Clinical Evaluation of a Multiplex PCR Panel for Simultaneous Detection of Bacteria, Viruses, and Parasites in Stool Specimens

#1580

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INTRODUCTION/BACKGROUND

The FilmArray™ Gastrointestinal (GI) Panel (BioFire Diagnostics, LLC, Salt Lake City, UT) is a rapid (~1 hr), user-friendly, highly-multiplexed test for 22 infectious agents of gastroenteritis from stool specimens in Cary Blair enteric transport media. The aim of this study was to establish clinical sensitivity and specificity for each panel member.

THE FILMARRAY GI PANEL

Simultaneous detection of 22 targets:

Bacteria
- Campylobacter (jejuni, coli and upsaliensis)
- Clostridium difficile
- Plesiomonas shigelloides
- Salmonella
- Vibrio (parahaemolyticus, vulnificus and cholerae)
- Vibrio cholerae
- Yersinia enterocolitica

Diarrheagenic E. coli/Shigella
- Enterotoxigenic E. coli (ETEC) lt/st
- Enteropathogenic E. coli (EPEC)
- Shiga-like toxin-producing E. coli (STEC)stx1/stx2
- Shigella/Enteroinvasive E. coli (EIEC)
- Enteroaggregative E. coli (EAEC)
- E. coli O157

Viruses
- Adenovirus F40/41
- Human Astrovirus
- Norovirus GI/GII
- Rotavirus A
- Sapovirus (I, II, IV, and V)

Parasites
- Cryptosporidium
- Cyclospora cayetanensis
- Entamoeba histolytica
- Giardia lamblia

Methods

Specimens meeting the following inclusion criteria were selected for the study at four geographically distinct study sites: the specimen was received by the laboratory in Cary Blair enteric transport media, was submitted for clinician ordered investigation of GI pathogen analysis (e.g. stool culture, C. difficile testing, or ova and parasite exam), was of sufficient volume for testing, and could be tested (FilmArray and bacterial culture) within 4 days of specimen collection. Specimens were collected under IRB approved protocols at each site.

Reference/comparator methods to evaluate the performance of the FilmArray GI Panel included stool culture for bacteria (performed at study sites using the media listed in Table 2) or PCR with bi-directional sequencing for C. difficile, diarrheagenic E. coli/Shigella, parasites, and viruses (performed at BioFire, two assays per analyte).

Table 1. Stool Culture Media (or their equivalent) for Use in the Study

Media	Primary Organism(s) Isolated[a]
Blood agar	General growth of fecal organisms (Plesiomonas and Vibrio may be found here amongst fecal flora)
MacConkey agar	Gram-negative enteric bacilli (non-lactose fermenters)
MacConkey-Sorbitol	E. coli O157
GN broth + Hektoen enteric (HEK)	Enrichment broth for enhanced recovery of Salmonella and Shigella before plating to HEK
Campylobacter agar	Campylobacter spp.[b]
Cefsulodin-Irgasan™-Novobiocin (CIN) agar	Yersinia enterocolitica
Thiosulfate Citrate Bile Salts (TCBS) agar	Vibrio spp.

[a] The primary organism isolated from each type of media is listed. However, some organisms may be found on multiple types of media (e.g. Vibrio can be recovered on blood, MacConkey, and TCBS agars).
[b] The clinical reference standard method is most effective for isolation of C. jejuni, C. coli, and C. upsalisens and may be less sensitive for other species.

Archived stool specimens were obtained from external medical facilities and reference laboratories worldwide where they had previously been tested by standard laboratory methods and found to contain analytes of interest.

Surrogate/contrived clinical specimens were prepared using residual specimens from the prospective clinical study that had previously tested negative for all GI panel analytes. Spiking was performed using five individual quantified strains for each organism such that half of the contrived positive specimens had analyte concentrations at 2 × the limit of detection (LoD) while the remaining specimens were tested at additional concentrations that spanned the clinically relevant range. Specimens were randomized and study personnel were blinded to their contents before testing.

