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Performance of the xTAG® Gastrointestinal Pathogen Panel, a Multiplex Molecular Assay for Simultaneous Detection of Bacterial, Viral, and Parasitic Causes of Infectious Gastroenteritis

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The xTAG® Gastrointestinal Pathogen Panel (GPP) is a multiplexed molecular test for 15 gastrointestinal pathogens. The sensitivity and specificity of this test were assessed in 901 stool specimens collected from pediatric and adult patients at four clinical sites. A combination of conventional and molecular methods was used as comparator. Sensitivity could be determined for 12 of 15 pathogens and was 94.3% overall. The specificity across all 15 targets was 98.5%. Testing for the pathogen identified was not requested by the physician in 65% of specimens. The simultaneous detection of these 15 pathogens can provide physicians with a more comprehensive assessment of the etiology of diarrheal disease.

Keywords: Luminex, xTAG GPP, multiplex, gastroenteritis, infectious diarrhea

Gastrointestinal infections are a significant cause of morbidity and mortality worldwide [16, 17]. Identification of enteric pathogens currently incorporates a range of different methods with limitations, including varying sensitivities, specificities, and turnaround times, raising the opportunity to complement traditional techniques with molecular testing. The xTAG® Gastrointestinal Pathogen Panel (GPP) is a new qualitative bead-based multiplexed molecular diagnostic test to simultaneously detect and identify 15 viral, bacterial, and parasitic pathogens commonly implicated in the etiology of acute and chronic gastroenteritis. The panel includes adenovirus 40/41, rotavirus A, norovirus GI/GII, *Salmonella* spp., *Campylobacter* spp. (*C. jejuni*, *C. coli*, *C. lari*), *Shigella* spp. (*S. boydii*, *S. sonnei*, *S. flexneri*, *S. dysenteriae*), *Clostridium difficile* Toxin A/B, enterotoxigenic *Escherichia coli* (ETEC) LT/ST, *E. coli* O157, Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2, *Yersinia enterocolitica, Vibrio cholerae*, *Giardia lamblia, Entamoeba histolytica*, and *Cryptosporidium* spp. (*C. parvum*, *C. hominis*). The assay procedure consists of four steps and can be completed in approximately 5 h.

The objective of this study was to evaluate the clinical performance characteristics of the xTAG GPP against traditional comparator methods found in diagnostic testing laboratories. The sensitivity and specificity of xTAG GPP were assessed using 901 stool specimens from both pediatric and adult patients collected at four clinical sites (Mount Sinai Hospital and University Health Network, Toronto, Ontario, Canada; St. Louis Children's Hospital and Barnes Jewish Hospital, St. Louis, Missouri, USA; Edinburgh Royal Infirmary, Edinburgh, UK; and Leiden University Medical Centre, Leiden, The Netherlands) from February to October 2010. Each participating laboratory analyzed specimens according to the routine diagnostic algorithm in place at that site, and as ordered by the referring physician. Bacterial culture was performed according to standard procedures at all sites [2]. Commercial enzyme immunoassays (EIA) for bacterial toxins were performed as per the

manufacturer's instructions at the North American sites. Parasites were detected using microscopy or EIA (North America), or microscopy or real-time PCR (Europe). Real-time PCR was used for viral detection at the European sites only. Clinical samples from each site were tested with xTAG GPP using standard laser-based flow cytometry on the Luminex® 100/200™ system. xTAG GPP testing was performed at Luminex Molecular Diagnostics (Toronto, Canada) according to the manufacturer's instructions. A subset of samples was also assessed by conventional PCR and bidirectional sequencing using validated primers targeting genomic regions distinct from those of the xTAG GPP (Table 1).

