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Corresponding Author：上原由紀
順天堂大学大学院医学研究科感染制御科学
〒113-8421 東京都文京区本郷 2-1-1
TEL：03-3813-3111 Fax：03-5684-7830
E-mail: yuuehara@juntendo.ac.jp
Performance evaluation of a novel fully automated real-time reverse transcriptase-polymerase chain reaction kit for the detection of norovirus

Authors

Tomohiro Hosoda, Yuki Uehara, Naoto Matsuda, Yukiko Kawase, Mika Tanei,
Yuichiro Haba, Ayako Nakamura, Yoko Tabe, Toshio Naito, and Akimichi Ohsaka

a Department of General Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan (2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan)
b Department of Infection Control Science, Juntendo University, Faculty of Medicine, Japan (2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan)
c Department of Clinical Laboratory, Juntendo University Hospital, Tokyo, Japan (3-1-3, Hongo, Bunkyo-ku, Tokyo 113-8431, Japan)
d Department of Clinical Laboratory Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan (2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan)
e Department of Transfusion Medicine and Stem Cell Regulation, Juntendo University Faculty of Medicine, Tokyo, Japan (2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan)
Abstract

Objective: Rapid and accurate detection of norovirus is essential for the prevention and control of the outbreaks. The aim of this study is to compare the fully automated real-time reverse transcriptase-polymerase chain reaction method (EV-kit) with the conventional immunochromatography method (IC) for diagnosis of norovirus, using one-tube reverse transcriptase polymerase chain reaction (RT-PCR) analysis as the gold standard.

Methods: Between November 2013 and March 2014, clinical data and fecal specimens (53 bulk stools, 41 rectal swabs) were collected from 94 patients who visited the Department of General Medicine, Juntendo University Hospital for acute diarrhea. The sensitivity and specificity of each study test was determined by comparing with RT-PCR, and reproducibility was analyzed by determining Cohen’s kappa coefficients.

Results: Of 94 specimens, 35 (37%, 26 bulk stools, 9 rectal swabs) were positive for norovirus antigen by RT-PCR. The sensitivity, specificity, and Cohen’s kappa coefficient of the EV-kit were 91% (32/35), 88% (52/59), and 0.778, respectively; those of the IC were 54% (19/35), 90% (53/59), and 0.468, respectively. For rectal swab testing, the sensitivity was 89% (8/9) for the EV-kit and 33% (3/9) for IC, and that for bulk stool testing was 92% (24/26) for the EV-kit and 62% (16/26) for IC.
Conclusions: Use of the EV-kit was significantly more sensitive than was IC testing, taking RT-PCR analysis as the gold standard. Rectal swab or bulk stool specimens may be adequate for the detection of norovirus antigen when the EV-kit is used.

Key words

Norovirus, automated real-time RT-PCR, immunochromatography, stool, rectal swab
For rapid detection of norovirus antigen from human stool, only immunochromatography method (IC) is approved by Japanese health insurance system. Several IC products are available, but their sensitivities vary and they are not adequate for use on a clinical basis\(^1\).

Molecular methods, such as reverse transcriptase-polymerase chain reaction (RT-PCR) analysis, have high sensitivity and specificity even for specimens with low viral load\(^2\); RT-PCR is often used as a standard method for the diagnosis of norovirus infection because of its high sensitivity\(^1\). Although a newer, one-tube RT-PCR is commercially available\(^3\), the RT-PCR technique is not routinely used in the clinical laboratory, probably due to the shortage of manpower and the lack of proficiency among medical technologists.

The Diagnode Enteric Viral Panel Real-Time PCR kit\(^{\text{®}}\) (EV-kit, Becton Dickinson, Franklin Lakes, NJ, USA) is a newly developed molecular test for use with the BD MAX\(^{\text{®}}\) system (Becton Dickinson). The BD MAX is a fully automated sample-in and answer-out instrument that combines sample extraction, RT, and real-time PCR on a walkaway platform\(^4\). Results are shown as images, eliminating human errors in reading.
In addition, use of the EV-kit with BD MAX results in less contamination and workload, compared with standard RT-PCR.