The clinical sensitivity (or positive percent agreement; PPA) and specificity (or negative percent agreement; NPA) for the FilmArray GI Panel assays were determined using standard binomial sampling statistics. A total of 1556 valid specimens were collected under IRB approved protocols at each site between May and September of 2013.

Study Enrollment and Demographics

Table 2. Overall and Per Site Demographic Analysis

		Overall	Site 1	Site 2	Site 3	Site 4
Sex	Male	718 (64%)	99 (39%)	139 (38%)	406 (51%)	74 (51%)
	Female	838 (54%)	154 (61%)	227 (62%)	387 (49%)	70 (49%)
Status	Outpatient	1350 (87%)	96 (38%)	328 (90%)	790 (99.9%)	136 (94%)
	Hospitalized	164 (11%)	157 (62%)	0 (0%)	1 (0%)	6 (4%)
	Emergency	42 (3%)	0 (0%)	38 (10%)	2 (0%)	2 (1%)
	Total	1556	253	366	793	144

Age Group - Overall

Age Group - By Site
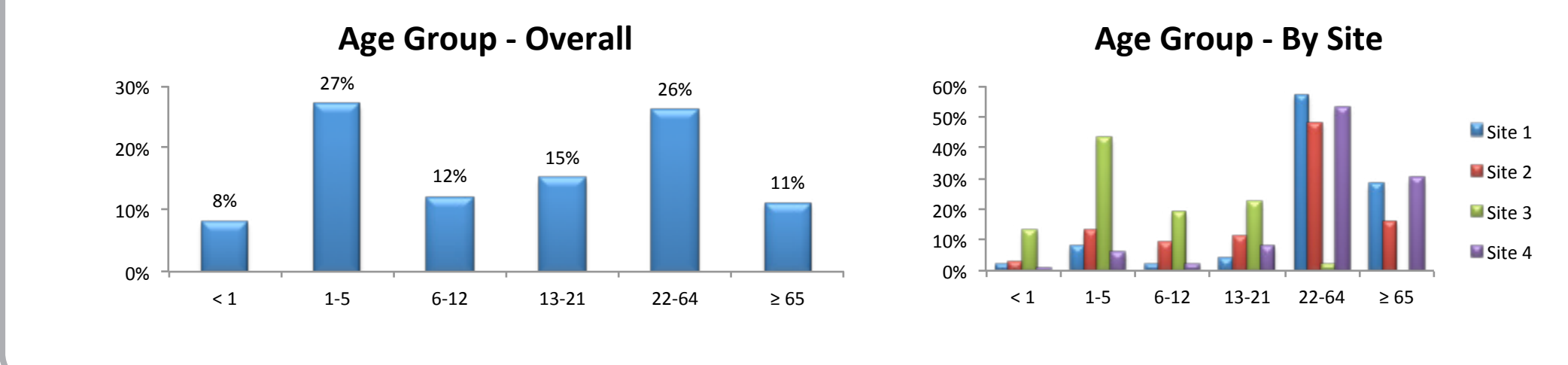
Expected Values

The three most prevalent organism results in the prospective study were for EPEC (22.4%; 348/1556), C. difficile (13.1%; 204/1556), and EAEC (7%; 109/1556). The remaining organism results were reported at a prevalence of 4.5% or less. E. histolytica was the only organism not detected in the prospective study. The prevalence of organisms was similar across all age groups, with the exception of C. cayetanensis (exclusive to Site 1 due to an outbreak) as well as toxigenic C. difficile and Adenovirus F 40/41, which were more prevalent in pediatric populations. The high prevalence of toxigenic C. difficile in children <1 year of age (40.5%; 49/121) is consistent with C. difficile being a known colonizer of neonates and with prevalence reported in the literature for this age group.

