JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Comparison of multiplex syndromic panel tests with conventional methods in the detection of gastroenteritis agents

Özlem Aydemir[1], Hande Toptan[2], Elif Ö Şahin[2], Hüseyin A Terzi[2], Gökçen Ormanoğlu[1], Mehmet Köroğlu[1], Mustafa Altındiş[1]

[1] Department of Medical Microbiology, Faculty of Medicine, Sakarya University, Sakarya, Türkiye
[2] Medical Microbiology Laboratory, Sakarya University Training and Research Hospital, Ministry of Health, Sakarya, Türkiye

Abstract

Introduction: We aimed to evaluate the performance of multiplex polymerase chain reaction (PCR)-based FTD gastroenteritis kit (Fast-Track Diagnostics, Esch-sur-Alzette, Luxembourg) and QIAstat-Dx gastrointestinal panel (Q-GP; Hilden, Germany) in the detection of different enteric pathogens.

Methodology: The molecular test results of 320 stool samples from patients with a preliminary diagnosis of infectious gastroenteritis between July 2019 and October 2023 were retrospectively examined, and compared with conventional test results.

Results: A single pathogen was detected in 144 samples, and more than 1 pathogen was detected in 22 samples with FTD and QIAstat-Dx GP. *Salmonella* was isolated by culture in 30% samples that were detected as *Salmonella*-positive by PCR. *Shigella*, *Campylobacter*, verotoxin producing *Escherichia coli*, Shiga-like toxin producing *E. coli*, enteropathogenic *E. coli*, enteroaggregative *E. coli*, and enterotoxigenic *E. coli* were detected by molecular tests; but could not be isolated in stool culture. Rotavirus was detected by PCR in 11.1% samples; antigen test was positive in 20% samples that were adenovirus-positive based on molecular tests. Five percent of the samples in which *C. difficile* was detected by molecular tests were determined to be toxin A/B positive by immunochromatographic test. *G. lamblia* trophozoites were seen in direct microscopic evaluation in samples that were identified as *G. lamblia* positive by PCR.

Conclusions: The multiplex gastrointestinal pathogen panel test is a simpler and faster test than traditional microbiology methods. However, the effect of these test results on the patient's diagnosis and treatment needs to be investigated. More studies are needed to compare standard and molecular methods.

Key words: infectious gastroenteritis; multiplex real-time PCR; conventional diagnostic techniques.

J Infect Dev Ctries 2025; 19(1):76-82. doi:10.3855/jidc.19386

(Received 12 October 2023 – Accepted 11 March 2024)

Introduction

Acute infectious gastroenteritis (AIGE) is the most common cause of morbidity and mortality worldwide after respiratory tract infections. It is reported to be the most common indication of hospitalization and cause of death in children under 5 years of age in developing countries [1].

Although acute enteric infections are mostly self-limited, such infections may sometimes cause more serious clinical manifestations and complications that require hospitalization. Diarrhea outbreaks in the community are usually caused by microorganisms found in water, food and the hospital environment. Infections can be transmitted from person to person through direct contact or fomites [1,2].

Bacteria and viruses are often the causative agents in infectious gastroenteritis. In recent years, diarrhea-causing strains of *Escherichia coli* have been identified more frequently due to the developments in diagnostic tests. Some parasites can also be a causal factor in gastroenteritis (GE) [3–5].

AIGE usually causes clinical presentations such as abdominal pain, cramping, fever, malaise, bloody stools, nausea, vomiting, and diarrhea. Therefore, differential diagnosis of causative pathogens based on clinical symptoms can be difficult. Treatment, isolation, and follow-up approaches may vary depending on the causative pathogen. Identification of the causative pathogen is also important for the management of diarrheal diseases in terms of public health. Therefore, rapid and accurate identification of the causal factor is important. Although the methods used in the diagnosis of AIGE vary according to the suspected agent, culture, immunological tests and microscopy continue to be the most frequently used methods [6].

