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Evaluation of the SD Bioline Norovirus rapid immunochromatography test using fecal specimens from Korean gastroenteritis patients

Hyun Soo Kim*, Jeongwon Hyun, Jae-Seok Kim, Wonkeun Song, Hee Jung Kang, Kyu Man Lee

Department of Laboratory Medicine, Hallym University College of Medicine, Seoul, Republic of Korea

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The analytical and clinical performance of a new rapid immunochromatography test, the SD Bioline Norovirus test, was evaluated for the detection of human norovirus in fecal specimens. The analytical performance studies were performed for detection limit, reproducibility, cross-reactivity, and interference. For comparison, 92 norovirus-positive stool samples and 126 norovirus-negative samples for which the results were confirmed by 2 different real-time PCR kits were used. The rapid immunochromatography test detected the equivalent of 4.48×10^6 copies/mL of the norovirus genome in stool samples. On performing the repeatability/reproducibility test, samples above this concentration all provided positive results (100%) and 97.8% of the samples slightly below this concentration (2.45×10^6 copies/mL) provided negative results. No cross-reactivity or interference was detected. Positive percent agreement (sensitivity), negative percent agreement (specificity), and overall percent agreement of the rapid immunochromatography test compared with testing by real-time PCR were 90.2%, 100%, and 95.9%, respectively. In addition, the rapid immunochromatography test was completed within 20 min. The SD Bioline Norovirus test was, therefore, easier and more rapid to perform and showed excellent reproducibility, no cross-reactivity, no interference, and high agreement compared with real-time PCR. Thus, this test is useful for rapid screening to identity norovirus infection.

1. Introduction

Norovirus is the most common causative organism responsible for acute gastroenteritis epidemics in all age groups worldwide (Fankhauser et al., 2002; Glass et al., 2009). In the US, norovirus was detected in 217 of 233 (93%) epidemic acute gastroenteritis cases between 1997 and 2000 (Fankhauser et al., 2002). In addition to epidemic infection, sporadic infection is also common, and the prevalence rate varies slightly by area and by time (Glass et al., 2009; Fankhauser et al., 2002; Park et al., 2010a,b). Norovirus infection is common in South Korea during autumn and winter, and its prevalence is reported to be 9.8–35.8% (Park et al., 2010a,b; Park et al., 2011; Chung et al., 2010).

Norovirus is an RNA virus of the *Caliciviridae* family and is classified into 5 genogroups (GI–GV) according to the polymerase and capsid protein sequences (Glass et al., 2009). Over 30 genotypes have been reported to date (Patel et al., 2009; Glass et al., 2009). Of these, several strains of GI, GII, and GIV have been identified in humans, and human norovirus infection is known to be caused primarily by 8 genotypes of the GI genogroup and 17 of the GII genogroup (Patel et al., 2009; Glass et al., 2009).

Many attempts have been made to establish diagnostic methods for norovirus infection, and detection of norovirus in stool specimens has been performed using the techniques of electron microscopy, enzyme immunoassay, immunochromatography (ICG), Western Blotting, RT-PCR, and real-time PCR (Atmar and Estes, 2001). Among these assays, RT-PCR and real-time PCR are recognized as the most sensitive methods currently available, but the drawbacks are that they require expensive special equipment and skilled technologists. Additionally, they are more time consuming and costlier than the rapid ICG test. This study was performed to evaluate the analytical and clinical performance of a newly developed rapid ICG test (SD Bioline Norovirus test) for detecting human norovirus genogroups GI and GII in stool specimens.

2. Materials and methods

2.1. Fecal samples

Two hundred and eighteen frozen fecal suspensions (10–20% fecal suspension diluted with saline) collected and stored at −70 °C from August 2010 to April 2011 were tested retrospectively in this study. These comprised 92 norovirus-positive stool samples and

* Corresponding author at: Department of Laboratory Medicine, Hallym University College of Medicine, 94-200 Yeongdeungpo-dong, Yeongdeungpo-gu, Seoul 150-719, Republic of Korea. Tel.: +82 2 2639 5562; fax: +82 2 2671 5270.
E-mail address: hskim0901@empal.com (H.S. Kim).

