Application of a screening test for antibody to hepatitis B core antigen

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SUMMARY Hepatitis B core antigen (HBcAg) was prepared from human liver tissue and used in an immunoelectro-osmophoresis screening test to detect antibody to HBcAg (anti-HBc) in patients with evidence of liver disease and in blood donors. With the exception of two immunosuppressed HBsAg carriers, anti-HBc was found in all cases of hepatitis B infection even when HBsAg was detectable only by radioimmunoassay. Non-specific reactions were observed in 'non-B' hepatitis but, in spite of this problem, anti-HBc screening was considered a useful addition to routine tests in the clinical hepatitis laboratory.

The appearance of antibody to hepatitis B core antigen (anti-HBc) during acute hepatitis B infection has been clearly documented in both man (Hoofnagle *et al.*, 1973) and the chimpanzee (Markenson *et al.*, 1975). It has also been found that antibody is regularly present in the sera of established carriers (Hoofnagle *et al.*, 1973). It therefore seems that determination of anti-HBc might provide a sensitive indication of active or recent hepatitis B infection, but so far its application in routine laboratories has been very limited.

Several methods for detecting anti-HBc have been described, including fluorescent antibody (Brzosko *et al.*, 1973), complement fixation (Hoofnagle *et al.*, 1973), immunoelectro-osmophoresis (Budkowska *et al.*, 1974), and radio-immune precipitation (Moritsugu *et al.*, 1975). Hepatitis B core antigen (HBcAg) for the tests has been obtained from either human or chimpanzee liver or, more rarely, from serum rich in Dane particles.

This paper describes a simple method of extracting HBcAg from human liver and the application of an immunoelectro-osmophoresis (IEOP) technique for testing clinical specimens for anti-HBc.

Material and methods

PREPARATION OF CORE ANTIGEN

A 10% extract of postmortem liver tissue from an immunosuppressed HBsAg carrier was prepared by grinding with sand and distilled water in a mortar. The extract was filtered through muslin, frozen,

thawed three times, and clarified by low-speed centrifugation (1500 *g* for 30 minutes). The supernate was then centrifuged at 200 000 *g* for two hours. The resulting pellet was resuspended in distilled water and the cycle of low- and high-speed centrifugations was repeated. The final high-speed pellet was resuspended in 1/10th of the original volume of distilled water and was used as the 'partially purified' HBcAg. Further purification was attained by CsCl density gradient centrifugation.

SEROLOGICAL TESTS FOR ANTI-HBC

IEOP was performed by the technique developed for HBsAg detection (Pesendorfer *et al.*, 1970). Screening tests for anti-HBc were carried out using both the 'partially purified' HBcAg (used at two to four times its IEOP titre) and 'normal' antigen control prepared from HBsAg negative liver tissue. All positive reactions were confirmed with CsCl gradient purified HBcAg, and selected reactions were further checked by electron microscopy of the precipitin line (Fig. 1).

Complement fixation (CF) (Grist *et al.*, 1975) and immune electron microscopy (IEM) (Almeida *et al.*, 1969) were performed by conventional methods.

HBsAg and anti-HBs tests were performed by IEOP and radioimmunoassay (RIA) using commercially available reagents (Abbott Laboratories).

HBeAg and anti-HBe were detected by immunodiffusion (Magnius and Espmark, 1972).

SPECIMENS EXAMINED FOR ANTI-HBC

Sera submitted for hepatitis B testing were selected in the following categories:

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Fig. 1 HBeAg particles aggregated with antibody in precipitin line from IEOP test ($\times 180\,000$).

- 1 HBsAg-positive acute hepatitis
- 2 HBsAg-negative acute hepatitis
- 3 HBsAg-positive chronic liver disease
- 4 HBsAg-negative chronic liver disease
- 5 Blood donors found to be negative for both HBsAg and anti-HBs on initial IEOP screening test.

Results

The 'partially purified' HBeAg had a titre of 1:32 by IEOP and 1:320 by CF when tested against a reference antiserum (National Institutes of Health, USA). EM examination revealed typical 27 nm particles free from antibody coating (Fig. 2). HBsAg could not be detected by CF or IEOP but the final extract had a RIA ratio of 2.5. It was negative for anti-HBs (RIA ratio < 2.1), HBeAg, and anti-HBe.

The results obtained using the IEOP screening test are given in Table 1. Anti-HBc was readily detectable in all specimens which contained HBsAg whether the antigen was present in high or low titre.

BLOOD DONORS

Very few persons without liver disease were found to possess anti-HBc. Of the seven detected out of 929 blood donors, one was found to have low titred HBsAg and three others anti-HBs. Of the three remaining positives by the screening test, one could not be confirmed by immune electron microscopy.