Stool culture is the gold standard method used to detect bacterial pathogens that cause gastrointestinal tract infections. However, it has low sensitivity rates, requires extended time, and is labor intensive [7]. Although fecal antigen tests are available for the diagnosis of some viral gastroenteritis, antigen tests are not available for all viruses, bacteria, parasites, and fungi that cause gastrointestinal (GI) infections. In addition, the sensitivity rates of antigen tests vary [7]. Microscopy is the most commonly used reference method for the identification of parasites. However, microscopy has low sensitivity, and requires skilled personel with specific training and experience to identify parasites. Antigen tests are available for the detection of *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica*; but not for all parasitic agents [5].

Conventional diagnostic methods require time, intensive labor, and experience; and the causative agent can be detected only in 60–70% of GE cases [2,6,8]. Multiplex-polymerase chain reaction (PCR)-based GI pathogen panel tests have been used in recent years to overcome the disadvantages and limitations of conventional methods. With these nucleic acid amplification tests (NAAT), many enteric pathogens (bacteria, viruses, and parasites) can be detected within a short time with high sensitivity and specificity [2,6,8].

Nucleic acid amplification-based gastroinstestinal system (GIS) pathogens panel tests are also used in our laboratory. In this study, we aimed to identify GI pathogens by multiplex real-time PCR method in patients with suspected GE, and compare the results with those identified using conventional microbiological methods.

Methodology

The molecular test results of 320 stool samples from patients with a pre-diagnosis of ınfectıous GE that were sent to our laboratory for molecular test panel identification between 2019 and 2023 were retrospectively examined. The conventional test results requested from the same patients were retrieved from the hospital automation system and were also analyzed retrospectively.

The Ethical Committee of Sakarya University School of Medicine approved the study (approval no: 050.01.04/243).

Conventional diagnostic techniques

Stool culture for bacterial agents responsible for infectious GE was performed using (1) immunochromatographic tests (ICT) detecting toxin A/B for the diagnosis of *Clostridium difficile* (*C. difficile* toxin A/toxin B combo rapid test cassette; Acro-Biotect, Montclair CA, USA); (2) direct microscopic methods for the detection of parasitic agents; (3) *Giardia*/*Cryptosporidium*/*Entamoeba* antigen lateral flow test (*Crypto-Entomoeba-Giardia* test; Mon Lab, Barcelona, Spain); and (4) rotavirus and adenovirus, ICT-based tests (Bio gold rotavirüs/adenovirüs rapid test cassette; Cites Diagnostic, Canada). All samples for stool culture were cultured in eosin methylene blue agar (EMB agar; RTA, İstanbul, Türkiye), and *Salmonella Shigella* agar (SS agar; RTA, İstanbul, Türkiye).

Multiplex PCR sample processing and data analysis

A total of 189 samples (59%) were tested with the multiplex PCR-based FTD gastroenteritis kit (Fast-Track Diagnostics, Esch-sur-Alzette, Luxembourg), and 131 samples (40.9%) were tested with the fully automatic QIAstat-Dx gastro-intestinal panel kit (QIAstat-DxGP; Q-GP; Hilden, Germany), following the manufacturers' protocol. Seventeen pathogens (*Campylobacter coli*/*jejuni*/*lari*, *Clostridium difficile*, *Salmonella* spp, *Shigella* spp., verocytotoxin-producing *E. coli*, *Yersinia enterocolitica*, human adenovirus, human astrovirus, norovirus GI, norovirus GII, rotavirus, sapovirus, enterovirus, *Cryptosporidium*, *Entamoeba histolytica*, and *Giardia lamblia* could be detected simultaneously with the FTD gastroenteritis kit. The QIAstat-Dx gastro-intestinal syndromic panel test could be used to detect 24 agents (*Clostridium difficile* toxin A/B, enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC)/*Shigella*, enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli*) (ETEC) It/st, *Campylobacter* spp. (*C. jejuni, C. upsaliensis, C. coli*), *Plesiomonas shigelloides*, *Salmonella* spp. (STEC) stx1/stx2, Shiga-like toxin producing *E. coli* (STEC) O157:H7, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Yersinia enterocolitica*, adenovirus F40/41, astrovirus, norovirus GI, norovirus GII, rotavirus A, *Sapovirus* (GI, GII, GIV, GV), spp., *Cyclospora cayetanensis*, *Entamoeba histolytica*, and *Giardia lamblia*).

