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DEVELOPMENT OF CYTOMEGALOVIRUS DETECTION IN URINE BY POLYMERASE CHAIN REACTION FOR THE FOLLOW-UP OF LIVER TRANSPLANTATION RECIPIENTS

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Cytomegalovirus infection is one of the major causes of morbidity in liver transplantation recipients (S. Kusne et al, 1988, ***Medicine, 67***: 132 - 143). Rapid and sensitive methods to detect viral infection are required to allow earlier treatment. Serological assays have shown inconclusive results in immunosupressed patients (C. S. Pannuti et al., 1987, ***Rev. Inst. Med. Trop. São Paulo, 29***: 317-322) and viral isolation is cumbersome and takes a long time. Polymerase Chain Reaction (PCR) appears as a very efficient technique to early diagnosis in these patients.

Primers covering the major immediate early (MIE) gene of CMV (Towne strain) were synthesized at Instituto Adolfo Lutz using an Applied Biosytems 380B DNA Synthesizer using reagents and procedures according to the manufacturers. These primers spans a 435bp segment within the exon 4 of MIE gene that contains a unique ***Ban I*** site. Primers with sequences chosen according to G. J. Demmler et al. (1988, *J. Inf. Dis., 158*: 1177 - 1184) are shown below.

Primer MIE-4: CCAAGCGGCCTCTGATA-ACCAAGCC

Primer MIE-5: CAGCACCATCCTCCTCTT-CCTCTGG

Probe MIE (GAGGCTATTGTAGCC-TACACTTTGG) was also synthesized in order to be used for hybridization to increase the specificity and sensitivity of the results if necessary.

Urine samples were obtained from liver transplantation recipients from the Department of Experimental Surgery, Fundação do Fígado, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil. Four patients were studied, one urine sample from each one was analized at least twice using the three different pre-treatments described in the next paragraph. These samples were previously submitted for viral isolation by conventional cell culture methods: in two of them it was possible to isolate CMV and in the other two no virus was found.

Three different sample conditions were used: 10μl treated with lysis buffer (10mM Tris-HCl pH 7.8, 10 mM EDTA, 0.5% SDS) and boiled for 10min; 1 and 10μl urine samples previously boiled for 10 min only.

Polymerase Chain Reactions were performed using the Perkin Elmer-Cetus DNA Thermal Cycler, and cycles were adjusted in the following way: 1 — denaturation: 94°C for 120 sec; 2 — annealing: 65°C for 90 sec; 3 — extension: 72°C for 60 sec. Each extension period was increased by 10 sec on each subsequent cycle so that the last extension period was 7.5 min. A total of 40 cycles were performed in 6.5 h.

The reaction products were detected by electrophoresis on 3.5% polyacrilamide/0.5% agarose gel, directly or previously digested with ***Ban I*** to check the restriction pattern. Pre and Post-PCR procedures were performed in differents rooms to avoid possible sources of contamination.

As seen in the Fig., positive samples showed the expected 435bp fragment after electrophoresis and no signal was detected in the negative control samples. In these first 4 samples studied, it was found total concordance between viral isolation and PCR results. Comparing the two volumes used, 1 μl sam-

