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Primer Pair p289-p290, Designed To Detect Both Noroviruses and Sapoviruses by Reverse Transcription-PCR, Also Detects Rotaviruses by Cross-Reactivity

Juan E. Ludert,* Ana C. Alcalá, and Ferdinando Liprandi

Centro de Microbiología y Biología Celular, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, Venezuela

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A primer pair (p289-p290) designed to detect both noroviruses and sapoviruses by reverse transcription-PCR (Jiang et al., J. Virol. Methods 83:145, 1999) cross-reacts with rotaviruses. The rotavirus amplicon corresponds to genome segment 1. Furthermore, primer pair p289-p290 detected rotaviruses as efficiently as rotavirus-specific primers directed to rotavirus gene 4.

Caliciviruses are recognized nowadays as a common cause of outbreaks as well as of sporadic cases of acute gastroenteritis in children and adults (3). Human caliciviruses are classified into two genera, *Norovirus* and *Sapovirus*, within the *Caliciviridae* family. These viruses are highly infectious and are transmitted by food and water and from person to person (3). Since caliciviruses have not been adapted to grow in tissue culture, diagnosis has traditionally been made by using electron microscopy. However, the successful cloning and sequencing of the genomes of several calicivirus strains paved the way for the development of more-sensitive assays such as enzyme immunoassay and reverse transcription (RT)-PCR (2). At the present time, RT-PCR is the tool most widely used for the detection of caliciviruses. During a molecular epidemiological study of enteric viruses in fecal samples collected from children with acute diarrhea, we found that primer pair p289-p290, a primer set widely used within the calicivirus community and designed to amplify a 319- or 331-bp fragment from the RNA polymerase region of noroviruses or sapoviruses, respectively (5), cross-reacts with rotaviruses.

A total of 30 fecal samples was collected from children who died of acute diarrhea between 1998 and 2003 in a large sentinel hospital in Venezuela. Fecal suspensions, 10% (wt/vol) in 50% freon:water were clarified by low-speed centrifugation. Nucleic acids were extracted from the clarified fecal suspensions with Trizol/LS (GIBCO BRL, Rockville, Md.) and examined for the presence of rotaviruses, caliciviruses, and astroviruses by RT-PCR (unpublished data). Caliciviruses were detected by using two primer sets. The primers GLPSG1-YGDD1 and GLPSG2-YGDD1, which amplify a 154-bp fragment from the RNA polymerase region, were used for the detection of *Norovirus* genogroup I and genogroup II, respectively (4). In addition, samples were analyzed by using the primer pair p289-p290. RT and PCR were carried out as previously described (4, 5) for each primer set. PCR products were analyzed by electrophoresis in 3% (wt/vol) agarose Tris-borate-EDTA gels and detected by UV illumination after being stained with ethidium bromide.

In three of the samples analyzed, primer pair p289-p290 generated amplicons with an apparent size of 300 bp, very close to the size of the expected products (319 to 331 bp). Two of the amplicons were purified and sequenced directly in both directions with an automatic sequencer. Comparison of the sequences with strains deposited in GenBank using the BLAST program (http:\\www.ncbi.nlm.nih.gov) clearly indicated that both amplicons corresponded to rotavirus gene segment 1, which encodes the RNA-dependent RNA polymerase. All three samples proved to be positive for rotaviruses by RT-PCR and by enzyme-linked immunosorbent assay and negative for noroviruses, as determined with primers GLPSG1-YGDD1 and GLPSG2-YGDD1. In addition, primer set p289-p290 generated amplicons of approximately 330 bp with human rotavirus strains Wa and 69M, porcine rotavirus strain YM, and bovine rotavirus strain UK but not with porcine strain OSU or simian strain SA11. The amplicons generated from the rotavirus tissue culture-adapted strains also correspond to gene segment 1. Of note, primer set p289-p290 was as efficient in detecting 10-fold serial dilutions of double-stranded RNA extracted from strain Wa as primers CON 1 and CON 2, specific to rotavirus gene 4 (1).

The extensive genetic diversity of noroviruses and sapoviruses has made it difficult to design primer sets with the sensitivity and specificity to detect all calicivirus strains (2). Several conserved regions of the genome have been targeted for amplification and primer design, but in general the RNA-dependent RNA polymerase region, which is the most-conserved region of the genome, has been the most widely used (2). RNA-dependent RNA polymerases of single- and double-stranded RNA viruses share several conserved motifs and probably have a common phylogenetic origin. Efforts toward the design of specific primers for the detection of a wide spectrum of caliciviruses may in turn result in specificity problems (Table 1). These results warn of potential nonspecific reactions and the need to confirm the results obtained by RT-PCR. Due to the inadequacy of any single primer pair to detect all human caliciviruses, analysis of samples with several primer sets is customarily done. This practice increases the cost

* Corresponding author. Mailing address: Centro de Microbiología y Biología Celular, Instituto Venezolano de Investigaciones Científicas (IVIC), Apdo. 21827, Caracas 1020-A, Venezuela. Phone: 58-212-5041655. Fax: 58-212-5041382. E-mail: jeludert@ivic.ve.

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TABLE 1. Regions of homology with primer pair p289–p290 of the RNA-dependent RNA polymerase gene of caliciviruses representative of *Norovirus* genogroups I and II, *Sapovirus*, and selected rotavirus strains[a]

Strain[b]	Primer 290 GATTACTCCAAGTGGGACTCCAC	Primer 289 TATGGTGATGATTACATTGTCA	Location	RT-PCR reactivity[c]
NORWALK	-----TA-AGCA--------A--ACAAAA	ATTT--------------G-G-----GT	4568–4886	+
HAWAII	-----T--TCGA--------A--ACAGCA	TTTC--------------G-A-----G-		+
HOUSTON/86	-----------A-----------ACAAAA	CACACG-----------C-G---GTA--		+
UK	ATA---G---G--ATA-TA----CAGTGA	CTATCT----------T-ACACGCT-TT	1314–1547	+
YM	ATA-------G---TT-TA--T-CAGTAA	TTGTCA--------C-T-ACG-GAT-TT		+
KU	-TA-------G---TT-T---T-CAGTGA	TTATCA--C-------T-ACG-GAT-TT		NT
SA11	ATAC--G--TG--ATA-TA----CAGTGA	TTGTCA--C--C----TAACGCG-T-TT		−

[a] Sequence alignments were made by using the program DNAman, version 5.2.2 (Lynnon, Bio Soft, Quebec, Canada).

[b] Norwalk, Hu/NV/GI/Norwalk/1968/US, GenBank accession no. M87661; Hawaii, Hu/NV/GII/Hawaii/1971/US, GenBank accession no. U07661; Houston/86, Hu/SV/Houston/1986/US, GenBank accession no. U65427; UK, bovine rotavirus strain, GenBank accession no. X55444; YM, porcine rotavirus strain, GenBank accession no. X76486; KU, human rotavirus strain, GenBank accession no. AB022765; SA11, simian rotavirus strain, GenBank accession no. X16830. The locations of the primers in the genome are based on the sequence of the full-length Norwalk genome and the complete sequence of UK VP1.

[c] +, reactive by RT-PCR; −, nonreactive by RT-PCR; NT, not tested.

and the complexity of the assay and adds difficulty to result comparison between laboratories (6). Our findings highlight once more the difficulties still existing for the detection of caliciviruses and the need for the international harmonization of methods (6).

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