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Verification of analytical bacterial spectrum of QIAstat-Dx® GI V2 and Novodiag® Bacterial GE+ V2-0 diagnostic panels

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Background: Implementing multiplex PCR or syndromic panel-based testing platforms to detect microbial species that cause acute diarrhoea may guide patient management more effectively and efficiently.

Objectives: To assess and compare the performance of two syndromic panel-based testing systems, QIAstat-Dx® Gastrointestinal Panel V2 (QGI) and the Novodiag® Bacterial GE+ V2-0 (NGE).

Methods: The QGI and NGE panels include 16 and 14 bacterial gastrointestinal pathogens, respectively. The performance of the panels was tested retrospectively using 141 positive clinical stool specimens, External Quality Assessment (EQA) panels and spiked faecal specimens.

Results: For *Campylobacter jejuni* and *coli* ($n = 20$), *Salmonella* ($n = 24$), *Shigella* ($n = 13$), *Yersinia enterocolitica* (non-1A biotypes) ($n = 8$), *Clostridioides difficile* ($n = 24$) and *Vibrio parahaemolyticus* ($n = 2$), QGI correctly verified 19/20, 20/24, 13/13, 8/8, 23/24 and 2/2, whereas NGE correctly verified 20/20, 17/24, 13/13, 8/8, 14/24 and 1/2. Among diarrhoeagenic *Escherichia coli* ($n = 29$), QGI reported one Shiga toxin-producing *E. coli* (STEC) *stx1a* O26:H11 as STEC serotype O157:H7 and NGE failed on one enteropathogenic *E. coli*, one enteroaggregative *E. coli* and one STEC (*stx2e*). *Y. enterocolitica* biotype 1A (non-pathogenic) ($n = 6$) were all positive in QGI, but negative in NGE.

Conclusions: Both QGI and NGE testing panels can improve laboratory workflow and patient management by providing user-friendly platforms that can rapidly detect a number of targets with one specimen. QGI was significantly more sensitive in identifying *C. difficile*. Both methods had suboptimal detection of *Salmonella* and this needs to be examined further. The short hands-on time and turnaround time are of value for on-demand testing and use in a high-throughput setting.

Introduction

Gastrointestinal illnesses caused by infectious pathogens exact a heavy burden on health care systems across the globe. WHO and the United Nations International Children's Emergency Fund (UNICEF) report a total of 2 billion cases of acute diarrhoea each year—with 1.9 million children under 5 years dying from diarrhoea annually—making acute diarrhoea the second leading cause of death after pneumonia for this age group.[1,2] The substantial number of cases presented each year takes a huge toll on global health care resources, necessitating a need for rapid diagnosis to manage patient care and to provide treatment in an efficient and effective manner.

Standard, conventional methods for the detection and identification of diarrhoeagenic bacteria involving stool culture, biochemical assays and serologic assays have been in use for years. However, these tests are both time- and labour-intensive.[3] A major shift in the field of clinical microbiology diagnostics came about with the introduction of commercial multiplex PCR panel-based testing platforms, which can detect more than one pathogenic species in a given specimen through amplification of bacterial species-specific DNA. These multiplex PCR panel-based testing systems, also known as syndromic panel-based testing systems, are designed to identify infectious pathogens that may cause similar symptoms or a syndrome. They offer a multitude of advantages over traditional routine techniques. A major advantage is the short turnaround times of these panel-based testing platforms, some of which can be as low as 1 h beginning from specimen preparation to analysis of results.[4] In addition, no dedicated PCR laboratory facility is needed, as nucleic acid extraction, nucleic acid amplification and analysis of the results are performed in closed cartridges. Furthermore, these testing panels do not require extensive hands-on time and are automated systems.[4]

Implementation of the panel-based testing system improves not only the workflow in clinical laboratories, but also the patient outcomes. Compared with conventional testing, health care workers at a London academic hospital detected an additional 221 cases of patients with infectious pathogens using the Luminex

