AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

BACTERIOLOGY

Comparative Evaluation of Enteric Bacterial Culture and a Molecular Multiplex Syndromic Panel in Children with Acute Gastroenteritis

Thomas Kellner,[a] Brendon Parsons,[b,c] Linda Chui,[b,c] Byron M. Berenger,[d,e,f] Jianling Xie,[g] Carey-Ann D. Burnham,[h] Phillip I. Tarr,[i] Bonita E. Lee,[j] Alberto Nettel-Aguirre,[k,l,m] Jonas Szelewicki,[b,c] Otto G. Vanderkooi,[n,o,p,q] Xiao-Li Pang,[b,c] Nathan Zelyas,[r,s] Stephen B. Freedman,[t,u] on behalf of the Alberta Provincial Pediatric EnTeric Infection TEam (APPETITE)

[a]Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada
[b]Public Health Laboratory (ProvLab), Alberta Public Laboratories, Edmonton, Alberta, Canada
[c]Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada
[d]Department of Pathology and Laboratory Medicine, Alberta Public Laboratories, Calgary, Alberta, Canada
[e]Calgary Laboratory Services, Calgary, Alberta, Canada
[f]Public Health Laboratory (ProvLab), Alberta Public Laboratories, Calgary, Alberta, Canada
[g]Section of Pediatric Emergency Medicine, Department of Pediatrics, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada
[h]Department of Pathology & Immunology, Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA
[i]Division of Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA
[j]Department of Pediatrics, Faculty of Medicine and Dentistry, Women and Children's Health Research Institute, University of Alberta, Edmonton, Alberta, Canada
[k]Department of Pediatrics, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada
[l]Department of Community Health Sciences, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada
[m]Faculty of Kinesiology, Alberta Children's Hospital Research Institute, O'Brien Population Health Institute, University of Calgary, Calgary, Alberta, Canada
[n]Department of Pediatrics, University of Calgary, Calgary, Alberta, Canada
[o]Department of Microbiology, Immunology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada
[p]Department of Pathology & Laboratory Medicine, University of Calgary, Calgary, Alberta, Canada
[q]Department of Community Health Sciences, Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada
[r]Department of Medical Microbiology and Immunology, University of Alberta Edmonton, Alberta, Canada
[s]Provincial Laboratory for Public Health, Edmonton, Alberta, Canada
[t]Section of Pediatric Emergency Medicine, Department of Pediatrics, Alberta Children's Hospital, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada
[u]Section of Pediatric Gastroenterology, Department of Pediatrics, Alberta Children's Hospital, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

ABSTRACT Although enteric multianalyte syndromic panels are increasingly employed, direct comparisons with traditional methods and the inclusion of host phenotype correlations are limited. Luminex xTAG gastrointestinal pathogen panel (GPP) and culture results are highly concordant. However, phenotypic and microbiological confirmatory testing raises concerns regarding the accuracy of the GPP, especially for *Salmonella* spp. A total of 3,089 children with gastroenteritis submitted stool specimens, rectal swab specimens, and clinical data. The primary outcome was bacterial pathogen detection agreement for shared targets between culture and the Luminex xTAG GPP. Secondary analyses included phenotype assessment, additional testing of GPP-negative/culture-positive isolate suspensions with the GPP, and in-house and commercial confirmatory nucleic acid testing of GPP-positive/culture-negative extracts. The overall percent agreement between technologies was >99% for each pathogen. *Salmonella* spp. were detected in specimens from 64 participants: 12 (19%) by culture only, 9 (14%) by GPP only, and 43 (67%) by both techniques. Positive percent agreement for *Salmonella* spp. was 78.2% (95% confidence interval [CI], 64.6%, 87.8%). Isolate suspensions from the 12 participants with specimens GPP negative/culture positive for *Salmonella* tested positive by GPP. Speci-

Citation Kellner T, Parsons B, Chui L, Berenger BM, Xie J, Burnham C-AD, Tarr PI, Lee BE, Nettel-Aguirre A, Szelewicki J, Vanderkooi OG, Pang X-L, Zelyas N, Freedman SB, on behalf of the Alberta Provincial Pediatric EnTeric Infection TEam (APPETITE). 2019. Comparative evaluation of enteric bacterial culture and a molecular multiplex syndromic panel in children with acute gastroenteritis. J Clin Microbiol 57:e00205-19. https://doi.org/10.1128/JCM.00205-19.

Editor Nathan A. Ledeboer, Medical College of Wisconsin

Address correspondence to Stephen B. Freedman, stephen.freedman@ahs.ca.

For a companion article on this topic, see https://doi.org/10.1128/JCM.00213-19.

Received 8 February 2019
Returned for modification 26 February 2019
Accepted 1 April 2019
Accepted manuscript posted online 10 April 2019
Published 24 May 2019

mens GPP positive/culture negative for *Salmonella* originated in younger children with less diarrhea and more vomiting. GPP-positive/culture-negative specimen extracts tested positive using additional assays for 0/2 *Campylobacter*-positive specimens, 0/4 *Escherichia coli* O157-positive specimens, 0/9 *Salmonella*-positive specimens, and 2/3 *Shigella*-positive specimens. For both rectal swab and stool samples, the median cycle threshold (C_T) values, determined using quantitative PCR, were higher for GPP-negative/culture-positive samples than for GPP-positive/culture-positive samples (for rectal swabs, 36.9 [interquartile range {IQR}, 33.7, 37.1] versus 30.0 [IQR, 26.2, 33.2], respectively [$P = 0.002$]; for stool samples, 36.9 [IQR, 33.7, 37.1] versus 29.0 [IQR, 24.8, 30.8], respectively [$P = 0.001$]). GPP and culture have excellent overall agreement; however, for specific pathogens, GPP is less sensitive than culture and, notably, identifies samples false positive for *Salmonella* spp.

KEYWORDS culture, *Salmonella*, enteric bacteria, nucleic acid technology, transmissible gastroenteritis virus

Bacterial enteric pathogens continue to cause substantial morbidity worldwide (1, 2). Although conventional stool culture is classically used to identify bacterial enteropathogens in clinical microbiology laboratories, such methods have drawbacks, including prolonged turnaround times and a reliance on diverse selective and enrichment media, identification tests, and expertise (3). In contrast, nucleic acid amplification techniques (NAAT) can detect multiple pathogens within hours (4). However, questions remain about their postmarket accuracy when evaluating a range of enteropathogens across diverse populations and regions (5). Published data have limited relevance to North American children, as most reports have emerged from low- and middle-income countries (5–7), focused primarily on adults (4, 6, 8–12), did not compare NAAT results to standard culture results (7–10, 13), applied inconsistent testing protocols (4, 8, 11, 14), or analyzed single pathogens (15). No studies have included host phenotypes in interpreting discordant results.

Here, we determine the agreement for the bacterial pathogens of interest between stool bacterial culture and the Luminex xTAG gastrointestinal pathogen panel (GPP; Luminex Molecular Diagnostics, Austin, TX, USA) NAAT platform in a systematically tested cohort of children with acute gastroenteritis in Alberta, Canada. Secondarily, we evaluate discordant samples by analyzing clinical phenotypes and determine GPP and culture accuracy through additional testing.

MATERIALS AND METHODS

Population. This prospective cohort study was conducted as part of the Alberta Provincial Pediatric EnTeric Infection TEam (APPETITE) project (16). Eligible children were enrolled through the Alberta Children's Hospital (Calgary, AB, Canada) and Stollery Children's Hospital (Edmonton, AB, Canada) emergency departments (EDs) and a provincial nursing triage telephone advice line (Health Link) (17).

