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Multicenter Evaluation of the BioFire FilmArray Gastrointestinal Panel for Etiologic Diagnosis of Infectious Gastroenteritis

Sarah N. Buss,[a]* Amy Leber,[b] Kimberle Chapin,[c] Paul D. Fey,[a] Matthew J. Bankowski,[d,e] Matthew K. Jones,[f] Margarita Rogatcheva,[f] Kristen J. Kanack,[f] Kevin M. Bourzac[f]

Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska, USA[a]; Department of Laboratory Medicine, Nationwide Children's Hospital, Columbus, Ohio, USA[b]; Departments of Pathology and Medicine, Lifespan Academic Medical Center, Providence, Rhode Island, USA[c]; Diagnostic Laboratory Services, Inc., Aiea, Hawaii, USA[d]; Departments of Pathology, Tropical Medicine, Medical Microbiology, and Pharmacology, John A. Burns School of Medicine and the University of Hawaii at Manoa, Honolulu, Hawaii, USA[e]; BioFire Diagnostics, LLC, Salt Lake City, Utah, USA[f]

The appropriate treatment and control of infectious gastroenteritis depend on the ability to rapidly detect the wide range of etiologic agents associated with the disease. Clinical laboratories currently utilize an array of different methodologies to test for bacterial, parasitic, and viral causes of gastroenteritis, a strategy that suffers from poor sensitivity, potentially long turnaround times, and complicated ordering practices and workflows. Additionally, there are limited or no testing methods routinely available for most diarrheagenic *Escherichia coli* strains, astroviruses, and sapoviruses. This study assessed the performance of the FilmArray Gastrointestinal (GI) Panel for the simultaneous detection of 22 different enteric pathogens directly from stool specimens: *Campylobacter* spp., *Clostridium difficile* (toxin A/B), *Plesiomonas shigelloides*, *Salmonella* spp., *Vibrio* spp., *Vibrio cholerae*, *Yersinia enterocolitica*, enteroaggregative *E. coli*, enteropathogenic *E. coli*, enterotoxigenic *E. coli*, Shiga-like toxin-producing *E. coli* (*stx*$_1$ and *stx*$_2$) (including specific detection of *E. coli* O157), *Shigella* spp./enteroinvasive *E. coli*, *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia lamblia*, adenovirus F 40/41, astrovirus, norovirus GI/GII, rotavirus A, and sapovirus. Prospectively collected stool specimens (n = 1,556) were evaluated using the BioFire FilmArray GI Panel and tested with conventional stool culture and molecular methods for comparison. The FilmArray GI Panel sensitivity was 100% for 12/22 targets and ≥94.5% for an additional 7/22 targets. For the remaining three targets, sensitivity could not be calculated due to the low prevalences in this study. The FilmArray GI Panel specificity was ≥97.1% for all panel targets. The FilmArray GI Panel provides a comprehensive, rapid, and streamlined alternative to conventional methods for the etiologic diagnosis of infectious gastroenteritis in the laboratory setting. The potential advantages include improved performance parameters, a more extensive menu of pathogens, and a turnaround time of as short as 1 h.

Infectious gastroenteritis (IGE) is a leading cause of global morbidity and mortality. It is estimated that IGE contributes to the death of 2,195 children each day (1). IGE also contributes to serious morbidities, such as malnutrition, stunting, and impaired cognitive function (2, 3). Diarrheal disease disproportionately affects developing nations, but IGE remains a significant problem in industrialized countries as well. For example, it is estimated that each year, approximately 178.8 million cases of gastrointestinal illness occur in the United States, resulting in 474,000 hospitalizations and 5,000 deaths (4). Although the etiologic agents responsible for about 80% of these illnesses are unidentified or otherwise unspecified (4), norovirus and *Salmonella* spp. are currently the most commonly identified pathogens associated with food-borne disease in the United States and account for 5.5 and 1.0 million cases each year, respectively (http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html). Health care- and antibiotic-associated diarrhea are also problematic, with the major causative pathogen being toxin-producing *Clostridium difficile*. In the United States, >300,000 cases of *C. difficile* are diagnosed annually, with associated costs of at least $1 billion (5, 6). In addition, recent studies have shown that hospitalized patients often harbor other diarrheal pathogens (e.g., norovirus, rotavirus, and adenovirus), which are not routinely tested for in these patient populations (7, 8).

In the United States and across the globe, IGE is associated with a diverse array of etiologic agents, including bacteria, viruses, and parasites. Clinical presentation does little to aid with specific diagnosis, because diarrhea is the predominant symptom of IGE, regardless of etiology (9, 10). Nonetheless, a common tactic used for patient management in developing nations is syndromic diagnosis, consisting of differentiation between acute watery, persistent, and bloody diarrhea (11). In contrast, industrialized countries typically employ a battery of tests for detecting the causative agent of IGE. Common diagnostic practice in the United States requires providers to choose among bacterial, viral, and parasitic pathogen groups, with associated test methodologies, including rapid antigen testing, culture, specialized microscopy, and singleplex PCR assays, for detecting the responsible organism or toxin. Often, the clinician is unsure of what is included with each test

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Address correspondence to Sarah N. Buss, sarah.buss@wyo.gov.

* Present address: Sarah N. Buss, Wyoming Public Health Laboratory, Cheyenne, Wyoming, USA.

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requested and, more importantly, may miss testing for specific pathogens of interest (e.g., *Cyclospora* or norovirus) (12, 13). In the clinical laboratory, these various test requests translate into laborious and often costly practices, especially if test requests must be forwarded to a reference laboratory. In addition, the turnaround times range from under an hour, to 2 to 4 days, or even weeks in some instances. The lengthy turnaround times associated with stool specimen testing diminish the value of an etiologic diagnosis with respect to patient management (12). Furthermore, even when specimens are collected and tested, the causative agent of IGE is often left unidentified for many reasons, including limited laboratory test menus, physician ordering practices, insensitive methodologies, the complexity of the stool matrix, and antibiotic usage (13–16).

The specific etiologic diagnosis of IGE provides important information for case management, infection control, and public health interventions. Therefore, effective diagnostic methodologies are critical for optimal treatment and prevention plans. Clinical laboratories are increasingly utilizing multiplexed molecular assays in order to detect a variety of pathogens from different sample matrices. In this study, the performance of one such multiplexed molecular assay, the BioFire FilmArray Gastrointestinal (GI) Panel, was examined at four geographically distinct clinical sites across the United States. The accuracy of the panel was compared to that of conventional stool culture for common bacterial pathogens (*Campylobacter* spp., *Escherichia coli* O157, *Plesiomonas shigelloides*, *Salmonella* spp., *Vibrio* spp., *V. cholerae*, and *Y. enterocolitica*) and to that of PCR with sequencing for detecting toxigenic *C. difficile*, diarrheagenic *E. coli* and *Shigella* spp., viruses, and parasites.