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126 norovirus-negative samples, confirmed using 2 different real-time PCR kits (RIDA GENE Norovirus V, R-Biopharm, Darmstadt, Germany; and AccuPower Norovirus Real-time RT-PCR kit, Bioneer, Daejeon, Korea). Of these, 129 samples (59.2%) were from male patients and 188 samples (86.2%) were from patients less than 5 years old. This study was approved by the Institutional Review Board of Hangang Sacred Heart Hospital. Real-time PCR assays using the stored frozen fecal suspensions were performed in June 2011, and the SD Bioline Norovirus test (Standard Diagnostics, Yongin, Korea) was performed on the same stored fecal samples during November 2011, that is, 5 months later.

2.2. SD Bioline Norovirus test

According to the SD Bioline Norovirus reagent package insert, SD Bioline Norovirus test is a quick, qualitative ICG test that detects human norovirus genogroups GI and GII in stool samples. The norovirus in the specimen reacts with colloidal gold-labeled norovirus-specific monoclonal antibodies recognizing both GI and GII strains, thus forming a complex of antigen-antibody colloidal gold conjugates. As this complex migrates along the result window by capillary action, it is captured by norovirus-specific monoclonal antibodies immobilized in the test line positioned across the result window, and a colored line is generated. In the absence of norovirus, a complex is not formed, and no colored line appears. Frozen fecal suspensions were warmed to room temperature, and 100 μL of fecal suspension was added to a tube containing 1 mL of diluent (1:10 dilution) and mixed well. Four to 5 drops (approximately 100–125 μL) were added to the sample well of the test device, and results were read after 15 min. In samples with negative ICG and positive real-time PCR results, 200 μL of fecal suspension was mixed with 200 μL diluent (1:1 dilution) instead of 1 mL diluent, and the test was repeated.

2.3. Norovirus real-time PCR assay

RNA extracts were prepared from fecal suspensions by using a QIAamp Viral RNA mini kit (Qiagen, Hilden, Germany) and a QIAcube platform (Qiagen). Two real-time PCR kits (RIDA GENE Norovirus V and AccuPower Norovirus Real-time RT-PCR kit) were used. For the RIDA GENE Norovirus V assay (R-Biopharm, Darmstadt, Germany), 5 μL of RNA extract was mixed with 20 μL of master mix and real-time RT-PCR was performed using a PCR thermocycler (Rotor-Gene Q, Qiagen) with the following program: reverse transcription at 50 °C for 15 min; 45 cycles of denaturation at 95 °C for 15 s and annealing/extension at 55 °C for 30 s. For the assay with the AccuPower Norovirus Real-Time RT-PCR kit (Bioneer, Daejeon, Korea), 5 μL of RNA extract was mixed with 45 μL of master mix, and real-time RT-PCR was performed using a PCR thermocycler (Exicycler 96 Real-Time Quantitative Thermal Block, Bioneer) with the following program: reverse transcription at 45 °C for 15 min; pre-denaturation at 95 °C for 5 min; 40 cycles of denaturation at 95 °C for 5 s and annealing/extension at 55 °C for 5 s. All the procedures were performed according to the manufacturer's instructions. To quantify the copy number of norovirus RNA, *in vitro* transcribed RNA calibrators were prepared as follows. The amplicon of the target region in the norovirus genome was cloned into the pGEM-T easy vector (Promega, Madison, WI, USA), and HIT-DH5α competent cells (RBC Bioscience, New Taipei City, Taiwan) were then used for transformation. The cloned plasmid was purified using the Accupower plasmid mini kit (Bioneer), and *in vitro* transcription of the target region was conducted by MAXIscript® SP6 Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The mass of *in vitro* transcribed RNA was determined using a NanoDrop 2000 system (Thermo Fisher Scientific, Wilmington, DE, USA) and converted to copy number ([X g/μL RNA/(transcript length in nucleotides × 340)] × 6.022 × 10^{23} = Y molecules/μL; http://www.qiagen.com/resources/info/guidelines_rtpcr/quantifying.aspx). The RNA copy number in the samples was calculated on the basis of the Ct (threshold cycle, the number of cycles at which the fluorescence exceeds the threshold) value, with the corresponding RNA copy number determined from calibration curves prepared using RNA calibrators (Yang et al., 2002).