Fig. 2 HBeAg particles free from antibody in 'partially purified' liver extract ($\times 180\,000$).

ACUTE HEPATITIS B

The admission specimen from all 50 patients who were HBsAg positive also contained anti-HBc by electrophoresis and the results could be confirmed by immune electron microscopy. The pattern of antibody response in five representative cases is illustrated in Fig. 3.

ACUTE HBsAg NEGATIVE HEPATITIS

Ten of the 20 admission specimens from patients who were HBsAg negative were anti-HBc positive by IEOP. Table 2 shows the results of confirmatory tests on these sera.

Four specimens were confirmed positive by IEM and they were also shown to contain anti-HBs by RIA. Therefore, the presence of anti-HBc in these HBsAg negative patients probably indicated past hepatitis B infection.

The remaining six positives from the IEOP screening test could not be confirmed by CF or IEM

Table 1 Results of anti-HBc in different categories of patients

Category	HBsAg status	Number tested	Anti-HBc positive
Acute hepatitis	RIA +	50	50 (100%)
Acute hepatitis	RIA -	20	10 (50%)
Chronic hepatitis	RIA +	20	20 (100%)
Chronic hepatitis	RIA -	66	14 (21%)
Blood donor	IEOP -	929	7 (0.75%)

Fig. 3 Pattern of anti-HBc response in five representative cases of acute hepatitis B.

and were negative for anti-HBs by RIA. Two of them reacted with an antigen from normal liver. In addition, follow-up samples obtained 18 months later from two of the six patients were negative for anti-HBc. No further samples could be obtained

Table 2 Investigations of anti-HBc positive sera from HBsAg negative patients with acute hepatitis

Patient	Age	Sex	Anti-HBs	Anti-HBc		
				RIA	IEOP	CF
1	34	M	+	Neat	A/C*	+
2	22	M	+	1:4	A/C	+
3	20	M	+	1:128	≥1:256	+
4	55	M	+	1:2	<1:8	+
5	23	M	-	1:2	<1:8	-
6	23	M	-	1:2†	<1:8	-
7	40	M	-	1:2	<1:8	-
8	2	M	-	1:4	<1:8	-
9	50	M	-	1:8‡	<1:8	-
10	50	F	-	1:2‡	<1:8	-

Note: *Anti-complementary

†Also reacted with 'normal' liver antigen

‡Negative in follow-up specimen

from the other four. It was concluded that six of the 10 positives in HBsAg negative patients were not actually due to the presence of anti-HBc. They may have been 'false-positives' due to non-specific reactions.

CHRONIC LIVER DISEASE

All 20 patients with HBsAg-positive chronic liver disease were found to be positive for anti-HBc (Fig. 4).

Of 66 patients with HBsAg-negative chronic liver disease 14 reacted in the IEOP screening test but the titres were low (Fig. 4). Results of confirmatory tests on the 14 sera are shown in Table 3. Because a high proportion (65%) of these patients were anti-HBs positive all the group were tested (Table 4). A much lower proportion (13%) of the anti-HBc negative group was anti-HBs positive but this is still higher than the 6% level found in normal adults in this country (Cossart, 1976).

Discussion

This study was undertaken firstly to establish the practicability of routine tests for anti-HBc and secondly to assess their value in clinical practice.

An electrophoresis test was chosen because many hepatitis laboratories have considerable experience with this method which is simple, quick, and economical. The problem of producing a substantial amount of HBcAg was solved by obtaining livers from postmortem examinations on HBsAg-positive subjects. Two of 24 were suitable, that is, contained abundant HBcAg but scanty HBsAg in patients with little, or no, detectable anti-HBc. In our experience, this combination was found only in the two patients who yielded the antigen preparations used for the tests. They were both heavily immunosuppressed.

The crude antigen from the simple extraction procedure was almost as satisfactory as the more highly purified one but both gave some non-specific precipitin lines in which antibody-coated cores could not be found by electron microscopy. A control antigen extracted from a normal liver also gave lines with some of the false positive sera, which were rare among blood donors (0.1%) but frequent in patients with acute hepatitis (30%) and not uncommon in chronic hepatitis (6%). Similar non-specific reactions have been reported when sera from the acute phase of hepatitis have been tested against other antigens (Almeida *et al.*, 1974). They were not eliminated by inactivating the HBcAg for 56° for 30 minutes.

Despite this problem, anti-HBc tests have proved very useful in evaluating equivocal HBsAg results.

Fig. 4 Anti-HBc titres in patients with chronic liver disease.

