Evaluation of the new RIDA®GENE Gastro Panel for the direct detection of stool pathogens in comparison to routine diagnostic methods

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Background

According to the WHO, gastrointestinal infections which can be caused by bacteria, viruses and parasites are among the most frequently reported diseases worldwide with about 2 billion cases. Diarrheal disease globally results in the second common cause of child deaths among children under 5 years. Bacteria are responsible for the majority of the infections with Campylobacter species as one of the most common causes of bacterial diarrhea worldwide with 400 – 500 million reported cases annually. Additionally, Escherichia coli also ranks as the major infectious agent and moreover accounts for most of all bacterial caused traveler’s diarrhea. To a great extent, non-bacterial, enteric etiologies are caused by noroviruses and the CDC estimates that more than 21 million cases of acute gastroenteritis, 700,000 hospitalisations and 800 deaths are caused by norovirus infections each year in the United States. Furthermore, directly significant viral gastroenteritis cases are induced by Astrovirus, Adenovirus or Rotavirus, mainly affecting infants under the age of five. Parasitic gastrointestinal infections are mainly caused by the pathogens: protozoa Giardia lamblia, Cryptosporidium spp., Entamoeba histolytica and Dientamoeba fragilis. The rapid identification of these diarrhea causing agents with specific and sensitive laboratory methods is important for prompt and precise patient therapy as well as the identification of the infectious source.

The RIDA®GENE Gastro Panel allows the detection of the most common gastrointestinal pathogens as illustrated in Figure 1. In recent years, syndrome-based PCR panels were introduced with promising sensitivity and specificity. In this study, we evaluated the syndrome-based RIDA®GENE Gastro Panel PCR assays (R-Biopharm AG) for the simultaneous, direct detection of the most important gastrointestinal pathogens in stool specimens.

Material and Methods

The RIDA®GENE Gastro Panel is composed of multiple real-time PCR assays for the qualitative detection of 10 pathogens known to cause gastrointestinal infections (Figure 3). 350 prospectively collected fecal samples with signs and symptoms of acute gastrointestinal infection were tested with the RIDA®GENE Gastro Panel on the LightCycler® 480 (Roche). Results were compared to the phenotypical and molecular laboratory methods which are used in the routine microbiology laboratory for the detection of bacterial, viral and parasitic pathogens. These were as follows: selective bacterial culture media (bacterial pathogens), sorbent methods (Shigella spp., Salmonella spp., Staphylococcus spp., Vibrio spp., Enterobacteriaceae), ELISA assays, lateral flow assays (Viral pathogens, Campylobacter, parasites), MAC/VIP WM, real-time PCR, PCR based assays (Giardia spp., Entamoeba histolytica, Cryptosporidium) and the BD Max System (Entamoeba histolytica, RIDA®MAX Gastro Panel, both from Dianova). The PCR multiplex panel (Seegene Allplex™ Gastrointestinal Full Panel Assay) was used in case of discordant results. All assays were performed according to manufacturer’s instructions.

Results

In total, the 350 stool samples contained 521 bacterial stool pathogens (Salmonella spp.; Y. enterocolitica; Enteroinvasive E. coli; Campylobacter spp.; EHEC; STEC; EAEC; ETEC; Shigella spp.; EPEC), 25 viruses (bromovirus n=9), adenovirus n=6, rotavirus n=5, adenovirus n=3 and 25 parasites (Dientamoeba fragilis n=10), Giardia lamblia n=10, Cryptosporidium n=4) were also detected (Figure 1). Of the 350 stool specimens, 24 patients showed multiple infections. The sensitivities and specificities of the RIDA®GENE Gastro Panel were 100.0% and 98.4%, respectively for bacteria, 100% and 100% for viruses and 100% and 100% for parasites. For bacteria, one specimen culture positive for Salmonella spp. and one enamechin- sensitive positive for ETEC were negative in RIDA®GENE PCR assays, but also negative with all other PCR assays used in the study (BD MAX, Sargent Diagnostics). It might be assumed that these steel specimens contained pathogen contaminations before the detection limit of the PCR tests. Three specimens were PCR-positive for EPEC and one for Campylobacter, but showed a negative result for culture. Five specimens were adenovirus-positive with the RIDA®GENE Gastro Panel and negative with the reference methods (LiO). All these results were considered as false-negative. Nevertheless, in contrast to the reference methods used, the RIDA®GENE panel detects all adenovirus types and not only adenovirus 41/46. This might explain the few false-positive results with the RIDA®GENE Gastro Panel (Tables 1-5).

Conclusions

The RIDA®GENE Gastro Panel multiple real-time PCR assay showed sensitive and specific results for the detection of bacterial, viral and parasitic pathogens in stool specimens. The pathogen composition of the RIDA®GENE assay provides a high degree of flexibility for the user and allows syndrome-based detection of the most relevant gastrointestinal infections based on symptoms and duration of complaints as well as of a travel or food related patient history. The assays contain all required components including all FDA and CE IVD relevant controls.

Table 1: Pathogens detection from 350 stool samples using the RIDA®GENE Gastro Panel in comparison to reference methods

<table>
<thead>
<tr>
<th>Pathogen/Target</th>
<th>RIDA®GENE Gastro Panel</th>
<th>Reference Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter spp.</td>
<td>24</td>
<td>±</td>
</tr>
<tr>
<td>Enteropathogenic E. coli (EPEC)</td>
<td>6</td>
<td>±</td>
</tr>
<tr>
<td>Enteroaggregative E. coli (EAEC)</td>
<td>2</td>
<td>±</td>
</tr>
<tr>
<td>Enterotoxigenic E. coli (ETEC)</td>
<td>24</td>
<td>±</td>
</tr>
<tr>
<td>Enterohemorrhagic E. coli (EHEC)</td>
<td>1</td>
<td>±</td>
</tr>
<tr>
<td>Shigella spp./Entromimeicosis E. coli (BEC)</td>
<td>3</td>
<td>±</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>2</td>
<td>±</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>1</td>
<td>±</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>2</td>
<td>±</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>2</td>
<td>±</td>
</tr>
<tr>
<td>ETEC</td>
<td>1</td>
<td>±</td>
</tr>
<tr>
<td>EAEC</td>
<td>1</td>
<td>±</td>
</tr>
<tr>
<td>STEC</td>
<td>2</td>
<td>±</td>
</tr>
<tr>
<td>EPEC</td>
<td>1</td>
<td>±</td>
</tr>
<tr>
<td>C. difficile</td>
<td>1</td>
<td>±</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>1</td>
<td>±</td>
</tr>
<tr>
<td>EHEC</td>
<td>1</td>
<td>±</td>
</tr>
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</table>

Table 2: Performance of the RIDA®GENE Bacterial Stool Panel I (350 stool specimens)

<table>
<thead>
<tr>
<th>Method</th>
<th>RIDA®GENE Gastro Panel</th>
<th>Reference Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>100/100</td>
<td>96/96</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>100/100</td>
<td>96/96</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>100/100</td>
<td>96/96</td>
</tr>
</tbody>
</table>

Table 3: Performance of the RIDA®GENE Viral Stool Panel I (350 stool specimens)

<table>
<thead>
<tr>
<th>Method</th>
<th>RIDA®GENE Gastro Panel</th>
<th>Reference Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Norovirus</td>
<td>100/100</td>
<td>96/96</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>100/100</td>
<td>96/96</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>100/100</td>
<td>96/96</td>
</tr>
</tbody>
</table>

Figure 8: Pathogens detected (n=3) in 350 steel specimens.

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