In this study, we compared the performance of the EV-kit with IC, using results of RT-PCR as the gold standard. Furthermore, we compared the sensitivity and specificity of both methods for use with bulk stools vs. rectal swabs. Our results suggest that the EV-kit is the more useful diagnostic method regardless of the specimen type.
II. Materials and Methods

A. Study population

The present study was conducted at the outpatient clinic of the Department of General Medicine of Juntendo University Hospital, which is a university-affiliated teaching hospital with 1,020 beds in Tokyo, Japan, from November 1, 2013 to March 31, 2014. This time period was an epidemic season of norovirus infection in Japan. During the study period, outpatients who visited the clinic with at least one diarrhea or loose stool within 24 hours were asked to participate in this study. Exclusion criteria were patients who were age under 20, who did not agree with participating in this study, who could neither collect bulk stool nor swab specimens, and who were given diagnoses other than infectious gastroenteritis.

B. Specimen collection and handling

Bulk stool specimens were collected in a cup in the outpatient ward as per routine, but if a patient could not collect a fecal specimen in a cup, three rectal swab specimens were collected by the patient using FLOQSwabs® (Copan Diagnostics Ins., Murrieta, CA, USA). All specimens were presented to the clinical laboratory. The IC method was
performed immediately after the collection of specimens; the remainder of the specimen was stored at -20 °C until the time of testing. For swab specimens, one swab was used for the IC method immediately, and the other two swabs were washed using 500 μL of sterile saline and stored at -20 °C until the time of testing.

As the standard, one-tube RT-PCR was performed using the Ampdirect® kit, which was sufficiently specific and sensitive for the detection of norovirus genogroups I and II (Shimadzu Biotech, Kyoto, Japan)³. The newly developed Diagnode Enteric Viral Panel Real-Time PCR kit (EV-kit) for use with the BD MAX® system (Becton Dickinson) was used as the automated real-time RT-PCR method, and the ImmunoCatch-Noro® (Eiken Chemical, Tokyo, Japan) system was used as the IC method. The EV-kit can detect norovirus genogroup I, II and rotavirus, though the manufacture does not recommend to report genogroups of Norovirus. These three tests were performed according to the manufacturer’s instructions. The total times required for the analysis of 10 specimens using the EV-kit and the RT-PCR method were recorded by the laboratory technicians.

C. Statistics
The sensitivity, specificity, positive predictive value, and negative predictive value were determined using the standard methods. Categorical variables were analyzed by Fisher’s exact test. JMP® version 10.0.0 was used to analyze these data. A difference having a p value <0.05 was defined as statistically significant. Concordance between the results of the EV-kit and the IC method with the results of RT-PCR was evaluated by determining the Cohen’s kappa coefficient (κ). Almost perfect concordance was defined as κ≥0.81, substantial concordance was defined as 0.80≥κ≥0.61, moderate concordance was defined as 0.60≥κ≥0.41, and fair concordance was defined as 0.40≥κ≥0.21.
III. Results

Of 144 outpatients with diarrhea, 50 patients were excluded for the following reasons: 30, no consent obtained; 11, no specimens; 7, other diagnoses; and 2, under 20 years old. Thus, 94 patients (45 male and 49 female) with a median age of 29 years (range 20-83 years) were included in this study.

Of 94 specimens analyzed, 35 (37.2%) were positive by RT-PCR and all of them were genogroup II. Thirty nine (41.4%) and 25 (26.6%) specimens were positive by the EV-kit and IC method, respectively. Figure 1 shows the results for all specimens. The sensitivity and specificity of the EV-kit were 91.4% (32/35) and 88.1% (52/59), whereas those of IC were 54.3% (19/35) and 89.8% (53/59), respectively. The Cohen’s kappa coefficient comparing RT-PCR and EV-kit results was 0.78, categorized as substantial concordance, and was 0.47 for RT-PCR and IC, categorized as moderate concordance. Overall, the EV-kit had significantly higher and more reliable sensitivity than did IC ($p=0.0009$). No specimens contained rotavirus RNA by the EV-kit.

