

Use of a multiplex molecular assay for the detection of pathogens in stools from diarrheic patients

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INTRODUCTION

Infectious diarrheas may be caused by a large variety of pathogens such as bacteria, viruses and parasites.

Diagnostic laboratories use frequently bacteriological classic cultures on specific medias, nucleic acid tests, immunological tests as immunochromatography and morphology observation after concentration techniques

The use of multiplex assays should significantly reduce hands on time and cost, allowing a rapid and exhaustive result.

One multiplex assay based on the Luminex Universal Array is the xTAG® Gastrointestinal Pathogen Panel from Luminex Molecular Diagnostics.

OBJECTIVES

1. Evaluation of the xTAG® Gastrointestinal Pathogen Panel (xTAG GPP) for the microbiological diagnosis of acute diarrhoeas
2. Comparison of the xTAG GPP results with those of classical microbiological techniques

PATIENTS and METHODS

PATIENTS SUFFERING FROM DIARRHEA

Immunocompromised adults (n=115)
 Haematological malignancies
 Solid organ transplant patients

Children (n=232)
 Immunocompromised children (n=53)
 Children attending the emergency unit (n=119)
 Newborns from neonatology unit (n=60)

xTAG® GASTROINTESTINAL PATHOGEN PANEL

Multiple detection for:

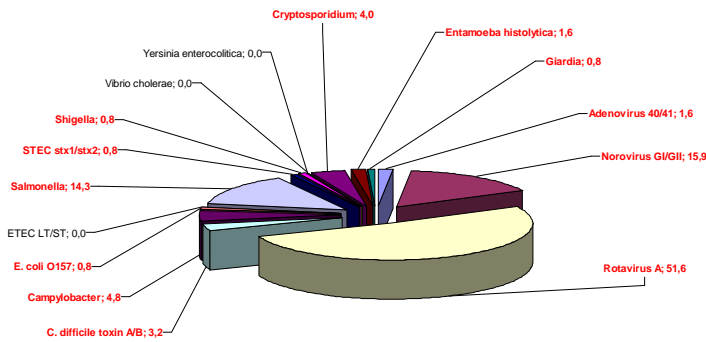
- **viruses:** Adenovirus 40/41, Rotavirus A, Norovirus GI/GII
- **bacteria:** *Salmonella*, *Shigella*, *Campylobacter*, *Clostridium difficile* toxin A/B, Enterotoxigenic *E. Coli* LT/ST, *E. Coli* O157, Shiga like toxin producing *E. Coli*, *Vibrio cholerae*, *Yersinia enterocolitica*
- **parasites:** *Giardia*, *E. histolytica*, *Cryptosporidium*

(Nucleic acids were extracted with the MagNA Pure 96 instrument using the MagNA Pure 96 DNA and viral RNA small volume kit (Roche diagnostics®))

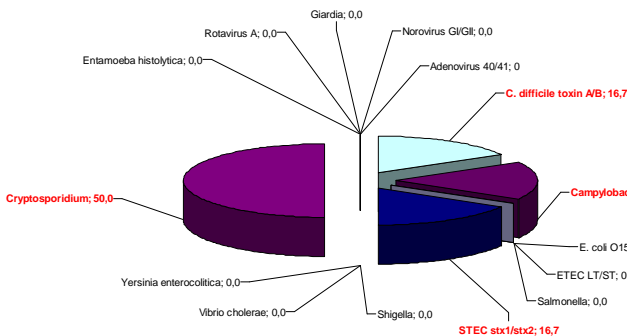
TECHNIQUES USED FOR COMPARISON

- Rapid immunochromatographic tests for viruses and *C. difficile* toxin A/B detections
- Conventional bacteriological stool cultures (*Salmonella*, *Shigella*, *Campylobacter*, *Vibrio cholerae*, *Yersinia enterocolitica*)
- Molecular detection for *E. coli* shiga toxin like gene, *E. coli* O157, Enterotoxigenic *E. coli* LT/ST
- Ova and parasite exam with or without coloration

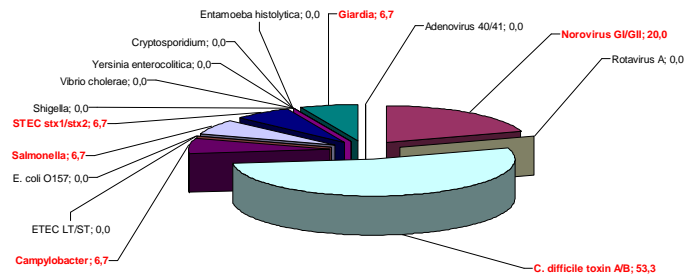
RESULTS



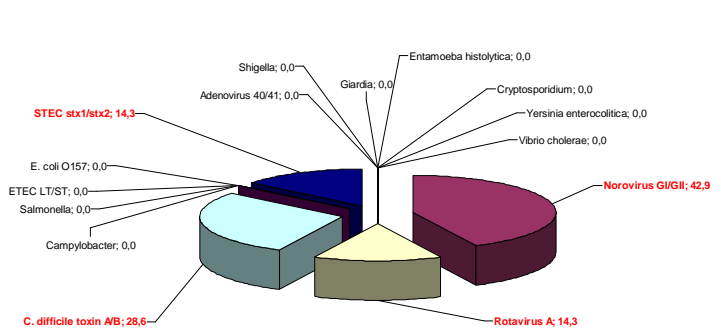
Detected pathogens (%) among children attending emergency unit
93 positive / 119 patients (32 coinfections)



Detected pathogens (%) among children from neonatology unit
8 positive / 60 patients (0 coinfections)



Detected pathogens (%) among immunocompromised adults
14 positive / 115 patients (2 coinfections)



Detected pathogens (%) among immunocompromised children
9 positive / 53 patients (0 coinfection)

The xTAG GPP assay was statistically more sensitive than culture for *Salmonella* detection ($p<0.0001$), *Campylobacter* detection ($p=0.02$), *C. difficile* Toxin detection ($p<0.0001$) and Rotaviruses, Noroviruses immunochromatographic assays ($p<0.0001$).

E. histolytica, *Giardia* and *Cryptosporidium* were detected with the xTAG whereas classical techniques were negative.

The xTAG GPP assay was statistically less sensitive ($p<0.01$) than monoplex PCR detection for shiga like toxin producing *E. coli*

CONCLUSION

The xTAG® Gastrointestinal Pathogen Panel performed well in comparison to conventional culture or immunochromatographic assays for the detection of gastrointestinal pathogens and provided useful informations in less than 5 hours.

Most of pathogens were detected among children attending the emergency unit and a high prevalence of coinfections was observed in such patients. Current results showed that pathogens were unfrequently detected in stools of diarrheic immunosuppressed patients and that coinfections were rare