Clinical performance of xTAG GPP was compared with real-time PCR assays for viruses and parasites in routine diagnostic use at the two European sites [14, 15]. Sensitivity versus real-time PCR was 100% for rotavirus A, *Giardia lamblia*, and *Entamoeba histolytica*, and >90% for norovirus GI/GII and *Cryptosporidium*. Sensitivity for adenovirus 40/41 versus real-time PCR was only 20% (4/20); however, because the real-time PCR primers did not distinguish between adenovirus species, it is likely that the sensitivity for types 40/41 is higher but could not be accurately determined in this study. This is further suggested by the results of sequence analysis performed on a subset of these samples, where the four specimens detected by xTAG GPP were confirmed as types 40/41 and seven of the specimens not detected were confirmed as types other than 40/41 (data not shown). The remaining nine specimens were not detected by the sequencing assay and generally had high real-time PCR Ct values, suggesting low titers or shedding, which may be detectable by a sensitive PCR assay [7]. The specificity of the xTAG GPP versus real-time PCR was >97% for the three viral and three parasitic pathogens listed above.

When microscopy was used as the comparator method, the sensitivity for detection of parasitic pathogens was 91.7% for *Cryptosporidium* and 100% for *Giardia lamblia*. Sensitivity versus microscopy was not determined for *Entamoeba histolytica* as there were no microscopy positive specimens in this sample set. Specificity versus microscopy was >96% for all three parasites.

Bacterial culture was performed on fresh or frozen specimens according to the standard procedures at each site using selective and nonselective media [2]. The sensitivity versus culture was >93% for *Campylobacter*, *Shigella*, and *E. coli* O157, and 82.7% for *Salmonella*. Further investigation by sequence analysis on available samples showed that the sensitivity for *Salmonella* was actually 96.7% (29/30), highlighting the reduced sensitivity of bacterial culture for diagnosis of this pathogen. *V. cholerae* and *Y. entercolitica* were not detected by xTAG GPP in this sample set, but were detected by culture in one specimen and by sequencing in two specimens, respectively; however, these samples were not available for further characterization and so the discrepancy could not be resolved (Table 1). Specificity versus culture was >96% for all bacterial targets tested. The overall high specificity demonstrated by xTAG GPP is likely due to the specificity of the sequences used in the primer design. For example, *Campylobacter* primers were designed to detect *C. jejuni*, *C. coli,* and *C. lari* only and do not target other *Campylobacter* species.

The data showed that sensitivity versus commercial EIAs for STEC (Premier EHEC, Meridian Bioscience, Inc.) and *Clostridium difficile* toxin A/B (*TOX A/B QUIK CHEK*®, TechLab, Inc., USA; Premier™ Toxins A&B, Meridian Bioscience, Inc., USA; or VIDAS® *Clostridium difficile* A & B, bioMerieux, France) was 100% and 96.7%, respectively. Specificity compared with EIA was >96%.

A greater number of specimens positive for viruses and parasites were found in the European samples, as more tests for viruses and parasites were requested at those sites; however, no differences in the positivity rates for specific pathogens detected by xTAG GPP were observed between sites (data not shown). In 65% of specimens positive by xTAG GPP, testing for the identified pathogen was not requested by the ordering physican, illustrating the difficulty in predicting a specific etiology and preselecting appropriate assays for pathogen detection (Table 2). Multiple positive results (co-infection) were detected by xTAG GPP in 86 (9.5%) of the specimens (Table 3). All enteric pathogens probed by xTAG GPP, with the exception of *Y. enterocolitica* and *V. cholerae*, were implicated in co-infections, and *C. difficile* was found to have the highest involvement in co-infections.

It is unclear if co-detection of *C. difficile* and another pathogen represents true co-infection or is indicative of asymptomatic *C. difficile* colonization combined with another enteric pathogen. As *C. difficile* testing is readily available at most centers, the physician might seek testing for this pathogen, where a positive result could be an incidental finding and the illness may be the result of something that cannot or is not readily tested for, possibly leading to overtreatment for *C. difficile* [6]. However, detecting *C. difficile* in a patient with diarrhea has important infection control implications, and a rapid and accurate diagnosis is essential for timely enactment of infection control and treatment practices [12]. The rapid identification of the

Table 1. xTAG GPP sensitivity and specificity per comparator methods[a].

