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White Paper

xTAG® Gastrointestinal Pathogen Panel – The Value of Molecular GI Testing

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Gastroenteritis

Diarrheal disease continues to be a worldwide problem with approximately 1.7 billion cases reported globally each year.[1] Diarrhea is a leading cause of malnutrition and the second leading cause of death in children under the age of five—killing 525,000 each year. In the U.S., roughly 48 million people become ill, 128,000 are hospitalized, and 3,000 die of foodborne diseases annually.[2] In 2014, the cost for hospitalization of these patients was estimated at over $6.2 billion.[3] Diarrheal disease can be caused by a variety of different pathogens including viruses, bacteria, and parasites. Due to similar clinical signs and symptoms, diagnosis of the causative pathogens can be difficult, and conventional diagnostic tests can take several days to provide results.

Table 1. Comparison of traditional stool tests

Method	Tests for	Turnaround Time	Percent Positive
Stool Culture	One to a few bacterial pathogens per test	2-3 days	Up to 6% (Slutsker, 1997)
Ova & Parasite (O & P) Exam	Parasitic pathogens	Several days – samples collected over several days	Up to 3% (Fotedar, 2007; Tuncay, 2007)
Rapid Tests	Single pathogen per test	20-30 minutes	Varies
Real-Time PCR	Typically 1-3 pathogens/ virulence factors per test	Fewer than 5 hours	Varies – depends on pathogen target, perfomance of assay, number of assays
ELISA	Single antigen/ antibody per test	6-24 hours	Varies

Slutsker L, et al. (1997) *Escherichia coli* O157:H7 diarrhea in the United States: clinical and epidemiologic features. Ann Intern Med, 505-513.

Fotedar R, et al. (2007) PCR detection of *Entamoeba histolytica, Entamoeba dispar*, and *Entamoeba moshkovskii* in stool samples from Sydney, Australia. J Clin Microbiol, 1035-1037.

Tuncay S, et al. (2007) DiŞkida Entamoeba histolytica'nin Saptanmasinda. TÜrkiye Parazitoloji Dergisi, 188-193.

Timely and accurate identification of suspected pathogens in stool specimens from gastroenteritis patients can be challenging for the clinical laboratory and typically requires multiple steps, including bacterial culture, enzyme-linked immunosorbant assay (ELISA), and microscopy. These conventional test methods can be time consuming, labor intensive, and exhibit varying clinical performance. Traditional workup for stool specimens may even be performed in different laboratories or require sending out to a reference laboratory, thus the results can be fragmented and filter in over several days (Table 1). Furthermore, since the tests are typically selected and performed sequentially based on presumed etiology, the cause is unidentified in approximately 80% of cases.[2] This could adversely affect patient management decisions and possible lead to inappropriate treatment.[4]

Recent advancements in sample processing, extraction, and molecular methodologies have led to increased adoption of molecular assays for gastrointestinal (GI) pathogen detection by clinical laboratories.[5,6] Though many molecular-based tests exist for a number of GI pathogens, they are often single-plex (i.e., one pathogen/target per test) and are often developed in house and validated as a laboratory developed test (LDT).[7] LDTs can present a problem in the clinical diagnostic setting since the time, cost, and labor resources required to perform a validation study can be overbearing. Recently, several molecular assays have received IVD clearance for the detection of pathogens from human stool.[8] The first multiplexed molecular diagnostic assay for GI pathogens to receive clearance by the U.S. FDA was the Luminex® xTAG® Gastrointestinal Pathogen Panel (GPP).[9] xTAG GPP detects up to 15 pathogens (depending on geographical region) responsible for >90% of infectious diarrhea from human stool samples, and can effectively rule out the majority of pathogens as the cause of diarrheal disease in roughly 80% of all samples tested. With its multiplexing capability and a short five hour turnaround time, xTAG GPP has the potential to revolutionize diagnosis and treatment of diarrheal disease.

xTAG GPP—The First Test of Its Kind

xTAG GPP is a qualitative molecular multiplex test intended for the simultaneous detection and identification of multiple bacterial, viral, and parasitic GI pathogens from individuals with signs and symptoms of infectious colitis or gastroenteritis. The assay uses xTAG Technology with the xMAP® Technology platform to detect multiple targets in a single sample.[10] Luminex's xMAP Technology combines advanced fluidics, optics, and digital signal processing with proprietary color-coded microspheres (beads) for performing multiplexed bioassays. Each bead set can be coated with a reagent specific to a particular bioassay, allowing the capture and detection of specific analytes from the sample. Inside the Luminex analyzer, a light source excites the internal dyes to identify each bead and measure any reporter dye captured during the assay. xTAG Technology is a proprietary universal tag sorting system comprised of unique capture oligonucleotides (oligos) optimized to be an isothermal set with minimal cross-reactivity. This system allows easy optimization, development, and expansion of molecular diagnostic assays. Together, these technologies were used to develop and validate the xTAG GPP assay on the Luminex® 100/200® and MAGPIX® systems.