MATERIALS AND METHODS

Clinical specimens. The 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 medium, was submitted with orders by the provider for stool culture, was of sufficient volume for testing, and could be tested via the FilmArray GI Panel and bacterial culture within 4 days of specimen collection (stored at 4°C). At the study sites, culture was set up as part of routine clinical testing (i.e., immediately upon receipt of the specimen), and to meet the inclusion criteria, culture was always set up within 4 days of specimen collection. The submitting physicians may have ordered testing in addition to stool culture; however, the results of such testing were not collected or utilized in this study for comparator analysis. The specimens were collected under institutional review board (IRB)-approved protocols at each site, which included a waiver of informed consent for the use of remnant deidentified specimens. A total of 1,556 specimens met these criteria during an enrollment period between May and September of 2013. The specimens were deidentified and assigned a study code number (SCN) linked to patient demographic information, including age, sex, hospitalization status (hospitalized, outpatient, or emergency department), and date of specimen collection. All study-specific testing was conducted on specimen aliquots labeled with only the SCN and no identifiable information.

Stool culture comparator testing. The specimens were tested for bacterial gastrointestinal pathogens using stool culture, which was performed at each study site using the standard procedure of each laboratory. All study specimens at all sites were plated to the following media or their equivalent: blood agar, blood agar with 10 μg/ml ampicillin, MacConkey agar, MacConkey-sorbitol agar, *Campylobacter* CVA agar (CVA), cefsulodin-irgasan-novobiocin agar (CIN), and thiosulfate-citrate-bile salts agar. Additionally, Gram-negative (GN) broth was inoculated, incubated overnight at 35 to 37°C, and used to inoculate Hektoen enteric or eosin methylene blue agar. The media were obtained from BD (Franklin Lakes, NJ) or Remel (Lenexa, KS), depending on the preference of each study site. All media were incubated at 35 to 37°C in ambient air, except for CIN, which was held at room temperature, and CVA, which was held at 42°C under microaerobic growth conditions. The plates were held for 2 days before being reported as negative, and the isolates were initially identified using standard procedures validated and utilized by each study site laboratory (e.g., biochemical and phenotypic analysis). Pathogenic isolates identified by the study sites were frozen in nutrient broth containing 20% glycerol and shipped to BioFire Diagnostics in case additional molecular analysis was required (e.g., incomplete laboratory identification or discrepant analysis), as described below and detailed in Table 1.

FilmArray GI Panel testing. An aliquot of each specimen was stored at 4°C until FilmArray GI Panel testing could be completed at the study sites. Two hundred microliters of specimen in Cary-Blair transport medium was subject to FilmArray GI Panel testing, according to the manufacturer's instructions. The FilmArray GI Panel test consists of automated nucleic acid extraction, reverse transcription, amplification, and analysis, with results available in 1 h per run per specimen.

This study was conducted with an investigational use only (IUO) version of the FilmArray GI Panel that detected seven bacteria (*Aeromonas* spp., *Campylobacter* spp. [*C. jejuni*, *C. coli*, and *C. upsaliensis*], *C. difficile* toxin A/B, *P. shigelloides*, *Salmonella* spp., *Vibrio* spp. [*V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae* with specific detection of *V. cholerae*], and *Y. enterocolitica*), six diarrheagenic *Shigella* spp./*E. coli* (enteroaggregative *E. coli* [EAEC], enteropathogenic *E. coli* [EPEC], enterotoxigenic *E. coli* [ETEC], enteroinvasive *E. coli* [EIEC]/*Shigella* spp., Shiga-like toxin-producing *E. coli* [STEC] [with specific detection of *E. coli* O157]), four parasites (*Cryptosporidium*, *Cyclospora cayetanensis*, *E. histolytica*, and *G. lamblia*), and five viruses (adenovirus F 40/41, astrovirus, norovirus GI/GII, rotavirus A, and sapovirus). The final FDA-cleared FilmArray GI Panel is identical to the IUO panel used in this study, with the exception that results from the *Aeromonas* target are not analyzed or reported. The performance characteristics of the IUO *Aeromonas* target were assessed in this study.

Each FilmArray GI Panel pouch contains an internal nucleic acid extraction control and a PCR control. The FilmArray GI Panel runs were considered valid if the run completed normally and internal controls passed. The FilmArray GI Panel software performs automated result analysis with each target in a valid run reported as detected or not detected. If either internal control fails, the software automatically provides a result of invalid for all panel analytes. All specimens having invalid results were retested, and the study inclusion criteria required that all testing be completed within 4 days of specimen collection. A result of not applicable is reported for EPEC when STEC is detected, because the EPEC target (*eae* gene) is present in some STEC strains. Not applicable is also reported for *E. coli* O157 when STEC is not detected, because otherwise the assay may identify *E. coli* O157 strains that lack stx_1 and stx_2 genes.

Real-time PCR and sequencing for comparator analysis. The specimen aliquots for PCR testing were frozen immediately (at ≤70°C) after enrollment and shipped to BioFire on a weekly basis. Nucleic acid was extracted from proteinase K-treated and bead-beaten specimens using a MagNA Pure LC 2.0 automated system with the total nucleic acid isolation–high-performance kit (Roche Diagnostics, Indianapolis, IN). Real-time PCR and sequence analysis were performed by BioFire personnel in a blinded manner for comparator testing (Table 1). Table 1 details the gene targets used in this study for real-time PCR-based comparison. Additionally, if a laboratory was unable to identify a particular bacterial isolate to the species level, a nucleic acid extract from the isolate was subjected to PCR and bidirectional sequencing (e.g., 16S rRNA gene or other appropriate target).