Class	Target	Culture		Microscopy		EIA/DFA		Real-time PCR		Sequencing[b]	
		Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Virus	Adenovirus 40/41	ND[c]	ND	ND	ND	ND	ND	20% (4/20)[d]	99.3% (628/632)[e]	100% (9/9)	100% (235/235)
	Rotavirus A	ND	ND	ND	ND	ND	ND	100% (18/18)	99.9% (633/634)[e]	0%[f] (0/1)	99.5% (219/220)
	Norovirus GI	ND	ND	ND	ND	ND	ND	100% (9/9)	100% (642/642)[e]	NA[g]	99.5% (220/221)
	Norovirus GII	ND	ND	ND	ND	ND	ND	92.5% (62/67)	97.6% (570/584)[e]	100% (1/1)	98.6% (217/220)
Bacteria	*Salmonella*	82.7% (62/75)	98.5% (408/414)	ND	ND	ND	ND	ND	ND	96.7% (29/30)	94.8% (181/191)
	Campylobacter	97.4% (111/114)	96.2% (382/397)	ND	ND	ND	ND	ND	ND	100% (36/36)	82.2% (152/185)
	Shigella	100% (40/40)	97.2% (452/465)	ND	ND	ND	ND	ND	ND	97.3% (36/37)	97.8% (180/184)
	Clostridium difficile Toxin A/B	ND	ND	ND	ND	96.7% (30/31)	96.9% (343/354)	ND	ND	100% (15/15)	91.1% (204/224)
	ETEC	ND	ND	ND	ND	NA	90.6% (68/75)	ND	ND	NA	99.5% (220/221)
	Escherichia coli O157	93.7% (15/16)	98.8% (407/412)	ND	ND	ND	ND	ND	ND	83.3% (5/6)	98.6% (212/215)
	STEC	ND	ND	ND	ND	100% (8/8)	99.4% (162/163)	ND	ND	100% (6/6)	99.5% (214/215)
	Yersinia enterocolitica	NA	100% (366/366)	ND	ND	ND	ND	ND	ND	0%[f] (0/2)	100% (219/219)
	Vibrio cholerae	0%[f] (0/1)	100% (194/194)	ND	ND	ND	ND	ND	ND	NA	100% (221/221)
Parasite	*Giardia*	ND	ND	100% (4/4)	96.9% (190/196)	NA	100% (11/11)	100% (22/22)	98.9% (628/635)	NA	96.4% (213/221)
	Entamoeba histolytica	ND	ND	NA	99% (198/200)	ND	ND	100% (6/6)	99.4% (647/651)	NA	99.5% (217/218)
	Cryptosporidium	ND	ND	91.7% (11/12)	100% (188/188)	NA	100% (11/11)	91.3% (21/23)	100% (634/634)	NA	100% (221/221)

[a]Specimens were analyzed according to the routine diagnostic algorithm per site as ordered by the referring physician. xTAG GPP testing was performed at Luminex Molecular Diagnostics (Toronto, Canada).

[b]A subset of specimens was assessed by PCR and sequencing using validated primers targeting regions distinct from those of xTAG GPP.

[c]Not determined.

[d]Adenovirus real-time PCR primers were not specific for adenovirus 40/41.

[e]Five specimens generated inhibited results for rotavirus, adenovirus, and norovirus (GI/GII) by real-time PCR. Results from these specimens were excluded from the calculations for these targets.

[f]Target not detected by xTAG GPP and sample not available for further analysis.