Gastrointestinal Pathogen Panel (Luminex Corp.). This panel-based increase in sensitivity guided the clinicians in making decisions that ultimately led to a better use of the hospital's isolation facilities.[5] In an evaluation of frozen faecal specimens from 158 in-patients who initially tested negative for *Clostridioides difficile* and/or rotavirus by conventional techniques, the FilmArray Gastrointestinal Panel (BioFire Diagnostics) tests revealed that 35 patients had at least one other infectious pathogen. Based on this finding, the investigators suggested that the utilization of a gastrointestinal panel-based testing system could alleviate nosocomial transmission.[6] Furthermore, another rationale for the implementation of the panel-based testing system is its cost effectiveness. Goldenberg and colleagues[7] analysed the economic impact of using the Luminex xTAG Gastrointestinal Pathogen Panel (Luminex Corp.) versus standard culture techniques to diagnose in-hospital patients in a London academic hospital. Their analysis indicated that although it was more expensive to run panel-based tests than conventional tests—which include microbial culture, biochemical assays, serological tests and microscopy—the additional costs incurred were offset by the decrease in patient isolation costs when the panel-based testing system was implemented.[7]

A number of commercial gastrointestinal pathogen panel-based testing platforms are now available.[4] The QIAstat-Dx® Gastrointestinal Panel V2 (QGI) (Qiagen), a testing platform that has received CE-IVD marking, is a multiplex PCR-based testing system that can detect 14 bacteria, 6 viruses, and 4 parasites. The QGI testing system provides its users with values of threshold cycle (C_t) for each target amplified in a given sample. Another multiplex PCR-based gastrointestinal testing platform that has CE-IVD certification is the Novodiag® Bacterial GE+ V2-0 (NGE) (Mobidiag), which can detect 13 bacteria. Additionally, the recently launched Novodiag® Stool Parasites CE-IVD panel includes 25 targets of protozoa, tapeworms, flatworms, roundworms and microsporidia spp. has been launched. However, this panel has to be run separately from the NGE. Both testing platforms operate as closed systems and are fully automated with low hands-on time. Despite the availability of these various gastrointestinal panel-based testing systems, not many studies have been conducted to compare the performance of these testing platforms.[8,9] In this study, we evaluated and compared the performance of QGI and NGE panel-based testing systems on positive clinical specimens, two External Quality Assessment (EQA) panels and spiked faecal specimens.

Materials and methods

Clinical specimens

All clinical specimens used in this study were from a collection of frozen faecal specimens obtained from in- and out-patients suspected to have infectious gastroenteritis. Specimens were collected on a continuous basis as raw faeces and kept at −80°C for up to 4 years. Using culture and PCR (in-house and commercial) testing methods, these specimens had been found to be positive for bacterial pathogens associated with infectious gastroenteritis. These methods include conventional culture methods for *Salmonella, Campylobacter, Yersinia, Plesiomonas* and *Vibrio* spp., a combination of culture and in-house PCR methods for *Shigella* spp. and diarrhoeagenic *Escherichia coli*, and real-time PCR (GeneXpert) for *C. difficile* (Cepheid, Palo Alto, USA) (Table 1). The routine tests were carried out using methods previously described.[10–12] Ethics approval and informed consent were not required as the specimens were requested routinely for analysis to detect bacterial gastrointestinal pathogens.

EQA panels

In this study, we also examined specimens from the External Quality Assessment (EQA) Bacterial Gastroenteritis (EQA Program number GastroB18S QAB124153_1) from Quality Control for Molecular Diagnostics (QCMD), an independent international organization dedicated to providing quality assessment services with a focus on infectious diseases and EQA

Table 1. Routine methods for detection of gastrointestinal pathogens and targets represented on two commercial multiplex PCR assays

		Target included on multiplex panel	
Target	Method(s) used for routine testing	QIAstat	Novodiag
Campylobacter	Culture	✓	✓
Salmonella	Culture	✓	✓
Yersinia enterocolitica	Culture	✓	✓
Yersinia pseudotuberculosis	Culture		✓
Shigella spp.	Culture, in-house PCR	✓	✓
STEC	Culture, in-house PCR	✓	✓
EIEC	Culture, in-house PCR	✓	✓
EPEC and AEEC	Culture, in-house PCR	✓	✓
ETEC	Culture, in-house PCR	✓	✓
EAEC	Culture, in-house PCR	✓	✓
Clostridioides difficile (tcdB)	Real-time PCR (GeneXpert)	✓	✓
Plesiomonas shigelloides	Culture	✓	
Vibrio cholerae	Culture	✓	✓
Vibrio parahaemolyticus	Culture	✓	✓
Vibrio vulnificus	Culture	✓	

EAEC, enteroaggregative *E. coli*; STEC, Shiga toxin producing *E. coli*, EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; AEEC, attaching and effacing *E. coli*.

panels from Statens Serum Institut (SSI, Copenhagen, Denmark) for diarrhoeagenic *E. coli*.

