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Short Communication

Evaluation of Immunochromatography Tests and New Enzyme Immunoassay for Detection of Novel GII.17 Norovirus in Stool Samples

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Running ahead: Rapid tests for detection of GII.17 norovirus
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SUMMARY

A novel GII.17 norovirus (NoV) Kawasaki 2014 has been spread in several regions worldwide. Rapid and reliable diagnostic tests are needed for the detection of this newly emerged NoV variant. In this study, the sensitivity of seven brands of immunochromatography (IC) test kits, 6 are on the market in Japan and 1 in Europe, were evaluated by testing with confirmed GII.17 NoV positive stool samples. The stool samples were also tested by bioluminescent enzyme immunoassay (BLEIA). Real-time RT-PCR was used as a gold standard method. Among seven brands IC kits, RIDA QUICK, was the most sensitive kit with the detection rate of $10^7$ copies/ml while those of other IC kits ranged from $10^8$-$10^9$ copies/ml. Nevertheless, it should be pointed out that the detection rate of BLEIA was about 100-1,000 times ($10^4$-$10^5$ copies/ml) higher than those of RIDA QUICK. However, the procedure for BLEIA took a longer time and required sophisticated equipment.
A novel GII.17 norovirus (NoV) variant emerged in several countries in Asia in the winter of 2014/15 and caused several outbreaks (1). Our previous report of 4 commercially available immunochromatography (IC) kits in Japan for the detection of GII.17 NoV revealed that lower limit of detection was almost 10^8 copies/ml (2), while lower limit of detection for GII.4 NoV was around 10^6 copies/ml (3). The study of 6 IC kits that were commercially available revealed a wide range of detection sensitivity. RIDA QUICK showed the highest sensitivity with the detection rate of 6.54 X 10^6 copies/g of stool, while those of the other IC kits were 4.88 X 10^8 copies/g of stools (4).

An ultrasensitive and fully automated bioluminescent enzyme immunoassay (BLEIA) has been developed for the detection of 6 NoV GI genotypes (1,3,4,7,8 and 12) and 8 NoV GII genotypes (1,2,3,4,5,6,12, and 13). The sensitivity of detection of BLEIA ranged from 10^5 to 10^6 copies/g stool samples (5). The sensitivity and specificity of the BLEIA was correlated well with those of real-time RT-PCR and the loop mediated isothermal amplification methods (6).

Here, we evaluated the sensitivity of 7 brands of IC kits for the detection of GII.17 NoV, 6 of which are on the market in Japan while the other one is commercially available in Europe. In addition, the GII.17 NoV positive specimens were tested by BLEIA, and real-time RT-PCR.

A total of 6 stool samples that were positive for GII.17 NoV, 5 (12860, 12868, 12870, 12880, HU-2015) were collected from children with diarrhea in Japan from January 2015 to March 2015, while R1-Thai was collected from a healthy adult in Thailand in December, 2014 (7). Ethical Committee of the Nihon University School of Medicine approved this study (No.25-13-0).

Seven brands of IC kits used in the present study were as follows: 1) Quick Navi-Noro2, Denka Seiken Co., Ltd.; 2) Quick Navi-Noro2 (version 2), Denka Seiken Co., Ltd.; 3) ImmunoChach-Noro, Eiken Chemical Co., Ltd.; 4) Quick Chaser-Noro, Mizuho
Medy Co., Ltd.; 5) GE test Noro Nissui, Nissui Pharmaceutical Co., Ltd.; 6) RIDA QUICK Norovirus (Version N1402), R-Biopharm AG, Germany; 7) Rapid SP Noro, DS Pharma Biomedical Co., Ltd.). In addition, the BLEIA (Eiken Chemical Co., Ltd.) had been evaluated for the detection of GII.17 NoV in comparison with those IC kits. All procedures for IC and BLEIA testing were based on the manufacturer’s instructions (5,6).

The NoVs were initially screened by RT-PCR and followed by direct sequencing to identify their genotypes (8). To evaluate the sensitivity of the seven NoV IC kits for the detection of GII.17 NoV in the clinical specimens, the virus copy numbers were quantified by real-time RT-PCR (9).

The supernatant of 10% stool sample suspensions in PBS were tested by all 7 IC kits. The viral loads in the samples were determined by real-time RT-PCR (9). The sample code 12868 and HU-2015 showed the strong positive results by all seven IC kits. Both specimens contained the GII.17 NoV viral loads more than $10^9$ copies/ml. The specimen code 12868 contained the viral loads of $8.06 \times 10^9$ copies/ml, and the sample code HU-2015 contained the viral loads of $1.90 \times 10^9$ copies/ml. It was observed that sample 12860, which contained the viral loads of $2.50 \times 10^8$ copies/ml could be detected only by the RIDA QUICK Norovirus and Rapid SP Noro IC kits. However, the R1-Thai ($1.82 \times 10^6$ copies/ml), 12880 ($6.46 \times 10^3$ copies/ml) and 12870 ($4.91 \times 10^3$ copies/ml) were negative by all 7 kits (Table 1).

In order to determine the sensitivity of IC kits, three stool samples which contained more than $10^8$ copies/ml were diluted 1:10, 1:100, and 1:1,000 with the diluting buffers supplied by the companies and each dilution was tested by those seven IC kits. The sample code 12868 and HU-2015 were positive until 1:100 dilution ($8.06 \times 10^7$ copies/ml and $1.90 \times 10^7$ copies/ml) by RIDA QUIK Norovirus. Both samples were positive for 1:10 dilution ($8.06 \times 10^6$ copies/ml and $1.90 \times 10^8$ copies/mL) by the other 5 IC kits except for
original Quick Navi-Noro2 was negative (Table 2).

