Luminex xTAG® Technology

Clinical Summary

The Clinical Performance and Value of xTAG® Gastrointestinal Pathogen Panel (GPP):

FDA CLEARED

Screen for 11 Common Bacterial, Viral, and Parasitic Causes of Gastroenteritis with a Single Test in the Same Day

Luminex® xTAG® Technology

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The Clinical Performance and Value of xTAG Gastrointestinal Pathogen Panel (GPP):

Screen for 11 Common Bacterial, Viral, and Parasitic Causes of Gastroenteritis with a Single Test in the Same Day

xTAG GPP Kit

Introduction

Globally, there are an estimated two billion cases of diarrheal disease every year which kill approximately 1.8 million people annually. Diarrheal disease is the second leading cause of death and the leading cause of malnutrition in children under five years old (WHO, 2009). Accurate treatment of diarrheal disease is dependent on diagnosis. Timely laboratory diagnosis is greatly needed to speed the delivery of treatment. Conventional methods, such as: culture, ova and parasite (O & P) exams, and enzyme-linked immunosorbent assays (ELISA) are widely used to detect gastrointestinal pathogens within clinical samples, but typically exhibit long turnaround times and poor sensitivity. In an effort to improve turnaround times and sensitivity, clinical laboratories increasingly have been seeking molecular-based methods as an aid in diagnosing diarrheal disease.

There are few examples of FDA-cleared molecular diagnostic assays for the detection of pathogens from human stool. Though molecular based tests do exist for a number of GI pathogens, they are often single -plex (i.e. one pathogen/target per test) and must be validated as a laboratory-developed test (LDT). Non-FDA-cleared tests/LDTs can present a problem in the clinical diagnostic arena because the cost, time, and labor resources required to perform a validation study can be overbearing. Luminex has recently received FDA clearance for a gastrointestinal pathogen panel (xTAG® GPP), which detects 11 pathogens from human stool samples in approximately five hours. The FDA-cleared xTAG GPP is able to effectively rule out the large majority of pathogens as the cause of diarrheal disease in roughly 80% of all samples tested. Due to the multiplexing capabilities and short turnaround time, xTAG GPP has the potential to revolutionize patient diagnosis and treatment for diarrheal disease.

The Burden of Gastroenteritis and Current Diagnostic Testing Limitations

Diarrheal disease inflicts a significant toll on the health care system and imparts a high degree of morbidity and mortality in select populations. In 2010, the cost for the 237,000+ hospitalized patients suffering from gastrointestinal infections in the U.S. was over $6 billion (U.S. Dept. of Health and Human Services, 2010). Due to similar symptoms it is difficult to differentiate among viral, bacterial and parasitic agents. Hence, about 80% of all causes of diarrhea currently go unidentified, often resulting in inappropriate treatment (CDC, 2012). Diarrheal disease is both preventable and treatable.

Current testing to diagnose the many potential causes of infectious diarrhea is spread across multiple laboratories, involves multiple testing methods with varying levels of clinical performance, and contributes to long turnaround times that hamper proper patient care. The stool specimen often arrives in the Microbiology laboratory where it is split for subsequent testing across Microbiology, Virology, and Molecular laboratories. Clinical laboratories may also send out specimens to a reference laboratory for additional tests. Personnel must be extensively trained to perform different types of assays (e.g. culture, EIA, PCR). Each individual test result does not contribute much diagnostic value, and the sensitivity and/or specificity is limited.

In addition, collection of results from these different laboratories and tests often takes multiple days, sometimes weeks.

xTAG GPP - The First Test of Its Kind

The use of xTAG chemistry on the Luminex platform has been well established in the field of molecular diagnostics. As with previous first-in-class *in vitro* diagnostic (IVD) tests incorporating these Luminex technologies, notably the xTAG Cystic Fibrosis Kit and the xTAG Respiratory Viral Panel, xTAG GPP was cleared through the de novo 510(k) process established by the U.S. FDA. The de novo 510(k) enables clearance of an IVD that represents the first device in this category to be reviewed by the FDA.

xTAG GPP has already been approved as an IVD in Canada and Europe. In these regions, the full multiplexed panel covering a total of 15 bacteria, viruses, and parasites is commercially available. This same panel was reviewed by the U.S. FDA. However, the FDA decided to limit the initial clearance to 11 of the 15 pathogens. The pathogens excluded from the U.S. clearance are *Entamoeba histolytica, Vibrio cholerae, Yersinia enterocolitica*, and adenovirus 40/41. Luminex will continue to engage in discussions with the U.S. FDA aimed at including the four analytes excluded from the de novo 510(k) clearance in future versions of the assay. It is important to note that these analytes were excluded from the U.S. IVD through a software mask and the reagents necessary to detect them are still included. The formulation of the reagents within the U.S. IVD kit is identical to those within the IVD products cleared for use in Canada and Europe. Therefore, inclusion of any of the four aforementioned analytes in future versions of the assay will likely not result in a change in chemical composition of the kit reagents. When compared to current testing methods, xTAG GPP IVD requires only one sample, covers more pathogens, provides faster turnaround times, and is more sensitive (Table 1).

