

Title

Evaluation of rapid immunochromatographic tests for norovirus in neonatal and infant fecal specimens

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Abstract

Objectives: The accuracy of rapid immunochromatographic tests for norovirus in neonatal and infant fecal specimens has not been sufficiently evaluated. This study evaluated the diagnostic performance of two norovirus rapid immunochromatographic kits (QuickNavi-Norovirus® and QuickNavi-Norovirus 2®; Denka Seiken, Niigata, Japan) for neonatal and infant fecal specimens.

Methods: We collected infant fecal specimens every month from 0 to 12 months old. Fecal specimens were tested using the two types of rapid immunochromatographic kit. Real-time reverse transcription polymerase chain reaction (RT-PCR) testing was employed for all specimens as the standard for norovirus detection. Diagnostic performances of the two kits were calculated and compared.

Results: A total of 346 fecal specimens were evaluated. Specificities of the QuickNavi-Norovirus and QuickNavi-Norovirus 2 were 80% (275/343) and 99% (339/343), respectively. Specificity of the QuickNavi-Norovirus in the neonatal period was only 33% (23/70), but this gradually increased with postnatal age. By 4 months old, specificity had increased to 93%. With regard to QuickNavi-Norovirus 2, specificity was over 94% from 0 through 12 months old.

Conclusions: QuickNavi-Norovirus 2 offered improved performance, and appears more useful than QuickNavi-Norovirus to diagnose norovirus infections in the neonatal and infant periods.

Introduction

Norovirus is a major pathogen in epidemic gastroenteritis. Rapid diagnosis of norovirus infection is important for the early treatment, prevention, and control of outbreaks. In neonatal intensive care units (NICUs), rapid diagnosis of norovirus is particularly important. Nosocomial norovirus outbreaks in NICUs have been reported [1] and the clinical course can vary among patients, occasionally resulting in necrotizing enterocolitis [2, 3].

Rapid immunochromatographic tests for norovirus are now being used with increasing frequency. RIDA® QUICK Norovirus (R-Biopharm, Darmstadt, Germany),

ImmunoCardSTAT!® (Meridian Bioscience, Inc., Ohio, United States), QuickNavi®-Norovirus (Denka Seiken, Niigata, Japan) were reported to offer a sensitivity of 92%, 92% and 82% and a specificity of 98%, 98% and 97%, respectively [4-6]. We previously experienced a norovirus pseudo-outbreak caused by the low specificity of QuickNavi-Norovirus in neonatal fecal specimens [7]. In that case, 18 out of 40 (45.0%) fecal specimens from 10 out of 14 (71.4%) neonates were false positives. Few studies have evaluated the effectiveness of rapid immunochromatographic tests for norovirus in neonatal fecal specimens. In particular, no studies have evaluated the effectiveness of QuickNavi-Norovirus and its newly developed successor (QuickNavi-Norovirus 2®; Denka Seiken) in neonatal fecal specimens.

This study compared the diagnostic performance of QuickNavi-Norovirus and QuickNavi-Norovirus 2 for neonatal and infant fecal specimens.

Materials and Methods

This prospective single-center study was conducted in the Department of Pediatrics at Fukuyama Medical Center, Hiroshima, Japan. We studied 81 healthy, term neonates (42 girls). Mean (\pm standard deviation) gestational age was 39.3 ± 1.4 weeks (range, 37.1-41.9 weeks), and mean birth weight was 3017 ± 311 g (range, 2480-3994 g). We asked all parents to bring infant fecal samples every month from 0 to 12 months old. We collected the fecal specimens from May 24, 2010 to March 29, 2012. Fecal specimens were numbered for later identification, stored in a container, kept frozen and transferred to Denka Seiken Kagamida Factory (Niigata, Japan).

Fecal specimens were tested using the two types of rapid immunochromatographic kit for norovirus (QuickNavi-Norovirus and QuickNavi-Norovirus 2). Both kits included monoclonal antibodies against norovirus genogroups I(GI) and II(GII) [6, 8]. Fecal specimens were prepared in dilution buffers supplied in the kits and assays were conducted in accordance with the manufacturer's instructions. Monoclonal antibodies and composition of the dilution buffer differed between QuickNavi-Norovirus and QuickNavi-Norovirus 2 [8].

Real-time reverse transcription polymerase chain reaction (RT-PCR) testing was

employed for all specimens as the standard for norovirus detection, in accordance with the method described by Kageyama et al. [9]. Primer sets and fluorescent probes for the Norovirus ORF1-ORF2 junction region were used in real-time RT-PCR. The primer pair COG1F-COG1R and a mixture of fluorescent probes, RING1(a)-TP and RING1(b)-TP, were used to detect GI. The primer pair COG2F-COG2R and the fluorescent probe RING2-TP were used to detect GII. We had committed the testing of two types of rapid immunochromatographic kit and the real-time RT-PCR testing to Denka Seiken Co., Ltd.. Diagnostic performances of the QuickNavi-Norovirus and QuickNavi-Norovirus 2 were calculated for each kit and compared.

