



First experiences of the xTAG® Gastrointestinal Pathogen Panel using the MAGPIX instrument

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Introduction

The xTAG® Gastrointestinal Pathogen Panel (xTAG® GPP) developed by Luminex Molecular Diagnostics is a new qualitative multiplex PCR assay to detect simultaneously 15 different pathogens in human stool samples. As diarrheal disease can be caused by different kinds of pathogens, the xTAG® GPP assay is able to detect 2 viruses, 3 parasites, and 9 bacteria (including toxin gene detection). In this study, we examined the performance of the xTAG® GPP assay using the MAGPIX instrument, which is based on CCD imaging technology. We analyzed 171 stool specimens which were originally sent in for Norovirus RT-PCR testing. During summer the majority of the received stool samples are negative for Norovirus in RT-PCR testing. Interestingly, 27% of these negative samples were positive for other pathogens using the xTAG® GPP assay including other viruses, bacteria, and parasites. Co-infections were also found in some cases.

Methods

171 stool samples received for Norovirus RT-PCR testing were used in evaluation of the xTAG® GPP assay. The samples underwent automated nucleic acid extraction using the BioRobot 9604 or the Abbott M2000sp instrument.

The pretreatment step described in the package insert was not performed. The RT-PCR and the following hybridization reaction was performed according to the xTAG® GPP manual.

Data acquisition and analysis was performed on the MAGPIX instrument using TDAS software.

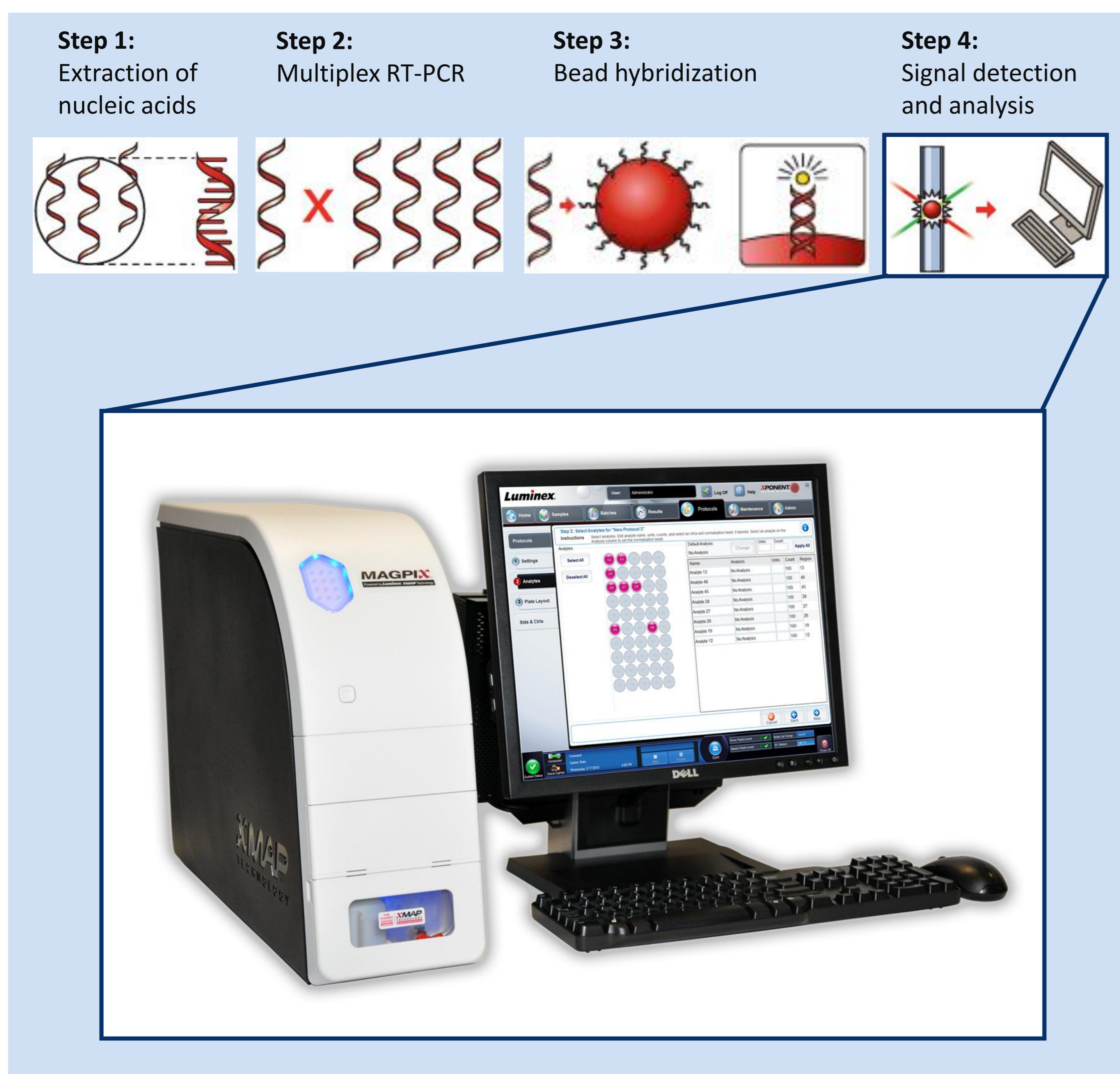
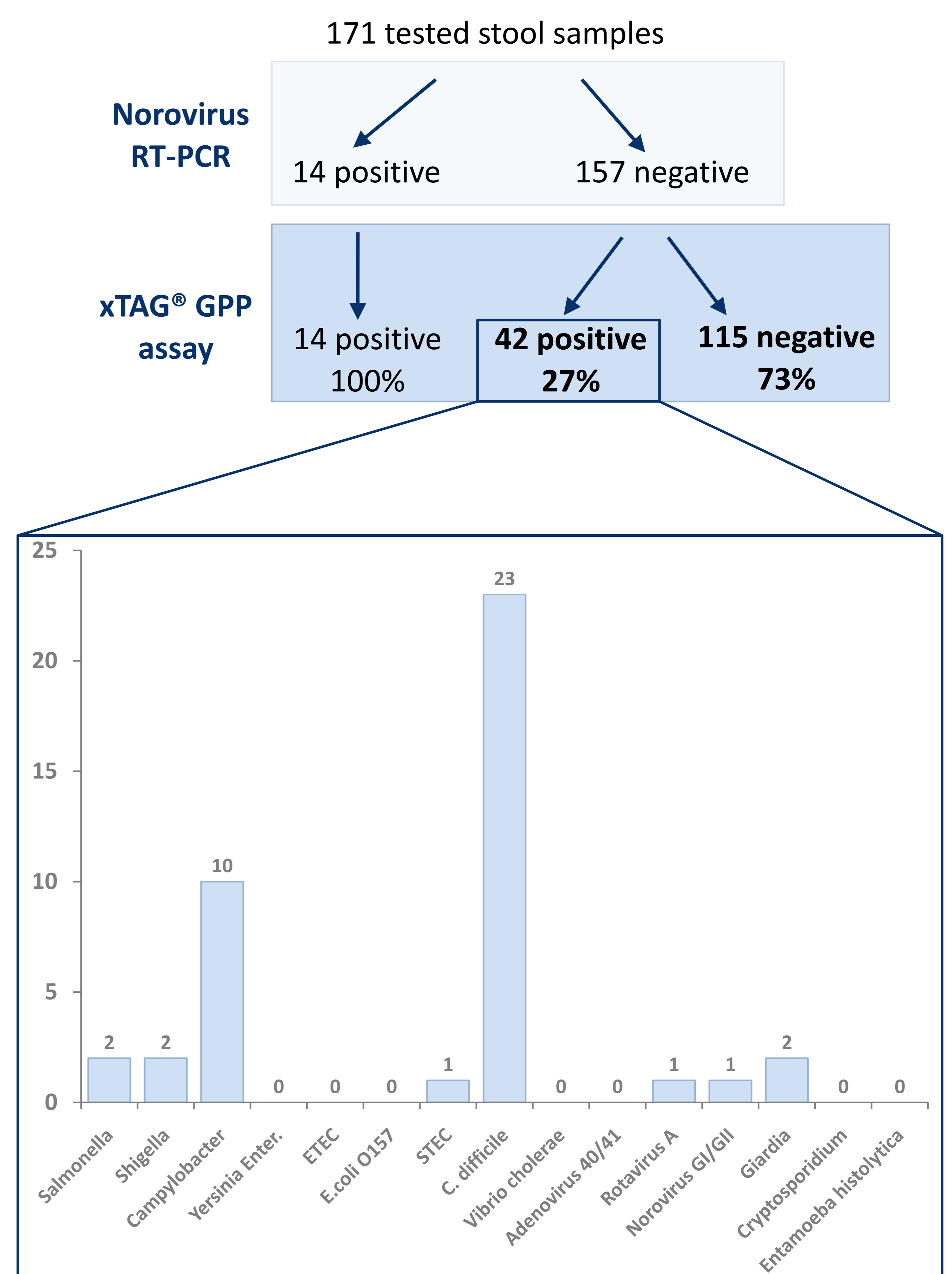


