R-Biopharm AG

RIDA®QUICK Norovirus: a New Dimension for Norovirus Detection

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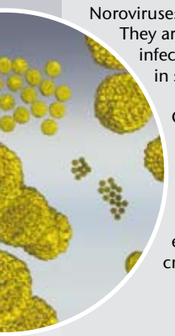
Objectives



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

Monoclonal antibodies raised against VLPs or against capsid protein preparations of noroviruses 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.
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 infected samples. However, dose dependant reactivity can be observed proving the specificity of the reaction.

Device description and functionality

Fig. 1

Fig. 2

Precision

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. The results are in absolute agreement with each other, demonstrating that the assay renders reproducible results with excellent precision.

Cross Reactivity

The antibodies used for the assay were tested for cross-reactivity against usual causes for gastroenteritis (a total of 64 viruses, bacteria or parasites). *Table 1* shows a list of the most relevant pathogens tested. No cross-reactivity was observed as well as no interference with substances commonly used to treat the symptoms of gastrointestinal diseases.

Pathogen	Sample Type	Result
Bacteria	**fecal spec.**	**neg.**
Citrobacter freundii	Culture	neg.
Listeria innocua	Culture	neg.
Salmonella Agona	Isolate	neg.
Samonella choleraesuis	Culture	neg.
Salmonella enteritidis sample	dil. fecal spec.	neg.
Salmonella infantis	Isolate	neg.
Salmonella Ohio	Isolate	neg.
Salmonella typhimurium	Isolate	neg.
Shigella flexneri	Culture	neg.
Shigella sonnei	Culture	neg.
E. coli (O157:H-)	Isolate	neg.
E. coli (O111:H-)	Isolate	neg.
E. coli (O26:H11)	Isolate	neg.
Clostridium difficile	Culture	neg.
Campylobacter sample	dil. fecal spec.	neg.

Pathogen	Sample Type	Result
Virus	**fecal spec.**	**neg.**
Adenovirus	cultrure supern.	neg.
Astrovirus	fecal spec.	neg.
Enterovirus	fecal spec.	neg.
Rotavirus	culture supern.	neg.
Sapovirus	fecal spec.	neg.
Parasites	**fecal spec.**	**neg.**
Cryptosporidium parvum >107	Culture	neg.
Giardia lamblia sample	dil. fecal spec.	neg.
Ent. histolytica	dil. fecal spec.	neg.

Table 1

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 baculoviruses used for heterologous protein expression were kindly provided by Kim Green (NIAID; Bethesda MD). Preliminary results confirm the detection of noroviruses of these genogroups and -types and additionally GII.5; GII.6; GII.7; GII.8; GII.10; GII.12; GII.13; GII.14 in fecal specimen.

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

Clinical performance of the assay was assessed using 113 stored stool specimens from the norovirus outbreak season 2007/2008. A realtime RT-PCR for norovirus based on the primer published by *Hoehne M et al. 2006* was used as reference method. The RIDASCREEN® Norovirus 3rd Gen. ELISA was used as a benchmark immunological assay.
The results from the retrospective study are summarized in tables 2 and 3. RIDA®QUICK Norovirus rendered a clinical sensitivity of 86 % and a specificity of 94.6 % using real-time RT-PCR as reference. In comparison, the RIDASCREEN® Norovirus 3rd Gen. rendered a clinical sensitivity of 76.8 % and a specificity of 98% with the same collective of samples. The overall results obtained with the RIDA®QUICK Norovirus are in excellent agreement with those obtained using the well established RIDASCREEN® Norovirus 3rd Gen. ELISA assay.

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

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