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Direct Quantitation of CMV DNA in leukocytes by bDNA Signal Amplification Assay. J.A. Kolberg, B. Hoo, R. Miner, R. Kelso, J. Wiegand, D. Chernoff, D. Jekic-McMullen, W.L. Drew. Chiron Corporation, Emeryville, CA and UCSF/Mt. Zion Medical Center, San Francisco, CA.

Objective: Develop an assay to directly quantitate levels of CMV in leukocytes for use in monitoring patients on anti-CMV therapies. Methods: An assay for direct quantitation of CMV DNA based on branched DNA (bDNA) signal amplification was used. A plasmid which contains the target probe binding sequence was utilized as the standard curve for quantitation. Results: The specificity of the bDNA assay is greater than 95% in seronegative blood donors. Fifty culture positive samples were tested and with our current quantitation limit, the sensitivity of the bDNA assay compared to culture is greater than 95%. The assay is highly reproducible and able to detect less than 1.5 fold differences in the mean quantitation of CMV DNA. Antivirals, antibiotics and other drugs that may be used in immunocompromised patients have no effect on assay performance. No cross reaction is observed with other herpesviruses, other blood-borne viruses or pathogenic bacteria when tested at levels 1000x above the quantitation limit. Because of the sensitive and reproducible quantitation of CMV DNA by this assay, we have initiated preliminary experiments to assess the utility of this assay to monitor patients on anti-CMV therapies. In most patients, significant changes in CMV DNA levels (greater than 3 fold) were detected after initiation of therapy. Conclusions: We have developed a sensitive reproducible assay for directly quantitating levels of CMV DNA which may be useful in monitoring the efficacy of anti-CMV therapies.

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CHARACTERIZATION OF THE GUINEA PIG CYTOMEGALOVIRUS UL97 GENE HOMOLOGUE. D.S. Fox and M.R. Schleiss, Department of Infectious Disease, Children's Hospital Research Foundation, Cincinnati, OH 45229

Human cytomegalovirus (HCMV) is a major pathogen in individuals who are immunosuppressed. One of the few drugs effective against HCMV is the nucleoside analog ganciclovir, which inhibits the function of the viral DNA polymerase. However, the increasing incidence of ganciclovir resistance presents a serious problem, especially among patients with acquired immune deficiency syndrome. Clinically, ganciclovir resistance is generally secondary to mutations within the UL97 gene, the gene responsible for phosphorylating ganciclovir to its active form. Alterations within the UL97 open reading frame (ORF) conferring ganciclovir resistance have been identified within a putative catalytic domain spanning amino acid residues 454 to 462 (Lurain *et al.* J.Virol. 68:4427-31. 1994), as well as within a region spanning amino acids 638 to 641 (Sullivan *et al.* Nature. 358:162-164. 1992). In contrast to HCMV, the guinea pig cytomegalovirus is relatively resistant to ganciclovir. Unfortunately, the molecular basis of this resistance is unknown. To test whether GPCMV encodes a UL97 homologue, the colinear region of the GPCMV genome was cloned and subjected to DNA sequence analysis. Sequencing identified an ORF originating in the EcoR1 S fragment of the viral genome and terminating in the adjacent EcoR1 F fragment. This ORF contains striking homology to UL97 of HCMV, and computer matrix analysis confirmed the ORF to be the GPCMV UL97 homologue. Comparison of the DNA sequence of this GPCMV ORF with the HCMV UL97 ORF identified a novel non-conservative amino acid change within the putative catalytic domain, resulting in the substitution of a cysteine residue for a valine (amino acid position 462 of the HCMV UL97 ORF). These observations suggest a possible molecular mechanism explaining the resistance of GPCMV to ganciclovir. Northern analysis confirmed that this region of the viral genome is transcriptionally active, with transcripts of 3.9, 3.3, and 2.7 kb; the 3.9 and 2.7 kb transcripts were present at 4 hours post-infection. This is the first molecular characterization of a UL97 gene homologue in a non-human cytomegalovirus and should provide insights to mechanisms of antiviral resistance as well as UL97 gene function in the viral life cycle.