Spiked faecal specimens

Faecal specimens spiked with clinical or reference strains were used to verify additional bacterial species, subspecies and subtypes not covered by clinical specimens. We generated a pooled negative specimen by pooling specimens that had been determined to be negative for all pathogens of the panel-based testing systems by the routine tests. Then, a total of 150 mL 0.9% NaCl was added to 50 g of the negative pooled faecal sample (without visible blood) and the suspension was homogenized by stirring with a wooden spatula at room temperature. The suspension was then filtered through a steel sieve to remove any large solid debris while any large soft debris was pushed through the mesh. The filtrate was diluted with extra 0.9% NaCl to a total volume of 150 mL. The resulting suspension was divided into 2 mL aliquots with regular homogenization to ensure a uniform suspension before being stored at −20°C.

Bacteria that were used to spike the faecal suspensions were prepared at a stock concentration of 10^5 cfu/mL. To prepare this bacterial stock concentration, bacteria grown on agar plates were added to 1 mL 0.9% NaCl until the turbidity of the resulting suspension was comparable to the turbidity of 0.5 McFarland turbidity standard (equivalent to 1.5×10^8 cfu/mL). Through serial dilution with 0.9% NaCl, the bacterial suspension was diluted to a concentration of 10^5 cfu/mL. A total of 222 μL of this diluted bacterial suspension was used to spike a 2 mL faecal suspension, giving rise to a spiked sample with a final bacterial concentration of 10^4 cfu/mL.

QGI testing

The multiplex PCR gastrointestinal panel assay was carried out according to manufacturer's instructions (Qiagen, Hilden, Germany). Approximately 50–200 mg of thawed faeces was collected with a FecalSwab (Copan) and resuspended in 2 mL of Cary Blair transport medium. 200 μL of this suspension was loaded into the liquid sample port of a QIAstat-Dx® Gastrointestinal Panel Cartridge with a transfer pipette. The sample barcode and the QIAstat-Dx® Gastrointestinal Panel Cartridge QR code were then subsequently scanned by the QIAstat-Dx® Analyzer. The cartridge containing the sample was then inserted into the QIAstat-Dx® Analyzer for the reactions to begin. The QGI testing system is a closed system that performs cell lysis, purification of nucleic acids, amplification of nucleic acid targets, measurements of fluorescence of the amplified PCR products, and generation of the amplification curves. The results are interpreted, and a test report is generated by the QIAstat-Dx® Analyzer Software. Quality control of the reactions for a given sample is monitored through the successful amplification of an internal control. If the control reaction is reported positive, all results are valid. If the control reaction is reported negative, only positive results for targets are valid while negative results for targets are invalid.

NGE testing

Using a FLOQSwab (Copan), the thawed faeces specimen was suspended thoroughly in a 2 mL eNAT medium (Copan) and the tube was subsequently vortexed for 5 s. The tube was left at room temperature for 30 min to allow the eNAT medium to inactivate microbes and to stabilize bacterial DNA in the specimen. After vortexing the tube for 5 s, 600 μL of eNAT solution was transferred to the Novodiag® Bacterial GE+ Cartridge. The cartridge was then inserted into the Novodiag® Bacterial GE+ Analyzer. The analyser performs nucleic acid extraction, nucleic acid amplification and analysis of the results. The results are reported by the Novodiag® Bacterial GE+ Analyzer Software. The analyser uses both fluorescent probes and a microarray (coupled with total internal reflection fluorescence-based detection) to measure amplification of targeted nucleic acids. An internal control is also subjected to amplification and used as a quality control for the reactions carried out for a given sample. If the control reaction is reported negative, then all results for a given sample are considered invalid.

Bacterial species identified by QGI and NGE

Table 1 summarizes the bacterial species that are targeted by QGI and NGE testing platforms. Although both multiplex PCR panel-based testing platforms can identify a broad range of bacterial pathogen species associated with infectious gastroenteritis, the two panel-based testing platforms do exhibit similarities and differences in targeting and reporting bacterial strains and biomarker genes as highlighted below.

Campylobacter spp.

Both panel-based systems can identify *Campylobacter jejuni* and *Campylobacter coli*. Furthermore, the QGI can target *Campylobacter upsaliensis*. Detected *Campylobacter* species are identified by species name with the NGE testing system whereas these species are only reported as '*Campylobacter* species' with the QGI testing system.

Salmonella spp.

The two panel-based testing platforms under investigation do not distinguish between *Salmonella* Typhi/Paratyphi and zoonotic *Salmonella* serotypes, reporting positive specimens as '*Salmonella* species'.

Yersinia spp.

