Development and validation of a non-radioactive DNA polymerase assay for studying cytomegalovirus resistance to foscarnet

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Résumé en anglais	Phenotypic characterisation of the human cytomegalovirus (HCMV) pUL54 DNA polymerase is a useful tool for testing for mutations in the UL54 gene thought to render HCMV resistant to foscarnet. In this study, an in-house non-isotopic method for assessing polymerase enzymatic activity in the presence and absence of foscarnet was developed and its utility for HCMV polymerase phenotyping evaluated. Polymerase activity was assessed by monitoring the incorporation of digoxigenin-labelled nucleotides into the growing DNA chain and foscarnet concentrations inhibiting enzymatic activity by 50% were determined. HCMV DNA polymerases were synthesised in vitro by expression of UL54 under the control of the T7 promoter. Mutations of interest were introduced into the wild-type UL54 gene by site-directed mutagenesis. Mutated polymerases and polymerases from HCMV reference strains were studied. The activity of polymerases containing mutations known to confer resistance to foscarnet (V715M, T700A and N495K) was inhibited by concentrations of foscarnet eight to 14 times higher than those required to inhibit wild-type polymerases. Our in-house non-radioactive phenotypic assay was sensitive and reproducible. It is also easy to perform and could provide a convenient method for characterising mutations conferring resistance to foscarnet in HCMV.
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