Implementation of Culture-independent Testing of Gastrointestinal Pathogens in the Clinical Laboratory

MO-209

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Modified Abstract

Background: Culture-independent testing such as the Biofire FilmArray™ GI Panel (GIP) has improved sensitivity for the identification of infectious causes of gastroenteritis. The GIP was implemented at our institution in January 2015 to replace traditional methods for the detection of gastrointestinal (GI) pathogens. The purpose of this study was to evaluate the detection rates of GI pathogens with the GIP and the incidence of GI pathogens detected by the GIP in 2015 compared to traditional methods in 2014. **Methods:** Stools submitted for GIP testing from January to December 2015 were evaluated. Stools with *Clostridium difficile* detected were also tested by EIA. The incidence of GI pathogens detected by the FGIP in 2015 was compared to those reported by traditional methods in 2014. Sapovirus, Astrovirus, diarrheagenic *Escherichia coli* (EAEC, EPEC, ETEC), and *C. difficile* were excluded from comparative analyses. **Results:** A total of 2256 stools were tested by the GIP with ≥ 1 pathogen detected in 911 (40.4%). Among the 911, coinfections were detected in 176 (19.3%) with 2 and ≥ 3 pathogens detected in 144 (15.8%) and 27 (3%) of the positive specimens, respectively. The highest rates of detection with the GIP were observed for *C. difficile* (342 at 15.2%), EPEC/EAEC (236 at 10.5%), Norovirus (200 at 8.9%), *Campylobacter* spp (52 at 2.3%), Sapovirus (45 at 2.0%), and Rotavirus A (36 at 1.6%). Each of the remaining GIP pathogens had a detection rate of ≤ 1.5%. Of the 342 *C. difficile* detected by the GIP, only 88 (25.7%) were toxin positive by EIA. Most GI pathogens showed an increased incidence from 2014 to 2015, respectively: *Campylobacter* spp. (18 and 52), *Salmonella* (16 and 30), *Shigella*/EIEC (3 and 15), shiga toxin producing *E. coli* (8 and 28), *Plesiomonas shigelloides* (1 and 11), *Vibrio* spp (0 and 4), *Yersinia enterocolitica* (0 and 10), Norovirus (115 and 200), Rotavirus (8 and 36), *Giardia lamblia* (7 and 17), and *Cryptosporidium* (2 and 28). **Conclusions:** Implementation of the GIP increased the cases of infectious gastroenteritis detected and provided increased awareness of coinfections. These data suggest that the GIP can be used to monitor trends in disease incidence and aid in clinical decision-making. Ongoing studies are being done to assess the impact the GIP has on public health practices and patient outcomes.

Introduction

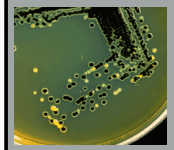
The introduction of culture-independent testing (CIDT) in clinical settings has improved turnaround time and decreased the number of tests performed to obtain a quicker diagnosis for improved patient care and infection control. Given the nonspecific presentation of symptoms in infectious gastroenteritis, often the etiology of infectious gastroenteritis is unknown.. However, this is no longer an issue with several multiplex panels having received FDA clearance in recent years to diagnosis infectious causes of gastroenteritis. Among these new CIDT, the FilmArray™ Gastrointestinal Panel (BioFire, Inc., Salt Lake City, UT) has the most comprehensive array of targets to include 22 bacteria, viruses, and parasites known to cause gastroenteritis. The use of CIDTs presents both opportunities and challenges to clinical and public health laboratories. A major challenge with using CIDTs is the absence of an isolate to perform strain typing, antimicrobial susceptibility testing, and other methods to identify molecular characteristics of the organism.

Methods

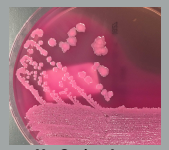
FilmArray™ GI Panel. All stool specimens submitted to the laboratory between January and December 2015 with an order for the FilmArray™ GI panel (GIP) were evaluated. Stools were collected in or transferred to Cary Blair or Enteric Plus transport media (ratio 2:15) prior to running the GIP Panel. The panels were run on the FilmArray and FilmArray 2.0 systems according to the manufactures instructions. In the event STEC was detected, EPEC was reported as not applicable, and in the absence of STEC detection, *E. coli* O157 was reported as not applicable.

Traditional Stool Culture. GIPs that resulted in the detection of bacterial pathogens of public health importance (e.g. *Campylobacter* spp, *Salmonella*, *Shigella*/Enteroinvasive *Escherichia coli* (EIEC), Shiga-like toxin-producing *E. coli* (STEC), *Yersinia enterocolitica*, and *Vibrio* spp) were reflexed to traditional stool culture for organism recovery. Briefly, stool specimens were plated on commercially prepared blood agar, MacConkey agar, Hektoen enteric agar, *Campylobacter* CVA agar, cefsulodin-irgasan-novobiocin agar (CIN), or thiosulfate-citrate-bile salts sucrose agar depending on the organism detected by the FilmArray GI Panel. Plates were held for 2 days at the appropriate conditions and suspicious colonies were identified using the Microscan NID panel, APIE, or APINE identification panels as described previously (Buss *et al.*, 2015).

