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

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Abstract

Objectives: To compare the diagnostic performance of two norovirus rapid immunochromatographic kits (QuickNavi®-Norovirus [QN] and QuickNavi®-Norovirus 2 [QN2]; Denka Seiken, Niigata, Japan) for neonatal and infant faecal specimens.

Methods: Monthly faecal samples were collected from infants from birth to 12 months of age, and tested for norovirus using QN and QN2. Real-time reverse transcription polymerase chain reaction (RT–PCR) was used as the gold standard for norovirus detection. The diagnostic performance of the kits was calculated.

Results: A total of 343 specimens from 81 infants were analysed. In all samples, the specificity of QN and QN2 was 80% (275/343) and 99% (339/343), respectively. In infants aged <1 month, the specificity of QN was 33% (23/70), increasing to 93% at 4 months of age. Specificity of QN2 was ≥94% in infants between 0 and 12 months of age.

Conclusions: QN2 offers improved performance and is more useful than QN for the diagnosis of norovirus infection in the neonatal and infant period.

Keywords

Norovirus, immunochromatography, rapid detection test, specificity, false positive, neonate

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Introduction

Norovirus is a major cause of epidemic gastroenteritis. Rapid diagnosis of norovirus infection is important for the early treatment, prevention and control of

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outbreaks, particularly in neonatal intensive care units (NICUs). Nosocomial norovirus outbreaks occur in NICUs,[1] and can occasionally result in necrotizing enterocolitis.[2,3]

Rapid immunochromatographic tests for norovirus include RIDA® QUICK Norovirus (R-Biopharm AG, Darmstadt, Germany), ImmunoCardSTAT!® (Meridian Bioscience, Inc., Ohio, United States) and QuickNavi®-Norovirus (Denka Seiken, Niigata, Japan). These tests have sensitivity of 92%, 92% and 82%, and specificity of 98%, 98% and 97%, respectively.[4–6] We have, however, reported a norovirus pseudo-outbreak resulting from the presumed reduced specificity of QuickNavi®-Norovirus for neonatal faecal specimens.[7] In that case, 18 of 40 (45.0%) faecal specimens from 10 of 14 (71.4%) neonatal patients were false positives.

Both QuickNavi®-Norovirus (QN) and its successor QuickNavi®-Norovirus 2 (QN2) include monoclonal antibodies against norovirus genogroups (G)I and II,[6,8] while specific monoclonal antibodies and the composition of the dilution buffer differ between the two kits.[8] QN2 has sensitivity, specificity and accuracy of 92%, 98% and 94%, respectively, in adults and children (excluding neonates).[8] The effectiveness of QN and QN2 has not been studied in neonatal faecal specimens.

The aim of the present study was to compare the diagnostic performance of QN and QN2 for neonatal and infant faecal specimens.

Patients and methods

Study population

This prospective, single-centre study was conducted in the Department of Paediatrics, Fukuyama Medical Centre, Hiroshima, Japan between May 24, 2010 and March 29, 2012. Faecal samples were collected monthly from all infants (with the exception of those admitted to the NICU) monthly, from birth to 12 months of age. Specimens were numbered for later identification, frozen and transferred to Denka Seiken Kagamida Factory (Niigata, Japan) for norovirus detection by immunochromatography and real-time reverse transcription polymerase chain reaction (RT–PCR).

The study was approved by the Institutional Ethics Committee, Fukuyama Medical Centre, Hiroshima, Japan, in accordance with the Declaration of Helsinki. The parents of each infant provided written informed consent prior to participation in the study.

Immunochromatography

Faecal specimens were tested for the presence of norovirus using QuickNavi®-Norovirus and QuickNavi®-Norovirus 2, according to the manufacturer's instructions. The diagnostic performance of each test was determined.

RT–PCR

All faecal specimens underwent real-time RT–PCR for norovirus detection, as described.[9]

Results

The study included faecal specimens from 81 healthy, full-term neonates (39 male/42 female; mean gestational age 39.3 ± 1.4 weeks [range 37.1–41.9 weeks]; mean birth weight 3017 ± 311 g [range 2480–3994 g]). A total of 362 faecal specimens were examined, 19 of which were excluded (three obtained by enema [resulting in a high probability of a false-positive result[6]]; 16 failed the QN test [due to absence of control lines]). The final analysis included 343 specimens. Real-time RT–PCR identified norovirus GII cDNA in three specimens from three separate asymptomatic infants. QN

Table 1. Diagnostic performance of QuickNavi®-Norovirus and QuickNavi®-Norovirus 2 (both Denka Seiken Co., Ltd., Niigata, Japan) for detection of norovirus in faeces specimens from healthy, full-term infants aged between 0 and 12 months (total 343 specimens from 81 infants). Real-time reverse transcription polymerase chain reaction was used as gold standard for norovirus detection.

Parameter	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)

Data presented as % (*n* specimens).

and QN2 tests were positive for all three specimens.

The overall sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of QN and QN2 are shown in Table 1. The specificity, PPV, NPV and accuracy of QN2 were superior to QN in infants aged 0–12 months. When stratified according to age, the specificity of QN was 33% (23/70) in the neonatal period (infants aged <1 month), rising to 93% (43/46) at 4 months of age (Figure 1). The specificity of QN2 was 94% (66/70) in the neonatal period and 100% each month, for infants between 1 and 12 months of age (Figure 1).

