# High percent of co-detections of viral versus bacterial gastrointestinal pathogens in stool samples calls for a different approach in analysis of molecular testing results

Melissa Kolb[1], Jasmin Köffer[1], Ulrike Betz[1], Charlene Eckel[1], Camilla Ulekleiv[2], Chunling Wang[2], Jelena Feenstra[2], Mary Singh[2], Ulrich Eigner[1]

[1]MVZ Labor Dr. Limbach & Kollegen eGbR, Heidelberg, Germany; [2]Thermo Fisher Scientific, South San Francisco, CA, USA

MVZ Labor Dr. Limbach HEIDELBERG

**ESCMID Global 2025, Vienna, Austria**

LIMBACH GRUPPE

## Introduction

Multiplex PCR has emerged as an alternative to traditional testing methods for gastrointestinal (GI) pathogen detection due to higher sensitivity and faster turnaround times. Co-detections of multiple GI pathogens are common and can complicate results interpretation. We evaluated the prevalence of co-detections in stool samples positive for viral and bacterial pathogens, as well as analytical performance of TrueMark™ Enteric Panels.

## Materials and methods

The panels assessed in this study included TrueMark™ Enteric Bacterial Select Panel I which was designed to detect and differentiate *Campylobacter (jejuni, coli and upsaliensis)*, *Salmonella*, *Shigella*/EIEC and Shiga toxin-producing *E. coli* (STEC) stx1/stx2 in a single PCR reaction; TrueMark™ Enteric Viral Select Panel - designed for detection and differentiation of rotavirus A, adenovirus F40/41 and astrovirus in a single reaction; and TrueMark™ Enteric Norovirus Select Panel designed for detection and differentiation of norovirus GI and GII in a single reaction. All 3 panels contain *Bacillus atrophaeus* as an internal process control. In this study, stool samples (n=315) were collected in Germany, France, and Ivory Coast. Following nucleic acids extraction with MagMAX™ Prime Viral/Pathogen NA Isolation Kit on KingFisher™ Flex, rotavirus, adenovirus, astrovirus, and norovirus were tested in 170 samples using TrueMark™ Enteric Viral Select Panel and TrueMark™ Enteric Norovirus Select Panel. In 145 samples TrueMark™ Enteric Bacterial Select Panel I was used to detect *Campylobacter*, *Salmonella*, *Shigella*/EIEC, and STEC. Results from the 3 TrueMark™ Panels for research use only were compared with two other real-time PCR-based tests.

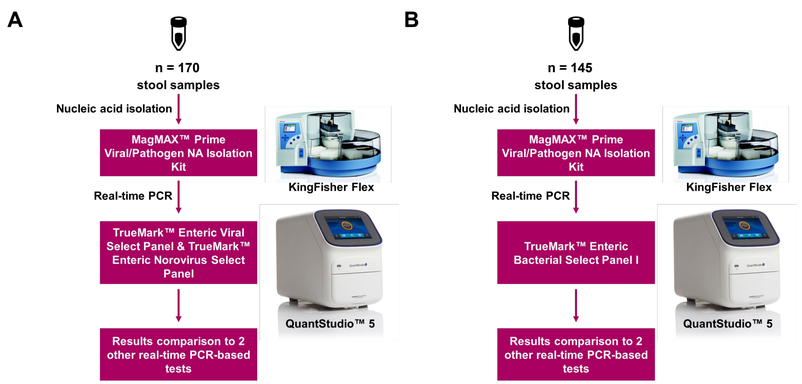


*Figure 1. Study design for A) viral panels analysis and B) bacterial panel analysis*

## Results

TrueMark™ Viral Select panels identified 47 norovirus GI/GII, 52 astrovirus, 48 rotavirus A and 55 adenovirus F40/41 positive samples in 170 samples tested. Negative percent agreements (NPA) to the comparators were all >93.65%, while positive percent agreements (PPA) were all >90.57% (Tables 1, 2). Co-infections were detected in 32.2% (n=32) of positive samples, with similar distributions of different combinations of four viruses (Figures 2, 4). In contrast, analysis of 145 samples included in evaluation of bacterial pathogens revealed a low rate of coinfections with only 5 samples showing co-detections: *Campylobacter*-STEC (n=2), *Shigella*/EIEC-STEC (n=2) and *Shigella*/EIEC-*Campylobacter* (n=1). Examples of Ct value distribution of samples positive for single viruses – adenovirus and astrovirus or co-infections with other viral pathogens are shown in Figures 3 and 4.

***Table 1. Concordance between TrueMark™ Enteric Viral Select Panel and composite comparator results for detection of astrovirus, adenovirus F40/41 and rotavirus A in stool samples***

| | | Composite comparator result | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Astrovirus | | | Adenovirus F40/41 | | | Rotavirus A | | |
| | | Positive | Negative | Total | Positive | Negative | Total | Positive | Negative | Total |
| **TrueMark™ Enteric Viral Select Panel** | Positive | 44 | 8 | 52 | 48 | 7 | 55 | 45 | 3 | 48 |
| | Negative | 0 | 118 | 118 | 5 | 110 | 115 | 2 | 120 | 122 |
| | Total | 44 | 126 | 170 | 53 | 117 | 170 | 47 | 123 | 170 |
| **Positive percent agreement** (95% CI) | | **100.00%** (91.97% - 100.00%) | | | **90.57%** (79.75% - 95.90%) | | | **95.74%** (85.75% - 98.83%) | | |
| **Negative percent agreement** (95% CI) | | **93.65%** (87.97% - 96.75%) | | | **94.02%** (88.16% - 97.07%) | | | **97.56%** (93.07% - 99.17%) | | |

