33	29960	6	34668	30	29866

Figure 4. Carry-Over Contamination. No Carry-over contamination was observed with the MagNA Pure 96.

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