

Evaluation of immunochromatography tests for detection of novel GII.17 norovirus in stool samples

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A novel GII.17 norovirus has emerged as a major cause of epidemic and endemic acute gastroenteritis in several countries in Asia. We used a small panel of stool samples in which GII.17 virus had been quantified by real-time RT-PCR to evaluate four commercially available norovirus immunochromatography (IC) kits. At least 10^8 copies/mL of GII.17 virus were required by each IC kit for a positive result, which is 1,000-fold more than that reported for these assays for GII.4 viruses.

In the winter of 2014–15, a novel GII.17 norovirus variant emerged in several countries in Asia [1]. From September 2014 to March 2015, 70% of all outbreaks in Guangdong and Jiangsu provinces in China were caused by a novel GII.17 virus [2,3]. A similar increase in the number of infections with this novel GII.17 virus has been reported in Japan [4] and Thailand since December 2014. In this study, we assessed whether current immunochromatography (IC) tests can detect these novel GII.17 noroviruses.

Laboratory investigation

For the rapid detection of norovirus, an IC test is one of the most convenient and accessible diagnostic tools commonly used in primary care units and private clinics in Japan [5]. However, these IC tests were developed mainly for the detection of genotypes such as GII.3 and GII.4. To evaluate if these IC tests are able to detect these novel GII.17 noroviruses, we tested four commercial IC kits available in Japan: GE test Noro Nissui, Nissui Pharmaceutical Co., Ltd.; ImmunoCatch-Noro, Eiken Chemical Co., Ltd.; Quick Navi-Noro 2, Denka Seiken Co., Ltd.; Quick Chaser-Noro, Mizuho Medy Co., Ltd.

A panel of six GII.17-positive stool samples from patients in Japan ($n = 5$) and Thailand ($n = 1$), in which the virus copy numbers had been quantified by real-time RT-PCR (reference values), were randomly selected from stool samples for which there was a large quantity available. Two of the six GII.17 stool samples tested positive by all four IC kits (Table).

Testing of the six specimens by real-time RT-PCR demonstrated that the two samples that were positive by IC test contained high virus titres (1.90×10^9 and 8.06×10^9 virus copies/mL). In contrast, the other four specimens that were negative in the IC test had virus titres ranging from 4.91×10^3 to 2.50×10^8 virus copies/mL. One of the two specimens positive in the IC tests (HU-2015) was re-tested using 1:10 and 1:100 stool dilutions, using three of the four kits (one was not available at the time of re-testing). The results demonstrated that at 1:10 dilution (virus titre 1.90×10^8 copies/mL) two of three tests still showed the positive results, with a weak positive band, while at 1:100 dilution (virus titre 1.90×10^7 copies/mL), all three IC tests were negative. These data demonstrated that the sensitivity of the IC kits for the detection of this novel GII.17 virus was about 10^8 copies/mL.

Discussion

Norovirus is one of the most common etiological agents of acute gastroenteritis in people of all ages in developing and developed countries [6]. The virus is transmitted mainly via food and water and by person-to-person. On the basis of sequence differences in the virus VP1 region, noroviruses can be divided into seven genogroups (GI to GVII): viruses from GI, GII and GIV cause disease in humans. GI is further divided into nine genotypes (GI.1 to GI.9) while GII contains at least 22 genotypes (GII.1 to GII.22) [7]. Of all genotypes,

TABLE

Virus copy numbers of six GII.17 noroviruses tested and results of four norovirus immunochromatography tests

Norovirus GII.17 sample ID	Date sample taken	Virus titre (copies/mL) ^a	Norovirus immunochromatography tests			
			ImmunoCatch-Noro	Quick Navi-Noro 2	GE test Noro Nissui	Quick Chaser-Noro
12860	2 Feb 2015	2.50 × 10 ⁸	Negative	Negative	Negative	Negative
12868	2 Mar 2015	8.06 × 10 ⁹	Positive	Positive	Positive	Positive
12870	4 Mar 2015	4.91 × 10 ³	Negative	Negative	Negative	Negative
12880	17 Mar 2015	6.46 × 10 ³	Negative	Negative	Negative	Negative
HU-2015	31 Jan 2015	1.90 × 10 ⁹	Positive	Positive	Positive	Positive
R1-Thai	19 Dec 2014	1.82 × 10 ⁶	Negative	Negative	Negative	Negative

^a Quantified by real-time RT-PCR (reference values).

GII.4 is the most common infection worldwide and new GII.4 variants emerge every two to three years [8]. Although based on a small sample size, our findings suggest that the commercial IC kits for the detection of norovirus available on the market in Japan are able to detect the novel GII.17 norovirus, but with relatively low sensitivity. Only samples that contained more than 10⁹ copies/mL were positive in all four IC tests. Previous data have shown that the minimal detection limit of an IC test for GII.4 norovirus was about 10⁶ virus copies/mL, which is a 1,000-fold more sensitive [9]. Therefore, redesign of the currently available norovirus IC tests may be required to detect the novel GII.17 noroviruses with the same sensitivity as for the more commonly circulating norovirus genotypes. Laboratories and physicians should be aware of these findings, in particular where the novel GII.17 norovirus has been shown to be circulating.

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Conflict of interest

None declared.

Authors' contributions

PK: conducted the laboratory characterisation of norovirus and drafted the manuscript; AT: conducted the laboratory characterisation of norovirus; ST: involved in laboratory investigation; SO: involved in the data interpretation and revised the manuscript; NM: revised the manuscript; SH: revised the manuscript; HU: conceptualised the study and revised the manuscript.

* Authors' correction

At the request of the authors, reference 9 was replaced on 20 July 2015.

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