In most instances, when real-time PCR was used as a comparator method (Table 1), two independent well-validated assays targeting each analyte were utilized in conjunction with bidirectional sequencing. The exceptions included the utilization of previously published assays without

TABLE 1 Methods of comparator testing and discrepancy analysis for all potential pathogens targeted by the FilmArray GI Panel

Analyte	Comparator method[a]	Discrepant analysis[b]
Campylobacter spp.	Culture	*cadF* and *gyrA* PCR, BTFA
C. difficile	*tcdA* and *tcdB* PCR	ECP, BTFA
P. shigelloides	Culture	*hugA* PCR, BTFA
Salmonella spp.	Culture	*stn* PCR
Vibrio spp.	Culture	*gyrB* PCR, BTFA
V. cholerae	Culture	*gyrB* PCR, BTFA
Y. enterocolitica	Culture	NA
EAEC	*aggR* and *aatA* PCR	ECP, BTFA
EPEC[c]	2 × *eae* PCR	ECP, BTFA
ETEC	*eltA*, *est1a*, and *est1b* PCR	ECP, BTFA
STEC	stx_1 and stx_2 PCR	ECP, BTFA
E. coli O157[d]	Culture (STEC positive only)	*rfbE* PCR
Shigella spp./EIEC	2 × *ipaH* PCR	ECP, BTFA
Shigella spp.	Culture	ECP, BTFA
Cryptosporidium spp.	DHFR and TRAPC1 PCR	ECP, 18S rRNA gene PCR, BTFA
C. cayetanensis	ITS2 and 18S rRNA gene PCR	NA
E. histolytica	18S rRNA gene and actin-like gene PCR	NA
G. lamblia	2 × 18S rRNA gene PCR (one published [18])	ECP, BTFA
Adenovirus F 40/41	*hexon* and *pol* PCR	ECP, BTFA
Astrovirus	ORF1a and ORF1b PCR	ECP, BTFA
Norovirus GI/GII	RdRp-capsid junction PCR (17)	PCR with multiple primer sets, BTFA
Rotavirus	VP4 and VP7 PCR	ECP, BTFA
Sapovirus	2 × RpRd-capsid junction PCR (one published [19])	ECP, BTFA

[a] All PCR tests were followed by amplicon sequencing, except for the published assays for *Giardia* spp., norovirus GI/GII, and sapovirus. ORF, open reading frame.
[b] Benchtop FilmArray test; ECP, enhanced comparator protocol.
[c] If stx_1 or stx_2 was detected by the comparator STEC assay, the comparator EPEC results for the specimen were considered not applicable (NA). The same algorithm is utilized by the FilmArray GI Panel software.
[d] *E. coli* O157 comparator results were considered only if STEC was detected. The same algorithm is utilized by the FilmArray GI Panel software.

sequencing for the detection of norovirus GI/GII (CDC CaliciNet assay [17]), *G. lamblia* (one of two assays used [18]), and sapovirus (one of two assays used [19]). These published assays produced amplicons of insufficient length for meaningful sequence analysis. When possible, the comparator real-time PCR targets differed from the FilmArray GI Panel targets, and in all cases, the primers utilized for comparator testing were different from the FilmArray GI Panel primers. Aside from the noted previously published assays, all real-time PCR comparator assays were designed and validated by BioFire. The PCR results were considered valid when the positive, negative, and specimen inhibition controls performed as expected. Macrogen (Rockville, MD) performed amplicon cleanup and sequencing, and the sequences were compared against the GenBank nucleotide database using the Nucleotide Basic Local Alignment Search Tool (BLASTn), with default settings. Sequencing-based comparator assays were considered positive only when a bidirectional sequencing result of adequate quality was found to match a sequence for the expected analyte with an E value of ≤1.0E−30. If the results from the two comparator assays were discordant from one another, nucleic acid was reextracted, and both comparator assays were repeated to ensure the discordant result was not due to contamination or specimen mix-up. The consensus result from two out of the three tests was considered the final result for each assay.

Results and discrepant analysis. A FilmArray GI Panel result was considered true positive (TP) or true negative (TN) only when it agreed with the result from the comparator method. When the results were discordant, discrepant analysis ensued. When sufficient specimen volume was available, all discordant specimens were first analyzed using PCR and sequencing assays that targeted different genes or gene regions than those of either the FilmArray GI Panel or the comparator method (if molecular), as shown in Table 1. If no alternative PCR assays were identified in the literature or developed internally, the FilmArray GI Panel false-positive (FP) or false-negative (FN) specimens were initially tested using the original molecular comparator assays (Table 1) with enhanced methods, including up to 10 additional PCR cycles and up to 10 additional replicate samples (enhanced comparator protocol). Amplicon sequencing and BLASTn analysis (as described above) were ultimately used following PCR. Any specimens that were not resolved by the initial round of discrepant analysis were tested with a benchtop version of the FilmArray GI Panel assay (i.e., FilmArray primers in a conventional real-time PCR) and amplicon sequencing.

Calculations and statistical analysis. Sensitivity and positive percent agreement (PPA) were calculated as 100 × (no. of TP/[no. of TP + no. of FN]), and specificity and negative percent agreement (NPA) were calculated as 100 × (no. of TN/[no. of TN + no. of FP]). As described, PPA and NPA are calculated in the same manner as sensitivity and specificity, respectively. The terms PPA and NPA are used instead of sensitivity and specificity to indicate that a non-gold-standard assay (e.g., PCR) was used for the original comparator analysis. The exact binomial two-sided 95% confidence interval (95% CI) was calculated according to the method of Clopper and Pearson (20).

RESULTS

Demographics. The demographic characteristics associated with the 1,556 specimens prospectively analyzed in this study are presented in Table 2. The majority of the specimens (86.8%) were collected from outpatients, with hospitalized and emergency room patients represented by 10.5% and 2.7% of the total study population, respectively. General adult (584 specimens from those >21 years old [38%]) and pediatric (972 specimens from those ≤21 years old [62%]) populations were represented with slightly more specimens collected from female patients (838 specimens [54%]) than from male patients (718 specimens [46%]).

Summary of FilmArray GI Panel findings. The FilmArray GI Panel detected at least one potential pathogen in 832 of the 1,556

TABLE 2 Demographic characteristics of study specimens

Patient subset	Data by patient sex and age (yr)[a]: <1 M	<1 F	1–5 M	1–5 F	6–12 M	6–12 F	13–21 M	13–21 F	22–64 M	22–64 F	>65 M	>65 F	Total no. (%) of specimens
Outpatient	70	48	216	187	102	83	97	133	115	186	41	72	1,350 (86.8)
Hospitalized	1	1	4	6	1	0	1	5	30	64	24	27	164 (10.5)
ER[b]	1	0	4	1	1	6	1	3	5	11	4	5	42 (2.7)
Total	72	49	224	194	104	89	99	141	150	261	69	104	1,556

[a] M, male; F, female.
[b] ER, emergency room.

specimens that were tested, yielding a positivity rate of 53.5% (Table 3). The prevalence of each potential pathogen detected by the FilmArray GI Panel during the testing period is presented in Table 4. The most prevalent organisms detected during this study were EPEC, toxigenic *C. difficile*, and EAEC, which were found in 22.4%, 13.1%, and 7.0% of the tested specimens, respectively. All other assay targets were detected in <5% of specimens, and *E. histolytica* was the only target not detected during this study.

