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ABSTRACT

Background: The xTAG® Gastrointestinal Pathogen Panel (GPP) from Luminex Molecular Diagnostics (Toronto, Canada) is a new qualitative nucleic acid multiplex assay designed for the simultaneous detection of 15 pathogens in human stool samples, including 3 viruses, 3 parasites and 9 bacteria/bacterial toxins. The assay is run on either the Luminex® 100/200™ or the recently introduced, MAGPIX® instrument that utilizes the CCD imaging technology. In this study, we examine the performance of the GPP test using the MAGPIX platform for the detection of 4 targets: Adenovirus (serotypes 40 and 41), Norovirus (GI /GII), Rotavirus A and *C. difficile* (Toxin A/B).

Methods: One hundred and fourteen stool specimens were selected for evaluation including 26 Rotavirus, 33 Norovirus, 15 Adenovirus, and 34 *C. difficile* positives. Nucleic acid was extracted from 140 µl of 10% stool suspensions using the bioMérieux easyMAG™ extractor. GPP testing was performed according to manufacturer's instructions. Briefly, reverse transcription and amplification was performed in a one-step reaction, amplified products were sorted on a bead-array incorporating the xTAG Universal Array coupled to magnetic microspheres, and reactions read on the MAGPIX instrument. Results were compared to reference methods, including the RIDA® Quick Rotavirus (R-Biopharm) for Rotavirus and in-house developed PCR assays for Norovirus, Adenovirus and *C. difficile*.

Results: Of 114 stools tested, the GPP assay detected 37/37 Norovirus positives (2 GI and 35 GII genotypes), 26/26 Rotavirus A positives and 5/5 Adenovirus serotypes 40/41. Ten Adenovirus positive stools that were confirmed to be non-40/41 serotypes by sequencing or genotype-specific PCR were negative when tested by the GPP assay as expected. For *C. difficile*, GPP detected 34/37 (91.9%) positive stools. Of the 3 *C. difficile* specimens that were missed by GPP, crossing points for the in-house assay were greater than 36 cycles, indicating low level positives. The GPP detected 7/114 (6.1%) stools positive for more than one pathogen including 1 Rotavirus/ *C. difficile*, 2 Norovirus/ *C. difficile*, 1 Adenovirus/ *C. difficile*, 1 Norovirus/ Rotavirus and 2 *Salmonella* sp. / *C. difficile*.

Conclusions: In this preliminary evaluation, the xTAG Gastrointestinal Pathogen Panel had excellent sensitivity and specificity. The overall sensitivity and specificity for these four targets was 97.4% (111/114) and 100% respectively. The assay was run on the less expensive MAGPIX instrument but can also be run on the Luminex 100/200. Further investigations are required to examine the performance of the other pathogens.

OBJECTIVE

To evaluate the performance of the Gastrointestinal Pathogen Panel (GPP) assay using the MAGPIX platform for the detection of 4 targets: Adenovirus (serotypes 40/41), Norovirus (GI/GII), Rotavirus A and *C. difficile*.

METHODS

Specimens – 114 archived stool specimens, including 26 Rotavirus A, 33 Norovirus, 15 Adenovirus, and 34 *C. difficile* positives, submitted to the Regional Virology Laboratory at St. Joseph's Healthcare (Hamilton, Canada) were used in the evaluation of the new xTAG Gastrointestinal Pathogen Panel (GPP) from Luminex Molecular Diagnostics (Toronto, Canada).

Antigen Testing – Rotavirus was tested with the RIDA Quick Rotavirus (R-Biopharm, Dermstadt, Germany) as per manufacturer's instructions.

Nucleic Acid Extraction- A 10% (wt/vol) suspension of stool specimen was prepared in DNase/RNase free water. Total nucleic acid was extracted from 140 µl of the stool suspension using the bioMérieux easyMAG automated extractor and eluted in 55 µl of buffer.

PCR Testing- Adenovirus, Norovirus GI, Norovirus GII and *C. difficile* were tested by in-house developed real time PCR assays, targeting the hexon, capsid, polymerase and tcdC/cdtA genes, respectively. All amplification reactions were set up using the QuantiTect® Multiplex RT-PCR Kit (Qiagen, Mississauga Canada) and run on the Rotor-Gene Q instrument (Qiagen, Germantown, MD).

Gastrointestinal Pathogen Panel (GPP)- This multiplex PCR based assay detects 15 pathogens in human stool samples, including 3 viruses, 3 parasites and 9 bacterial/bacterial toxins.