Eligible participants were $<$18 years of age and had had $\geq$3 episodes of vomiting and/or diarrhea in the preceding 24 h and $<$7 days of symptoms. Children were excluded if they were enrolled in the previous 14 days, unable to participate in follow-up, had significant psychiatric comorbidities or neutropenia, or were critically ill. Children recruited through Health Link did not require medical attention, and supportive care at home was recommended.

Approval was granted by the University of Calgary and University of Alberta research ethics boards. Caregivers provided informed consent; assent was obtained when appropriate.

Outcome measures. The primary outcome was agreement, measured as overall percent agreement, positive percent agreement (PPA), and Cohen's κ, between stool bacterial culture and the GPP for bacterial pathogens sought by both detection methods: *Campylobacter* spp., *Escherichia coli* O157, *Salmonella* spp., and *Shigella* spp. *Yersinia enterocolitica* was not included because of negligible detection rates.

Secondary outcomes focused on *Salmonella* spp. and consisted of a comparison of the clinical phenotypes of patients with concordant and discordant results and three confirmatory tests. First, GPP-negative/culture-positive isolates were tested using the GPP. Second, an in-house real-time quantitative PCR (RT-qPCR) assay was performed on nucleic acid extracts of specimens GPP positive/culture positive and GPP negative/culture positive for *Salmonella* spp. to confirm the initial concordant result and to evaluate the relationship between the cycle threshold (C_T) value and pathogen identification. Third, GPP-positive/culture-negative specimens were tested with the in-house RT-qPCR and a different commercial assay (the Prodesse ProGastro SCSS assay; Hologic Inc., San Diego, CA) (18) on a SmartCycler

II instrument (Cepheid, Sunnyvale, CA) to confirm or refute the presence of *Salmonella* spp., *Shigella* spp., and Shiga toxin-producing *E. coli* (STEC) (see "Confirmatory testing" below for details).

Specimen acquisition. Two rectal swab specimens were obtained from each ED participant (FLOQSwab; Copan Italia, Brescia, Italy) (19): one was placed in 2 ml of modified Cary-Blair transport medium and used for bacterial culture, and the other was placed into a sterile tube without medium and tested (off-label) using the GPP. Stool samples were collected in sterile containers (V302-F; Starplex Scientific Inc., ON, Canada). If they were not provided prior to discharge, stool samples were collected by the parents at home and placed in the same sterile containers described above, which were retrieved by a courier service.

Health Link participants received specimen collection kits by courier. Specimens were collected per ED protocols and were returned to the clinical microbiology laboratory by a study-funded courier service within 12 h of collection. All specimens were placed in coolers with ice packs while in transit to the laboratory. Bacterial cultures were inoculated per Public Health Laboratory (ProvLab) protocols as soon as possible following specimen arrival, after which both the rectal swabs and stool specimens were stored at −80°C until molecular testing was performed.

Specimen processing. (i) Culture. The FLOQSwab rectal swab specimens were vortexed for 30 s, and 100 μl of transport medium was transferred onto MacConkey agar with crystal violet (Dalynn Biologicals, Calgary, AB, Canada), sheep blood agar (Oxoid, Thermo Fisher Scientific, Waltham, MA, USA), Hektoen agar (Dalynn Biologicals, Calgary, AB, Canada), cefsulodin, irgasan, and novobiocin agar (Dalynn Biologicals, Calgary, AB, Canada), CHROMagar O157 with 2.5 mg/liter potassium tellurite (CHROMagar, Paris, France), and *Campylobacter* blood-free selective agar (Dalynn Biologicals, Calgary, AB, Canada) plates. Overnight enrichment was performed by adding ~200 μl of bulk stool to mannitol selenite broth followed by culture on *Salmonella-Shigella* (Dalynn Biologicals, Calgary, AB, Canada) and Wilson-Blair (ProvLab) agar. *Campylobacter* plates were incubated microaerobically (in 6.0% O_2, 7.1% CO_2, 3.6% H_2, and 83.3% N_2 at 42°C for up to 72 h); all other media were incubated in atmospheric oxygen (35°C, 24 h). A quantity of stool samples of <1 g was considered insufficient and not subjected to culture. Identification of isolates was performed per routine laboratory confirmation protocols using an API 20E system (bioMérieux Inc., USA) in ProvLab, Calgary, AB, Canada, while a Vitek MS system (bioMérieux, St-Laurent, QC, Canada) and supplemental biochemical methods were used in ProvLab, Edmonton, AB, Canada. *Salmonella* serotyping was performed using the Check & Trace *Salmonella* assay (Check-Points, Netherlands) (20). All positive cultures were skimmed and frozen at −80°C.

(ii) Molecular diagnostics. The GPP is a qualitative multiplex molecular-based syndromic panel that identifies nine bacterial targets (*Campylobacter* spp., *Clostridioides* [formerly *Clostridium*] *difficile* toxin A/B, *E. coli* O157, enterotoxigenic *E. coli* [ETEC], Shiga toxin-producing *E. coli* [STEC] stx_1 and stx_2, *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, *Yersinia* spp.) (8, 21). GPP testing was performed at a single site (ProvLab, Edmonton, AB, Canada). Immediately before nucleic acid extraction, stools were thawed and aliquots were made, and the aliquots were then refrozen at −80°C. Rectal swab samples remained frozen until extraction. Samples were batch tested two to three times a week, depending on specimen volumes.

The FLOQSwab rectal swab specimens were vortexed in 500 μl of NucliSENS lysis buffer, and 300 μl of this lysate was added to 700 μl of lysis buffer in Bertin SK38 solid grinding lysis bead tubes with 10 μl of bacteriophage MS2 (each from Luminex Molecular Diagnostics, Toronto, ON, Canada). Similarly, 100 to 150 mg or 100 μl of solid or liquid stool, respectively, was suspended in a final volume of 1 ml of lysis buffer. Two hundred microliters of prepared lysates was extracted using a NucliSENS easyMAG system (bioMérieux, Marcy l'Etoile, France). Total nucleic acids were eluted in a volume of 70 μl and tested per the recommendations for the xTAG GPP kit. All extracts were stored at −80°C until confirmatory testing.

Confirmatory testing. (i) Testing of GPP-negative/culture-positive *Salmonella* isolates. *Salmonella* species isolates were cultured overnight (37°C) on sheep blood agar, and a single colony was transferred into Bertin SK38 tubes containing 1 ml of NucliSENS lysis buffer. Lysis and extraction were performed per the recommendations for the testing of stool by the xTAG GPP.

We also performed RT-qPCR using a TaqMan chemistry-based quantitative PCR (qPCR) targeting a conserved region of the *Salmonella invA* gene. The forward primer (CTGCGGTACTGTTAATTAC), reverse primer (GAACGTGGCGATAATTTC), and dual-quenched probe (6-FAM-CGGCATCGG/ZEN/CTTCAATCAAG A-Iowa Black FQ; where 6-FAM is 6-carboxyfluorescein) (Integrated DNA Technologies [IDT], Coralville, IA, USA) were designed using Beacon Designer (version 8.20) software (Premier Biosoft, Palo Alto, CA, USA). Five-microliter nucleic acid extracts were used in a 20-μl total qPCR volume with 2× PrimeTime gene expression master mix (IDT, Coralville, IA, USA) with a 0.222 μM final probe concentration and a 0.333 μM final primer concentration. A fast cycling protocol with an initial 95°C polymerase activation step for 3 min, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s, was performed on an Applied Biosystems 7500 Fast instrument. Positive controls with DNA extracted from *Salmonella enterica* serovar Enteritidis and no-template controls were integrated into each qPCR run. qPCR assay optimization included evaluation against a specificity panel of four clinical *Salmonella* serotypes (*Salmonella Enteritidis*, *Salmonella* Heidelberg, *Salmonella* Typhimurium, *Salmonella* Newport), *Yersinia enterocolitica* (ATCC 9610), *Shigella sonnei* (clinical isolate A79), and *Shigella flexneri* (ATCC 12022).