Table 3. Prevalence of Detected Analytes Stratified by Age Group # = Number; EV= Expected Value

Organism	Overall (n=1556) #	EV	<1 (n=121) #	EV	1-5 (n=418) #	EV	6-12 (n=193) #	EV	13-21 (n=240) #	EV	22-64 (n=411) #	EV	65+ (n=173) #	EV
Campylobacter	58	3.7%	1	0.8%	11	2.6%	12	6.2%	6	2.5%	19	4.6%	9	5.2%
Clostridium difficile	204	13.1%	49	40.5%	66	15.8%	18	9.3%	33	13.8%	29	7.1%	9	5.2%
Plesiomonas shigelloides	18	1.2%	0	0%	7	1.7%	4	2.1%	4	1.7%	3	0.7%	0	0%
Salmonella	37	2.4%	5	4.1%	7	1.7%	5	2.6%	5	2.1%	11	2.7%	4	2.3%
Vibrio	2	0.1%	0	0%	0	0%	0	0%	0	0%	2	0.5%	0	0%
Vibrio cholerae	1	0.1%	0	0%	0	0%	0	0%	0	0%	1	0.2%	0	0%
Yersinia enterocolitica	1	0.1%	1	0.8%	0	0%	0	0%	0	0%	0	0%	0	0%
Enteroaggregative E. coli (EAEC)	109	7.0%	9	7.4%	34	8.1%	20	10.4%	17	7.1%	25	6.1%	4	2.3%
Enteropathogenic E. coli (EPEC)	348	22.4%	30	24.8%	155	37.1%	45	23.3%	46	19.2%	55	13.4%	17	9.8%
Enterotoxigenic E. coli (ETEC)	31	2.0%	1	0.8%	5	1.2%	7	3.6%	5	2.1%	9	2.2%	4	2.3%
Shiga-like toxin-producing E. coli (STEC)	38	2.4%	1	0.8%	24	5.7%	2	1.0%	4	1.7%	5	1.2%	2	1.2%
E. coli O157	4	0.3%	0	0%	3	0.7%	1	0.5%	0	0%	0	0%	0	0%
Shigella / Enteroinvasive E. coli (EIEC)	49	3.1%	0	0%	31	7.4%	7	3.6%	5	2.1%	6	1.5%	0	0%
Cryptosporidium	24	1.5%	0	0%	9	2.2%	3	1.6%	6	2.5%	5	1.2%	1	0.6%
Cyclospora cayetanensis	19	1.2%	0	0%	0	0%	0	0%	0	0%	13	3.2%	6	3.5%
Entamoeba histolytica	0	0.0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Giardia lamblia	27	1.7%	1	0.8%	6	1.4%	5	2.6%	2	0.8%	13	3.2%	0	0%
Adenovirus F 40/41	55	3.5%	12	9.9%	36	8.6%	5	2.6%	0	0%	2	0.5%	0	0%
Astrovirus	8	0.5%	1	0.8%	4	1.0%	0	0%	1	0.4%	2	0.5%	0	0%
Norovirus GI/GII	70	4.5%	15	12.4%	31	7.4%	5	2.6%	7	2.9%	9	2.2%	3	1.7%
Rotavirus A	18	1.2%	11	9.1%	2	0.5%	1	0.5%	1	0.4%	2	0.5%	1	0.6%
Sapovirus	59	3.8%	12	9.9%	31	7.4%	7	3.6%	1	0.4%	5	1.2%	3	1.7%

The FilmArray GI Panel detected at least one potential pathogen in 832 of the 1556 specimens that were tested, yielding a positivity rate of 54%. Multiple pathogens were detected in 18% of specimens (31.5% of the positive specimens) and the greatest number of potential pathogens detected in a single specimen was six (Campylobacter, EAEC, EPEC, ETEC, Giardia, and Norovirus).

