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# HUMAN CYTOMEGALOVIRUS IN THE URINE OF PATIENTS WITH CHRONIC RENAL FAILURE

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## Introduction

Congenital infections of the newborn bear the risk of severe disease and mental retardation [1–3]. The dosage of immunosuppressive drugs must be reduced in renal transplant patients when active infection with the cytomegalovirus (CMV) occurs [4,5]. Conventional serological tests for antibodies to cytomegalovirus are not suitable for rapid diagnosis of cytomegalovirus infection. We therefore established a method to detect shedding of the cytomegalovirus in the urine using molecular DNA hybridization.

## Material and methods

*Patients studied* 52 haemodialysis patients and 53 renal transplant recipients were tested for the presence of CMV DNA in the urine.

*Tissue culture* The specimens were tested in cultures of human embryonic lung fibroblasts (HEL Wi-38), which were weekly re-fed and monitored for the development of cytopathic effects.

*Determination of cytomegalovirus-specific antibodies* Virus-specific antibodies were determined by complement fixation test, and enzyme immunoassay for immunoglobulin G and M class of antibodies.

*Determination of CMV DNA by molecular hybridization* 10ml aliquots of urine were centrifuged at 30,000 rpm at −4°C. The pellets were resuspended and digested with proteinase K (200 μg/ml) for three hours. After two organic extractions the aqueous phases were heated to 100°C for five minutes and then filtered through nitrocellulose paper. The dried filters were soaked with Denhardt' solution and subsequently prehybridized with 30 μg/ml yeast t-RNA.

The final hybridization was performed with the cloned and $^{32}$P-radioactively labelled Eco RI-J-fragment of the cytomegalovirus strain Ad 169 (clone pCM 5018). The specific activity of the probe after nick-transplation was 5 x $10^8$ cpm/$\mu$g DNA [7].

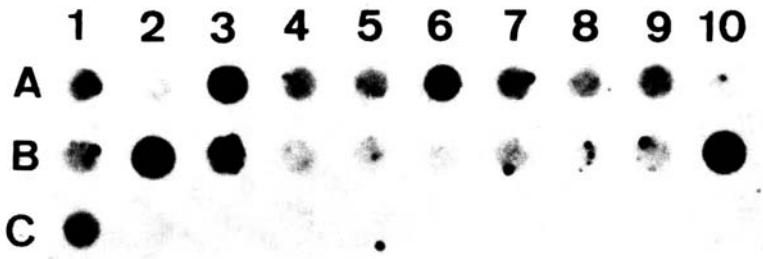


Figure 1. Autoradiographic signals of a nitrocellulose membrane

## Results

The Eco RI-J-fragment of the strain Ad 169 has a nucleotide sequence of 10.6 kb and represents 4.7% of the total CMV DNA sequence. The detection limit of serial dilutions of cloned DNA was 1pg per spot, which corresponds to about 8 x $10^4$ genomes of the cytomegalovirus.

Thirty-three of the 52 haemodialysis patients and 12 of the 53 renal transplants were positive for CMV DNA in the urine. Antibodies of the immunoglobulin M class to CMV antigens could not be detected in the renal transplant patients with urines positive for CMV DNA. There was no correlation between the detection of CMV DNA in the urine and presence of antibodies in serum. Values of SGPT were elevated in 10 of 28 patients with chronic renal failure (=35.7%) with positive CMV DNA in the urine, whereas only seven of 54 CMV DNA-negative patients (=12.9%) had elevated values of transaminases.

## Discussion

The Eco RI-J-fragment of the strain Ad 169 of the human cytomegalovirus has been shown to exhibit a constant sequence of nucleotides without cross-hybridizsation to the cellular genome [6,8]. The determination of CMV DNA in the urine was faster, more sensitive and probably more reliable than the conventional methods of virus isolation from the urine [9]. Serologic tests are not more reliable than virus isolation in patients who are treated by immunosuppressive drugs for organ transplantation [10]. Diagnosis of an infection with the human cytomegalovirus could be performed more rapidly and in a sensitive manner by molecular DNA hybridization. This method may be a suitable alternative for rapid diagnosis of CMV infection.

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