Evaluation of VIASURE real-time PCR assays for detection of rotavirus and norovirus GI and GII in fecal samples

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INTRODUCTION

Rotaviruses (RVs) and noroviruses (NoVs) are the main etiological agents of nonbacterial acute gastroenteritis (AGE) in both children and adults. They can cause nosocomial infections in healthcare settings, particularly in vulnerable hospitalized patients. A rapid and sensitive detection is crucial to accurately diagnose these infections and to implement control measures to avoid their transmission. The sensitivity and specificity of the VIASURE Rotavirus assay was compared with those of the Rotavirus-Adenovirus immunochromatographic (ICG) test (Certest Biotec), an in-house conventional RT-PCR for rotavirus detection and the RIDA®GENE Viral Stool Panel II real-time RT-PCR (R-Biopharm AG). The performance of the VIASURE Norovirus GI and GII assays was also compared with an in-house conventional RT-PCR for norovirus detection and the RIDA®GENE Norovirus I & II real-time RT-PCR (R-Biopharm AG).

OBJECTIVE

To evaluate the performance characteristics of the newly launched tests “VIASURE Rotavirus Real Time PCR Detection Kit”, the “VIASURE Norovirus GI Real Time PCR Detection Kit” and the “VIASURE NoV GII Real Time PCR Detection Kit” (Certest Biotec, Spain) for their diagnostic application in a clinical laboratory.

MATERIAL AND METHODS

Fecal samples from children and adults with AGE were collected and diluted in PBS to prepare 10% fecal extracts. The presence of RV was tested in 210 samples and NoV GI and GII were analyzed in 181 samples. RNA was extracted from 200 µl of fecal suspensions by using the VIASURE RNA/DNA Extraction Kit (Certest Biotec). The immunochromatographic (ICG) test for rapid RV detection was performed following the manufacturers instructions. Conventional RV RT-PCR reactions for VP7, VP4 and/or VP6 viral genes were carried out according to the protocols described by the European Rotavirus Network (http://www.eurorota.net/). A conventional NoV RT-PCR was performed using primers JV12/JV13 targeting the polymerase gene and eventual capsid gene amplification. The real-time RT-PCR assays evaluated in this study were performed according to the manufacturer´s instructions, using the StepOne real-time PCR equipment (Applied Biosystems) for the VIASURE kits and the ABI 7500 real-time PCR System (Applied Biosystems) for the RIDAGENE kits. Samples with a Ct value ≤ 35 were considered positive, and Ct values of ≤ 40 and ≤ 45 were evaluated.

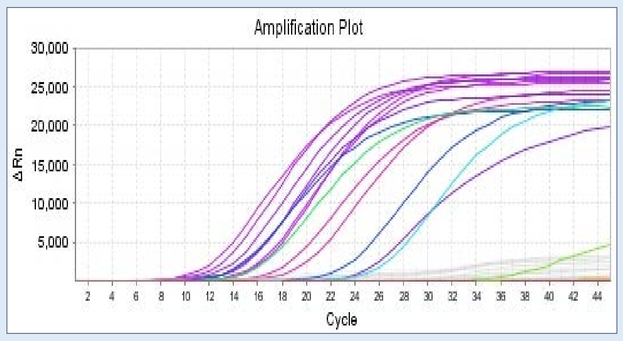

Fig. 1. Analysis of fecal samples for rotavirus by the VIASURE Rotavirus Real Time PCR Detection Kit. Graph was obtained with the StepOne software.

RESULTS

Regarding RV detection, ICG detected the presence of RV in 31.7% (72/227) samples and end-point RT-PCR in 32.2 (73/227), whereas both the VIASURE Rotavirus real time PCR assay and the RIDAGENE Viral Stool Panel II real-time RT-PCR detected 39.2% (89/227) positive specimens when considering a Ct value of ≤35. NoV GI and GII were detected by conventional RT-PCR in 10.7% (23/214) and in 32.7% (70/214) samples, respectively. The analysis of the same specimens by the VIASURE real-time assays yielded 11.2% (24/214) NoV GI-positive and 34.6% (74/214) NoV GII-positive samples when considering a Ct ≤35, whereas the RIDAGENE tests yielded 11.2% (24/214) NoV GI-positive and 33.6% (72/214) NoV GII-positive results. As shown in the tables, when considering higher Ct values the number of positive samples increased. The VIASURE Rotavirus assay detected P[4] and P[8] VP4 genotypes and G1, G2, G3, G6, G9, G10 and G12 VP7 genotypes. The VIASURE Norovirus GI and GII assays detected the following genotypes: GI.1, GI.2, GI.3, GI.4, GI.5, GI.7, GI.9, GI.Pb, GII.1, GII.2, GII.4 (5 variants), GII.6, GII.7, GII.17, GII.21 and GII.Pg.