Multiple pathogens were detected in 31.5% of the positive specimens (262/832), and the greatest number of potential pathogens detected in a single specimen was six (*Campylobacter*, EAEC, EPEC, ETEC, *G. lamblia*, and norovirus GI/GII). All pathogens detected by the FilmArray GI Panel in this study were observed, to some degree, as part of coinfections. The single cases of *Y. enterocolitica* and *V. cholerae* that were detected were identified as part of coinfections with norovirus GI/GII plus rotavirus A and EPEC plus sapovirus, respectively. A majority of the *P. shigelloides* (88.9%), ETEC (83.9%), adenovirus F 40/41 (61.8%), EAEC (61.5%), norovirus GI/GII (61.4%), sapovirus (55.9%), rotavirus A (55.6%), *C. difficile* (53.4% total, 49% from patients >1 year old), *G. lamblia* (51.9%), *Campylobacter* spp. (51.7%), astrovirus (50%), and *Vibrio* spp. (50%) detections in this study were associated with coinfections (Table 4). *C. cayetanensis* (10.5%) was least associated with coinfection, followed by *E. coli* O157 (25%), STEC (34.2%), *Shigella* spp./EIEC (34.7%), *Salmonella* spp. (40.5%), EPEC (45.7%), and *Cryptosporidium* spp. (45.8%). No statistically significant associations were found with any particular combination of pathogens.

FilmArray GI Panel performance. For 1,544/1,557 specimens (99.4%), FilmArray GI Panel testing was completed with one attempt; the user aborted one initial run (0.06%), three runs had software errors (0.19%), and one of the internal controls failed in nine instances (0.58%). Of the 13 specimens that did not yield initial results, 12 specimens produced valid results after a single retest. One of the specimens could not be retested within 4 days of collection and was therefore excluded from the study (resulting in 1,556 valid specimens analyzed of the 1,557 specimens tested). During testing, 22/1,591 (1.4%) pouches were discarded due to pouch manufacturing defects resulting in specimen loading failures prior to testing. In all cases in which the pouches experienced a failure of internal controls, the specimens were retested successfully without specimen dilution, indicating that no failures were due to PCR inhibition.

The performance characteristics for individual FilmArray GI Panel targets are presented in Table 5. Sensitivity/PPA and specificity/NPA were calculated with respect to the comparator methods defined in Table 1 without the use of data from discrepant analysis; these unresolved data were also utilized for the final submission to the FDA and appear in the test's package insert. The FilmArray GI Panel demonstrated a sensitivity/PPA of 100% for 12/22 targets: *P. shigelloides*, *Salmonella* spp., *Y. enterocolitica*, ETEC, STEC, *E. coli* O157, *Cryptosporidium* spp., *C. cayetanensis*, *G. lamblia*, astrovirus, rotavirus A, and sapovirus. For all other detected and FDA-cleared targets, the FilmArray sensitivity/PPA was ≥94.5% (*Campylobacter* spp., *C. difficile*, EAEC, EPEC, *Shigella* spp./EIEC, adenovirus F 40/41, and norovirus GI/GII). When the 95% confidence interval (95% CI) could be calculated (i.e., for 18/22 targets), the lower boundary typically met or exceeded 80.0% (i.e., for 14/18 targets), but low prevalence (<10 detections) did negatively affect the 95% CI for four analytes detected during this study (*P. shigelloides*, *E. coli* O157, astrovirus, and rotavirus A), as shown in Table 5. It was not possible to assess the sensitivity/PPA of the FilmArray GI Panel for detecting *Vibrio* spp., *V. cholerae*, or *E. histolytica*, as these organisms were not detected by comparator methods, or at all in the case of *E. histolytica*. The specificity/NPA of the FilmArray GI Panel was ≥97.1% for all analytes. The lower boundaries of the 95% CI for specificity were ≥96.0% for all targets, with the exception of STEC O157, which had a lower 95% CI boundary of 85.1% due to its low prevalence ($n = 3$) in this study.

TABLE 3 Total number of FilmArray GI Panel-positive specimens by number of detections

No. of potential pathogens in FilmArray GI Panel result	No. of specimens ($n = 1,556$)	% of total (% positives)
Detected (at least one)	832	53.47 (100)
One	570	36.63 (68.51)
Two	199	12.79 (23.92)
Three	50	3.21 (6.01)
Four	9	0.58 (1.08)
Five	3	0.19 (0.36)
Six	1	0.06 (0.12)

Discrepant analysis. The comparator methods identified 957 potential pathogens in the 1,556 specimens that were tested. The FilmArray GI Panel detected 943 of these pathogens; 14 (0.9% of all specimens) FilmArray GI Panel false-negative (FN) detections occurred. The discrepant specimens were analyzed with adjunct molecular methods, and the results are presented in Table 6. During the discrepant analysis, nucleic acid from the particular pathogen in question was identified in the single FN *Campylobacter* sp., one FN *C. difficile* (note that one FN *C. difficile* specimen could not

TABLE 4 Total number of FilmArray GI Panel detections by type of pathogen and age group

Potential pathogen	Total no.	No. (% of total) associated with coinfection	No. of detections in age group (yr):					
			<1 (n = 121)	1–5 (n = 418)	6–12 (n = 193)	13–21 (n = 240)	22–64 (n = 411)	≥65 (n = 173)
Campylobacter spp.	58	30 (51.7)	1	11	12	6	19	9
C. difficile	204	109 (53.4)	49	66	18	33	29	9
P. shigelloides	18	16 (88.9)	0	7	4	4	3	0
Salmonella spp.	37	15 (40.5)	5	7	5	5	11	4
Vibrio spp.	2	1 (50)	0	0	0	0	2	0
V. cholerae	1	1 (100)	0	0	0	0	1	0
Y. enterocolitica	1	1 (100)	1	0	0	0	0	0
EAEC	109	67 (61.5)	9	34	20	17	25	4
EPEC	348	159 (45.7)	30	155	45	46	55	17
ETEC	31	26 (83.9)	1	5	7	5	9	4
STEC	38	13 (34.2)	1	24	2	4	5	2
E. coli O157	4	1 (25)	0	3	1	0	0	0
Shigella spp./EIEC	49	17 (34.7)	0	31	7	5	6	0
Cryptosporidium spp.	24	11 (45.8)	0	9	3	6	5	1
C. cayetanensis	19	2 (10.5)	0	0	0	0	13	6
E. histolytica	0	0 (0)	0	0	0	0	0	0
G. lamblia	27	14 (51.9)	1	6	5	2	13	0
Adenovirus F 40/41	55	34 (61.8)	12	36	5	0	2	0
Astrovirus	8	4 (50)	1	4	0	1	2	0
Norovirus GI/GII	70	43 (61.4)	15	31	5	7	9	3
Rotavirus A	18	10 (55.6)	11	2	1	1	2	1
Sapovirus	59	33 (55.9)	12	31	7	1	5	3
Total	1,180	607	149	462	147	143	216	63