xTAG GPP identifies the following GI pathogen types, subtypes and toxin genes: adenovirus 40/41, norovirus GI/GII, rotavirus A, *Campylobacter* (*C. jejuni*, *C. coli*, and *C. lari* only), *Clostridium difficile* toxin A/B, *Escherichia coli* O157, enterotoxigenic *E. coli* (ETEC) LT/ST, Salmonella, shiga-like toxin producing *E. coli* (STEC) *stx1/stx2*, *Shigella* (*S. boydii, S. sonnei, S. flexneri,* and *S. dysenteriae*), *Vibrio cholerae* cholera toxin gene *(ctx), Yersinia enterocolitica* (outside of the U.S. only), *Cryptosporidium* (*C. parvum* and *C. hominis* only), *Entamoeba histolytica, Giardia* (*G. lamblia* only, also known as *G. intestinalis* and *G. duodenalis*)—see Table 2. The laboratory can select/deselect which clinical targets are detected and reported on a per sample (i.e., per patient) basis. xTAG GPP was cleared through the de novo 510(k) process which enables clearance of an IVD that represents the first device in this category to be reviewed by the U.S. FDA. When compared to conventional testing methods, xTAG GPP requires only one sample, covers more pathogens, provides a faster turnaround time, and is more sensitive.[9]

xTAG GPP incorporates multiplex reverse-transcriptase PCR (RT-PCR) with the xTAG universal tag sorting system on the Luminex xMAP platform. The assay also detects an internal control (bacteriophage MS2) that is added to each sample prior to extraction. For each pre-treated and extracted sample, the extracted nucleic acid is amplified in a single multiplex RT-PCR/PCR reaction. An aliquot of the RT-PCR product is then added to a hybridization/detection reaction containing the universal tag-coupled beads and the reporter dye. Each bead set detects a specific target through hybridization of the specific TAG to the complementary anti-TAG on the bead. Following hybridization, the Luminex analyzer reads each sample and the xTAG Data Analysis Software for the Gastrointestinal Pathogen Panel (TDAS GPP) interprets the raw signal and provides a report summarizing which pathogens are present for those pathogens selected by the laboratory and/or physician. The assay workflow is illustrated in Figure 1.

Table 2. xTAG® GPP targets (region-specific clearances).

	U.S. FDA Cleared	Health Canada Licensed	CE Marked
Bacteria and Bacterial Toxins			
Campylobacter	•	•	•
Clostridium difficile, Toxin A/B	•	•	•
Escherichia coli O157	•	•	•
Enterotoxigenic ***E. coli*** (ETEC) LT/ST	•	•	•
Shiga-like Toxin producing ***E. coli*** (STEC) ***stx1/stx2***	•	•	•
Salmonella	•	•	•
Vibro cholerae	•	•	•
Yersinia enterocolitica		•	•
Viruses			
Adenovirus 40/41	•	•	•
Norovirus GI/GII	•	•	•
Rotavirus A	•	•	•
Parasites			
Cryptosporidium	•	•	•
Entamoeba histolytica	•	•	•
Giardia	•	•	•
Bacteriophage MS2 (Internal Control	•	•	•

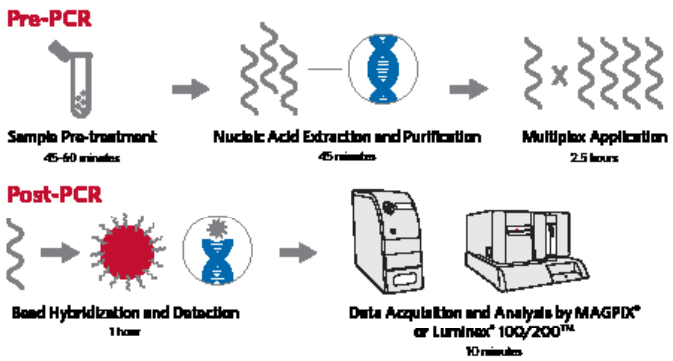
Excellent Clinical Performance with Exceptional Negative Predictive Value

Recent studies have shown that xTAG GPP has a higher sensitivity and specificity as compared to RT-PCR and traditional assay methods.[9,11-15] (See summary in **Appendix A**.) Clinical performance of the test was first assessed in a multicenter clinical study (conducted for CE marking and Health Canada Licensing) consisting of 901 stool specimens collected from pediatric and adult patients at four clinical sites in North America and Europe.[11] Using a combination of conventional and molecular methods as comparator, sensitivity could be determined for 12 of the 15 targets and was 94.3% overall. Specificity across all 15 targets was 98.5%. Subsequently, a multicenter conducted in North America (for U.S. FDA 510(k) clearance) evaluated 1407 stool specimens prospectively collected from pediatric and adult patients.[9] Sensitivity (or positive agreement) could be determined for 11 targets and was >92% (92.3–100%) for 10/11. Specificity (or negative agreement) was >89% (89.8–99.9%) for all 15 targets. A retrospective study consisting of 203 pre-selected positive specimens was conducted for low prevalence targets and demonstrated positive agreement >88% (88.9–100%). And, a supplemental clinical study using 313 pediatric stool specimens prospectively collected in Botswana, Africa was conducted to further evaluate performance for adenovirus 40/41,

rotavirus, ETEC, *Cryptosporidium* and *Giardia*. As a result of these studies, xTAG GPP was 510(k)-cleared in the U.S. in January 2013 for 11 of the 15 targets in raw (unpreserved) stool specimens.

Due to the limited number of *E. histolytica* and *V. cholerae* clinical samples, an additional study of contrived specimens was performed, and a separate study of stool specimens in Cary-Blair medium was conducted to establish diagnostic accuracy of xTAG GPP for Cary-Blair specimens.[12] These additional studies led to U.S. 510(k) clearance of three additional targets and Cary-Blair stool specimens in September 2014.

Several independent studies report similar results for the clinical performance of xTAG GPP.[13-15] Mengelle and coworkers found a statistically significant improvement in sensitivity for rotavirus, noroviruses, *Salmonella* spp., *Campylobacter* spp., and toxigenic *C. difficile* by xTAG GPP, as compared to conventional tests.[13] Beckmann et al. evaluated GPP for testing stool specimens from pediatric patients and tropical travelers, and found 100% sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) for adenovirus, rotavirus, *C. difficile*, and *Cryptosporidium*.[14] A study by Patel et al. using 211 clinical specimens determined the sensitivity, specificity, positive and negative agreement values for the assay to be 96.4%, 99.7%, 90.1%, and 99.5%, respectively, as compared to routine test methods.[15]



**Time estimate of within 5 hours is for a maximum of one extraction (24 samples).*

Figure 1
xTAG® GPP Assay Workflow*

xTAG GPP Improves Diagnostic Yield

Depending on methodology, generally less than 10% of stool specimens submitted for routine GI pathogen testing yield a positive result.[16-18] However, molecular testing with xTAG GPP can provide a more than two-fold improvement in stool pathogen detection when compared to conventional methods.[13,19-22] (See summary in **Appendix B**.)