The NGE testing system detects the *virF* gene found in virtually all pathogenic *Yersinia enterocolitica*. Occasionally this gene may also be found in the non-pathogenic 1A subtype. The gene target for *Y. enterocolitica* with the QGI testing system is not described in kit insert. The NGE test system also detects *Yersinia pseudotuberculosis*.

Clostridioides difficile

Unlike the NGE testing system, which identifies specimens with *tcdB* gene, the QGI testing system targets both *tcdB* and *tcdA* genes.

E. coli and Shigella spp.

Shiga toxin-producing E. coli (STEC) (stx1/stx2). The NGE testing system can distinguish between the stx_1 and stx_2 targets in its reports and further reports the presence of the *eae* gene, if detected, in positive specimens. The presence of *eae* gene in a given sample indicates either co-infection with an enteropathogenic *E. coli* (EPEC) or attaching and effacing *E. coli* (AEEC) strain or that the STEC strain also possesses the *eae* gene. In contrast, the QGI testing system does not distinguish between stx_1 and stx_2 positive targets and this testing system specifically reports STEC serotype O157:H7.

Enteroinvasive E. coli (EIEC). Both testing platforms target the invasive plasmid gene (*IpaH*) shared by both *Shigella* spp. (*S. sonnei, S. flexneri, S. boydii* and *S. dysenteriae*) and EIEC, identifying positive specimens as '*Shigella*/EIEC'.

Enteropathogenic E. coli (EPEC). Both panel-based testing systems detect the *eae* gene, which encodes proteins responsible for the attaching and effacing (A/E) lesions within intestinal epithelial cells. This gene is also found in AEEC and in some STEC strains.

Enterotoxigenic E. coli (ETEC) (eltA/estA). Although the two multiplex PCR panel-based platforms target *eltA* gene and the two major

ST variants (*estAh* and *estAp*), both testing platforms do not distinguish between these gene variants encoding for enterotoxins in their reports.

Analysis

For clinical and EQA specimens, negative samples were considered negative if initially negative in order to reflect a routine clinical situation. Samples with failure, invalid or error results were re-tested until a positive or negative result was obtained (maximum of three attempts).

Spiked faecal specimens with clinical or reference strains were tested up to three times before being reported negative. The re-testing option was decided before the study to counter potential errors independent of the testing that might be introduced by using spiked specimens.

The main objective of this study was to verify the ability of testing platforms to identify a high number of bacterial species, subspecies, subtypes and biotypes in positive samples. Therefore, the sample size for each target was small. The analysis did not include sample size calculations. Sensitivity calculations were performed by aggregation by organism with the corresponding 95% CI, using R version 4.05 and the method for calculations of confidence intervals of two independent proportions (Epi: ci.pd).

Analytical specificity was only assessed with a minor number of specimens: faecal samples with *Campylobacter lari* ($n = 1$), *Campylobacter fetus* subsp. *fetus* ($n = 2$) and *Campylobacter concisus* ($n = 1$) were negative on both assays. In addition, faecal samples with *Aeromonas* spp. ($n = 3$) tested as negative for *Aeromonas* on both assays. The EQA, QCMD GastroB18S-09 negative sample was negative by both assays.

Results

Performance of QGI and NGE testing systems

In total, 141 positive samples were tested on both systems. For *C. jejuni* and *C. coli* ($n = 20$), *Salmonella* ($n = 24$), *Shigella* ($n = 13$), *Y. enterocolitica* (non-1A biotypes) ($n = 8$), *C. difficile* ($n = 24$) and *Vibrio parahaemolyticus* ($n = 2$), QGI correctly verified 19/20, 20/24, 13/13, 8/8, 23/24 and 2/2, whereas NGE correctly verified 20/20, 17/24, 13/13, 8/8, 14/24 and 1/2 (Table 2).

QGI tended to exhibit higher sensitivity than NGE in detecting *Salmonella* spp. but the difference was not significant. Three samples with expected *S.* Anatum, *S.* Legon and one of the *S.* Newport were repeatedly negative on both assays and on an attempt to re-culture. In contrast, both assays found the samples were positive for *C. jejuni* plus enteroaggregative *E. coli* (EAEC), EPEC plus enterotoxigenic *E. coli* (ETEC), and ETEC, respectively.

Ten *C. difficile* positive samples were not detected by NGE (Table 2). These were in particular samples positive for Toxin B (*tcdB*) plus the binary toxin (*cdtA*/*cdtB*), but also in samples with the *tcdC*Δ117, characteristic of the virulent CD027 ribotype. QGI failed to detect a single sample (a CD027 positive sample). The sample was also negative by NGE. The difference in sensitivity between the two testing systems was significant.

