European Journal of Clinical Microbiology & Infectious Diseases (2019) 38:2103–2112
https://doi.org/10.1007/s10096-019-03646-4

ORIGINAL ARTICLE

Multicenter evaluation of the new QIAstat Gastrointestinal Panel for the rapid syndromic testing of acute gastroenteritis

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Received: 5 June 2019 / Accepted: 15 July 2019 / Published online: 27 July 2019

Abstract

In acute gastroenteritis (AGE), identification of the infectious agent is important for patient management. Since symptoms do not reliably identify the agent, microbiological diagnostics are important. Conventional methods lack sensitivity and often take days. Multiplex PCR panels offer fast and sensitive alternatives. Our aim was to assess the performance of the new QIAstat Gastrointestinal Panel (GIP) detecting 24 different gastroenteric pathogens from stool in Cary-Blair transport medium (Adenovirus F 40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, Sapovirus, *Campylobacter* spp., *Clostridium difficile*, *Plesiomonas shigelloides*, *Salmonella* spp., *Vibrio cholera*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Yersinia enterocolitica*, enteroaggregative *Escherichia coli*, enteropathogenic *E. coli*, enterotoxigenic *E. coli*, Shiga-toxin-producing *E. coli* (stx1 and stx2) (including specific detection of *E. coli* O157), *Shigella* spp./enteroinvasive *E. coli*, *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Entamoeba histolytica* and *Giardia lamblia*). We tested both prospective ($n = 163$) and retrospective ($n = 222$) stool samples sent for routine diagnostics by the QIAstat GIP comparing it to the FDA-approved BioFire FilmArray GIP. Seegene Allplex GIP was used for discrepancy testing. After discrepancy testing, QIAstat GIP detected 447 of 455 pathogens (98.2%, 95% confidence interval (CI) 96.6–99.1%). There were eight false positive detections. Multiple pathogens were detected in 32.5% of positive samples. The QIAstat GIP detected a large range of AGE pathogens with a high sensitivity. It offers an easy-to-use system for GI pathogen detection in stool within 70 min. An advantage of the QIAstat is the availability of cycle threshold (CT) values to aid in interpretation of results.

Keywords Diarrhoea · Gastrointestinal pathogens · Laboratory diagnosis · Molecular assay · Multiplex PCR · Syndromic testing

Introduction

Acute gastroenteritis (AGE) is a common, transient disorder usually caused by an infection with viruses, bacteria or parasites. AGE causes considerable morbidity and mortality with an estimated 1.4 million deaths worldwide every year [1, 2]. Though most AGE infections are self-limited, they may result in more severe illness requiring hospitalization. In addition, sequelae can be serious and include Guillain-Barré syndrome, reactive arthritis, post-infection irritable bowel syndrome, post-infection malabsorption syndrome or haemolytic uremic syndrome [3]. The Centers for Disease Control and Prevention estimate nearly 48 million cases annually in the USA, accounting for a large number of hospitalizations and outpatient visits, and significant direct and indirect costs [4].

A wide variety of pathogens can cause AGE. In the developed world, viruses and bacteria are predominant, some of them emerging because of newer diagnostics, such as

Irene Hannet and Anne Line Engsbro are to be considered joint first authors.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10096-019-03646-4) contains supplementary material, which is available to authorized users.

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Aeromonas, *Plesiomonas* and the diarrheagenic pathotypes of *Escherichia coli* (DEC) [5]. Parasite-caused AGE is typically seen as more a burden in resource-restricted settings. However, even in developed countries, it is estimated that only 1% of *Cryptosporidium* cases are diagnosed, mainly due to lack of suspicion and unavailability of diagnostic modalities [2, 6]. Due to increasing importance of gastroenteritis parasites, both *Giardia lamblia* and *Cryptosporidium* were included in 2004 in the WHO Neglected Diseases Initiative [7–9].

The diagnostic landscape for AGE has changed dramatically with the availability of highly multiplexed PCR (hM-PCR) stool tests, resulting in important new insights regarding the epidemiology of AGE [10]. Multiplexed PCR stool tests vary in the number of on panel pathogens, but overall are reported to have similar performance characteristics. Yalamanchili et al. [11] stated a sensitivity and specificity of greater than 94 and 97% respectively for five FDA-approved hM-PCR stool tests, and Yoo et al. [12] reported overall positive percent agreements respectively of 94 and 92% for the Seegene Allplex and Luminex x-Tag Gastrointestinal pathogen panels in a retrospective sample set. Axelrad et al. [13] report on their findings of 9403 hM-PCR stools tested with the FilmArray GIP. A positive result was found in 25.9%, and of the detected pathogens 31.5% were viruses, 62.7% were bacteria and 5.8% were parasites; the coinfection rate was 28.5%. The most commonly identified pathogens were enteropathogenic *E. coli* (EPEC) (22.5%), Norovirus (17.3%) and enteroaggregative *E. coli* (EAEC) (13.7%). In comparison, during an equal previous period using conventional stool methods, a positivity rate of 4.1% had been found, and 15.4% viruses, 82.1% bacteria and 4.3% parasites had been detected, with *Campylobacter* and *Salmonella* species as the most commonly identified pathogens.

Besides changes in insights in the occurrence and incidence of certain pathogens, the understanding of the roles of these various agents in gastrointestinal (GI) disease is also advancing [14]. In a study using a hM-PCR stool test, two or more DEC, most commonly EPEC or EAEC, were detected in 16% (116/709) of AGE stool samples [15]. The detection of multiple positive targets from specimens however also leads to some difficulty in attributing disease to particular organisms [5].