2.4. Norovirus genotyping

Norovirus genotyping was conducted as described previously (Kim et al., 2008). For 1-step RT-PCR, the specific primers GI-F1M (nt 5342)/GI-R1M (nt 5671) and GII-F1M (nt 5058)/GII-R1 M (nt 5401) targeting open reading frame 2 (ORF2)-encoding capsid protein (VP1) were used. Nested PCR was also performed using the primers GI-F2 (nt 5357)/GI-R1M (nt 5671) and GII-F3 (nt 5088)/GII-R1M (nt 5401).

The products from the nested PCR were purified using a MEGA quick-spin PCR kit (INTRON Biotechnology, Seongnam, Korea), and then cloned into the pGEM T-easy vector and analyzed by DNA sequencing. The nucleotide sequences were analyzed using ABI Prism BigDye Terminator version 3.1 (Applied Cosmogenetech, Seoul, Korea) and an automated norovirus genotype tool was used to determine norovirus genotypes (available at: http://www.rivm.nl/mpf/norovirus/typingtool) (Kroneman et al., 2001).

2.5. Reproducibility and limit of detection

Twofold serial dilutions of pooled positive fecal suspension prepared in saline were used to evaluate the reproducibility and the detection limit for the ICG assay. Each diluted sample was tested in triplicate, over a period of 10 days, using SD Bioline kits from 3 different lots (lot numbers 189001, 189002, and 189003). The positive rates of repeated tests for each concentration were calculated to determine the reproducibility. The norovirus concentration in each sample was measured by real-time PCR (Bioneer), and the number of norovirus RNA copies/mL for each sample was recorded.

2.6. Cross-reactivity

Cross-reactivity was examined with multiple strains of viruses, bacteria, and fungi, which have been listed below. For viruses, the virus culture supernatant was used to evaluate cross-reactivity. For bacteria and fungi, the colonies were diluted with saline to prepare a suspension with a density equivalent to 0.5 McFarland by using a densitometer (Vitek Densichek; bioMérieux, Marcy L'Etoile, France).

Viruses: Rotavirus from the American Type Culture Collection ([ATCC] Number VR-2018; http://www.atcc.org); adenovirus type 40 (ATCC VR-931), type 41(ATCC VR-930), type 31 (ATCC VR-1109), type 11 (ATCC VR-12), type 8 (ATCC VR-1604), type 37 (ATCC VR-929), type 1 (KBPV-VR-57 from Korea Bank for Pathologic Viruses [KBPV]; http://knrrb.knrrc.or.kr), type 2 (KBPV-VR-58), type 3 (KBPV-VR-2), type 4 (KBPV-VR-60), type 8 (KBPV-VR-3), type 18 (KBPV-VR-4), type 23 (KBPV-VR-5); enterovirus type 71 (ATCC VR-784); cytomegalovirus (ATCC VR-538); poliovirus type 1 (ATCC VR-58); Coxsackievirus A type 9 (ATCC VR-186); Coxsackievirus B type 3 (ATCC VR-688); Coxsackievirus B type 5 (ATCC VR-1036); Coxsackievirus B type 6 (ATCC VR-1037); BK virus (ATCC VR-837); herpes simplex virus type 1 (ATCC-VR-733); respiratory syncytial virus (ATCC VR-26); parainfluenza virus type 2 (ATCC VR-92); parainfluenza virus type 3 (ATCC VR-93); rhinovirus type 14 (ATCC VR-284); echovirus type 7 (ATCC VR-37); coronavirus (ATCC VR-740, -759); and mumps virus (ATCC VR-106).

