

Evaluation of immunochromatographic tests for the rapid detection of the emerging GII.17 norovirus in stool samples, January 2016

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A novel GII.17 norovirus emerged in Asia in the winter of 2014/15. A worldwide spread is conceivable and norovirus diagnostic assays need to be evaluated to investigate if they adequately detect this emerging genotype. Seven immunochromatographic kits commercially available in Europe were evaluated on ten stool samples where GII.17 virus had been quantified by real-time reverse transcription-polymerase chain reaction. All the kits detected GII.17 with various sensitivities, partly depending on the virus titre.

We report that seven commercially available norovirus immunochromatographic (IC) tests available in Europe, have the capacity to detect strains of genogroup GII.17 in stool. Sensitivities vary however, and partly depend on the viral load in the samples.

Laboratory investigation

The following seven IC tests were evaluated on the same panel of stool samples: Actim Noro (Medix Biochemica, Kauniainen, Finland), Immunocatch Norovirus (Eiken Chemical Co., Ltd., Tokyo, Japan), Immunoquick Norovirus (Biosynex S.A., Strasbourg, France), Nadal Norovirus I+II (Nal van minden, Regensburg, Germany), SD Bioline Norovirus (Standard Diagnostics, Inc., Yongin-si, Republic of Korea), Simple Norovirus (Operon, S.A., Cuarte de Huerva, Zaragoza, Spain), and RidaQuick Norovirus (R-Biopharm AG, Darmstadt, Germany).

The assays were performed on 10 frozen stool samples from our collection. These samples had been collected from patients affected by five different gastroenteritis outbreaks. One outbreak was related to oyster consumption, while the four others were caused by person-to-person transmission. Three of the four latter outbreaks had occurred in nursing homes and the fourth in a hospital. All 10 samples had been previously shown to be GII.17 norovirus-positive by sequencing of the RNA-dependent RNA polymerase (RdRp) region

and the N-terminal/shell (N/S) region. The samples had also been genotyped on the Norovirus Automated Genotyping Tool [1]. The capsid sequences displayed 95.8% to 99.7% nt identity with the reference strain Hu/GII/JP/2014/GII.P17_GII.17/Kawasaki323 (GenBank accession number: AB983218).

The stool samples were conserved at -40°C and were thawed on the day of the evaluation. All the commercial IC tests were performed according to the manufacturers' instructions. Due to limited amounts of samples, one assay per test was done, except when the results were negative, in which case they were controlled by a second assay when possible. The virus copy numbers were quantified by real-time reverse transcription-polymerase chain reaction (RT-PCR) on the same day.

Evaluation of the IC tests showed that all were able to detect GII.17 norovirus present in the stool samples. Sensitivities of the tests varied however, ranging from detection in one sample of the 10, to seven samples of the 10 (Table).

Measurements of the virus titres in each sample allowed to demonstrate that a positive result could be obtained with most of the IC tests (five in seven) if the samples presented a minimal load of 4.88×10^8 virus copies/g of stool. The lowest viral load detected by an IC test was 6.54×10^6 copies/g of stool and this gave a weak positive signal with the RidaQuick Norovirus test. Of note however, three samples (E13289, E12908, E12909) with relatively high viral loads of 1.35×10^9 , 1.34×10^{10} and 3.51×10^{10} copies/g of stool gave negative results with four, three and two IC tests, respectively. These samples originated from two separate outbreaks. Another set of samples from two different outbreaks, namely E13289 and E12990 (6.89×10^8 copies/g of stool) also yielded different results throughout the IC tests. While E12990 was positive in

TABLE

Results of seven immunochromatographic tests on ten stool samples containing GII.17 norovirus at various concentrations

Sample ID ^a	Virus titre (copies/g of stool) ^b	Norovirus immunochromatographic tests						
		RidaQuick Norovirus	Simple Norovirus	Immunocatch Norovirus	Actim Noro	Immunoquick Norovirus	SD Bioline Norovirus	NADAL Norovirus I+II
E12909	3.51×10^{10}	Positive	Positive	Positive	Positive	Positive	Negative ^c	Negative
E12905	1.55×10^{10}	Positive	Positive	Positive	Positive	Positive	Positive	Positive
E12908	1.34×10^{10}	Positive	Positive	Positive	Positive	Negative ^c	Negative ^c	Negative
E13289	1.35×10^9	Positive	Positive	Positive	Negative ^c	Negative ^c	Negative ^c	Negative
E12990	6.89×10^8	Positive	Positive	Positive	Positive	Positive	Positive	Negative
E11161	4.88×10^8	Positive	Positive	Positive	Positive	Positive	Negative ^c	Negative
E12989	9.39×10^6	Negative ^c	Negative ^c	Negative	Negative ^c	Negative ^c	Negative ^c	Negative
E13290	6.54×10^6	Positive	Negative ^c	Negative	Negative ^c	Negative ^c	Negative ^c	Negative
E12991	4.90×10^5	Negative ^c	Negative ^c	Negative	Negative ^c	Negative ^c	Negative ^c	Negative
E12972	1.12×10^4	Negative ^c	Negative ^c	Negative	Negative ^c	Negative ^c	Negative ^c	Negative

ID: identity.

