Real-Time Reverse Transcription-PCR for Detection of Rotavirus and Adenovirus as Causative Agents of Acute Viral Gastroenteritis in Children

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Viral pathogens are the most common cause of gastroenteritis in developed countries. Human rotavirus and adenovirus infections are major causes of acute outbreaks and sporadic cases of gastroenteritis, occurring primarily among children less than 2 years of age. Patient hospitalization is often required, with enormous infection control implications. This work describes the development of real-time PCR assays for the detection of group F adenovirus, rotavirus A, and rotavirus C from stool specimens. Two hundred twenty stool samples from pediatric patients exhibiting symptoms of diarrhea and/or vomiting were examined. PCR results were compared with those of virus detection by electron microscopy and latex agglutination antigen detection. The incorporation of an internal-control RNA that was spiked into individual stool extracts functioned as an internal validation for the reporting of PCR-negative results. Rotavirus C was not detected by real-time PCR in the patient stool samples examined. Real-time reverse transcription-PCR resulted in 175% and 111% increases in the rates of detection of adenovirus F and rotavirus A, respectively, compared with latex agglutination testing. Molecular detection increased the number of stool specimens in which causative agents of gastroenteritis were identified by 155% compared to electron microscopy. Genotyping of a proportion of the rotavirus and adenovirus strains identified only genotype G1 rotavirus and both adenovirus genotypes 40 and 41 in circulation within the patient cohort examined. The results highlight the significance of rapid molecular methods for the routine screening of stool samples in hospital laboratories to provide rapid definitive diagnoses.

Viral pathogens are the most common cause of gastroenteritis in developed countries (10, 11, 27). Worldwide, gastrointestinal rotavirus infections result in an estimated 440,000 deaths in children <5 years of age (18). Adenoviruses are second only to rotavirus as the most important causative agents of acute infantile gastroenteritis (31).

Rotaviruses are double-stranded RNA viruses, and serogroups A, B, and C are known to cause gastrointestinal infections in humans. Group A rotaviruses are predominant and result in severe diarrheal diseases in infants and young children (19). Group A rotaviruses can be classified on the basis of their viral protein 4-associated P type and viral protein 7 (VP7)-associated G type, of which G types G1, G2, G3, G4, G8, and G9 are most commonly detected (6). Group B rotaviruses cause adult diarrhea and are reportedly geographically confined, having been first identified in a large waterborne epidemic in China (5). Group C rotaviruses, an emerging cause of gastroenteritis in children over 2 years old and in adults, have now been identified as causative agents of gastroenteritis in both sporadic cases and outbreaks worldwide (8, 14, 23, 25, 28).

Human adenoviruses are double-stranded DNA viruses and are classified into six subgenera, A to F (30). Subgenus F (types 40 and 41) consists of the enteric adenoviruses and is an important cause of viral gastroenteritis among children less than 2 years of age (29), although older children and adults may also be infected.

Electron microscopy (EM) can be used to simultaneously detect and identify most enteric viral agents from a single stool specimen. However, the method is labor intensive and is relatively insensitive, requiring up to $10^6$ intact viral particles per ml stool (22). Commercially available antigen detection kits, such as enzyme immunoassay (EIA) and latex agglutination (LA) tests, are often the method of choice for the diagnosis of adenovirus and rotavirus infection in stool samples in routine microbiology hospital laboratories. These kits are easy to use and highly specific; however, due to the levels of strain diversity among circulating gastrointestinal viruses, the estimated sensitivity for many antigen detection kits is low.

Various molecular techniques have been exploited for the development of highly sensitive and rapid assays for the detection of causative agents of viral gastroenteritis (16, 20, 26). Reverse transcription-PCR has reportedly increased the detection rate of rotavirus A by up to 48% compared to EIA or electron microscopy (2, 17). Simpson et al. report that the detection of adenovirus was increased by more than 200% using PCR compared to results with EM (27). The screening of stool specimens for a range of gastrointestinal viruses using highly sensitive molecular methods would provide an indication of the true prevalence of the causative agents of acute gastroenteritis in the pediatric patient cohort. In particular, rotavirus C prevalence remains largely unknown due to the
absence of commercially available diagnostic tests. Routine diagnosis of rotavirus C is often inferred by an EM test positive for rotavirus but a negative group A rotavirus EIA result. A recent study showed that 43% of a population in the United Kingdom were seropositive for group C rotavirus, but a report investigating the occurrence of the virus in Irish patients has not been published (7).

