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xMAP GPP - Gastrointestinal Pathogen Panel

Experience of a multiplex nucleic acid test for the detection of gastrointestinal pathogens in faecal samples

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Method and Material

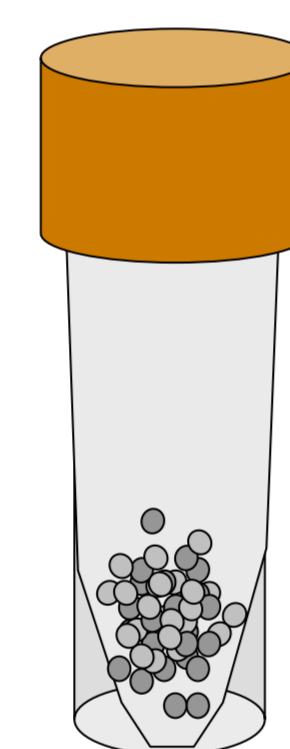
Experimental set up:

In this ongoing study we here show the latest results of analyses of fecal samples from gastroenteritis patients tested with the xMAP GPP.

Samples underwent a bead-beating pre-treatment step prior to total nucleic acid extraction. Reverse transcription and amplification was performed in one-step and amplified products were sorted on a bead-array incorporating the xTAG® Universal Array coupled to magnetic microspheres. Detection was carried out on a Luminex® 200™ xMAP® instrument. Total turn-around-time was less than five hours.

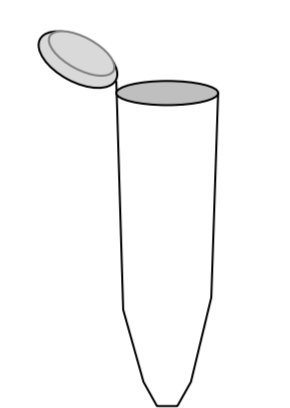
1. Extraction of nucleic acid

- 100-150 mg/100 µL stool
- 10 µL MS2 (internal extraction control)
- 1 mL easyMag® lysis buffer

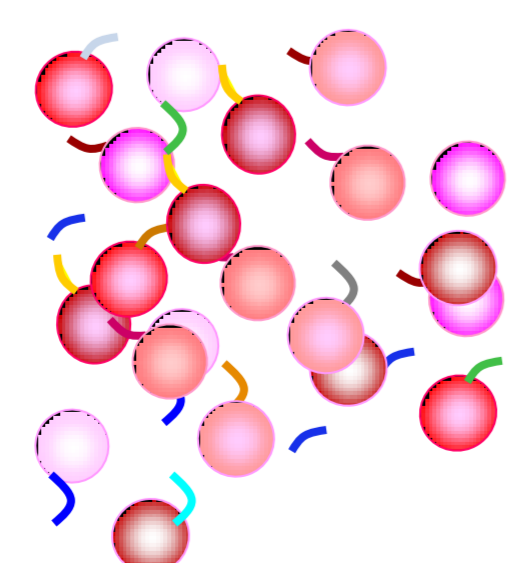
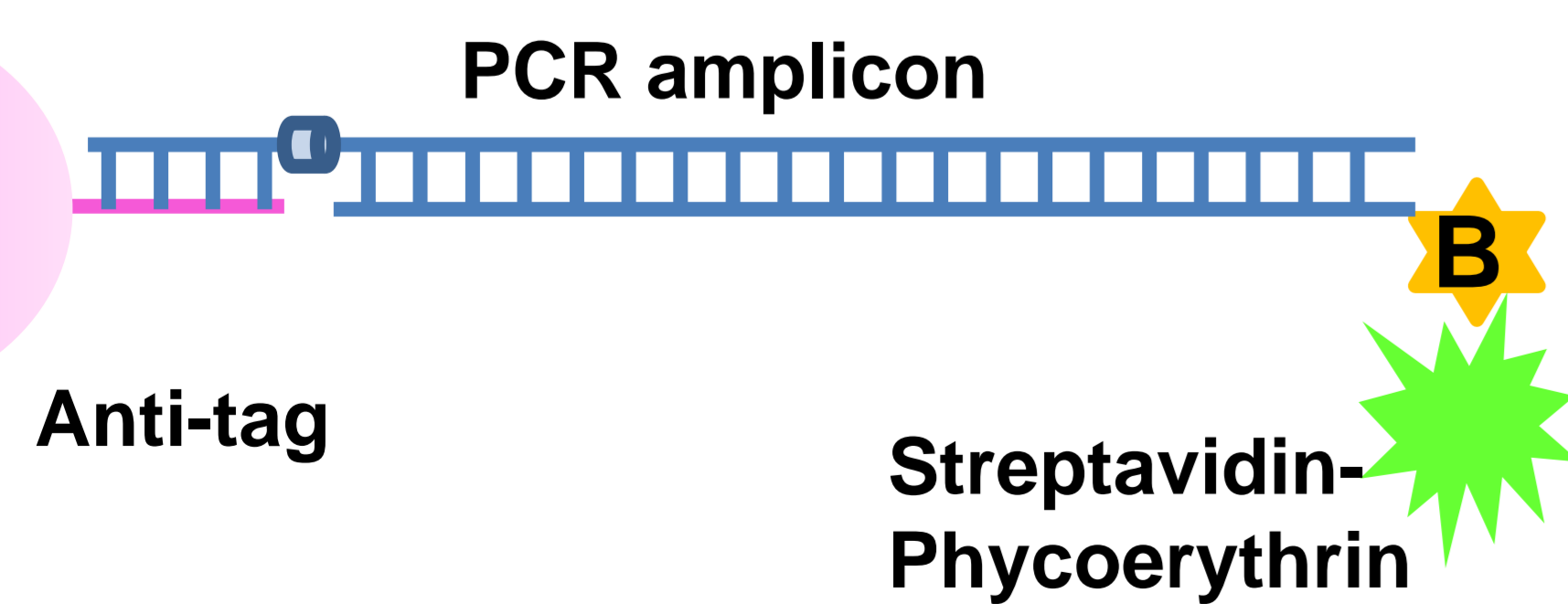