Table 1: Traditional Methods Comparison

Method	Tests For	Turn-Around Time	Percent Positive
Stool culture	Single or a few bacterial pathogens per test	2–3 days	Up to 6% (Slutsker, 1997)
Ova & Parasite (O&P) exam	Parasitic pathogens	Several days – multiple samples collected days apart from each other	Up to 3% (Fotedar, 2007), (Tuncay, 2007)
Rapid tests	Single pathogen per test	20–30 min	Varies
Real-time PCR	Typically 1-3 pathogens/virulence factors per test	Under 5 hours	Varies; depends on the pathogen target, performance of assay, and number of assays
ELISA	Single antigen/ antibody per test	6–24 hours	Varies
xTAG GPP	11 bacterial, viral, and parasitic pathogens in a single test	Under 5 hours including extraction** < 24 samples per easyMag run	~35% - All 11 targets ~20% - minus *C. difficile* results (Luminex Corp., 2013)

xTAG Gastrointestinal Pathogen Panel (GPP) Product Description

xTAG GPP is a qualitative molecular multiplex test intended for the simultaneous detection and identification of multiple gastrointestinal pathogens including bacteria, viruses, and parasites from individuals with signs and symptoms of infectious colitis or gastroenteritis. The assay uses the proprietary Luminex xTAG Technology and the xMAP® Technology platform to detect multiple targets in a single sample. xTAG GPP can detect agents causing hospital acquired infections (HAIs) such as *C. difficile* and norovirus, foodborne illness agents like *Escherichia coli* and *Salmonella*, and common causes of diarrhea in infants and children under five years of age, such as rotavirus A, *Campylobacter*, and *Shigella*. The following pathogen types, subtypes and toxin genes are identified using the xTAG GPP:

Table 2: xTAG GPP U.S. Kit

IVD Product (Cat. No. I032C0316) detects the following pathogens.

Bacteria & Bacterial Toxins	Viruses	Parasites
Campylobacter (C. jejuni, C. coli and *C. lari* only)	Norovirus GI/GII	*Giardia lamblia*
Clostridium difficile, Toxin A/B	Rotavirus A	*Cryptosporidium* (*C. parvum* and *C. hominis* only)
Escherichia coli O157		
Enterotoxigenic *E. coli* (ETEC) LT/ST		
Shiga-like Toxin producing *E. coli* (STEC) stx1/stx2		
Salmonella		
Shigella (*S. boydii, S. sonnei, S. flexneri* and *S. dysenteriae*)		

Summary of Clinical Data Reviewed by the U.S. FDA

Several clinical studies were reviewed by the U.S. FDA in the de novo 510(k) application:

1. **A prospective clinical study in symptomatic patients** to determine clinical performance of the xTAG GPP assay compared to reference/comparator methods.
2. **A retrospective clinical study in symptomatic patients using pre-selected specimens** to test for low prevalence targets.
3. **Supplemental clinical studies** in asymptomatic volunteers and symptomatic pediatric patients.

The results of these studies and supporting analytical studies are summarized in detail in the package insert. An overview of clinical data is provided below.

Prospective and Retrospective Clinical Studies in Symptomatic Patients

The clinical performance of xTAG GPP for each analyte probed by the assay was evaluated in clinical specimens (stool) prospectively collected between June 2011 and February 2012. A total of 1407 clinical specimens were collected from pediatric and adult patients and submitted for microbial testing at six independent laboratories. Four of the laboratories were located in the United States (Arizona, Missouri, Tennessee and Texas) and two were in Southern Ontario (Canada). Demographic details for this prospective data set are summarized in Table 3.