Evidence of a positive health economic impact of hM-PCR testing is rapidly growing. Axelrad [16] reported that the implementation of hM-PCR stool testing was associated with a reduction in the utilization of endoscopy ($p < 0.05$) and abdominal radiology ($p < 0.05$) within 30 days, as well as antibiotic prescribing ($p < 0.001$) within 14 days following stool testing. Beal et al. [17] concluded that patients tested on hM-PCR GIP had shorter length of stay ($p = 0.04$), fewer other infectious stool tests ($p < 0.01$), fewer numbers of days on antibiotics ($p = 0.06$), fewer imaging studies ($p < 0.01$) and had an overall estimated reduction in healthcare costs.

HM-PCR stool testing may contribute to antimicrobial stewardship [18]. Cybulski et al. [19] found that patients diagnosed by a hM-PCR stool test compared to those diagnosed by culture were more likely to receive targeted rather than empirical therapy ($p = 0.02$). Positive Shiga toxin like *E. coli* (STEC) results were reported 47 h faster greatly facilitating the discontinuation of empirical antimicrobials, while the detection of mixed bacterial and/or parasitic infections generally led to initiation of appropriate combination therapy.

While the above are examples of tangible cost savings, there are also other less tangible benefits of a fast diagnosis, including improved patient experience [20]. Procedures such as endoscopy may be cancelled if there is a risk of transmitting infection. A fast diagnosis might reduce the amount of time spent in isolation for some patients if the primary reason for isolation is infection risk rather than symptom management.

The QIAstat GIP assay (Qiagen, Hilden, Germany) is a new rapid easy-to-use, hM-PCR stool assay that can simultaneously detect and identify 24 gastroenteritis pathogens. This study reports on findings of a multicentre study that compared its performance against the FilmArray GIP assay.

Materials and methods

Clinical specimens

Residual de-identified stool samples in Cary-Blair (CB) medium using Fecal swab™ (Copan Diagnostics Inc., Brescia, Italy) received for routine testing of bacterial, viral and/or parasites from 385 patients were tested in the study. All samples had been collected from patients that had presented with signs and symptoms of a gastrointestinal infection.

The specimens meeting the following protocol inclusion criteria were selected for the study at two study sites: the specimen had been received by the laboratory as raw stool or CB medium, had been sent by the provider for routine bacterial and/or viral/parasite testing, was of sufficient residual volume for testing (> 700 μL), and was tested via the QIAstat GIP and comparator method within 4 days of collection when stored at 2–8 °C, and within 90 min after thawing when stored frozen. In case of error, comparator or QIAstat GIP testing was repeated depending on residual volume.

The specimens were de-identified and assigned a study number linked to patient demographic information, including age, sex and hospitalization status (general practice, hospitalized). The specimens had been collected in the study under an institutional review board (IRB)-approved protocol, which included a waiver of informed consent for the use of residual de-identified samples.

Study design and sites

The study was designed as observational, prospective-retrospective aiming to test all QIAstat GIP pathogens from fresh or frozen samples. All samples were tested using the QIAstat GIP and were compared to the FilmArray GIP assay (bioMerieux, Marcy l'Étoile, France), which served as the reference method. Testing in both clinical laboratories was performed according to the manufacturer's instructions by trained laboratory personnel, who had shown proficiency with both methods.

The University Hospital of Hvidovre (Copenhagen, Denmark) tested 163 protocol-compliant prospectively collected specimen during December–February 2019.

The University Hospital of Bonn, Germany, tested 222 protocol-compliant retrospective clinical samples during June–August 2018. The vast majority of samples had tested positive for at least one gastrointestinal pathogen by routine methods in the previous 18 months, and residual sample had been stored as either raw stool or Cary-Blair at − 80 °C after routine testing.

Resolution of discordant results and differentiation of Norovirus GI/GII

Samples that yielded discordant results were retested using the applicable Seegene Allplex™ GIP assays, 1–4 (Seegene, Seoul, South Korea), depending on available residual volume. In addition to resolution testing, Allplex GIP panel 1 was also used to differentiate Norovirus GI from Norovirus GII detections. Allplex GIP panel 2 was used to resolve discordant *Clostridium difficile* results.

