POTENTIAL CLINICAL APPLICATION OF THE xTAG® GPP MULTIPLEX ASSAY IN DIAGNOSIS OF GATROENTERITIS: A MULTICENTRIC ITALIAN STUDY

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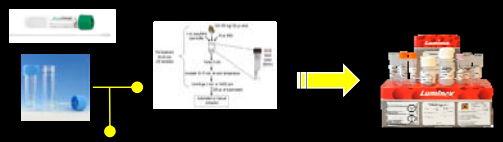
INTRODUCTION AND PURPOSE

Gastroenteritis are significant cause of morbidity and mortality worldwide. Their diagnosis is often associated with the use of different diagnostic systems with variable sensitivity and specificity. xTAG®GPP, produced by Luminex Molecular Diagnostics, is a new qualitative beads-based multiplex PCR assay to detect simultaneously 15 different pathogens responsible for community and hospital acquired infection. Here the evaluation in Multicentric Study of the potential clinical application of the GPP assay to detect the most clinically relevant gastrointestinal targets in patients with diarrhea.

METHODS

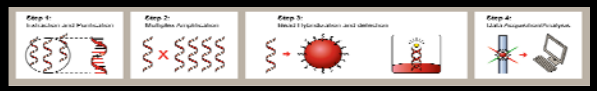
195 raw stool/stool in cary-blair media, collected from hospitalized symptomatic patients aged between 0 and 16 years (mean age = 3,12 years) at participating sites, were extracted using NucliSENS EasyMAG® method (BioMérieux®, France) and tested per site laboratory as described in the package insert for the GPP assay (Fig.1).



Reagents Supplied in the Kit (Sufficient for 96 tests)

xTAG® GPP **Primer Mix**
xTAG® **OneStep Enzyme Mix**
xTAG® **OneStep Buffer, 5X**
xTAG® **RNase-Free Water**
xTAG® **BSA**
xTAG® **MS2**
xTAG® GPP **Bead Mix**
xTAG® **Reporter Buffer** (contains 0.15 M NaCl)
xTAG® **0.22** SAPE (shipped in separate box)

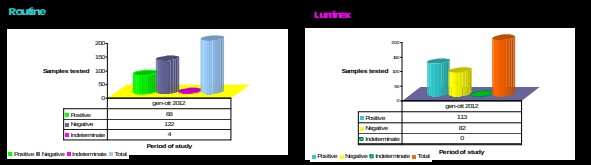
5 hoursworkflow

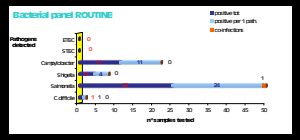


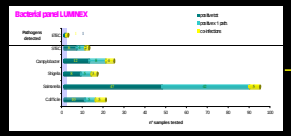
The GPP assay includes a RNA based internal control (MS2 bacteriophage) which was spiked into each samples prior to extraction, in order to helps users troubleshoot and assess assay performance. The RT-PCR and the following hybridization reaction were performed according to the xTAG® GPP manual by a single trained operator. We use the recommended thermal cycler Mastercycler® gradient (Eppendorf®, Germany). Analysis of signal and data acquisition were carried out using TDAS Software. Sensitivity and specificity of the assay were established confirming the xTAG®GPP results with routine diagnostic procedures: a standard culture for bacteria detection, EIA assay and real-time PCR for viruses detection and microscopic assay for parasites identification. The same protocol was applied to the follow-up samples when available.

RESULTS

The first sampling of 195 patients tested, gave a positive results with xTAG®GPP for 113/195 (57,9%) against 68/195 (34,9%) tested routinely, in order to determine the potential clinical application of the xTAG®GPP assay.



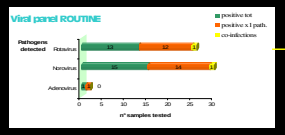


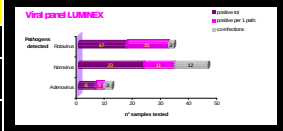


PATHOGENS	N° of POSITIVE	N° of COINFECTION	RANGE AGE
C.difficile	10	5 3 Noro-1 Shiga-1 Adeno	
Salmonella	47	5 1Rota/Noro- 1Giar-1 Noro- 1Adeno-1 Campylo	
Shigella	8	5 2 Giardia- 1 C.difficile	
Campylobacter	12	4 2 Noro-1 Adeno-1 Salmonella	
STEC	6	2 1 colI0157-1 Noro	
ETEC	1	1 Norovirus	

PATHOGENS	N° of POSITIVE	N° of COINFECTION	RANGE AGE
Salmonell a	25	1 Nov/Rot	1 years

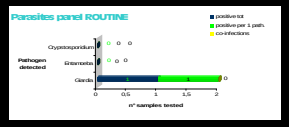
PATHOGENS	N° of POSITIVE	N° of COINFECTION	RANGE AGE
Adenovirus	1	0	
Norovirus	15	1 Rota/Salmonella	
Rotavirus	13	1 Nov/Salmonella	

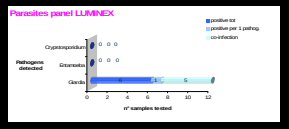




PATHOGENS	N° of POSITIVE	N° of COINFECTION	RANGE AGE
Adenovirus	6	5 1Salm-1Cdiff-1Campylo	
Norovirus	23	12 ***	
Rotavirus	17	2 1Nov/Salmonella 1Nov	

*** 1STEC 1 Rotavirus 3 C.difficile 1 Rotavirus/Salmonella 2 Giardia 2 Campylobacter 1 Salmonella 1 ETEC





PATHOGENS	N° of POSITIVE	N° of COINFECTION	RANGE AGE
Giardia	6	5 2 Norovirus- 2 Shiga-1 Salmonella	
Entamoeba	0	0	
Cryptosporidium	0	0	

42/113 (37,16%) of positives were co-infected with 2 different targets while 1 sample showed the presence of 3 simultaneous different pathogens. The percentage of positivity in first sampling tested has increased by 23% using the Luminex method. A follow up samples were collected only in 10,3% of cases to assess the possibility of any colonization.

CONCLUSION

xTAG®GPP is highly sensitive and specific multiplex panel and shows an excellent clinical performance against the reference method routinely used for detection and identification of gastroenteritis pathogens. xTAG®GPP can be considered a suitable tools to identify undiagnosed infection as well as co-infection for hospitalized patients in order to improve the management of diarrhea.

..thank you for Luminex Corporation..