(ii) RT-qPCR with nucleic acid extracts of GPP-positive/culture-positive specimens. As described above for GPP-negative/culture-positive *Salmonella* isolates, we performed RT-qPCR to confirm the result for GPP-positive/culture-positive specimens and to validate our assay.

(iii) Evaluation of GPP-positive/culture-negative specimens. The frozen DNA extracts used on the Luminex xTAG GPP were thawed and tested using the Prodesse ProGastro SCSS assay (Hologic Inc., San Diego, CA) on a SmartCycler II instrument (Cepheid, Sunnyvale, CA) per manufacturer guidelines. The latter tests for *Salmonella*, *Shigella*, and *Campylobacter* nucleic acids and Shiga toxin 1 and 2 genes (18).

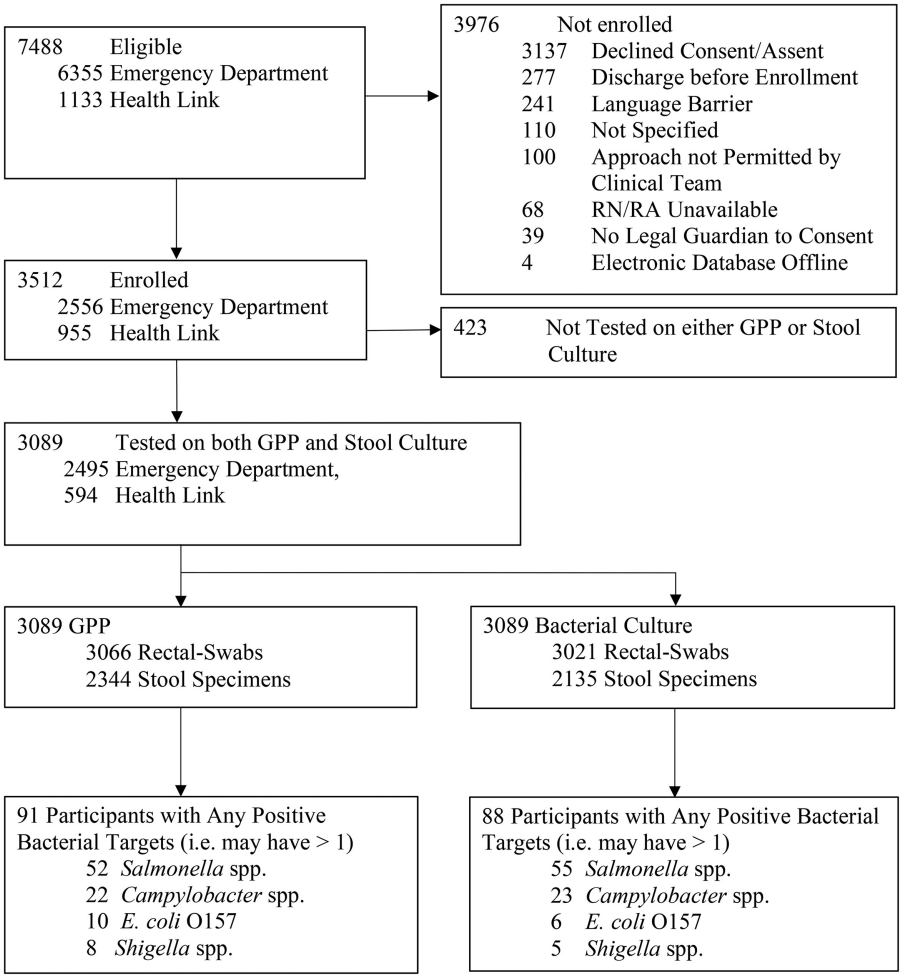

FIG 1 Flow diagram of potential study participants and enteropathogen detection methods. GPP, gastrointestinal pathogen panel.

As described above, we performed RT-qPCR to evaluate specimens GPP positive/culture negative for *Salmonella* spp. We performed a similar evaluation for *Shigella* spp., STEC, and *E. coli* O157. The primers and probes used are described in Appendix S1 in the supplemental material.

Statistical analysis. Test results were categorized as positive or negative, with no indeterminant readings. We did not differentiate between specimen types because rectal swabs have diagnostic capabilities similar to those of stool samples (19). Only participants whose specimens underwent culture and GPP testing were analyzed.

Overall percent agreement between the GPP and bacterial culture was determined for the pathogens of interest (22). For assessing positive response rates, PPA was calculated as $[(A)/(A + C)] \times 100\%$, where A is the number of specimens with concordant positive results and C is the number of specimens with GPP-negative/culture-positive results (22). PPA and overall percent agreement were chosen over sensitivity and specificity per U.S. Food and Drug Administration guidelines when evaluating diagnostic tests in the absence of a gold standard (22). Cohen's κ value was calculated to measure the extent of agreement (23). Clinical characteristics were compared using a chi-square test and the Kruskal-Wallis H test, as appropriate. The Mann-Whitney U test was used to compare RT-qPCR C_T values of the rectal swab and stool specimen groups GPP positive/culture positive and GPP negative/culture positive for *Salmonella* spp.

We did not use imputation because data from only seven participants were incomplete; we did include all available data from these participants in our analysis. SPSS (version 24.0; IBM Corp., Armonk, NY) was used to perform analyses. A single 2-tailed P value was used to assess differences among all three groups, and the significance level was set at 0.05.

RESULTS

Specimens from 3,089 (88.0%) of the 3,511 participants enrolled between 12 December 2014 and 31 March 2018 underwent GPP and bacterial culture (Fig. 1).

TABLE 1 PPA and overall percent agreement between the Luminex xTAG GPP and bacterial culture of stool and rectal swab specimens[a]

Bacterial targets	Luminex xTAG GPP result	No. of specimens with the following bacterial culture result:		PPA (95% CI)	Overall % agreement (95% CI)	Cohen's κ
		Positive	Negative			
Campylobacter spp.	Positive	20	2	87.0 (65.3, 96.6)	99.8 (99.6, 99.9)	0.89
	Negative	3	3,064			
Escherichia coli O157	Positive	6	4	100 (51.7, 100)	99.9 (99.6, 99.9)	0.75
	Negative	0	3,079			
Salmonella spp.	Positive	43	9	78.2 (64.6, 87.8)	99.3 (98.9, 99.6)	0.80
	Negative	12	3,025			
Shigella spp.	Positive	5	3	100 (46.3, 100)	99.9 (99.7, 99.97)	0.77
	Negative	0	3,081			
Total	Positive	73	18	83.0 (73.1, 89.8)	98.9 (98.5, 99.25)	0.81
	Negative	15	2,983			

[a]The bacterial targets included in this table were identified by both the Luminex xTAG gastrointestinal pathogen panel (GPP) and bacterial culture.

Ninety-one (3.0%) and 88 (2.9%) of these specimens, respectively, were positive for at least one of *Campylobacter* spp., *E. coli* O157, *Salmonella* spp., or *Shigella* spp.