QIAstat-Dx system and gastrointestinal cartridge

The QIAstat Analyzer (Qiagen, Hilden, Germany) is a rapid multiplex PCR platform by which integrated nucleic acid extraction, real-time PCR and fluorescence amplicon detection can be performed in a closed system in approximately 1 h. To load the QIAstat GIP cartridge, 200 μL stool resuspended in CB is transferred into the assay cartridge using the provided transfer pipette. Upon completing the test and ejecting the cartridge, the analyser interprets results and displays a test summary. Amplification curves and cycle threshold (CT) values can be viewed for detected pathogen(s) and for the sample internal control (IC). The QIAstat GIP cartridge used in this study, an investigational use only version identical to the CE marked version, simultaneously detects and identifies 24 diarrheagenic bacterial, viral and protozoal agents/genotypes by using pathogen-specific virulence genes or species-specific regions in housekeeping genes. Toxigenic *C. difficile* detection was based on identification of the genes that encode an enterotoxin (tcdA) and a cytotoxin (tcdB). The pathotypes of diarrheagenic *E. coli* were identified using pathotype-specific genetic markers: EAEC by pAA virulence plasmid-carried genes encoding the AAF biogenesis transcription regulator (aggR) or outer membrane protein (aatA); enteroinvasive *E coli* (EIEC) and *Shigella* by the invasion plasmid antigen H gene (ipah) (note that EIEC and *Shigella* cannot be distinguished); EPEC by detection of the intimin gene (eae) in the absence of stx1 and/or stx2; enterotoxigenic *E coli* (ETEC) by genes encoding heat-labile (lt) or heat-stable (st) enterotoxins; STEC by the detection of Shiga toxin 1 and/or 2 genes (stx1 or stx2) in association with the intimin gene (eae). The QIAstat GIP also identifies *Campylobacter* (*C. jejuni*, *C. coli* and *C. upsaliensis*, non-differentiated), *Plesiomonas shigelloides*, *Salmonella* spp., pathogenic species of *Vibrio* (*V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, differentiated) and *Yersinia enterocolitica*. The viral pathogens detected include Adenovirus F 40/41, Astrovirus, Norovirus GI/GII (differentiated), Rotavirus A and Sapovirus. Detected parasites include *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Entamoeba histolytica* and *G. lamblia*.

The QIAstat GIP tests were considered valid if the test completed and all controls passed. When detected, Rotavirus A can act as the sample IC, validating the test results. EPEC results are not applicable and not reported when STEC is detected in the sample because detection cannot be differentiated from a coinfection of EPEC and eae-containing STEC. Serotype *E. coli* O157 results are only reported when the STEC signal is also detected in the sample.

QIAstat GIP testing was performed solely for the study and no results were returned to the treating physician.

Statistical analysis

True positive (TP), false positive (FP), true negative (TN) and false negative (FN) were evaluated comparing QIAstat GIP versus FilmArray GIP detection status (Table S1) followed by resolution testing of discrepancies by Allplex GIP for final status determination. Results were considered true positives if (i) comparator testing and QIAstat GIP were both positive; (ii) comparator testing was positive, QIAstat GIP was negative and discrepancy analysis was positive (QIAstat GIP false negative); and (iii) comparator testing was negative, QIAstat GIP was positive and discrepancy testing was positive. On the contrary, results were considered true negatives if (i) comparator testing and QIAstat GIP were both negative; (ii) comparator testing was negative, QIAstat GIP was positive and discrepant testing was negative (QIAstat GIP false positive); and (iii) comparator testing was positive, QIAstat GIP was negative and discrepant testing was negative.

Positive percent agreement (PPA) or sensitivity and negative percent agreement (NPA) or specificity for each analyte were calculated as 100% × (TP/(TP + FN)) and 100% × (TN/

TN + FP), respectively. Binomial two-sided 95% confidence intervals (95% CI) for PPA/sensitivity and NPA/specificity were calculated using the Wilson Score Method. The categorical variables are given as numbers and percentages, and where performed, the between-group data were compared with Yates' corrected χ^2 test. *P* values of ≤ 0.05 were considered statistically significant.

Results

Patient demographics

One hundred eighty-nine (49.1%) of samples were collected from female patients. Forty-three samples (11.2%), 45 samples (11.7%), 33 samples (8.6%), 16 samples (4.2%), 155 samples (40.3%) and 93 samples (24.2%) of samples were respectively from age categories, 0–1, 1–5, 5–12, 13–21, 22–64 and 65 years or older. The overall hospitalization rate was 41.6% (160/385) and 207 samples (53.8%) had been referred for testing by a general practitioner (GP). Eighteen samples (4.7%) containing *E. histolytica* were obtained from another laboratory for which no hospitalization status was available.

Summary of QIAstat Gastrointestinal Panel findings

The QIAstat GIP assay detected at least one pathogen in 311 of the 385 tested specimens, in 94/163 (57.7%) in Hvidovre, Denmark and in 217/222 (97.7%) in Bonn, Germany. Multiple pathogens were detected in 32.5% of the positive specimens (101/311), and the highest number of pathogens detected in a single specimen was five (EAEC, EIEC, EPEC, ETEC, Sapovirus). The majority of multiple infection samples—66/101 samples or 65.3%—contained two pathogens (Table 1).

The number of each potential pathogen detected by QIAstat GIP and its association with co-detection by age category is presented in Table 2.

The diarrheagenic *E. coli*—EAEC, EPEC, ETEC and EIEC—were most frequently associated with coinfections, in respectively 88.6, 75, 75 and 69.2% of detections, and were furthermore often detected together.

C. difficile, *Campylobacter* and *Salmonella* were associated with coinfections in roughly one in three samples. *Y. enterocolitica* was detected in five monoinfection and one coinfection sample.

The QIAstat GIP viruses were roughly equally split between mono and co-detections.

QIAstat GIP detected 75 parasites in 73 samples. Forty-one parasite detections were in single infection samples, and 32 in coinfections (43.8%). In 25 of 32 (78.1%) coinfection samples, one or more DEC, most frequently EPEC (15/32, 46.9%), were co-detected with the parasite. Although from all parasites *Cryptosporidium* was most frequently associated with coinfections (11/22, 55%).

Of all viruses, 41.5% (44/106) were detected in children of 5 years or younger, the majority being Rotavirus A and Adenovirus F40/41, respectively, 15 and 13 out of 44 viral detections. Of the bacteria, 79.4% were detected in samples from patients older than 21 years of age, *C. difficile* (41/49 detections; 83.7%), *Campylobacter* (46/57 detections; 80.7%), and the DECs as an aggregated group (97/148 detections; 65.5%).

