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Research Note

DETECTION OF HEPATITIS A VIRAL GENOME IN STOOL SAMPLES OF PATIENTS WITH RELAPSED HEPATITIS A BY THE POLYMERASE CHAIN REACTION

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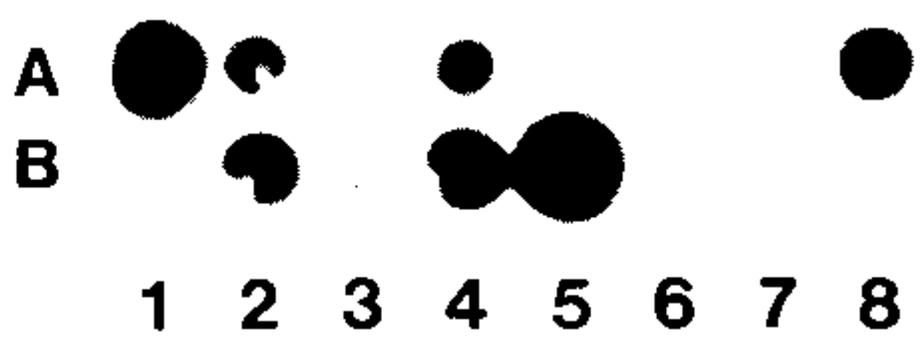
Hepatitis A is a disease usually resolved in a few weeks. However, there have been reports of relapse, characterized by both clinical and biochemical means. Recently, one group reported the presence of the HAV virus in 7 cases of relapsed Hepatitis A by molecular hybridization (M. H. Sjogren et al., 1987, *Ann. Int. Med., 106:* 221), whereas other authors obtained negative results with this same method (N. C. Tassopoulos et al., 1986, *J. Infec. Dis., 154:* 231). In this work we show the presence of the HAV genome in the stools of two out of three patients clinically diagnosed as relapsed Hepatitis A, through the amplification of a segment of the viral genome by the polymerase chain reaction (PCR) (R. K. Saiki et al., 1988, *Science, 239:* 4874).

The sera of the three patients, collected 3-6 months after the acute phase, were positive for IgM anti-HAV and their stools negative for the HAV antigen by radioimmunoassay, as well as for the HAV genome, when tested by molecular hybridization with a cDNA probe.

The RNA from stool suspensions in PBS-azide was extracted with phenol: chloroform in the presence of guanidinium thiocyanate. Following precipitation of the RNA, the first strand of cDNA was synthesized by AMV reverse transcriptase, using as a primer an oligonucleotide 24 bp long, starting at position 2565 of the viral genome (J. I. Cohen et al., 1987, *J. Virol., 61:* 50). A second primer was added to the reaction mix, together with PCR buffer and the enzyme Taq polymerase. The second primer was also a 24-mer from nucleotide position 2043 to 2066, defining a 546 bp long fragment overlapping part of the VP1 and VP3 genes, that was amplified by 30 cycles of PCR. The PCR was carried out manually and the conditions of each cycle component were: denaturation at 94 C for 1.5 min, annealing at 48 C for 2 min and elongation at 72 C for 3 min 10 μl aliquots of the PCR products were run on ethidium bromide stained agarose gels, transferred to nitrocellulose and hybridized to an HAV radioactively labelled probe to ascertain the specificity of the reaction. As this is an extremely sensitive technique, all care was taken during the manipulations to avoid contamination by HAV sequences. The figure shows that all negative controls indeed resulted to be negative by PCR. These controls were processed using the same reaction components and mixes that were used for the tested stools and positive controls. The results presented in this work were repeated in three independent experiments. As a means to further increase the sensitivity of the assay, the PCR products were spotted on dot blots for hybridization, shown in the figure.



Positive controls: A1, tissue culture RNA; A4, B2, B3 and B4, acute Hepatitis A; B5, HAV DNA. Negative controls: A7, stools from a healthy person; B1, reaction mix components. Relapsed Hepatitis A patients: A2, A3 and A8.

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