Discussion

The present study is the first to evaluate QuickNavi®-Norovirus and QuickNavi®-Norovirus 2 in faecal specimens from neonates and infants, and found that QN2 offers superior specificity to QN, particularly during the neonatal period (aged <1 month). Others have reported false-positive results using alternative norovirus rapid detection tests in samples of neonatal faeces. It was shown that 25/37 NICU patients tested positive for norovirus using an enzyme-linked immunosorbent assay with 73% sensitivity and 100% specificity, and 13 of these positive cases were negative when tested via RT–PCR.[10] In another report, 22/43 NICU patients were norovirus positive using an enzyme immunoassay (EIA) with 77% sensitivity and 86% specificity, but 11 of the positive samples were negative via RT–PCR.[11] Similar findings were reported in a NICU where five patients who were positive for norovirus (using an immunochromatographic test with 74% sensitivity and 100% specificity) were all shown to be negative for norovirus when tested using RT–PCR.[12] These data underscore the need for confirmation of infection by molecular assays, in conjunction with rapid detection tests, when diagnosing norovirus infection in the neonatal period.

Rapid-detection tests for viruses other than norovirus have also been shown to generate high numbers of false positives in neonatal samples. These include a pseudo-outbreak of respiratory syncytial virus in a NICU that was determined to be due to cross reactivity between a lung surfactant drug and the EIA used.[13] False positives have also been reported for adenovirus[14] and rotavirus.[15] The specificity of the tests used in these studies is thought to be lower in neonates than in adults and children.[16-18] Particular care should be taken when using these kits in the NICU setting. In addition, variations in specificity should be considered when new rapid detection tests are developed.

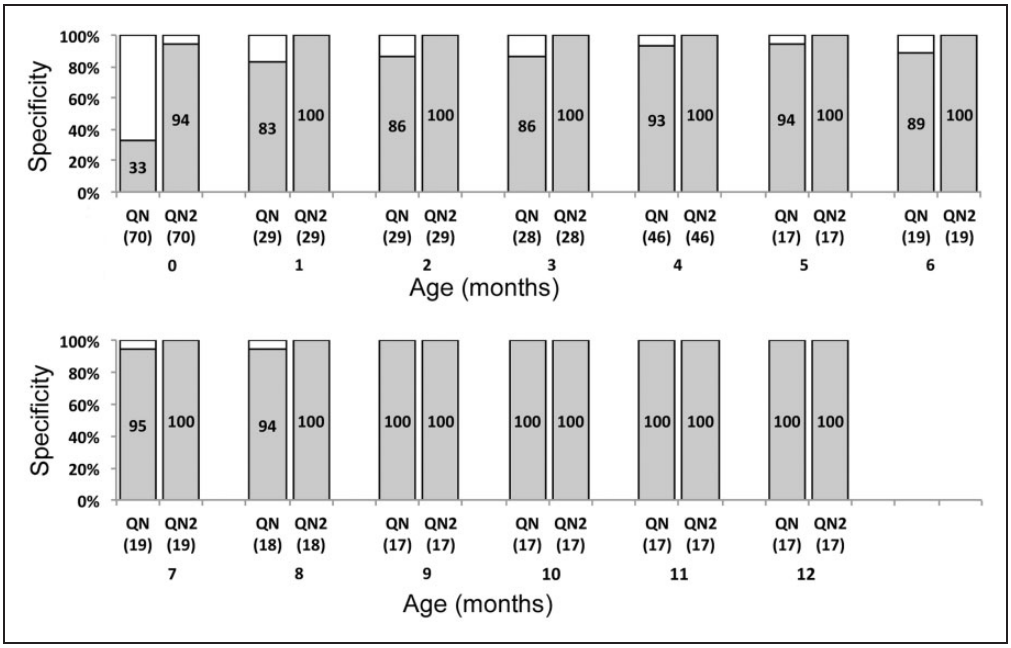

Figure 1. Specificity of QuickNavi®-Norovirus (QN) and QuickNavi®-Norovirus 2 (QN2; both Denka Seiken Co., Ltd., Niigata, Japan) for detection of norovirus in faecal specimens taken monthly from healthy, full-term infants aged between 0 and 12 months, stratified by age (total 343 specimens from 81 infants). Real-time reverse transcription polymerase chain reaction was used as the gold standard for norovirus detection.

The present study has several limitations. It was not possible to evaluate the sensitivity of QN and QN2, because only three faecal specimens were confirmed positive by real-time RT–PCR. It is difficult to collect norovirus-positive faeces from neonates because of the low prevalence of the infection. The sensitivity of these tests for neonatal and infant faeces must be re-evaluated in future. In addition, it was not possible to determine the cause of the false positives in the neonatal specimens. It is likely that unknown substances unique to neonatal and early infant faeces cross-react and cause false-positive results with QN specifically. The use of enemas or suppositories, and specialized diets that include thickening agents, may cause false-positives.[6] Such samples, however, were excluded from the present study. The specificity of QN2 was determined using rectal swab samples, the use of which is known to reduce specificity in QN.[8] QN2 offers sufficient specificity with rectal swab samples, but no substances present in both rectal swabs and neonatal faecal specimens have been shown to cause cross reactions.

In conclusion, QN2 appears to offer improved performance and be more useful than QN for the diagnosis of norovirus infection in the neonatal and infant period.

Declaration of conflicting interest

Denka Seiken Co., Ltd. provided rapid detection kits and performed the immunochromatography and real-time RT–PCR testing for this study. Denka Seiken Co., Ltd. had no role in 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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