Kahlau et al. used xTAG GPP in a field test of 347 stool specimens from adult hospitalized patients and observed that 157 samples (45%) were positive for at least one pathogen.[19] In 19 samples, xTAG GPP detected pathogens for which tests were not even requested by the physician. Claas et al. noted that testing for the pathogen identified by xTAG GPP was not requested in 65% of the specimens tested.[11] Mengelle et al. also reported that xTAG GPP provided the diagnosis for gastroenteritis cases of unknown origin and this was especially true for norovirus, *Salmonella* spp., *Campylobacter* spp., and *Cryptosporidium* spp.[13]

Coste and coworkers observed that multiplexed PCR assays, including xTAG GPP, helped detect one or more enteric pathogens in 72% of specimens from symptomatic adult kidney transplant patients, as compared to only 23% by classical microbiological methods.[20] Similar results were found by Zboromyrska et al. in an evaluation of xTAG GPP for the diagnosis of traveler's diarrhea.[21] Of the 185 samples tested, xTAG GPP detected 86 pathogens in 67 samples (36.2% samples positive), whereas only 16 were detected by routine methods. xTAG GPP also detected 60 additional pathogens that conventional methods failed to detect or that were not requested by the clinician.

A study conducted by Perry and coworkers tested 1,000 retrospective and 472 prospective stool specimens with xTAG GPP and found positive detection rates for pathogens and toxins of 24.8% and 32.6%, respectively, as compared to less than 10% by the conventional methods.[22] Another recent study that evaluated the healthcare economic impact of xTAG GPP also found that xTAG GPP detected a pathogen in nearly twice as many patients as the routine methods.[23] For 409 isolated patients, only 81 (20%) had one or more agents of infectious gastroenteritis identified by the conventional testing pathway, as compared to 152 (37%) by xTAG GPP. For 391 patients not isolated, conventional tests revealed a pathogen in 20 (5%) vs. 48 (12%) by xTAG GPP. The overall detection rates were increased from 13% to 25% by xTAG GPP.

xTAG GPP Excels in Detection of Coinfections

Identification of coinfections or secondary infections can be important for effective treatment of infectious diseases as it can provide information to help guide the most appropriate pathogen-specific therapy. Multiplexed assays have the advantage of identifying multiple pathogens simultaneously from a single specimen, and xTAG GPP has dramatically improved the diagnosis of GI coinfections as compared to conventional tests.[11,13-15,20-22] (See summary in **Appendix C**.)

Claas et al. observed detection of coinfections in 9.5% of the specimens tested by xTAG GPP and all of the enteric pathogens probed, except *Y. enterocolitica* and *V. cholerae*, were implicated.[11] *C. difficile* was found to have the highest involvement in coinfections, although it is not clear if this represents true coinfection or asymptomatic colonization by *C. difficile* with another enteric pathogen. Mengelle and coworkers reported that xTAG GPP efficiently detected coinfections in 7% of diarrheic stool samples tested and noted that these results were in agreement with other published studies.[13] Furthermore, Coste et al. found that molecular tests identified coinfections in 38% of specimens from renal transplant patients, none of which were detected by the classical methods.[20] Zboromyrska et al. detected 14 coinfections in 70 positive samples with xTAG GPP as compared to only one by conventional tests.[21] In combination, xTAG GPP and routine methods identified a total of 20 coinfections (28.6%) in patients with traveler's diarrhea.

Clinical Utility—The Value of xTAG GPP to the Patient, Physician, and Hospital

Diarrhea caused by infectious agents has been shown to disproportionately affect particular patient populations, specifically pediatric, immunocompromised, and travelers. In the U.S., norovirus illness in children under five years of age was estimated to have led to a $273 million healthcare burden in 2009 and 2010.[24] In the UK, adenovirus has been recognized as an increasingly important pathogen in pediatric bone marrow transplant patients and in Europe, the clinical impact of nosocomial rotavirus infections in children under five can cost up to 2,500 euro per infection.[25,26] Depending on destination and season, 30–70% of travelers acquire diarrhea while traveling, resulting in an estimated cost of $1 billion USD worldwide.[27,28]

As such, numerous studies have evaluated the clinical utility of xTAG GPP for diagnosis of infectious diarrhea in a variety of different patient populations and environments, including travelers, pediatrics, transplant and immunosuppressed patients, hospitals, outpatients, and during outbreaks.[13-14,20,29-32] (See Table 3.)