Table 3: Demographic Data for Prospective Data Set (n=1407)

Sex	Number of Subjects
Male	632 (44.9%)
Female	775 (55.1%)
Age (years)	**Number of Subjects**
0 - 1	6 (0.4%)
>1 - 5	20 (1.4%)
>5 - 12	25 (1.8%)
>12 - 21	51 (3.6%)
>21 - 65	879 (62.5%)
>65	426 (30.3%)
Subject Status	**Number of Subjects**
Outpatients	421 (29.9%)
Hospitalized	804 (57.1%)
Emergency Department	118 (8.4%)
Long Term Care Facility	18 (1.3%)
Not Determined	46 (3.3%)
Immune Status	**Number of Subjects**
Immuno-compromised	493 (35.0%)
Immuno-competent	758 (53.9%)
Not Determined	156 (11.1%)

All prospective clinical specimens were analyzed by reference/comparator methods at central laboratories independent of xTAG GPP testing sites. For *Campylobacter, E. coli* O157, *Salmonella* and *Shigella*, all specimens were assessed from the fresh state by bacterial culture. For *Giardia* and *Cryptosporidium*, fixed stool was assessed for the presence of cysts by microscopy. All specimens were assessed by FDA-cleared cytotoxicity assay (using diluted stool filtrate processed directly from clinical specimen) and FDA-cleared EIA (using broth enrichment processed directly from clinical specimen) for *C. difficile* Toxin A/B and STEC, respectively. ETEC performance was calculated against a composite comparator method consisting of four analytically validated nucleic acid amplification tests (NAATs) followed by bi-directional sequencing. If at least one of the four NAATs was positive by PCR/bi-directional sequencing, the specimen was considered to be positive by comparator. If all four NAATs were negative, then the specimen was coded as negative by comparator. For rotavirus A, a composite reference method was also used as comparator; this method consisted of one FDA-cleared EIA and one analytically validated NAAT followed by bi-directional sequencing. If either the EIA or NAAT results were positive, the clinical specimen was coded as positive for rotavirus A by comparator. Comparator negative rotavirus A required that both the EIA and NAAT results be negative. Norovirus comparator results were calculated against a composite method consisting of CDC norovirus real-time RT-PCR assay (region C & D) and CDC conventional RT-PCR (region C & D) followed by bi-directional sequencing.

Clinical runs and re-runs using xTAG GPP were carried out for clinical specimens that had been extracted from the fresh or frozen state using the NucliSENS® easyMAG® method (BioMérieux, Inc., Durham, NC) according to the manufacturer's instructions. Total extracted nucleic acid material was stored at -70°C prior to testing with xTAG GPP at each of the clinical sites.

Diagnostic sensitivity and specificity (or positive and negative agreement, respectively) were determined based on the fraction of comparator positive or negative results relative to those obtained by xTAG GPP. Sensitivity (or positive agreement) was calculated by dividing the total number of "true positive" xTAG GPP results (TP) by the sum of the TP and "false negative" (FN) xTAG GPP results.

Specificity (or negative agreement) was calculated by dividing the total number of "true negative" xTAG GPP results (TN) by the sum of the TN and "false positive" (FP) xTAG GPP results. An xTAG GPP result was considered to be a TP or TN result only in the event that it agreed with the comparator method result for the analyte in question. 95% confidence intervals were calculated using the Wilson score method. A large number of negative samples were collected in the prospective arm of the study. Results for each of the analytes from 1407 samples tested in the prospective arm of the study are summarized in Table 4 below along with results from a retrospective study of 203 archived positive stool specimens by reference/comparator methods for pathogens that were of low prevalence in the prospective cohort. This retrospective study included specimens collected at multiple sites in North America and Europe. The range of analyte concentrations in these pre-selected specimens represented the clinically relevant range of concentrations observed in patients with gastrointestinal infections.

Table 4: Summary of Diagnostic Accuracy Observed In the Prospective and Retrospective Clinical Studies

Analyte	Prospective Cohort (n=1407)			Retrospective Cohort (n=203)			Prospective Cohort (n=1407)		
	Sensitivity			Positive Agreement			Specificity		
	TP/(TP+FN)	Percent	95%CI	TP/(TP+FN)	Percent	95%CI	TN/ (TN+FP)	Percent	95%CI
Campylobacter	3/3	100%	43.9% - 100%	40/41	97.6%	87.4% - 99.6%	1155/1176	98.2%	97.3% - 98.8%
Cryptosporidium	12/13	92.3%	66.7% - 98.6%	12/12	100%	75.7% - 100%	1131/1184	95.5%	94.2% - 96.6%
E. coli O157[1]	2/2	100%	34.2% - 100%	14/14	100%	78.5% - 100%	1158/1167	99.2%	98.5% - 99.6%
Giardia	4/4	100%	51.0% - 100%	15/16	93.7%	71.7% - 98.9%	1132/1171	96.7%	95.5% - 97.6%
Salmonella	10/10	100%	72.2% - 100%	24/27	88.89%	71.9% - 96.1%	1143/1161	98.4%	97.6% - 99.0%
Shigella	2/2	100%	34.2% - 100%	20/20	100%	83.9% - 100%	1154/1171	98.5%	97.7% - 99.1%