Figure 1: Schematic work flow of the xTAG® GPP assay.

<http://www.luminexcorp.com/Products/Assays/ClinicalDiagnostics/xTAGGPP/index.htm>
http://www.millipore.com/content-page.do?db=corporate/press.nsf&id=pressrelease_08092010_emd

Results



Using the xTAG® GPP assay 42 samples out of 157, which were first tested negative for Norovirus, were positive for other pathogens. Mostly, *Clostridium difficile* and *Campylobacter* were identified. *Clostridium difficile* infection were confirmed in representative sample using the RealStar® *Clostridium difficile* PCR Kit (Astra).

In 4 samples co-infections of two pathogens were observed.

We compared the sensitivity of our *in house* RT-PCR and the xTAG® GPP assay and detect no significant differences.

sample	<i>in house</i> test (RT-PCR)		xTAG® GPP assay	
	Norovirus	Ct-values	Pathogen	MFI
undiluted	pos	20,44	Norovirus GI/GII	3030
10 ⁻¹	pos	23,48	Norovirus GI/GII	3084
10 ⁻²	pos	26,95	Norovirus GI/GII	2907
10 ⁻³	pos	29,9	Norovirus GI/GII	1866
10 ⁻⁴	pos	32,62	Norovirus GI/GII	694
10 ⁻⁵	neg	-	neg	-
10 ⁻⁶	neg	-	neg	-

Conclusion

- The procedure of the xTAG® GPP assay is rather simple with a hands-on time of 5 hours
- Using the xTAG® GPP assay we were able to identify undiagnosed infection as well as co-infections
- The xTAG® GPP assay is a very helpful tool to identify gastroenteritis causing pathogens and infected patients can be quickly identified and appropriately managed