Table 3. Clinical Utility of xTAG GPP

Patient Population/ Clinical Setting	References
Adult	Mengelle et al., 2013[13]
Pediatric	Mengelle et al., 2013[13]; Beckmann et al., 2014[14]
Immunocompromised/ Transplant	Mengelle et al., 2013[13]; Coste et al., 2013[20]
Outpatients	Beckmann et al., 2014[14]
Travelers	Beckmann et al., 2014[14]; Zboromyrska et al., 2014[21]
Outbreaks	Malecki, Schildgen et al., 2012[29]; Malecki, Mattner et al., 2012[30]; Valcin et al., 2013[31]
Epidemiology/Monitoring	Zboromyrska et al., 2014[21]; Valcin et al., 2013[31]; Rouzier et al., 2013[32]

xTAG GPP in Patient Care

Mengelle and coworkers evaluated xTAG GPP in 440 stool samples (329 patients) from four patient populations presenting with acute GI infection: 102 immunosuppressed adults, 50 immunosuppressed children, 56 children in the neonatal unit, and 121 children in the emergency unit.[13] Most of the samples collected from the children in the emergency unit tested positive for GI pathogens (92.6%) and most of the rotavirus (98.6%) and norovirus (53.3%) cases were found in these patients. Fewer positive samples were found in the other patient populations, with positivity rates of 17% (immunosuppressed adults), 25.3% (immunosuppressed children), and 19% (children in the neonatal unit). Norovirus was detected in 26.7% of the immunosuppressed children and in 20% of the immunosuppressed adults. *Salmonella* spp. was detected most frequently in children attending the emergency unit (61.9%), while toxigenic *C. difficile* was most common in immunocompromised adults (61.1%). The authors concluded that xTAG GPP is a very sensitive and convenient method for detecting multiple GI pathogens from a single stool sample and may be easily used in routine daily practice.

Beckmann et al. assessed xTAG GPP in pediatric gastroenteritis patients presenting to the emergency room and in outpatients with recent tropical travel history (with GI symptoms or suspected parasite infections). Overall, pathogens were identified in 28% of the stool samples. The detection rate in pediatric patients was 52%, which was significantly higher than the 11% found in travelers (mostly adult patients). The pathogen distribution found in the pediatric samples corresponded to that expected for diarrhea in young children, with predominantly rotavirus (27%). This study demonstrated broad detection of relevant GI pathogens by xTAG GPP and the investigators perceive the major role of the assay to be for immunocompromised patients and patients with a broad differential diagnosis, since results could be provided quickly and have an impact on clinical management. They recommended performing direct antigen detection for children with gastroenteritis first, followed by xTAG GPP if negative or if additional pathogens are clinically suspected. For patients returning from the tropics, they recommended restricting xTAG GPP to patients with a clinical diagnosis of gastroenteritis. xTAG GPP testing can be selectively complemented with tests for additional suspect pathogens and for antimicrobial resistance testing as needed.

Zboromyrska and colleagues also evaluated xTAG GPP for etiological diagnosis of traveler's diarrhea.[21] xTAG GPP detected 76 pathogens in 185 stool samples (a 31.9% detection rate), including 60 pathogens that were not detected by conventional methods or were not requested for testing. The primary pathogens causing traveler's diarrhea were *Shigella* (24.2%), ETEC (23.2%), enteroaggregative *E. coli* (14.7%), and *Giardia* (13.7%). Significant regional differences were observed for ETEC with 19.4% of cases acquired in Africa, 11.3% in Asia, and none in South Central (SC) America. *Giardia* was found in 14.1% of those who had traveled to Asia, 3% of those who had traveled to SC America, and only 1.5% of those who had traveled to Africa. They concluded that xTAG GPP significantly improved the detection of enteropathogens and allowed better assessment of the etiology of traveler's diarrhea.

Diarrhea is a complication that occurs frequently in transplant patients and is usually attributed to adverse effects of immunosuppressive therapy when microbiological examination is negative. A recent publication by Coste et al. describes the improvement of microbiological diagnosis and management of diarrheic kidney transplant patients through implementation of molecular testing for infectious gastroenteritis.[20] For 54 severe diarrhea events occurring in 49 adult kidney transplant recipients, molecular methods identified one or more enteric pathogens in 39 stool specimens. Enteropathogenic *E. coli*, *Campylobacter* spp., and noroviruses were the most frequent cause of diarrhea in these patients. Statistical analysis of the data revealed that the

post-transplantation term of diarrhea onset due to norovirus was significantly prolonged compared to that of other causes of infectious and noninfectious diarrhea. Moreover, immunosuppressive therapy combining cyclosporine and mycophenolate mofetil was associated with a significantly higher risk of developing viral gastroenteritis, particularly that caused by norovirus. Thus, this study demonstrated xTAG GPP as a powerful technique for the microbiological diagnosis of enteric pathogens in kidney transplant patients.

xTAG GPP in Gastroenteritis Outbreaks

Malecki and coworkers reported on implementation of rapid screening diagnostic methods during the 2011 outbreak of enterohemorrhagic *E. coli* (EHEC) in Germany and their test of xTAG GPP for this purpose.[29-30] xTAG GPP was capable of identifying the novel EHEC strain, *E. coli* O104:H4 (which is Shiga toxin 1 negative but Shiga toxin 2 positive). Twenty patients suffering from hemorrhagic diarrhea or suspected to be infected with the new EHEC strain were tested by xTAG GPP and four patients were found to be positive for the EHEC O104:H4 strain. Two patients suffered from severe *Campylobacter* infections but were negative for EHEC, and one patient was positive for another EHEC strain (that produced both Shiga toxins 1 and 2). They concluded that xTAG GPP is useful for prescreening patients suffering from the new EHEC strain. Additional benefits are that only a preselected cohort of clinical samples would have to be analyzed for confirmation of suspected strains, and patients negative for EHEC but positive for other pathogens can be administered the correct antibiotic therapy. With the capability for high throughput testing, they found that xTAG GPP is able to cover the peaks in an outbreak situation.

Similarly, xTAG GPP was used to assess the etiology of acute diarrheal illness in patients seen at a cholera treatment center in Haiti during the 2010 cholera outbreak.[31] Cholera, which was previously unrecognized in Haiti, spread through the country in the fall of 2010 and an analysis was conducted to understand the epidemiological characteristics, clinical management, and risk factors for disease severity. xTAG GPP was used in parallel with culture for *V. cholerae* and *Salmonella* for confirmation of cholera cases. Subsequently, oral cholera vaccine (OCV) was introduced by the Haitian Ministry of Health as a pilot project in urban and rural areas.[32] xTAG GPP and culture were used as part of the post-vaccination follow-up to confirm cholera cases in patients presenting with acute watery diarrhea.