This paper describes the design and development of a method for the routine detection of three gastrointestinal viruses from a single stool sample, namely group F adenovirus, rotavirus A, and rotavirus C. This research has developed a method for the efficient extraction of total nucleic acids from stool samples, with the incorporation of an internal positive control. In conjunction with the design of highly sensitive real-time reverse transcription (RT)-PCR assays, this has resulted in increased levels of detection of causative agents of gastroenteritis in the 220 pediatric stool samples examined compared to results obtained following electron microscopy and/or latex agglutination testing.

**MATERIALS AND METHODS**

**Specimen collection.** A total of 220 stool samples were collected from patients exhibiting symptoms of diarrhea and/or vomiting received at the Microbiology laboratory, Our Lady’s Hospital for Sick Children, from February 2004 to April 2005, divided into aliquots, and stored at −80°C until analyzed using molecular methods. Twenty-five stool samples received at the laboratory from patients not exhibiting symptoms of gastroenteritis were similarly divided into aliquots and stored and used as controls for the purpose of this study.

**Latex agglutination detection of rotavirus and adenovirus antigens and electron microscopy.** All 220 stool samples were tested for the presence of rotavirus and adenovirus antigen using the Diarlex Rota-Adeno kit (Orion Diagnostica, Espoo, Finland). Specimens were processed as described in the kit protocol, using the centrifugation technique.

One hundred thirty-nine of the 220 stool specimens requesting additional viral investigations were sent to an external laboratory for examination by electron microscopy.

**Nucleic acid extraction from stool specimens.** Total nucleic acids were extracted using a single protocol similar to that published by O’Neill et al., which coextracts RNA and DNA for the isolation of viral nucleic acids from stool samples (16). All specimens were extracted in a dedicated class 2 laminar flow hood using dedicated pipettes and aerosol-resistant pipette tips. Fecal specimens were prepared as 10% suspensions with STAR buffer (Roche Diagnostics GmbH, Mannheim, Germany). Chloroform aliquots of 0.1× volume were added, and following mixing, the samples were centrifuged at 8,000 × g for 10 min. The aqueous layer (stool extract) was removed to a fresh tube and stored at −80°C, or the extraction was completed before freezing. Stool extracts of 200 μl were further purified with the QIAJEN QIAamp DNA Blood mini kit as described for DNA extraction from whole blood in the handbook. Total nucleic acids were eluted from the spin columns using 50 μl of nuclease-free water. Extracted nucleic acids were stored at −80°C. Stool samples were coextracted with a negative extraction control consisting of 200 μl of STAR buffer to which no stool had been added.

**Development of internal positive control system as a measure of the efficiency of removal of PCR inhibitors from stool samples.** To determine the efficiency of the extraction protocol in removing the high levels of PCR inhibitors present in stool samples, total mouse RNA was introduced to samples following stool clarification and carried through the specimen preparation, amplification, and detection protocols. Approximately 25 ng of mouse RNA was spiked into stool extracts. Following nucleic acid extraction and reverse transcription, each sample was assayed using the TaqMan rodent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) control reagents (Applied Biosystems, Warrington, United Kingdom), a predesigned real-time PCR assay for the detection of rodent GAPDH genes. PCR was carried out on the ABI Prism 7000 sequence detector (Applied Biosystems) using SDS software version 1.2. The ΔRn value of each sample (the normalized reporter signal minus the baseline signal), an indication of the magnitude of the signal generated in a given reaction, was determined. An extracted stool sample was considered inhibitory if the ΔRn value was at least 20% lower than the average ΔRn value for the extraction control.

**Reverse transcription reactions.** RT reactions were carried out in a final volume of 40 μl using the TaqMan reverse transcription kit (Applied Biosystems) on a GeneAmp 9700 thermocycler from Applied Biosystems. The reactions contained 1× RT buffer, 5.5 mM MgCl₂, 0.5 mM each deoxynucleoside triphosphate (dNTP), 2.5 μM random hexamer, 0.4 U/μl RNase inhibitor, 1.25 U/μl Multiscribe reverse transcriptase, and 17.5 μl of extracted nucleic acids. Immediately before testing, extracted nucleic acids were removed from −80°C storage and added to 200-μl thin-walled PCR tubes containing the RT buffer, dNTPs, and MgCl₂. To facilitate the reverse transcriptase activity of the double-stranded RNA of rotaviruses, the reaction components were denatured by heating at 95°C for 5 min and then snap-chilled on ice for 5 min. Random hexamer, RNase inhibitor, and Multiscribe reverse transcriptase were then added to the reaction. Thermal cycling parameters for the RT reactions were as follows: 10 min at 25°C, 30 min at 42°C, 20 min at 48°C, and 5 min at 95°C. Completed RT reactions were stored at −20°C until required for further analysis.