The demographic data of the patients were analyzed retrospectively from the laboratory automation system records of our hospital.

Statistical analysis

Descriptive analyses were performed to provide information on general characteristics of the study population. Categorical variables were compared by Chi square test. Cohen's Kappa (κ) coefficients were

calculated to determine the concordance between PCR and other methods. Categorical variables were presented as a count and percentage. A p value < 0.05 was considered significant. Analyses were performed using the statistical software SPSS (IBM SPSS Statistics, version 23.0; IBM Corp, Armonk, NY, USA).

Results

In this study, 320 stool samples were collected from 46 (45.6%) female and 174 (54.4%) male patients. Sixty-four (20%) of the patients were children.

Evaluation of molecular panel results

A single pathogen was detected in 144 (45%) of 320 stool samples studied with QIAstat-Dx GP and FTD. More than one pathogen was detected in 22 samples. (Table 1)

The identified pathogens included *Salmonella* spp in 20 (6.3%) samples, verotoxin producing *E. coli* (VTEC)/STEC O157:H7 in 16 (12.2%) samples, EPEC in 14 (10.4%) samples, *Campylobacter* spp in 13 (4.4%) samples, *Clostridium difficile* in 12 (3.8%) samples, EAEC in 9 (6.8%) samples, ETEC in 5 (1.6%) samples, *Shigella* in 4 (1.3%) samples, norovirus GI/GII in 24 (7.5%) samples, rotavirus in 18 (5.3%) samples, sapovirus in 10 (3.1%) samples, human astrovirus in 9 (2.8%) samples, and human adenovirus in 5 (1.6%) samples (Table 1).

Comparison of conventional methods and molecular panel results

Stool culture and molecular testing panel were requested simultaneously for 112 patients. *Salmonella* was detected by the molecular panel in 20 patients and stool culture detected *Salmonella* in 6 (6.3%) of these patients. *Shigella*, *Campylobacter*, VTEC/STEC, EPEC, EAEC, and ETEC were identified by molecular tests, but could not be isolated by stool culture.

When 95 stool samples in which bacterial agents were detected by molecular tests were examined with microscopy, leukocytes were seen in 38 (40.5%) and erythrocytes were seen in 16 (16.8%).

Antigen testing was requested for 64 stool samples to identify rotavirus/adenovirus. The antigen test results were positive in 2 (11.1%) of the 18 samples that were positive for rotavirus based on PCR panel results. Adenovirus antigen test was positive in 2 of 5 samples (20%) that were identified as positive for adenovirus based on PCR results (Tables 2 and 3).

Table 1. Distribution of samples identified as positive with FTD and QiaStat Dx.

Pathogens	FTD, n	Qiastat Dx, n	Total, n (%)
Salmonella spp	13	7	20 (6.3)
Campylobacter spp	3	10	13 (4.4)
Shigella spp	4	-	4 (1.3)
VTEC/STEC	4	12	16 (12.2)
EPEC	-	14	14 (10.6)
EAEC	-	9	9 (6.8)
ETEC	-	5	5 (1.6)
Yersinia enterocolitica	1	1	2 (1.5)
Clostridium difficile	2	10	12 (3.8)
Rotavirus	1	17	18 (5.3)
Norovirus	13	11	24 (7.5)
Astrovirus	6	3	9 (2.8)
Adenovirus	3	2	5 (1.6)
Sapovirus	6	4	10 (3.1)
Giardia lamblia	1	1	2 (0.63)
Cryptosporidium parvum	2	1	3 (0.94)
Total	57	107	166

EAEC; enteroaggregative *E. coli;* EPEC; enteropathogenic *E. coli*; ETEC; enterotoxigenic *E. coli*; FTD; Fast-Track Diagnostics; QiaStat Dx; QIAstat-Dx gastrointestinal panel; VTEC/STEC; verotoxin producing *E. coli/* Shiga-like toxin producing *E. Coli.*

Table 2. Pathogens and positivity rates detected in stool samples by FTD/Qiastatdx and conventional microbiological methods.

