Acta virol. **37:** 106–108. 1993

A RAPID DNA EXTRACTION METHOD FROM CULTURE AND CLINICAL SAMPLES. SUITABLE FOR THE DETECTION OF HUMAN CYTOMEGALOVIRUS BY THE POLYMERASE CHAIN REACTION

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Received February 3, 1992

Summary. – We propose an one-step DNA extraction method suitable for the polymerase chain reaction. This procedure utilizes Chelex 100, a chelating in exchange resin. This technique was compared with a traditional technique (proteinase K lysis, phenol-chloroform extraction and ethanol precipitation) for isolation of human cytomegalovirus DNA from clinical samples. The procedure using Chelex 100 appeared to be a simple and fast extraction method for human cytomegalovirus DNA.

Key words: DNA extraction; Chelex 100; human cytomegalovirus; polymerase chain reaction

Human cytomegalovirus (HCMV) is a major pathogen in congenitally infected infants, allograft recipients and patients infected with human immunodeficiency virus.

The recently developed DNA amplification technique by the polymerase chain reaction (PCR) has already been applied with succes for the clinical diagnosis of the HCMV infections.

Traditional DNA extraction methods from clinical samples and infected cell cultures require several steps: proteinase K lysis, separation and purification utilizing phenol-chloroform extractions and/or ethanol precipitation (Maniatis, 1989). Although these methods are successful in recovering purified DNA, they are time consuming, require multiple tube transfers and increase opportunities for introduction of contaminants.

We propose an one-step DNA extraction method that yields preparations, which can be directly used for PCR. This procedure utilizes Chelex 100 (BIO-RAD), a chelating ion exchange resin (Walsh, 1991). The alkalinity of Chelex suspensions and the exposure to 100 °C result in disruption of the cell membrane and denaturation of DNA. After 30 min, a DNA solution suitable for PCR is obtained.

In this study, we compared DNA extraction using Chelex 100 with the traditional procedure. Seventy samples were treated in duplicate: 30 bronchoalveolar lavages, 20 urine samples, 10 saliva samples and 10 heparinized blood samples. Non-infected human embryonic lung fibroblasts (HEL) served as negative controls and fibroblasts inoculated with wild HCMV strains were used as positive controls.

Specimens of urine and saliva were ultracentrifugated and the virus containing pellets were then extracted; 500 μl of bronchoalveolar lavage were directly used; for blood samples, buffy-coat white blood cells were taken.

Routine virus isolation had previously been performed on all specimens in HEL fibroblasts. Monoclonal antibodies directed against early and immediate early antigens were additionally added 48 hr after inoculation of fibroblasts for detection of viral antigen expression.

Traditional DNA extraction technique was as follows: sample cells were heated to 56 °C for 1 hr in 400 µl of lysis solution containing 10 mmol/l Tris HCl pH 8.3, 50 mmol/l KCl, 2.5 mmol/l $MgCl_2$ and 100 µg/ml proteinase K (Boehringer Manheim). Then, DNA was extracted twice with phenol-chloroform, precipited with ethanol, and dissolved in 10 mmol/l Tris HCl pH 8.0, 0.05 mmol/l EDTA buffer (Innis, 1990).

The same samples and cell cultures were also carefully mixed with a suspension containing 20 % Chelex 100 in 0.1 % SDS, 1 % Nonidet P40 (NP40) and 1 % Tween 20 aqueous solution. The mixture was then heated to 100 °C for 20 min, and centrifuged at 12 000 x g for 10 min. The supernatant was directly used for PCR.

For the PCR procedure, we amplified a 139 bp segment of the late antigen gp 64. Primers have been described elsewhere (Shibata, 1988). Amplification was performed in a total volume of 50 µl. The reaction mixture consisted of 10 µl of the HCMV DNA solution, and 45 µl of the following solution: KCl 50 mmol/l, Tris HCl pH 8.3 10 mmol/l, $MgCl_2$ 1.5 mmol/l, gelatin 0.001 %, dATP, dCTP, dGTP, dTTP 200 µmol/l each, HCMV primers 1 µmol/l each and 2.5 U of Taq polymerase (Perkin-Elmer-Cetus). The PCR reaction was performed in a DNA thermal cycler (HYBAID) for 40 cycles (95 °C for 30 sec, 66 °C for 40 sec, 74 °C for 40 sec, after a 7 min denaturation step at 95 °C.

20 µl aliquots of each sample were then subjected to electrophoresis in 2 % agarose gel, stained with ethidium bromide and photographed.

As shown in Table 1, PCR amplification of HCMV-DNA from tissue cultures and 64 clinical samples gave similar results after the classic extraction or the Chelex procedure. We found only four negative PCR results with the first method. The presence of Taq polymerase inhibitors was suspected because all specimens were culture-positive and the β - globin gene was not amplified. After the Chelex procedure, the mentioned four samples were positive. We observed two false positive results due to contamination in one urine sample and one

Table 1. Comparison of the traditional extraction and Chelex procedure

Samples	Number	PCR results	
		Traditional extraction	Chelex extraction
Tissue cultures	3	+	+
Negative controls	3	–	–
Bronchoalveolar lavage	20	+	+
	8	–	–
	1	–	+
	1	+	–
Urine	12	+	+
	6	–	–
	1	–	+
	1	+	–
Saliva	7	–	–
	3	+	+
Blood	5	+	+
	3	–	–
	2	–	+

broncho-alveolar lavage after the classic extraction; the two patiens were culture-negative and sero-negative for HCMV. No contamination was noted after the Chelex extraction.

In the present study, the Chelex procedure proved to be a simple and rapid extraction method for HCMV DNA in different clinical samples. This technique can be used in the laboratory on routine basis. Moreover, space it seems to decrease the risk of contamination and the presence of inhibitors. Subsequent studies will be required to evaluate the suitability of the DNA Chelex 100 extraction for other clinical samples and other viruses.

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