

## Early Identification of Human Cytomegalovirus Strains by the Shell Vial Assay Is Prevented by a Novel Amino Acid Substitution in UL123 IE1 Gene Product

Early human cytomegalovirus (HCMV) identification by the shell vial assay (SVA) is a widespread approach to rapid virus detection in cell cultures and is based upon use of monoclonal antibody (MAb) to the major immediate-early 1 (IE1) antigen (IE1A) p72 (3, 4). The present report illustrates the need for using a pool of MAbs in the SVA in order to avoid false-negative results, which could prevent a correct diagnosis of congenital HCMV infection.

In a pregnant woman, primary HCMV infection was diagnosed based upon increase in immunoglobulin G (IgG) level (from 2.5 to 5.7 IU/ml), decrease in IgM index (from 2.84 to 0.94), and increase in IgG avidity index (from 10 to 44%) between 8 and 21 weeks of gestation (6). At 16 weeks of gestation, quantitative PCR (1) showed the presence of viral DNA (3 genome equivalents [GE]/10  $\mu$ l), while HCMV antigenemia (2) and viremia (3) results were negative. Simultaneously, HCMV was recovered from amniotic fluid (VR7772), where HCMV DNA was present abundantly (12,500 GE/10  $\mu$ l). At 21 weeks of gestation, diagnosis of fetal HCMV infection was confirmed by the presence of HCMV IgM and viral DNA in fetal blood and virus recovery (VR7796) and viral DNA detection (75,000 GE/10  $\mu$ l) in amniotic fluid. However, when the two HCMV isolates were reacted in the SVA with the MAb (5D2) currently used for HCMV identification, no virus could be identified. Sequencing of UL123 from viral DNA extracted from the two sequential amniotic fluid samples as well as from the two sequential virus isolates evidenced a mutation in codon 20 of exon 2 of UL123 (TCC→TTC; Ser→Phe), which was also detected in viral DNA extracted from urine of the mother, thus showing that mutated virus had been transmitted vertically.

In the past, Zipeto et al. identified another virus strain (VR4414) harboring a mutation (TCC→CCC) in the same codon of UL123, inducing a different amino acid substitution (Ser→Pro) not recognized by the same MAb 5D2 (7). As shown in Table 1, when the mutated virus strains were tested

in the SVA using a panel of IE1A-specific MAbs developed in the laboratory, along with a commercial MAb (E13) reactive with the same epitope as 5D2 (5) and a MAb reactive with a late antigen, it was found that while both MAbs 5D2 and 6B1 did not recognize any of the three mutated viruses, MAb E13 did not recognize VR4414 but identified both VR7772 and VR7796. All the other MAbs reacted with the three mutated virus strains similarly.

Substitution of serine (in reference strain AD169) with proline (in VR4414) rendered this virus strain unrecognizable by all IE1A MAbs reactive with the same epitope (Table 1), while substitution of serine with phenylalanine in VR7772 and VR7796 allowed the virus to be recognized by MAb E13 but not 5D2 and 6B1. This differential reactivity of different MAbs with the same epitope may be explained by the conformational change conferred to the epitope by the proline substitution, which makes the epitope inaccessible to any MAb, while phenylalanine, allowing increased epitope flexibility, may be recognized by some but not all MAbs of the same epitope specificity. In conclusion, to avoid false-negative results of SVA, use of a pool instead of single MAbs is advised and highly desirable. Quantitative PCR has high sensitivity and specificity, offering a cost-effective method and short turnaround time. However, prenatal diagnosis of congenital HCMV infection is a critical issue and should never rely on a single assay, either conventional or molecular. Thus, PCR and SVA should be mutually confirmatory.

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TABLE 1. MAb reactivity in the SVA for early identification of HCMV reference strain AD169 and the three HCMV isolates mutated in codon 20 of exon 2 of UL123, VR4414 (Ser → Pro), VR7772, and VR7796 (Ser → Phe)

MAb (reactivity)	IE1A epitope class	Virus identification by the SVA							
		AD169		VR4414		VR7772		VR7796	
		24 h	96 h	24 h	96 h	24 h	96 h	24 h	96 h
5D2 (IE1A)	A	+	+	–	–	–	–	–	–
6B1 (IE1A)	A	+	+	–	–	–	–	–	–
1A1 (IE1A)	B	+	+	+	+	+	+	+	+
5B2 (IE1A)	C	+	+	+	+	+	+	+	+
2A1 (IE1A)	D	+	+	+	+	+	+	+	+
E13 (IE1A) <sup>a</sup>	A	+	+	–	–	+	+	+	+
5A11 (LA) <sup>b</sup>	NA <sup>c</sup>	–	+	–	+	–	+	–	+

<sup>a</sup> E13 was from Biosoft, Paris, France.

<sup>b</sup> LA, late antigen.

<sup>c</sup> NA, not applicable.

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