QGI and NGE identified all eight samples with *Y. enterocolitica* non-1A-biotypes. It was remarkable that *Y. enterocolitica* biotype 1A (non-pathogenic) ($n = 6$) were all positive in QGI, but all negative in NGE (Table 2).

Among diarrhoeagenic *E. coli* ($n = 29$), QGI detected all the subtypes found in the specimens tested, with one exception: it reported an STEC *stx1a* O26:H11 as STEC O157:H7 (Table 3). NGE did not detect one specimen containing *E. coli* STEC, one *E. coli* EAEC and one *E. coli* EPEC. Both testing platforms identified additional unexpected *E. coli* types (Table 3).

Although both testing platforms share a number of bacterial targets implicated in infectious gastroenteritis, the QGI panel also detects certain bacterial species that are not part of the NGE's target list and vice versa. We found that the QGI testing system could detect specimens containing *Plesiomonas shigelloides* (3/3). In addition, this testing system also verified specimens containing *Vibrio vulnificus* (2/2) and *C. upsaliensis* (1/1). The NGE testing system correctly identified specimens with *Y. pseudotuberculosis* (2/2) (Table 4).

Analytical specificities of both testing systems were also tested to evaluate the potential of cross-reactivity. Both testing platforms yielded negative results for specimens containing *C. lari* ($n = 1$), *C. fetus* subsp. *fetus* ($n = 2$) and *Aeromonas* spp. ($n = 3$). Furthermore, an EQA, QCMD GastroB18S-09 negative specimen was also found to be negative in both testing platforms.

Technical hands-on time and instrument time

Being closed, automated systems, both testing platforms, QGI and NGE, were comparable in terms of workflow timing, requiring 2 min of hands-on time and slightly more than 1 h of instrument time for each run. QGI and NGE took up 75 and 73 min, respectively, for each run.

Discussion

The use of multiplex PCR panel-based testing systems to detect infectious microbial pathogens for clinical diagnosis has revolutionized the field of clinical microbiology. Clinical laboratories are increasingly adopting this technology and, with rising demands for multiplex PCR-based testing systems, a number of companies have designed and marketed their own systems. In this investigation, we have evaluated and compared the performance of two newly CE-IVD-certified multiplex PCR panel-based testing systems for gastrointestinal bacterial pathogens, namely QGI and NGE testing systems in a hospital setting. Overall, we found that both testing systems could detect and identify all of their targets, but the systems exhibited different sensitivities. QGI tended to be more sensitive than NGE; however, the sample sizes are too small to draw clear conclusions. In an aggregation by organism, only *C. difficile* reached significance level.

For *Salmonella*, three samples with expected *S.* Anatum, *S.* Legon and one of the *S.* Newport were repeatedly negative on both assays and on an attempt to re-culture. In contrast, both assays found the samples were positive for *C. jejuni* plus EAEC, EPEC plus ETEC, and ETEC, respectively. This raises the concern that the samples might never have been positive for *Salmonella*. Regardless of this, the suboptimal performance needs to be further addressed with additional testing or by improvement of kits prior to clinical use of both platforms. A new version, NGE V3-0 is now on the market. Whether the sensitivity is improved for these pathogens must be determined in a new clinical study. Both testing platforms are closed systems requiring very short hands-on time (2 minutes) and turnaround times of approximately 75 minutes for each run. Testing specimens with the QGI platform revealed the presence of non-pathogenic *Y. enterocolitica* 1A in six specimens. In routine settings, the detection of *Y. enterocolitica* warrants further analysis in order to distinguish between non-pathogenic and pathogenic strains. This can be accomplished through culture

Table 2. Organisms identified by QIAstat and Novodiag in historical clinical faecal sample collection, EQA and spiked negative faecal samples with strains: classic GI bacteria and *Clostridioides difficile*

