R-Biopharm AG

# Evaluation of a novel multiplex real-time PCR assay for the detection of *Campylobacter* spp., *Salmonella* spp. and *Y. enterocolitica* in stool specimen

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## Objectives

Gastroenteritis is a major cause of morbidity and mortality worldwide, mostly in developing countries. Each year 2 billion diarrhoeal diseases occur worldwide. About 1.9 million children younger than 5 years die from diarrhoea each year, more than AIDS, malaria and measles combined. Gastrointestinal infections are caused by viruses, bacteria and intestinal parasites.[1,2] Common causes of bacterial diarrhoeal disease are *Campylobacter* spp., *Salmonella* spp. and *Y. enterocolitica*. Culture is the primary method and "the gold standard" for establishing the laboratory diagnosis of bacterial diarrhoea, but requires several days. We evaluated the RIDA®GENE Bacterial Stool Panel multiplex real-time PCR assay for the qualitative, direct detection and differentiation of *Campylobacter* spp., *Salmonella* spp. and *Y. enterocolitica* in human stool specimen.

**Picture 1:**
RIDA®GENE Bacterial Stool Panel multiplex real-time PCR

## Methods

Evaluation of the assay was performed on 294 prospective collected stool specimens from symptomatic patients' with signs and symptoms of acute gastroenteritis suspected of being caused by bacteria. Stool samples were homogenized and tested by culture (*Campylobacter*: Karmali Agar, Oxid; *Salmonella*: XLD Agar, BD; *Yersinia*: CIN-Agar, BD) followed by MALDI-TOF (MALDI Biotyper, Bruker, Daltonics) while an aliquot for real-time PCR was frozen until extraction. Nucleic acid extraction was performed with the Corbett X-tractor Gene.
Before extraction the stool samples were diluted 1:3 with water, intensely vortexed and centrifuged at 3,000 rpm for 30 sec.
An appropriate volume of the supernatant was transferred for extraction. The RIDA®GENE Bacterial Stool Panel multiplex real-time PCR was carried out on the Roche LightCycler® 480II (Fig. 1).
The RIDA®GENE Bacterial Stool Panel is a qualitative multiplex real-time PCR assay targeting the ttr-gene for *Salmonella* spp., 16s-rDNA for *Campylobacter* spp. and the ail-gene for *Y. enterocolitica* with fluorogenic target-specific hydrolysis probes (Fig. 2).
Samples with a Ct-value ≤ 45 are interpreted positive (Fig. 6, 7, 8).
An Internal Control DNA (ICD), which can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as a PCR inhibition control, ensures reliable results. RIDA®GENE Bacterial Stool Panel can be run on commonly used real-time PCR instruments such as LightCycler®480II, SmartCycler®, ABI 7500, Mx3005P or Rotor-Gene Q.

**Fig. 1:** Study design

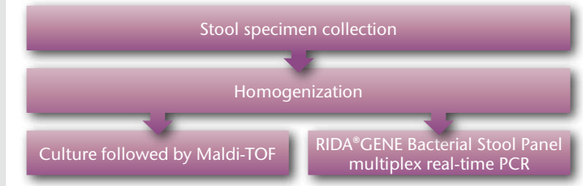



**Fig. 2:** Analyte target gene and detection channel overview

| Analyte | Target gene | Detection Channel LightCycler® 480II |
|---|---|---|
| *Salmonella* spp. | ttr | 465/510 |
| *Yersinia enterocolitica* | ail | 533/610 |
| *Campylobacter* spp. | 16s-rDNA | 618/660 |
| ICD | synthetic target DNA sequence | 533/580 |

**References:**
[1] World Gastroenterology Organisation Global Guidelines: Acute diarrhea in adults and children: a global perspective.
[2] UNICEF/WHO, Diarrhoea: Why children are still dying and what can be done, 2009.

## Results

Of the 294 stool specimens, 51 (17 %) specimens were positive for *Campylobacter* spp., 44 (15 %) were positive for *Salmonella* spp. and 11 (4 %) were positive for *Y. enterocolitica* by MALDI-TOF.
Using culture followed by MALDI-TOF as gold standard the sensitivity, specificity, PPV, NPV of the RIDA®GENE Bacterial Stool Panel were 100 %, 100 %, 100 % and 100 % for *Salmonella* spp., 100 %, 98.4 %, 94.4 % and 100 % for *Campylobacter* spp., and 100 %, 100 %, 100 % and 100 % for *Y. enterocolitica* (Fig. 3, 4, 5). Calculated PPV and NPV show the probability that a positive or negative result accurately indicates the presence or absence of infection, respectively. Calculation is not based on prevalence of the infection.
However, 3 samples negative by culture for *Campylobacter* spp. were detected positive by real-time PCR. The analytical sensitivity was determined with ≤ 5 DNA copies per reaction by dilution series of *Salmonella* spp., *Campylobacter* spp. and *Yersinia enterocolitica* standards.

No cross-reaction was found with a wide range of common pathogenic organisms of the intestinal tract (Data not shown).

**Fig. 3:** RIDA®GENE Bacterial Stool Panel (*Campylobacter spp.*) vs. Culture/MALDI-TOF

| | | Culture/MALDI-TOF + | Culture/MALDI-TOF - | Total |
|---|---|---|---|---|
| RIDA®GENE Bacterial Stool Panel | + | 51 | 3 | 54 |
| | - | 0 | 240 | 240 |
| | Total | 51 | 243 | 294 |

**Fig. 4:** RIDA®GENE Bacterial Stool Panel (*Salmonella spp.*) vs. Culture/MALDI-TOF

| | | Culture/MALDI-TOF + | Culture/MALDI-TOF - | Total |
|---|---|---|---|---|
| RIDA®GENE Bacterial Stool Panel | + | 44 | 0 | 44 |
| | - | 0 | 250 | 250 |
| | Total | 44 | 250 | 294 |

**Fig. 5:** RIDA®GENE Bacterial Stool Panel (*Y. enterocolitica*) vs. Culture/MALDI-TOF

| | | Culture/MALDI-TOF + | Culture/MALDI-TOF - | Total |
|---|---|---|---|---|
| RIDA®GENE Bacterial Stool Panel | + | 11 | 0 | 11 |
| | - | 0 | 283 | 283 |
| | Total | 11 | 283 | 294 |

**Fig. 6:** Example of *Campylobacter* spp. run on the LightCycler® 480II

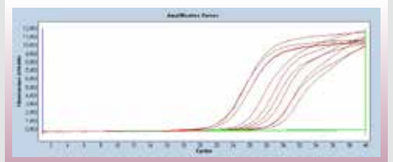


**Fig. 7:** Example of *Salmonella* spp. run on the LightCycler® 480II

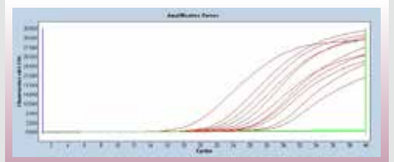
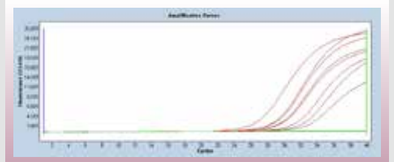


**Fig. 8:** Example of *Yersinia enterocolitica* run on the LightCycler® 480II



## Conclusion

The RIDA®GENE Bacterial Stool Panel is a rapid, high sensitive and specific assay and is an alternative for the conventional culture-based laboratory diagnosis of *Campylobacter* spp., *Salmonella* spp. and human pathogenic *Y. enterocolitica*.

Results are available in less than 2 hours.

An Internal Control DNA (ICD) ensures reliable results.

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