RIDA®GENE Sapovirus – A new qualitative real-time PCR Assay for the detection of Sapovirus in human stool samples

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Objectives

Noro-, rota- and adenovirus infections are well-known major causes of diarrhea. Whereas those viruses are often identified as cause of gastrointestinal infections, many patient samples remain without diagnosis. Sapoviruses belong to the family of *Caliciviridae* and are also major causative agents of gastroenteritis worldwide. Clinical symptoms are similar to those of norovirus infections including diarrhea and vomiting and hence the pathogenic cause of such an infection is difficult to determine on the basis of clinical features alone. Until today, little epidemiologic studies were conducted and sapovirus was rarely detected due to lack of sensitive detection methods.

The development of a real-time PCR assay displays a major advantage in detection of gastroenteritis-causing sapovirus infections.

Picture 1: RIDA®GENE Sapovirus
Art. No. PG1605

Methods

RIDA®GENE Sapovirus is a qualitative and quantitative assay targeting the ORF1 region of Sapovirus with fluorogenic target-specific hydrolysis probes.
Overall, 90 stool samples were retrospectively analysed with the RIDA®GENE Sapovirus real-time PCR assay and compared to the in-house reference end-point real-time PCR method of the CNR.

30 samples were known Sapovirus-positive samples and 60 samples were Sapovirus-negative, of which 10 were positive for norovirus, rotavirus, adenovirus, astrovirus and other viruses. Stool samples were extracted with the NucliSENS®easyMag™ according to CNR standard protocol (Figure 1). Samples positive for Sapovirus were subsequently genotyped by the CNR.

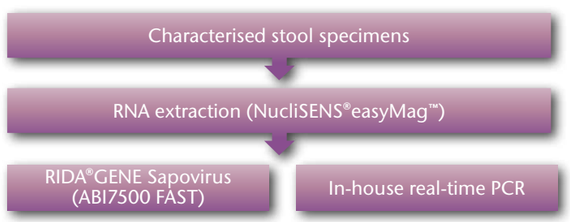

Figure 1: Study design

Results

All 30 positive Sapovirus samples were correctly identified by the RIDA®GENE Sapovirus real-time PCR assay (Table 1).
Of the 60 negative stool samples, 56 were in concordance with the results from the in house real-time PCR (Table 1), whereas 4 samples showed inhibition and will be retested.
Overall, 86 tested samples (97.5 %) showed concordant results with both real-time PCR methods, where 30 samples were found to be positive and 56 samples were found to be negative (Table 1). The positive, negative and overall agreement was 100 %, respectively (Table 2). Of the 30 sapovirus-positive samples, genotyping was retrospectively performed (Table 3).
Here, four different genotypes were identified: GI.1, GI.2, GII.1 and OH08021/2008/JP.
20 samples were of the GI.2 genotype whereas two samples of each of the other genotypes were identified. Four samples were non-typable due to lack of sufficient material.

Table 1: In-house real-time PCR vs. RIDA®GENE Sapovirus real-time PCR

		In-house real-time PCR		
		+	-	Total
RIDA®GENE Sapovirus	+	30	0	30
	-	0	56	56
	Total	30	56	86

Table 2: Clinical performance of the RIDA®GENE Sapovirus real-time PCR assay

Positive agreement: 100 %
Negative agreement: 100 %
Overall agreement: 100 %

Table 3: Analytical reactivity of the RIDA®GENE Sapovirus real-time PCR assay

Sapovirus Genotypes				
GI.1	GI.2	GII.1	OH08021/2008/JP	Non typable
2	20	2	2	4

[1] *Chiba S et al.* Sapporo Virus: History and recent findings. J. Infect. Dis. 2000, 181(Suppl 2): S303-S308.

Conclusion

The RIDA®GENE Sapovirus real-time PCR assay shows good correlation with an established in-house real-time PCR method from the French reference center for viral gastroenteric pathogens. Four of 60 negative samples showed inhibition and will be re-analysed with the RIDA®GENE Sapovirus real-time PCR assay. The new RIDA®GENE Sapovirus assay proved to be a sensitive and specific real-time PCR assay for the diagnosis of sapovirus infections. The RIDA®GENE Sapovirus real-time PCR assay detects different sapovirus genotypes including GI.1, GI.2, and GII.1.

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