Clinical Performance Characteristics of the xTAG® Gastrointestinal Pathogen Panel (GPP): A Multi-Center Clinical Evaluation

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Introduction

xTAG GPP is a qualitative molecular multiplex diarrhea test intended for the simultaneous detection and identification of multiple gastrointestinal pathogens including bacteria, viruses, and parasites. 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 Hospital Acquired Infections (HAI) such as *C. difficile* or Norovirus, foodborne illness agents like *E. coli* or *Salmonella* and common pediatric diarrhea causatives such as Rotavirus.

The purpose of this multi-site study was to establish the clinical performance of the GPP assay in detecting the clinically relevant gastrointestinal targets in patients with diarrhea.

Materials and Methods

Samples

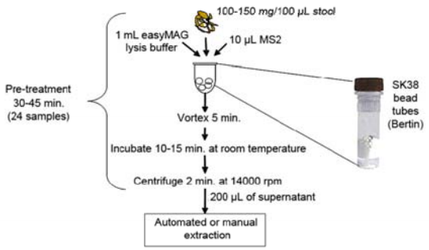
901 raw stool and stool in Cary-Blair media from symptomatic patients were collected and tested from the four clinical sites: Mount Sinai Hospital, Toronto, Ontario; St Louis Children's Hospital, Missouri; Leiden University, The Netherlands and Edinburgh Royal Infirmary, United Kingdom.

Methods

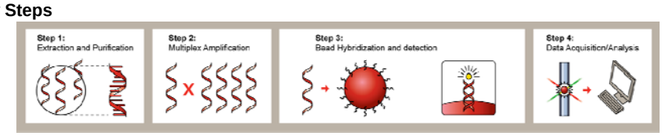
To establish clinical performance all specimens were run on GPP and various comparator methods. All clinical specimens were tested per site laboratory routine algorithm or as ordered by the referring physician, using reference method routinely used at each site and demographic & site comparator results collected on all specimens. European viral and parasitic samples were assessed by qPCR and all North American samples were assessed by sequencing with primers that targeted different genomic regions than xTAG GPP primers. Additional sequencing was performed as part of discordance/confirmatory analysis for *C. difficile* and Adenovirus.

GPP testing was carried out in accordance with the package insert for the GPP assay (see Figure 1) and by a single trained operator at each of the clinical sites. The operators at all sites had equivalent competency levels for running the assay. Nucleic acids were extracted using the NucliSENS EasyMAG® method (BioMérieux®, Inc., Durham, NC) or QIAamp® MinElute® Virus Spin kit (Qiagen®, Valencia, CA), according to the manufacturer's instructions.. Analysis of signals from the Luminex® 100/200™ and MAGPIX® systems was carried out using the TDAS software.

Pretreatment



Key Steps



Luminex 100/200 or **MAGPIX**

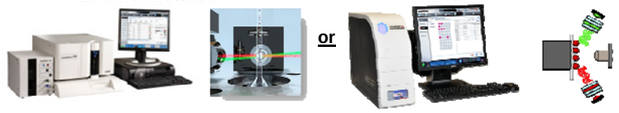


Figure 1. GPP Procedure

The GPP assay includes a RNA based internal control (MS2 Bacteriophage) which is spiked into each sample prior to extraction. Results from MS2 helps users troubleshoot and assess assay performance.

The thermal cycler temperature should be pre-heated to 53°C and set as BLOCK Temperature with the heated lid enabled.
**Recommended thermal cycler Ramp Speeds: ABI: MAX, Bio-Rad DNAEngine (MJ PTC 200): 2.5°C/sec, Eppendorf: 25% Ramp Speed (1.5°C/sec)*

Figure 2. Assay Cycling Conditions

Results

Table 1: General Demographic Details for the Prospective Data set (N=901)

SEX	NUMBER OF SUBJECTS (Percent of Total)
Male	458 (50.8%)
Female	442 (49.1%)
Not Determined	1 (0.1%)
AGE (years)	**NUMBER OF SUBJECTS (Percent of Total)**
0 - 1	120 (13.3%)
>1 - 5	79 (8.8%)
>5 - 21	134 (14.9%)
>21 - 65	382 (42.4%)
>65	182 (20.2%)
Not Determined	4 (0.4%)
SUBJECT STATUS	**NUMBER OF SUBJECTS (Percent of Total)**
Outpatients	161 (17.9%)
Hospitalized	237 (26.3%)
Emergency Department	115 (12.8%)
Not Determined	388 (43.0%)