Pathogens	FTD/Qiastat Dx		Conventional methods		*p* value
	Number of Samples Inspected, n	Positive, n (%)	Number of Samples Inspected, n	Positive, n (%)	
Salmonella spp	320	20 (6.3)	115	6 (5.2)	0.864
Campylobacter spp	320	13 (4.4)	-	-	-
Shigella spp.	320	4 (1.3)	115	0	0.577
VTEC/STEC	131	16 (12.2)	-	-	-
EPEC	131	14 (10.6)	-	-	-
EAEC	131	9 (6.8)	-	-	-
ETEC	320	5 (1.6)	-	-	-
Yersinia enterocolitica	131	2 (1.5)	-	-	-
Clostridium difficile	320	12 (3.8)	20	1 (5)	0.552
Rotavirus	320	18 (5.3)	64	2 (3.1)	0.549
Norovirus	320	24 (7.5)	-	-	-
Astrovirus	320	9 (2.8)	-	-	-
Adenovirus	320	5 (1.6)	64	2 (3.1)	0.330
Sapovirus	320	10 (3.1)	-	-	-
Giardia lamblia	320	2 (0.63)	64	2 (3.1)	0.131
Cryptosporidium parvum	320	3 (0.94)	-	-	-

EAEC; enteroaggregative *E. coli*; EPEC; enteropathogenic *E. coli*; ETEC; enterotoxigenic *E. coli*; FTD; Fast-Track Diagnostics; QiaStat Dx; QIAstat-Dx gastrointestinal panel; VTEC/STEC; *v*erotoxin producing *E. coli/* Shiga-like toxin producing *E. coli.*

Table 3. FTD/Qiastat Dx and other tests results.

		PCR		κ	*p* value
		Positive	Negative		
ICT rotavirus antigen test	Positive	2	0	0.152	0.076
	Negative	16	46		
ICT adenovirus antigen test	Positive	1	0	0.316	0.078
	Negative	4	59		
Salmonella stool culture	Positive	6	0	0.415	< 0.001
	Negative	14	95		
C. difficile toxin A/B ICT test	Positive	1	0	0.068	1,000
	Negative	11	8		

FTD; Fast-Track Diagnostics; ICT; immunochromatographic test; κ; Kappa coefficient; QiaStat Dx; PCR; polymerase chain reaction; QIAstat-Dx gastrointestinal panel.

C. difficile toxin A/B was analyzed in 64 stool samples. Only 1 of the 12 stool samples in which *C. difficile* was detected by molecular tests was determined to be toxin A/B positive by the ICT method. (Table 3).

Direct stool microscopy was performed on 64 samples that were also tested by the PCR panel. *G. lamblia* trophozoites were observed in 2 stool samples, and these samples were also identified as positive for *G. lamblia* by PCR. *Giardia*/*Cryptosporidium*/*Entamoeba* antigen test was positive in only 1 stool sample.

Since *Campylobacter* culture was not routinely performed in the laboratory, molecular test results could not be compared with this conventional method.

Analysis of pathogens involved in coinfections

More than 1 pathogen was detected simultaneously with molecular tests in 22 (12.7%) of the samples in which bacterial pathogen were isolated in stool culture. More than one viral agent was detected by molecular methods in 8 (4.8%) of stool samples in which pathogen was detected. The PCR panel identified bacterial + viral pathogens in 10 (6%) samples, parasite + bacteria in 3 (1.2%) samples, and parasite (*Giardia*) + viral (sapovirus) pathogens in 1 (0.6%) sample (Table 4). Among the samples that were positive for more than one pathogen, 59.3% belonged to pediatric patients and 40.7% belonged to adult patients.

Discussion

Direct microscopy, culture-based tests and immunochromatographic test/lateral flow assay tests are traditional methods used to identify pathogens that cause gastrointestinal infections. Traditional methods are limited in their effectiveness because they require more time, can detect only a limited number of agents, and have low sensitivity/specificity [7–9]. Therefore, molecular panel tests based on multiplex PCR are used for the rapid diagnosis of GI tract infectious agents. These tests enable the detection of many bacteria, viruses and parasites within a short time [8–10]. They may also play an important role in determining the frequency and spread of GI tract infection agents in the future. Sensitivity and specificity of NAAT depends on the targeted pathogens, whether the stool samples are fresh or frozen, and inhibitors that may be present in the stool [11–16]. However, it is reported to have high sensitivity and specificity of > 90% when used in samples from symptomatic patients [16]. Although commercially available multiplex PCR tests vary depending on the number of pathogens in the panel, they generally show similar performance and sensitivity rates ranging between 92–97% [12,16,17]. In our study, PCR panel tests detected one or more pathogens in 51.8% of the samples. A single pathogen was detected in 144 stool samples, and > 1 pathogens were detected in 22 samples. One or more viral pathogens were detected in 66 (20.3%) stool samples, at least 1 bacterial pathogen was detected in 95 (29.6%) samples, and parasites were detected in 5 samples.