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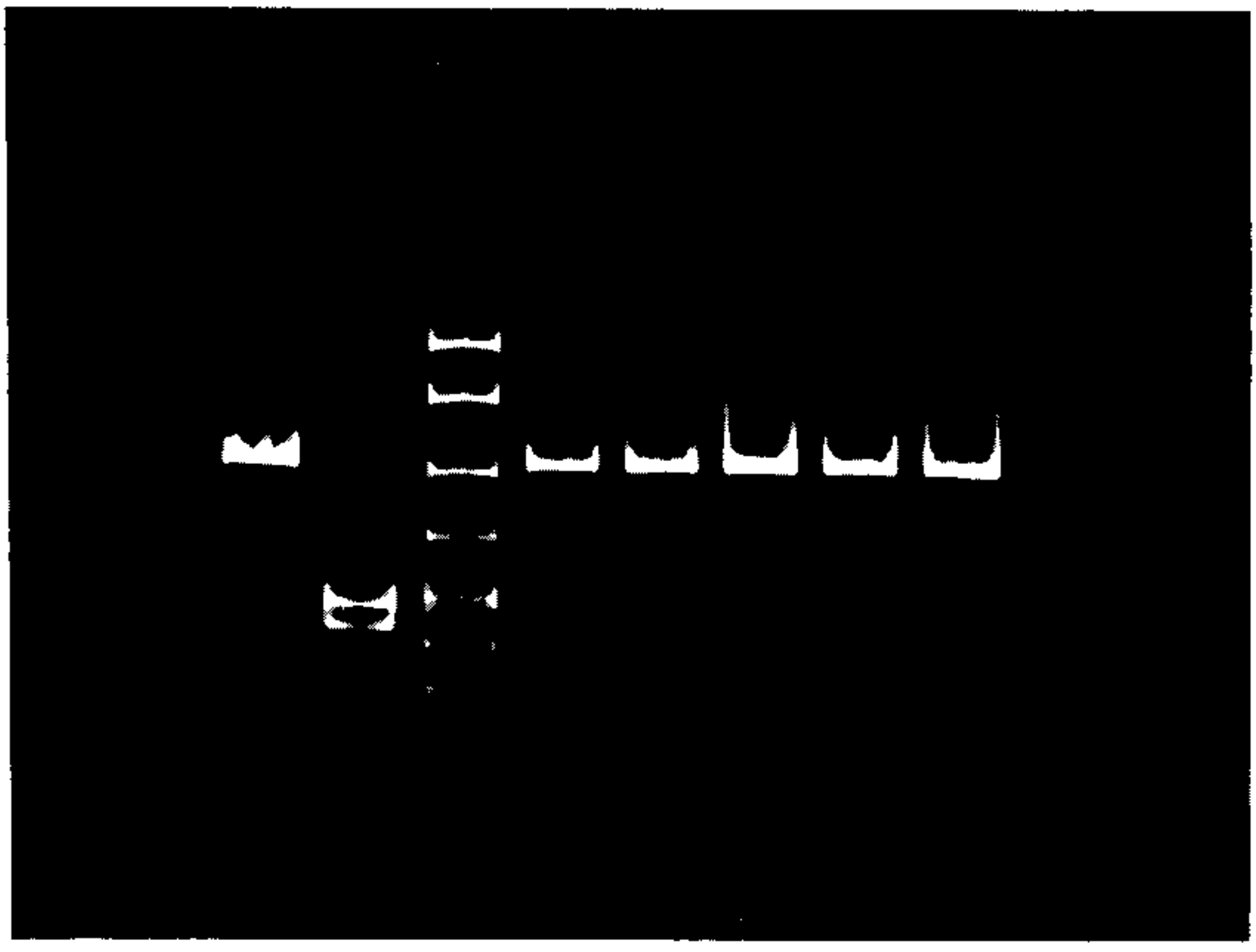
ples gave better results than 10 μl ones. The later showed irregular results, and sometimes were resistant to restriction digestion probably caused by the presence of enzyme inhibitors. No difference was observed with samples pre-treated with lysis buffer. Specificity of PCR products was verified by *Ban I* digestion showing the typical 215 and 220 bp bands pattern. On the other hand, the 435bp band or even other odd size bands were never observed in negative samples, implying in absence of non-specific amplification (see lanes 9 and 10 in the Fig.). These features allow easy distinction between negative and positive samples.

Although a larger number of samples should be analized and results further compared with conventional CMV detection methods, PCR appear as a suitable, specific and sensitive technique for rapid detection of CMV, mainly in immunosupressed hosts with very confusing serological results.



3.5% Polyacrilamide/0.5% agarose gel electrophoresis of PCR products from urine of liver transplantation recipients — 1: MIE containing plasmid. 2: 1μl urine (digested with Ban 1). 3: pBR322 digested with Msp I (622, 527, 404, 309, 242, 238, 217, 201, 190, 180, 147, 123 and 110bp). 4, 5: 1 μl urine. 6: 10μl urine pretreatec with lysis buffer. 7, 8: 10 μl urine. 9, 10: 10μ urine (negative controls).