Primary outcome. The overall percent agreement was >99% for each individual bacterial target and 98.9% (95% confidence interval [CI], 98.5%, 99.3%) for all targets combined (Table 1). PPA ranged from 78.2% for *Salmonella* spp. (95% CI, 64.6%, 87.8%) to 100% for *E. coli* O157 (95% CI, 51.7%, 100%). Overall, PPA was 83.0% (73/88; 95% CI, 73.1%, 89.8%). Cohen's κ was >0.70 for *E. coli* O157, *Shigella* spp., and *Salmonella* spp. and almost perfect for *Campylobacter* spp. ($\kappa = 0.89$).

Secondary outcomes for *Salmonella*. The most frequently identified pathogen, *Salmonella* spp., was detected in 64 participants (Fig. 2). *Salmonella* spp. were detected in 80% (51/64) of submitted rectal swabs (positive results, $n = 5$ for GPP only, $n = 12$ for culture only, $n = 34$ for both) and 84% (43/51) of submitted stool specimens (positive results, $n = 7$ for GPP only, $n = 8$ for culture only, $n = 28$ for both). *Salmonella Enteritidis* accounted for 48% (31/64) of the *Salmonella* spp. identified by culture (Table 2). Sixty-seven percent (43/64) of the participants with *Salmonella* spp. detected in swab or stool produced concordant GPP and culture test results; 19% (12/64) were GPP negative/culture positive, and 14% (9/64) were GPP positive/culture negative. Rectal swabs and stool samples had similar detection rates for *Salmonella* spp., with the exception of GPP-positive/culture-negative specimens, where only 33% (3/9) of the rectal swabs but 88% (7/8) of the stool samples were positive (Table 3).

Participants in the group GPP positive/culture positive for *Salmonella* spp. were older (median age, 38 months [interquartile range {IQR}, 13, 63 months]) than those in the GPP-positive/culture-negative group (median age, 7 months [IQR, 5, 16 months]) (Table 4). The latter group of children had significantly more vomiting episodes in the 24 h before enrollment (median, 6 [IQR, 2, 13]) than those with concordant positive specimens (median, 1 [IQR, 0, 3]) or GPP-negative/culture-positive specimens (median, 1 [IQR, 0, 4]). All 43 GPP-positive/culture-positive specimens were provided by children who presented with diarrhea; only 44% (4/9) of GPP-positive/culture-negative children had had diarrhea prior to enrollment ($P < 0.001$). The maximum number of diarrheal episodes per 24-h period before enrollment was greater in children with specimens with concordant results (median, 10 [IQR, 7, 15]) than in children with GPP-positive/culture-negative specimens (median, 0 [IQR, 0, 9]) ($P = 0.002$).

Confirmatory testing. Five of 5 (100%) stool specimens and 8/8 (100%) rectal swab specimens GPP negative/culture positive for *Salmonella* isolates were positive by confirmatory testing by GPP (Fig. 2). *Salmonella* qPCR of GPP-negative/culture-positive extracts was positive for 4/12 (25%) rectal swab specimens and 1/5 (20%) stool specimens. Of the concordant GPP-positive/culture-positive specimens, 36/38 (94.7%)

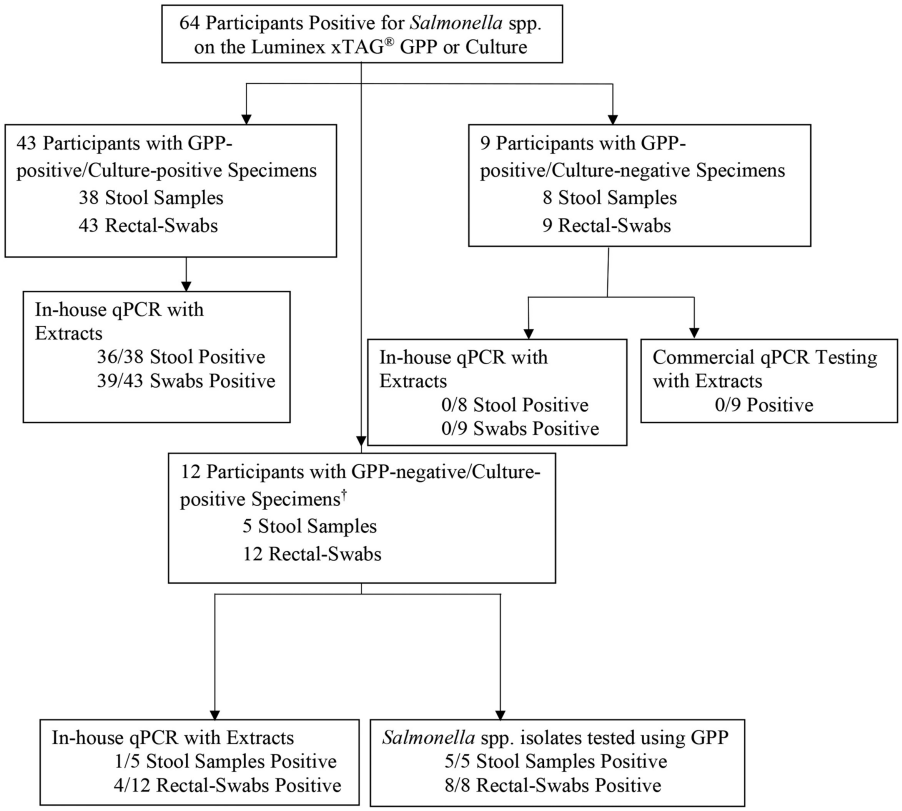

FIG 2 Confirmatory testing flow diagram of *Salmonella* species-positive specimens. All available specimens were tested. Participants may have submitted both a swab and a stool sample. GPP, gastrointestinal pathogen panel; qPCR, quantitative PCR; †, GPP-negative/culture-positive samples were not retested with the commercial assay.

stool samples and 39/43 (90.7%) rectal swab specimens were positive on qPCR, with median cycle threshold (C_T) values being 29.0 (IQR, 24.8, 30.8) for stool and 30.0 (IQR, 26.2, 33.2) for rectal swabs ($P = 0.02$ for the 32 paired specimens). Median C_T values were higher for GPP-negative/culture-positive than for GPP-positive/culture-positive *Salmonella* rectal swab (36.9 [IQR, 33.7, 37.1] versus 30.0 [IQR, 26.2, 33.2]; $P = 0.002$) and stool specimen extract (36.9 [IQR, 33.7, 37.1] versus 29.0 [IQR, 24.8, 30.8]; $P = 0.001$).

Retesting of GPP-positive/culture-negative specimen extracts yielded the same results on both the Prodesse ProGastro SCSS and in-house qPCR assays (except for *Campylobacter* spp., for which in-house primers were not developed): 0/4 *E. coli* O157, 0/9 *Salmonella* species, and 2/3 *Shigella* species isolates were positive for the bacteria detected initially on the GPP. Both *Campylobacter* species-positive specimens were negative on commercial PCR testing (Table 5).

DISCUSSION

We found excellent overall agreement between the GPP and bacterial culture. For *Salmonella* spp., however, GPP-positive/culture-negative children differed significantly from those for whom the results were concordant. Confirmatory analyses demonstrated that GPP-positive/culture-negative specimens likely are false positive, while GPP-negative/culture-positive specimens reflect the inability of the assay to detect *Salmonella* spp. when small quantities of nucleic acid are present.