**Primer and probe design for real-time PCR.** DNA sequences from highly conserved target genes were used for real-time PCR assay design as outlined in Table 1. Nucleic acid sequences were retrieved from GenBank (www.ncbi.nlm.nih.gov) and aligned using the ClustalW (www.ebi.ac.uk/clustalw) multiple se-

<table>
<thead>
<tr>
<th>Reaction (target gene)</th>
<th>Primer or probe (5’ label)</th>
<th>Sequence (5’→3’)</th>
<th>Concn (μM)b</th>
<th>Positionc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus F (hexon)</td>
<td>Adeno.fwd</td>
<td>TTCACGCAATAAACTCWWGGCTTTTG</td>
<td>0.60</td>
<td>2374–2398</td>
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<td></td>
<td>Adeno.rev</td>
<td>AATTCTTTGTCAGCGCTTGG</td>
<td>0.60</td>
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<tr>
<td></td>
<td>Adeno.probe1 (FAM)</td>
<td>CATACCCCCTTATTGG</td>
<td>0.10</td>
<td>2452–2468</td>
</tr>
<tr>
<td></td>
<td>Adeno.probe2 (FAM)</td>
<td>CTTACCCCCTTATTGG</td>
<td>0.10</td>
<td>2452–2468</td>
</tr>
<tr>
<td>Rotavirus A (VP6)</td>
<td>RotaA-fwd1</td>
<td>GGTAGTCTCTGACCTCTTGGCAAAA</td>
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<tr>
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<td>RotaA.probe1 (VIC)</td>
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<td>RotaA.probe2 (VIC)</td>
<td>ATATGAATGACCTGCAACAAT</td>
<td>0.25</td>
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</tr>
<tr>
<td>Rotavirus C (VP7)</td>
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<td>TGCTGACAGGATGTTTATCGTGT</td>
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<td>655–684</td>
</tr>
<tr>
<td></td>
<td>RotaC.rev</td>
<td>TGGGTGCATTTGATCATACCTCA</td>
<td>0.60</td>
<td>731–708</td>
</tr>
<tr>
<td></td>
<td>RotaC.probe (FAM)</td>
<td>CGTCTAGTACGAAACTCT</td>
<td>0.20</td>
<td>689–706</td>
</tr>
</tbody>
</table>

a All probes are MGBNFQs with FAM or VIC as the reporter dye coupled on the 5’ end of the oligonucleotide as indicated. Mixed bases in degenerate primers are as follows: W = A/T.
b Oligonucleotide concentration in real-time PCR amplification reactions.
c The position of each primer is relative to the numbering of the following gene sequences: adenovirus, relative to sequence with accession no. X51782; rotavirus A, relative to sequence with accession no. X94617; and rotavirus C, position to sequence with accession no. X77257.

**TABLE 1. Nucleotide base sequences of primers and probes designed for development of real-time RT-PCR assays**
DNA from each plasmid used as a DNA standard for real-time PCR was prepared following the manufacturer's instructions. The nucleotide base sequence of the insert DNA from each plasmid was determined by nucleotide sequencing of both DNA strands (Lark Technologies, Inc., Essex, United Kingdom).

**RNAse and DNase digestion of nucleic acids extracted from stool specimens.** RNase and DNase digestion of a number of extracted stool samples was performed to verify the source of nucleic acid responsible for PCR amplification. Extracted stool samples that had been identified as positive for adenovirus F (DNA genome) or rotavirus A (double-stranded RNA genome) were examined. The RNase and DNase digestions were performed separately at 37°C for 30 min using RNase Cocktail and TURBO DNA-free (Ambion (Europe) Ltd., Cambridge, United Kingdom), respectively, according to the manufacturer's instructions. Following reverse transcription, samples were examined by real-time PCR with the adenovirus F and rotavirus A real-time PCR assays.

**Differentiation of virus genotypes by PCR amplification and base sequencing.** A number of stool samples identified as positive for gastrointestinal virus(s) by real-time PCR were also examined with broadly reactive solution phase PCR assays (performed in a final volume of 50 μl) for examination of the circulating virus strain diversity.