be retested due to insufficient volume), one FN adenovirus F 40/41, and one FN norovirus GI/GII specimen, indicating that the FilmArray GI Panel assay failed to detect the analyte in these samples. The remaining nine FN specimens (one adenovirus F 40/41, one EAEC, three EPEC, two norovirus GI/GII, and two *Shigella* spp./EIEC specimens) were presumed positive for the conventionally detected pathogen and retested using the FilmArray GI Panel. Of these nine, one norovirus GI/GII sample was positive upon retest, suggesting the remaining specimens contained analyte levels below the detectable range of the FilmArray GI Panel assays.

In total, the FilmArray GI Panel identified 1,180 potential pathogens in the 1,556 specimens tested in this study. The comparator methods also detected 943 of these pathogens, yielding 237 FilmArray GI Panel false-positive (FP) detections (20.1% of all FilmArray GI Panel detections). All 237 FP specimens were analyzed by at least one additional molecular method as a means of discrepant analysis (Table 1), with the results shown in Table 6. After discrepant analysis, 33 FilmArray GI Panel detections remained unresolved (2.8% of all FilmArray GI Panel detections) and could not be linked to cross-reactivity between the FilmArray GI Panel primers and nontarget organisms. Cross-reactivity was detected in five specimens (0.42% of all FilmArray GI Panel detections); three FP ETEC detections (9.7% of all ETEC detections) were linked to cross-reactivity between the FilmArray GI Panel ETEC assays and commensal organisms (*Hafnia alvei* and *Citrobacter koseri*) that contain variants of the *fliP* gene (a gene within the flagellar biosynthesis operon). This low-frequency potential for false-positive ETEC results due to cross-reactivity is noted in the FilmArray GI Panel package insert. Additionally, for two FP *G. lamblia* detections (7.4% of all *Giardia* sp. detections), the sequencing data obtained from the conventional PCR using the FilmArray GI Panel *Giardia* primers indicated FilmArray GI Panel cross-reactivity with commensal bacteria (*Bifidobacterium longum* and *Ruminococcus callidus*). This limitation is also outlined in the test's package insert. In the remaining 199 of the initial 237 discrepant FP specimens (84% of all FilmArray GI Panel FP results), nucleic acid from the targeted pathogen was identified using the adjunct methods, thus confirming the original FilmArray GI Panel-positive result. Overall, the FilmArray GI Panel correctly detected more positive specimens than did the comparator testing methods for all detected pathogens, except *Y. enterocolitica*, EIEC/*Shigella* spp., and *C. cayetanensis*, for which the FilmArray GI Panel detected the same number of positives as that of the comparator methods.

Aeromonas. The IUO panel utilized here targeted *Aeromonas* spp. in addition to the 22 pathogens discussed above. In this study, 25 specimens were culture positive for *Aeromonas* species. The FilmArray GI Panel detected *Aeromonas* DNA in 20 of these 25 specimens, yielding 5 FN specimens and 80% sensitivity for the target (95% CI, 59.3 to 93.2%). In addition, the FilmArray GI Panel detected *Aeromonas* DNA in 26 culture-negative specimens, and the specificity for the target was 98.3% (95% CI, 97.5 to 98.9%) compared to culture. Adjunct analysis of the discrepant specimens showed that 5/5 of the FN samples and 26/26 of the FP samples contained *Aeromonas* DNA, confirming the FilmArray GI Panel sensitivity of 80% and yielding a resolved specificity of 100%.

DISCUSSION

These data demonstrate that the FilmArray GI Panel is capable of accurately detecting bacterial, viral, and parasitic pathogens di-

TABLE 5 Performance summary and characteristics of the FilmArray GI Panel versus those of comparator assays (stool culture or PCR and sequencing)

Analyte	No. of detections[a]		Sensitivity/PPA[b]			Specificity/NPA[b]		
	C	FA	TP/(TP+FN)	%	95% CI (%)	TN/(TN+FP)	%	95% CI (%)
Campylobacter spp.	35	58	34/35	97.1	85.1–99.9	1,497/1,521	98.4	97.7–99.0
C. difficile	165	204	163/165	98.8	95.7–99.9	1,350/1,391	97.1	96.0–97.9
P. shigelloides	3	18	3/3	100	29.2–100	1,538/1,553	99.0	98.4–99.5
Salmonella spp.	31	37	31/31	100	88.8–100	1,519/1,525	99.6	99.1–99.9
Vibrio spp.	0	2	0/0			1,554/1,556	99.9	99.5–100
V. cholerae	0	1	0/0			1,555/1,556	99.9	99.6–100
Y. enterocolitica	1	1	1/1	100	NA[c]	1,555/1,555	100	99.8–100
EAEC	83	109	82/83	98.8	93.5–100	1,446/1,473	98.2	97.3–98.8
EPEC	317	348	314/317	99.1	97.3–99.8	1,167/1,201	97.2	96.1–98.0
ETEC	22	31	22/22	100	84.6–100	1,525/1,534	99.4	98.9–99.7
STEC	33	38	33/33	100	89.4–100	1,518/1,523	99.7	99.2–99.9
E. coli O157	3	4	3/3	100	29.2–100	34/35	97.1	85.1–99.9
Shigella spp./EIEC (culture)[d]	49 (31)	49	47/49	95.9	86.0–99.5	1,505/1,507	99.9	99.5–100
Cryptosporidium spp.	18	24	18/18	100	81.5–100	1,532/1,538	99.6	99.2–99.9
C. cayetanensis	19	19	19/19	100	82.4–100	1,537/1,537	100	99.8–100
E. histolytica	0	0	0/0			1,556/1,556	100	99.8–100
G. lamblia	20	27	20/20	100	83.2–100	1,529/1,536	99.5	99.1–99.8
Adenovirus F 40/41	44	55	42/44	95.5	84.5–99.4	1,499/1,512	99.1	98.5–99.5
Astrovirus	7	8	7/7	100	59.0–100	1,548/1,549	99.9	99.6–100
Norovirus GI/GII	55	70	52/55	94.5	84.9–98.9	1,483/1,501	98.8	98.1–99.3
Rotavirus A	6	18	6/6	100	54.1–100	1,538/1,550	99.2	98.7–99.6
Sapovirus	46	59	46/46	100	92.3–100	1,497/1,510	99.1	98.5–99.5

