Speeding up Norovirus Diagnostics: The RIDA®QUICK Norovirus Detection Kit

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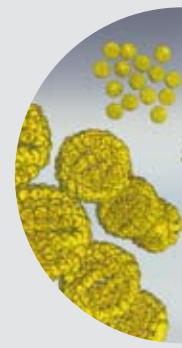
Objectives

Noroviruses are commonly associated with large outbreaks in recreational or institutional settings. They are highly infective and spread from person to person easily. An outbreak of norovirus infections not only results in a health burden for the respective individuals living or working in such settings but also in a true financial burden for the respective institution. Current approaches such as the PCR detection of the viral genome and the use of sensitive ELISA test kits still require several hours to confirm a suspicion of norovirus infection.

In order to initiate appropriate hygiene measures efficiently, however, information about the possible causes for the enteritic symptoms is required as quickly as possible. We here describe the development of an assay format that will detect norovirus in less than 20 minutes.

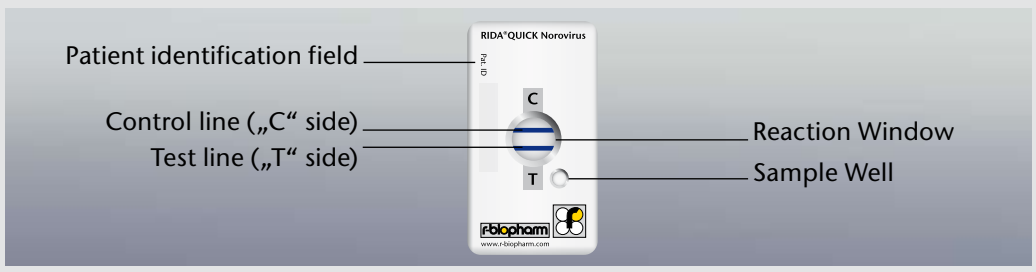
The RIDA®QUICK Norovirus Assay is a flow through enzyme linked immunoassay based solely on the use of virus specific but genotype cross-reactive monoclonal antibodies for the detection of norovirus.



Methods and Results

Device description and functionality

Fig. 1



Monoclonal antibodies raised against virus-like particles (VLPs) or against capsid protein preparations of noro-viruses from various genogroups and -types are bound to a filter membrane. The membrane is embedded in a respective device allowing sample application and further processing. Stool sample dilutions are incubated with the conjugate mix and subsequently applied to the membrane using the sample well. After migration of the suspension through the membrane, the reaction window is used for the following washing and development steps. Final step is the addition of the substrate to the reaction window. Results are recorded within 3 minutes.

Fig. 2

A serial dilution of a norovirus containing stool specimen was applied to the described device. The results are illustrated in Fig 2. The intended use of the device is the qualitative detection of norovirus in stool samples. However, dose dependant reactivity can be observed proving the specificity of the reaction.

Precision

Sample ID	Replicate	Day 1; 1. shift			Day 1; 2. shift			Day 2; 1. shift			Day 2; 2. shift			Day 3; 1. shift			Day 3; 2. shift			Agree-ment
		Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3	
241008-1 neg	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%
241008-2 neg	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%
VI1125-mpr	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
1-VI1125-lpr	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
2-VI1125-lpr	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%

Table 1:

Precision of the assay was assessed using 3 positive stool samples with medium and low virus load respectively. Two negative stool samples were added as control specimen. Three operators determined the infection status in three different labs on three consecutive days with two runs per day. As can be seen convincingly from table 1, the results are in absolute agreement with each other, demonstrating that the assay renders reproducible results with excellent precision.

Cross Reactivity

No cross-reactivity with the usual causes for gastroenteritis was observed as well as no interference with substances commonly used to treat the symptoms of gastrointestinal diseases.

Clinical Performance

The RIDA®QUICK Norovirus feasibility study was run using recombinant VLPs from a series of norovirus genotypes. Recombinant norovirus capsid protein coded by the ORF2 of the norovirus genome was expressed in infected insect cells. The purified VLPs from the following norovirus genogroups and –types were tested during the assay development: GI.1; GI.3; GII.1; GII.2; GII.3; GII.4. Recombinant baculovirus used for heterologous protein expression was kindly provided by Kim Green (NIAID; Bethesda MD).

RIDA®QUICK vs. rt RT-PCR [1]

		Realtime RT-PCR		
RIDA®QUICK		Dis +	Dis -	Total
Test +		49	3	52
Test -		8	53	61
Total		57	56	113
		95% confidence interval		
		lower	upper	
Sens:	86,0%	84,9%	86,6%	
Spec:	94,6%	93,5%	95,3%	
PPV:	94,2%	93,0%	94,9%	
NPV:	86,9%	85,9%	87,5%	
Prev:	50,4%	50,0%	50,9%	
Efficiency:	90,3%	89,7%	90,6%	

Table 2: RIDA®QUICK Norovirus vs. rt RT-PCR

RIDASCREEN® vs. rt RT-PCR

		Realtime RT-PCR		
RIDASCREEN®		Dis +	Dis -	Total
Test +		43	1	44
Test -		13	55	68
Total		56	56	112
		95% confidence interval		
		lower	upper	
Sens:	76,8%	75,7%	77,5%	
Spec:	98,2%	97,0%	98,8%	
PPV:	97,7%	96,2%	98,4%	
NPV:	80,9%	80,0%	81,5%	
Prev:	50,0%	49,6%	50,4%	
Efficiency:	87,5%	86,9%	87,8%	

Table 3: RIDASCREEN® Norovirus 3rd Gen. vs. rt RT-PCR

Conclusion

The RIDA®QUICK Norovirus detection assay opens a new dimension for the rapid analysis of compromised specimen. The assay can be run without the need of sophisticated laboratory equipment. It reliably renders results in approximately 20 minutes. Thus the assay is a valuable device for the sensitive detection of this hazardous health burden and it is an important tool for the prevention of gastrointestinal outbreaks in common settings where timely reactions are essential.

[1] **References:** Hoehne M, Schreier E: Detection of Norovirus genogroup I and II by multiplex real-time RT-PCR using a 3'-minor groove binder-DNA probe. BMC Infectious Diseases 2006, 6: 69

For research use only. The RIDA®QUICK Norovirus kit is not intended for use in diagnostic procedures.

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