Axelrad *et al*. used multiplex PCR to identify pathogens in stool samples [17]. They obtained positive results in 25.9% of 9,403 stool samples; 62.7% of the detected pathogens were bacteria, 31.5% were viruses, and 5.8% were parasites. The coinfection rate as 28.5%. The most frequently identified pathogens in this study were EPEC (22.5%), norovirus (17.3%), and EAEC (13.7%). When using conventional methods, total positive samples rate remained at 4.1% [17]. Castany-

Table 4. Analysis of pathogens identified in coinfections.

	n (%)
Rotavirus + Adenovirus	4 (1.25)
Sapovirus + Norovirus	2 (0.6)
Norovirus + Adenovirus,	1 (0.3)
Norovirus GI + Norovirus GII	1 (12.5)
Salmonella + Rotavirus,	1 (0.3)
Salmonella + Sapovirus	1 (0.3)
Enteroaggregative *E. coli*+ Rotavirus + Astrovirus	2 (0.6)
Norovirus + Enteropathogenic *E. coli*	2 (0.6)
Clostridium + Norovirus + Rotavirus	2 (0.6)
Campylobacter + Astrovirus	1 (0.3)
Enteroaggregative *E. coli* + Astrovirus	1 (0.3)
Cryptosporidium spp.+ *Salmonella*	2 (0.6)
Cryptosporidium spp +*Yersiniae*	1 (0.3)
Giardia + Sapovirus	1 (0.3)
Total	22 (12.7)

Feixas *et al*. detected 1 or more pathogens in 68.8% of the samples with a multiplex PCR panel, while they were able to detect pathogens in 35.2% of the samples using conventional methods (culture, microscopy, and antigen tests) [18]. In our study, the molecular GI system test panel was designed based on a retrospective review of the patient reports. Therefore, traditional methods could not be applied to all samples. This is the most important limiting aspect of our study

In developed countries, *Salmonella* spp., *Campylobacter* spp., EPEC, and EAEC are the most common pathogens causing bacterial acute gastroenteritis [19–23]. In our study, these three bacteria were found to be the most common causative agents.

Bacteria could be isolated by culture in only 6 of the 20 samples in which *Salmonella* was detected by a molecular panel test. Since *Campylobacter* culture is not routinely performed in our laboratory, *Campylobacter* detected in 13 samples by PCR could not be isolated by culture. In our laboratory, stool cultures were directly planted without being kept in selective media, and this could be the reason for the low *Salmonella* isolation rates detected in culture. We consider this to be a limiting aspect of our study.

In a multicenter study in the USA, 14% of *Salmonella* spp detected by PCR could not be confirmed by gold standard methods [9]. Kellner *et al.* reported that there was over 99% agreement between molecular GI panel tests and bacterial culture [6].

In our study, *C. difficile* positivity was detected in 12 patients. Only 1 of the isolates detected by the molecular panel could be detected by the toxin A/B ICT test. However, when interpreting *C. difficile* positivity detected by molecular tests, the patient's age, risk factors, and the patient's clinical condition should be taken into account, and the diagnosis of *C. difficile* infection should not be made exclusively by PCR [24].

In recent years, norovirus infections have become more common after the introduction of the rotavirus vaccine in children [25–30]. Norovirus was detected in 7.5% of the samples in our study, rotavirus in 5.3% samples, sapovirus in 3.1% samples, astrovirus in 2.8% samples, and adenovirus in 1.6% samples.