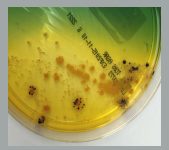
Other Detection Methods. Norovirus GI/GII was detected from stool specimens using the Cepheid Xpert® Norovirus (Sunnyvale, CA). Rotavirus and *Giardia/Cryptosporidium* were detected using Immuno*Card* STAT!® Rotavirus (Meridian Biosciences, Cincinnati, OH) and the GIARDIA/CRYPTOSPORIDIUM CHEK® (TechLab, Blacksburg, VA), respectively. A two-step algorithm was used to detect *C. difficile*: 1) antigen and toxin were tested by EIA (Alere, Waltham, MA) and 2) antigen positive/toxin negative specimens were tested for the presence of the *tcdB* gene using a toxigenic *C. difficile* assay (Great Basin Scientific, Salt Lake City, UT). All commercial tests were performed according to manufacturer's instructions.



Hektoen Enteric Agar



MacConkey Agar



Thiosulfate-Citrate-Bile Salts Sucrose Agar

Sorbitol-Maconkey Agar

Results

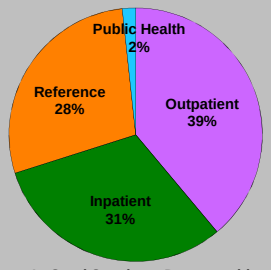


Figure 1. Stool Specimen Demographics

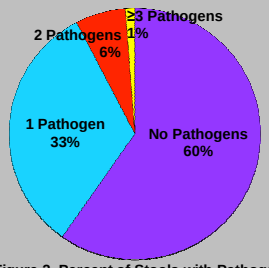


Figure 2. Percent of Stools with Pathogens

Table 1. Detection of diarrheal pathogen using traditional and FilmArray GI testing

	Method(s) used to detect GI Pathogens	
	Conventional	FilmArray GI
Target	2014	2015
Bacteria		
Campylobacter	18	52
Plesiomonas shigelloides	1	11
Salmonella	16	30
Yersinia enterocolitica	0	10
Vibrio spp.	0	2
Vibrio cholerae	0	1
EAEC	NA	76
EPEC	NA	160
ETEC	NA	32
STEC (non-O157)	8	23
STEC O157	4	5
EIEC/*Shigella*	3	15
Viruses		
Astrovirus	NA	17
Adenovirus 40/41	22	22
Norovirus GI/GII	115	200
Rotavirus A	8	36
Sapovirus	NA	45
Parasites		
Cryptosporidium	2	28
Cyclospora cayetanensis	3	3
Entamoeba histolytica	0	0
Giardia lamblia	7	17
Number of Positive Tests (%)	203 (5.1%)	569 (25.2%)
Number of Negative Tests (%)	4001 (94.9%)	1345 (73.6%)
Total Number of Tests	4204	2256

Abbreviations: EAEC, enteroaggregative *Escherichia coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; GI, gastrointestinal; NA, conventional testing not available; STEC, Shiga toxigenic *E. coli*.
*Methods not available to isolate EIEC.
Total number of tests representing combined stool culture, *Giardia/Cryptosporidum* EIA, and Norovirus PCR; excluding ova and parasite testing.

Table 2. Comparison of methods used to diagnose *C. difficile* Infection

	C. DIFF QUIK CHEK COMPLETE™		*C. difficile*	Total	
FilmArray GI	Antigen	Toxin	Assay[a]	No.	(%)
+	+	+	NP[b]	88	25.7
+	+	-	+	102	29.8
+	-	-	NP	47	13.7
+	+	-	-	16	4.7
+	No additional testing ordered			86	25.1

[a]Abbreviations: NP, not performed.
[b]Detects *tcdB* gene (Toxin B) using toxigenic *C. difficile* assay by Great Basin.

Table 3. Culture recovery of bacteria of public health importance detected by FilmArray GI

	FilmArray GI	Culture
Target	Number Detected	Number Recovered (%)
Campylobacter	52	35 (67.3)
Shigella/EIEC	15	10 (66.7)
STEC (non-O157)	23	9 (39.1)*
STEC O157	5	3 (60)
Salmonella	30	23 (76.7)
Yersinia enterocolitica	10	3 (30)
Vibrio spp.	2	1 (50)
Vibrio cholerae	1	1 (100)

Abbreviations: EIEC, enteroinvasive *E. coli*; GI, gastrointestinal; STEC, Shiga toxigenic *E. coli*.
*Methods not available to isolate EIEC.

Detection of coinfections by the GIP

- 19% (176/911) of positive GIPs had multiple pathogens detected (Figure 2).
- Common pathogens detected as coinfections were:
  - EPEC (43.2% [76/176])
  - *C. difficile* (37.5% [66/176])
  - Norovirus (30.7% [54/176])
  - EAEC (26.7% [47/176])
  - ETEC (14.2% [25/176])
  - Sapovirus (13.1% [23/176])

UNMC FilmArray GI Panel Guidance Document

Conclusions

- CIDT resulted in increased positivity rates of GI pathogens compared to traditional methods.
- Low recovery rates of organisms from GIP positive stools was likely due to the enhanced sensitivity of the CIDT, the detection of nonviable organisms in antibiotic-treated patients prior to testing, and/or suboptimal collection, transport, or culture methods of stool specimens.
- CIDT can guide laboratorians to select appropriate method(s) for organism recovery.
- CIDT can be used to monitor trends in disease incidence and clinical decision-making.

Acknowledgements

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References

Buss, S. N., Leber, A., Chapin, K., Fey, P. D., Bankowski, M. J., Jones, M. K., ... & Bourzac, K. M. (2015). Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. *Journal of clinical microbiology*, *53*(3), 915-925.