QIAstat Gastrointestinal Panel performance

For 369/385 specimens (95.8%), QIAstat GIP testing was completed in the first attempt; the analyser aborted three initial runs (0.8%), three runs had software errors (0.8%), and the sample IC failed to amplify in 10 instances (2.6%). Of the 16 specimens that did not yield initial results, 15 specimens produced valid results after a single retest and 1 specimen was successfully retested by a second retest attempt.

The performance characteristics for individual QIAstat GIP targets before discrepancy resolution are presented in Table S1 and the performance characteristics after discrepancy resolution are presented in Table 3.

Before resolution by discrepancy testing, QIAstat GIP and FilmArray GIP agreed on the detection of 438 of 483 totally detected pathogens from 302 samples (Table S1). Of 385 samples, 345 (89.6%) had fully concordant results. After resolution by discrepancy testing on the Allplex GIP, a total of 455 pathogen results were considered as true positive (Table 3), of which QIAstat GIP detected 447,

Table 1 Total number of QIAstat Gastrointestinal Panel-positive specimens by number of detections

No. of pathogens	No. of specimens	% of total specimens	% of total positive specimens
Detected at least one	311	80.8%	(100%)
1	210	54.5%	67.5%
2	66	17.1%	21.2%
3	28	7.3%	9.0%
4	6	1.6%	1.9%
5	1	0.3%	0.3%

Table 2 Total number of QIAstat Gastrointestinal Panel detections by type of pathogen, co-detection status and age group

Pathogen	No..	No. associated with coinfections (%)	Total no./no. associated with coinfections					
			< 1 year ($n = 43$)	1–5 years ($n = 45$)	6–12 years ($n = 33$)	13–21 years ($n = 16$)	22–64 years ($n = 155$)	> 64 years ($n = 93$)
Campylobacter	57	19 (33.3%)	2/2	2/1	3/3	4/0	37/10	9/3
Clostridium difficile	49	16 (32.7%)	7/3	1/0	0/0	0/0	13/3	28/10
Plesiomonas shigelloides	1	1 (100.0%)	0/0	0/0	0/0	0/0	1/1	0/0
Salmonella	13	5 (38.5%)	1/0	0/0	3/0	1/1	5/2	3/2
Yersinia enterocolitica	6	1 (16.7%)	0/0	0/0	0/0	2/0	3/0	1/1
Enteroaggregative *E. coli* (EAEC)	35	31 (88.6%)	0/0	4/4	9/8	1/1	20/17	1/1
Enteropathogenic *E. coli* (EPEC)	52	39 (75.0%)	6/3	10/8	5/4	1/1	16/14	14/9
Enterotoxigenic *E. coli* (ETEC) lt/st	12	9 (75.0%)	1/1	0/0	0/0	0/	7/7	4/1
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	22	11 (50.0%)	2/0	0/0	5/2	0/0	6/2	9/7
E. coli O157 serogroup	1	1 (100.0%)	0/0	0/0	0/0	1/1	0/0	0/0
Shigella/enteroinvasive *E. coli* (EIEC)	26	18 (69.2%)	1/1	3/3	4/3	0/0	13/9	5/2
Cryptosporidium	20	11 (55.0%)	1/0	0/0	2/2	0/0	11/6	6/3
Cyclospora cayetanensis	1	1 (100.0%)	0/0	0/0	0/0	0/0	1/1	0/0
Entamoeba histolytica	19	8 (42.1%)	1/1	7/3	6/0	/0	2/2	2/2
Giardia lamblia	35	14 (40.0%)	2/1	11/6	5/1	0/0	13/6	4/0
Adenovirus F40/41	26	15 (57.7%)	6/4	7/3	4/2	0/0	6/3	3/3
Astrovirus	4	2 (50.0%)	0/0	2/2	0/0	0/0	2/0	0/0
Norovirus GI	6	4 (66.7%)	3/3	0/0	0/0	0/0	2/0	1/1
Norovirus GII	29	14 (48.3%)	3/0	3/2	3/2	0/0	12/5	8/5
Rotavirus A	25	17 (68.0%)	10/4	5/5	2/1	0/9	5/4	3/3
Sapovirus	16	8 (50.0%)	1/0	5/3	1/0	0/0	9/5	0/0

for an overall sensitivity of 98.2% (95% CI 96.6–99.1%). After discrepancy testing, the overall specificity of the QIAstat GIP was 99.9% (95% CI 99.8–99.9).

Twenty-nine pathogens detected by FilmArray GIP were not detected by QIAstat GIP (Table S1). Twenty-one of these were also not detected by Allplex GIP and were considered TN, leaving eight pathogen detections as FN (Table 3). QIAstat GIP detected 16 pathogens that were not detected by FilmArray GIP (Table S1). Eight of these were also not detected by Allplex GIP and were considered FP (Table 3).

Two of the 29 pathogens by FilmArray GIP but not detected by QIAstat GIP—a Rotavirus A and a Norovirus—were in QIAstat GIP negative samples. The Rotavirus A was detected by the discrepancy method (ct value 32.0) but the Norovirus was not. The remaining 27 FilmArray GIP discordant negative QIAstat results were from 25 samples. In 7 of these 25 samples QIAstat GIP detected a single pathogen, and 2 or more pathogens were detected by QIAstat GIP in the remainder 18 samples.

