# A Multi-Center Clinical Evaluation of the Luminex xTAG® Gastrointestinal Panel (xTAG® GPP) In Patients with Clinical Signs and Symptoms of Gastrointestinal Infection

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## Introduction

There are an estimated two billion cases of diarrheal disease globally every year, which kill approximately 1.8 million people (mostly children) annually. Conventional methods, such as culture, O&P exams, and ELISA are widely used to detect viral, bacterial and parasitic gastrointestinal pathogens, but typically these methods exhibit long turnaround times and poor sensitivity. In contrast, molecular-based methods can provide more timely and accurate diagnosis to help health care providers make appropriate patient management and infection control decisions. In this study, we assessed the clinical performance of the Luminex® xTAG® Gastrointestinal Pathogen Panel (xTAG GPP), a multiplex nucleic acid assay which detects 11 viral, bacterial and parasitic pathogens in human stool samples collected from patients with signs and symptoms of gastrointestinal infection.

## Materials and Methods

### Sample Collection

A total of 1407 clinical specimens were collected between June 2011 and February 2012 from pediatric and adult patients presenting at six North American laboratories with signs and symptoms of gastrointestinal infection.

### Reference Method Testing

All clinical specimens were assessed for Norovirus GI/GII by CDC real-time RT-PCR (region C & D) and conventional RT-PCR (region C & D) followed by bi-directional sequencing. For Rotavirus A, a composite reference method consisting of Premier™ Rotaclone® EIA (Meridian Bioscience) and one well-characterized nucleic acid amplification test (NAAT) followed by bi-directional sequencing, was used as comparator. Since xTAG GPP also detects non-viral pathogens, all specimens were assessed by bacterial culture for *Campylobacter (C. jejuni*, *C. coli* and *C. lari)*, *E. coli* O157, *Salmonella spp. and Shigella (S. boydii*, *S. sonnei*, *S. flexneri* and *S. dysenteriae)*. Fixed stools were assessed for the presence of *Giardia* (*G. lamblia)* and *Cryptosporidium* (*C. parvum* and *C. hominis)* cysts by microscopy. All specimens were also tested by Bartels® Cytotoxicity Assay (Diagnostic Hybrids) for *C. difficile* Toxin A/B and broth enrichment followed by ImmunoCard STAT!® EHEC (Meridian BioScience) for STEC. ETEC comparator results were calculated against a composite consisting of four well-characterized NAATs followed by bi-directional sequencing. ETEC comparator positive specimens required at least one of the four NAATs to be positive. Reference method results were therefore available for all 11 pathogens tested by xTAG GPP on all clinical specimens included in the prospective study.

### xTAG GPP Testing

xTAG GPP runs and re-runs were carried out on clinical specimens that had been extracted using the NucliSENS® EasyMAG® method (BioMérieux, Inc.). Total extracted nucleic acid material was stored at -70$^0$C prior to testing with xTAG GPP at each of the clinical sites. The prospective sample set was supplemented with archived stool specimens that were positive by comparator for pathogens that were of low prevalence in the prospective study. All pre-selected specimens were tested in a randomized fashion with xTAG GPP at 4 clinical sites.

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## Results

**Table 1: General Demographic Data for Prospective Data Set (n=1407)**

| SEX | # SUBJECTS |
|---|---|
| Male | 632 (44.9%) |
| Female | 775 (55.1%) |
| **AGE (yrs)** | **# 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%) |
| **IMMUNE STATUS** | **# SUBJECTS** |
| Immuno-compromised | 493 (35.0%) |
| Immuno-competent | 758 (53.9%) |
| Not Determined | 156 (11.1%) |

**Table 2: xTAG GPP Positivity Rate by Pathogen**

| VIRAL PATHOGENS | POSITIVITY RATE |
|---|---|
| Norovirus GI/GII | 170 (12.1%) |
| Rotavirus A | 4 (0.3%) |
| **BACTERIAL PATHOGENS** | |
| *Salmonella* | 28 (2.0%) |
| *Shigella* | 19 (1.4%) |
| *Campylobacter* | 24 (1.7% |
| *C. difficile* Toxin A/B | 220 (15.6%) |
| ETEC ST/LT | 6 (0.4%) |
| *E. coli* O157 | 11 (0.8%) |
| STEC (stx 1/stx 2) | 17 (1.2%) |
| **PARASITIC PATHOGENS** | |
| *Giardia lamblia* | 43 (3.1%) |
| *Cryptosporidium* | 65 (4.6%) |