A meta-analysis comparing a variety of multiplex gastrointestinal panels with standard microbiology methods reported that PPA ranged from 68% when NAAT methods provide the benchmark to 93% when conventional methods are considered the gold standard (24). However, the high PPA obtained when conventional methods served as the benchmark was inconsistent across pathogens, a concern that we identified as it

TABLE 2 Serotype analysis of *Salmonella* species-positive specimens on the Luminex xTAG GPP and bacterial culture

Salmonella serovar	No. (%) of specimens with the following result:			
	Total	Luminex xTAG GPP positive/culture negative	Luminex xTAG GPP negative/culture positive	Luminex xTAG GPP positive/culture positive
Untyped or nontypeable[a]	10 (15.6)	9	0	1
Salmonella Agona	1 (1.6)	0	0	1
Salmonella Alachua	1 (1.6)	0	0	1
Salmonella Anatum	1 (1.6)	0	1	0
Salmonella Bareilly	1 (1.6)	0	0	1
Salmonella enterica subsp. *enterica*	4 (6.3)	0	0	4
Salmonella enterica subsp. *salamae* (II)55:k:z39	1 (1.6)	0	0	1
Salmonella Enteritidis	31 (48.4)	0	7	24
Salmonella Heidelberg	1 (1.6)	0	0	1
Salmonella Infantis	2 (3.1)	0	1	1
Salmonella Newport	1 (1.6)	0	0	1
Salmonella Reading	2 (3.1)	0	0	2
Salmonella Rubislaw	1 (1.6)	0	0	1
Salmonella Schwarzengrund	1 (1.6)	0	0	1
Salmonella Thompson	1 (1.6)	0	0	1
Salmonella Typhi	1 (1.6)	0	0	1
Salmonella Typhimurium	3 (4.7)	0	2	1
Salmonella Virchow	1 (1.6)	0	1	0
Total	64 (100)	9 (14.1)	12 (18.8)	43 (68.8)

[a]Of these, isolates were detected in only 9/10 specimens by the Luminex xTAG GPP. The final specimen was positive for a *Salmonella* sp. on GPP and culture, but the serovar was not identified.

relates to *Salmonella* spp. A Vietnamese report identified a lack of specificity of *Salmonella* species detection using the GPP (5), potentially due to a high rate of *Salmonella* carriage (25) or amplification of DNA from non-*Salmonella* strains. In a U.S. multicenter study, 14% of *Salmonella* spp. detected by GPP could not be confirmed by gold standard methods (26).

Given that specimens GPP negative/culture positive for *Salmonella* had higher C_T values than GPP-positive/culture-positive specimens, our data do not support the hypothesis that culture fails to detect pathogens because it is less sensitive. While prolonged storage under suboptimal conditions may permit nucleic acid degradation (27) and, hence, a lower yield on NAAT platforms, in our study, all samples were processed within 7 days of receipt and both aliquots and extracts were stored at −80°C at all times. This approach led to our finding a PPA of 78%, which is aligned with previous reports (27, 28). While the inability to detect some pathogens on the GPP could reflect inefficient nucleic acid extraction or inhibition, many of the same nucleic acid extracts yielded positive results with our in-house assay and the alternate platform. Our RT-qPCR results and our retesting of culture-positive *Salmonella* isolates on the GPP lead us to believe that the discrepancies represent a sensitivity threshold, with there

TABLE 3 Comparison of rectal swab and stool specimen results for *Salmonella* spp. grouped by diagnostic testing approach[b]

Salmonella species-positive group	Specimen type	No. of specimens with the indicated result/total no. of specimens tested (%)		
		Luminex xTAG GPP positive	Culture positive	qPCR positive
GPP positive/culture positive	Stool	34/38 (89.5)	31/38 (81.6)	36/38 (94.7)
	Rectal swab	36/43 (83.7)	38/43 (88.4)	39/43 (90.7)
GPP positive/culture negative	Stool	7/8 (87.5)[a]	0/8 (0)	0/8 (0)
	Rectal swab	3/9 (33.3)[a]	0/9 (0)	0/9 (0)
GPP negative/culture positive	Stool	0/5 (0)	5/5 (100)	1/5 (20.0)
	Rectal swab	0/12 (0)	8/12 (66.7)	4/12 (33.3)

[a]$P = 0.05$. No other comparisons achieved statistical significance.
[b]GPP, gastrointestinal pathogen panel; qPCR, quantitative PCR.

TABLE 4 Clinical characteristics and enteropathogen codetection among participants with concordant and discordant *Salmonella* species test results[g]

Clinical feature	Luminex xTAG GPP positive/culture positive		Luminex xTAG GPP positive/culture negative		Luminex xTAG GPP negative/culture positive		*P* value
	No. of specimens	Value	No. of specimens	Value	No. of specimens	Value	
Age (mo)[b]	43	38 (13, 63)	9	7 (5, 16)	12	31 (11, 66)	0.006
Time since symptom onset (h)[b,c]	43	84 (45, 102)	9	49 (13, 66)	12	56 (31, 87)	0.05
No. (%) of patients with vomiting[a]	43	23 (54)	9	7 (78)	12	6 (50)	0.44
Maximum no. of vomiting episodes in any 24-h period[b]	43	1 (0, 3)	9	6 (2, 13)	12	1 (0, 4)	0.02
Duration of vomiting at time of enrollment (h)[b]	43	2 (0, 75)	9	16 (2, 66)	12	0.2 (0, 37)	0.47
No. (%) of patients with diarrhea[a]	43	43 (100)	9	4 (44)	12	9 (75)	<0.001
Maximum no. of diarrhea episodes in any 24-h period[b]	43	10 (7, 15)	9	0 (0, 9)	12	7 (1, 12)	0.002
Duration of diarrhea at time of enrollment (h)[b]	43	83 (37, 101)	9	0 (0, 65)	12	34 (1, 87)	0.02
No. (%) of patients with fever[a]	43	32 (74)	9	6 (67)	12	6 (50)	0.14
Maximum pain level in prior 24 h[b,d]	39	7 (5, 8)	7	4 (0, 8)	11	8 (5, 10)	0.15
No. (%) of specimens with non-*Salmonella* pathogens present[a,e]	43	11 (26)	9	6 (67)	12	3 (25)	0.05
Median (IQR) qPCR C_T value							
Rectal swab	39	30 (26, 33)	0	NA	4	37 (34, 37)	0.002[f]
Stool	36	29 (25, 31)	0	NA	1	38	0.05[f]

[a]*P* value by the chi-square test across all three groups.
[b]*P* value by the Kruskal-Wallis H test across all three groups.
[c]Defined by the time of onset of either vomiting or diarrhea, whichever was greater.
[d]Pain level was reported by the parents of the participants on a scale ranging from 0 (no pain at all) to 10 (worst pain imaginable) in the 24 h prior to enrollment (only for patients enrolled in the emergency departments).
[e]This included detection of any of the following targets by GPP, the gastroenteritis virus panel, and/or stool culture in any of the specimens (i.e., swab, stool): adenovirus, astrovirus, norovirus, rotavirus, sapovirus, *Aeromonas*, *Campylobacter*, *C. difficile*, *V. cholerae*, *E. coli* O157, ETEC, *Shigella*, STEC, *Yersinia*, *Cryptosporidium*, *Entamoeba*, or *Giardia*.
[f]Mann-Whitney U test for comparison of two groups.
[g]GPP, gastrointestinal pathogen panel; qPCR, quantitative PCR; NA, not available.

being less target pathogen nucleic acid present in the GPP-negative/culture-positive group.