QIAstat GIP detected 16 pathogens not detected by FilmArray GIP in 15 samples. Four of these pathogens were in negative samples by FilmArray GIP, and were an ETEC (ct value 33.4), a *Campylobacter* (CT value 33.7), a *C. difficile* (CT value 37.5) and a Norovirus GII (CT value 21.4). Only Norovirus GII was also detected by the discrepancy method, resulting in three false positive single infection samples by QIAstat GIP. Seven of the remaining 12 QIAstat GIP detected/FilmArray GIP not detected pathogens were detected by the discrepancy method and considered TP. Table 4 shows the eight samples with FP pathogens detected by QIAstat GIP, together with sample infection status, additional pathogens and pathogen's and sample IC CT values.

It was not possible to assess the sensitivity/PPA of the QIAstat GIP for any of the *Vibrios* (*cholera*, *parahaemolyticus* or *vulnificus*) as these organisms were not detected by any of the methods. Nor was it possible to assess the sensitivity/PPA for *C. cayetenensis* as it was detected only once by QIAstat GIP (CT value 35.3) but not by any other method (FP result).

Table 3 Performance summary and characteristics of the QIAstat GIP versus the FilmArray GIP after resolution of discordant results by Allplex GIP assays

Pathogen	FN no.	FP no.	TP no.	TN no.	PPA/ sensitivity	95% CI	NPA/ specificity	95% CI
Campylobacter	3	1	56	325	94.9%	86.1–98.3%	99.7%	98.3–99.9%
Clostridium difficile	0	1	48	336	100.0%	92.6–100%	99.7%	98.3–99.9%
Plesiomonas shigelloides	0	0	1	384	100.0%	20.7–100%	100.0%	99.0–100%
Salmonella	0	0	13	372	100.0%	77.2–100%	100.0%	99.0–100%
Yersinia enterocolitica	0	0	6	379	100.0%	61.0–100%	100.0%	99.0–100%
Vibrio cholera	0	0	0	385	NA	NA	100.0%	99.0–100%
Vibrio parahaemolyticus	0	0	0	385	NA	NA	100.0%	99.0–100%
Vibrio vulnificus	0	0	0	385	NA	NA	100.0%	99.0–100%
Enteroaggregative *E. coli* (EAEC)	0	1	34	350	100.0%	89.8–100%	99.7%	98.4–99.9%
Enteropathogenic *E. coli* (EPEC)	0	1	51	333	100.0%	93.0–100%	99.7%	98.3–99.9%
Enterotoxigenic *E. coli* (ETEC) lt/st	2	1	11	371	84.6%	57.8–95.7%	99.7%	98.5–100%
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	0	0	22	363	100.0%	85.1–100%	100.0%	99.0–100%
E. Coli O157 serogroup	0	0	1	384	100.0%	20.7–100%	100.0%	99.0–100%
Shigella/enteroinvasive *E. coli* (EIEC)	0	0	26	359	100.0%	87.1–100%	100.0%	98.9–100%
Cryptosporidium	0	0	20	365	100.0%	83.9–100%	100.0%	99.0–100%
Cyclospora cayetanensis	0	1	0	384	NA	NA	99.7%	98.5–100%
Entamoeba histolytica	0	0	19	366	100.0%	83.2–100%	100.0%	99.0–100%
Giardia lamblia	0	0	35	350	100.0%	90.1–100%	100.0%	98.9–100%
Adenovirus F40/41	0	0	26	359	100.0%	87.1–100%	100.0%	98.9–100%
Astrovirus	0	0	4	381	100.0%	51.0–100%	100.0%	99.0–100%
Norovirus GI	0	0	6	379	100.0%	61.0–100%	100.0%	99.0–100%
Norovirus GII	1	2	27	355	96.4%	82.3–99.4%	99.4%	98.0–99.8%
Rotavirus A	2	0	25	358	92.6%	76.6–97.9%	100.0%	98.9–100%
Sapovirus	0	0	16	369	100.0%	80.6–100%	100.0%	99.0–100%
Grand total	8	8	447	NA	98.2%	96.6–99.1%	NA	NA

Table 4 False positive detections by QIAstat GIP, CT values of sample IC and pathogen

False positive pathogen	CT value		Sample infection status Additional pathogen/CT value
	Pathogen	Sample IC	
Enterotoxigenic *E. coli* (ETEC) lt/st	33.4	29.6	Single
Campylobacter	33.7	30.2	Single
Norovirus GII	28.8	31.2	Double *Campylobacter*/32.0
Cyclospora Cayetanensis	35.5	30.2	Double *C. difficile*/19.0
Enteropathogenic *E. coli* (EPEC)	32.3	30.5	Triple Sapovirus/17.4 *Cryptosporidium*/27.2
Enteroaggregative *E. coli* (EAEC)	35.5	33	Triple *C. difficile*/31.3 *Cryptosporidium*/20.7
Clostridium difficile	37.5	32.5	Single
Norovirus GII	32.8	33.9	Triple *E. histolytica*/19.7 EPEC/30.8