Table 1
Comparison of the results of rapid immunochromatography test and real-time PCR assays for the detection of norovirus in stools.

Immunochromatography	Real-time PCR	No. of specimen (%)
Positive	Positive	83 (38.1%)
Negative	Positive	9 (4.1%)
Positive	Negative	0 (0.0%)
Negative	Negative	126 (57.8%)
Total		218 (100%)

Percent positive agreement of rapid immunochromatography test compared to PCR: 90.2% (83/92) (95% confidence interval, 82.2–95.4%).
Percent negative agreement of rapid immunochromatography test compared to PCR: 100% (126/126) (95% confidence interval, 97.1–100%).
Overall percent agreement of rapid immunochromatography test compared to PCR: 95.9% (209/218) (95% confidence interval, 92.3–98.1%).

Bacteria: *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella oxytoca* (ATCC700432), *Pseudomonas aeruginosa* (ATCC 27853), *Neisseria gonorrhoeae* (ATCC 49226), Methicillin-resistant *Staphylococcus aureus* (ATCC 43300), *Clostridium perfringens* (KCCM 40946 from the Korean Culture Center of Microorganisms [KCCM]; http://kccm.or.kr), *Klebsiella pneumoniae* (KCCM 41285), *Aeromonas hydrophila* (KCCM 32586), *Enterobacter cloacae* (KCCM 12178), and *Vibrio parahaemolyticus* (KCCM 11965). *Salmonella* group B, *Salmonella* group C, *Salmonella* group D, *Salmonella* group E, *Shigella* group D, *Staphylococcus epidermidis*, *Serratia marcescens*, *Yersinia enterocolitica*, *Yersinia pseudoenterocolitica*, *Vibrio vulnificus*, *Salmonella typhi*, and *Clostridium difficile* were obtained from clinical isolates from patients.

Fungi: *Candida albicans* and *Candida parapsilosis* were obtained from clinical isolates from patients.

2.7. Interference testing

Interference tests were performed with the following substances: human blood from a patient sample, barium sulfate (contrast medium), loperamide (anti-diarrhea drug; Janssen, Korea), metronidazole (antibiotic; CJ Pharma, Korea), hemoglobin (Sigma–Aldrich, USA), bilirubin (Sigma–Aldrich, USA), and triglyceride mix (Sigma–Aldrich, USA). Each substance (5 mg) was dissolved in 1 mL of solvent (triglyceride in ether; bilirubin in chloroform; all others in distilled water), and 50 μL of solution was mixed with 950 μL of negative stool suspension (negative base pool) and 950 μL of low-positive stool suspension (low-positive base pool). For substances in liquid form (*e.g.*, blood), 50 μL of the substance was mixed with 950 μL of the negative and the low positive base pools.

2.8. Procedure time

The time required to complete the ICG test was determined for 3 operators by using fresh fecal samples. Each operator performed the ICG test 3 times, and the mean and standard deviation value of the procedure time (in minutes) for all 3 operators was calculated.

3. Results

3.1. Sensitivity, specificity, and agreement rate based on real-time PCR results

Eighty-three of the 92 real-time PCR-positive samples (90.2%) were positive by the ICG test, and all 126 real-time PCR-negative samples were negative by the ICG test (Table 1). The positive percent agreement (PPA, or sensitivity), negative percent agreement (NPA, or specificity), and overall percent agreement of the rapid ICG test relative to real-time PCR were 90.2% (95% confidence interval, 82.2–95.4%), 100% (95% confidence interval, 97.1–100%), and 95.9% (95% confidence interval, 92.3–98.1%), respectively. The ICG assay detected the GI.3, GII.3, GII.4, GII.6 and GII.12 genotypes by open reading frame 2 classifications in this study (Table 2). Nine samples were negative by ICG but positive by real-time PCR, and of these, 8 showed a low positive signal (Ct > 30) in the RIDA GENE Norovirus V assay, indicating a low concentration of norovirus (Table 3). The relationship between Ct values as determined by the RIDA GENE Norovirus V assay and ICG results is shown in Fig. 1.