Figure 1. Positivity Rate and Number of Organisms Per Sample
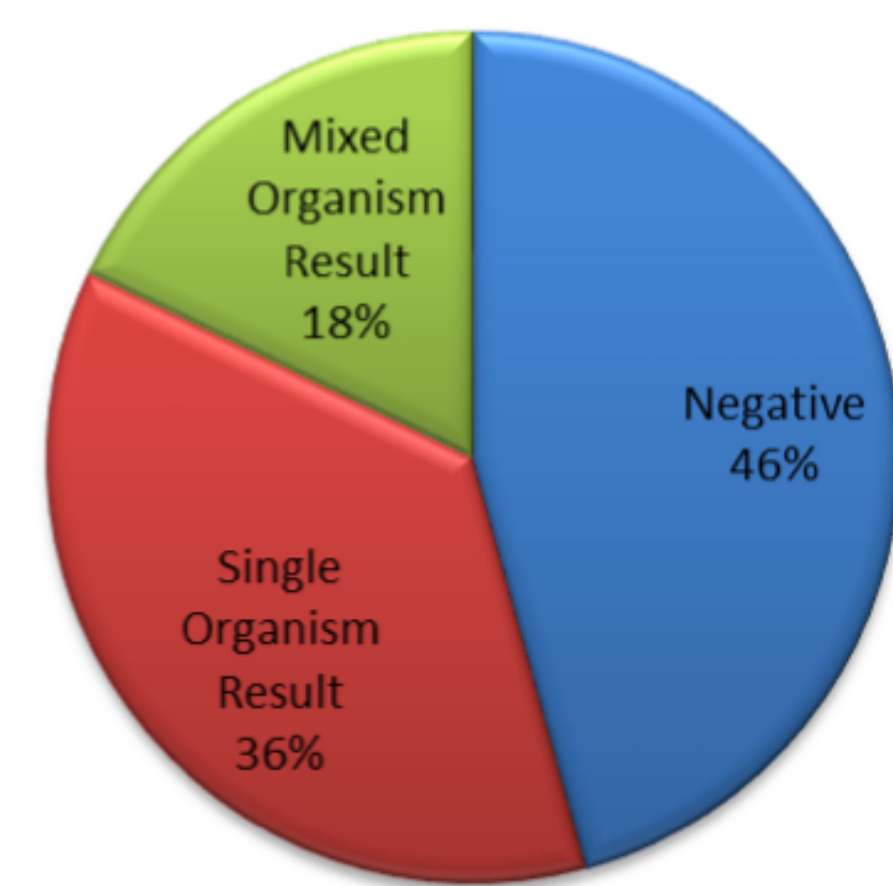
Analysis of Instrument and Test Performance

The overall success rate on the initial test of the enrolled specimens was 99.4% (1544/1557); three tests failed due to software errors, one test was aborted by the user, and nine tests failed due to pouch control failures. No instrument errors were observed. A single specimen was lost due to the inability to retest it within the required 4-day test window; all others were successfully retested, resulting in a final success rate of 99.9% (1556/1557).

The very low failure rate of the internal controls indicate that the FilmArray GI Panel is adept at removing inhibitory substances from stool specimens without the need for specimen dilution or pretreatment.

FilmArray GI Assay Performance

Table 4. FilmArray GI Panel Assay Performance - Prospective Clinical Study

Bacteria	Sensitivity/PPA[a] TP/(TP + FN)	%	95% CI	Specificity/NPA[a] TN/(TN + FP)	%	95% CI
Campylobacter (C. jejuni/C. coli/C. upsalisens)	34/35	97.1	85.1-99.9	1497/1521	98.4	97.7-99.0
Clostridium difficile toxin A/B[a]	163/165	98.8	95.7-99.9	1350/1391	97.1	96.0-97.9
Plesiomonas shigelloides	3/3	100	29.2-100	1538/1553	99.0	98.4-99.5
Salmonella	31/31	100	88.8-100	1519/1525	99.6	99.1-99.9
Vibrio (V. parahaemolyticus/V. vulnificus/V. cholerae)	0/0	-	-	1554/1556	99.9	99.5-100
Vibrio cholerae	0/0	-	-	1555/1556	99.9	99.6-100
Yersinia enterocolitica	1/1	100	N/A	1555/1555	100	99.8-100

