Prospective Application of the Luminex xTAG® GPP multiplex PCR in diagnosing infectious gastroenteritis

Wessels E, K.T. Zlateva, L.Rusman, M. Van Bussel, E.C.J. Claas
Department of Medical Microbiology, Leiden University Medical Center, The Netherlands

Introduction

Gastrointestinal infections are a significant cause of morbidity and mortality worldwide. Identification of enteric pathogens currently incorporates a range of different methods with varying sensitivities, specificities, and turn-around times. Here the clinical performance of the xTAG® Gastrointestinal Pathogen Panel (xTAG® GPP), a new qualitative bead-based multiplexed molecular diagnostic test, is established. It includes 21 molecular targets that detect and identify 15 bacterial, viral, and parasitic pathogens responsible for gastrointestinal infections in a 5 hour workflow.
Sensitivity and specificity of the assay were established in multicenter study in comparison to routine diagnostic procedures.
Subsequently, the assay was prospectively compared to the routine diagnostic procedure of the Leiden University Medical Centre, in which the clinician usually does not request the whole diagnostic package (i.e. bacteria, viruses and parasites) but focuses on clinical picture and formation of the stool.

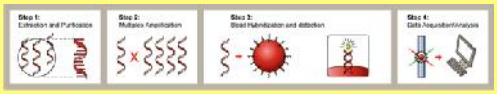
Methods

Samples
A total of 901 clinical stool samples were collected from symptomatic and asymptomatic pediatric and adult patients for routine testing at the participating sites in Toronto, Canada; St. Louis, USA; Edinburgh, UK and Leiden, The Netherlands.
In the prospective study, all fecal samples (n=465) received in the LUMC diagnostic lab for diagnosis of gastroenteritis (Nov-Dec 2011), were also subjected to xTAG®-GPP analysis.

Laboratory Testing
All samples were subjected to the routine diagnostic algorithm at the sites. Apart from conventional diagnostic procedures, the two European labs used real-time PCR for detection of parasites and viruses in stool samples.
For the prospective study, real-time PCR was used for parasites and viruses and conventional culture for bacterial pathogens.

xTAG®-GPP testing
Aliquots of extracted material were tested using xTAG® GPP on standard laser –based/flow cytometry Luminex 100/200™ system. PCR-inhibited specimens were re-tested following 1 in 10 dilution of nucleic acid extracts.

Figure 1: xTAG® GPP workflow and Turn Around Time



45 min 2.5hr 1hr 20 min

Data Analysis
For the multicenter study a composite comparator method approach was used to calculate clinical sensitivity and specificity. This comprised of the conventional reference methods used at each site, real-time PCR for detection of viruses and parasites at the European sites and sequencing of all targets from the North American sites (with other primers than GPP). Further sequencing was performed for additional discrepant analyses.
For each enteric pathogen were taken into account results obtained through the routine algorithm used at each site as well as results of bi-directional sequencing. If at least one of the comparator test results was positive, the sample was coded as comparator positive for the purpose of the composite analysis. Likewise, if all comparator test results were negative, the sample was coded as comparator negative for that target.

Results I Multicenter Study

Sensitivity and specificity
Sensitivity and specificity determinations were based on results obtained in 901 unique patient specimens. General demographic details for this patient cohort are summarized in Table 1.

Table 1: General demographic data

SEX	NUMBER OF SUBJECTS
Male	408 (90.8%)
Female	442 (49.1%)
Not Determined	1 (0.1%)
AGE (yrs)	
0 - 1	120 (13.3%)
>1 - 5	79 (8.8%)
>5 - 21	134 (14.9%)
>21 - 65	382 (42.4%)
>65	182 (20.2%)

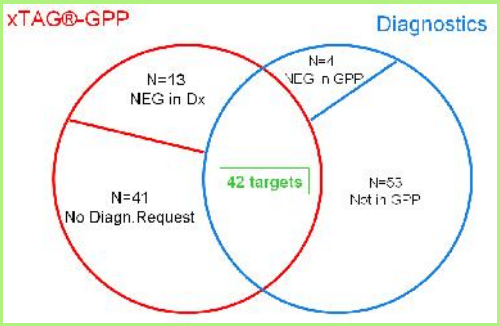
Table 2: Overall Sensitivity and Specificity xTAG®-GPP on LX100/200

Analyte	Sensitivity	Specificity
Salmonella	84.6% (66/78)	98.8% (423/428)
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[1]	97.3% (288/296)
Escherichia coli 0157	88.2% (15/17)	98.8% (407/412)
STEC stx1/stx2	100% (14/14)	99.0% (292/295)
Yersinia enterocolitica	N/A[1]	100% (500/500)
Vibrio cholerae	N/A[1]	100% (414/414)
Giardia	100% (22/22)	97.5% (835/856)
Entamoeba histolytica	100% (6/6)	98.9% (644/651)
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)	97.0% (771/795)

1) Not assessed due to low nr. of positives

Results II Prospective Study LUMC

Table 3 Overall comparison routine diagnostic (Dx) results to xTAG
In 42 samples the pathogen detected in routine diagnosis was confirmed by xTAG® GPP.



Discrepant results
N=13 (xTAG pos/ Dx neg): 2 *Campylobacter jejuni*, *2 Clostridium difficile*, 1 *Shigella*, and 2 Noroviruses were confirmed after repeated testing by real-time PCR.
For 4 *Salmonella* and 2 *Entamoeba histolytica* unconfirmed positives, further analysis is pending.
N=41 (xTAG pos/ No Dx): comprising of viruses, bacteria and parasites, almost all confirmed as true positives by real-time PCR. Also in this group further analysis of unconfirmed *E. histolytica and Salmonella sp.* positives is being performed.
N=4 (xTAG neg/ Dx pos): 2 Noroviruses, 1 Rotavirus, and 1 Cryptosporidium with Ct value> 34.9.
N=53 (not in xTAG, Dx pos): The pathogens detected are not part of xTAG® GPP. The majority is *Dientamoeba fragilis (n=18)* and the non-pathogenic *E. dispar (n=12)*. In addition, 7 Enteroviruses and 5 Parechoviruses were detected. In December 2011 6 positive Sapovirus samples (3 from 1 outbreak) were detected.

Conclusions

xTAG GPP is a highly sensitive and specific multiplex panel that can detect the most important causes of infectious gastroenteritis. By combining 21 targets into a multiplex reaction, this assay has the potential to improve diagnosis of gastrointestinal infections by increasing diagnostic yield and decreasing the time to a result compared to the current standard of care.
The prospective application showed the added value of the general xTAG® GPP approach in detecting the most common causes in infectious gastroenteritis.The samples missed by xTAG-GPP either have high Ct values, or are not present in the panel.

Acknowledgements

Luminex MD provided the xTAG®-GPP kits for the study.

Contact: e.claas@lumc.nl