The clinical presentations of participants GPP positive/culture negative for *Salmonella* spp. were at variance with those of individuals with classic *Salmonella* infection (29). Retesting of these specimens using commercial and in-house assays provided negative results, indicating that the GPP results were likely false positive. All specimens GPP positive/culture negative for *E. coli* O157 were also negative when tested with an alternate commercial and in-house assay, suggesting an overall suboptimal specificity for these targets. Concerns regarding false positives due to cross-reactivity with commensal bacteria have also been reported with the BioFire FilmArray gastrointestinal panel (4). Other possible causes for false positives on the NAAT include cross contamination, amplification of a target from a non-*Salmonella* strain, or detection of *Salmonella* spp. in a child who is a carrier. Contamination can be addressed by engineering or procedure modifications, and non-*Salmonella* species amplification can be minimized by including corroborative loci. For example, in the case of *E. coli* O157, the presence of conserved loci for intimin (*eae*) (30) and a component of the O157 side chain synthesis cluster (*rfbE*) (31) increases confidence that a Shiga toxin gene signal originates in a bona fide pathogen. Nonetheless, these findings highlight the concern regarding false-positive results when a multiplex NAAT is employed

TABLE 5 Analysis of specimens Luminex xTAG GPP positive/bacterial culture negative for *Campylobacter* spp., *Escherichia coli* O157, *Salmonella* spp., and *Shigella* spp. on commercial and in-house PCR assays

Bacteria with positive test result on GPP	No. of specimens	Commercial assay retest result	In-house PCR result
Campylobacter spp.	2	Negative	NA[a]
Escherichia coli O157	4	Negative	Negative
Salmonella spp.	9	Negative	Negative
Shigella spp.	2	Positive	Positive
Shigella spp.	1	Negative	Negative

[a]NA, not applicable (our in-house assay did not include primers for *Campylobacter* spp.).

and the importance of considering such results with the clinical presentation and codetected pathogens in mind (3, 12).

Although we might be underpowered to detect problems with the identification of *E. coli* O157 and *Shigella* spp. by the GPP, the panel identified all culture-positive participants. The rapid identification of these two pathogens is of considerable value. Detecting *E. coli* O157 infection would prompt the withholding or discontinuation of antibiotics (32) and the reversal of dehydration using parenteral fluids (33–35); the detection of *Shigella* would appropriately prompt antibiotic therapy (36, 37). Evidence supporting this assertion was provided by a study of the BioFire FilmArray GI panel, which reduced the time to initiation of antimicrobial therapy by 50 h and the time to the discontinuation of therapy in STEC-infected children by 47 h (12).

Selenite enrichment broth was employed as part of our stool culture protocol to enhance the identification of *Salmonella* spp. (38). By comparison, false-negative results by NAAT may relate to technical issues, including nucleic acid extraction efficiency. Future iterations of NAAT-based multianalyte syndromic panels should consider protocol modifications that enhance diagnostic accuracy, including alternative approaches to extraction, modified amplification or cycling conditions, and the incorporation of additional pathogen loci to reduce the number of panel-positive/culture-negative results.

Given our data, the existing literature, and the adjustments required for NAAT assays, one needs to consider how postmarket monitoring of the performance of multianalyte assays should be performed. Specifically, if laboratories abandon culture detection of enteric pathogens in favor of multianalyte assays, there is little opportunity to compare recovery rates between the two methodologies. Hence, the failure to detect pathogens by multianalyte assays will go unverified. The U.S. Food and Drug Administration has postmarket surveillance mechanisms for drug safety and efficacy, especially for those drugs approved via accelerated pathways (39, 40), but no analogous mechanism exists for diagnostic devices. Our findings urge continuing assessment of the accuracy of multianalyte assays for bacterial enteric pathogens.

To our knowledge, this is the first study to compare GPP with bacterial culture employing samples from an entirely pediatric population in a high-income country. It is also the only study to incorporate the clinical phenotype into the interpretation of discordant specimens. Our study nonetheless has limitations. Despite our large sample size, we detected only 64 participants with *Salmonella* species-positive specimens, and *Campylobacter* species-, STEC-, and *Shigella* species-positive specimens were even less common. The low prevalence of bacterial enteropathogens has implications for our reported percent agreement and κ calculations. Participants were recruited from only two western Canadian cities, so our findings cannot be automatically generalized to locations with a different epidemiology. We, unfortunately, do not have data to enable calculation of the precise time interval between specimen receipt at the laboratory and culture setup. It should also be noted that sampling error may have occurred due to the small volume of nucleic acid extract used and that all specimens underwent a freeze-thaw cycle; both of these operational elements could explain negative GPP and qPCR results in the setting of low bacterial loads.

In conclusion, overall GPP results had excellent concordance with those of bacterial culture, but the PPA was suboptimal for the shared bacterial targets. In particular, *Salmonella* species identification with the GPP was prone to false positives and negatives. These results have clinical and public health implications. While the GPP platform and other NAAT assays have the potential to provide valuable and credible results, their current accuracy requires additional validation before universal abandonment of culture diagnostics. Careful consideration of the context of the illness in patients whose stool tests yield positive results is required.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.00205-19.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

We thank the patients and their families for cooperating with our study; Bryanne Crago and Christina Ferrato (Public Health Laboratory [ProvLab], Alberta Public Laboratories, Calgary, AB, Canada), Judy Qui (Department of Laboratory Medicine and Pathology, University of Alberta), DynaLIFE Dx Diagnostic Laboratory Services, community laboratories, as well as Public Health Laboratory (ProvLab), Alberta Public Laboratories, Edmonton and Calgary, especially the bacteriology staff, for their assistance with receiving, handling, and processing specimens; the emergency department research nurses and PEMRAP at the Alberta Children's Hospital for recruiting study participants; the emergency department bedside nurses for assisting with rectal swab performance; Nadia Dow and Manasi Rajagopal, as well as the research assistants, research nurses, and the Little Bit of Help research volunteer program, for their assistance with participant recruitment at the Stollery Children's Hospital; the nurses at Health Link who responded to calls from across the province for their assistance with participant recruitment; and Laurel Ryan for her role as a patient adviser. We extend special thanks to Marie Louie for building the connections that have made our endeavors possible. No compensation for the assistance of any aforementioned individuals was provided.

This work was supported by the Alberta Provincial Pediatric EnTeric Infection TEam (APPETITE), which was funded by a grant from the Alberta Innovates-Health Solutions Team Collaborative Research Innovation Opportunity. APPETITE is also supported through partnership awards by the Alberta Children's Hospital Research Institute (Calgary, AB, Canada) and the Women and Children's Hospital Research Institute (Edmonton, AB, Canada). S.B.F. is supported by the Alberta Children's Hospital Foundation Professorship in Child Health and Wellness. P.I.T. is also supported by grant number NIH P30DK052574 (ARAC Core, Digestive Diseases Research Core Center). The Pediatric Emergency Medicine Research Associate Program (PEMRAP) is supported by a grant from the Alberta Children's Hospital Foundation. T.K. was supported by summer studentship grants from the Alberta Health Services Emergency Strategic Clinical Network and the Alberta Children's Hospital Research Institute.

The APPETITE team receives financial support from Luminex Molecular Diagnostics, Austin, TX, USA, and bioMérieux Canada, Inc., St-Laurent, QC, Canada.