	Target
Virus	Adenovirus (subtype 40, 41), Norovirus (subtype GI, GII), Rotavirus A
Bacteria	<i>Campylobacter</i> , <i>Clostridium difficile</i> toxin A/B, <i>E. Coli</i> O157, <i>Enterotoxigenic E. Coli</i> (ETEC) LT/ST, <i>Salmonella</i> , <i>Shiga-like Toxin producing E. Coli</i> (STEC) stx1/ stx 2, <i>Shigella</i> , <i>Vibrio cholerae</i> , <i>Yersinia enterocolitica</i>
Parasites	<i>Cryptosporidium</i> sp, <i>Entamoeba histolytica</i> , <i>Giardia</i>

METHODS

Gastrointestinal Pathogen Panel (GPP)- 10µl of extracted nucleic acid was amplified in a single multiplex RT-PCR reaction to generate amplicons ranging in size from 58 to 242bp (not including the 24-mer tag). The RT-PCR product (5µl) was then added to a hybridization/detection reaction containing magnetic beads with its universal tag and the Streptavidin, R-Phycoerythrin conjugate. Each bead population detects a specific bacterial, viral or parasitic target through a specific anti-tag/tag hybridization. After a 45 minute incubation at 45°C, the reactions were read on the MAGPIX instrument (Figure 1) where the beads were interrogated with both green LED (525nm) and red LED (635nm). The red LED excites the internal bead dyes to identify the analyte being measured and the green LED excites the detection fluorophores to measure quantity of expression. The identity and quantity of each target is captured with a CCD imager (Figure 2). Results are expressed as median fluorescence intensity (MFI).



Figure 1: MAGPIX Platform

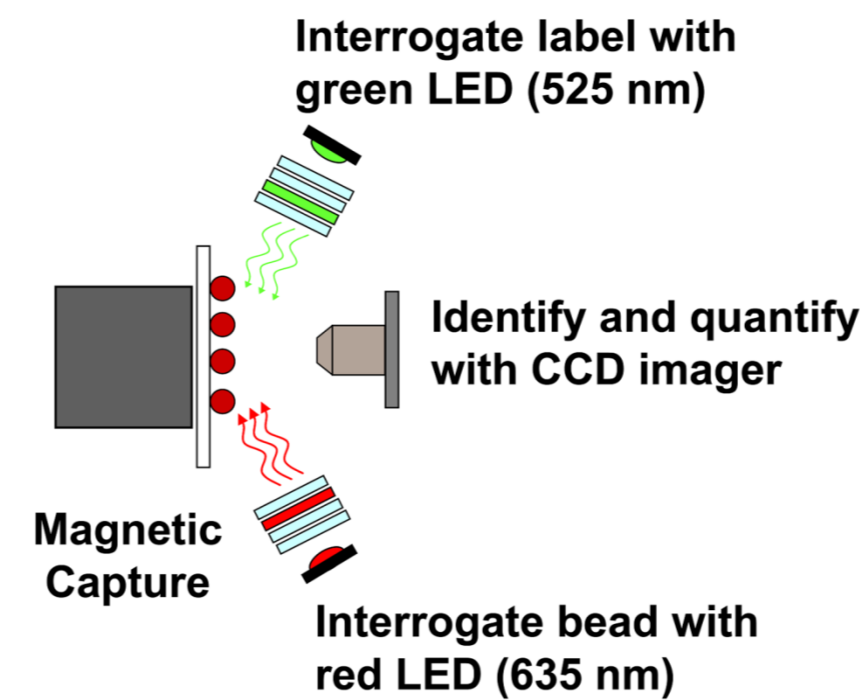


Figure 2: Interrogation of Beads

Discordant Testing- All stools with a GPP result that was discordant with its reference assay outcome were tested in a second independent uniplex PCR. The stools were considered positive or negative based on the results from 2 separate assays. Adenovirus discordant samples were further sequenced by the Institute of Molecular Biology and Biotechnology (Mobix) at McMaster University (Hamilton, Canada), using an ABI sequencer (Applied Biosystems, Foster City, CA) to determine its subtype.

RESULTS

Of the 114 stool samples tested, the GPP assay detected 37/37 Norovirus positives (2 GI, 35 GII serotypes), 26/26 Rotavirus A positives and 5/5 Adenovirus serotypes 40/41. Ten Adenovirus positive stools that were confirmed to be non-40/41 serotypes by sequencing or genotype-specific PCR were negative by the GPP assay as expected. The specificity of the GPP for the viral targets was 100% as no false positives were detected.

	Reference Result	
	+	-
Norovirus	37	0
	0	77

Sensitivity: 37/37 (100%) Specificity: 77/77 (100%)

	Reference Result	
	+	-
Rotavirus A	26	0
	0	88

Sensitivity: 26/26 (100%) Specificity: 88/88 (100%)

	Reference Result	
	+	-
Adenovirus (subtypes 40/41)	5	0
	0	109

Sensitivity: 5/5 (100%) Specificity: 109/109 (100%)

For *C. difficile*, the GPP had a sensitivity of 91.9% (34/37) and a specificity of 100% (77/77). Of the 3 *C. difficile* specimens that were missed by GPP, crossing points for the in-house assay were greater than 36 cycles, indicating low level positives.