Organisms (no. samples)	Species (no. samples)	Material (no. samples)	Detection rate		Difference (95% CI)
			QIAstat	Novodiag	
Campylobacter (*n* = 21)	*C. jejuni/coli* (20)	–	19/20	20/20	5% (−0.24 to 0.12)
	C. jejuni (18)	Clinical samples (15)	14/15	15/15	
		EQA (2), QCMD (GastroB18S-01 and 02)	2/2	2/2	
		ATCC 33560	1/1	1/1	
	C. coli (2)	EQA (1), QCMD (GastroB18S-03)	1/1	1/1	
		ATCC 33559	1/1	1/1	
	C. upsaliensis (1)	CCUG 23626	1/1	NI	
Salmonella[a] (*n* = 24)	*Salmonella* spp.[a] (*n* = 24)	–	20/24	17/24	13% (−0.11 to 0.35)
		Clinical samples (16)	12/16[b]	10/16[b]	
		EQA (2), QCMD (GastroB18S-02 and 05)	2/2	1/2	
		Clinical strains (4)	4/4	4/4	
		ATCC 13076 and ATCC 14028	2/2	2/2	
Yersinia (*n* = 16)	*Y. enterocolitica* (non-1A biotypes) (8)		8/8 (100)	8/8 (100)	-
	Y. enterocolitica (14)	Clinical samples (7)	7/7	6/7[c]	
		EQA (1), QCMD (GastroB18S-08)	1/1	0/1[c]	
		Clinical strains (5)	5/5	2/5[c]	
		ATCC 23715	1/1	0/1[c]	
	Y. pseudotuberculosis (2)	Clinical samples (2)	NI	2/2	
Shigella (*n* = 13)	*Shigella* spp.	–	13/13 (100)	13/13 (100)	-
	S. sonnei (7)	Clinical samples (6)	6/6	6/6	
		ATCC 25931	1/1	1/1	
	S. flexneri (4)	Clinical samples (2)	2/2	2/2	
		EQA (2), QCMD (GastroB18S-07)	1/1	1/1	
		ATCC 12022	1/1	1/1	
	S. boydii (1)	NCTC 9359	1/1	1/1	
	S. dysenteriae (1)	Clinical sample	1/1	1/1	
Clostridioides difficile (*n* = 24)	*Clostridioides difficile* (*n* = 24)	–	23/24 (96)	14/24 (58)	38% (0.14–0.57)
	Toxin B (*tcdB*) (9)	Clinical samples (9)	9/9	8/9	
	Toxin B (*tcdB*) plus binary toxin (*cdtA*/*cdtB*) (7)	Clinical samples (7)	7/7	3/7	
	Toxin B (*tcd*B) plus binary toxin (*cdtA*/*cdtB*) and *tcdC*Δ117 (8)	Clinical samples (6)	5/6	3/6	
		Clinical strains (1)	1/1	0/1	
		EQA (1), QCMD (GastroB18S-04)	1/1	0/1	
Diarrhoeagenic *E. coli* (*n* = 29)	Diarrhoeagenic *E. coli* (*n* = 29)	–	29/29 (100)[d]	26/29 (90)	10% (−0.03 to 0.26)
	STEC (15)	Clinical samples (9)	9/9[d]	8/9	
		EQA (6) (SSI EQA 7&8)	6/6	6/6	
	ETEC (3)	Clinical samples (2)	2/2	2/2	
		EQA (1) (SSI EQA 8)	1/1	1/1	
	EPEC (3)	Clinical samples (3)	3/3	2/3	
	EIEC (2)	Clinical samples (2)	2/2	2/2	

Continued

Table 2. *Continued*

Organisms (no. samples)	Species (no. samples)	Material (no. samples)	Detection rate		Difference (95% CI)
			QIAstat	Novodiag	
	AEEC (1)	Clinical samples (1)	1/1	1/1	
	EAEC (1)	Clinical samples (1)	1/1	0/1	
	Multiple types (4)	Clinical samples (2)	2/2	2/2	
		EQA (2) (SSI EQA 6&8)	2/2	2/2	

NI, not included in panel.
[a]*Salmonella* Enteritidis [8 clinical samples + 2 EQA, QCMD (GastroB18S-02 and 05) + ATCC 13076], *S.* Typhimurium (1 clinical sample, ATCC 14028), *S.* Typhi (2 spiked clinical strains), *S.* Paratyphi A (2 spiked clinical strains), *S.* Paratyphi B (1 clinical sample), *S.* Newport (2 clinical samples), *S.* Bareilly (1 clinical sample), *S.* Enterica O4,12H:i:− (1 clinical sample), *S.* Anatum (1 clinical sample), *S.* Legon (1 clinical sample).
[b]The samples with *S.* Anatum, *S.* Legon and one of the *S.* Newport were repeatedly negative on both assays as attempt to re-culture. In contrast, both assays found the samples were positive for *C. jejuni* plus EAEC, EPEC plus ETEC, and ETEC, respectively. One *S.* Enteritidis clinical sample was negative on both assays (not re-tested due to lack of more faecal material). An additional three *S.* Enteritidis (2 clinical samples and 1 EQA) were repeatedly negative on Novodiag.
[c]Biotype 1A not included in Novodiag. False negatives belong to biotype 1A.
[d]QGI reported one STEC *stx1a* O26:H11 as STEC serotype O157:H7.