The sensitivity of BLEIA was determined by testing with five stool samples at the dilution of $10^{-6}$. The sample codes 12868 and the HU-2015 were positive until $10^5$ dilution (8.06X$10^3$ copies/ml and 1.90X$10^4$ copies/ml). The 12860 was positive at 1:1000 dilution (2.50 X $10^5$ copies/ml). However, the 12880 and 12870 were negative even at undiluted samples. The R1-Thai was not examined in this experiment because of the shortage of the sample (Table 3).

We analyzed the same samples which we used in the previous article (2). However, we added more commercial kits and tested with different sample dilution. In addition, we examined the sensitivity of BLEIA for GII.17 NoV detection which has not been reported previously.

We reported previously that the lower detection limit of IC kit for GII.3 and GII.4 NoV was $10^6$-$10^7$ copies/ml by using rabbit polyclonal antibody for stool samples (3). The instruction of Quick Chaser-Noro, Mizuho Medy Co., Ltd. described the detection limit of 6.25X$10^6$ copies/ml for GI and GII NoV but other kits are not well described the detection limits. Our previous report demonstrated that IC tests for the detection of GII.17 NoV ($10^6$-$10^9$ copies/ml) were less sensitive than the detection of GII.4 ($10^6$-$10^7$ copies/ml) (2). This study clearly demonstrated that the RIDA QUICK Norovirus and Rapid SP Noro could detect GII.17 NoV at around $10^7$-$10^8$ copies/ml, respectively. The reasons to explain the difference in the detection limits for each kit may related to 1) the characteristics of monoclonal antibodies used by different kits, 2) RIDA QUICK Norovirus use streptavidin and biotin for coating the antibody and particle captures at the positive line, respectively (10). For the development of NoV IC kit, using broad reactive and high quality antibody will increase the sensitivity for the detection of a wide range of NoV genotypes. In addition, combination of another new technique may facilitate to improve high sensitivity of the kits.
such as silver amplification immunochromatography system which was used for rapid diagnosis of influenza (Mizuho Medy Co., Ltd.) (11). This kind of technology may be useful for rapid diagnosis of norovirus.

The BLEIA use luciferase reaction and automatic machine for performing test and it takes 46 min to get the results. It is interesting to observe that the sensitivity of BLEIA is much higher (10^4 to 10^5 copies/g of stools) than those of IC kits. Surprisingly, the sensitivity of BLEIA for the detection of GII.17 is higher than the detection of other NoVs (10^5 to 10^6 copies/g) (5), including genotypes of GI.1, 3, 4, 7, 8, 12 and GII.1, 2, 3, 4, 5, 6, 12, 13 genotypes. The BLEIA is one of a useful test for a rapid detection of NoV, including novel GII.17 NoV.

Although the IC kits and BLEIA are proved to be the useful tools for rapid diagnosis of NoV infection especially at clinics, the sensitivity of detection which is the limitation of the methods should be considered.

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Declaration of Interest:
No conflict of interest is declared.
References


Table 1. Evaluation the sensitivity of immunochromatography test kits for the detection of GII.17 NoV.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Virus titer (copies/mL) by real-time RT-PCR</th>
<th>Norovirus immunochromatography tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Quick Navi-Noro2</td>
</tr>
<tr>
<td>12868</td>
<td>8.06 x 10^9</td>
<td>+</td>
</tr>
<tr>
<td>HU-2015</td>
<td>1.90 x 10^9</td>
<td>+</td>
</tr>
<tr>
<td>12860</td>
<td>2.50 x 10^8</td>
<td>-</td>
</tr>
<tr>
<td>R1-Thai</td>
<td>1.82 x 10^6</td>
<td>-</td>
</tr>
<tr>
<td>12880</td>
<td>6.46 x 10^3</td>
<td>-</td>
</tr>
<tr>
<td>12870</td>
<td>4.91 x 10^3</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2. Evaluation the sensitivity of immunochromatography test kits for the detection of GII.17 NoV.

<table>
<thead>
<tr>
<th>IC tests</th>
<th>12868 (8.06 x 10⁹)</th>
<th>HU-2015 (1.90 x 10⁹)</th>
<th>12860 (2.50 x 10⁸)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Undiluted 1:10 1:100 1:1000</td>
<td>Undiluted 1:10 1:100 1:1000</td>
<td>Undiluted 1:10 1:100 1:1000</td>
</tr>
<tr>
<td>Quick Navi-Noro2</td>
<td>+ - - - + - - - - - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quick Navi-Noro2 (revise)</td>
<td>+ + - - + + - - - - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ImmunoCatch-Noro</td>
<td>+ + - - + + - - - - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quick Chaser-Noro</td>
<td>+ + - - + + - - - - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GE test Noro Nissui</td>
<td>+ + - - + + - - - - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIDA QUICK Norovirus</td>
<td>+ + + - + + + - + + - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid SP Noro</td>
<td>+ + - - + + - - + + - - - -</td>
<td></td>
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</table>
Table 3. Evaluation the sensitivity of BLEIA for the detection of GII.17 NoV.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Undiluted</th>
<th>1:10</th>
<th>1:10²</th>
<th>1:10³</th>
<th>1:10⁴</th>
<th>1:10⁵</th>
<th>1:10⁶</th>
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<tbody>
<tr>
<td>12868</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>HU-2015</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12860</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R1-Thai</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>12880</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12870</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

NT = not tested