POLYMERASE CHAIN REACTION (PCR) FOR DIAGNOSIS OF VIRUS INFECTION: HUMAN CYTOMEGALOVIRUS

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A rapid, sensitive and precise procdure of detecting cytomegalovirus (CMV) DNA in human tissue and biological liquid by means of the thermostable DNA-polymerase from Thermus thermophilus is described. The procedure consists of two consecutive PCRs. The first one includes 40 cycles of denaturation, primer annealing and chain extension with two 20-base primers chones from the conserved sequence of MIE (major immediate early) gene of CMV. The second reaction includes from 20 up to 50 cycles consisting of the same steps but with 20-base primers chosen to amplify the internal part of the fragment amplified during the first reaction. The final fragment is detected by the ethidium bromide staining after polyacrylamide gel electrophoresis. Verification of the band includes restriction enzyme analysis. The use of pCM5018 plasmid bearing the CMV MIE gene shows that the proposed procedure can detect as little as several (from 1 to 5) molecules of DNA.