Figure 2A shows the results for analysis of bulk stool specimens submitted by 53 patients. The sensitivity and specificity for the EV-kit were 92.3% (24/26) and 96.3% (26/27), whereas those for IC were 61.5% (16/26) and 88.9% (24/27), respectively. The
Cohen’s kappa coefficient for RT-PCR and the EV-kit was 0.89, categorized as almost perfect concordance, whereas it was 0.51 for RT-PCR and IC, categorized as moderate concordance. For bulk stool specimens, the EV-kit had significantly higher and more reliable sensitivity than did IC ($p=0.0188$).

Figure 2B shows the results of rectal swab specimens submitted by 41 patients. The sensitivity and specificity for the EV-kit were 88.9% (8/9) and 81.3% (26/32), whereas those for IC were 33.3% (3/9) and 90.6% (29/32), respectively. The Cohen’s kappa coefficient for RT-PCR and the EV-kit was 0.59, categorized as moderate concordance, and for RT-PCR and IC was 0.27, categorized as fair concordance. For rectal swab specimens, the EV-kit had significantly higher sensitivity than did IC ($p=0.0498$). The total amounts of time required to analyze 10 specimens by the EV-kit and by RT-PCR were about 210 minutes and 335 minutes, respectively.
The present study compared use of the first fully automated real-time RT-PCR method (EV-kit) with IC, using the RT-PCR method as the gold standard. This study had two major findings. First, the sensitivity of the EV-kit for detection of norovirus was significantly higher and more reliable than that of the IC method. Secondly, the sensitivity of the EV-kit for analysis of rectal swab specimens was almost equivalent to that for use with bulk stool specimens.

Previous studies reported that the sensitivity of real-time RT-PCR ranged from 83.5% to 100%, and the specificity ranged from 78% to 100%\(^7\text{-}^{11}\); other studies noted the sensitivity of IC tests ranging from 17.0% to 83.3%, and specificity ranging from 47% to 100\(^1,^{12},^{13}\). In this study, the EV-kit had significantly higher sensitivity than did IC, similar to the previous reports of real-time RT-PCR methods. In particular, the EV-kit had substantial concordance with RT-PCR overall, so the present results confirm the high sensitivity and specificity of the EV-kit to reliably detect norovirus. The three specimens showed pseudo-negative results of EV-kit. Two stool samples were collected more than 2 days after onset of the disease, and one sample was collected by rectal swab. These might cause low quantity of extracted RNA and low sensitivity, though overall
sensitivity of the EV-kit with bulk stool specimens and with rectal swab specimens in this study was high enough as 92.3% and 88.9%, respectively. Two previous studies noted that the sensitivity of real-time RT-PCR for rectal swab specimens was almost equal to that for bulk stool specimens\(^\text{14, 15}\)). In the present study, the EV-kit had results compatible with those previously reported for real-time RT-PCR, suggesting the possibility that rectal swab sampling could substitute for bulk stool specimens when either immediate collection or a large number of specimens is desirable. In addition, IC for both rectal swab specimens and bulk stool specimens had lower sensitivity than did the EV-kit for rectal swab specimens. This result indicates the greater usefulness and reliability of the EV-kit compared to IC methods, regardless of specimen type. The shorter time required for the EV-kit than for RT-PCR is an additional advantage for its use for detection of norovirus infection in the clinical laboratory.