[a] C, comparator method as defined in Table 1; FA, FilmArray GI Panel.
[b] Targets that utilized clinical reference standard comparator methods (i.e., stool culture) are reported in terms of sensitivity and specificity. However, the terms positive and negative percent agreement (PPA and NPA, respectively) are typically used to describe performances for analytes that used PCR as a reference. As detailed in Materials and Methods, sensitivity is calculated in the same manner as is PPA, and specificity is calculated in the same manner as is NPA. CI, confidence interval.
[c] NA, not applicable.
[d] Molecular methods targeting both *Shigella* spp. and EIEC were utilized for comparator testing and calculation of FilmArray GI Panel performance characteristics. However, culture was used by the clinical study sites to identify *Shigella* spp. for informational purposes, and the culture results are noted in parentheses.

rectly from a stool specimen in Cary-Blair enteric transport medium in about 1 h per specimen/instrument. There are several limitations of this study that are important to point out. For example, there were low numbers of positive specimens obtained for some FilmArray GI Panel targets, most notably *E. histolytica*, *Vibrio* spp., *V. cholerae*, and *Y. enterocolitica*. The sensitivities for these four pathogens could not be determined at all (or with confidence for *Y. enterocolitica*) due to their low prevalences during the study, but the specificity of the FilmArray GI Panel for these targets was ≥99.9%. Another limitation of the study includes the fact that specimens were obtained during the spring and summer months only, and the prevalences of some seasonal pathogens may be misrepresented. In addition, samples were collected from clinical centers in the United States only, and it is not known how the FilmArray GI Panel performs in developing countries where coinfections are known to be common. The abundance of gastrointestinal coinfections in the U.S. population is not well studied, as this is the largest study of its kind in the U.S. population, to our knowledge. However, this study showed an appreciable amount of coinfections in U.S. patients (31.5% of all positive specimens), especially in pediatric populations. A final limitation of this study includes the fact that all study specimens were originally submitted to the clinical centers based on a health care provider's request for stool culture, not necessarily for viral or parasitic pathogen testing. Thus, prevalence might be biased to favor organisms detected by traditional stool culture. This seems unlikely, as the most common pathogens detected in this study (EPEC, *C. difficile* toxin A/B, EAEC, norovirus GI/GII, and sapovirus) would not be detected by routine culture.

Culture is known to be less sensitive than molecular methods for the detection of enteric bacterial pathogens (21, 22), and our results suggest that conventional culture methods have difficulties with the recovery of *P. shigelloides*, *Salmonella* spp., *Vibrio* spp., *V. cholerae*, *C. upsaliensis*, and *Aeromonas* species. During this study, there were no FilmArray GI Panel FN *P. shigelloides*, *Salmonella* spp., *Vibrio* spp., or *V. cholerae* detections, and when the FilmArray GI Panel detected these organisms in culture-negative specimens (a total of 23 samples), nucleic acid from the appropriate organism was always detected upon discrepant analysis. An important caveat is that the FilmArray GI Panel cannot differentiate between live and dead organisms. Overall, the FilmArray GI Panel also identified more positive *Aeromonas* specimens than did culture. However, the FilmArray GI Panel did miss *Aeromonas* spp. in 5 culture-positive specimens, yielding 80% sensitivity for the target. In contrast, a retrospective study (23) recently found that the FilmArray GI Panel had an *Aeromonas* sensitivity of only 23.8% compared to that of culture. The discrepancy between those findings and ours likely relates to the fact that the *Aeromonas*-positive specimens utilized by Khare et al. (23) had been frozen for >1 month and were freeze-thawed prior to testing, whereas the specimens in this study were tested within 4 days of collection and never frozen. Regardless, due to the low sensitivity compared to that of the gold standard of culture, *Aeromonas* is not included as a reportable analyte in the FDA-cleared test.

TABLE 6 Results of discrepant analysis

Analyte by FilmArray GI Panel result	Total no.	No. with investigation outcome[a]:		
		Inconclusive	FA correct	FA incorrect
False negatives				
Campylobacter spp.	1			1[b]
C. difficile toxin A/B	2	1		1
EAEC	1	1		
EPEC	3	3		
Shigella spp./EIEC	2	2		
Adenovirus F 40/41	2	1		1
Norovirus GI/GII	3	1		2[c]
Total	14	9	0	5
False positives				
Campylobacter spp.	24	5	19[d]	
C. difficile toxin A/B	41		41	
P. shigelloides	15		15	
Salmonella spp.	6		6	
Vibrio spp.	2		2[e]	
V. cholerae	1		1[f]	
EAEC	27		27	
EPEC	34	11	23	
ETEC	9		6	3[g]
STEC	5		5	
STEC *E. coli* O157	1		1	
Shigella spp./EIEC	2	2		
Cryptosporidium spp.	6		6[h]	
G. lamblia	7	1	4	2[i]
Adenovirus F 40/41	13	2	11	
Astrovirus	1		1	
Norovirus GI/GII	18	10	8	
Rotavirus A	12	1	11	
Sapovirus	13	1	12	
Total	237	33	199	5

[a] Inconclusive, discrepant analysis did not help identify the underlying cause of discrepancy. FA correct, the result of discrepant analysis agreed with original the FilmArray (FA) result. FA incorrect, the discrepant analysis disagreed with the original FilmArray result.
[b] One FA false negative was due to missed detection of *C. jejuni* subsp. *doylei*.
[c] Two FA false negatives were due to missed detection of norovirus GI.
[d] Culture failed to identify 10 *C. upsaliensis*, eight *C. jejuni*, and one *C. jejuni* subsp. *doylei* organism.
[e] One specimen was determined to contain *V. parahaemolyticus*, and one contained *V. cholerae*.
[f] Specimen was determined to contain *V. cholerae*.
[g] Three FA false positives were identified as cross-reactivity with *C. koseri* (2) and *H. alvei* (1).
[h] FilmArray detected two *C. felis*, two *C. ubiquitum*, one *C. parvum*, and one *Cryptosporidium* spp. organisms for which species was not determined.
[i] Two FA false positives were identified as cross-reactivity with *B. longum* and *R. callidus* (one each).

