Evaluation of a rapid immunochromatographic test for the detection of norovirus in stool samples

M. J. Bruins, M. J. H. M. Wolfhagen, J. Schirm, G. J. H. M. Ruijs

Editorial Manager(tm) for European Journal of Clinical Microbiology & Infectious Diseases

Manuscript Draft

Manuscript Number: EJCMID-D-09-00603R1

Title: Evaluation of a rapid immunochromatographic test for the detection of norovirus in stool samples.

Article Type: Brief Report

Keywords: norovirus; feces/virology; gastroenteritis; sensitivity/specificity; rapid test

Corresponding Author: Ms Marjan J Bruins,

Corresponding Author's Institution: Isala klinieken

First Author: Marjan J Bruins

Order of Authors: Marjan J Bruins; Maurice J Wolfhagen, MD, PhD; Jurjen Schirm, PhD; Gijs J Ruijs, MD, PhD

Abstract: Worldwide noroviruses are an important cause of gastroenteritis and are major agents of both sporadic as well as epidemic infection. Because of the rapid transmission of the virus, early detection is essential. Until recently the available test methods for the detection in stool were enzyme immunoassays and real time reverse transcription PCR (RT-PCR), which both take several hours to perform. We evaluated the rapid immunochromatographic test RIDA®QUICK Norovirus for the detection of norovirus in stool of patients with acute gastroenteritis. This test is easy to perform and read and only takes 20 minutes. The sensitivity and specificity compared to RT-PCR results and the positive and negative predictive values were 57.1%, 99.1%, 93.3% and 91.2% respectively. The rapid test is useful for quick screening, but a negative result should be followed up by RT-PCR.

European Journal of Clinical Microbiology and Infectious Diseases - Brief report

Evaluation of a rapid immunochromatographic test for the detection of norovirus in stool samples.

Marjan J. Bruins[1]

M.J.H.M. Wolfhagen[1]

J. Schirm[2]

G.J.H.M. Ruijs[1]

[1] Isala klinieken, Laboratory of Clinical Microbiology and Infectious Diseases, Zwolle, The Netherlands

[2] Laboratory for Infectious Diseases, Groningen, The Netherlands

Corresponding author:

Marjan J. Bruins

Isala klinieken

Laboratory of Clinical Microbiology and Infectious Diseases

Stilobadstraat 3

8021 AB Zwolle

The Netherlands

Tel +31384243111

Fax +31384243146

Email m.j.bruins@isala.nl

Key words: norovirus; feces/virology; gastroenteritis; sensitivity/specificity; rapid test

Abstract

Worldwide noroviruses are an important cause of gastroenteritis and are major agents of both sporadic as well as epidemic infection. Because of the rapid transmission of the virus, early detection is essential. Until recently the available test methods for the detection in stool were enzyme immunoassays and real time reverse transcription PCR (RT-PCR), which both take several hours to perform. We evaluated the rapid immunochromatographic test RIDA®QUICK Norovirus for the detection of norovirus in stool of patients with acute gastroenteritis. This test is easy to perform and read and only takes 20 minutes. The sensitivity and specificity compared to RT-PCR results and the positive and negative predictive values were 57.1%, 99.1%, 93.3% and 91.2% respectively. The rapid test is useful for quick screening, but a negative result should be followed up by RT-PCR.

Noroviruses (NoVs) have a leading role in causing gastroenteritis worldwide, causing high morbidity rates among patients of all ages [1]. Formerly identified as 'small round structured viruses' and 'Norwalk like virus', NoVs are now known as single-stranded RNA viruses, belonging to the genus *Norovirus* and part of the family *Caliciviridae* [2]. The genus comprises five genogroups I-V (GI-GV), of which GI, GII and GIV are human pathogens [3]. Strains of GI and GII are the most common agents of gastroenteritis [1]. Gastroenteritis caused by NoV has an incubation period of 24-48 hours, although earlier onset does occur. Symptoms usually last 2-3 days and include nausea, vomiting, abdominal cramps, non-bloody diarrhoea and sometimes fever [1]. NoV may be acquired from contaminated food or water and is excreted in stool and vomit. Since very low doses of viral particles are infectious, rapid spreading through faecal-oral or air-borne transmission easily causes an epidemic, especially in institutions such as hospitals and nursing homes [1]. Rapid detection of NoV in stool is essential in both sporadic cases of gastroenteritis and in outbreak prevention and management. Until recently the available test methods were reverse transcription PCR (RT-PCR) and enzyme immunoassays (EIAs) for the detection of viral RNA and antigen respectively. Both methods take several hours to perform however [4, 5], whereas the newly developed rapid antigen test RIDA®QUICK Norovirus (RQN assay; R-Biopharm, Darmstadt, Germany) gives a result in approximately 20 minutes.

We evaluated the RQN assay by testing all stool samples submitted to the Laboratorium voor Medische Microbiologie en Infectieziekten (LMMI), Zwolle, The Netherlands, from patients suspected of NoV infection in the period of January-April 2009 and compared the results to those of a real-time RT-PCR.