Previous studies with multiplex PCR have reported coinfection rates of 20–80%. The most common cause of diarrhea in coinfections has been reported to be *E. coli* strains [14,15,17,18,22]. In our study, the rate of coinfection was 12.7%. EPEC/EHEC were detected in 23.8% of 21 samples with coinfection. Coinfections are generally seen more frequently in pediatric patients [18,31,32]. In our study, 53% of the samples in which coinfection was detected belonged to pediatric patients.

Other factors that may affect the results include: whether the sample was collected properly, transfer and waiting conditions, whether the sample was fresh or frozen, choice of medium used for culture, and incubation conditions. These factors may be responsible for inconsistency between molecular tests and culture results [32]. Studies have reported that culture is accepted as the gold standard, and panel tests may give false positive results [6].

Despite their advantages, nucleic acid-based panel tests can also cause clinical situations that are difficult to interpret. These tests have high sensitivity and even very low numbers of enteric pathogens can be detected in samples [16]. In some cases, the clinical significance of a detected microorganisms may be unclear [16]. At the same time, positive results can be obtained in the presence of colonization or in asymptomatic carriers. This may lead to unnecessary use of antibiotics. [16,33]. For example, bacteria may be excreted with the stool for weeks for or months after *Salmonella* infection [16,33,34], leading to confusion in clinical interpretation. It has been suggested that patients with negative culture and positive molecular panel tests are generally asymptomatic carriers [7,16]. It has been observed that there is a significant difference in symptoms and laboratory findings in children with gastroenteritis, in terms of bacterial agents in PCR negative and positive patients [24]. Therefore, PCR testing for certain bacterial agents should be performed according to the preliminary diagnosis in patients with suspected bacterial AIGE to benefit from the diagnostic accuracy of the test, while keeping the costs low, making optimum use of the workforce, and preventing unnecessary treatments [24].

Questions remain about the clinical benefits and cost-effectiveness of molecular test panels [8]. In a multicenter study bacterial agents were detected in 8% of the patients with conventional diagnostic methods, and 37% of the patients with molecular tests. The authors reported that it was beneficial to detect diarrheagenic *E. coli* strains (STEC, EPEC, EAEC and ETEC) which cause acute gastroenteritis by PCR, since these cannot be identified by traditional culture methods. PCR-based tests had a rapid turnaround time and targeted therapy could be applied instead of empirical therapy, which contributed to clinical benefit and cost-effectiveness [8]. In another study, it was suggested that the overall healthcare cost may be lower due to the reduced length of hospital stay when GI multiplex NAAT are used [10]. Although an initial

investment is required to use this type of multiplex panel test, it can significantly reduce cost by reducing the time patients stay in contact isolation [11].

Conclusions

GI multiplex nucleic acid amplification tests provide an opportunity to efficiently and sensitively identify potential causative agents in patients with gastroenteritis. However, these tests should be used selectively because of their high cost and the results of these tests should be interpreted with caution, taking into account the patients' clinical symptoms, diagnosis, and pathogens identified.

Corresponding author

Ozlem Aydemir, MD.
Department of Medical Microbiology, Sakarya University Education and Research Hospital,
Adnan Menderes Cd Sağlık Sk No: 195, Sakarya, Türkiye.
Tel: +090 5056369400
Fax: +90 264 2759192
Email: ozlemaydemir@sakarya.edu.tr

Conflict of interests

No conflict of interests is declared.