Analyte	Positive Agreement			Positive Agreement			Negative Agreement		
	TP/(TP+FN)	Percent	95%CI	TP/(TP+FN)	Percent	95%CI	TN/ (TN+FP)	Percent	95%CI
C. difficile Toxin A/B[2]	107/114	93.9%	87.9% - 97.0%	N/A	N/A	N/A	922/1027	89.8%	87.8% - 91.5%
ETEC[3]	2/8	25.0%	7.1% - 59.1%	38/39	97.4%	86.8% - 99.5%	1156/1160	89.8%	99.1% - 99.9%
Norovirus GI/GII	74/78	94.9%	87.5% - 98.0%	N/A	N/A	N/A	1023/1119	91.4%	89.6% - 92.9%
Rotavirus A	2/2	100%	34.2% - 100%	28/28	100%	87.9% - 100%	1162/1164	99.8%	99.4% - 100%
STEC[4]	1/1	100%	20.7% - 100%	18/18	100%	82.4% - 100%	1153/1169	98.6%	97.8% - 99.2%

[1] 8/8 *E. coli* O157 in the retrospective cohort were also positive for STEC by xTAG GPP. Sample remnants of all eight *E. coli* O157 specimens were tested for the presence of stx1 and stx2 genes by bi-directional sequencing and the results added to those obtained for STEC.

[2] A total of 95 specimens in the prospective cohort generated a "Nonspecific reaction, not characteristic of *Clostridium difficile* toxin. A titration test was performed on all 95 specimens and it was determined that in each case, the cytotoxicity reaction was not typical of *C. difficile* toxin. **A total of 48 *C. difficile* Toxin A/B xTAG GPP positive specimens that were negative by the reference method were confirmed as positive by either FDA cleared *C. difficile* toxin molecular assays or bi-directional sequencing analysis** using analytically validated primers that targeted genomic regions distinct from the xTAG GPP.

[3] ETEC comparator results were calculated against a composite consisting of four well characterized nucleic acid amplification tests (NAATs) followed by bi-directional sequencing. All six specimens that were false negative by xTAG GPP for ETEC were positive by only one of four comparator NAATs. **Repeat sequencing of these six specimens were negative by all four NAATs.**

[4] 6/10 STEC specimens in the retrospective cohort were also positive for *E. coli* O157 by xTAG GPP. Sample remnants of all 10 STEC specimens were assessed by bi-directional sequencing for *E. coli* O157 and the results added to those obtained for *E. coli* O157.

Based on the prospective data summarized above, the expected positivity rate for xTAG GPP is around 13% when considering all analytes except for norovirus and *C. difficile*. Positivity rates for these two analytes were much higher in this cohort when compared to all other analytes on the panel. Positivity rates (expected values) for this study are summarized in Table 5.

Table 5: Expected Values (Positivity Rates) as Determined Prospectively by xTAG GPP between June 2011 and February 2012

Target (Analyte)	Number of Positive Specimens in the Entire Cohort (n=1407)		0 - 1 year (n=6)		>1 - 5 years (n=20)		>5 - 21 years (n=76)		>21 - 65 years (n=879)		>65 years (n=426)	
	No.	Exp. Value	No.	Exp. Value	No.	Exp. Value	No.	Exp. Value	No.	Exp. Value	No.	Exp. Value
Rotavirus A	4	0.3%	0	0.0%	0	0.0%	2	2.6%	1	0.1%	1	0.2%
Norovirus GI/GII	170	12.1%	1	16.7%	6	30.0%	11	14.5%	101	11.5%	51	12.0%
Salmonella	28	2.0%	0	0.0%	1	5.0%	3	3.9%	18	2.0%	6	1.4%
Shigella	19	1.4%	0	0.0%	0	0.0%	0	0.0%	12	1.4%	7	1.6%
Campylobacter	24	1.7%	0	0.0%	2	10.0%	0	0.0%	15	1.7%	7	1.6%
C. difficile Toxin A/B	220	15.6%	2	33.3%	2	10.0%	13	17.1%	120	13.7%	83	19.5%
ETEC LT/ST	6	0.4%	0	0.0%	0	0.0%	1	1.3%	3	0.3%	2	0.5%
E. coli O157	11	0.8%	1	16.7%	0	0.0%	2	2.6%	6	0.7%	2	0.5%
STEC (stx 1/stx 2)	17	1.2%	0	0.0%	0	0.0%	3	3.9%	8	0.9%	6	1.4%
Giardia lamblia	43	3.1%	0	0.0%	0	0.0%	2	2.6%	26	3.0%	15	3.5%
Cryptosporidium	65	4.6%	0	0.0%	4	20.0%	2	2.6%	46	5.2%	13	3.1%
All Calls	607		4		15		39		356		193	