The RQN assay is an immunochromatographic test which detects NoV GI and GII in stool. The membrane in the test cassette contains a test line with attached to it monoclonal antibodies to NoV and a control line with anti-mouse IgG. A stool suspension is prepared in

dilution buffer. The first conjugate containing biotinylated anti-NoV is added to the supernatant of the suspension and the mix is pipetted into the sample well on the test cassette. The sample migrates along the membrane, crossing the test line and the control line. If NoV is present, antigen-antibody complexes bind to the test line. Unbound anti-NoV binds to the control line. The second conjugate containing streptavidin-peroxidase is added to the reaction window on the test cassette, allowing the streptavidin to bind to the biotin. Excess biotin is washed away and the substrate (H_2O_2) with the chromophore tetramethylbenzidine (TMB) is added. Reaction between substrate and TMB turns both test and control lines blue when conjugate is bound to biotinylated antibody. If no NoV is present in the stool sample only the control line turns blue.

The real-time RT-PCR for the detection of NoV GI and GII was performed at the Laboratory for Infectious Diseases, Groningen, The Netherlands. Stool suspensions were prepared in phosphate-buffered saline and NoV RNA was extracted from the supernatant using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics, Almere, The Netherlands) according to the manufacturer's instructions.

First reverse transcription was performed in a 20 µl reaction containing 5 µl RNA using the TaqMan® Reverse Transcription Reagents kit (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) by incubation at 25°C for 10 min., at 48°C for 30 min. and at 95°C for 5 min. Of the obtained cDNA 10 µl was amplified using an Applied Biosystems 7500 Real-Time PCR System, using primer and probe sets as described by Hoehne *et al*. [4], in a 25 µl reaction containing 12.5 µl TaqMan® Universal PCR Master Mix (Applied Biosystems), 15 pmol of each primer and 10 pmol probe by incubation at 50°C for 2 min., at 95°C for 10 min., and subsequently 40 cycles at 95°C for 15 seconds and 60°C for 1 min. Appropriate control reactions were included in each RT-PCR run.

In total 538 stool samples from 420 patients (98 hospitalized patients, 39 nursing home inhabitants, 25 general practice patients and 258 outpatients) were tested. Of these, one sample showed inhibition of the PCR and was excluded. Of the remaining 537 samples, 98 (18.2%) were PCR-positive and 60 (11.2%) were positive by the RQN assay (Table 1). Using the RT-PCR as the gold standard the sensitivity, specificity, positive and negative predictive values of the RQN assay were 57.1% (56/98), 99.1% (435/439), 93.3% (56/60) and 91.2% (435/477) respectively.

The sensitivity of the RQN assay was low, but the specificity was excellent. The test allows single sample testing within 20 minutes and is easy to perform and interpret.

Apostel *et al*. tested 113 stored stool specimens with the RQN assay and RT-PCR [6]. Sensitivity and specificity of the RQN assay were 86.0% and 94.6% respectively. They found similar results when the same samples were tested with the RIDASCREEN® Norovirus EIA (R-Biopharm), in which NoV GI and GII antigen in stool is detected in a microwell plate (76.8 and 98.2% respectively). Other studies comparing the RIDASCREEN® Norovirus EIA to RT-PCR reported sensitivities of the EIA between 44 and 76% and specificities between 47 and 96%, which are more consistent with our findings for the RQN assay [7-10]. The difference between Apostel's and our RQN assay sensitivity results may be explained by differences between the two patient populations, the prevalent viral genotypes in these populations and the stage of infection at which sampling took place.

The RQN assay and the RIDASCREEN® Norovirus EIA seem to perform equally well, but the rapid RQN assay saves valuable test time compared to the EIA. The lower sensitivities of both the rapid test and the EIA compared to RT-PCR may be explained by the inability of both tests to detect low viral loads. It is important to test well homogenized stool as soon as possible after the onset of symptoms, when viral shedding is highest.

In addition to the most frequently detected GII strains, also strains belonging to GIV have been reported to cause diarrhoea. La Rosa *et al*. recently found GIV strains in sewage and in stools from patients with acute gastroenteritis [11]. This indicates that future techniques must be able to detect a wider range of NoV variants.

The rapid immunochromatographic RQN assay is a valuable tool for the rapid screening of NoV infection. Its high specificity enables early confirmation of an outbreak, and easy follow-up of positive patients. A negative test result, however, should be followed up by RT-PCR.