References

1. Freeman K, Mistry H, Tsertsvadze A, Royle P, McCarthy N, Taylor-Phillips S, Manuel R, Mason J (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. doi: 10.3310/hta21230.
2. Balsalobre-Arenas L, Alarcón-Cavero T (2017) Rapid diagnosis of gastrointestinal tract infections due to parasites, viruses, and bacteria. Enferm Infecc Microbiol Clin 35: 367–376. doi: 10.1016/j.eimc.2017.01.002.
3. 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. doi: 10.3201/eid1701.P11101.
4. Schuetz AN (2019) Emerging agents of gastroenteritis: *Aeromonas*, *Plesiomonas*, and the diarrheagenic pathotypes of *Escherichia coli*. Semin Diagn Pathol S0740–2570. doi: 10.1053/j.semdp.2019.04.012.
5. 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. doi: 10.1097/INF.0000000000001794.
6. Kellner T, Parsons B, Chui L, Berenger BM, Xie J, Burnham CA, Tarr PI, Lee BE, Nettel-Aguirre A, Szelewicki J, Vanderkooi OG, Pang XL, Zelyas N, Freedman (2019) Comparative evaluation of enteric bacterial culture and a molecular multiplex syndromic panel in children with acute gastroenteritis. J Clin Microbiol 57: e00205–19. doi: 10.1128/JCM.00205-19.
7. 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. doi: 10.1007/s10096-005-1373-z.
8. 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. doi: 10.1093/cid/ciy357.
9. 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. doi: 10.1128/JCM.01393-14.
10. Beal SG, Tremblay EE, Toffel S, Velez L, Rand KHA (2018) Gastrointestinal PCR panel improves clinical management and lowers health care costs. J Clin Microbiol 56: e01457–17. doi: 10.1128/JCM.01457-17.
11. Goldenberg SD, Bacelar M, Brazier P, Bisnauthsing K, Edgeworth JD (2015) A cost benefit analysis of the Luminex x TAG gastrointestinal pathogen panel for detection of infectious gastroenteritis in hospitalised patients. J Infect 70: 504–511. doi: 10.1016/j.jinf.2014.11.009.
12. Yoo J, Park J, Lee HK, Yu JK, Lee GD, Park KG, Oak HC, Park YJ (2019) Comparative evaluation of Seegene allplex gastrointestinal, Luminex x TAG gastrointestinal pathogen panel, and BD MAX enteric assays for detection of gastrointestinal pathogens in clinical stool specimens. Arch Pathol Lab Med 143: 999–1005. doi: 10.5858/arpa.2018-0002-OA.
13. 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. doi: 10.1016/j.cmi.2015.04.007.
14. 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. doi: 10.1128/JCM.02674-14.
15. 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. doi: 10.1007/s10096-016-2858-7.
16. 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.
17. Axelrad JE, Joelson A, Nobel Y, Whittier S, Lawlor G, Riddle MS, Green PHR, Lebwohl B (2018) The distribution of enteric infections utilizing stool microbial polymerase chain reaction testing in clinical practice. Dig Dis Sci 63: 1900–1909. doi: 10.1007/s10620-018-5087-3.
18. Castany-Feixas M, Simo S, Garcia-Garcia S, Fernandez de Sevilla M, Launes C, Kalkgruber M, Gene A, Muñoz-Almagro C, Brotons P (2021) Rapid molecular syndromic testing for aetiological diagnosis of gastrointestinal infections and targeted antimicrobial prescription: experience from a reference paediatric hospital in Spain Eur J Clin Microbiol Infect Dis 40: 2153–2160. doi: 10.1007/s10096-021-04266-7.