[g]No positive samples identified in this sample set.

causative agent in infective diarrheal disease is crucial for timely treatment decisions, as the appropriate antimicrobial therapy can shorten illness and reduce morbidity in some bacterial and parasitic infections, and can be life-saving in invasive infections [8]. For example, selective antimicrobial therapy is indicated for toxigenic *E. coli*, *Shigella*, and

Table 2. Unrequisitioned bacteria and parasites detected by xTAG GPP.

Target	No. not requested by physician	% of total additional positives
Salmonella	6	46
Campylobacter	10	40
Shigella	4	24
Clostridium difficile Toxin A/B[a]	32	74
ETEC	12	63
E. coli O157	6	55
STEC	13	81
Yersinia enterocolitica	1	100
Giardia	31	79
Entamoeba histolytica	10	71
Cryptosporidium	12	100
Overall	137	65

[a]18/32 positive specimens collected from subjects <3 years old.

Campylobacter, but is not routinely recommended for *Salmonella*, and antimotility agents should not be used in cases of *E. coli* O157. Using this assay to simultaneously detect 15 gastrointestinal pathogens in one test will provide physicians with a more comprehensive assessment of the etiology of diarrheal disease, which could improve the management of these patients.

Current methodologies for the diagnosis of gastrointestinal pathogens suffer from various limitations. For example, EIAs for *C. difficile* and norovirus have been shown to have low sensitivity [3, 13]. In a recent study, the two-step GDH/EIA (glutamate dehydrogenase/EIA) method for detecting *C. difficile* lacked sufficient sensitivity (42.3%) to be recommended [3]. As a consequence, some clinicians assume an initial negative result using EIA for *C. difficile* may represent a false-negative test, and repeat testing is often performed [13]. A study using EIA for the detection of norovirus reported the sensitivity to be 57.6% [4]. The sensitivity for detecting norovirus in outbreak samples could be improved from 44.1% to 76.9% when five samples were tested instead of three, leading the authors to conclude that although norovirus EIA may be useful during outbreak investigations, routine or sporadic samples should be tested by molecular methods. Recent reports indicate that molecular methods exhibit high sensitivity and improve the detection of gastrointestinal pathogens when used for stool testing [1, 5, 10, 11]. The sensitivity and specificity values observed in this study demonstrate that the xTAG GPP assay has comparable performance even with the high level of multiplexing, and can surpass the limitations of nonmolecular tests, providing diagnostically actionable results for all analytes tested.

Table 3. Targets implicated in co-infections.

Target	No. of co-infections	% of co-infections
Clostridium difficile Toxin A/B[a]	26	30.2
Norovirus	23	26.7
Campylobacter	22	25.6
Shigella	21	24.4
Salmonella	18	20.9
Giardia	18	20.9
ETEC	13	15.1
E. coli O157/STEC	11	12.8
Entamoeba histolytica	7	8.1
Cryptosporidium	7	8.1
Adenovirus 40/41	4	4.6
Rotavirus A	4	4.6

[a]12/26 positive specimens collected from subjects <3 years old.

Another limitation of current methodologies can be turnaround time, which for bacterial culture can take several days to complete. Diagnosis of parasites by microscopy is time-consuming and can be challenging, due to the small size or intermittent shedding of organisms. Thus, an examination of multiple consecutive specimens may be required, which can also increase the time to diagnosis [9]. The findings in this study suggest that inclusion of the xTAG GPP into routine diagnostic algorithms can enable a more timely identification of the causative agent in cases of gastroenteritis, compared with the current standard of care.

In summary, the study described herein evaluated a novel multiplexed PCR-based test (xTAG GPP) designed to simultaneously detect and identify 15 enteric pathogens commonly implicated in infective diarrheal disease. This assay is the first commercially available diagnostic test with the ability to detect viruses, bacteria, and parasites on total nucleic acid isolated from a single stool specimen. Furthermore, this is achieved in a 5 h turnaround time and with an overall sensitivity and specificity greater than 94% and 98%, respectively. It has the potential for optimization of patient management and infection control practices, especially for hospitalized patients and those who are immunocompromised.

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