Table 3 Investigations of anti-HBc positive serum from HBsAg negative patients with chronic liver disease

Patient	Age	Sex	Illness	Anti-HBs RIA	Anti-HBc		
					IEOP	CF	IEM
1	54	F	Cirrhosis	+	1:4	1:8	+
2	43	F	Cirrhosis	+	1:4	1:16	+
3	63	M	Cirrhosis	+	1:8	1:64	+
4	65	F	Chronic active hepatitis	+	1:4	1:8	+
5	49	F	Cirrhosis	+	1:2	1:8	+
6	21	F	Chronic active hepatitis	+	1:16	1:128	+
7	32	M	Persistent hepatitis	+	1:2	A/C	+
8	47	F	Chronic active hepatitis	+	Neat	A/C	+
9	19	M	Chronic active hepatitis	-	1:8	1:32	+
10	63	F	Chronic active hepatitis	+	Neat	<1:8	-
11	70	F	Chronic active hepatitis	-	Neat	<1:8	+
12	70	M	Chronic liver disease	-	1:2	<1:8	-
13	61	F	Hepatic failure	-	1:8	<1:8	-
14	50	F	Cirrhosis	-	1:2	<1:8*	-

Note: *Reacted with 'normal' liver antigen $\geq 1:1024$

Table 4 Chronic hepatitis HBsAg RIA negative

	Anti-HBs RIA +	Anti-HBs RIA -	Total
Anti-HBc IEOP +	9	5	14
Anti-HBc IEOP -	7	45	52
Total	16	50	66

A negative anti-HBc result in patients who are not immunosuppressed virtually excludes the presence of HBs antigenaemia.

A larger study of anti-HBc tests for screening blood donors is needed. Our results suggest that it is much cheaper, simpler, and quicker than radioimmunoassay or enzyme-immunoassay for HBsAg, yet it detected all the hepatitis B carriers detected by these cumbersome methods. It is not known whether persons with anti-HBc who are HBsAg-negative are infective. We were unable to obtain specimens from the four patients transfused with material in this category, but it was possible to establish that none developed clinical hepatitis during the ensuing nine months.

It is difficult to interpret the anti-HBc results in patients with HBsAg-negative chronic liver disease since little was known about their country of origin or previous history. However, they certainly differ from the general population as regards presence of both anti-HBs (23 % positive; compared with normal 6 %) and anti-HBc (21 % positive; compared with 0.75 % normal). This suggests that hepatitis B infection may have been the initial liver injury in a significant proportion of these patients.

In general, our findings show that routine anti-HBc tests can be introduced relatively easily, and that they offer useful additional information about patients with suspected hepatitis B infection.

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References

- Almeida, J. D., Zuckerman, A. J., Taylor, P. E., and Waterson, A. P. (1969). Immune electron microscopy of the Australia-SH (serum hepatitis) antigen. *Microbios*, **1**, 117-123.
- Almeida, J. D., Gay, F. W., and Wreghitt, T. G. (1974). Pitfalls in the study of hepatitis A. *Lancet*, **2**, 748-750.
- Brzosko, W. J., Madaliński, K., Krawczyński, K., and Nowoslawski, A. (1973). Duality of hepatitis B antigen and its antibody. I. Immunofluorescence studies. *Journal of Infectious Diseases*, **127**, 424-428.
- Budkowska, A., Walicka, B., Domaniewska, G., and Brzosko, W. J. (1974). Immuno-electro-osmophoresis for the detection of antibody to hepatitis B core antigen. *New England Journal of Medicine*, **290**, 1489-1490.
- Cossart, Y. E. (1976). Unpublished.
- Grist, N. R., Ross, C. A. C., and Bell, E. J. (1975). *Diagnostic Methods in Clinical Virology*, 2nd edition. Blackwell Scientific Publications, Oxford.
- Hoofnagle, J. H., Gerety, R. J., and Barker, L. F. (1973). Antibody to hepatitis-B-virus core in man. *Lancet*, **2**, 869-873.
- Magnius, L. O. and Espmark, J. A. (1972). New specificities in Australia antigen positive sera distinct from the le Bouvier determinants. *Journal of Immunology*, **109**, 1017-1021.
- Markenson, J. A., Gerety, R. J., Hoofnagle, J. H., and Barker, L. F. (1975). Effects of cyclophosphamide on hepatitis B virus infection and challenge in chimpanzees. *Journal of Infectious Diseases*, **131**, 79-87.
- Moritsugu, Y., Gold, J. W. M., Wagner, J., Dodd, R. Y., and Purcell, R. H. (1975). Hepatitis B core antigen. Detection of antibody by radioimmunoprecipitation. *Journal of Immunology*, **114**, 1792-1798.
- Pesendorfer, F., Krassnitsky, O., and Wewalka, F. (1970). Immuno-electrophoretic methods. *Bulletin of the World Health Organization*, **42**, 974-975.