**Table 3: Sensitivity and Specificity of xTAG GPP for Viral Pathogens**

| Pathogen | Prospective Cohort (n=1407) | | Retrospective Cohort (n=203) | | Prospective Cohort (n=1407) | |
|---|---|---|---|---|---|---|
| | Sensitivity | 95% CI | Positive Agreement | 95% CI | Specificity | 95% CI |
| Norovirus GI/GII | 94.9% (74/78) | 87.5% - 98.0% | N/A | N/A | 91.4% (1023/1119) | 89.6% - 92.9% |
| Rotavirus A | 100% (2/2) | 34.2% - 100% | 100% (28/28) | 87.9% - 100% | 99.8% (1162/1164) | 99.4% - 100% |

**Table 4: Sensitivity and Specificity of xTAG GPP for Bacterial Pathogens**

| Pathogen | Prospective Cohort (n=1407) | | Retrospective Cohort (n=203) | | Prospective Cohort (n=1407) | |
|---|---|---|---|---|---|---|
| | Sensitivity | 95% CI | Positive Agreement | 95% CI | Specificity | 95% CI |
| *Salmonella* | 100% (10/10) | 72.2% - 100% | 88.9% (24/27) | 71.9% - 96.1% | 98.4% (1143/1161) | 97.6% - 99.0% |
| *Shigella* | 100% (2/2) | 34.2% - 100% | 100% (20/20) | 83.9% - 100% | 98.5% (1154/1171) | 97.7% - 99.1% |
| *Campylobacter* | 100% (3/3) | 43.9% - 100% | 97.6% (40/41) | 87.4% - 99.6% | 98.2% (1155/1176) | 97.3% - 98.8% |
| *C. difficile* Toxin A/B | 93.9% (107/114) | 87.9% - 97.0% | N/A | N/A | 89.8% (922/1027) | 87.8% - 91.5% |
| ETEC ST/LT | 25% (2/8) | 7.1% - 59.1% | 97.4% (38/39) | 86.8% - 99.5% | 99.7% (1156/1160) | 99.1% - 99.9% |
| *E. coli* O157 | 100% (2/2) | 34.2% - 100% | 100% (14/14) | 78.5% - 100% | 99.2% (1158/1167) | 98.5% - 99.6% |
| STEC (stx 1/stx 2) | 100% (1/1) | 20.7% - 100% | 100% (18/18) | 82.4% - 100% | 98.6% (1153/1169) | 97.8% - 99.2% |

**Table 5: Sensitivity and Specificity of xTAG GPP for Parasitic Pathogens**

| Pathogen | Prospective Cohort (n=1407) | | Retrospective Cohort (n=203) | | Prospective Cohort (n=1407) | |
|---|---|---|---|---|---|---|
| | Sensitivity | 95% CI | Positive Agreement | 95% CI | Specificity | 95% CI |
| *Giardia lamblia* | 100% (4/4) | 51.0% - 100% | 93.7% (15/16) | 71.7% - 98.9% | 96.7% (1132/1171) | 95.5% - 97.6% |
| *Cryptosporidium* | 92.3% (12/13) | 66.7% - 98.6% | 100% (12/12) | 75.7% - 100% | 95.5% (1131/1184) | 94.2% - 96.6% |

## Discussion

- Clinical sensitivity of xTAG GPP was 94.9% for Norovirus GI/GII and 100% for Rotavirus A. An additional 28 retrospective Rotavirus A comparator positive specimens were tested. xTAG GPP correctly identified 100% of these pre-selected specimens.
- Clinical specificity of xTAG GPP was 91.4% for Norovirus GI/GII and 99.8% for Rotavirus A.
- An additional 344 bacterial or parasitic positive specimens were included in the study (both prospective and retrospective specimens). xTAG GPP accurately detected 94.2% of these specimens.
- All 6 specimens that were false negative by xTAG GPP for ETEC were positive by only 1/4 comparator NAATs. Repeat sequencing of these 6 specimens were negative by all 4 NAATs.
- Clinical specificity of xTAG GPP for bacteria ranged from 89.8% to 99.7%. Clinical specificity of the assay for parasites ranged from 95.5% to 96.7%.
- 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 xTAG GPP.
- NPV values estimated on the prospective cohort were >99% for all 11 pathogens.
- Because of stool volume requirements for reference testing on all 11 pathogens, not all specimens submitted to the clinical sites were eligible for the study. As a result, positivity rate in the prospective cohort was low for most of the organisms. This design limitation prevented formal assessment of PPV.
- 91 specimens (6.5%) were identified as co-infected in the prospective study. In most cases, viruses presented with bacteria (N=29, 31.9%). The single most common co-infection (N=24, 26.4%) was Norovirus GI/GII with *C. difficile* Toxin A/B. Out of the 91 co-infections, 86 contained one or more analytes that had not been detected with the reference/comparator methods.

## Conclusion

xTAG GPP is a sensitive and specific multiplex panel that can simultaneously detect the most important causes of infectious gastroenteritis. By combining 11 tests into a single multiplex reaction, this assay is a valuable tool to diagnostic and public health laboratories.