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Errors in Published Sequences of Human Cytomegalovirus Primers and Probes: Do We Need More Quality Control?

Over the past decade, many authors have focused on PCR as a powerful technique for the evaluation of human cytomegalovirus (CMV). The key to PCR lies in the design of oligonucleotides, as the specific sequences largely affect PCR's efficacy and sensitivity. This study was designed to examine the quality of published sequences of CMV primers and probes.

PubMed was searched in the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov) for English peer-reviewed articles using CMV and PCR as keywords. Articles reporting on virus genotyping or species-level identification, as well as letters to editors and reviews were excluded. The full texts of 91 papers published between 1993 and 2004 were studied. Of these, 34 papers did not describe the detailed nucleotide sequence, including 17 papers using commercially available kits. The remaining 57 papers with a total of 199 CMV-specific oligonucleotides were examined. Oligonucleotides with identical sequences or with one additional nucleotide at either the 3′ or 5′ end of the sequence were identified as synonymous.

Using The Sequence Manipulation Suite web-based programs (written by Paul Stothard, University of Alberta, Canada), the binding sites of all 199 oligonucleotides were identified using GenBank strain AD169 genome sequence (GenBank accession no. X17403 and NC_001347). Mismatches to all published sequences of CMV were analyzed by the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/). Papers containing oligonucleotide errors were studied for technical reasons for employment of mutated oligonucleotides or subsequently published errata. Moreover, information on identified errors was conveyed to all corresponding authors and coauthors.

Ten oligonucleotides did not match any CMV strain, and reasons were specified neither in the respective articles nor in authors' replies (Table 1). Primers R2, F6, and F9 were incorrectly transferred from previous publications (2, 5, 7) as indicated by the authors (V. H. Aquino and X. L. Pang, personal communication). The oligonucleotides F1, R3, and P10 were apparently also incorrectly transferred from prior publications (14). Moreover, primers F4 and R5 possessed mismatches at their 3′-end triplets, which may reduce PCR efficiency (21). Furthermore, the two degenerated probes (P7 and P8) contained Y (C/T) instead of R (A/G) in P7 and vice versa in P8. Obviously, these mismatches may drastically affect the efficacies of these probes (17).

In summary, we show that 5% of the CMV oligonucleotides included mismatches to all published sequences half of which were incorrectly reported mostly in secondary publications. Moreover, since we considered all sequences correct when they matched at least one CMV strain, even when the authors used a different strain for design or admitted an error, the number of errors is underestimated by our approach. These observations point to the possibility that the report of erroneous primers and probes is a widespread problem irritating other researchers. It might therefore be advisable for authors and reviewers alike to pay special attention to the verification of such sequences and for researchers citing or following published work.

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TABLE 1. Published CMV oligonucleotides that showed errors with all published CMV strains

Oligonucleotide[a]	Sequence (5′-3′)[b]	Position[c]	Stated correctly[d]
F1 (11)	GAA ACG CGC G**C**GC AAT CGG	81873–81890	2, 3, 9, 12, 22
R2 (9, 12, 22)	TAC G**CT GCA GTT CAC/CCC AG**	82055–82036	2
R3 (11)	T–G AA**C** TGG AAC GTT TGG C	82174–82156	2, 8, 9, 12, 13, 22
F4 (18, 19)	CGG AAA CGA TGG TGT AGT T**G**G	82571–82591	
R5 (18, 19)	TCC AAC ACC CAC **T/AG** ACC **G**GT	82838–82818	
F6 (15)	ATA GGA GGC GCC ACG TAT TC$^{\mathbf{T}}$	82975–82994	1, 5, 6, 20
P7 (16, 20)	ACA CCA CTT ATC T**Y**C TGG GCA GC	83023–83001	
P8 (16, 20)	CGT TTC GTC GTA GCT ACG C**R**T ACA T	83050–83026	
F9 (2)	TGG TGT TTT $^{\mathbf{T}}$CAC GCA GGA A	109961–109979	7
P10 (4)	CCT CCC GCT CCT **GAG/C–T**	171149–171166	10, 14

[a] Oligonucleotides coded (F, forward primer; R, reverse primer; P, probe); reference(s) of the erroneous oligonucleotide shown in parentheses.
[b] Mismatch position is bold and underlined. Y, C/T; R, G/A; –, missing nucleotide. An added nucleotide is shown as a bold superscript nucleotide. Exchanged nucleotides are shown in bold underlined type with a slash.
[c] Nucleotide number according to GenBank accession no. X17403.
[d] Paper(s) in which the oligonucleotide was stated correctly.

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