RIDA®GENE Norovirus V
real-time PCR

Art. Nr.: PG1405V
100 Reaktionen

Für die in-vitro Diagnostik.

-20 °C
1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Norovirus V is a real-time RT-PCR for the qualitative detection of norovirus (Genogroup I and II) in human stool samples (1, 2). RIDA®GENE Norovirus V real-time RT-PCR can be used as a tool for the diagnosis of gastroenteritis caused by Noroviruses.

2. Explanation of the test

Noroviruses cause by far the most cases of non-bacterial gastroenteritis outbreaks (3, 4, 5). A gastroenteritis caused by norovirus is manifested by severe nausea, vomiting and diarrhea. Noroviruses are egested by stool and with the vomit. An airborne transmission through aerosols containing the virus is often the cause of a very rapid spreading in shared facilities (6, 7, 8).

Noroviruses belong to the family of *Caliciviridae*. Because of their morphology (small round structured virus (SRSV)), they can be distinguished from the traditional calicivirus. The SRSVs were named after the place of their isolation. Thus, the name Norwalk-like stood for all viruses which have been isolated during outbreaks of gastroenteritis. The name originated from the first SRSV isolation in the city of Norwalk, Ohio, in the US in 1972. Later, other isolates like Snow Mountain agent, Hawaii agent and Montgomery County agent were named in a similar way. Noroviruses are small, non-enveloped viruses with a single-stranded RNA (ssRNA). They can be grouped in 5 Genogroups with currently 29 genotypes and a multiplicity of clades. So far, human pathogens have only been described from Genogroup 1 (GGI) with 8 genotypes and from Genogroup 2 (GGII) with 17 genotypes. The remaining 4 genotypes are distributed between Genogroups 3 to 5.

3. Test principle

The RIDA®GENE Norovirus V real-time RT-PCR is a molecular diagnostic test for the detection of norovirus (Genogroup GGI and GGII) in human stool samples. The detection is done in a one step real-time RT-PCR format where the reverse transcription is followed by the PCR in the same reaction tube. The RNA from human stool samples is transcribed into cDNA by a reverse transcriptase. Gene fragments specific for norovirus GGI and GGII are subsequently amplified by real-time PCR. Probes which are labeled with a fluorescent dye and quencher hybridize to the multiplied amplicons if these are properly amplified. Reporter and quencher get
separated. The reporter emits a fluorescent signal which is detected by the optical
unit of a real-time PCR cycler. The fluorescence signal increases with the amount of
formed amplicons.

The norovirus-specific amplicon is measured at the FAM channel (522 nm). In each
PCR reaction the internal amplification control is co-amplified and detected to
determine possible RT-PCR-inhibitions. The internal amplification control is
measured at the VIC channel (553 nm).

4. Reagents provided

Tab.1: Reagents provided (Reagents provided in the kit are sufficient for 100 determinations)

<table>
<thead>
<tr>
<th>Kit code</th>
<th>Reagent</th>
<th>Amount</th>
<th>Lid color</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PP-Mix</td>
<td>1x</td>
<td>770 µl</td>
</tr>
<tr>
<td>2</td>
<td>Reaction Mix</td>
<td>2x</td>
<td>650 µl</td>
</tr>
<tr>
<td>3</td>
<td>Positive Control</td>
<td>1x</td>
<td>100 µl</td>
</tr>
<tr>
<td>4</td>
<td>Enzyme Mix</td>
<td>1x</td>
<td>65 µl</td>
</tr>
<tr>
<td>5</td>
<td>PCR Water</td>
<td>1x</td>
<td>500 µl</td>
</tr>
</tbody>
</table>

5. Storage instructions

- Protect all reagents from light and store at -20 °C. All reagents can be used until
  the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw reagents before using (e.g. in a refrigerator at 2 - 8 °C).
- Avoid thawing and freezing reagents several times (e.g. after the first thawing
  separate in aliquots and freeze immediately).
- During PCR preparation all the reagents should be stored cold in an appropriate way.
6. Additional equipment and materials required

- RNA-Extraction kit for stool samples (e.g. QIAGEN, QIAamp Viral RNA Mini Kit)
- real-time PCR Cycler with two detection channels for 522 nm and 553 nm:
  Applied Biosystems: ABI 7000, 7300, 7500, 7700, 7900 SDS, StepOne
  Eppendorf: ep realplex², ep realplex⁴
  Stratagene: Mx Serie
  QIAGEN: Rotor-Gene Q
- real-time PCR consumables (plates, tubes, foil)
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips

7. Precautions for users

- For in vitro diagnostic use only.
- RIDA®GENE Norovirus V is licensed by Roche. No further accessorical charges have to be paid.
- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- This test must only be performed by laboratory personnel trained in molecular biology methods.
- Strictly follow the working instructions.
- When handling samples, wear disposable gloves. After finishing the test, wash your hands.
- Do not smoke, eat or drink in areas where samples or test reagents are being used.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.

8. Test procedure

8.1 RNA-Extraction
For RNA extraction of human stool samples use a commercially available RNA isolation kit (e.g. QIAGEN, QIAamp Viral RNA Mini Kit). Extract viral RNA according to the manufacturer’s instructions.
We recommend to dilute the stool sample before extraction 1:10 with water. Vortex intensely. Centrifuge for 1 min at 12,000 rpm. According to the manufacturer’s instructions pipette this volume for the supernatant.