Table 2
Detectability of norovirus genotypes by the immunochromatography (ICG) test.

Genotype	No. of samples	No. of ICG-positive samples	Detection rate
GI.3	1	1	100%
GII.3	14	14	100%
GII.4	65	59	90.8%
GII.6	1	1	100%
GII.12	2	1	50%
GII, untyped	9	7	77.8%
Total	92	83	90.2%

3.2. Reproducibility and detection limit

The reproducibility between replicate samples, days, and lots was 100% in positive fecal samples diluted 1:80, 1:160, and 1:1280, and in all negative fecal samples (Table 4). However, 95.6% and 2.2% positivity was observed for the positive fecal samples diluted 1:320 and 1:640, respectively. Lot no. 189003 was slightly more sensitive compared to the others, but the inter-lot variability for the detection limit of all 3 lots was less than that for 1-fold dilution. The ICG test could detect norovirus up to a dilution of 1:320, corresponding to a viral load of 4.48×10^6 copies/mL by the AccuPower Norovirus Real-time RT-PCR kit (Table 4).

3.3. Cross-reactivity

No cross-reactivity was observed for any of the 30 viruses, 24 bacteria, or 2 fungi tested (see Section 2 for details).

3.4. Interference

There was no interference by any of following substances: human blood, barium sulfate (contrast medium), loperamide

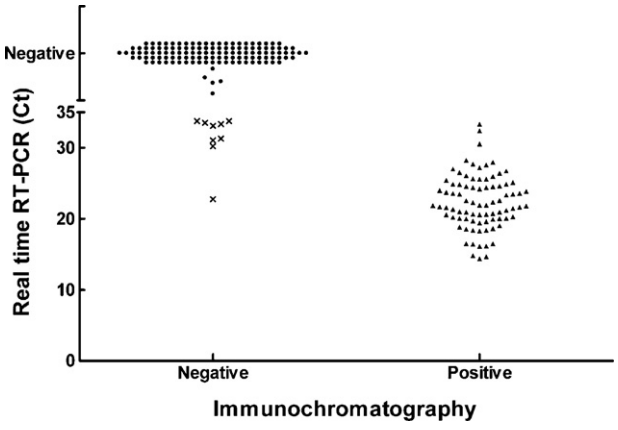

Fig. 1. Correlation of rapid immunochromatography test results with real time PCR results.
Abbreviation: Ct, threshold cycle. The specimens showing discrepant results among the rapid test and real-time PCR assay are presented as "x".

Table 3
Specimens showing discrepant results between the rapid ICG test and the real-time PCR assays for the detection of norovirus in stool.

Specimen	ICG	Real-time PCR (Ct by A kit[a])	Real-time PCR (Ct by B kit[a])	Genotype	Age/Sex	Clinical characteristics
1	N	P (30.23)	P (35.09)	GII.4	12 mo/M	AGE
2	N	P (22.76)	P (24.36)	GII.4	24 mo/M	AGE
3	N	P (31.29)	P (25.89)	GII.4	27 mo/F	AGE
4	N	P (33.75)	P (33.04)	GII	3 mo/M	AGE
5	N	P (33.09)	P (34.63)	GII.4	11 mo/F	AGE
6	N	P (33.75)	N (37.02)	GII.4	3 d/M	Fever
7	N	P (31.06)	P (22.51)	GII.12	6 y/M	AGE
8	N	P (33.52)	P (29.05)	GII.4	74 y/M	AGE
9	N	P (33.34)	P (15.31)	GII	8 y/M	AGE

Abbreviations: ICG, immunochromatography; Ct, threshold cycle; P, Positive; N, negative; G2, norovirus genogroup II; M, male; F, female; AGE, acute gastroenteritis.
[a] A kit is RIDA GENE Norovirus V assay and B kit is AccuPower Norovirus Real-time RT-PCR assay.

