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Diagnosis of gastroenteric infections: comparison of traditional methods with the new molecular technologies

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Introduction

Complete diagnosis of infectious gastroenteritis implies the detection of pathogenic bacteria, viruses and/or parasites what requires a specific microbiological procedure for each one, being time consuming. New microbiological molecular tools allow the detection of multiple and different kind of pathogens in the same sample.

Material and Methods

A total of 387 human stool clinical samples collected in 2010 and 2011, 233 retrospective (-80°C frozen samples) and 154 prospective, were analysed for enteric pathogens.

Routine enteropathogen detection included:

- standard stool-culture for bacteria
- ELISA (ProSpect, OXOID) or an in-house PCR for rotavirus
- in-house real-time PCR for norovirus
- microscopy for *Giardia lamblia*
- microscopy and /or an in-house PCR for detection of *Entamoeba*.

All samples were tested with the Luminex xTAG-GPP® capable of simultaneous detection of 15 enteropathogens: 9 bacteria, 3 viruses and 3 parasites. Samples were read using a Luminex 200 analyzer.

Automated total nucleic acid extraction was done using the Nuclisens Easymag (bioMérieux) without any pervious treatment. 82% samples were rectal swabs. Rectal swabs and stools were resuspended in B199 medium prior to extraction. Some of the discordant samples were also studied with the Seeplex® PCR (Seegene), panels V, B1, and B2.

Results

- ✔ 189/225 (84%) positive stools for enteropathogens by standard techniques (173 retrospective and 52 prospective, including 16 positive to more than one pathogen) showed agreement with the Luminex xTAG-GPP®.
- ✔ Most *Campylobacter* (61/63), rotavirus (63/65) and norovirus (25/26) were detected. However 7/42 *Salmonella* were not detected with the Luminex xTAG-GPP®, but they could neither be detected using another commercial PCR. In 6 *Salmonella* positive cultures the Luminex xTAG-GPP® detected the *Salmonella* together with an *Entamoeba*.
- ✔ Of the 162 negative samples (60 retrospective and 102 prospective) agreement was observed in 138 (85%). In 12/24 negative samples, the Luminex xTAG-GPP® identified a rotavirus, most of them confirmed by an in-house rotavirus PCR.
- ✔ Performance time for Luminex xTAG-GPP® was about 5 hours, working in batches of 24 samples. By standard technologies, more than 48 hours were needed to obtain final results.

Stool analysis result	Total 387	Luminex xTAG-GPP® Concordant	Luminex xTAG-GPP® Discordant
Single pathogen			
Campylobacter	**63**	**59** 57 *Campylobacter* 2 *Campylobacter* + *Giardia*	**4** 1 *Campylobacter* + norovirus 1 *Campylobacter* + *Salmonella* 1 rotavirus 1 neg
Salmonella 20 S. Enteritidis 17 S. Typhimurium 5 other salmonellas	**42**	**33** 27 *Salmonella* 6 *Salmonella* + *Entamoeba* (PCR *Entamoeba*: 6 neg)	**9** 1 *Salmonella* + norov 7 neg (**Seeplex**: 3 neg + 4 ND) 1 *Salmonella* + *Yersinia*
Yersinia enterocolitica	**3**	**3** 3 *Yersinia*	**0**
E. coli O157	**2**	**0**	**2** 1 neg (**Seeplex**: 1 neg) 1 ND
Shigella	**1**	**1** 1 *Shigella*	**0**
Rotavirus	**65**	**59** 57 rotavirus 2 rotavirus + ADV	**6** 2 neg (**Seeplex**: 1 neg + 1 ND: **PCR**: 1 neg + 1 ND) 3 rotavirus + norovirus (**Seeplex**: 1 rotavirus alone + repeated: 2 norovirus neg) 1 rotavirus + *Campylobacter* (**Seeplex**: 1 rotavirus alone)
Norovirus	**30**	**24** 20 norovirus 2 norovirus + ETEC 1 norovirus + ADV 1 norovirus + ADV + *Giardia* (**Seeplex**. norovirus + ADV)	**6** 3 neg (**Seeplex**: 2 neg + 1 ND) 1 ADV (**Seeplex**: ADV) 1 rotavirus (**Seeplex**: 1 rotavirus) 1 norovirus + rotavirus (**Seeplex**: 1 norovirus alone)
Giardia	**2**	**2** 2 *Giardia*	
Entamoeba-liver abscess	**1**	**1** 1 *Entamoeba* + rotavirus	
Mixed pathogens			
Campylobacter + norovirus	**2**	**1** 1 *Campylobacter* + norovirus	**1** 1 *Campylobacter* + *Salmonella*
Campylobacter + rotavirus	**6**	**5** 5 *Campylobacter* + rotavirus	**1** 1 *Campylobacter* + *Salmonella*
Salmonella + rotavirus + norovirus	**1**		**1** 1 *Salmonella* + rotavirus
Salmonella + *Giardia*	**1**		**1** 1 *Giardia*
Rotavirus + norovirus	**6**	**1** 1 rotavirus + norovirus	**5** 2 rotavirus (**Seeplex**: 1 rotavirus + 1 ND) 2 rotavirus (**Seeplex**: 2 rotavirus + norovirus) 1 rotavirus + ETEC
Negative			
Neg	162	138 132 neg (16 Clostridium A/B) 3 ADV (Seeplex: 3 ND) 2 ETEC (Seeplex: ND) 1 Cryptosporidium	24 8 rotavirus (**Seeplex**: 6 rotavirus +1 neg +1 ND) 2 *Campylobacter* (**Seeplex**: 1 *Campylobacter* + 1 ND) 1 *Campylobacter* + rotavirus (**Seeplex**: Campylobacter + rotavirus) 5 norovirus (**Seeplex**: 1 neg, 4 ND) 2 rotavirus+ ETEC (**Seeplex**: 2 neg) 1 Salmonella (**Seeplex**: ND) 1 Salmonella + rotavirus (**Seeplex**: ND) 1 E. coli O157 (**Seeplex**: ND) 1 *Entamoeba* (PCR *Entamoeba*: neg) 1 *Giardia* (microscopy: neg) 1 *Giardia* + Cryptosporidium. (microscopy: neg)

Conclusions

The Luminex xTAG-GP® technology demonstrated good and quick results in the screening of human enteropathogens.

The lack of a gold standard technology makes difficult to assess the complete performance of this new technology.