Evaluation of Luminex xTAG® -GPP multiplex PCR for the diagnosis of traveller's diarrhoea

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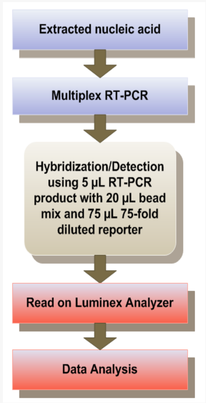
OBJECTIVES:

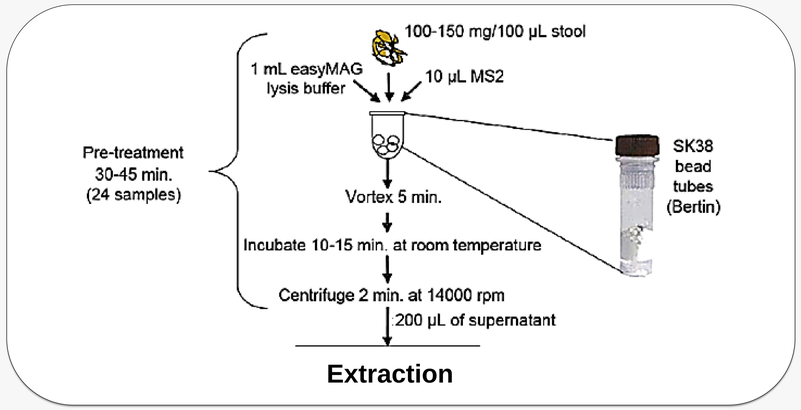
Traveller's diarrhoea is the leading cause of illness in people travelling abroad and may affect up to 80% of travellers to high-risk destinations. Diagnosis implies the detection of bacteria, virus and parasites with different techniques required for each. The xTAG® Gastrointestinal Pathogen Panel (GPP, Luminex Molecular Diagnostics) is a qualitative multiplex PCR assay for simultaneous detection of 15 most common pathogens causing gastrointestinal infections, including 9 bacterial targets, 3 virus and 3 parasites. The objective of the present study was to evaluate the usefulness of this assay for microbiological diagnosis of traveller's diarrhoea compared with conventional methods.

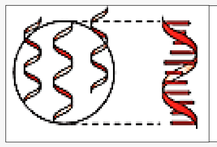
MATERIALS AND METHOD:

Samples:
Stool samples were obtained from symptomatic patients with traveller's diarrhoea from the Tropical Medicine Unit of the Hospital Clinic of Barcelona. Two hundred seventy four stool samples were collected between November 2011 and November 2012. Samples were assayed to detect enteropathogens using the GPP panel and routine methods.

Method:
The additional pre-treatment step was performed to ensure maximum extraction efficiency.





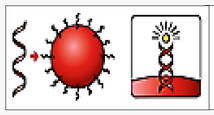


Extraction and purification

Two hundred microliters of the supernatant were used for extraction using EZ1 extractor (Qiagen) and Virus mini kit, according to the manufacturer´s recommendations. The extracted nucleic acid was eluted in 60 µl of Buffer AVE.

Multiplex RT-PCR

Ten microliters of extracted nucleic acid were used for each RT-PCR reaction**.**



Hybridization

During the incubation with the bead mix and reporter each Luminex bead population detects specific bacterial, viral, parasitic target and/or internal control.

Detection and data analysis

The fluorescence generated for each bead population was read with the MAGPIX® instrument. The data was analysed with xTAG Data Analysis Software for the Gastrointestinal Pathogen Panel (TDAS GPP)

RESULTS:

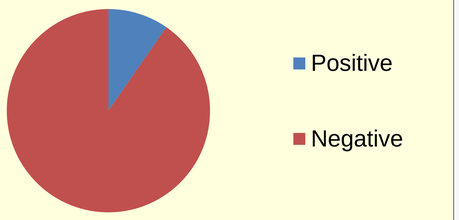
Of a total of 274 samples tested valid results were obtained in 269 and 5 showed inhibition. 88/269 (32.71%) samples were positive: 62/88 (70.45%) were detected only by GPP assay, 6/88 (6.81%) only by routine methods and 20/88 (22.72%) by both techniques. In 16 (19.51%) samples more than one pathogen was detected by the Luminex method.
It must be emphasized that there was not any clinician request for virus detection, as well as *C. difficile* toxins testing for any stool sample processed as traveller's diarrhoea .

Table 1. Pathogens detected by GPP-panel compared to the routine diagnostic methods.

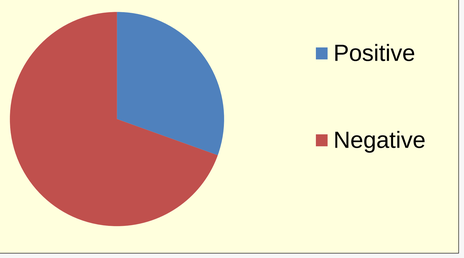
Pathogen*	No. samples with positive result			
	Only routine methods	Only GPP-panel	Both	Total
Shigella spp.	0	25	1	26
Salmonella spp.	0	0	2	2
ETEC ST/LT	4	24	5	33
Campylobacter spp.	0	2	2	4
Yersinia enterocolítica	0	0	1	1
E. coli H7:O157	0	1	0	1
STEC stx1/stx2	0	1	0	1
C. difficile toxin A/B	n.r.	3	0	3
Vibrio cholerae	0	0	0	0
Entamoeba histolytica	2	3	1	6
Giardia lamblia[1]	0	11	8	19
Cryptosporidium spp.	0	0	0	0
Norovirus GI/GII	n.r.	9	0	9
Rotovirus A	n.r.	2	0	2
Adenovirus 40/41	n.r.	0	0	0

*Only pathogens included in the GPP-panel
[1]There was no request (n.r.) for parasitological testing in 2 stool samples positive by the GPP-panel

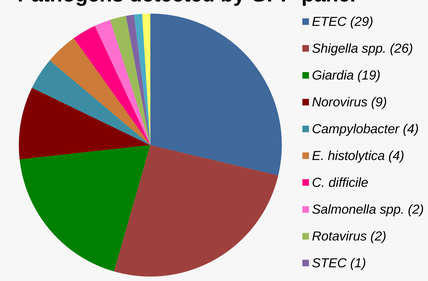
The rate of positivity using routine methods (9,6%)



The rate of positivity using the GPP-panel (30,4%)



Pathogens detected by GPP-panel



CONCLUSIONS:

The xTAG® GPP test demonstrated greater sensitivity than conventional methods such as culture and microscopy to detect multiple pathogens causing traveller's diarrhoea. Moreover, with the Luminex assay final results were obtained in 5 hours while routine methods required 24-48 hours.

The increase in positivity using the GPP assay was about 70%. It is important to highlight that 72.72% of enterotoxigenic *Escherichia coli* and 96.15% of *Shigella spp.* were detected by the GPP and not by routine methods.