Table 4
Positive rates obtained in the repeatability/reproducibility test performed by immunochromatography (ICG) assay.

	Real-time PCR (copies/mL)	ICG (Lot 1: 189001) (n = 30, triplicate for 10 days)	ICG (Lot 2: 189002) (n = 30, triplicate for 10 days)	ICG (Lot 3: 189003) (n = 30, triplicate for 10 days)	Total (n = 90)
Pooled fecal suspension (1:80 dilution)	Positive (2.08×10^7)	100%	100%	100%	100%
Pooled fecal suspension (1:160 dilution)	Positive (1.25×10^7)	100%	100%	100%	100%
Pooled fecal suspension (1:320 dilution)	Positive (4.48×10^6)	86.7%	100%	100%	95.6%
Pooled fecal suspension (1:640 dilution)	Positive (2.45×10^6)	0%	0%	6.7%	2.2%
Pooled fecal suspension (1:1280 dilution)	Positive (1.09×10^6)	0%	0%	0%	0%
Pooled fecal suspension (Negative sample)	Negative	0%	0%	0%	0%

(anti-diarrhea drug), metronidazole (antibiotic), hemoglobin, bilirubin, or triglyceride mix.

3.5. Procedure time

The mean ± standard deviation value for 9 process times by 3 operators was 16.33 ± 0.25 min.

4. Discussion

In this study, the performance of a newly developed norovirus detection kit that uses the ICG technique was evaluated. If consistent positive or negative results from 2 different real-time PCR assays are regarded as "true positives" or "true negatives," the sensitivity of the new assay is 90.2% and the specificity is 100%. Commercial norovirus antigen test kits can be broadly classified into 2 types: the ICG type and the ELISA type. The RIDAQUICK Norovirus assay from R-Biopharm is the most widely used ICG assay, and the QuickNavi-Noro kit (Denka Seiken, Japan), IP-NORO kit (Immuno-Probe, Japan), and IC kit (Morinaga Milk Industry, Japan) have also been introduced. For ELISA testing, the RIDASCREEN Norovirus kit, IDEIA Norovirus kit (Oxoid, UK), and Denka ELISA kit (Denka Seiken, Japan) are currently being used. However, various sensitivities and specificities have been reported for these kits, even for different studies using a single test kit. The sensitivity of 90.2% (95% confidence interval, 82.2–95.4%) obtained in this study for the SD Bioline ICG assay is similar to or higher than the reported sensitivities of the RIDAQUICK (57.1–83%), QuickNavi-Noro (88.6%), IP-NORO (73.7–78.9%), IC Kit (75.4%), RIDASCREEN (36–92%), IDEIA (38–76.9%), and Denka ELISA (80–90.4%) tests (Bruggink et al., 2011; Bruin et al., 2006; Bruins et al., 2010; Burton-MacLeod et al., 2004; Costantini et al., 2010; Derrington et al., 2009; Khamrin et al., 2008, 2009; Kim et al., 2011; Kirby et al., 2010; Nguyen et al., 2007; Siqueira et al., 2011; Wilhelmi de Cal et al., 2007). Its specificity of 100% (95% confidence interval, 97.1–100%) is the same as or better than that of existing ICG tests (89.5–100%) and ELISA methods (69–100%) (Bruin et al., 2006; Bruins et al., 2010; Burton-MacLeod et al., 2004; Castriciano et al., 2007; Costantini et al., 2010; Derrington et al., 2009; Khamrin et al., 2008, 2009; Kim et al., 2011; Kirby et al., 2010; Nguyen et al., 2007; Park et al., 2012; Siqueira et al., 2011; Wilhelmi de Cal et al., 2007). Furthermore, there were no cross-reactions with various types of viruses, bacteria, or fungi, and no interference by substances that can appear in the feces, such as blood, hemoglobin, bilirubin, triglyceride, anti-diarrhea drug (loperamide), and antibiotics (metronidazole).