19. Friesema IH, de Boer RF, Duizer E, Kortbeek LM, Notermans DW, Norbruis OF, Bezemer DD, van Heerbeek H, van Andel RN, van Enk JG, Fraaij PL, Koopmans MP, Kooistra-Smid AM, van Duynhoven YT (2012) Etiology of acute gastroenteritis in children requiring hospitalization in the Netherlands. Eur J Clin Microbiol Infect Dis 31: 405–415. doi: 10.1007/s10096-011-1320-0.
20. Xie J, Nettel-Aguirre A, Lee BE, Chui L, Pang XL, Zhuo R, Parsons B, Vanderkooi OG, Tarr PI, Ali S, Dickinson JA, Hagen E, Svenson LW, MacDonald SE, Drews SJ, Tellier R, Graham T, Lavoie M, MacDonald J, Freedman SB, Pediatric Emergency Research Canada (PERC), Alberta Provincial Pediatric EnTeric Infection TEam (APPETITE Team) (2019) Relationship between enteric pathogens and acute gastroenteritis disease severity: a prospective cohort study.Clin Microbiol Infect 25: 454–461. doi: 10.1016/j.cmi.2018.06.016.
21. Kim Y, Kim HJ, Lim S, Bae KS, Han SB, Jeong DC, Kang JH, Shin GJ, Lee GD, Park YJ (2017) Community-acquired *Escherichia coli* enteritis in Korean children: the clinical application of a stool polymerase chain reaction assay. Infect Chemother 49: 275–281. doi: 10.3947/ic.2017.49.4.275.
22. 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. doi: 10.1016/j.cmi.2015.04.007.
23. Shane AL, Mody RK, Crump JA, Tarr PI, Steiner TS, Kotloff K, Langley JM, Wanke C, Warren CA, Cheng AC, Cantey J, Pickering LK (2017) 2017 Infectious Diseases Society of America clinical practice guidelines for the diagnosis and management of infectious diarrhea. Clin Infect Dis 65: e45–e80. doi: 10.1093/cid/cix669.
24. Lee HW, Han SB, Rhim JW (2021) Application of a multiplex polymerase chain reaction test for diagnosing bacterial enteritis in children in a real-life clinical setting. Children (Basel) 8: 538. doi: 10.3390/children8070538.
25. Hebbelstrup Jensen B, Jokelainen P, Nielsen ACY, Franck KT, Rejkjær Holm D, Schønning K, Petersen AM, Krogfelt KA (2019) Children attending day care centers are a year-round reservoir of gastrointestinal viruses. Sci Rep 9: 3286. doi: 10.1038/s41598-019-40077-9.
26. Hassan F, Kanwar N, Harrison CJ, Halasa NB, Chappell JD, Englund JA, Klein EJ, Weinberg GA, Szilagyi PG, Moffatt ME, Oberste MS, Nix WA, Rogers S, Bowen MD, Vinjé J, Wikswo ME, Parashar UD, Payne DC, Selvarangan R (2019). Viral etiology of acute gastroenteritis in < 2-year-old US children in the post-rotavirus vaccine era. J Pediatric Infect Dis Soc 8: 414–421. doi: 10.1093/jpids/piy077.
27. Payne DC, Vinjé J, Szilagyi PG, Edwards KM, Staat MA, Weinberg GA, Hall CB, Chappell J, Bernstein DI, Curns AT, Wikswo M, Shirley SH, Hall AJ, Lopman B, Parashar UD (2013) Norovirus and medically attended gastroenteritis in US children. N Engl J Med 368: 1121–1130. doi: 10.1056/NEJMsa1206589.
28. Nicholson MR, Van Horn GT, Tang YW, Vinjé 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. doi: 10.1016/j.jpeds.2016.05.068.
29. Hall AJ, Lopman BA, Payne DC, Patel MM, Gastañaduy PA, Vinjé J, Parashar UD (2013) Norovirus disease in the United States. Emerg Infect Dis 19: 1198–1205. doi: 10.3201/eid1908.130465.
30. Wikswo ME, Desai R, Edwards KM, Staat MA, Szilagyi PG, Weinberg GA, Curns AT, Lopman B, Vinjé J, Parashar UD, Payne DC, Hall AJ (2013) Clinical profile of children with norovirus disease in rotavirus vaccine era. Emerg Infect Dis 19: 1691–1693. doi: 10.3201/eid1910.130448.
31. Stockmann C, Pavia AT, Graham B, Vaughn M, Crisp R, Poritz MA, Thatcher S, Korgenski EK, Barney T, Daly J, Rogatcheva M (2017) Detection of 23 gastrointestinal pathogens among children who present with diarrhea. J Pediatric Infect Dis Soc 6: 231–238. doi: 10.1093/jpids/piw020.
32. Beckmann C, Heininger U, Marti H, Hirsch HH (2014) Gastrointestinal pathogens detected by multiplex nucleic acid amplification testing in stools of pediatric patients and patients returning from the tropics. Infection 42: 961–970. doi: 10.1007/s15010-014-0656-7.
33. Murata T, Katsushima N, Mizuta K, Muraki Y, Hongo S, Matsuzaki Y (2007) Prolonged norovirus shedding in infants ≤ 6 months of age with gastroenteritis. Pediatr Infect Dis J 26: 46–49. doi: 10.1097/01.inf.0000247102.04997.e0.
34. Buchwald DS, Blaser MJ (1984) A review of human salmonellosis: II. Duration of excretion following infection with nontyphi *Salmonella*. Rev Infect Dis 6: 345–356. doi: 10.1093/clinids/6.3.345.