Key Measures		
Overall Positivity Rate per Sample (accounting for mixed infections)	486 GPP Positive Samples /1407 Total Samples Tested	34.5%
Positivity Rate per Sample (minus *C. difficile*)	314 GPP Pos. Samples (minus *C. difficile*) /1407 Total Samples Tested	22.3%
Positivity Rate per Sample (minus *C. difficile* and norovirus)	189 GPP Pos. Samples (minus *C. difficile* and norovirus) / 1407 Total Samples Tested	13.4%
Percent of Samples Tested with Two or More Pathogens per Sample	91 GPP Pos. Samples w/ Mixed Infections /486 All GPP Positive Samples	18.7%

Supplemental Clinical Studies

In order to determine baseline levels for each analyte included in xTAG GPP for individuals who were not exhibiting signs and symptoms of infectious gastroenteritis, 200 clinical stool samples were collected from healthy, asymptomatic donors from various age groups. PCR inhibition was observed in 23 of the 200 samples tested (11.5%), as determined by results for the internal control used with xTAG GPP (bacteriophage MS2). After re-running these specimens in accordance with the instructions for use, PCR inhibition was still observed in eight samples (4%). The absence of a detectable internal control signal in these samples meant that negative results for the indicated microbial targets could not be reported. Therefore, the final data analysis was conducted on 192 of the 200 samples collected for this study. The false positive rate in these 192 specimens ranged from 0%-2.6%, depending on the analyte (see Table 6).

Table 6: Summary of xTAG GPP Performance in Asymptomatic Volunteers

Target	False Positive Rate FP / (TN+FP)
Campylobacter	0.0% (0/192)
C. difficile toxin A/B	1.6% (3/192)
Cryptosporidium	0.0% (0/192)
E. coli O157	0.0% (0/192)
ETEC LT/ST	0.0% (0/192)
Giardia	1.0% (2/192)
Norovirus GI/GII	1.6% (3/192)
Rotavirus A	0.0% (0/192)
Salmonella	2.6% (5/192)
STEC stx1/stx2	0.0% (0/192)
Shigella	0.0% (0/192)

The clinical performance of xTAG GPP for rotavirus A, ETEC, *Cryptosporidium* and *Giardia* was also evaluated in a set of pediatric stool specimens (N=313) prospectively collected between February 2011 and January 2012 from symptomatic pediatric patients admitted to two referral hospitals in Botswana, Africa. All pediatric patients included in this evaluation presented with diarrhea and/or vomiting. General demographic details for these patients are summarized in the table below.

Table 7: General demographic details of Botswana Sample Set

Sex	Number of Subjects
Male	186 (59.4%)
Female	127 (40.6%)
Total	313
Age (yrs.)	**Number of Subjects**
< 1	231 (73.8%)
1	62 (19.8%)
2	11 (3.5%)
3	3 (0.9%)
4	3 (0.9%)
> 4	3 (0.9%)
Total	313

All specimens were shipped frozen to a testing site located in Southern Ontario (Canada) for xTAG GPP testing. Comparator testing by nucleic acid amplification followed by bi-directional sequencing using analytically validated primers was performed on samples positive for rotavirus A, ETEC, *Cryptosporidium* and *Giardia* by xTAG GPP. In order to minimize bias, a random subset of the Botswana cohort that tested negative by xTAG GPP was assessed by the same nucleic acid amplification followed by bi-directional sequencing for rotavirus A, ETEC, *Cryptosporidium*, and *Giardia*. Summarized below are the positive and negative agreement between bi-directional sequencing results and xTAG GPP for rotavirus A, ETEC, *Cryptosporidium*, and *Giardia*.

Table 8: Supplemental Clinical Data for Rotavirus A, ETEC, *Cryptosporidium* and *Giardia* In a Set of Pediatric Stool Specimens

Analyte	Positive Agreement			Negative Agreement		
	TP / (TP+FN)	Percent	95% CI	TN / (TN+FP)	Percent	95% CI
Rotavirus A	175/193	90.7%	85.7% - 94.0%	108/111	97.3%	92.4% - 99.1%
ETEC	26/27	96.3%	81.7% - 99.3%	26/29	89.7%	73.6% - 96.4%
Cryptosporidium	11/12	91.7%	64.6% - 98.5%	12/12	100%	75.7% - 100%
Giardia	9/9	100%	70.1% - 100%	10/11	90.9%	62.3% - 98.4%

Additionally, nucleic acid amplification followed by bi-directional sequencing using analytically validated primers was performed on all available clinical specimens that were positive by xTAG GPP for the seven other analytes (i.e. *Campylobacter*, *C. difficile* Toxin A/B, *E. coli* O157, norovirus, *Salmonella*, *Shigella*, and STEC). Results for these targets are available in the package insert for further review. Generally, results for the other seven targets were mixed because not all samples were available for comparator method testing, and for those samples that were tested, the comparator methods used may not have had optimal sensitivity.