Table 2: GPP Sensitivity & Specificity per Target on MAGPIX

Target (Analyte)	Sensitivity	Specificity
Salmonella	84.6% (66/78)	98.4% (421/428)
Shigella	97.7% (43/44)	97.8% (451/461)
Campylobacter	97.5% (120/123)	97.8% (438/448)
Clostridium difficile Toxin A/B	97.7% (44/45)	94.9% (424/447)
ETEC LT/ST	N/A	97.0% (287/296)
Escherichia coli 0157	94.1% (16/17)	98.8% (406/411)
STEC stx1/stx2	100% (14/14)	98.6% (291/295)
Yersinia enterocolitica	N/A	100% (500/500)
Vibrio cholerae	N/A	100% (414/414)
Giardia	100% (22/22)	97.5% (835/856)
Entamoeba histolytica	100% (6/6)	98.8% (643/651)
Cryptosporidium	91.7% (22/24)	99.9% (853/854)
Rotavirus A	94.7% (18/19)	99.8% (852/854)
Adenovirus 40/41	100% (9/9)	100% (235/235)
Norovirus GI/GII	93.5% (72/77)	96.4% (766/795)

(1) Due to low sample size, clinical sensitivity was not assessed for ETEC, *Yersinia* and *Vibrio cholerae*. However, analytical accuracy for these analytes was demonstrated in the limit of detection and reactivity studies with cultural isolates or plasmids.
(2) *Entamoeba* sequencing primers were designed to detect all species of *Entamoeba* (including *Entamoeba dispar*). Therefore, real-time PCR was used as comparator for this target.
(3) Adenovirus real-time PCR primers were designed to target all Adenovirus serotypes. Therefore, bi-directional sequencing using primers specific to Adenovirus type 40/41 was used as comparator for this target.
(4) 9 specimens were confirmed as Norovirus GI. The remaining specimens were confirmed as Norovirus GII.

Table 3: Co-infection Occurrences/ Pathogen Type

Co-infection Type	Occurrences
Viral / Bacterial	26
Viral / Parasitic	3
Bacterial / Bacterial	27
Bacterial / Parasitic	28
Viral / Bacterial / Parasitic	2
TOTAL	**86**

Table 4: Co-infection Occurrences/ GPP Target

Target	N	Percentage
Adenovirus 40/41	**4**	**4.6%**
Rotavirus A	**4**	**4.6%**
Norovirus	23	26.7%
Salmonella	18	20.9%
Shigella	21	24.4%
Campylobacter	22	25.6%
C. difficile Toxin A/B	31	36.4%
ETEC LT/ST	**13**	**15.1%**
E. coli O157/ STEC stx1/stx2	**11**	**12.8%**
Yersinia enterocolitica	**0**	**N/A**
Vibrio cholerae	**0**	**N/A**
Giardia	18	20.9%
Entamoeba histolytica	7	8.1%
Cryptosporidium	7	8.1%

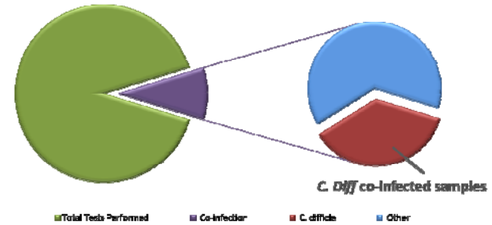


Figure 3. *C. Difficile* Co-infections

Summary

GPP has very good sensitivity and specificity as most targets exhibited >95% sensitivity and specificity. GPP shows excellent performance against singleplex qPCR and very good performance vs. culture. Close to 10% of clinical specimens were co-infected by 2 or more targets; and 36% of the co-infected samples were positive for Clostridium difficile. Overall and individual positivity rates are 4-5 fold higher than current testing algorithms.

Conclusion

xTAG GPP is a rapid and comprehensive tool for detection and identification of gastroenteritis pathogens that provides fast and accurate data for patient management and epidemiological surveillance.

Acknowledgements

Luminex would like to thank its collaborators at the four trial sites for exemplary work and continued cooperation. We would also like to acknowledge our team of scientists and clinicians who made this work possible.

†**Note:** IVD GPP is IVD in EU and Canada. Products are region specific and may not be approved in your country of residence. Canadian panel does not include *E. coli* & *C. difficile* targets. Contact Luminex for more information at www.luminexcorp.com/gpp