Streamlined Workflow with xTAG GPP

xTAG GPP has a shorter hands on time and faster turnaround time as compared to classical methods, due to its ability to simultaneously detect multiple pathogens and its efficient, streamlined workflow. Claas and coauthors, who first described the performance characteristics of xTAG GPP, postulated that with a four step procedure and five hour turnaround time, xTAG GPP has the potential for optimizing patient management and infection control practices, particularly for hospitalized and immunocompromised patients.[11] Several subsequent reports in the published literature describe the efficiency of xTAG GPP when compared to other diagnostic methods.[13-15,19,21,23,30] (See summary in **Appendix D**.)

Kahlua and coworkers reported that with optimal transport and laboratory logistics, results from xTAG GPP could be available within 6–10 hours.[19] This rapid turnaround time would be most important for initiating more specific therapy, quickly setting up hygiene countermeasures, or for deisolating patients which could save economic resources due to false or unnecessary blockades of hospital beds. Mengelle et al. described a dramatic reduction in turnaround time with xTAG GPP.[13] In approximately four hours, xTAG GPP provides results for 15 pathogens, as compared to the conventional techniques, which take a minimum of two days, involve many varying technical steps, and require a range of technical competences to perform culture, PCR, and microscopy. Zboromyrska and colleagues also found a significant reduction in test turnaround time, with approximately five hours for xTAG GPP as compared to 24–48 hours for culture—the most time-consuming conventional techniques.[21] Thus, xTAG GPP allows microbiologists to provide answers to the clinician in a single working day. The investigators believe this may be very important for initiating appropriate antimicrobial treatment or for outbreak management.

Similarly, Beckmann and colleagues found that xTAG GPP had a total turnaround time of six hours, after which the results for the 15 infectious agents were available in the electronic lab information system.[14] In contrast, conventional culture and microscopy can take several days and has a high hands on time. As xTAG GPP allows parallel testing for multiple pathogens, testing of 20–40 patients can be easily handled. Combined with automated extraction, they estimated the hands-on time to be two hours to generate 300 results for 20 patients.

Malecki and coworkers, who implemented xTAG GPP as a frontline screening test during the 2011 EHEC outbreak in Germany, stated that with the ability to test up to 96 samples per run and a workflow time of about five hours, the test is suitable for high throughput analyses in outbreak situations.[30] Further, because bloody diarrhea is a common first symptom of many diseases caused by various pathogens, rapid identification of the causative agent as an *E. coli* was essential for the appropriate handling of each patient and the outbreak itself.

In a parallel clinical evaluation conducted at two sites, Patel et al. assessed the feasibility, clinical utility, and acceptability of xTAG GPP as a highly sensitive, specific, and reproducible method to improve turnaround time, treatment measures, and outbreak response for infectious gastroenteritis.[15] They addressed improvements in workflow, cost benefits, and increased efficiencies provided by xTAG GPP to the laboratory by comparing cost per specimen, labor cost, turnaround time, and hands-on time to that of the conventional methods. The investigators estimated a 75% reduction in labor cost per specimen identification, a 75% reduction in hands on time and a 93% reduction in turnaround time. They also estimated the potential scalability of the methods and found that more than twice as many specimens (22 vs. 10) could be handled by xTAG GPP without additional labor cost (a 45.5% efficiency improvement).

While reagent cost would increase (+63%) with xTAG GPP, this was more than offset by the potential savings for all of the other

categories. The authors determined that the additional reagent cost was not significant when compared to the overall savings across the other categories and concluded that high throughput detection of multiple GI pathogens improved turnaround time, consolidated laboratory workflow, and simplified stool culture practices, thus reducing the overall cost and the number of specimens processed.

A comparative time-and-motion study of xTAG GPP vs. conventional methods was recently conducted at two large tertiary healthcare systems in the UK and U.S.[33] The two study sites utilize similar conventional testing methodologies but with some distinct operational differences. Activity-based cost analysis was performed to account for labor, overhead, and material costs to determine the benefits of multiplex molecular testing for gastroenteritis diagnosis and how it can be best integrated in the laboratory. The key parameters considered were total hands on time, comparison of throughput, time from sample receipt to reporting of all results, quality of result (positivity rates for all methods), and resources used (labor and material costs).

While the total cost per stool was similar to current costs when testing for all pathogens (+/- 22% on average), xTAG GPP reduced the labor time and associated cost by 87–92%. Also striking was the difference in total time to results, which could be reduced from 25–90 hours to 6–7.25 hours (Figure 2). Thus, a complete set of clinical results from a single test order can be delivered back to the physician in the same day, allowing for better-informed patient management and treatment. In addition, the substantial reduction in hands on time for technologists opens the possibility for laboratory staff to focus on additional value-added activities, such as expanding the test menu.

Health Economics—Cost Savings with xTAG GPP

Gastroenteritis is a major cost burden to health services since cases of suspected infectious diarrhea coming to or developing in hospitals are typically placed in isolation and handled with contact precautions. Clinicians are challenged to pragmatically use a usually limited number of isolation rooms while waiting for laboratory confirmation of communicable GI infections. Further inefficiencies could result from unnecessarily isolating patients who do not have infectious gastroenteritis and from prematurely removing infectious patients from isolation due to an incorrect diagnosis, with the potential for subsequent disease transmission and potential outbreaks.

A recent publication by Goldenberg et al. reports the results of a health economic study to assess the potential clinical and cost benefits afforded by of xTAG GPP for diagnosis of infectious gastroenteritis.[23] The investigators hypothesized that faster, more sensitive and more comprehensive testing of hospitalized patients with suspected infectious gastroenteritis by multiplexed molecular methods, such as xTAG GPP, could result in significant efficiencies in the utilization of isolation facilities, better patient management and overall cost savings to the hospital.