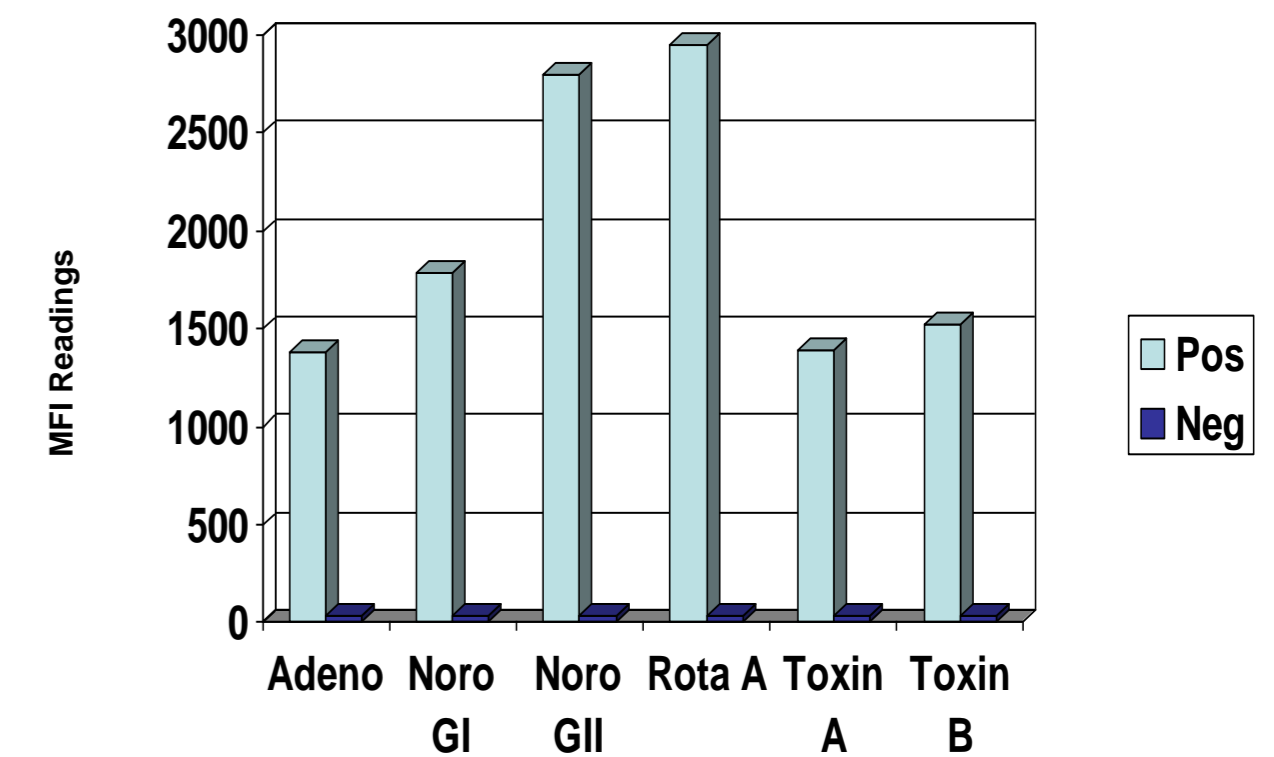
	Reference Result	
	+	-
<i>C. difficile</i>	34	0
	3	77

Sensitivity: 34/37 (91.9%) Specificity: 77/77 (100%)

The GPP assay detected 7/114 (6.1%) stools positive for two pathogens, including 1 Rotavirus/ *C. difficile*, 2 Norovirus/ *C. difficile*, 1 Adenovirus/ *C. difficile*, 1 Norovirus/ Rotavirus and 2 *Salmonella*/ *C. difficile*.

Representative MFI readings generated with the GPP assay show signal to noise ratios ranging from 34 to 82:1.

Sample	MFI Readings of GPP						
	Adeno	Noro GI	Noro GII	Rota A	Toxin A	Toxin B	Salmonella
CD633	30	38	31	36	1209.5	1944.5	37
VR6427	29	35	31	3014	36	75	31
VR4347	29	41	2520	62	38	46	41.5
VR11694	1508	44	30	33.5	1835	2312	34
MD1412	33	2710	34	35	53	47	30
CD591	27.5	34	29	32	2381	2073.5	2852



SUMMARY

- The xTAG® Gastrointestinal Pathogen Panel (GPP) had an overall sensitivity of 97.4% (111/114) and specificity of 100% for the 4 targets evaluated: Adenovirus (40/41), Norovirus (GI, GII), Rotavirus A, and *C. difficile*.
- The GPP detected 7/114 (6.1%) dual positives (1 Rotavirus/ *C. difficile*, 2 Norovirus/ *C. difficile*, 1 Adenovirus/ *C. difficile*, 1 Norovirus/ Rotavirus and 2 *Salmonella*/ *C. difficile*).
- The GPP assay can be run on either the Luminex 100/200 or the recently introduced, less expensive MAGPIX® instrument that utilizes the CCD imaging technology.
- Further investigations are required to examine the performance of the other pathogens.