Vortex, Incubate, Centrifuge, Extract

2. Multiplex RT-PCR



3. Hybridization

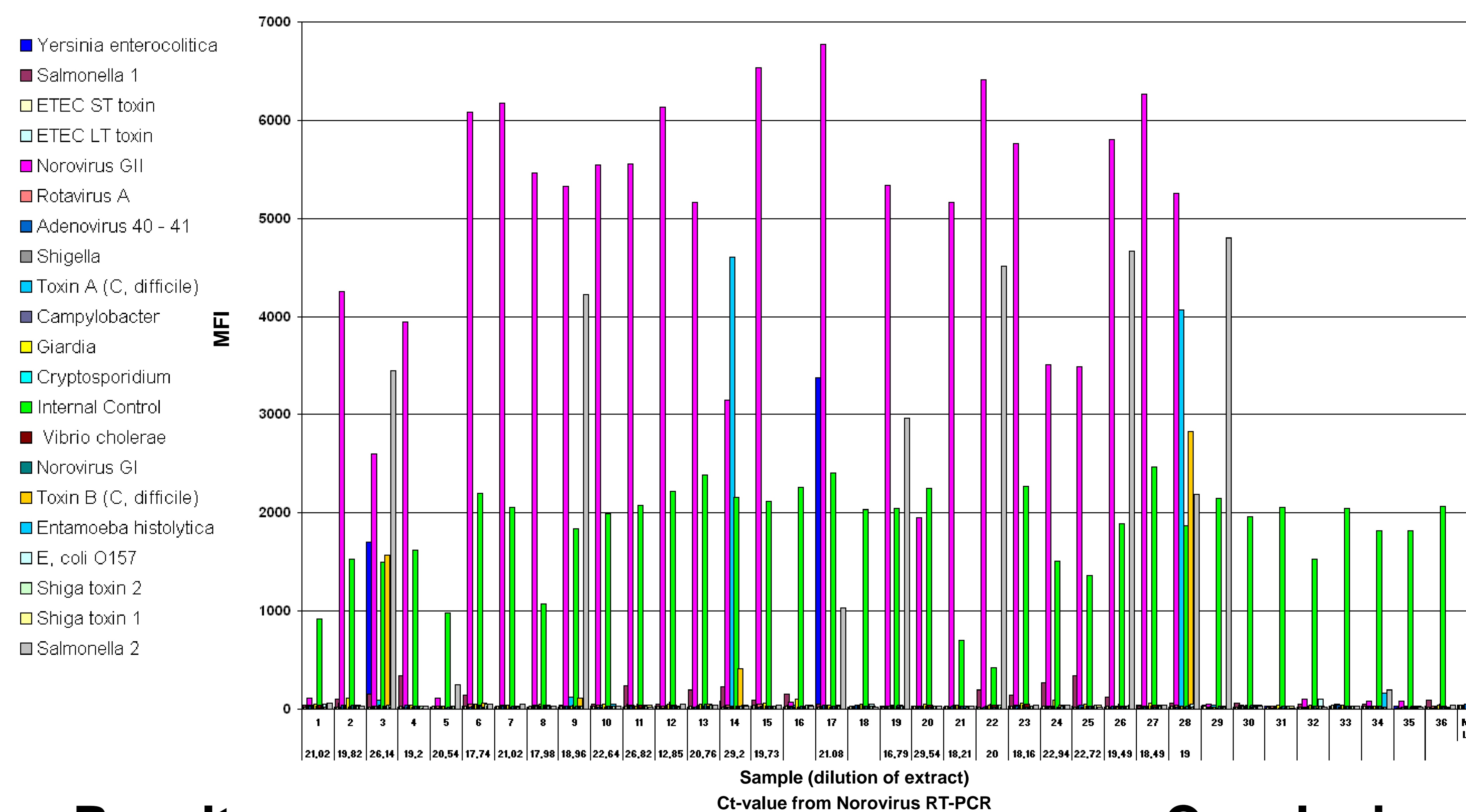


4. Reading in Luminex®200™

5. Data Analysis

Introduction

xMAP GPP is a new multiplex method for simultaneous detection of virus, bacteria and parasites. Multiplex molecular diagnostics of infectious diseases are often rapid and cost-effective. They also have the benefit of detecting co-infections and can reveal unexpected pathogens. Here we describe a first experience of this multiplex panel with fecal samples from patients with gastroenteritis sent to Clinical Microbiology in Uppsala, Sweden. The samples were originally sent in for Norovirus GGI and GGII RT-PCR testing.



Results

The results of the 36 fecal samples of gastroenteritis patients described here are from an ongoing study.

- 24 of 26 qPCR positive samples for Norovirus (Ct range 12.85-29.54) were detected within a MFI range of 1955-6771, while two of them were very weak with MFI 111 and 116 (sample 1 and 5).

The panel detected several co-infections, which were confirmed by bi-directional sequencing.

-Sample 14: Norovirus GII (MFI 3153, Ct 29.2) and the *C. difficile* toxins A (MFI 4606) and B (MFI 415)

-Sample 17: Norovirus GII (MFI 3378, Ct 21.08) and *Y. enterocolitica* (MFI 6771)