Diarrheagenic E. coli/Shigella	Positive Percent Agreement (PPA)[a] TP/(TP + FN)	%	95% CI	Negative Percent Agreement (NPA)[a] TN/(TN + FP)	%	95% CI
Enteroaggregative E. coli (EAEC)	82/83	98.8	93.5-100	1446/1473	98.2	97.3-98.8
Enteropathogenic E. coli (EPEC)	314/317	99.1	97.3-99.8	1167/1201	97.2	96.1-98.0
Enterotoxigenic E. coli (ETEC) lt/st	22/22	100	84.6-100	1525/1534	99.4	98.9-99.7
Shiga-like toxin-producing E. coli (STEC) stx1/stx2	33/33	100	89.4-100	1518/1523	99.7	99.2-99.9
E. coli O157[a]	3/3	100	29.2-100	34/35	97.1	85.1-99.9
Shigella/Enteroinvasive E. coli (EIEC)	47/49	95.9	86.0-99.5	1505/1507	99.9	99.5-100

Parasites	Positive Percent Agreement (PPA)[a] TP/(TP + FN)	%	95% CI	Negative Percent Agreement (NPA)[a] TN/(TN + FP)	%	95% CI
Cryptosporidium	18/18	100	81.5-100	1532/1538	99.6	99.2-99.9
Cyclospora cayetanensis	19/19	100	82.4-100	1537/1537	100	99.8-100
Entamoeba histolytica	0/0	-	-	1556/1556	100	99.8-100
Giardia lamblia	20/20	100	83.2-100	1529/1536n	99.5	99.1-99.8

Viruses	Positive Percent Agreement (PPA)[a] TP/(TP + FN)	%	95% CI	Negative Percent Agreement (NPA)[a] TN/(TN + FP)	%	95% CI
Adenovirus F 40/41	42/44	95.5	84.5-99.4	1499/1512	99.1	98.5-99.5
Astrovirus	7/7	100	59.0-100	1548/1549	99.9	99.6-100
Norovirus GI/GII	52/55	94.5	84.9-98.9	1483/1501	98.8	98.1-99.3
Rotavirus A	6/6	100	54.1-100	1538/1550	99.2	98.7-99.6
Sapovirus (Genogroups I, II, IV, and V)	46/46	100	92.3-100	1497/1510	99.1	98.5-99.5

[a]C. difficile performance is reported as positive percent agreement and negative percent agreement, and E. coli O157 performance is reported as sensitivity/specificity, in contrast to the headings of their respective sections. The performance measures of sensitivity and specificity only refer to those analytes for which the gold-standard bacterial culture was used as the reference method; Campylobacter, E. coli O157, Plesiomonas shigelloides, Salmonella, Vibrio, Vibrio cholerae, and Yersinia enterocolitica. Performance measures of positive percent agreement (PPA) and negative percent agreement (NPA) refer to all other analytes, for which PCR/sequencing assays were used as comparator methods.

Investigation of Discrepant Results

Discrepant results between the FilmArray GI Panel and comparator methods were investigated with supplementary molecular methods, and the results are outlined in Table 5.

Table 5. Results of Discrepancy Investigation

Analyte	Total	Inconclusive	FA Correct	FA Incorrect
False Negatives				
Bacteria	3	1	-	2[a]
Diarrheagenic E. coli/Shigella	6	6	-	-
Viruses	5	2	-	3[b]
Total (%)	14	9 (64%)	0 (0%)	5 (36%)
False Positives				
Bacteria	89	5	84[c,d]	-
Diarrheagenic E. coli/Shigella	78	13	62	3[e]
Parasites	13	1	10[f]	2[e]
Viruses	57	14	43	-
Total (%)	237	33 (14%)	199 (84%)	5 (2%)