References

1. Patel MM, Hall AJ, Vinjé J, Parashar UD (2009) Noroviruses: a comprehensive review. J Clin Virol 44:1-8
2. Green KY (2007) Caliciviridae: The Noroviruses. In: Knipe DM, Howley PM (eds) Fields' virology, 5th edn. Lippincott Williams and Wilkins, Philadelphia, pp 949-978
3. Zheng DP, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS (2006) Norovirus classification and proposed strain nomenclature. Virology 15:312-323
4. Hoehne M, Schreier E (2006) Detection of norovirus genogroup I and II by multiplex real-time RT-PCR using a 3'-minor groove binder-DNA probe. BMC Infectious Diseases 6:69
5. Farkas J, Jiang X (2007) Rotaviruses, caliciviruses, astroviruses, enteric adenoviruses, and other diarrheic viruses. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA (eds) Manual of clinical microbiology, 9th edn. American society for microbiology, Washington, DC, pp 1453-1469
6. Apostel F, Dressler D, Leidinger H, Fischer R, Sander P (2009) RIDA®QUICK Norovirus: a new dimension for norovirus detection. Clin Microbiol Infect 15(Suppl 4):123
7. Dimitriadis A, Marshall JA (2005) Evaluation of a commercial enzyme immunoassay for detection of norovirus in outbreak specimens. Eur J Clin Microbiol Infect Dis 24:615-618
8. González GG, Liprandi F, Ludert JE (2006) Evaluation of a commercial enzyme immunoassay for the detection of norovirus antigen in fecal samples from children with sporadic acute gastroenteritis. J Virol Methods 136:289-291
9. Gray J.J, Kohli E, Ruggeri FM, Vennema H, Sánchez-Fauquier A, Schreier E, Gallimore CI, Iturriza-Gomara M, Giraudon H, Pothier P, Di Bartolo I, Inglese N, de

Bruin E, van der Veer B, Moreno S, Montero V, de Llano MC, Höhne M, Diedrich SM (2007) European multicenter evaluation of commercial enzyme immunoassays for detecting norovirus antigen in fecal samples. Clin Vaccine Immunol 14:1349-1355

10. Okitsu-Negishi S, Okame M, Shimizu Y, Phan TG, Tomaru T, Kamijo S, Sato T, Yagyu F, Müller WE, Ushijima H (2006) Detection of norovirus antigens from recombinant virus-like particles and stool samples by a commercial norovirus enzyme-linked immunosorbent assay kit. J Clin Microbiol 10:3784-3786

11. La Rosa G, Pourshaban M, Iaconelli M, Muscillo M (2008) Detection of genogroup IV noroviruses in environmental and clinical samples and partial sequencing through rapid amplification of cDNA ends. Arch Virol 153:2077-2083

Table

Table 1. Results of the RQN assay compared with RT-PCR

	No. of samples with PCR result		
RQN assay result	Positive	Negative	Total (%)
Positive	56	4	60 (11,2)
Negative	42	435	477(88,8)
Total (%)	98 (18,2)	439 (81,8)	537 (100)

Authors' Response to Reviewers' Comments

Click here to download Authors' Response to Reviewers' Comments: Bruins et al Reply to reviewers comments.doc

Sir,
Below you find our responses to the Reviewers' comments to the first submission of our manuscript EJCMID-D-09-00603R1:

Reviewer #1:
A few editorial comments.
P 3, L 7: invert word order - "... family Caliciviridae."
Answ: Done.

P 4, L 15: "... both test and control lines blue when conjugate is bound to biotinylated antibody."
Answ: Done.

P 4, L 49: " ... 49 cycles at ..."
Answ: Done.

Reviewer #2:

Major comments:

1. Page 5. Line 15: "Apostel et al. tested 113 stored stool specimens with the RQN assay and RT-PCR [5]. Sensitivity and specificity of the RQN assay were 86.0% and 94.6% respectively".
-The authors should give an explanation why did the sensitivity of the same kit differ much between two studies (86% vs 57%).
Answ: An explanation has been added to the text.

2. A paragraph on how could this assay be helpful for the diagnosis of norovirus outbreaks and the clinical management of the disease should be described in the concluding remarks.
Answ: A remark on the usefulness of the test has been added to the text.

3. Quantifying the viral load of the samples would be helpful to assess if viral load had impact on the detection level of the assay.
Answ: We agree and will keep this in mind for future studies.

Minor comments:

1. Abstract: line 11 "real time reverse transcription PCR (rRT-PCR)"
Answ: The abstract has been adapted.

2. Page 3, line 37, "Both methods..20 minutes"
-provide references
Answ: Two references have been added.

3. Page 3, line 44: "We evaluated the RQN...to our laboratory."
-mention the laboratory name
Answ: Laboratory name and location have been added.
4. Page 4, Real time RT-PCR methodology should be shortened.

Answ: We prefer this detailed description for the benefit of others.

5. Page 5, line 46, "La Rosa et al. recently found GIV strains in sewage and in stool from patients with acute gastroenteritis"
-stools
Answ: Done.

6. Page 5, line 49, "Possibly in the near future detection techniques must be modified to include more NoV variants".
-rephrase
Answ: The sentence has been rephrased.

Kind regards,
Ms. Marjan J. Bruins
Zwolle
The Netherlands