Rotavirus detection:

	ICG	End-point RT-PCR	VIASURE			RIDAGENE		
			Ct 35	Ct 40	Ct 45	Ct 35	Ct 40	Ct 45
POSITIVE	72	73	89	116	116	89	113	114
NEGATIVE	155	154	138	111	111	138	114	113
TOTAL	227	227	227	227	227	227	227	227
% POSITIVE	31.7	32.2	39.2	51.1	51.1	39.2	49.8	50.2

Norovirus GI detection:

	VIASURE			RIDAGENE			End-point RT-PCR
	Ct 35	Ct 40	Ct 45	Ct 35	Ct 40	Ct 45	
POSITIVE	24	32	33	24	30	30	23
NEGATIVE	190	182	181	190	184	184	191
TOTAL	214	214	214	214	214	214	214
% POSITIVE	11.2	15.0	15.4	11.2	14.0	14.0	10.7

Norovirus GII detection:

	VIASURE			RIDAGENE			End-point RT-PCR
	Ct 35	Ct 40	Ct 45	Ct 35	Ct 40	Ct 45	
POSITIVE	74	78	78	72	76	76	70
NEGATIVE	140	136	136	142	138	138	144
TOTAL	214	214	214	214	214	214	214
% POSITIVE	34,6	36,4	36,4	33,6	35,5	35,5	32,7

Comparison of the performance of the VIASURE and the RIDAGENE assays

			VIASURE					
			ROTAVIRUS		NOROVIRUS GI		NOROVIRUS GII	
			POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
RIDAGENE	Ct ≤ 35	POSITIVE	89	0	24	0	72	0
		NEGATIVE	0	138	0	190	2	140
		% AGREEMENT	100 (K=1)		100 (k=1)		99,1 (k=0,98)	
	Ct ≤ 40	POSITIVE	113	0	30	0	76	0
		NEGATIVE	3	111	2	182	2	136
		% AGREEMENT	98,7 (K=0,97)		99,1 (k=0,96)		99,1 (k=0,98)	
	Ct ≤ 45	POSITIVE	114	0	30	0	76	0
		NEGATIVE	2	111	3	181	2	136
		% AGREEMENT	99,1 (K=0,98)		98,6 (k=0,94)		99,1 (k=0,98)	

Performance characteristics of the VIASURE assays

VIASURE		SENSITIVITY* ± 95% CI	SPECIFICITY** ± 95% CI	PPV ± 95% CI	NPV ± 95% CI
Rotavirus	Ct ≤ 35	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	Ct ≤ 40	100 ± 0	98,2 ± 1,7	98,3 ± 1,6	100 ± 0
	Ct ≤ 45	100 ± 0	99,1 ± 1,2	99,1 ± 1,2	100 ± 0
Norovirus GI	Ct ≤ 35	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	Ct ≤ 40	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	Ct ≤ 45	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Norovirus GII	Ct ≤ 35	100 ± 0	97,8 ± 1,9	96,2 ± 2,5	100 ± 0
	Ct ≤ 40	100 ± 0	97,8 ± 1,9	96,2 ± 2,5	100 ± 0
	Ct ≤ 45	100 ± 0	97,8 ± 1,9	96,2 ± 2,5	100 ± 0

* Sensitivity was referred to the RIDAGENE assay. ** Specificity was assayed by sequencing PCR products

CONCLUSIONS

The sensitivity of the VIASURE Rotavirus Real Time PCR assay increases those of the ICG assay and the end-point RT-PCR. Rotavirus was detected by both evaluated real-time RT-PCR assays with a total agreement rate above 98.7%. Norovirus GI and GII were detected by the VIASURE and RIDAGENE assays with a total agreement of >98.6% and 99.1%, respectively. The VIASURE tests are fast, easy to use, and show high sensitivity and specificity values, making them suitable for diagnostic purposes.