The FilmArray GI Panel failed to detect *Campylobacter* DNA in one culture-positive specimen, and only 19 of 24 *Campylobacter* FP specimens were proven to contain *Campylobacter* DNA during discrepant analysis. In general, the observed *Campylobacter* discrepancies could be attributed to difficulties with the detection or recovery of specific species and subspecies. The FilmArray GI Panel FN *Campylobacter* sp. specimen was shown via discrepant analysis to contain *C. jejuni* subsp. *doylei* DNA. *C. jejuni* subsp. *doylei* is known to have higher limits of detection in the FilmArray GI Panel, and this limitation is outlined in the test's package insert. One FilmArray GI Panel FP specimen was also shown, upon discrepant analysis, to contain *C. jejuni* subsp. *doylei* DNA, indicating that the FilmArray GI Panel can detect this subspecies from clinical specimens even when culture does not. All three of the *Campylobacter* species recognized by the FilmArray GI Panel were detected by stool culture: *C. jejuni* ($n = 31$), *C. coli* ($n = 2$), and *C. upsaliensis* ($n = 1$). Interestingly, *C. upsaliensis* was overrepresented in the FP specimens that were resolved during discrepant analysis (10 *C. upsaliensis* and 9 *C. jejuni*) compared to in the TP samples (31 *C. jejuni* and 1 *C. upsaliensis*). *Campylobacter* spp. are fastidious organisms, and culture methods are biased toward the detection of *C. jejuni* and *C. coli* (24, 25). The relatively high number of FilmArray GI Panel-positive culture-negative *C. upsaliensis* specimens identified in this study may reflect the fact that *C. upsaliensis* strains, which are known human pathogens (26, 27), are susceptible to the antibiotics used in selective *Campylobacter* media and are often not recoverable in culture (27–29).

The conventional methods used to detect protozoan parasites from stool specimens also suffer from a lack of sensitivity compared to that of molecular tests (30). The traditional microscopic ova and parasite exam of stool is insensitive and labor-intensive, and laboratories may have difficulties maintaining technologist proficiency, further reducing the efficacy of the test (16). Antigen detection tests are more sensitive and less laborious than microscopy (31) but have reduced sensitivity compared to that of molecular methods (32). Consequently, molecular tests were utilized in this study as the comparator method for detecting protozoa, with no FN detections but six FP *Cryptosporidium* and seven FP *G. lamblia* detections. Four of the seven FilmArray GI Panel FP *G. lamblia* specimens were shown to contain *G. lamblia* DNA during discrepant analysis, one sample was left unresolved, and cross-reactivity between the FilmArray *G. lamblia* assay and commensal organisms was detected in two specimens (i.e., 7.4% of all FilmArray *G. lamblia* detections). All six FP *Cryptosporidium* specimens tested negative for *Cryptosporidium* spp. during discrepant analysis using the enhanced comparator protocols that targeted the DHFR and TRAPC1 genes. A limitation of the DHFR and TRAPC1 assays is that information in the NCBI database is only available for a subset of *Cryptosporidium* species, so it is not known if these assays are inclusive for all *Cryptosporidium* species. Therefore, additional testing was conducted using real-time PCR targeting the 18S gene or conventional PCR using the FilmArray GI Panel primers, followed by sequencing. *Cryptosporidium* DNA was identified in all six specimens using the additional molecular testing. Species discrimination was possible in five of the six cases, and two *Cryptosporidium ubiquitum*, two *Cryptosporidium felis*, and one *Cryptosporidium parvum* organism were identified. *C. ubiquitum* and *C. felis* are rare/emerging *Cryptosporidium* spp. that were recently characterized as human pathogens (33–35). The organisms may be detected in stool by conventional modified acid-fast stain, but the extent to which these species are recognized by commercially available *Cryptosporidium* antigen detection tests is not well understood (36). Regardless, the FilmArray GI Panel was able to detect these emerging pathogens.

The commonly used methods to detect viruses from stool include commercial antigen detection (norovirus GI/GII, rotavirus A, and adenovirus F) and molecular methods. Antigen detection tests are consistently less sensitive than are molecular methods for the detection of norovirus GI/GII (37–39), rotavirus A (40), and adenovirus F (41). Therefore, this study utilized molecular tests as the comparator method for all viral FilmArray GI Panel targets. The FilmArray GI Panel correctly detected more viruses than did

the comparator methods, with a total of five FN viral detections and 57 FP detections, with 43/57 confirmed by sequencing to contain virus-specific nucleic acid. While laboratories do not commonly test for astrovirus and sapovirus, the prevalences of these pathogens can be relatively high in certain populations (41–44). In the present study, astrovirus was detected in 0.5% of the specimens tested, whereas sapovirus was the fifth most common pathogen detected by the FilmArray GI Panel (after EPEC, *C. difficile* toxin A/B, EAEC, and norovirus GI/GII) and was found in 3.8% of all study specimens. Sapovirus was particularly prevalent in young children and was detected in 10% of all specimens from children >1 year old and 7.4% of all specimens from children between 1 and 5 years of age. In fact, all the targeted viruses were found to be more prevalent in young children than in adults (Table 4). Like norovirus GI/GII (45, 46), both astrovirus (47, 48) and sapovirus (49) are thought to be more prevalent during winter months. Interestingly, both viruses were detected during this study during the spring and summer months. As laboratories implement the FilmArray GI Panel, it will be epidemiologically valuable to confirm the true prevalences of these underdiagnosed pathogens.

Similarly, most laboratories do not currently test for all of the diarrheagenic *E. coli* types targeted by the FilmArray GI Panel (i.e., EAEC, EPEC, ETEC, and EIEC). The diarrheagenic *E. coli* strains are considered agents of endemic childhood diarrhea in developing nations, as well as infantile and traveler's diarrhea in the developed world (50). The FilmArray GI Panel detected EPEC, EAEC, and ETEC in 22.4%, 7%, and 2% of all study specimens, respectively, indicating that these organisms are commonly associated with patients that seek medical care due to IGE in the United States. Note that the FilmArray GI Panel EPEC assay does not differentiate between typical and atypical strains. The FilmArray also detected *Shigella* spp./EIEC in 49 (3.1%) specimens; 31/49 specimens were culture positive for *Shigella* spp., and 18/49 were culture negative. It is likely that some of the culture-negative, FilmArray-positive specimens contained *Shigella* spp., because the organism can be difficult to recover in culture (51–53). However, it is also possible that some of the culture-negative, FilmArray-positive specimens contained EIEC and would likely have been missed by standard stool culture protocols.