Adenovirus genotyping was performed using the method and primers (AD1 and AD2) of Sarantis et al. (24), a primer pair targeting conserved segments bracketing the hypervariable region 7 (HRV-7) of the hexon gene and suitable for the generic amplification of all adenovirus genotypes. Examination of the strain diversity of rotavirus A samples was performed using oligonucleotide primers described by DiStefano et al. targeting a region of known hypervariability in the VP7 gene (1). Amplified products were analyzed on 3% agarose ethidium bromide-stained gels. All RT-PCR products of the appropriate size were purified using the Qiagen QIAQuick PCR purification kit and cloned into the pCR2.1-TOPO vector using the TOPO TA cloning system (Invitrogen). The possibility of multiple genotypes of the same virus occurring in individual samples was not investigated. The nucleotide base sequence of the insert DNA from each plasmid was determined by nucleotide sequencing of both DNA strands, using the universal M13 forward and M13 reverse primers (Lark Technologies, Inc., Essex, United Kingdom). Sequence data generated from forward- and reverse-reverse sequencing reactions was assembled, and a consensus sequence was imported into MEGA version 3.1 software for molecular typing determination by sequence homology (9). Phylogenetic analysis of sequence data was performed following multiple DNA sequence alignments of data obtained internal to the amplifying primers using ClustalW. A consensus phylogenetic tree was obtained using the neighbor-joining algorithm with a bootstrap value of 1,000 replications.

**Type assignment was based on the shortest distance from a prototype strain, as deduced from the phylogenetic tree.**

**Nucleotide sequence accession numbers.** The nucleotide sequence data were submitted to GenBank. Partial VP7 gene sequences (rotavirus) were assigned accession numbers DQ498989 to DQ498997. Partial hexon gene sequences (adenovirus) were assigned accession numbers DQ498998 to DQ499005.

**RESULTS**

Development of an internal positive control protocol as a measure of the efficiency of removal of PCR inhibitors from stool samples. All 220 stool samples examined by real-time PCR amplification using the TaqMan rodent GAPDH assay...
were shown to have a ΔRn value of greater than 87% of that obtained for the corresponding extraction control. These data confirmed the efficient removal of PCR inhibitors from each individual sample.

**PCR specificity, sensitivity, and detection limits.** The specificity of each real-time primer and probe set used in this study was examined. Plasmid DNA standards (10⁵ copies of adenovirus F, rotavirus A, and rotavirus C) were examined with all three real-time PCR assays. Cross-reactivity of primer and probes was not observed when plasmid DNA standards were used as a template. Real-time PCR did not detect group F adenovirus, rotavirus A, or rotavirus C in the 25 control stool specimens examined.

To validate the real-time PCR assays prior to application to clinical samples, the detection limit and amplification efficiency of each reaction were determined. Standard curves with 10-fold serial dilutions of plasmid DNA controls (from 10⁶ to 10⁴ copies) were prepared and assayed in duplicate. The resulting standard curves (adenovirus group F, slope = -3.88 and Y-intercept = 43.74; rotavirus group A, slope = -3.50 and Y-intercept = 44.34; and rotavirus group C, slope = -3.56 and Y-intercept = 38.75), with strong correlation coefficients (r² ≥ 0.980), indicated strong linear relationships over the 7-log range examined. PCR amplification efficiency for each assay was calculated from the slope of the standard curves (% efficiency = (10⁻¹slope - 1) × 100) and ranged from 81.16% to 93.07%. A detection limit of 10 copies of target DNA per reaction was determined for all PCR assays, indicating high assay sensitivity.

**RNase and DNase digestion of nucleic acids extracted from stool specimens.** Total nucleic acids extracted from two stool samples were examined by real-time PCR prior to and following separate RNase and DNase treatment to confirm the source of nucleic acid responsible for PCR amplification. An adenovirus-positive sample (cycle threshold [C_T] = 17.37) showed no change in C_T following RNase treatment (C_T = 17.23), while the sample was undetected by adenovirus real-time PCR following DNase treatment. Similarly, a rotavirus-positive sample (C_T = 27.20) was undetectable by real-time rotavirus PCR assays following RNase treatment, while DNase treatment did not significantly affect the C_T of the sample (C_T = 27.79).

**Detection of target viruses in stool specimens from patients with acute gastroenteritis.** The results of assaying all 220 pediatric stool specimens by real-time PCR (group F adenovirus, rotavirus A, and rotavirus C) and latex agglutination and of electron microscopy examination of 139 stool specimens are tabulated in Table 3.