-Sample 28: Norovirus GII (MFI 3258, Ct 19) and the *C. difficile* toxins A (MFI 4068) and toxin B (MFI 2833)

-Sample 3: Norovirus GII (MFI 2598, Ct 26.14), *Y. enterocolitica* (MFI 1699) and toxin B (MFI 1574) of *C. difficile*

Conclusion

New undiagnosed infections and co-infections were found in some samples, which were then confirmed by sequencing or by RT-PCR. No sample was judged as salmonella positive, since both probes have to bind.

Traditionally, the cause of gastroenteritis, be it a bacterium, parasite or a virus, must be decided already when the sample is taken. This leads to missed diagnoses. The evaluation shows that multiplexed nucleic acid detection using xTAG®/xMAP®, targeting viruses, bacteria, bacterial toxins and parasites, complements traditional gastroenteritis diagnostic methods. It detects coinfections, and viral pathogens in samples sent for detection of bacteria and vice versa. Multiplexing takes away some of the guesswork in today's microbiological diagnostics.

xTAG® Gastrointestinal Pathogen Panel

Viruses
Adenovirus 40/41
Rotavirus A
Norovirus GI/GII

Bacteria and bacterial toxins
Salmonella
Shigella
Campylobacter
Clostridium difficile toxin A/B
Enterotoxigenic *E. coli* (ETEC) LT/ST
E. coli O157
Shiga-like Toxin producing *E. coli* (STEC) stx 1/stx 2
Vibrio cholerae
Yersinia enterocolitica

Parasites
Giardia
Entamoeba histolytica
Cryptosporidium