Very few studies have measured the detection limit of norovirus antigen test kits. The detection limit (analytical sensitivity) of the kit used in this study was 4.48×10^6 copies/mL, which is similar to the detection limit of another ICG method (Takanashi et al., 2008) and the IDEIA ELISA kit (Costantini et al., 2010), which was determined to be 10^6–10^7 copies/g of stool.

As mentioned previously, different studies using the same test kit have reported differing sensitivities and specificities. Therefore, the detection limit of different kits can be accurately compared using serial dilutions of the same clinical specimen, as shown in Table 4. In this study, the ICG test had inferior analytical sensitivity (*i.e.*, a higher detection limit) than real time PCR tests. This is supported by the observation that (1) no specimens tested positive in ICG and negative in PCR, whereas 9 specimens tested positive in PCR and negative in ICG; and (2) the Ct values of 8 of these specimens were >30, indicating low norovirus concentrations.

Several reasons may contribute to the differences in sensitivity and specificity among the samples analyzed using the same kit. The first reason is the characteristics of the specimen, including whether the specimen is from an adult or a child, whether it is a specimen taken during an outbreak period, whether it has been taken from a subject strongly suspected to be infected with norovirus or from a healthy subject, whether it is a stored or fresh specimen, and the conditions of specimen storage or treatment (if any). The second reason is that the sensitivity and specificity of the test method can vary depending on the sensitivity and specificity of the comparative method, real-time PCR. The third reason

is the diversity of genotypes within norovirus-positive specimens or the distribution of norovirus concentrations within samples. As norovirus comprises genogroups I–V and different genogroups or subtypes can be detected with different efficiencies by different kits, the sensitivity or specificity of the kit can differ depending on the range of genotypes in the samples. According to the manufacturers' data, the test kits used in this study could detect the GI.1, 4, and 11, and GII.1, 2, 3, 4, 5, 6, 10, 11, 14, and 16 genotypes. Another important factor in determining sensitivity is the virus concentration in the positive specimen group. The Ct values for real-time PCR for most PCR-positive samples in this study were 15–30, indicating that they contain high concentrations of norovirus. The fact that the specimens used were identified as positive or negative in 2 different PCR tests could increase the sensitivity and specificity of the assays in this study. Another factor that influenced the sensitivity and specificity in this study was the use of pre-diluted (10–20% fecal suspension; 1:5–1:10 dilution) frozen fecal suspension. According to the manufacturer's instruction, fresh, refrigerated, or frozen fecal specimens were recommended, not pre-diluted frozen fecal suspensions. In this study, therefore, in the case of samples with negative ICG and positive real-time PCR results, 200 μL of fecal suspension was mixed with 200 μL diluent instead of 1 mL diluent (total dilution titer was 1:10–1:20 dilution, which is similar to that of the original procedure of this assay using stool), and the test was repeated. Because of the similar dilution titer in the repeated test, this modified procedure involving pre-dilution might not decrease the sensitivity of the assay. However, the protocol in this study could increase the specificity of the assay due to the absence of repeated measurement with pre-diluted samples showing negative results.

An important characteristic of this study is that the detection limit, reproducibility, and inter-lot variability were evaluated by using serially diluted fecal samples, although the test kit is designed to provide qualitative and not quantitative results. The reproducibility was nearly 100% at concentrations above or below the detection cutoff. Although lot 1 (lot no. 189001) exhibited the least sensitivity and lot 3 (lot no. 189003) showed the greatest sensitivity, the difference in sensitivity between the lots was within 1-fold dilution. The difference between lots may explain the varying sensitivities that have been reported although the same test kit was used, and continuous improvements in the kits may explain the increased sensitivity in more recent reports.

In conclusion, the SD Bioline Norovirus test showed good analytical and clinical sensitivity, excellent specificity, no interference, no cross-reactivity, and excellent reproducibility. Therefore, this test is recommended as a fast and easy-to-use method for detecting and diagnosing norovirus infection.

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