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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.

1 European Journal of Clinical Microbiology and Infectious Diseases - Brief report

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

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

1 **Abstract**

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1 Noroviruses (NoVs) have a leading role in causing gastroenteritis worldwide, causing high
2 morbidity rates among patients of all ages [1]. Formerly identified as ‘small round structured
3 viruses’ and ‘Norwalk like virus’, NoVs are now known as single-stranded RNA viruses,
4 belonging to the genus *Norovirus* and part of the family *Caliciviridae* [2]. The genus
5 comprises five genogroups I-V (GI-GV), of which GI, GII and GIV are human pathogens [3].
6
7 Strains of GI and GII are the most common agents of gastroenteritis [1]. Gastroenteritis
8 caused by NoV has an incubation period of 24-48 hours, although earlier onset does occur.
9
10 Symptoms usually last 2-3 days and include nausea, vomiting, abdominal cramps, non-bloody
11 diarrhoea and sometimes fever [1]. NoV may be acquired from contaminated food or water
12 and is excreted in stool and vomit. Since very low doses of viral particles are infectious, rapid
13 spreading through faecal-oral or air-borne transmission easily causes an epidemic, especially
14 in institutions such as hospitals and nursing homes [1]. Rapid detection of NoV in stool is
15 essential in both sporadic cases of gastroenteritis and in outbreak prevention and
16 management. Until recently the available test methods were reverse transcription PCR (RT-
17 PCR) and enzyme immunoassays (EIAs) for the detection of viral RNA and antigen
18 respectively. Both methods take several hours to perform however [4, 5], whereas the newly
19 developed rapid antigen test RIDA[®]QUICK Norovirus (RQN assay; R-Biopharm, Darmstadt,
20 Germany) gives a result in approximately 20 minutes.
21

22 We evaluated the RQN assay by testing all stool samples submitted to the Laboratorium voor
23 Medische Microbiologie en Infectieziekten (LMMI), Zwolle, The Netherlands, from patients
24 suspected of NoV infection in the period of January-April 2009 and compared the results to
25 those of a real-time RT-PCR.
26

27 The RQN assay is an immunochromatographic test which detects NoV GI and GII in stool.
28 The membrane in the test cassette contains a test line with attached to it monoclonal
29 antibodies to NoV and a control line with anti-mouse IgG. A stool suspension is prepared in
30

1 dilution buffer. The first conjugate containing biotinylated anti-NoV is added to the
2 supernatant of the suspension and the mix is pipetted into the sample well on the test cassette.
3
4 The sample migrates along the membrane, crossing the test line and the control line. If NoV is
5 present, antigen-antibody complexes bind to the test line. Unbound anti-NoV binds to the
6 control line. The second conjugate containing streptavidin-peroxidase is added to the reaction
7 window on the test cassette, allowing the streptavidin to bind to the biotin. Excess biotin is
8 washed away and the substrate (H₂O₂) with the chromophore tetramethylbenzidine (TMB) is
9 added. Reaction between substrate and TMB turns both test and control lines blue when
10 conjugate is bound to biotinylated antibody. If no NoV is present in the stool sample only the
11 control line turns blue.
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14 The real-time RT-PCR for the detection of NoV GI and GII was performed at the Laboratory
15 for Infectious Diseases, Groningen, The Netherlands. Stool suspensions were prepared in
16 phosphate-buffered saline and NoV RNA was extracted from the supernatant using the
17 MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics, Almere, The
18 Netherlands) according to the manufacturer's instructions.
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21 First reverse transcription was performed in a 20 µl reaction containing 5 µl RNA using the
22 TaqMan® Reverse Transcription Reagents kit (Applied Biosystems, Nieuwerkerk a/d IJssel,
23 The Netherlands) by incubation at 25°C for 10 min., at 48°C for 30 min. and at 95°C for 5
24 min. Of the obtained cDNA 10 µl was amplified using an Applied Biosystems 7500 Real-
25 Time PCR System, using primer and probe sets as described by Hoehne *et al.* [4], in a 25 µl
26 reaction containing 12.5 µl TaqMan® Universal PCR Master Mix (Applied Biosystems), 15
27 pmol of each primer and 10 pmol probe by incubation at 50°C for 2 min., at 95°C for 10 min.,
28 and subsequently 40 cycles at 95°C for 15 seconds and 60°C for 1 min. Appropriate control
29 reactions were included in each RT-PCR run.
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1 In total 538 stool samples from 420 patients (98 hospitalized patients, 39 nursing home
2 inhabitants, 25 general practice patients and 258 outpatients) were tested. Of these, one
3 sample showed inhibition of the PCR and was excluded. Of the remaining 537 samples, 98
4 (18.2%) were PCR-positive and 60 (11.2%) were positive by the RQN assay (Table 1). Using
5 the RT-PCR as the gold standard the sensitivity, specificity, positive and negative predictive
6 values of the RQN assay were 57.1% (56/98), 99.1% (435/439), 93.3% (56/60) and 91.2%
7 (435/477) respectively.
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10 The sensitivity of the RQN assay was low, but the specificity was excellent. The test allows
11 single sample testing within 20 minutes and is easy to perform and interpret.
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14 Apostel *et al.* tested 113 stored stool specimens with the RQN assay and RT-PCR [6].
15
16

17 Sensitivity and specificity of the RQN assay were 86.0% and 94.6% respectively. They found
18 similar results when the same samples were tested with the RIDASCREEN[®] Norovirus EIA
19 (R-Biopharm), in which NoV GI and GII antigen in stool is detected in a microwell plate
20 (76.8 and 98.2% respectively). Other studies comparing the RIDASCREEN[®] Norovirus EIA
21 to RT-PCR reported sensitivities of the EIA between 44 and 76% and specificities between 47
22 and 96%, which are more consistent with our findings for the RQN assay [7-10]. The
23 difference between Apostel's and our RQN assay sensitivity results may be explained by
24 differences between the two patient populations, the prevalent viral genotypes in these
25 populations and the stage of infection at which sampling took place.
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27

28 The RQN assay and the RIDASCREEN[®] Norovirus EIA seem to perform equally well, but
29 the rapid RQN assay saves valuable test time compared to the EIA. The lower sensitivities of
30 both the rapid test and the EIA compared to RT-PCR may be explained by the inability of
31 both tests to detect low viral loads. It is important to test well homogenized stool as soon as
32 possible after the onset of symptoms, when viral shedding is highest.
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1 In addition to the most frequently detected GII strains, also strains belonging to GIV have
2 been reported to cause diarrhoea. La Rosa *et al.* recently found GIV strains in sewage and in
3 stools from patients with acute gastroenteritis [11]. This indicates that future techniques must
4 be able to detect a wider range of NoV variants.
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10 The rapid immunochromatographic RQN assay is a valuable tool for the rapid screening of
11 NoV infection. Its high specificity enables early confirmation of an outbreak, and easy
12 follow-up of positive patients. A negative test result, however, should be followed up by RT-
13 PCR.
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Table 1. Results of the RQN assay compared with RT-PCR

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

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."

Answer: Done.

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

Answer: Done.

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

Answer: 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%).

Answer: 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.

Answer: 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.

Answer: We agree and will keep this in mind for future studies.

Minor comments:

1. Abstract: line 11 "real time reverse transcription PCR (rRT-PCR)"

Answer: The abstract has been adapted.

2. Page 3, line 37, "Both methods..20 minutes"

-provide references

Answer: Two references have been added.

3. Page 3, line 44: "We evaluated the RQN...to our laboratory."

-mention the laboratory name

Answer: 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