relatively new but fashionable 1,5-benzodiazepine, clobazam is not. The TLC procedure relies on the hydrolysis of benzodiazepines or their metabolites to aminobenzophenones which are detected by their yellow colour, fluorescence characteristics and, in the case of primary aminobenzophenones, the formation of an azo dye. Whereas the 1,4-benzodiazepines readily undergo cleavage to the corresponding open-ringed benzophenone, no such similar reaction occurs with clobazam or its pharmacologically similar metabolite, N-desmethyloclobazam.

We conclude that TLC of the acid hydrolysate will lead to false negative results when clobazam has been ingested, and laboratories using this methodology for benzodiazepine screening should note this exception.

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Reference

- Widdop B. ACP Broadsheet 119: Simple tests to detect poisoning. *J Clin Pathol* 1988;41: 996-1004.

Hepatitis B (HBsAg and HBcAg) hepatic markers in HBsAg positive chronic liver disease

Patients without circulating hepatitis B surface antigen (HBsAg) can none the less have positive viral markers in liver tissue. Usually such patients have high serum titres of anti-hepatitis B core (anti-HBc) antibodies. Several explanations for this phenomenon have been suggested, including low HBsAg concentrations undetectable by traditional diagnostic methods and immunocomplex formation with circulating antibodies.

Recently Cuccurullo *et al*¹ showed that 17% of alcoholic patients and 21% of non-alcoholic patients were positive for HBsAg or HBcAg (core antigen) in liver tissue, despite being seronegative for all serum markers of hepatitis B virus. We studied liver markers (HBsAg and HBcAg) on a total of 60 patients with chronic liver disease and seronegative for HBsAg—30 with alcohol-induced hepatic disease and 30 with non-alcohol-induced hepatic disease. In all cases hepatitis serum B virus markers were studied using ELISA techniques (HBsAg, anti-HBs, anti-HBc, HBcAg and anti-HBe), and liver

tissue markers (HBsAg and HBcAg) with immunoperoxidase.

Fourteen patients had anti-HBs associated with anti-HBc; only a single patient was seropositive for anti-HBc alone. The rest were all hepatitis B virus seronegative. None had positive tissue markers for hepatitis B virus (HBsAg, HBcAg).

Our findings suggest that it is highly unlikely that a seronegative patient will display HBsAg or HBcAg in the liver. It is also unlikely that such a patient will have liver disease related to hepatitis B. These findings contradict, to some extent, those of Cuccurullo *et al*,¹ but the difference is unlikely to be due to geographical factors or methodological variables, because our serum HBsAg positive patients showed highly sensitive and specific hepatic markers.

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Reference

- Cuccurullo L, Rambaldi M, Iaquinio G *et al*. Importance of showing HBsAg positivity in the liver for better aetiological definition of chronic liver disease. *J Clin Pathol* 1987;40:167-71.

Dr Rimbaldi *et al* comment:

Salmerón Escobar *et al* report that none of the 60 patients with chronic liver disease seronegative for HBsAg studied showed positive tissue markers for hepatitis B virus (HBsAg and HBcAg), which is contrary to our previous data.¹ Many authors have reported positive tissue markers for HBsAg or HBcAg in the absence of HBsAg or HBcAg in both alcoholic and non-alcoholic patients. Further observations, conducted in a wider range of cases (n = 164) confirm our previous data (table). In all cases we verified

the specificity of the method as reported elsewhere.¹

In contrast to the findings of the Spanish authors, we maintain that geographical factors could have had a certain effect on the discrepancy found between their data and those of the other authors, ours included. Indeed, only 15 of the 60 hepatic patients (25%) that they studied showed anti-HBs or anti-HBc, or both, in the serum, which probably reflects a low percentage of hepatitis B infection in their population. The spectrum of this infection does, indeed, vary in different parts of Europe—for example, in alcoholics with chronic liver disease the positive percentage oscillates between 10 and 40%; in Northern Europe²⁻³ it is 45% in a French survey⁴ and reaches 75% in an Italian survey.⁵ This most probably reflects varying risks of exposure in different countries.

One could also hypothesise that at least a part of chronic liver disease with HBsAg and HBcAg negative tissue could be the result of infection with the non-A, non-B hepatitis virus.

References

- Cuccurullo L, Rambaldi M, Iaquinio G, *et al*. Importance of showing HBsAg and HBcAg positivity in the liver for better aetiological definition of chronic liver disease. *J Clin Pathol* 1987;40:167-71.
- Hislop W, Follet E, Bouchier I, MacSween R. Serological markers of hepatitis B in patients with alcoholic liver disease. A multicentre survey. *J Clin Pathol* 1981;34:1017-19.
- Orholm M, Aldershvile J, Tage-Jensen U, *et al*. Prevalence of hepatitis B virus infection among alcoholic patients with liver disease. *J Clin Pathol* 1981;34:1378-80.
- Buffet C, Attali P, Papoz L, Chaput JC, Etienne JP. Evidence for previous hepatitis B virus infection in alcoholic cirrhosis. *Dig Dis* 1982;27:473-4.
- Adelascio L, Monarca A, Dantes M, *et al*. Features of chronic hepatitis in alcoholics. survey in Milan. *Liver* 1987;7:283-9.

Distribution of serum and tissue positivity in alcoholic and non-alcoholic patients

Liver HBsAg or HBcAg, or both	At least one marker	Alcoholics		Non-alcoholics	
		n	(%)	n	(%)
Tissue positive	Serum positive	15/71	(21.12)	15/93	(16.13)
Tissue negative	Serum negative	30/71	(42.25)	28/93	(30.10)
Tissue positive	Serum negative	13/71	(18.30)	20/93	(21.50)
Tissue negative	Serum positive	13/71	(18.30)	30/93	(32.25)