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Detection of Cytomegalovirus DNA in Human Specimens by LightCycler PCR: Melting Point Analysis Is Mandatory To Detect Virus Strains with Point Mutations in the Target Sequence of the Hybridization Probes

In our recently published paper we described the application of the LightCycler technology for the detection of cytomegalovirus (CMV) DNA in human plasma and urine (1). We are now able to give further information gained since September 2000, when this PCR procedure was introduced as the standard assay for the detection of CMV DNA in our diagnostic laboratory.

As of June 2001, we had tested 912 specimens using this assay. According to the described test protocol, 185 specimens (20.3%) were positive for CMV DNA. A melting point (T_m) of 59.2°C was observed for the hybridization probes with PCR amplicons of all positive specimens. Another 12 specimens (1.3%) were negative in quantitative analysis but generated a significant peak, with a T_m of 53.1°C, in the melting point analysis. Gel electrophoresis revealed a distinct PCR product of about 250 bp with these specimens that corresponds to the targeted 254-bp amplicon. DNA sequencing of the PCR products with the decreased melting point confirmed the specific amplification of CMV DNA using these specimens as a template. The decline of the melting point is caused by a point mutation in position 630 of the CMV glycoprotein B gene (GenBank accession no. A13758), resulting in a shift from cytosine to thymidine. This strain variant is not included in the databases and causes a mismatch with position 5 of the LC-Red 640-labeled acceptor fluorophore probe. However, the existence of further strain variations in the amplified region cannot be excluded.

The melting point analysis allows the reliable detection of CMV strain variants harboring this point mutation. Specificity of the signal should be further confirmed by gel electrophoresis. Melting point analysis is mandatory to detect virus strain variants in the target sequence of the hybridization probes with the described LightCycler CMV PCR assay.

REFERENCES

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