Informed consent was obtained from the parents of all subjects prior to participation in the study, which was approved by the Institutional Ethics Committee in accordance with the Helsinki Declaration.

Results

A total of 362 fecal specimens were examined. Among these, 343 specimens were evaluated; 3 specimens that were obtained by enema, which gives false positive results with high probability [6], and 16 specimens that could not be determined by QuickNavi-Norovirus due to the absence of control line signals were excluded. For QuickNavi-Norovirus 2, no specimens were excluded.

Three specimens from three different infants with no symptoms were found to be positive for norovirus by real-time RT-PCR testing, and norovirus GII cDNA was detected in all three specimens. These three specimens also yielded positive results from both the QuickNavi-Norovirus and QuickNavi-Norovirus 2.

Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the QuickNavi-Norovirus and QuickNavi-Norovirus 2 are shown in Table 1. The specificity of QuickNavi-Norovirus in the neonatal period was only 33% (23/70), but specificity tended to increase with postnatal age (Fig. 1). On the other hand, specificity of QuickNavi-Norovirus 2 was 94% (66/70) in the neonatal period and 100% from 1 through 12 months old (Fig. 1).

Discussion

Noroviruses were first identified by electron microscopy. Subsequently, molecular assays such as RT-PCR have been developed for their detection. Rapid detection tests have recently been developed based on enzyme immunoassay (EIA), enzyme-linked immunosorbent assay (ELISA), and immunochromatographic methods, such as QuickNavi-Norovirus and QuickNavi-Norovirus 2. QuickNavi-Norovirus was reported to offer a sensitivity, specificity, and accuracy of 82%, 97%, and 89%, respectively, in adults and children excluding neonates [6], while QuickNavi-Norovirus 2 was reported to show values of 92%, 98%, and 94%, respectively, in adults and children excluding neonates [8].

However, several studies have reported false-positive results using norovirus rapid detection tests in neonatal feces. Köhler et al. [10] reported that 25 of 37 patients in a NICU tested positive for norovirus according to ELISA tests with 73% sensitivity and 100% specificity. Thirteen samples from the positive cases showed negative results from RT-PCR testing. Weichers et al. [11] reported that 22 of 43 patients in a NICU tested norovirus positive using an EIA method offering 77% sensitivity and 86% specificity. Among the positive samples, 11 were tested with RT-PCR and all were confirmed negative. Niizuma et al. [12] reported 5 patients in a growing care unit who tested norovirus-positive on immunochromatographic tests with 74% sensitivity and 100% specificity. All 5 samples were confirmed as negative by RT-PCR testing. Such findings suggest the need for confirmation by molecular assays in conjunction with rapid detection tests when diagnosing norovirus infection in the neonatal period.

There are limitations that must be considered when interpreting the results of this study. Sensitivities of the QuickNavi-Norovirus and QuickNavi-Norovirus 2 could not be evaluated because only 3 fecal specimens were confirmed positive by real-time RT-PCR testing. Collecting norovirus-positive feces from neonates is difficult because of the low prevalence of the infection. The sensitivities of tests for neonatal and infant feces therefore need to be reevaluated in the future. We could not elucidate what caused the false positive in the neonatal samples. We speculate that unknown substances unique to neonatal and early infant feces cross-react and cause false-positive results with

QuickNavi-Norovirus. The use of enemas or suppositories, and specialized diets such as thickening agents may cause false-positives [6]. Such samples, however, were excluded from the present study. Saito et al. [8] evaluated the specificity of the QuickNavi-Norovirus 2 using rectal swab samples, which may have led to a decrease in specificity for QuickNavi-Norovirus. They reported that rectal swab samples can be used for QuickNavi-Norovirus 2, which offers sufficient specificity with rectal swab samples. However, no substances present in both rectal swabs and neonatal fecal specimens have yet been shown to cause cross-reactions.

False-positive results obtained by rapid detection tests in neonatal samples have previously been discussed for several viruses other than norovirus. Dawn et al. [13] reported a pseudo-outbreak of respiratory syncytial virus infection in a NICU due to cross-reactivity between a lung surfactant drug and the enzyme immunoassay offering 91% of sensitivity and 80% of specificity [14]. Margareta et al. [15] evaluated false-positive cases in a NICU using the latex agglutination test for adenovirus offering 46% of sensitivity and 99% of specificity [16]. Kenneth et al. [17] discussed rotavirus false-positive cases in a NICU using the enzyme immunoassay offering 98% of sensitivity and 92% of specificity [18]. The specificity of those rapid detection tests in neonates was thought to be lower than in adults and children. Similar findings have been reported in terms of false-positive results for rapid detection tests using neonatal samples. Special attention about lower specificities is needed when using these kits in the NICU settings. In addition, when new rapid detection tests are developed, possible decreases in specificity need to be considered.

