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ORIGINAL ARTICLE

# Comparison of three multiplex real-time PCR assays for detection of enteric viruses in patients with diarrhea

Jari J. Hirvonen[1]

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## Abstract

In this study, the usability and performance of three commercially available multiplex real-time RT-PCR assays for the detection of major enteric viruses were investigated. In total, 481 fecal specimens were analyzed using the Allplex™ GI Virus Assay, the Rida®Gene Viral Stool Panel I, and the FTD Viral Gastroenteritis. The overall agreement between the assays was 99.9%. Despite convergent analytical performance, differences between the multiplex RT-PCR assays were apparent when considering their suitability for routine diagnostics.

**Keywords** Gastroenteritis · Enteric viruses · Diarrhea · Molecular diagnostics

## Introduction

Viral gastroenteritis is a major health problem across the world [1–3]. In developing countries, it is a crucial cause of morbidity and mortality and in developed countries, where mortality is low, morbidity and economic consequences are significant [1].

The most common viral pathogens known to cause human acute gastroenteritis are rotaviruses (RoV), human caliciviruses (HuCaV), including noroviruses (NoV) and sapoviruses (SaV), enteric adenoviruses (AdV), and human astroviruses (AsV) [4]. Traditionally, viral culture, electron microscopy, and latex agglutination tests have been used to detect these viral pathogens. In recent years, though, conventional and real-time reverse transcription (RT)-PCR protocols have been published to enhance the detection [5–10].

In this study, the usability and performance of three real-time multiplex RT-PCR assays, Allplex™ GI Virus Assay (Seegene Inc., Seoul, Korea) [10], Rida®Gene Viral Stool Panel I (R-biopharm AG, Darmstadt, Germany), and FTD Viral Gastroenteritis (Fast-Track Diagnostics Ltd., Qui-Si-Sana Seafront, Malta) [9], for the detection and differentiation of the major diarrheagenic viruses was investigated.

✉ Jari J. Hirvonen
hirvonen.j.jari@gmail.com

[1] Fimlab Laboratories, Arvo Ylpön katu 4, FI-33520 Tampere, Finland

## Materials and methods

A total of 481 clinical stool specimens, one specimen per patient, were prospectively collected from patients presenting with acute diarrhea during 2015 and 2017 in the region of Pirkanmaa and Kanta-Häme Hospital District, and Central Finland Health Care District. Specimens were stored at 4 °C and tested within 48 h after being receiving in the laboratory. An aliquot was stored at − 70 °C for later testing in case of result discrepancy.

To minimize variation in the results caused by the extraction method, nucleic acids were extracted using only one automated platform, the *m*2000sp (Abbott, Abbott Park, IL, USA) [11]. It should be noticed though that the extraction platform was not validated by any of the assay manufactures. Three hundred microlitres of liquid feces were pipetted or approximately a same amount of semi-solid feces swabbed to 2.5 mL Tris-EDTA buffer tubes and mixed thoroughly. Then, modified Sample Prep Kit for co-purification of DNA and RNA (m2000-RNA-LL-200-70-V71708 Vers. 1.03) was used for the extraction. Processing volume was 200 μL and elution volume 70 μL. After nucleic acid extraction all eluates were analyzed directly or frozen at − 70 °C until analysis.

Nucleic acid amplification and target detection was performed using the Allplex™ GI Virus Assay (Seegene Inc., Seoul, Korea), the Rida®Gene Viral Stool Panel I (R-biopharm AG, Darmstadt, Germany), and the FTD Viral Gastroenteritis (Fast-Track Diagnostics Ltd., Qui-Si-Sana Seafront, Malta) with Bio-Rad CFX96 real-time PCR

instrument (Bio-Rad Laboratories Inc., Hercules, CA, USA). Allplex™ GI Virus Assay and FTD Viral Gastroenteritis were validated on the CFX96 platform but Rida®Gene Viral Stool Panel I was validated on LightCycler 480II (Roche) and Stratagene Mx3005P (Agilent) only. However, suitable qPCR setup was able to be set also on Bio-Rad CFX96 for the Rida®Gene Viral Stool Panel I.

According to the data provided by the manufacturers, the Allplex™ GI Virus Assay and the FTD Viral Gastroenteritis allow the detection and differentiation of six virus targets (AdV, AsV, NoV GI and NoV GII, RoV, and SaV), whereas the Rida®Gene Viral Stool Panel I detects and differentiates four virus targets (AdV, AsV, NoV [including genogroups I and II], and RoV).

All assays were performed according to the manufacturer's instructions. Shortly, one-step real-time RT-PCR was performed on 5 μL of sample extract with Allplex™ GI Virus Assay master mix, and on 5 μL of sample extract with Rida®Gene Viral Stool Panel I master mix, both in 25 μL reaction volume. Contrary to Allplex and Rida®Gene assays, FTD Viral Gastroenteritis consisted of reagents for three different master mixes each detecting three different target set. For FTD Viral Gastroenteritis, 10 μL of sample extract were mixed with each master mix in 25 μL reaction volume in three separate PCR tubes. All three RT-PCRs were done on the exact same eluate. Kit-specific internal controls (IC) were added, according to the manufacturer's instructions, during extraction for Allplex and Rida®Gene assays to check the entire process from nucleic acid extraction to PCR, but not for FTD Viral Gastroenteritis as the IC were included in master mix 1.

