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...
manufacter’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. enterocolitica 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 physician, 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