Clinical Performance Summary

Key Points

- Overall, the clinical trials demonstrated a high degree of accuracy.
- Challenges included: study design requirements for large volumes of stool, low prevalence for many targets in the study population, complexity and performance of comparator methods, and higher than anticipated false positivity rate observed.
- The high estimated Negative Predictive Value (NPV) and comprehensiveness of the xTAG GPP assay will enable laboratories to re-state the way in which they rule-in and rule-out infectious gastroenteritis with a single same day assay.

The clinical performance of the xTAG GPP assay was assessed in a prospective multi-site clinical study and supplemented with two additional studies for low prevalence targets. Overall, results demonstrated a high degree of accuracy, particularly when considering the constraints associated with some of the comparator methods. For example, four independent NAATS were used as the comparator method for ETEC, but a comparator positive call required only one NAAT to yield a positive result. For *C. difficile*, an IVD cleared cytotoxic assay was used, yet this assay was unable to generate a result for many samples that were positive by xTAG GPP and other molecular methods. While these results were promising, the prospective study design was also challenged with low prevalence for all targets except for norovirus and *C. difficile* given the FDA's requirement of large volumes of raw stool to ensure that each sample could be tested by all comparator methods required for the study.

A retrospective study using pre-selected specimens (i.e. known positives by appropriate comparator methods) was conducted to assess the clinical performance of low prevalence targets. In addition, a prospective study was conducted in Botswana to supplement xTAG GPP testing on pediatric samples to determine clinical accuracy for rotavirus A, ETEC, *Cryptosporidium*, and *Giardia*. Many pediatric samples were excluded in the North American multi-site clinical study due to the requirement for large volumes of stool for comparator testing. The FDA assessed the results of these studies together with those from the prospective cohort and deemed the collective data sufficient for clearance of 11 of the 15 analytes assessed in the studies.

The FDA decision to exclude four targets from the de novo 510(k) clearance (*Entamoeba histolytica, Vibrio cholerae, Yersinia enterocolitica*, and adenovirus 40/41) does not have a significant impact on the ability of xTAG GPP to identify acute gastroenteritis-causing agents prevalent in the U.S. There were not enough clinical specimens available with *E. histolytica, V. cholerae,* or *Y. enterocolitica* to demonstrate clinical performance as these analytes occur infrequently and are much rarer than the other targets in the test. For adenovirus 40/41, the sensitivity versus an FDA-cleared EIA test

met FDA requirements. For samples that were not tested by EIA, an analytically validated in-house NAAT was used as the comparator method. When considering that 1) this comparator method generated more positive results than xTAG GPP; 2) there is currently no other nucleic acid test cleared as an aid in the diagnosis of gastroenteritis caused by adenovirus 40/41; and 3) this virus is known to shed in the GI tract after the infection has subsided, FDA deemed that more data would be required before considering inclusion of adenovirus 40/41 in the IVD test. Additional data will be required for inclusion of the four analytes masked by software, and Luminex will continue to pursue these discussions with the FDA. The panel of 11 bacteria, viruses and parasites cleared encompasses 90.2% of infectious gastroenteritis. If the four masked pathogens had been included in the initial clearance, the test would have been capable of detecting 90.6% of causative agents.

The design of the prospective study did not allow for a formal assessment of positive predictive value (PPV) and NPV. Positive and negative predictive values associated with diagnostic tests are highly dependent on prevalence. The PPV that can be estimated from this cohort is not necessarily reflective of the PPV that will be observed when the test is implemented into routine diagnostic use where all stool specimens may be tested, regardless of sample volume. Nonetheless, labeling claims are based on the performance evaluation results obtained from clinical trials designed and conducted to meet FDA review criteria (where in addition to xTAG GPP testing, all comparator methods for every pathogen had to also be tested for every sample). In this particular study population, prevalence for most pathogens was 2% or less and, accordingly, the estimated PPV was relatively low. In fact, the causative agents in the vast majority of positive samples were *C. difficile* or norovirus, resulting in the ~30% positivity rate in the prospective study.