In conclusion, we evaluated the diagnostic performance of two types of rapid immunochromatographic kits for norovirus (QuickNavi-Norovirus and QuickNavi-Norovirus 2) in neonatal and infant fecal specimens. Our findings suggest that QuickNavi-Norovirus 2 offers improved performance, and is more useful than QuickNavi-Norovirus to diagnose norovirus infections in the neonatal and infant periods.

Declaration of Conflicting Interest

A portion of this study was supported by Denka Seiken Co., Ltd., which provided the two types of rapid detection kit for norovirus (QuickNavi-Norovirus® and QuickNavi-Norovirus 2®) and performed the kits and real-time RT-PCR testing for this study, but played no role in the study design, experimental assays, data collection and analysis of the experimental data, interpretation of the experimental results, decision to publish, or preparation of the manuscript. Denka Seiken Co., Ltd. did not suggest any qualitative alterations to the experimental data throughout this study.

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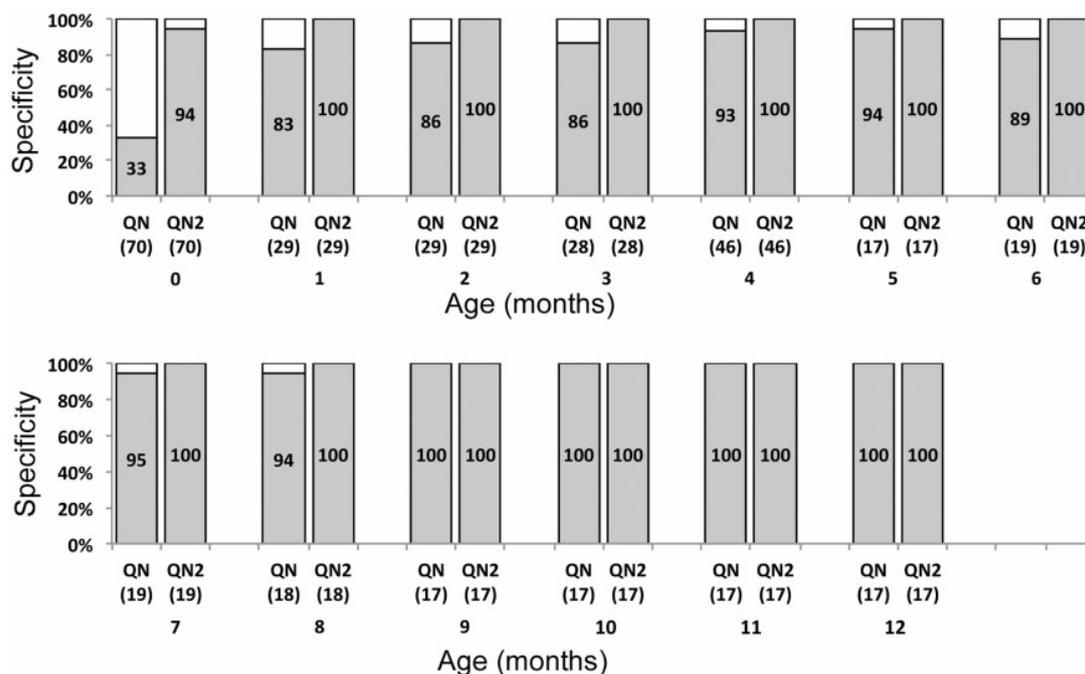
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Table 1. Diagnostic performances of QuickNavi-Norovirus and QuickNavi-Norovirus 2 in infants aged 0-12 months

	QuickNavi-Norovirus	QuickNavi-Norovirus2
Sensitivity	- (3/3)	- (3/3)
Specificity	80% (275/343)	99% (339/343)
Positive predictive value	4% (3/71)	43% (3/7)
Negative predictive value	100% (275/275)	100% (339/339)
Accuracy	80% (278/346)	99% (342/346)

Figure 1.



Specificities of QuickNavi-Norovirus (QN) and QuickNavi-Norovirus 2 (QN2) were compared from the neonatal period to 12 months old. Specificity of QN in the neonatal period was only 33% (23/70), compared to 93% by 4 months of age. The specificity of QN2 was 94% (66/70) in the neonatal period and 100% from 1 through to 12 months. QN2 showed high specificity even in neonates or early infants.