Discussion

The wide range of pathogens and the co-detection rate (32.5%) found by QIAstat GIP in the 385 tested samples is in line with the findings by others who reported on the use of hM-PCR tests in diarrheagenic stool samples [13, 15, 21, 22]. DECs were most frequently associated with coinfections. At least 1 DEC was found in 81/101 (80.2%) coinfection samples, and in 23 samples (22.7%), 2 or more DECs were co-detected. Whether indeed multiple pathotypes are present or whether horizontal transfer of target genes located on plasmids or pathogenicity islands may account for the detection of multiple strains in a single sample has been questioned [23]. Metagenomic sequencing has eluded to a vast genetic diversity of pathogenic *E. coli*, and many of their defining genes may not be restricted to a particular pathotype [14]. The high DEC co-detection association in this study is in line with findings by Spina [15] who reported that among the samples with multiple pathogens present, 84% were positive for EAEC or EPEC. Several reports highlight that high coinfection rates and emerging gastroenteritis pathogens detected by hM-PCR stool test has created a clinical conundrum in that health care providers are now faced with results that were not previously reported, and for which current guidelines provide no direction as to their management (treatment, clinical significance or the need for additional or repeat testing) [24]. As the detection of one or more potential pathogens in a faecal sample out of clinical context does not indicate which (if any) of the detected potential pathogens are clinically relevant, whether such detections represent true coinfections of viable organisms or colonizers remains a question [14]. This is especially true for some of the DECs as illustrated by the findings of Dutch investigators that asymptomatic infections with enteropathogens in day care attending toddlers (0–4 years) are not a rare event, and that 19.9% of stools from mostly asymptomatic children were positive for EPEC [25]. Pending studies that demonstrate clinical significance of semi-quantitative detection of co-detection of potential gastrointestinal pathogens, the QIAstat GIP pathogen CT values may be of help to assess whether co-detected pathogens, particularly EAEC and EPEC, are true findings, and in general may help to distinguish also from contamination or false positive detections.

In line also with other reports is the finding in this study that viruses were more frequently detected in the stool of young children presenting with AGE [26, 27] compared to other age groups. The predominantly detected viruses differed between the very young and other ages, with Rotavirus A and Adenovirus f40/41 more predominant in young children and mainly Norovirus GII in the samples of older patients.

A total of 49 *C. difficile* pathogens were detected by QIAstat GIP, the majority (73.5%; 36/49) in the stool of patients aged 49 years or older. In total, 30/49 patients (61.2%) with a detected *C. difficile* pathogen were hospitalized and 38.8% were referred for stool testing by a GP. As described, *C. difficile* can be associated with both nosocomial and community-associated diarrhoea. The interpretation of the finding of a positive result for *C. difficile* in patients in whom this organism may not have been historically looked for can be challenging and highlights the need for diagnostic stewardship principles to guide the use of multiplex molecular assays. Stool density is an important parameter for reliable results

and the treatment guidance for *C. difficile* disease (CDI) recommends the use of Bristol score 5–7 samples for CDI testing [28]. However, with labs receiving increasingly more CB stool instead of raw stool, the original stool density cannot always be assessed. In cases of an unexpected *C. difficile* results compared to pre-test probability, IC CT values could potentially support the lab in assessment of the appropriateness and quality of the provided sample.

The high number of parasite positive samples in this dataset tested with a multiplex PCR is unique and may expand insights in parasite AGE. The co-detection rate in parasite positive samples was 43.8%, and the vast majority of coinfection samples (78.1%) harboured one or more DEC, including in all *E. histolytica* (8/8, 100%) and in the majority of *G. lamblia* (12/14; 85,7%) coinfection samples. Although *Cryptosporidium* had the highest co-detection rate (55%) among the parasites, it was least associated with a DEC (6/11, 54.5%). An explanation for this finding may lay in the age distribution, as most *Cryptosporidium* parasites were detected in stool from adult patients. Thirteen of the 35 detected *G. lamblia* (37.1%) were found in children younger than 6 years of age, and may indicate that its occurrence may be more frequent in this age group than suspected and/or reported.

The Copenhagen lab found 192 gastroenteritis pathogens in the 163 prospective samples. The wide range of pathogens and frequent occurrence of certain pathogens that are considered emerging in this subset coincides with the findings of others [13]. However, some results might be FPs from a clinical and/or analytical perspective. The consequences of a FP result are not insignificant. They include inappropriate treatment, which may lead to adverse drug effects and the selection of antimicrobial resistance, unnecessary public health investigations and premature closure of the diagnostic work-up [29]. As a remedy, several authors have referred to a need for quantitation when using hM-PCR panels [3, 11, 28] and the pathogen CT values reported by QIAstat maybe a step in the direction. QIAstat GIP reported eight pathogens that were not detected by two other highly sensitive PCR methods and should thus be seen as true false positives (Table 4). One FP AGE pathogen was a *Cyclospora caytenensis*, detected with a CT value of 35.5. The low pre-test probability of this pathogen and the high CT value would likely have alerted a clinical laboratory to be careful reporting it out as a clinically significant finding. The same approach would potentially be taken with a *C. difficile* finding with a CT value of 37.5 in a single infection sample. All positive results from any test should be interpreted in the clinical context, and a study by Hitchcock [30] that non-reproducible results may be more likely to occur in settings where there is a low pre-test probability of infectious gastroenteritis, especially in patients with non-specific presentations in low-prevalence settings. Prompted by cases of non-reproducible results for *Y. enterocolitica* and positive results for targets rarely positive at their institution, these authors reported lower reproducibility rates for Adenovirus F40/41 (70%, 38/54), *Y. enterocolitica* (72%, 36/50), and non-cholerae *Vibrio* spp. (80%, 8/10) and very poor rates for *V. cholerae* (37.5%, 3/8) with their multiplex PCR test (FilmArray GI).

