

Improved detection of HBV DNA by PCR after microwave treatment of serum

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We have developed PCR detection of HBV DNA on serum samples.

However, serum treatment before the amplification reaction is a major limiting step and involves a high risk of contamination between samples.

Such a problem is probably linked either to the temperature resistance of the virus or to serum inhibition.

Microwaves have been previously applied to *in situ* hybridization for DNA denaturation of cellular slides (2). In our laboratory, the hypothesis was made that microwaves could also act as a denaturing or chaotropic agent for the HBV or certain serum components.

The present report describes a very simple and rapid microwave irradiation treatment of serum samples which enables direct PCR on desiccated serum.

Briefly, sera are treated by microwaves as follows: 10 µl serum samples are first distributed in the bottom of 0.5 ml PCR tubes which are closed and arranged in a 850 watt microwave oven (Arthur Martin, model 506.41) to be subjected to radiations at maximum power until the sera are desiccated. The time required to complete desiccation may vary, depending on the position of the tubes in the oven, and according to radiation heterogeneity. Positions were chosen to complete this step in the minimum time, of about 2–4 minutes. It can be noted that serum water condenses at the top of closed tubes and does not dissipate contaminant aerosols in the oven.

The tubes are then briefly centrifuged and samples are subjected to 35 cycles of PCR in a 100 µl reaction with 2 U Taq polymerase (Perkin Elmer-Cetus) 0.5 µM of each of the core region specific primers (1).

Table 1 illustrates the results of selected discriminating experiments.

After microwave treatment, a higher sensitivity is obtained either for HBV DNA or viral particles (Figure 1).

To ensure that the effect of microwave irradiation is mediated by serum desiccation, a 98°C temperature incubation was performed on viral particles in a 10 µl sample until the serum was desiccated. Results revealed that temperature and desiccation are insufficient to explain the microwave action.

Hepatitis virus was purified by ultracentrifugation, and directly subjected to PCR without microwave treatment.

High sensitivity level of this detection indicates that irradiation is not required in the absence of serum. Thus, it would appear that microwaves only mediate denaturation of serum inhibitory factors.

Protoporphyrin ring or polysaccharides such as heparin have been described as inhibiting the PCR (3, 4). An attempt was made to apply microwave treatment to heparin solution (30 U/ml); the results clearly indicate that the strong inhibition due to heparin can be partially prevented.

It is possible that several categories of molecules, present in the serum, are sensitive to this treatment.

REFERENCES

1. Kaneko,S., Miller,R.H., Feinstone,S.M. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 312–316.
2. Coates,P.J., Hall,P.A., Butler,M.G. and D'Ardenne,A.J. (1987) *J. Clin. Pathol.* **40**, 865–869.
3. De Franchis,R., Cross,N.C.P., Foulkes,N.S. and Cox,T.M. (1988) *Nucl. Acids Res.* **16**, 10355.
4. Beutler,E., Gelbart,T. and Kuhl,W. (1990) *Biotechniques* **9**, 166.

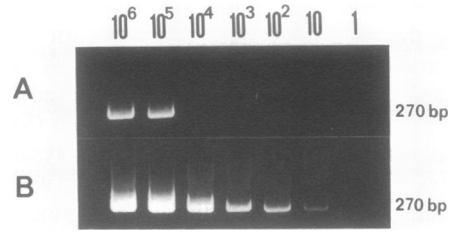

Figure 1. PCR on HBV particles diluted in serum. Without treatment (A); microwave treatment (B). The 270 bp product is revealed by U.V. fluorescence after ethidium bromide staining.

Table 1. Sensitivity level of HBV DNA detection. Results are expressed as viral DNA copy number detected per reaction.

Substrate	Sample Composition	Substrate Treatment: Direct	Microwave Irradiation	98°C Water bath Incubation
Viral particles	PBS	1–10	1–10	1–10
	Serum	10^5	1–10	10^5
Purified DNA	Water	1–10	1–10	1–10
	Serum	10^5	1–10	10^5
	Heparin solution	10^6	10^3	10^6