^a E12909, E12905 and E12908 were collected from one single gastroenteritis outbreak; E13289 and E13290 were collected from a second gastroenteritis outbreak; E12990, E12989 and E12991 were collected from a third gastroenteritis outbreak; all three outbreaks occurred in nursing homes. E12972 was collected from an outbreak in a hospital. All four outbreaks were due to a person-to-person transmission, and GII.17 norovirus was the only strain detected. E11161 was collected from an oyster-related gastroenteritis outbreak; GII.17 was the only viral strain detected in this sample.

^b Quantified by real-time reverse transcription-polymerase chain reaction.

^c The result was controlled by a second assay.

all but one of the tests, E13289 was negative in four of the IC tests.

On the other hand, some samples, which were common to an outbreak (E12909, E12905 and E12908), and presumably all carrying the same GII.17 strain, also did not react in the same way in three IC tests despite relatively high virus titres ($\geq 1.34 \times 10^{10}$ copies/g of stool). While they all gave positive results with four IC tests, only two of the three samples gave a positive signal with Immunoquick Norovirus, and only one of the three with SD Bioline Norovirus and Nadal Norovirus I+II.

Discussion

In the winter of 2014/15 a novel GII.17 norovirus emerged as a major cause of epidemic and endemic acute gastroenteritis in Asia, replacing the previously dominant GII.4 genotype [2-5]. In other parts of the world, GII.17 was up to then only sporadically detected [6-8]. Because noroviruses can spread rapidly around the world, as has been previously observed for GII.4 [9,10], it is possible that GII.17 will emerge in Europe in this season. Thus the public health community and surveillance systems need to be prepared. As emphasised by de Graaf et al. [6], contemporary norovirus diagnostic assays may not have been developed to detect GII.17, since this genotype was rarely found at the time. Therefore they need to be evaluated and adapted if necessary to adequately detect GII.17 norovirus.

For medical laboratories not equipped to carry out molecular investigations, easy-to-perform tests providing rapid results present an advantage. Khamrin et al. [11] tested four IC kits available in Japan for their

sensitivity to detect GII.17 strains in faecal specimens. Here we checked the performance of several tests commercially available in Europe.

We found that most of the IC tests could confirm the presence of the GII.17 norovirus, if the samples presented a minimal load of 4.88×10^8 virus copies/g of stool. A study by Takanashi et al. [12] showed that a minimal viral load of 4.6×10^6 copies/g of stool was sufficient to detect GII.4 norovirus by immunochromatography, while a 100-fold higher detection limit was found for GII.17. A similar observation for GII.17 was made by Khamrin et al. [11].

Three samples in our study, all of which had relatively high viral loads ($\geq 1.35 \times 10^9$ copies/g of stool) nevertheless gave negative results with some of the tests. Apart from viral load, which is clearly essential for the reactivity of the IC tests against GII.17, other factors may influence the tests' performance. The failure of some IC tests to detect some GII.17 strains could be due to the particular antibodies used in these tests, which may react differently when strains present antigenic variation. This could explain why sample E13289, despite its higher viral load (1.35×10^9 copies/g of stool), was positive in only three of the IC tests, while sample E12990 with lower viral load (6.89×10^8 copies/g of stool) was positive in all but one the IC tests. Indeed these two samples originated from different outbreaks and thus may potentially have had antigenic differences. Sequencing the genetic regions coding for the antigens involved in the tests' reactions could further assess this supposition.

Three samples (E12909, E12905 and E12908) originating from the same outbreak however, and thus assumed to contain the same GII.17 strain, reacted differently in three of the IC tests. Therefore, viral loads and antigenic differences cannot explain these results. One alternative reason could be the consistence of the stool samples paired with the sample collection devices. Indeed, the sample collection devices of the seven IC tests are not the same, and it is possible that some of them are not appropriate to collect certain stool samples. This hypothesis nevertheless remains to be confirmed.

Some limitations of this study should be mentioned. In particular, the possibility of false negatives for samples not controlled by a second assay cannot be excluded. Furthermore, even if this was not the objective of this study, it should be noted that a precise estimation of the detection limit of each IC test by serial dilutions of the samples was not performed.

In conclusion, the seven IC tests evaluated were able to detect GII.17 with various sensitivities due to virus titre, and possibly antigenic differences and kit design. Therefore some IC tests may need to be optimised for the detection of GII.17.

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Conflict of interest

None declared.

Authors' contributions

LT: conducted the laboratory investigation; MB: conducted the laboratory investigation; PP: revised the manuscript; KAB: conceptualised the study and drafted the manuscript.

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