An eight month parallel diagnostic study compared xTAG GPP with conventional laboratory testing where laboratory testing costs and patient isolation costs were measured or estimated for 800 patients. Clinicians ordered on average 4.5 conventional tests per patient episode as compared to testing once by xTAG GPP in a five day period. In addition to an almost two-fold improvement in positive detection rates, xTAG GPP provided actionable results in about half the time as conventional methods. Consequently, using xTAG GPP could reduce isolation days by 34% (from 2202 to 1447), saving approximately $105,000 (£66,765) in hospital isolation costs. The analysis was based on the reduction in laboratory turnaround time plus knowledge of the average time to deisolate patients after receipt of negative results. Although laboratory testing costs would increase by about $35,000 (£22,283), the overall hospital savings provided by xTAG GPP would more than offset the additional costs and produce an overall savings of $70,000 (£44,482) over the eight month period.

Breakeven analysis showed that in this hospital setting, xTAG GPP would be cost neutral at a mere 11.4% reduction in isolation days. In addition, sensitivity analysis showed that the xTAG GPP testing pathway would generate considerable net savings even if the average time in isolation were three days ($33,000/£20,816), and further optimization of workflow and turnaround time (from testing to acting on negative results) could result in additional savings ($107,000/£68,208). These estimated savings are exclusive of additional savings due to change/discontinuation of antimicrobial therapy or shorter length of stay. The authors concluded that syndromic testing against a broad panel of organisms using xTAG GPP can both improve detection rates and allow better laboratory workflow, and deisolation of patients testing negative could result in significant patient isolation savings.

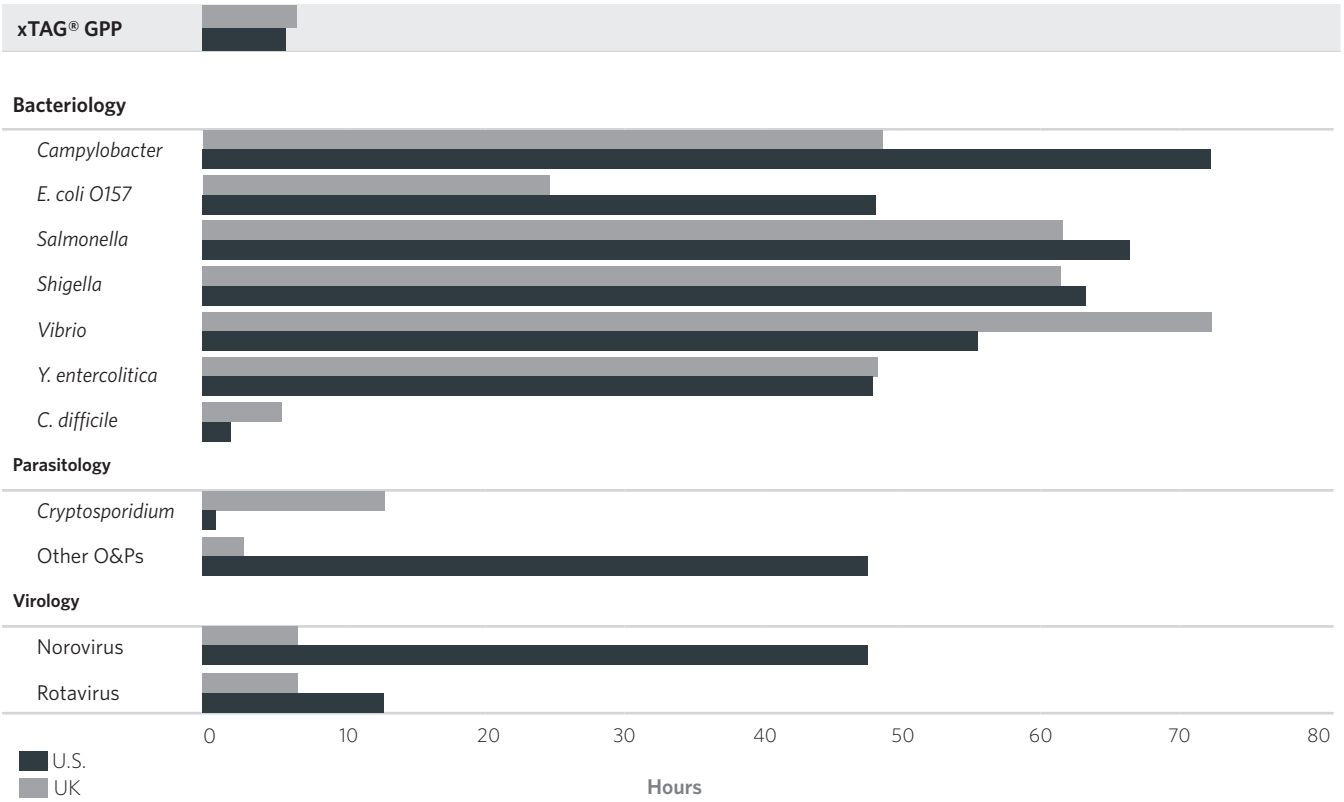


Figure 2
Comparison of total time to result.