and subsequent MALDI-TOF/MS biotyping. In addition, PCR-based detection of the *ail* gene, a virulence gene encoding a 17 kDa attachment-invasion locus protein,[13] is another approach. Reporting of non-pathogenic *Y. enterocolitica* 1A cases may have led to over-reporting of *Y. enterocolitica* infections. In 2018, the European Centre for Disease Prevention and Control (ECDC) reported that biotyping information, which is crucial in determining the pathogenicity of *Y. enterocolitica* strains, was provided only for 20% of *Y. enterocolitica* infections.[14] Phenotyping techniques are laborious and interpreting biotyping results is highly subjective, resulting in misidentification. Whole genome sequencing is increasingly being utilized to subtype *Y. enterocolitica* isolates in outbreak investigations.

Another feature of the QGI testing system is its ability to provide information on C_t values and amplification curves for each target it amplifies. The C_t values obtained for the various targets can be used as indicators of pathogen load.[15]

Implementation of the multiplex PCR panel-based testing systems has benefitted and improved workflow procedures in the laboratory, clinical outcomes, patient management and care. Our evaluation of both testing systems revealed that the assays were easy to perform, with little waste being generated, and the set-up did not require a large amount of space. A number of targets can be identified with just a single specimen. Given the relatively short turnaround times, these assays are highly flexible and can be performed on-demand. In addition, these testing systems can be potentially scaled-up for high-throughput testing facilities. It has been well-documented that diagnosing infectious gastroenteritis with a multiplex PCR panel-based approach resulted in reduced usage of antibiotics. O'Neal *et al.*[16] examined the initiation of antibiotics among patients who had undergone multiplex PCR gastrointestinal panel-based tests at a community teaching hospital. They showed that patients with negative test results were started on antibiotics significantly less frequently than patients with positive test results (62.5% versus 80.2%). Another advantage of the multiplex PCR panel-based approach is its ability to detect in stool specimens bacterial species that are difficult to culture. Using multiplex PCR gastrointestinal panel-based tests, a total of 20 out of 185 (11%) stool specimens from children were determined to be positive for *Campylobacter* spp.,[17] bacterial species noted for their difficulty to culture.[18] The panel-based diagnostic platform can also distinguish between non-pathogenic and pathogenic bacterial strains. For instance, the NGE seems to detect only pathogenic *Y. enterocolitica* strains in specimens. Despite the advantages of a panel-based testing method, clinicians should take into consideration the patient's condition, including severity and duration of symptoms, when interpreting the outcome of a multiplex PCR panel-based test.[19]

There are limitations associated with this study. Although it is a small study with limited number of specimens, it is nevertheless useful for verification purposes. The QGI can detect viruses and parasites in addition to bacteria species, while the NGE can only detect bacteria species. Although a comparison was carried out between these two panel-based systems, no assessment was made of QGI's performance in detecting viruses or parasites in clinical specimens. A third limitation is that the rate of false positives cannot be determined for the two multiplex PCR panel-based systems under comparison, because all the specimens in this study had been found to be positive for bacterial species by standard routine techniques involving culture, in-house PCR, and real-time PCR.

In conclusion, both QGI and NGE testing panels can improve laboratory workflow and patient management by providing user-friendly platforms that can rapidly detect a number of targets from one specimen. We found that both testing systems could detect and identify all of their targets, but the systems exhibited different sensitivities. QGI was significantly more sensitive in identifying *C. difficile* and tended to be more sensitive than NGE for other bacteria. However, the sample size was too small to draw firm conclusions. For both methods, the suboptimal performance on *Salmonella* needs to be addressed by additional testing or by improvement of kits prior to clinical use.