The Diarex Rota-Adeno kit identified 4 of the 220 stool samples examined as positive for adenovirus antigen (Table 3). Group F adenovirus was detected using real-time PCR in 11 of the 220 stool specimens, including the 4 specimens positive for adenovirus antigen. All 11 adenovirus-PCR positive specimens were from patients less than 2 years old.

Electron microscopy detected adenovirus particles in 4 of the 139 stool samples examined. Only one of these EM-positive samples was detected by real-time PCR for group F adenovirus (C_T = 13.99), a sample that was also positive for adenovirus by latex agglutination. However, real-time PCR detected an additional seven samples as adenovirus positive that were not detected by electron microscopy (Table 3). Interestingly, two of these seven samples were also adenovirus positive by antigen detection.

Eighteen of 220 stool samples examined were identified as positive for rotavirus by latex agglutination testing (Table 3). Real-time PCR detected an additional 20 specimens as positive for rotavirus A. The patient age for rotavirus PCR-positive samples ranged from less than 1 month to 6.6 years old, with a mean patient age of 15 months. Over 80% (31 of 38) of rotavirus A PCR-positive patients were less than 2 years old. Dual adenovirus-rotavirus infection was detected by PCR in 2 of the 220 specimens.

Seven of the 139 stool samples examined by electron microscopy were observed to contain rotavirus on examination. Six of these seven stool specimens were positive for rotavirus A using the real-time PCR assay designed as part of this study. In addition to the 6 EM-positive samples detected by rotavirus PCR assay, 14 specimens that were not detected by EM were identified as rotavirus A PCR positive (Table 3).

Rotavirus C was not detected by RT-PCR in the 220 stool specimens examined.

**Analyses of discrepant results.** Three of the 139 stool specimens examined by EM reported as containing adenovirus on examination by EM were repeatedly negative for group F adenovirus by real-time PCR (Table 3), including one specimen from a patient of >15 years of age. The possibility that adenovirus particles, perhaps serotypes other than type 40 or 41, were present was further investigated. Using the primers of Sarantis et al. that detect all known serotypes of human adenovirus with high sensitivity, solution-phase PCRs were performed. All three specimens failed to produce a PCR amplicon.

Six of the seven specimens positive for rotavirus by EM were detected with the rotavirus A real-time PCR assay designed in this study. The PCR negative, EM-positive stool specimen was reextracted and following reverse transcription reexamined by real-time PCR. No amplification signal was observed with the

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**TABLE 3. Detection of adenovirus and rotavirus in clinical samples by real-time RT-PCR**

<table>
<thead>
<tr>
<th>Assay and parameter</th>
<th>Adenovirus</th>
<th>Rotavirus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive</td>
<td>No. negative</td>
</tr>
<tr>
<td></td>
<td>No. positive</td>
<td>No. negative</td>
</tr>
<tr>
<td><strong>Real-time PCR result</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latex agglutination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. positive</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>No. negative</td>
<td>7</td>
<td>209</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>209</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. positive</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>No. negative</td>
<td>7</td>
<td>128</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>131</td>
</tr>
</tbody>
</table>

*Results of adenovirus group F and rotavirus group A detection by real-time PCR are compared to detection of rotavirus and adenovirus antigen by latex agglutination (220 stool specimens examined) and detection of rotavirus and adenovirus by electron microscopy examination (139 stool specimens examined).*
A total of eight stool samples identified as
rotavirus serotypes also failed.

All clone sequences clearly cluster with G1 serotype rotavirus
specimens corresponding to sequences S5 to S9 were positive for
latex agglutination testing, are indicated with a white square. Stool
responding to clinical specimens identified as negative for rotavirus by
cession number and serotype number. DNA sequences obtained from GenBank are listed as the ac-
sequence alignment of cloned amplicons generated by rotavirus geno-
typing method of DiStefano et al. (1). The cloned amplicons
(internal to the primers) were 307 bp and displayed 91 to 100%
sequence identity with each other. Blast analysis of all nine
sequences identified entries in the GenBank database with
≥98 to 100% sequence identity. Phylogenetic analysis of VP7
gene sequences currently in GenBank corresponding to rota-
virus G types 1, 2, 3, 4, 8, and 9 clearly identified each serotype
as a distinct cluster, with strains of the same serotype clustering
together (Fig. 1). All nine of clone sequences S1 to S9 (with
corresponding GenBank accession numbers DQ498998 to
499004) were assigned as adenovirus type 40 (GenBank accession number
DQ499005).