***Table 2. Concordance between TrueMark™ Enteric Norovirus Select Panel and composite comparator results for detection of norovirus in stool samples***

| | | Composite comparator result | | |
|---|---|---|---|---|
| | | Norovirus GI/GII | | |
| | | Positive | Negative | Total |
| **TrueMark™ Enteric Norovirus Select Panel** | Positive | 47 | 0 | 47 |
| | Negative | 5 | 118 | 123 |
| | Total | 52 | 118 | 170 |
| **Positive percent agreement** (95% CI) | | **90.38%** (79.39% - 95.82%) | | |
| **Negative percent agreement** (95% CI) | | **100.00%** (96.85% - 100.00%) | | |

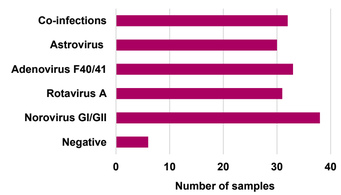


*Figure 2. Detection of single viruses or co-infections in the analyzed sample cohort*

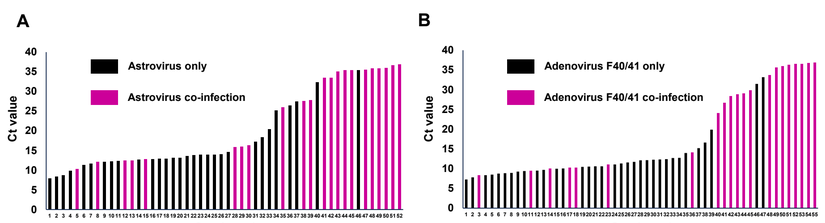


*Figure 3. TrueMark™ Enteric Viral Select Panel Ct-value distribution of A) Astrovirus and B) Adenovirus F40/41 positive samples*

## Results (contd.)

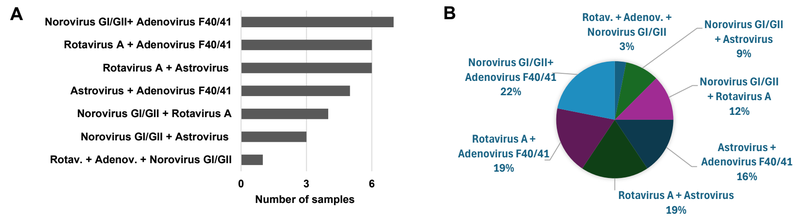


***Figure 4. Number (A) and relative prevalence (B) of co-infections detected with TrueMark™ Enteric Panels in the sample cohort analyzed for viral enteric pathogens***

TrueMark™ Enteric Bacterial Select Panel I demonstrated high concordance with comparators: 100% PPA for *Salmonella* and *Shigella*/EIEC, 97.4% for *Campylobacter*, and NPA >95.3% for all targets.(Table 3). The PPA for STEC was 70%, but the limitation was the small sample size and inclusion of only 10 positive samples. The 3 discordant STEC samples had Ct>31.8 on comparator test indicating that they are likely at the limit of detection.

***Table 3. Concordance between TrueMark™ Enteric Bacterial Select Panel I and composite comparator results for detection of Salmonella, Shigella/EIEC, Campylobacter and STEC in stool samples***

| | | Composite comparator result | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Salmonella* | | | *Shigella*/EIEC | | | *Campylobacter* | | | STEC | | |
| | | Positive | Negative | Total | Positive | Negative | Total | Positive | Negative | Total | Positive | Negative | Total |
| **TrueMark™ Enteric Bacterial Select Panel I** | Positive | 38 | 5 | 43 | 28 | 2 | 30 | 37 | 2 | 39 | 7 | 0 | 7 |
| | Negative | 0 | 102 | 102 | 0 | 115 | 115 | 1 | 105 | 106 | 3 | 135 | 138 |
| | Total | 38 | 107 | 145 | 28 | 117 | 145 | 38 | 107 | 145 | 10 | 135 | 145 |
| **Positive percent agreement** (95% CI) | | **100.00%** (90.82% - 100.00%) | | | **100.00%** (87.54% - 100.00%) | | | **97.37%** (86.50% - 99.53%) | | | **70.00%** (39.68% - 89.22%) | | |
| **Negative percent agreement** (95% CI) | | **95.33%** (89.52% - 97.99%) | | | **98.29%** (93.98% - 99.53%) | | | **98.13%** (93.44% - 99.49%) | | | **100.00%** (97.23% - 100.00%) | | |

## Conclusions

- **TrueMark™ Enteric Panels offer a reliable tool for identification of infections and co-infections of viral and bacterial pathogens for GI pathogen research.**
- **Use of molecular testing can provide rapid results, which is important for timely surveillance.**
- **High rate of co-infections with viral GI pathogens warrants further research into use of Ct values for pathogen prioritization and improved results interpretation.**

**For Research Use Only. Not for use in diagnostic procedures.**



ThermoFisher SCIENTIFIC
The world leader in serving science