8.2 Master Mix preparation

Calculate the total number of RT-PCR reactions (sample and control reactions) needed. Positive control, negative control and extraction control are recommended for each run. The internal amplification control (inhibition control) is included in the Master Mix.

It is recommended to calculate an additional volume of 10 % to compensate imprecise pipetting (see Tab.2). Thaw, vortex and centrifuge the Reaction Mix, the PP-Mix and the PCR Water before using. Keep reagents appropriately cold during working step.

Tab.2: Calculation and pipetting example for 10 reactions of the Master Mix

<table>
<thead>
<tr>
<th>Master Mix components</th>
<th>Volume per reaction</th>
<th>10 reactions (10 % extra)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Mix</td>
<td>12.5 µl</td>
<td>137.5 µl</td>
</tr>
<tr>
<td>PP-Mix (Primer-Probe-Mix)</td>
<td>7.0 µl</td>
<td>77.0 µl</td>
</tr>
<tr>
<td>Enzyme Mix</td>
<td>0.6 µl</td>
<td>6.6 µl</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td><strong>20.1 µl</strong></td>
<td><strong>221.1 µl</strong></td>
</tr>
</tbody>
</table>

Mix the components of the Master Mix gently and spin down.

8.3 Preparation of the RT-PCR-Mix

Pipette 20 µl of the Master Mix in each reaction vial (tube or plate).

Negative control: Add 5 µl PCR Water as negative control to the pre-pipetted Master Mix.

Sample: Add 5 µl RNA-Extract to the pre-pipetted Master Mix.

Positive control: Add 5 µl positive control to the pre-pipetted Master Mix.

Cover tubes or plate. Spin down and place in the real-time PCR cycler. The RT-PCR reaction should be started according to the PCR Cycler Set up (see Tab.3).
8.4 Programming

Tab.3: PCR Cycler Set up

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse Transcription</td>
<td>15 min, 50 °C</td>
</tr>
<tr>
<td>Initial Denaturation</td>
<td>5 min, 95 °C</td>
</tr>
<tr>
<td>Cycles</td>
<td>45 Cycles</td>
</tr>
<tr>
<td>PCR Denaturation</td>
<td>15 sec, 95 °C</td>
</tr>
<tr>
<td>Annealing/Extension</td>
<td>30 sec, 55 °C</td>
</tr>
<tr>
<td>Detection</td>
<td>Target gene: FAM Channel 522 nm</td>
</tr>
<tr>
<td></td>
<td>Internal amplification control: VIC Channel 553 nm</td>
</tr>
<tr>
<td></td>
<td>Quencher: no fluorescence</td>
</tr>
<tr>
<td>Temperature Transition Rate</td>
<td>Maximum</td>
</tr>
<tr>
<td>Ramp Rate</td>
<td></td>
</tr>
</tbody>
</table>

9. Result interpretation

The analysis of the samples is done by the software of the used real-time PCR cycler according to the manufacturer`s instructions. Positive and negative controls have to show correct results (see Fig.1).

Fig.1: Correct run of the positive and negative control

The positive control has a concentration of $10^3$ copies/µl. In each PCR run it is used in a total amount of $5 \times 10^3$ copies and generates an amplification signal with a ct-value of 27 (+/- 1). With each 1:10 dilution the ct-value increases in 3.3.
The result interpretation is done according to Table 4.

Tab.4: Sample interpretation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Internal Amplification Control</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>+</td>
<td>Sample negative</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Sample positive</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>Sample positive, Competition</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>PCR inhibition</td>
</tr>
</tbody>
</table>

A sample is evaluated negative if the sample RNA shows no amplification signal in the detection system but the internal amplification control is positive. An inhibition of the PCR reaction can be excluded by the detection of the internal amplification control (see Fig.2).

A sample is evaluated positive if both the sample RNA and the internal amplification control show an amplification signal in the detection system.

A sample is evaluated positive if the sample RNA shows an amplification signal in the detection system but the internal amplification control is negative. The detection of the internal amplification control is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the internal amplification control.

A sample is evaluated invalid if both the sample RNA and the internal amplification control show no amplification signal in the detection system. The sample contained a RT-PCR inhibitor. The extracted sample needs to be further diluted (1:10) and re-amplified, or the isolation and purification of the sample have to be improved.
10. Test characteristics

10.1 Analytical sensitivity
The RIDA® GENE Norovirus V real-time RT-PCR has a detection limit of $\leq 50$ RNA copies (Fig.2).

Fig.2: Dilution series ($10^5$ – $10^1$ RNA copies / µl)

The detection limit of the whole procedure depends on the sample matrix, RNA-extraction and RNA-concentration.

10.2 Analytical specificity
The RIDA® GENE Norovirus V real-time RT-PCR is specific for Norovirus of the genogroups I and II in human samples. No cross-reaction could be detected for the following species:

Tab.5: Cross-reactivity testing

<table>
<thead>
<tr>
<th>Listeria monocytogenes</th>
<th>Escherichia coli</th>
<th>Escherichia coli O157/H7</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yersinia enterocolitica</td>
<td>Staphylococcus aureus</td>
<td>Shigella boydii</td>
<td>-</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>Campylobacter jejuni</td>
<td>Salmonella</td>
<td>-</td>
</tr>
</tbody>
</table>

11. Limitations of the method
The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
12. Literature