References

1. Diarrhoeal disease. Fact sheet No 330. World Health Organization (Internet). Cited 2017 July. Available from: http://www.who.int/mediacentre/factsheets/fs330/en/index.html.
2. CDC estimates of foodborne illness in the United States. Centers for Disease Control and Prevention (Internet). Cited 2014 December. Available from: http://www.cdc.gov/foodborneburden/PDFs/FACTSHEET_A_FINDINGS_updated4-13.pdf.
3. National statistics on intestinal infection. U.S. Department of Health and Human Services (Internet). Cited 2017 July. Available from: http://hcupnet.ahrq.gov/.
4. Guerrant RL, Van Gilder T, Steiner TS, et al. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis 2001;32(3):331-351.
5. Pawlowski SW, Warren CA, Guerrant R. Diagnosis and treatment of acute or persistent diarrhea. Gastroenterology 2009;136(6):1874-1886.
6. Tang YW, Persing DH. Advances in the clinical microbiology of enteric infections. In: Blaser MJ, Smith PD, Ravdin JI, et al., editors. Infections of the gastrointestinal tract. 2nd edition. Lippincott Williams & Wilkins, Philadelphia, PA; 2002. pp. 1185-1197.
7. Dunbar SA, Zhang H, Tang YW. Advanced techniques for detection and identification of microbial agents of gastroenteritis. Clin Lab Med 2013;33(3):527-552.
8. Janda JM, Abbott SA. Culture-independent diagnostic testing: have we opened Pandora's box for good? Diagn Microbiol Infect Dis 2014;80(3):171-176.
9. Luminex Molecular Diagnostics, Inc. xTAG® GPP (US) Kit Insert. MLD-032-KPI-001.
10. Dunbar, SA. Bead-based suspension arrays for the detection and identification of respiratory viruses. In Y-W Tang and CW Stratton, (eds), Advanced Techniques in Diagnostic Microbiology, 2nd ed., Springer Science+Business Media, New York; 2013. p. 813-833.
11. Claas EC, Burnham CD, Mazzulli T, et al. Performance of the xTAG® gastrointestinal pathogen panel, a multiplex molecular assay for simultaneous detection of bacterial, viral, and parasitic causes of infectious gastroenteritis. J Microbiol Biotechnol 2013;23(7):1041-1045.
12. Luminex Molecular Diagnostics, Inc. xTAG® Gastrointestinal Pathogen Panel Package Insert IVD. MLD-032-KPI-001 Rev C, 2014.
13. Mengelle C, Mansuy JM, Prere MF, et al. Simultaneous detection of gastrointestinal pathogens with a multiplex Luminex-based molecular assay in stool samples from diarrhoeic patients. Clin Microbiol Infect 2013;19(10):E458-465.

14. Beckmann C, Heininger U, Marti H, et al. Gastrointestinal pathogens detected by multiplex nucleic acid amplification testing in stools of pediatric patients and patients returning from the tropics. Infection 2014;42(6):961-970.

15. Patel A, Navidad J, Bhattacharyya S. Site-specific clinical evaluation of the Luminex xTAG gastrointestinal pathogen panel for detection of infectious gastroenteritis in fecal specimens. J Clin Microbiol 2014; 52(8):3068-3071.

16. Johnston SP, Ballard MM, Beach MJ, et al. Evaluation of three commercial assays for detection of *Giardia* and *Cryptosporidium* organisms in fecal specimens. J Clin Microbiol 2003;41(2):623-626.

17. Voetsch AC, Angulo FJ, Rabatsky-Ehr T, et al. Laboratory practices for stool-specimen culture for bacterial pathogens, including *Escherichia coli* O157:H7, in the FoodNet sites, 1995-2000. Clin Infect Dis. 38 2004;(Suppl 3):S190-197.

18. Fotedar R, Stark D, Beebe N, et al. PCR detection of *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* in stool samples from Sydney, Australia. J Clin Microbiol 2007;45(3):1035-1037.

19. Kahlau P, Malecki M, Schildgen V, et al. Utility of two novel multiplexing assays for the detection of gastrointestinal pathogens – a first experience. SpringerPlus 2013;2:106. doi:10.1186/2193-1801-2-106.

20. Coste JF, Vuiblet V, Moustapha B, et al. Microbiological diagnosis of severe diarrhea in kidney transplant recipients by use of multiplex PCR assays. J Clin Microbiol 2013;51(6):1841-1849.

21. Zboromyrska Y, Hurtado JC, Salvador P, et al. Aetiology of traveller's diarrhoea: evaluation of a multiplex PCR tool to detect different enteropathogens. Clin Microbiol Infect 2014;20(10):O753-O759.

22. Perry MD, Corden SA, Howe RA. Evaluation of the Luminex xTAG Gastrointestinal Pathogen Panel and the Savyon Diagnostics Gastrointestinal Infection Panel for the detection of enteric pathogens in clinical samples. J Med Microbiol 2014;63(Pt 11):1419-1426.

23. Goldenberg SD, Bacelar M, Brazier P, et al. A cost benefit analysis of the Luminex xTAG Gastrointestinal Pathogen Panel for detection of infectious gastroenteritis in hospitalised patients. J Infect. 2014 Nov 29. pii: S0163-4453(14)00353-3. doi: 10.1016/j.jinf.2014.11.009. [Epub ahead of print].

24. Payne DC, Vinjé J, Szilagyi PG, et al. Norovirus and medically attended gastroenteritis in U.S. children. N Engl Med 2013;368;1121-30.

25. Walls T, Shankar AG, Shingadia D. Adenovirus: an increasingly important pathogen in paediatric bone marrow transplant patients. Lancet Infect Dis 2003;3(2):79-86.

26. Gleizes O, Desselberger U, Tatochenko V, et al. Nosocomial rotavirus infection in European countries: a review of the epidemiology, severity and economic burden of hospital-acquired rotavirus disease. Pediatr Infect J 2006;25(1 Suppl):S12-21.

27. Travelers' diarrhea. Centers for Disease Control and Prevention (Internet). Cited 2017 July. Available from: https://wwwnc.cdc.gov/travel/yellowbook/2018/the-pre-travel-consultation/travelers-diarrhea.

