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Letter to the Editor

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Failure of 2nd- and 3rd-Generation HCV ELISA and RIBA to Detect HCV Polymerase Chain Reaction-Positive Donations

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In the early 1980s, several countries introduced alanine aminotransferase (ALT) testing of blood donors as a surrogate test for non-A non-B hepatitis. During the period 1980–1984, an ALT study was performed on 10,521 West of Scotland blood donors including 5,057 donors from prisons in West Scotland. A total of 54 donations, 50 from prison donors and 4 from other donors, were found to have ALT values in excess of 2.5 times the upper limit of normal – criteria used by many to indicate hepatitis. All 54 donations were HBsAg negative by radioimmunoassay (Austria 2; Abbott Laboratories). Thirty-two of the 54 plasma donations were stored at –20°C and aliquots were tested for the presence of anti-HCV, HCV RNA and anti-HBc in 1993. All 32 were male donors and all but 1 were prisoners, all fulfilling the 1984 criteria for donors.

Anti-HCV screening was performed using 2nd- and 3rd-generation Abbott and Ortho Diagnostics HCV ELISAs. Twenty-six of the 32 donations were reactive with all 4 ELISAs whilst the remaining 6 were non-reactive with all 4 ELISAs. In the RIBA-2 assay (Chiron Corp.) 23 of the reactives were positive and 3 were indeterminate (1 c22; 2 c33 only). The 6 ELISA non-reactives were negative. All samples were retested by RIBA-3. The 3 RIBA-2 indeterminates became positive suggesting that all 26 of the donations that were reactive by 2nd- and 3rd-generation anti-HCV ELISAs were potentially infective for HCV. The 6 RIBA-2- and ELISA-negative donations remained RIBA-3 negative.

HCV RNA was measured using the polymerase chain reaction (PCR) with nested primers from the 5′ non-coding region [1]. Twenty-one samples were shown to be PCR positive (table 1) – 19 of the 26 RIBA-3 positives and 2 of the 6 ELISA/RIBA negatives. The latter 2 donations were re-aliquoted and sent to the SNBTS PCR Reference Laboratory who confirmed HCV RNA positivity with one donation being HCV type 1 and the other HCV type 3.

Fifteen of the 32 donations were anti-HBc positive (Corek; Sorin Biomedica) and 1 was positive for IgM anti-HBc indicating recent acute HBV infection. Neither of the above 2 RIBA and HCV ELISA-negative but PCR-positive donors had anti-HBc but a sample from 1 donor 5 years later was shown to have HBsAg, HBeAg, IgG anti-HBc and anti-HDV confirming the high-risk activity of this donor. This sample reacted strongly in 2nd- and 3rd-generation HCV ELISAs (Abbott) and RIBA-3 (4+ intensity for all bands) suggesting that the earlier seronegative but PCR-positive result was most likely because the donor was in the very early period of the acute HCV infection before antibody develops, i.e. 'window period'.

Whilst others [2, 3] have reported HCV PCR-positive samples in donations found to be negative by 1st- or 2nd-generation HCV ELISAs, we report that some PCR-positive donations may not be detected even when using 3rd-generation HCV ELISAs. Although the introduction of HCV antibody screening of all blood has dramatically reduced the number of cases of post-transfusion hepatitis previously labelled as non-A, non-B [4], theoretically, there remain potentially infectious donors who are at an early point in the infection process, which the best currently available serological tests will not detect. As the SNBTS discontinued prison sessions in 1984, such donations are rare in this country.

In the USA, ALT and anti-HBc testing has been used as surrogate markers of non-A, non-B hepatitis with a loss of around 3% of donations. It has been argued that should surrogate testing be stopped, potentially infective 'window' period donors would not be identified. Our data would confirm this and suggests that ALT but not anti-HBc screening would have detected the above 2 infections, but seronegative donors. In countries where surrogate testing has not been introduced the decision to proceed to ALT testing in addition to HCV antibody testing will be governed by the prevalence of HCV infection and risk activity in a particular donor population. In addition the existence of these two PCR-positive yet serological negative donations highlights the need to continue developing even more sensitive serological HCV assays.

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Table 1. RIBA-3, RIBA-2 and HCV RNA PCR results on 32 donations with elevated ALT levels

	Positive		Indeterminate		Negative		Total
	RIBA-3	RIBA-2	RIBA-3	RIBA-2	RIBA-3	RIBA-2	
HCV RNA positive	19	17	0	2	2[1]	2	21
HCV RNA negative	7	6	0	1	4	4	11
Total	26	23	0	3	6	6	32

[1] Both donations were re-aliquoted and confirmed PCR positive by our other PCR laboratory.

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