Finally, the ability to provide Norovirus genotype information by QIAstat GIP may be useful in detecting outbreaks and monitoring epidemiologic trends in norovirus disease [24]. While prolonged faecal shedding that can last for weeks (norovirus) or for months (*Salmonella*) is well documented [11], a recent study in Danish day care centres reported a high frequency (33%, 229/688) of year-round viral shedding by both symptomatic and asymptomatic children [31]. Shedding was highest for Sapovirus and lowest for Norovirus GI, and the authors concluded that children in day care centres may act as a reservoir for AGE viruses. Of interest was that the shedding with Norovirus GI was significantly less frequently observed compared to Norovirus GII shedding ($p = 0.006$).

Limitations

The data in this study may be epidemiologically biased as only 163 samples were collected prospectively while 222 samples were frozen retrospective positives. For this reason, general positivity rates do not apply. Also, an overall specificity is not indicated. However, none of the retrospective samples had been routinely tested with a multiplex PCR test and the occurrence of other gastroenteric pathogens was not known for the vast majority of samples.

Conclusion

Recent health economic outcome reports strongly support the cost effectiveness of hM-PCR tests in AGE. The QIAstat GIP detected a large range of AGE pathogens in diarrheic stool samples with a high level of concordance compared to other highly multiplexed GI panels. It is an easy-to-use system, requires the addition of a 200 μL volume aliquot of Cary-Blair stool into a fully self-contained test cartridge by a single pipetting step and reports results in 70 min. Pending studies that demonstrate clinical significance of semi-quantitative detection of potential gastrointestinal pathogens, the availability of CT values for the pathogen and IC result provide a level of semi-quantitation which may be helpful to assess pre-test probabilities. The high number of parasite positive samples in the study may provide further insights in the epidemiology of parasite AGE. Overall, it is important for health care providers and laboratory professionals to consider all aspects of the patient's condition (e.g., symptom duration and severity

and prior antimicrobial treatment) when interpreting the results of multiplex GI panels.

Acknowledgements Irene Hannet and Anne Line Engsbro are to be considered joint first authors. The authors would like to thank laboratory technicians Danah Knudsen and Robert Jozíc, who participated in the testing at Copenhagen University Hospital Hvidovre and University Hospital Bonn, respectively.

Funding The study was sponsored by STAT-Dx Life (now a Qiagen company).

Compliance with ethical standards

Conflict of interest Marijo Parčina has received funding for travel accommodations for a congress from STAT-Dx. Jan Gorm Lisby has received travel support from Qiagen. Irene Hannet and Josep Pareja were employed by STAT-Dx Life (now a Qiagen company) when the study was conducted and the manuscript was written.

Ethical approval and informed consent The specimens included in the study were collected under an institutional review board (IRB)-approved protocol, which included a waiver of informed consent for the use of residual de-identified samples.