Specimens were defined as true positive or negative when at least two of the three assays yielded a similar result. Since the Rida®Gene Viral Stool Panel I did not include primers and probes for the detection of SaV, the Rida®Gene Sapovirus RT-PCR assay was used when SaV-positive result was observed by Allplex or FTD assay. Fisher's exact test was used to determine the statistical significance of the differences between the various test methods.

## Results

The age of patient ranged from 2 to 91 years (median age 50 years). Of the 481 diarrheal stool specimens, all three assays detected 167 (34.7%) samples as positive for NoV (GII being more prevalent [93.3%] than GI [6.7%]), 22 (4.6%) samples positive for SaV, four (0.8%) samples for RoV, three (0.6%) samples for AsV, and two (0.4%) samples for AdV (Fig. 1). In addition, three discordant results were seen. Of these, one specimen yielded positive AdV result with FTD Viral Gastroenteritis and Rida®Gene Viral Stool Panel I assays but not with Allplex GE Viral Assay, one specimen yielded positive AdV result with FTD Viral Gastroenteritis only, and one specimen yielded positive AsV with Rida®Gene Viral Stool Panel I only. Coinfections were detected in four (2.1%) of the 195 true positive specimens. Of these, two were NoV GI and SaV coinfections, and two RoV and AsV coinfections.

The overall agreement rates between Allplex, FTD, and Rida®Gene Viral Stool Panel I assays were 100% for NoV, 100% for RoV, 99.8% for AsV, and 99.6% for AdV. The overall agreement rate between Allplex and FTD assays for SaV was 100%, and positive agreement rate between Allplex, FTD, and Rida®Gene Sapovirus assays was 100%.

Assay (RT-PCR) run times were as follows: 2 h and 44 min for the Allplex GI Virus Assay, 1 h and 31 min for the RidaGene Viral Stool Panel I, and 1 h and 23 min for the FTD Viral Gastroenteritis assay (Table 1). Calculated hands-on time for the preparation of each assay (for full 96-well qPCR plate) was nearly identical, approximately 20 min (Table 1). Total turnaround time for processing 96 samples, with one Abbott *m*2000sp nucleic acid extraction platform and one Bio-Rad CFX96 real-time PCR instrument, was 4.5 h for

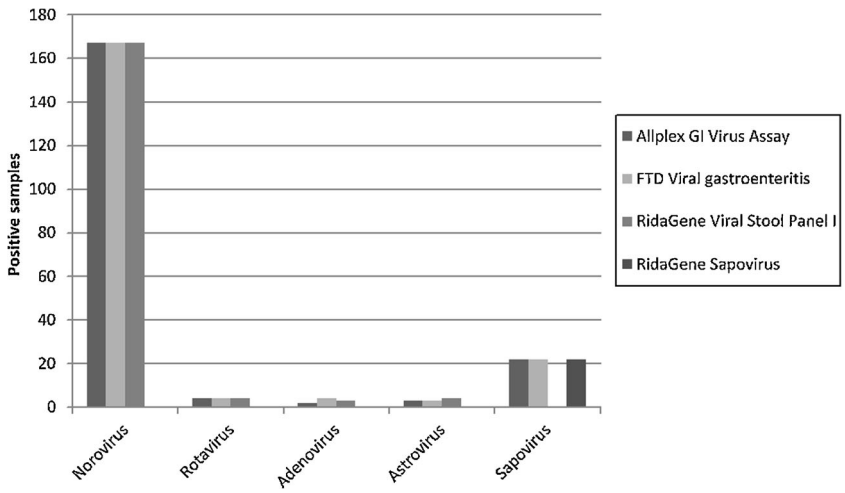


**Fig. 1** Overview of positives results of Allplex GI Virus Assay, FTD Viral Gastroenteritis, RidaGene Viral Stool Panel, and RidaGene Sapovirus assays

**Table 1** Assay run times, hands-on times and total turnaround times for Allplex™ GI Virus Assay, Rida®Gene Viral Stool Panel I, and FTD Viral Gastroenteritis

| Test | Assay run time for 30 samples[a] (min) | Assay run time for 94 samples[b] (min) | Hands-on time for one full plate assay run (min) | Total turnaround time[c] for 30 samples[a] (hours) | Total turnaround time[d] for 94 samples[b] (hours) |
|---|---|---|---|---|---|
| Allplex GI Virus Assay | 164 | 164 | 20 | 4.5 | 5.5 |
| RidaGene Viral Stool Panel I | 91 | 91 | 20 | 3.5 | 4.5 |
| FTD Viral Gastroenteritis | 83 | 332 | 20 | 3.5 | 9.5 |

[a] The maximum number of patient samples per one 96-well qPCR platform assay run (controls excluded) with FTD Viral Gastroenteritis

[b] The maximum number of patient samples per one 96-well qPCR platform assay run (controls excluded) with Allplex GI Virus Assay and RidaGene Viral Stool Panel I

[c] Extraction and purification of 30 samples with Abbott *m*2000sp takes approximately 100 min. Only one *m*2000sp and one Bio-Rad CFX96 real-time PCR instrument was used for the time measurement

[d] Extraction and purification of 94 samples using Abbott *m*2000sp takes approximately 150 min. Only one *m*2000sp and one Bio-Rad CFX96 real-time PCR instrument was used for the time measurement

the RidaGene Viral Stool Panel I, 5.5 h for the Allplex GI Virus Assay, and 9.5 h for the FTD Viral Gastroenteritis assay.