APPETITE collaborators include Samina Ali, Department of Pediatrics, University of Alberta, Edmonton, AB, Canada, and Kimberley Simmonds, Department of Community Health Sciences, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada, and Alberta Ministry of Health, Edmonton, AB, Canada.

REFERENCES

1. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. 2011. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis 17:7–15. https://doi.org/10.3201/eid1701.091101p1.
2. Crim SM, Iwamoto M, Huang JY, Griffin PM, Gilliss D, Cronquist AB, Cartter M, Tobin-D'Angelo M, Blythe D, Smith K, Lathrop S, Zansky S, Cieslak PR, Dunn J, Holt KG, Lance S, Tauxe R, Henao OL, Centers for Disease Control and Prevention (CDC). 2014. Incidence and trends of infection with pathogens transmitted commonly through food—Foodborne Diseases Active Surveillance Network, 10 U.S. sites, 2006–2013. MMWR Morb Mortal Wkly Rep 63:328–332.
3. Anderson NW, Tarr PI. 2018. Multiplex nucleic acid amplification testing to diagnose gut infections: challenges, opportunities, and result interpretation. Gastroenterol Clin North Am 47:793–812. https://doi.org/10.1016/j.gtc.2018.07.006.
4. Buss SN, Leber A, Chapin K, Fey PD, Bankowski MJ, Jones MK, Rogatcheva M, Kanack KJ, Bourzac KM. 2015. Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. J Clin Microbiol 53:915–925. https://doi.org/10.1128/JCM.02674-14.
5. Duong VT, Phat VV, Tuyen HT, Dung TT, Trung PD, Minh PV, Tu Le TP, Campbell JI, Le Phuc H, Ha TT, Ngoc NM, Huong NT, Tam PT, Huong DT, Xang NV, Dong N, Phuong Le T, Hung NV, Phu BD, Phuc TM, Thwaites GE, Vi LL, Rabaa MA, Thompson CN, Baker S. 2016. Evaluation of Luminex xTAG gastrointestinal pathogen panel assay for detection of multiple diarrheal pathogens in fecal samples in Vietnam. J Clin Microbiol 54:1094–1100. https://doi.org/10.1128/JCM.03321-15.
6. Albert MJ, Rotimi VO, Iqbal J, Chehadeh W. 2016. Evaluation of the xTAG gastrointestinal pathogen panel assay for the detection of enteric pathogens in Kuwait. Med Princ Pract 25:472–476. https://doi.org/10.1159/000447698.
7. Eibach D, Krumkamp R, Hahn A, Sarpong N, Adu-Sarkodie Y, Leva A, Kasmaier J, Panning M, May J, Tannich E. 2016. Application of a multiplex PCR assay for the detection of gastrointestinal pathogens in a rural African setting. BMC Infect Dis 16:150. https://doi.org/10.1186/s12879-016-1481-7.
8. Claas EC, Burnham CA, Mazzulli T, Templeton K, Topin F. 2013. Performance of the xTAG(R) gastrointestinal pathogen panel, a multiplex molecular assay for simultaneous detection of bacterial, viral, and parasitic causes of infectious gastroenteritis. J Microbiol Biotechnol 23:1041–1045. https://doi.org/10.4014/jmb.1212.12042.
9. Spina A, Kerr KG, Cormican M, Barbut F, Eigentler A, Zerva L, Tassios P, Popescu GA, Rafila A, Eerola E, Batista J, Maass M, Aschbacher R, Olsen KE, Allerberger F. 2015. Spectrum of enteropathogens detected by the FilmArray GI panel in a multicentre study of community-acquired gastroenteritis. Clin Microbiol Infect 21:719–728. https://doi.org/10.1016/j.cmi.2015.04.007.