Nine stool samples identified as positive for group A rota-
virus by real-time PCR were examined using the rotavirus VP7
typing method of DiStefano et al. (1). The cloned amplicons
responding to clinical specimens identified as negative for rotavirus by
latex agglutination testing, are indicated with a white square. All clone sequences clearly cluster with G1 se-
rotype rotavirus sequences.

DISCUSSION

This work describes the development of molecular assays for
the routine detection of three agents responsible for viral gas-
troenteritis from a single stool sample, namely group F ade-
navirus, group A rotavirus, and group C rotavirus. An assay for
the detection of group B rotavirus was not developed due to
the geographic confinement of the virus. A novel method was
developed for the prevention of false negatives due to the high
levels of PCR inhibitors present in stool samples with the
incorporation of an internal control RNA that is spiked into
individual stool extracts. The inclusion of a random hexamer-
primed reverse transcription reaction in the protocol devel-
oped, while not necessary for the amplification of DNA tem-
plates, allows a single protocol for the analysis of stool samples
for differing nucleic acids. Degenerate primers and in some
cases a mix of primers and/or probes were used in PCRs to
ensure maximum sensitivity of the assays. The alternative of
lowering PCR temperature stringency conditions could have
compromised assay specificity.

Rotavirus C was not detected by real-time PCR in the 220
stool samples examined, suggesting that the virus is not cur-
rently circulating as a commonly occurring causative agent of
viral gastroenteritis in this Irish pediatric cohort.

Real-time PCR resulted in 175% and 111% increases in the
rate of detection of adenovirus and rotavirus, respectively,
compared to latex agglutination testing. Real-time RT-PCR
detected all specimens identified as positive for rotavirus or
adenovirus by latex agglutination or antigen detection.

Adenovirus serotypes 40 and 41 were identified, while all
rotavirus specimens examined were assigned genotype G1. Genotyping studies of rotavirus samples recovered from in-
fected children in Ireland have previously identified G types 1,
2, 3, 4, 8, and 9 (12, 13, 15, 21), with all studies identifying G1

![Figure 1](http://jcm.asm.org/)
as the predominant G type. The finding of only G1-type rotavirus strains in this study is therefore surprising, albeit only nine patient specimens were genotyped, all of which were received at the hospital over a period of less than 12 months.

Serotype specificity could not account for the failure of latex agglutination testing to detect adenovirus or rotavirus antigen in a number of PCR-positive stool specimens. Three of seven adenovirus specimens assigned to serotype 41 were negative for adenovirus by LA. Similarly, four of nine genotypic G1 rotavirus specimens were negative for rotavirus by LA (Fig. 1). The viral protein-4-associated P type of rotavirus specimens was not examined.

Real-time PCR resulted in 100% and 186% increases in the rate of detection of adenovirus and rotavirus, respectively, compared to electron microscopy testing of the 139 stool specimens examined. These results compare favorably with those of previous studies, which report significantly increased levels of rotavirus and adenovirus detection by PCR compared to EM (17, 27).

Four stool specimens deemed positive for adenovirus or rotavirus by electron microscopy were not detected by real-time PCR or LA testing. All four samples also failed to produce an amplicon using the appropriate universal genotyping primers for adenovirus and rotavirus detection. The four specimens were subsequently screened using real-time RT-PCR assays for the detection of human astrovirus, norovirus, and sapovirus. One specimen was real-time PCR positive for norovirus group 2 (C. Logan, J. J. O'Leary, and N. O'Sullivan, unpublished data).

Failure to amplify rotavirus and adenovirus EM-positive specimens both with the real-time PCR assays designed in this study and with PCR assays capable of detecting all serotypes of the viruses is unlikely, in particular given the relatively low sensitivity of EM. The possibility that these samples contained virus particles morphologically similar to rotavirus or adenovirus, but with little nucleotide sequence homology, cannot be ruled out. In particular, this highlights the problems associated with the determination of a specimen as rotavirus C positive based on a positive EM but negative rotavirus LA result.

In conclusion, this work reports the design, development, and application of real-time PCR assays for the rapid, specific, and highly sensitive detection of group F adenovirus and rotavirus groups A and C as causative agents in gastrointestinal infection. The results reported in this study highlight the inadequacies of nonmolecular methods currently used for the routine detection of gastrointestinal viruses and emphasize the importance of the application of rapid molecular methods to clinical services in hospital laboratories to provide definitive diagnoses. In conjunction with genotyping protocols, highly sensitive molecular methods could provide valuable information on the incidence of currently circulating viral strains, therefore lending itself to surveillance of viral transmission.

REFERENCES