Table 3. Diarrheagenic *E. coli* identified by QIAstat and Novodiag in ($n = 29$) historical clinical faecal sample collection, EQA and spiked negative faecal samples with strains. Unexpected additional identified *E. coli* targets are presented

Organisms	Serotype	Targets	QIAstat	Novodiag
EAEC, ETEC, AEEC	NA	*eae, eltA, aggR*	EPEC, ETEC (*lt/st*), EAEC	EPEC (*eae*), ETEC, EAEC
AEEC	NA	*eae* (no *eltA, estA* primary)[a]	EPEC, ETEC (*lt/st*)	EPEC (*eae*)
ETEC	NA	*estA*	ETEC (*lt/st*)	ETEC
EPEC	NA	*eae*	EPEC	EPEC (*eae*)
STEC	O27:H30	*stx2b*	STEC (*stx1/stx2*)	EHEC (*stx2*)
EPEC	O111	*eae*	EPEC	EPEC (*eae*)
EAEC	NA	*aggR* (no *eae* primary)[a]	EPEC, EAEC	EPEC (*eae*)
STEC	O26:H11	*eae, stx1a*	STEC (*stx1/stx2*)	EHEC (*eae, stx1*)
EIEC	NA	*ipaH*	EIEC/*Shigella*	*Shigella* spp./EIEC
STEC	O26:H11	*stx2a, eae*	STEC (*stx1/stx2*)	EHEC (*eae, stx2*)
ETEC, EAEC	NA	*eltA, estA, aggR*	ETEC, EAEC (*lt/st*)	ETEC, EAEC
ETEC	NA	*eltA, estA*	ETEC (*lt/st*)	ETEC
STEC	O26:H11	*eae, stx1a*	STEC (*stx1/stx2*)	*E. coli* (*eae, stx1*)
EIEC	O96:H19	*ipaH*	EIEC/*Shigella*	*Shigella* spp./EIEC
STEC	O157:H7	*eae, stx1a, stx2c*	STEC O157: H7	EHEC (*eae, stx1, stx2*)
STEC	O26:H11	*stx1a*	STEC O157: H7	EHEC (*eae, stx1*)
STEC	O153, O178:H7	*stx1c* (no *eae* primary—two serotypes—double infection?)[a]	STEC (*stx1/stx2*)	EHEC (*eae, stx1, stx2*)
STEC	O145:H−	*stx2a*	STEC (*stx1/stx2*)	EHEC (*eae, stx2*)
STEC	O157:H7	*eae, stx1a, stx2a*	STEC O157: H7	EHEC (*eae, stx1, stx2*)
STEC	O154:H31	*stx1d*	STEC (*stx1/stx2*)	EHEC (*stx1*)
STEC with *estAp*	OX187:O28	*stx2g, estAp*	ETEC (*lt/st*), STEC (stx1/stx2)	EHEC (*stx2*), ETEC
STEC	O8:H9	*stx2a, stx2d*	STEC (*stx1/stx2*)	EHEC (*stx2*)
STEC	O63:H6	*stx2f*	STEC (*stx1/stx2*)	EHEC (*eae, stx2*)
STEC	O145:H34	*stx2f*	STEC (*stx1/stx2*)	EHEC (*eae, stx2*)
STEC	O156:H4	*stx2d*	STEC (*stx1/stx2*)	EHEC (*stx2*)
STEC with *eltA*	O166:H15	*stx2d, eltA*	STEC (*stx1/stx2*), ETEC (*lt/st*)	EHEC (*stx2*), ETEC
STEC	O9:H9	*stx2e*	STEC (*stx1/stx2*)	Negative
ETEC	O6:H16	*eltA, estAh*	ETEC (*lt/st*)	ETEC
EPEC	O157:HNA	*eae*	EPEC	Negative

AEEC, attaching and effacing *E. coli*; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; STEC, Shiga toxin-producing *E. coli*.
STEC subtypes: *stx1a* (3), *stx1c* (1), *stx1d* (1), *stx2a* (4), *stx2b* (1), *stx2c* (1), *stx2d* (3), *stx2e* (1), *stx2f* (2), *stx2g* (1).
Subtype *stx2a* and *stx2d* are HUS-associated subtypes.
ETEC subtypes: *eltA, estAh,* and *estAp*.
[a]Not retested.

Table 4. Organisms identified by QIAstat and Novodiag in historical clinical faecal sample collection, EQA and spiked negative faecal samples with strains: GI bacteria with fresh/saltwater origin

			Detection rate	
Organisms (no. samples)	Species/subtypes (no. samples)	Material (no. samples)	QIAstat	Novodiag
Plesiomonas ($n = 3$)	*P. shigelloides*	Clinical samples (1)	1/1	NI
		EQA, QCMD (GastroB18S-06)	1/1	
		ATCC 33560	1/1	
Vibrio spp. ($n = 4$)	*V. parahaemolyticus*	Clinical strains (2)	2/2	1/2
	V. vulnificus	Clinical strains (2)	2/2	NI
	V. cholerae	NA		

NI, not included in panel; NA, not available.

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