10. Becker SL, Chatigre JK, Gohou JP, Coulibaly JT, Leuppi R, Polman K, Chappuis F, Mertens P, Herrmann M, N'Goran EK, Utzinger J, von Muller L. 2015. Combined stool-based multiplex PCR and microscopy for enhanced pathogen detection in patients with persistent diarrhoea and asymptomatic controls from Cote d'Ivoire. Clin Microbiol Infect 21: e1–e10. https://doi.org/10.1016/j.cmi.2015.02.016.
11. Wessels E, Rusman LG, van Bussel MJ, Claas EC. 2014. Added value of multiplex Luminex gastrointestinal pathogen panel (xTAG((R)) GPP) testing in the diagnosis of infectious gastroenteritis. Clin Microbiol Infect 20:O182–O187. https://doi.org/10.1111/1469-0691.12364.
12. Cybulski RJ, Jr, Bateman AC, Bourassa L, Bryan A, Beail B, Matsumoto J, Cookson BT, Fang FC. 2018. Clinical impact of a multiplex gastrointestinal polymerase chain reaction panel in patients with acute gastroenteritis. Clin Infect Dis 67:1688–1696. https://doi.org/10.1093/cid/ciy357.
13. Nicholson MR, Van Horn GT, Tang YW, Vinje J, Payne DC, Edwards KM, Chappell JD. 2016. Using multiplex molecular testing to determine the etiology of acute gastroenteritis in children. J Pediatr 176:50–56.e2. https://doi.org/10.1016/j.jpeds.2016.05.068.
14. Murphy CN, Fowler RC, Iwen PC, Fey PD. 2017. Evaluation of the BioFire FilmArray(R) gastrointestinal panel in a midwestern academic hospital. Eur J Clin Microbiol Infect Dis 36:747–754. https://doi.org/10.1007/s10096-016-2858-7.
15. Qin X, Klein EJ, Galanakis E, Thomas AA, Stapp JR, Rich S, Buccat AM, Tarr PI. 2015. Real-time PCR assay for detection and differentiation of Shiga toxin-producing Escherichia coli from clinical samples. J Clin Microbiol 53:2148–2153. https://doi.org/10.1128/JCM.00115-15.
16. Freedman SB, Lee BE, Louie M, Pang XL, Ali S, Chuck A, Chui L, Currie GR, Dickinson J, Drews SJ, Eltorki M, Graham T, Jiang X, Johnson DW, Kellner J, Lavoie M, MacDonald J, MacDonald S, Svenson LW, Talbot J, Tarr P, Tellier R, Vanderkooi OG. 2015. Alberta Provincial Pediatric EnTeric Infection TEam (APPETITE): epidemiology, emerging organisms, and economics. BMC Pediatr 15:89. https://doi.org/10.1186/s12887-015-0407-7.
17. Letourneau S. 2009. Health Link Alberta: a model for successful health service integration. Healthc Q 13(Spec No):56–60.
18. Buchan BW, Olson WJ, Pezewski M, Marcon MJ, Novicki T, Uphoff TS, Chandramohan L, Revell P, Ledeboer NA. 2013. Clinical evaluation of a real-time PCR assay for identification of Salmonella, Shigella, Campylobacter (Campylobacter jejuni and C. coli), and Shiga toxin-producing Escherichia coli isolates in stool specimens. J Clin Microbiol 51: 4001–4007. https://doi.org/10.1128/JCM.02056-13.
19. Freedman SB, Xie J, Nettel-Aguirre A, Lee B, Chui L, Pang X-L, Zhuo R, Parsons B, Dickinson JA, Vanderkooi OG, Ali S, Osterreicher L, Lowerison K, Tarr PI, Chuck A, Currie G, Eltorki M, Graham T, Jiang J, Johnson D, Kellner J, Lavoie M, Louie M, MacDonald J, MacDonald S, Simmonds K, Svenson L, Tellier R, Drews S, Talbot J. 2017. Enteropathogen detection in children with diarrhoea, or vomiting, or both, comparing rectal flocked swabs with stool specimens: an outpatient cohort study. Lancet Gastroenterol Hepatol 2:662–669. https://doi.org/10.1016/S2468-1253(17)30160-7.
20. Ferrato C, Chui L, King R, Louie M. 2017. Utilization of a molecular serotyping method for Salmonella enterica in a routine laboratory in Alberta Canada. J Microbiol Methods 135:14–19. https://doi.org/10.1016/j.mimet.2017.01.018.
21. Anonymous. 2018. xTAG gastrointestinal pathogen panel (GPP) (CE-IVD), Luminex Corporation, Austin, TX. Accessed 8 December 2018.
22. U.S. Food and Drug Administration. 2007. Statistical guidance on reporting results from studies evaluating diagnostic tests—guidance for industry and FDA staff. https://www.fda.gov/RegulatoryInformation/Guidances/ucm071148.htm#top. Accessed 31 July 2018.
23. Feuerman M, Miller AR. 2008. Relationships between statistical measures of agreement: sensitivity, specificity and kappa. J Eval Clin Pract 14: 930–933. https://doi.org/10.1111/j.1365-2753.2008.00984.x.
24. Freeman K, Tsertsvadze A, Taylor-Phillips S, McCarthy N, Mistry H, Manuel R, Mason J. 2017. Agreement between gastrointestinal panel testing and standard microbiology methods for detecting pathogens in suspected infectious gastroenteritis: test evaluation and meta-analysis in the absence of a reference standard. PLoS One 12:e0173196. https://doi.org/10.1371/journal.pone.0173196.
25. Thompson CN, Phan MV, Hoang NV, Minh PV, Vinh NT, Thuy CT, Nga TT, Rabaa MA, Duy PT, Dung TT, Phat VV, Nga TV, Tu LT, Tuyen HT, Yoshihara K, Jenkins C, Duong VT, Phuc HL, Tuyet PT, Ngoc NM, Vinh H, Chinh NT, Thuong TC, Tuan HM, Hien TT, Campbell JI, Chau NV, Thwaites G, Baker S. 2015. A prospective multi-center observational study of children hospitalized with diarrhea in Ho Chi Minh City, Vietnam. Am J Trop Med Hyg 92:1045–1052. https://doi.org/10.4269/ajtmh.14-0655.
26. Patel A, Navidad J, Bhattacharyya S. 2014. Site-specific clinical evaluation of the Luminex xTAG gastrointestinal pathogen panel for detection of infectious gastroenteritis in fecal specimens. J Clin Microbiol 52: 3068–3071. https://doi.org/10.1128/JCM.01393-14.
27. Khare R, Espy MJ, Cebelinski E, Boxrud D, Sloan LM, Cunningham SA, Pritt BS, Patel R, Binnicker MJ. 2014. Comparative evaluation of two commercial multiplex panels for detection of gastrointestinal pathogens by use of clinical stool specimens. J Clin Microbiol 52:3667–3673. https://doi.org/10.1128/JCM.01637-14.
28. Huang RS, Johnson CL, Pritchard L, Hepler R, Ton TT, Dunn JJ. 2016. Performance of the Verigene(R) enteric pathogens test, Biofire FilmArray gastrointestinal panel and Luminex xTAG(R) gastrointestinal pathogen panel for detection of common enteric pathogens. Diagn Microbiol Infect Dis 86:336–339. https://doi.org/10.1016/j.diagmicrobio.2016.09.013.
29. Liu LJ, Yang YJ, Kuo PH, Wang SM, Liu CC. 2005. Diagnostic value of bacterial stool cultures and viral antigen tests based on clinical manifestations of acute gastroenteritis in pediatric patients. Eur J Clin Microbiol Infect Dis 24:559–561. https://doi.org/10.1007/s10096-005-1373-z.
30. Jerse AE, Yu J, Tall BD, Kaper JB. 1990. A genetic locus of enteropathogenic Escherichia coli necessary for the production of attaching and effacing lesions on tissue culture cells. Proc Natl Acad Sci U S A 87: 7839–7843. https://doi.org/10.1073/pnas.87.20.7839.
31. Bilge SS, Vary JC, Jr, Dowell SF, Tarr PI. 1996. Role of the Escherichia coli O157:H7 O side chain in adherence and analysis of an rfb locus. Infect Immun 64:4795–4801.
32. Freedman SB, Xie J, Neufeld MS, Hamilton WL, Hartling L, Tarr PI, Alberta Provincial Pediatric Enteric Infection Team, Nettel-Aguirre A, Chuck A, Lee B, Johnson D, Currie G, Talbot J, Jiang J, Dickinson J, Kellner J, MacDonald J, Svenson L, Chui L, Louie M, Lavoie M, Eltorki M, Vanderkooi O, Tellier R, Ali S, Drews S, Graham T, Pang XL. 2016. Shiga toxin-producing Escherichia coli infection, antibiotics, and risk of developing hemolytic uremic syndrome: a meta-analysis. Clin Infect Dis 62: 1251–1258. https://doi.org/10.1093/cid/ciw099.
33. Ake JA, Jelacic S, Ciol MA, Watkins SL, Murray KF, Christie DL, Klein EJ, Tarr PI. 2005. Relative nephroprotection during Escherichia coli O157:H7 infections: association with intravenous volume expansion. Pediatrics 115:e673–e680. https://doi.org/10.1542/peds.2004-2236.
34. Hickey CA, Beattie TJ, Cowieson J, Miyashita Y, Strife CF, Frem JC, Peterson JM, Butani L, Jones DP, Havens PL, Patel HP, Wong CS, Andreoli SP, Rothbaum RJ, Beck AM, Tarr PI. 2011. Early volume expansion during diarrhea and relative nephroprotection during subsequent hemolytic uremic syndrome. Arch Pediatr Adolesc Med 165:884–889. https://doi.org/10.1001/archpediatrics.2011.152.
35. Grisaru S, Ruhl M, Vanderkooi O, Berenger B, Freedman SB. 2018. Detection of Shiga toxin producing pathogens in stool samples of children with hemolytic uremic syndrome—a single center quality assurance project. Abstr 10th Int Symp Shiga Toxin (Verocytotoxin) Producing Escherichia coli Infect, Florence, Italy.
36. American Academy of Pediatrics. 2018. Shigella infections, p 723–727. *In* Kimberlin DW, Brady MT, Jackson MA, Long SS (ed), Red book: 2018: report of the Committee on Infectious Diseases, 31st ed, vol 2018. American Academy of Pediatrics, Itasca, IL.
37. Haltalin KC, Kusmiesz HT, Hinton LV, Nelson JD. 1972. Treatment of acute diarrhea in outpatients. Double-blind study comparing ampicillin and placebo. Am J Dis Child 124:554–561. https://doi.org/10.1001/archpedi.1972.02110160092010.
38. Boer MD, de Boer RF, Lameijer A, Sterne E, Skidmore B, Suijkerbuijk AWM, Heck M, van der Zanden A. 2019. Selenite enrichment broth to improve the sensitivity in molecular diagnostics of Salmonella. J Microbiol Methods 157:59–64. https://doi.org/10.1016/j.mimet.2018.12.018.
39. Institute of Medicine. 2007. The future of drug safety: promoting and protecting the health of the public. National Academies Press, Washington, DC.
40. U.S. Food and Drug Administration. 2017. Postmarket drug and biologic safety evaluations. https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Surveillance/ucm204091.htm. Accessed 16 March 2019.