28. Wang M, Szucs TD, Steffen R. Economic aspects of travelers' diarrhea. J Travel Med 2008;15(2):110-118.

29. Malecki M, Schildgen V, Kamm M, et al. Rapid screening method for multiple gastroenteric pathogens also detects novel enterohemorrhagic *Escherichia coli* O104:H4. Am J Infect Control 2012;40:82-83.

30. Malecki M, Mattner F, Schildgen O. Short review: molecular diagnosis of *Escherichia coli* O104: H4. Rev Med Microbiol 2012;23(2):14-17.

31. Valcin CL, Severe K, Riche CT, et al. Predictors of disease severity in patients admitted to a cholera treatment center in urban Haiti. Am J Trop Med Hyg 2013;89(4):625-632.

32. Rouzier V, Severe K, Juste MAJ, et al. Cholera vaccination in urban Haiti. Am J Trop Med Hyg. 2013;89(4):671-681.

33. Anson J, Corless CE, Jackson B, et al. Operational impact of implementing a multiplex molecular gastrointestinal pathogen screening assay – a UK and US laboratory time-and-motion study of gastroenteritis diagnostic testing. Manuscript in preparation.

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Appendix A. Clinical Performance Studies with xTAG® GPP

Reference	Region	Study Design	Sensitivity	Specificity	PPV	NPV
Luminex Molecular Diagnostics, Inc. (November 2014)[12]	North America	Prospective, 1407 specimens, adult and pediatric patients	>92%	>97%	ND*	ND*
	North America, Africa, Europe	Retrospective, 207 preselected positive specimens	>97%	ND	ND	ND
	Botswana	Supplemental Prospective, 313 specimens, pediatric patients	>86%	>96%	ND	ND
Claas EC, et al. (2013) J Microbiol Biotechnol.[11]	Europe and North America	Prospective, 901 specimens, adult & pediatric patients	>95%b	>97%**	ND	ND
Patel A, et al. (2014) J Clin Microbiol.[15]	North America	Prospective, 211 specimens, adult and pediatric patients	96.4%	99.7%	90.1%	99.5%
Beckmann C, et al. (2014) Infection.[14]	Switzerland	Prospective, 312 specimens, pediatric patients and travelers	100% (7/7 targets)	>91% (6/7 targets)	100% (4/7 targets)	100% (7/7 targets)

**Not Determined*

***Compared to Sequencing*

Appendix B. Positivity Rates Reported for xTAG® GPP

Reference	Study Design	Positive Rate with Conventional Methods	Positive Rate with xTAG® GPP	Key Findings
Claas EC, et al. (2013) J Microbiol Biotechnol.[11]	Prospective, 901 specimens, adult and pediatric patients	ND*	ND	xTAG GPP detected pathogens not requested by physicians in 65% of specimens
Mengelle C, et al. (2013) Clin Microbiol Infect.[13]	Prospective, 440 specimens, immunosuppressed adult, pediatric and immunosuppressed pediatric patients	ND	40% overall: • Virus: 23% • Bacteria: 13.9% • Parasites: 3%	Multiplex molecular tests afford high positive rates.
Zboromyrska Y, et al. (2014) Clin Microbiol Infect.[21]	Retrospective, 185 specimens, international travelers	18.4%	31.9%	13.5% increase in positive rate with xTAG GPP; a 73% increased detection over conventional
Perry MD, et al. (2014) J Med Microbiol.[22]	Retrospective, 1000 specimens,	9%	24.8%	15.8% increase in positive rate with xTAG GPP; a 276% increased detection over conventional
	Prospective, 472 specimens,	9%	32.6%	23.6% increase in positive rate with xTAG GPP; a 362% increased detection over conventional
Goldenberg SD, et al. (2014) J Infect.	Prospective, 409 isolated patients	13% overall 20%	25% overall 37%	12% increase in positive rate with xTAG GPP; a 208% increased detection over conventional
	Prospective, 391 non-isolated patients	5%	12%	

**Not Determined*

Appendix C. Coinfection Rates Detected by xTAG® GPP

Reference	Study Design	Coinfections Detected by Conventional Methods	Coinfections Detected by xTAG® GPP	Increase in Detection of Coinfections with xTAG® GPP
Claas EC, et al. (2013) J Microbiol Biotechnol.[11]	Prospective, 901 specimens, adult and pediatric patients	ND*	9.5%	ND
Coste JF, et al. (2013) J Clin Microbiol.[20]	Retrospective, 54 specimens, adult kidney transplant patients	0%	38%	38%
Mengelle C, et al. (2013) Clin Microbiol Infect.[13]	Prospective, 440 specimens, immunosuppressed adult, pediatric and immunosuppressed pediatric patients	ND	7%	ND
Zboromyrska Y, et al. (2014) Clin Microbiol Infect.[21]	Retrospective, 185 specimens, international travelers	1%	7.6%	6.6%

**Not Determined*

Appendix D. Reduction in Turnaround Time (TAT) Reported with xTAG® GPP

Reference	Conventional Methods TAT	xTAG® GPP TAT
Malecki M, et al. (2012) Rev Med Microbiol.[25]	48 hours	5 hours
Mengelle C, et al. (2013) Clin Microbiol Infect.[13]	>72 hours	4 hours
Kahlau P, et al. (2013) SpringerPlus.[19]	Median of 72 hours	Median of 24 hours*
Zboromyrska Y, et al. (2014) Clin Microbiol Infect.[21]	24–48 hours	5 hours
Patel A, et al. (2014) J Clin Microbiol.[15]	72 hours	5 hours
Beckmann C, et al. (2014) Infection.[14]	>48 hours	6 hours
Goldenberg SD, et al. (2014) J Infect.[23]	Median of 17–66 hours	Median of 41.8 hours*

**Time includes specimen transport.*