The high rate of EPEC found in this study was not anticipated, as EPEC has historically been associated with developing countries (54), and the true prevalences of these organisms in the United States are unknown. Recent literature does suggest that EPEC may be more prevalent in industrialized nations than was originally thought (55, 56). EPEC strains are a known cause of both acute and persistent diarrhea in young children (57) and were identified in this study in 24.8% of all samples collected from children <1 year of age and 37.1% of all the samples collected from children between the ages of 1 and 5 years. EPEC strains were also identified in the specimens collected from older patients in this study, but prevalence declined with age after the age of five years (Table 3). While EPEC strains are known to cause childhood diarrhea, they can also be found in healthy children and adults (55, 57), with unidentified host and organism specific factors likely involved in the outcome of infection. Consequently, a careful consideration of a patient's clinical presentation will be necessary to fully understand the significance of identifying EPEC in stool. Fecal leukocyte testing may assist providers in determining this significance, because fecal leukocytes have been associated with symptomatic EPEC infections (58).

The interpretation of *C. difficile* toxin A/B detection is also complicated, especially in children <1 year old, in whom the rate of asymptomatic colonization with toxigenic *C. difficile* is high and clinical illness is rarely reported (59). The American Academy of Pediatrics does not recommend routine testing for *C. difficile* in children <1 year of age and suggests that positive *C. difficile* results be interpreted with suspicion in children <3 years old (60). In this study, toxigenic *C. difficile* was identified in 40.5% (49 of 121) of the stool specimens from infants under the age of one. Laboratories that implement the FilmArray GI Panel should consider in advance how positive *C. difficile* toxin A/B results in infants and children will be handled, and laboratories may choose to blind such results or utilize disclaimers in their reporting. In fact, laboratories may choose not to utilize the FilmArray GI Panel at all for routine *C. difficile* testing due to the well-defined clinical situations that warrant *C. difficile* testing, in conjunction with the increased cost of multiplex molecular assays and a lack of consensus related to the most appropriate *C. difficile* testing methodology with respect to the outcome of infection (i.e., molecular detection of the toxin genes versus detection of the toxin) (61–63).

Current stool testing algorithms typically require physicians to consider which specific pathogens might be associated with individual cases of IGE and then choose a testing scheme that ensures that all the appropriate pathogens are targeted. Given the diverse array of pathogens associated with IGE and the diversity of specific testing methodologies, it is not surprising that such a piecemeal approach often fails to yield positive results. A national outbreak of cyclosporiasis originated in Iowa and Nebraska during the course of this study (http://www.cdc.gov/parasites/cyclosporiasis/outbreaks/investigation-2013.html) and highlighted the benefit of a panel-based approach to diagnostic testing. *C. cayetanensis* was detected by the FilmArray GI Panel in a study specimen from Nebraska about a week before *Cyclospora* was detected in the state using conventional methods (13). Modified acid-fast staining was not ordered by the providers on the first six (eight in total) *Cyclospora*-positive study specimens, as the outbreak had not yet been identified. The potential impact of the FilmArray GI Panel was particularly evident during the *Cyclospora* sp. outbreak because the organism is difficult to test for using conventional methods (i.e., modified acid-fast staining) and is not endemic in the United States.

In contrast to *C. cayetanensis*, *Shigella* spp. are culturable and relatively prevalent pathogens in the United States. Nevertheless, the FilmArray GI Panel was shown to be useful during an outbreak of *Shigella* that occurred during this study (64). During the outbreak, the FilmArray GI Panel correctly identified more positives than did culture on the subset of specimens tested by both methods. Thus, the FilmArray GI Panel does have the potential to positively impact the detection of pathogens associated with outbreaks. However, bacterial isolates currently remain essential for public health surveillance, outbreak investigations, and antimicrobial susceptibility testing. Laboratories that implement culture-independent diagnostic technologies must consider their state regulations and should ensure that their protocols sufficiently address the potential need for further workup of certain pathogens.

Given that the *Aeromonas* target is not reported on the FDA-cleared version of the FilmArray GI Panel, laboratories that implement the panel must also consider whether they will offer *Aeromonas* culture. *Aeromonas* spp. are known gastrointestinal

pathogens in children and adults (65, 66), and in this study, *Aeromonas* spp. were present in 3.3% of all specimens (51/1,556). When implementing the FilmArray GI Panel, laboratories that have traditionally reported *Aeromonas* spp. from stool culture should consider local prevalence in order to determine whether or not to continue to offer *Aeromonas* culture from stool. The need to additionally culture for *Aeromonas* spp. in some settings may create a challenge for laboratories, but BioFire may revisit the issue of inclusion of *Aeromonas* spp. in the FilmArray GI Panel in the future.

In general, the number of targeted pathogens in the FilmArray GI Panel is a unique strength of the assay; however, this represents an added challenge when considering the utilization of the test. There are a multitude of possible pathogen combinations that can be detected by the FilmArray GI Panel, and in this study, 32.9% of the FilmArray GI Panel-positive specimens were found to contain more than one potential pathogen. Khare et al. (23) similarly identified mixed infections in 27% of the FilmArray GI Panel-positive specimens. The FilmArray GI Panel is a closed system, and the high specificities demonstrated in this study illustrate that the FilmArray GI Panel is not prone to contamination. Regardless, the significance of detected coinfections may be difficult to understand, as the clinical implications of specific pathogen combinations are not well documented or understood. Furthermore, many GI pathogens can be shed asymptomatically or for prolonged periods of time after symptoms subside, further complicating the interpretation of positive results. For example, norovirus (67) and *Salmonella* spp. (68) can both be shed for weeks to months after symptoms subside, and asymptomatic infection with *Cryptosporidium* spp. or *G. lamblia* is not uncommon, especially in children (69–71). The identification of any combination of these organisms might then confound interpretation of positive FilmArray GI Panel results. Nevertheless, rapidly determining which potential pathogens a patient harbors is an important step in formulating an effective treatment plan and applying appropriate infection control measures.

In summary, our results indicate that the FilmArray GI Panel is a sensitive and specific multiplex assay designed for the simultaneous detection of 22 different pathogens directly from stool specimens. These data were submitted to the FDA as a part of the application for 510(k) clearance of the FilmArray GI Panel. Multiplex molecular assays have the potential to greatly simplify the current algorithms utilized for the etiologic diagnosis of IGE, in terms of both ordering practices and laboratory workflow. Due to the extensive panel of targeted pathogens and the rapid turnaround time, the FilmArray GI Panel has the potential to direct appropriate therapy and infection control precautions. Moreover, the rapid etiologic diagnosis of IGE provided by the FilmArray GI Panel and other multiplex molecular assays has the potential to more quickly identify and reduce further transmission in an outbreak of enteric pathogens.

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