[a] One Campylobacter FA false negative was due to a missed detection of C. jejuni subsp. doylei.
[b] Two Norovirus FA false negatives were due to missed detection of Norovirus GI.
[c] Culture failed to identify 10 C. upsaliensis, 8 C. jejuni, 1 C. jejuni subsp. doylei.
[d] Culture failed to identify one V. parahaemolyticus and one V. cholerae.
[e] Three ETEC FA false positives were identified as cross-reactivity with Citrobacter koseri (2) and Hafnia alvei (1).
[f] FilmArray detected two C. felis, two C. ubiquitum, one C. parvum, and one Cryptosporidium for which species was not determined that were not detected by the PCR comparator method.
[g] Two Giardia FA false positives were identified as cross-reactivity with Bifidobacterium longum and Ruminococcus callidus (one each).

Archived specimens that had been previously identified as positive for analytes of interest were tested to supplement prospective study data for low-prevalence analytes.

Table 6. FilmArray GI Panel Assay Performance - Archived Specimens

Analyte	PPA TP/(TP + FN)	%	95% CI	NPA TN/(TN + FP)	%	95% CI
Bacteria						
Plesiomonas shigelloides	12/12	100	73.5-100	107/107	100	96.6-100
Vibrio	1/1	100	2.5-100	127/127	100	97.1-100
Yersinia enterocolitica	8/8	100	63.1-100	117/117	100	96.9-100
Diarrheagenic E. coli/Shigella						
E. coli O157	19/19	100	82.4-100	0/0	-	-
Parasites						
Cryptosporidium	29/30	96.7	82.8-99.9	66/66	100	94.6-100
Entamoeba histolytica	2/2	100	15.8-100	123/123	100	97-100
Giardia lamblia	26/26	100	86.8-100	66/66	100	94.6-100
Viruses						
Astrovirus	31/32	96.9	83.8-99.9	91/91	100	96-100
Rotavirus	29/29	100	88.1-100	65/65	100	94.5-100

Despite extensive collection efforts, an insufficient number of archived specimens were available to demonstrate system performance for P. shigelloides, Vibrio, V. cholerae, Y. enterocolitica, and E. histolytica. Surrogate clinical specimens were contrived and tested with the FilmArray GI Panel. Spiking levels spanned clinically-relevant levels (when known), and the specimens sets included approximately 50% of the specimens spiked near the assays' LoDs.

Table 7. FilmArray GI Panel Assay Performance - Contrived Clinical Specimens

Analyte	PPA TP/(TP + FN)	%	95% CI	NPA TN/(TN + FP)	%	95% CI
Bacteria						
Entamoeba histolytica[a]	44/50	88.0	75.7-95.5	75/75	100	95.2-100
Plesiomonas shigelloides	70/70	100	94.9-100	105/105	100	96.5-100
Vibrio[b]	112/115	97.4	92.6-99.5	60/60	100	94.0-100
V. cholerae[c]	55/65	84.6	73.5-92.4	110/110	100	96.7-100
Yersinia enterocolitica	65/65	100	94.5-100	110/110	100	96.7-100

[a] Six unexpectedly negative specimens may be attributable to varying ratios of trophozoite and cyst forms of the organism in individual spiking events (trophozoites contain one nucleus, while cysts contain four nuclei).
[b] This includes 48/50 non-cholerae and 64/65 V. cholerae.
[c] Ten (10) of these specimens were spiked with an isolate which was found to have a highly divergent toxR gene that was not present in the NCBI database and non-reactive with the FilmArray GI Panel V. cholerae assay. The FilmArray GI Panel Vibrio assay was positive for nine of these specimens.

CONCLUSION

FilmArray GI Panel is a highly sensitive and specific test for infectious agents of gastrointestinal illness. The test has the capability of identifying a greater number of pathogens associated with gastrointestinal illness while being faster, more sensitive, more specific, and simpler to perform than standard methods.

ACKNOWLEDGEMENTS

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