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Comparison of different non-isotopic methods for hepatitis B virus detection in human serum

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Methods to detect the hepatitis B virus DNA (HBV DNA) by molecular hybridization in serum are becoming useful tools in the routine diagnostic laboratory.We have tested and evaluated three different non isotopic techniques in comparison with the isotopic method regarding sensitivity, specificity and overall detection time for the HBV detection in human serum.

Dot blot hybridization assays were performed in parallel with sera of 65 patiens and sera from control subjects. DNA labeling: HBV DNA (isolated EcoRI fragment from pCF80 plasmid, ref. 1) was labeled as follows: ^{32}PαdATP or biotin-11-dUTP were incorporated into DNA by nick-translation (BRL), photobiotin-labeled DNA was obtained by visible lamp irradiation (Mercury lamp,400W,20min) while digoxygenin-dUTP was incorporated by random priming (Boehringer).Hybridization was performed in 5xSSC,20mM Na phosphate pH 6,0,50% formamide, 42ºC overnight and washes to 0,1xSSC,0,1%SDS,65ºC.Detection.Positive hybridization with biotin-labeled probes was visualized by incubation with strep tavidin/alkaline phosphatase conjugate followed by a coloured enzymatic reaction (5Br4Cl3indolylphosphate and nitroblue tetrazolium salt). Detection of digoxygenin-labeled DNA was carried out by enzyme-linked immunoassay using an antidigoxygenin alkaline phosphatase conjugate (Boehringer kit) and the above described colorimetric reaction. The time required for detection was 2-3h, for non isotopic probes and 18h for the isotopic probe (autoradiography). Limits of sensitivity obtained for each technique (evaluated by serial dilutions of pCF80 DNA processed like sera)were:1-5pg with ^{32}P and the digoxygenin labeled probe, 5-10pg with the biotin-labeled probe and 50pg with the photobiotin-labeled DNA.A significant non-isotopic hybridization is observed with biotin-labeled probes (23% sera gave false positive signals) although we have observed that it can be completely eliminated if HBV DNA is previously purified from sera (phenol extraction, ethanol precipitation).

In conclusion, non isotopic test systems can be considered a suitable alternative for HBV detection in human serum. They give faster results than the isotopic method being stability of DNA probes and the avoidance of radioactive materials their most important advantage.The most suitable non isotopic method in our hands has been the immunological detection of digoxygenin-labeled DNA, which delivers a sensitivity comparable to the isotopic technique, and better specificity than biotin-labeled DNAs.

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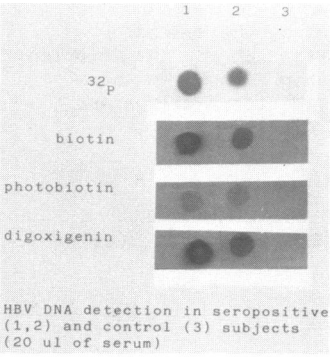
(1) Charnay, P. et al (1979) P.N.A.S.USA 65,2222-2226.



HBV DNA detection in seropositive (1,2) and control (3) subjects (20 ul of serum)