References

1. Riddle MS, Dupont HL, Connor BA (2016) ACG Clinical Guideline: diagnosis, treatment, and prevention of acute diarrheal infections in adults. Am J Gastroenterol 111:602–622. https://doi.org/10.1038/ajg.2016.126
2. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M, Roy SL et al (2011) Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis 17:7–15. https://doi.org/10.3201/eid1701.P11101
3. Piralla A, Lunghi G, Ardissino G, Girello A, Premoli M, Bava E et al (2017) FilmArray ™ GI panel performance for the diagnosis of acute gastroenteritis or hemorragic diarrhea. BMC Microbiol 12:111. https://doi.org/10.1186/s12866-017-1018-2
4. Kim HS, Rotundo L, Nasereddin T, Ike A, Song D, Babar A et al (2017) Time trends and predictors of acute gastroenteritis in the United States: results from National Health and Nutrition Examination Survey 2005-2014. J Clin Gastroenterol 51:693–700. https://doi.org/10.1097/MCG.0000000000000907
5. Schuetz AN (2019). Emerging agents of gastroenteritis: *Aeromonas*, *Plesiomonas*, and the diarrheagenic pathotypes of *Escherichia coli*. Semin Diagn Pathol S0740–2570. https://doi.org/10.1053/j.semdp.2019.04.012
6. Skovgaards DM, Hartmeyer GN, Skov MN, Hoegh SV, Kemp M (2017) *Cryptosporidium* species are frequently present but rarely detected in clinical samples from children with diarrhea in a developed country. Pediatr Infect Dis J 37:e138–e140. http://insights.ovid.com/crossref?an=00006454-900000000-96889. https://doi.org/10.1097/INF.0000000000001794. Accessed May 2018
7. Savioli L, Smith H, Thompson A (2006) *Giardia* and *Cryptosporidium* join the "Neglected Diseases Initiative". Trends Parasitol 22:203–208. https://doi.org/10.1016/j.pt.2006.02.015
8. Khurana S, Chaudhary P (2018) Laboratory diagnosis of cryptosporidiosis. Trop Parasitol 8:2–7. https://doi.org/10.4103/tp.TP
9. Hooshyar H, Rostamkhani P, Arbabi M, Delavari M (2019) *Giardia lamblia* infection : review of current diagnostic strategies. Gastroenterol Hepatol Bed Bench 12:3–12
10. Tack DM, Marder EP, Griffin PM, Cieslak PR, Dunn J, Hurd J et al (2019) Preliminary incidence and trends of infections with pathogens transmitted commonly through food—foodborne diseases active surveillance network. MMWR Morb Mortal Wkly Rep 68:369–373
11. Yalamanchili H, Dandachi D, Okhuysen PC (2018) Use and interpretation of enteropathogen multiplex nucleic acid amplification tests in patients with suspected infectious diarrhea. Gastroenterol Hepatol 14:646–652
12. Yoo J, Park J, Lee HK, Yu JK, Lee GD, Park KG et al (2019) Comparative evaluation of Seegene Allplex gastrointestinal, Luminex xTAG gastrointestinal pathogen panel, and BD MAX enteric assays for detection of gastrointestinal pathogens in clinical stool specimens. Arch Pathol Lab Med. https://doi.org/10.5858/arpa.2018-0002-oa
13. Axelrad JE, Joelson A, Nobel Y, Whittier S, Lawlor G, Riddle MS et al (2018) The distribution of enteric infections utilizing stool microbial polymerase chain reaction testing in clinical practice. Dig Dis Sci 63:1900–1909. https://doi.org/10.1007/s10620-018-5087-3
14. Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB (2013) Recent advances in understanding enteric pathogenic *Escherichia coli*. Clin Microbiol Rev 26:822–880. https://doi.org/10.1128/CMR.00022-13
15. Spina A, Kerr KG, Cormican M, Barbut F, Eigentler A, Zerva L et al (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
16. Axelrad JE, Freedberg DE, Whittier S, Greendyke W, Lebwohl B, Green DA (2019) Impact of gastrointestinal panel implementation on health care utilization and outcomes. J Clin Microbiol 57:e01775–e01718. http://jcm.asm.org/content/57/3/e01775-18.abstract. https://doi.org/10.1128/JCM.01775-18. Accessed 27 Feb 2019
17. Beal SG, Tremblay EE, Toffel S, Velez L, Rand HA (2017) Gastrointestinal PCR panel improves clinical management and lowers health care costs. J Clin Microbiol 56:e01457–e01417
18. Keske S, Zabun B, Aksoy K, Palaog E (2018) Rapid molecular detection of gastrointestinal pathogens and its role in antimicrobial stewardship. J Clin Microbiol 56:p00148–p00118. https://doi.org/10.1128/JCM.00148-18
19. Cybulski RJ Jr, Bateman AC, Bourassa L, Bryan A, Beail B, Matsumoto J et al (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
20. Freeman K, Mistry H, Tsertsvadze A, Royle P, Mccarthy N, Taylor-phillips S et al (2017) Multiplex tests to identify gastrointestinal bacteria, viruses and parasites in people with suspected infectious gastroenteritis: a systematic review and economic analysis. Health Technol Assess 21:1–188. https://doi.org/10.3310/hta21230
21. Buss SN, Leber A, Chapin K, Fey PD, Bankowski MJ, Jones MK et al (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
22. Murphy CN, Fowler RC, Iwen PC, Fey PD (2017) Evaluation of the BioFire FilmArray ® 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
23. Frost LS, Leplae R, Summers AO, Toussaint A (2005) Mobile genetic elements: the agents of open source evolution. Nat Rev Microbiol 3:722–732. https://doi.org/10.1038/nrmicro1235
24. Ramanan P, Espy MJ, Khare R, Binnicker MJ (2017) Detection and differentiation of norovirus genogroups I and II from clinical stool

specimens using real-time PCR. Diagn Microbiol Infect Dis 87: 325–327. https://doi.org/10.1016/j.diagmicrobio.2017.01.009

25. Enserink R, Scholts R, Bruijning-verhagen P, Duizer E, Vennema H, De Boer R et al (2014) High detection rates of enteropathogens in asymptomatic children attending day care. PLoS One 9:e89496. https://doi.org/10.1371/journal.pone.0089496
26. Stockmann C, Pavia AT, Graham B, Vaughn M, Crisp R, Poritz MA et al (2017) Detection of 23 gastrointestinal pathogens among children who present with diarrhea. J Pediatric Infect Dis Soc 6:231–238. https://doi.org/10.1093/jpids/piw020
27. Calderaro A, Martinelli M, Buttrini M, Montecchini S, Covan S, Rossi S et al (2018) International Journal of Medical Microbiology contribution of the FilmArray ® Gastrointestinal Panel in the laboratory diagnosis of gastroenteritis in a cohort of children : a two-year prospective study. Int J Med Microbiol 308:514–521. https://doi.org/10.1016/j.ijmm.2018.04.007
28. Debast SB, Bauer MP, Kuijper EJ (2014) European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for *Clostridium difficile* infection. Clin Microbiol Infect 20:1–26. https://doi.org/10.1111/1469-0691.12418
29. Graber ML, Franklin N, Gordon R (2005) Diagnostic error in internal medicine. Arch Intern Med 165:1493–1499
30. Hitchcock MM, Gomez CA, Banaei N, City SL, Cross M (2018) Low yield of FilmArray GI Panel in hospitalized patients with diarrhea: an opportunity for diagnostic stewardship. J Clin Microbiol 56:e01558–e01517. https://doi.org/10.1128/JCM.01558-1
31. Jensen BH, Jokelainen P, Christian A, Nielsen Y, Franck KT, Rejk D et al (2019) Children attending day care centers are a year-round reservoir of gastrointestinal viruses. Sci Rep 9:3286. https://doi.org/10.1038/s41598-019-40077-9