In contrast to PPV, NPV values can be estimated based on the prospective cohort in this study as they are likely to approximate NPV values that will be observed in the intended use population. Since xTAG GPP is a multiplex test, it generates a result for all pathogens in every sample that is assayed. Thus, the statistical representation of negative results was very high in this patient population. When using the data summarized above to calculate NPV, the following is observed:

Table 9: Estimated Negative Predictive Values observed in 1407 prospectively collected specimens (without discordance resolution)

Analyte	True Negative (TN) Specimens (n)	False Negative (FN) Specimens (n)	Estimated NPV: TN / (TN + FN)
Campylobacter	1155	0	100.0%
Cryptosporidium	1131	1	99.9%
E. coli O157	1158	0	100.0%
Giardia	1132	0	100.0%
Norovirus GI/GII	1023	4	99.6%
Salmonella	1143	0	100.0%
Shigella	1154	0	100.0%
C. difficile Toxin A/B	922	7	99.2%
ETEC	1156	6	99.5%
Rotavirus A	1162	0	100.0%
STEC	1153	0	100.0%
All Analytes	**12289**	**18**	**99.9%**

The high estimated NPV of xTAG GPP affords great clinical utility. Considering the breadth of the panel and the fact that the majority of patients tested for gastrointestinal infections will generate a negative result, there is a high degree of certainty in a negative call made by xTAG GPP.

The open platform, plate-based design of the Luminex 100/200 system and the highly multiplexed nature of the reverse transcription/polymerase chain reactions incorporated in xTAG GPP necessitates that certain controls are in place to mitigate the risk of false positive results stemming either from contamination or non-specific reactions. Luminex has found that proper training, maintenance, cleaning and control strategies when running the assay effectively mitigates these risks. As a result, Luminex has developed a rigorous xTAG GPP proficiency program to properly train and certify clinical laboratories in order to mitigate the risk of false positive results when running the assay.

When the FDA considered the false positive rate observed in the clinical study together with the fact that identification of any of the 11 cleared pathogens requires notification to public health authorities, they felt it was prudent to have positive results confirmed by an alternative method. This "presumptive positive" claim is part of the xTAG GPP labeling, which means that laboratories who choose not to confirm a positive result would be utilizing the test "off-label". It is important to note that there is no labeling requirement to confirm a negative result generated by xTAG GPP.

Intended Use/Indications for Use

The xTAG Gastrointestinal Pathogen Panel (GPP) is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of multiple viral, parasitic, and bacterial nucleic acids in human stool specimens from individuals with signs and symptoms of infectious colitis or gastroenteritis. The following pathogen types, subtypes and toxin genes are identified using the xTAG GPP:

- *Campylobacter* (*C. jejuni, C. coli* and *C. lari* only)
- *Clostridium difficile* (*C. difficile*) toxin A/B
- *Cryptosporidium* (*C. parvum* and *C. hominis* only)
- *Escherichia coli* (*E. coli*) O157
- Enterotoxigenic *E. coli* (ETEC) LT/ST
- *Giardia* (*G. lamblia* only, also known as *G. intestinalis* and *G. duodenalis*)
- Norovirus GI/GII
- Rotavirus A
- *Salmonella*
- Shiga-like Toxin producing *E. coli* (STEC) stx 1/stx 2
- *Shigella* (*S. boydii, S. sonnei, S. flexneri* and *S. dysenteriae*)

The detection and identification of specific gastrointestinal microbial nucleic acid from individuals exhibiting signs and symptoms of gastrointestinal infection aids in the diagnosis of gastrointestinal infection when used in conjunction with clinical evaluation, laboratory findings and epidemiological information. A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.

xTAG GPP positive results are presumptive and must be confirmed by FDA-cleared tests or other acceptable reference methods.

The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out co-infection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative xTAG Gastrointestinal Pathogen Panel results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

xTAG GPP is not intended to monitor or guide treatment for *C. difficile* infections.

The xTAG GPP is indicated for use with the Luminex® 100/200™ instrument.

Examples of Diagnostic Tests with Presumptive Claims

When the FDA considered the false positive rate observed in the xTAG GPP clinical study together with the fact that identification of any of the 11 cleared pathogens requires notification to public health authorities, they felt it was prudent to have positive results confirmed by an alternative method. The requirements regarding presumptive positive results may not be familiar to some laboratory directors. However, the xTAG GPP IVD is not the first test to contain such wording. The following are a few examples of diagnostic tests with current or past presumptive claims.

