Exon 4 of the Human Cytomegalovirus (CMV) Major Immediate-Early Gene as a Target for CMV Real-Time PCR

Lengerova et al. recently published on the failure of a real-time human cytomegalovirus (HCMV) PCR caused by nucleotide variability within exon 4 of the major immediate-early (MIE) gene (3). For more than 6 years, we and other laboratories have routinely used an HCMV in-house real-time PCR assay with primers and probe different from those used by Lengerova et al. but amplifying a sequence also located in the exon 4 of the MIE gene. When we designed this PCR assay, we were aware of the diversity of this gene (1) and we first compared previously published sequences of laboratory strains (Ad169, Toledo, and Towne) and clinical strains using the Clustal software in order to choose the primers and probe in a strictly conserved sequence, as shown in Fig. 1. The efficiency of our in-house test for correct detection and quantification of clinical HCMV strains was demonstrated by comparing it to commercial assays. We first validated the in-house assay by comparing it with the pp65 antigenemia assay. Five hundred eighty-eight consecutive blood samples from 50 allogeneic bone marrow transplant recipients were tested with the two techniques, with significantly correlated results (Spearman’s $r = 0.609; P = 0.0001$) and the HCMV PCR being much more sensitive in all cases (4). More recently, the in-house assay was compared with a new commercial real-time PCR assay: the CMV R-gene (Argene, France), which targets the pp65 gene. We first compared the two techniques with 100 plasma samples from 35 CMV-infected transplant recipients. Eighty samples were found to be positive with the two assays, and the HCMV DNA loads in those samples were significantly correlated (Spearman’s $r = 0.72; P < 0.001$). Twenty discordant results were obtained; HCMV DNA detection was positive with the in-house PCR but negative with CMV R-gene in 16 samples and positive with the CMV R-gene but negative with the in-house PCR in 4 samples. The HCMV DNA loads in the samples with discordant results were low, with a mean of 2.8 (range of 2.4 to 3.3) log$_{10}$ copies/ml and therefore near the threshold values of the two techniques (2.4 and 2.7 log$_{10}$ copies/ml for the CMV R-gene and for the in-house PCR, respectively). Finally, our in-house assay and the CMV R-gene were compared in another laboratory. In this study, 212 whole-blood samples from transplant recipients were tested in parallel with the two techniques. The sensitivity levels of the two assays were comparable, and the HCMV DNA loads in positive whole-blood samples obtained with the two assays were highly correlated (Spearman’s $r = 0.99; P < 0.0001$) (2).

We therefore do not agree with the conclusions of Lengerova et al. that “not using exon 4 of the MIE gene” as a template for routine HCMV PCR diagnosis should be strongly recommended. Indeed, our real-time PCR assay, which amplifies a conserved sequence of MIE exon 4, has proved to be highly efficient for clinical strain detection. However, we agree that a real-time PCR assay for routine diagnostic purposes should be designed after cautious study of previously published sequences of the targeted gene and compared with other available techniques.

REFERENCES


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fig. 1. Clustal alignment of the MIE gene sequences, used as a target for the in-house HCMV PCR, from three laboratory strains, Ad169, Towne, and Toledo (GenBank accession numbers M21295, AY446869, and AY446871, respectively), and six clinical strains (GenBank accession numbers M95634 to M95639). The primers and probe are in boldface and located from nucleotides 2792 to 2086, whereas the primers used by Lengerova et al. (3) were located from nucleotides 2719 to 2919 (strain Ad169; accession number M21295). The asterisks indicate homology between sequences.
failure caused by nucleotide variability within exon 4 of the human cyto-


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