There are some limitations of the present study. One is that this study was carried out for 5 months in one season in a single hospital. In addition, the analytical sensitivity of the EV-kit was reported to be influenced by the epidemic genotypes and the condition of the stool specimens used (data shown in the manufacturer’s instructions). A multicenter study spanning multiple years is required to determine whether the results of this study are generally applicable.
V. Conclusion

The sensitivity and specificity of the EV-kit are high enough, and the test is reliable enough, to use as a primary test for detection of norovirus. In particular, the EV-kit can be used for not only bulk stool specimens but also rectal swab specimens with equivalent sensitivity and specificity. The EV-kit is also an automated process which requires less working time than RT-PCR.

Acknowledgement

None.

Ethical approval

The present study was conducted in accordance with the ethics guidelines of the Declaration of Helsinki and in agreement with the Ethics Committee of Juntendo University. We informed each potential subject of the details of our study, and once informed consent had been received, stool specimens and medical data were collected and analyzed anonymously. The whole process did not harm patients’ health, safety or privacy. This consent procedure has been approved by the Ethics Committee of
Juntendo University Hospital (Approval number 13-109).

**Declaration of Interest**

This study was supported by Becton Dickinson, New Jersey, USA for providing test reagents for the real-time RT-PCR kit and lending us the real-time PCR instrument: BD MAX™ System.
References


Figure Legend

Figure 1.

Comparison of EV-kit and IC results for all specimens. The sensitivity of the EV-kit for detection of norovirus was significantly higher than that of IC, using RT-PCR as the gold standard ($p=0.0009$). EV-kit; Diagnode Enteric Viral Panel Real-Time PCR kit®, IC; immunochromatographic method, PPV; positive predictive value, NPV; negative predictive value, K; Cohen’s kappa coefficient. *$p<0.01$.

Figure 2A, 2B.

Higher sensitivity of the EV-kit for both bulk stool specimens (n=53, 2A) and rectal swab specimens (n=41, 2B) than IC, using RT-PCR as the gold standard ($p=0.0188$ and 0.0498, respectively). EV-kit; Diagnode Enteric Viral Panel Real-Time PCR kit®, IC; immunochromatographic method, PPV; positive predictive value, NPV; negative predictive value, K; Cohen’s kappa coefficient. **$p<0.05$
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Sensitivity of EV-kit = 91.4%*
Specificity of EV-kit = 88.1%
PPV of EV-kit = 82.1%
NPV of EV-kit = 94.5%
Kappa of EV-kit = 0.78

Sensitivity of IC = 54.3%*
Specificity of IC = 89.8%
PPV of IC = 76.0%
NPV of IC = 76.8%
Kappa of IC = 0.47
Figure 2a

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Figure 2b

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<td></td>
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Sensitivity (EV-kit): 88.9%**
Sensitivity (IC): 33.3%**
Specificity (EV-kit): 81.3%
Specificity (IC): 90.5%
PPV (EV-kit): 57.1%
PPV (IC): 50.0%
NPP (EV-kit): 96.3%
NPP (IC): 82.9%
K (EV-kit): 0.59
K (IC): 0.27
レイアウト調整
Kを小数第2位までにした
Figure 1  All specimens (n=94)

<table>
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Sensitivity=91.4%*
Specificity=88.1%
PPV=82.1%
NPV=94.5%
K=0.78

Sensitivity=54.3%*
Specificity=89.8%
PPV=76.0%
NPP=76.8%
K=0.47
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**Sensitivity=92.3%**

**Specificity=96.3%**

**PPV=96.0%**

**NPP=92.8%**

**K=0.89**

**Sensitivity=61.5%**

**Specificity=88.9%**

**PPV=84.2%**

**NPP=70.6%**

**K=0.51**
## Figure 2b  Rectal swab specimens (n=41)

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Sensitivity=88.9%**
Specificity=81.3%
PPV=57.1%
NPP=96.3%
K=0.59

Sensitivity=33.3%**
Specificity=90.6%
PPV=50.0%
NPP=82.9%
K=0.27