

Prospective application of the Luminex xTAG-GPP® multiplex PCR in diagnosing infectious gastro-enteritis

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INTRODUCTION

Infectious gastro-enteritis (GE) is a major diagnostic challenge as it can be caused by parasites, bacteria and viruses. The Luminex Gastro-enteritis Pathogen Panel (xTAG®-GPP) detects 18 most common GE causing pathogens including viruses (norovirus GI and GII, adenovirus, and rotavirus), bacteria (Campylobacter, Salmonella, Shigella, E. coli O157, Vibrio, and Yersinia), toxins (Clostridium toxins A/B, Shiga toxins 1 and 2, and ETEC toxins) and parasites (Giardia, Cryptosporidium and Entamoeba histolytica). This study shows the first prospective application of the first version of this new assay.

MATERIALS & METHODS

Materials

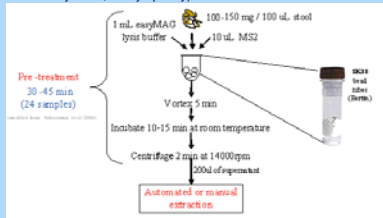
200 consecutive faecal samples submitted to the Clinical Microbiology Lab from June to August 2010 for routine diagnosis of gastroenteritis. All samples, irrespective whether the request was for bacterial culture, or molecular testing for viruses or parasites were included.

Due to seasonal differences in the aetiology of diarrhoea, an additional 100 selected samples that were previously analyzed and resulted in a range of positive results were included to cover the width of targets of the GPP assay.

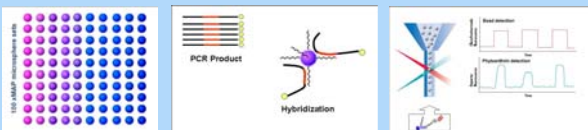
The samples were analyzed by routine diagnostic procedures in the lab, which is bacterial culture and real-time PCR for viral and parasitic GE pathogens and by the xTAG-GPP® assay.

Method

> Pretreatment of faeces using Bertin SK38 soil kit bead tubes + RNA/DNA extraction (e.g. Qiagen columns, BioMerieux EasyMAG, Qiasymphony)



- > One-step multiplex PCR.
- > Hybridization of PCR products to beads with specific oligo's.
- > Detection using a Luminex system Lx200

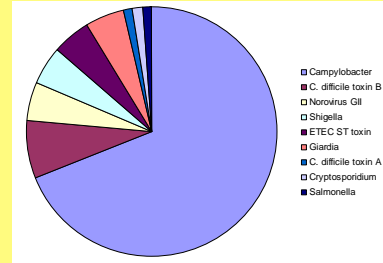


RESULTS

A) Proseptively collected samples

62 out of 200 prospectively collected samples were found positive in the GPP® assay, and 80 pathogens were detected.

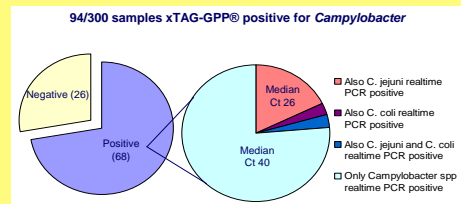
In 55 cases Campylobacter was detected. Other targets were C. difficile (n=7), Giardia (4), Norovirus GII (4), Shigella (4) and the ETEC ST toxin (4).



C) Campylobacter follow-up

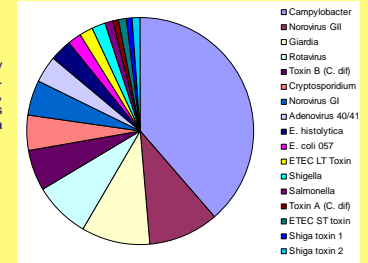
A total of 94 positive Campylobacter samples were detected using the GPP® assay, 10 of which were confirmed by bacterial culture (NB: not all samples were subjected to culture). Confirmation of the Campylobacter results was performed by real-time PCR targeting *Campylobacter* spp. (16s rRNA gene), *C. jejuni* (MapA gene) and *C. coli* (CeuE gene).

Sixteen of the Campylobacter positives were confirmed to be *C. jejuni* and/or *C. coli* with relatively low Ct values. In 26 cases, real-time PCR remained negative and in 52 cases, only the *C. species* real-time PCR was positive with median Ct values of 40, indicating a low bacterial load. Most likely these low positives represent apathogenic Campylobacters.



B) Selected samples

74 of the 100 selected samples were positive by GPP® and 101 pathogens were detected. Campylobacter was the most prevalent (n=39), followed by Giardia and Norovirus (n=10), rotavirus (n=8), *C. difficile* (n=7), Cryptosporidium (n=6), and a wide range of other pathogens.



D) Concordance with conventional diagnostics.

The x-TAG GPP® assay was superior to conventional bacterial culture. In comparison with real-time PCR, the assay performed well. For HAoV, Norovirus GII, Cryptosporidium (n=2), and *E. histolytica* (n=2) some samples with real-time PCR Ct values > 35 were not detected. In one case an *E. histolytica* with Ct = 29 was missed. In one sample, a Giardia positive result by GPP® was not detected by real-time PCR.

Major advantage of the assay is the complete diagnostic package that is being offered, resulting in pathogens being detected that have not been requested by the physician.

Specificity and sensitivity of xTAG-GPP® with real-time PCR as gold standard.

	n=	Specificity	Sensitivity
Adenovirus	4	100%	75%
Norovirus GI	5	100%	100%
Norovirus GII	14	99.6%	84%
Rotavirus	8	100%	100%
Cryptosporidium	6	100%	67%
<i>E. histolytica</i>	6	100%	50%
Giardia	14	99.7%	100%

CONCLUSIONS

The 18-target multiplex of the xTAG®-GPP assay was superior to bacterial culture and showed a high concordance with our current diagnostic molecular methods. For some targets a slight decrease of sensitivity in comparison to real-time PCR was observed. However, in comparison to our regular diagnostic procedures, more pathogens have been detected by the xTAG-GPP®. This is mainly caused by limitations in the diagnostic request of the physician to either bacteria, parasites or viruses, based on the clinical picture.

Following the results of the first prospective analyses, Luminex Molecular Diagnostics has adapted the x-TAG GPP® assay. Most important change is that the 16S rRNA based general Campylobacter assay has been replaced by three Campylobacter specific assays, targeting *Campylobacter jejuni*, *C. coli*, and *C. lari*. This adapted assay is currently being evaluated in our lab using the same panel of materials.

Diagnosis of patients with gastro-enteritis will be improved using the xTAG-GPP® assay.

We thank Peter de Man (St. Franciscus Hospital, Rotterdam, the Netherlands) for his selection of 50 faecal samples, and Jaco Verweij (LUMC, Leiden, the Netherlands) for his contribution on the Campylobacter real-time PCR assays.