Focus Technologies West Nile Virus IgM Capture ELISA

"…In conjunction with the Focus Technologies West Nile Virus ELISA IgG, the test is indicated for testing persons having symptoms of meningioencephalitis, as an aid in the presumptive laboratory diagnosis of West Nile virus infection. Positive results must be tested using the background subtraction method (either on the initial test or on a repeat test). Positive results must be confirmed by neutralization test or by using the current CDC guidelines for diagnosing West Nile encephalitis…" (FDA, Focus Technologies - West Nile Virus IgM Capture ELISA, 2004)

CDC Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set

"…Intended for the *in vitro* qualitative detection of Influenza A/H5 (Asian lineage) virus RNA either directly in patient respiratory specimens or in viral cultures for the presumptive laboratory identification of Influenza A/H5 (Asian lineage) virus… The definitive identification of influenza A/H5 (Asian lineage) either directly from patient specimens or from viral cultures requires additional laboratory testing…" (FDA, CDC - Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set, 2006)

Original xTAG RVP Clearance (confirmatory testing requirement has since been removed)

"…It is recommended that specimens found to be negative after examination using RVP be confirmed by cell culture…" (FDA, Luminex - ID-TAG Respiratory Viral Panel, 2008)

Prodesse ProParaflu+ Assay

"…Negative test results are presumptive and should be confirmed by cell culture…" (FDA, Prodesse - Parainfluenza multiplex nucleic acid assay, 2009)

Cepheid Xpert® *C. difficile*/Epi Assay

"The Cepheid Xpert® *C. difficile*/Epi Assay is a qualitative *in vitro* diagnostic test for rapid detection of toxin B gene sequences and for presumptive identification of 027/NAP1/BI strains of toxigenic *Clostridium difficile* from unformed (liquid or soft) stool specimens collected from patients suspected of having *C. difficile* infection (CDI)…Detection of 027/NAP1/BI strains of *C. difficile* by the Xpert *C. difficile*/Epi Assay is presumptive and is solely for epidemiological purposes and is not intended to guide or monitor treatment for *C. difficile* infections…" (FDA, Cepheid - *C. difficile* nucleic acid amplification test assay, 2011)

BD Veritor™ System For Rapid Detection of Flu A+B

"…A negative test is presumptive and it is recommended that these results be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay…" (FDA, BD Veritor™ System For Rapid Detection of Flu A+B, 2012)

Confirmatory Testing for GPP Positives Results

As mentioned in the FDA cleared package insert for the xTAG GPP IVD assay, the laboratory is required to confirm any GPP positive result with an acceptable reference method (either in-house or via a reference laboratory). The laboratory can still report presumptive positive results to the physician provided that the report includes appropriate presumptive language and that positive results are promptly confirmed with an appropriate test. Though positivity trends for each target will vary depending on patient demographics and geography, clinical trial data suggest that approximately 30% (or approximately 20%, if not including *C. difficile* results) of all samples tested with the xTAG GPP IVD assay will yield a positive result. It is important to remember that negative GPP results do not require any additional confirmatory testing given the excellent negative predictive value of the xTAG GPP assay.

Many of the pathogens targeted by the xTAG GPP IVD assay (e.g. *Shigella, Salmonella, Campylobacter, E. coli* O157, and shiga toxin-expressing *E. coli*) must be isolated and submitted to state health laboratories due to state and/or federal requirements. The FDA carefully considered the impact that the introduction of xTAG GPP would have on the public health system, particularly with respect to positive results. These considerations are an underlying factor in the requirement for confirmatory testing. Proactively establishing a procedure for how to report and transfer xTAG GPP positive samples to the state public health lab and/or state epidemiologist will assist in this regard.

The Value of xTAG GPP to the Lab, Hospital and Patient

xTAG GPP is a fast and comprehensive test that rules out the majority of pathogens as the cause of diarrheal disease in roughly 80% of all samples tested. In as little as five hours, laboratories will obtain results for 11 pathogens in a quick and streamlined workflow that eliminates the complexity of managing multiple samples and test methods.

The xTAG GPP IVD represents a valuable tool to diagnostic and public health laboratories. Extensive analytical and clinical validations were required to support the de novo 510(k) clearance of 11 disease-causing pathogens. The established performance characteristics together with the comprehensiveness of the panel will enable laboratories to re-state the way in which they rule-in and rule-out infectious gastroenteritis.

Arrange an on-site trial of xTAG GPP by speaking with your local Luminex representative.

E-mail: info@luminexcorp.com
Phone: 512-381-4397
Toll Free: 877-785-2323

Learn more about the xTAG GPP Assay at www.luminexcorp.com/gpp

Learn more about the burden of gastroenteritis at www.gastroenteritis.com

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IVD For In Vitro Diagnostic Use Only. Products are region specific and may not be approved in some countries/regions. Contact Luminex to obtain details for your country.

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