## Discussion

Here, the utility of three commercially available real-time multiplex RT-PCR assays (Allplex GI Virus Assay, Rida®Gene Viral Stool Panel I, and FTD Viral Gastroenteritis assay) for detection of NoV, RoV, AdV, AsV, and SaV was assessed. By minimizing technical, extraction and qPCR instrument-related variation, three discordant results between the assays were observed. Of these, one specimen was negative with Allplex assay being, however, AdV positive with FTD and Rida®Gene assays, one was AdV positive with FTD assay only, and one specimen was AsV positive with Rida®Gene Viral Stool Panel I only. The Ct values of the discrepant results varied from 32 to 40, indicating that these were low positive samples. Although the main limitation of this study was a small number of positive findings other than NoV and SaV, similar assay performance results have been also published in previous studies [10, 12].

During the study period, the most common finding was NoV, particularly viruses belonging to the GII. Most of these cases were outbreaks in hospitals and nursing homes. Even though NoV is recognized as the most common cause of gastroenteritis [1, 13–16], other enteric viruses, such as SaV, have also shown to play a role in such infections [17–19]. Here, one water-related outbreak was observed, in which the only causative agent was SaV. In Finland, SaV seems to be detected most commonly from waterborne outbreaks, where failures in the drinking water distribution system cause gastroenteritis [4, 20]. The SaV outbreak was rapidly noticed with the Allplex GI Virus Assay and the FTD Viral Gastroenteritis assay but not with the Rida®Gene Viral Stool Panel I assay, since the SaV detection is not included into the test. After a separate assay run with the Rida®Gene Sapovirus assay, concordant positive results were seen. Hence, a major disadvantage of the RidaGene Viral Stool Panel I is that, contrary to the Allplex and FTD assays, it lacks the detection of SaV and differentiation of NoV genogroups I and II. Therefore, the usage of the RidaGene Viral Stool Panel I in gastroenteritis outbreaks and epidemic situations is limited.

RoV, which is still in some countries the leading cause of acute gastroenteritis among children [21–23], was detected from a few specimens (0.6%) only. This result is explained by the universal RoV vaccination which was introduced into National Immunization Programme (NIP) of Finland in 2009. As concluded by Hemming et al., RoV vaccination has led to a major reduction of RoV gastroenteritis cases and a high coverage of RoV vaccination will maintain RoV activity at a low level, although, it will not eliminate wild-type RoV circulation [24, 25]. Similar to RoV, AdV, and AsV findings were sparse in this study.

Differences between the multiplex RT-PCR assays became more apparent when considering their suitability for routine diagnostics. The FTD Viral Gastroenteritis assay enabled the fastest analysis time. However, since the FTD assay consists of three different PCR assay mixes, two mixes for three different virus targets in each and one single-plex mix, one assay run requires three separate qPCR wells or tubes. Due to this, the analytical capacity of the assay is limited, as compared to Allplex and Rida®Gene assays, as only 30 samples (excluding controls) can be analyzed in one 96-well qPCR platform. However, this limitation can be overcome by using several PCR instruments or by using 384-well qPCR platform. Moreover, since IC is included only in assay mix 1 together with NoV targets, the FTD assay mix 2 (AdV, AsV, and RoV) and assay mix 3 (SaV) cannot be used alone, in accordance with the clinical laboratory standards, although this feature would be otherwise beneficial if targets are needed to be screened individually. Unlike FTD Viral Gastroenteritis assay, Allplex GI Virus Assay proved to be a true multiplex test enabling the detection of multiple (six) viral targets (and IC)

in one reaction. The disadvantage of the Allplex GI Virus Assay is, however, that the assay (qPCR) run time is approximately 2 h and 40 min which is 80 min slower as compared with the other two assays used in this study.

In conclusion, all commercial assays investigated here (Allplex GI Virus Assay, RidaGene Viral Stool Panel I, and FTD Viral Gastroenteritis assay) proved to be fast and accurate methods for detection of most common viruses causing gastroenteritis. Although each assay had its own advantages and disadvantages, which each laboratory must take into account when considering their usage in routine practise, all three multiplex assays are compatible for a wide variety of molecular platforms and, thus, can be readily implemented in the clinical microbiology laboratory. It should be noticed though that the assay performance and total turnaround time is dependent also on the nucleic acid extraction method used. Here, the Abbott *m*2000sp system was successfully used with consistent amplification results.

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## Compliance with ethical standards

**Conflict of interest** The author reports no conflict of interests.

**Ethical approval** This article does not contain any studies with human participants or animals performed by the author. There were no requirements for informed consent.

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