HCV testing in low-risk population

SIR,—The significance of samples which are reactive in the Chiron/Ortho C100-3 ELISA for hepatitis C virus (HCV) antibody has been raised in *The Lancet*'s correspondence columns.[1-5] We have evaluated specificity by studying 67 C100-3 ELISA reactive samples from volunteer blood donors with a sensitive assay for HCV RNA[6] and with the Chiron/Ortho recombinant immunoblot assay (RIBA C100). Surrogate markers for non-A, non-B hepatitis (NANBH), such as raised alanine aminotransferase (ALT) activities and antibody to hepatitis B core (HBc) antigen, were also measured.

9998 volunteer blood donors were screened with the ELISA. 53 samples were clearly above the cut-off and were designated reactive and 15 samples were borderline. All samples were retested twice. The 53 reactive samples were all repeatedly reactive, whereas the borderline samples fluctuated between borderline reactive and non-reactive (1 of these was not available for further analysis). Samples with surrogate markers for NANBH but which were non-reactive in the ELISA were not available for analysis.

Polymerase chain reaction (PCR) assays were done on RNA extracted from the equivalent of 20 µl plasma or sera.[6] Different samples of plasma (sera) from the same individual were extracted by separate investigators in different locations, and the order of repeat PCR reactions was randomised in an attempt to eliminate false-positive results. PCR was done two to four times.

The RIBA C100 test includes the three recombinant antigens superoxide dismutase (SOD), HCV fusion polypeptides 5-1-1 (synthesised in *Escherichia coli*) and C100-3 (synthesised in yeast), and SOD alone (synthesised in yeast), applied to a nitrocellulose strip. High and low levels of IgG are positive controls. Samples reactive with both 5-1-1 and C100-3 are "reactive"; those reactive with only one antigen (or with SOD and one or both of the HCV antigens) are "indeterminate"; and samples that react with the SOD and/or IgG controls only are "non-reactive".

45% (30/67) of ELISA reactive samples, including the fluctuating borderline samples, were confirmed by RIBA; 13% (9/67) were indeterminate and 42% (28/67) were RIBA non-reactive. 25 (37%) of the 67 ELISA reactive samples and 21 (70%) of the 30 samples reactive with both ELISA and RIBA contained detectable levels of viral RNA, as did 3 of the 9 RIBA indeterminate and 1 of the 28 of RIBA negative samples.

24% (16/67) of ELISA repeat reactive samples had surrogate markers, and 14 of these were RIBA reactive. Of the other 2, 1 was RIBA C100 non-reactive and had undetectable viral RNA and the other was RIBA indeterminate and had detectable HCV RNA. 11 of 14 ELISA/RIBA reactive samples with surrogate markers had detectable levels of HCV RNA.

Analysis of ELISA optical density (OD) ratios in relation to RIBA/PCR data showed that 12 of 14 fluctuating borderline samples were PCR and RIBA negative. The frequency of RIBA reactive samples and PCR positive samples seems to increase with increasing OD (table).

Since 70% of the ELISA/RIBA reactive samples have HCV RNA and are most likely to transmit HCV and only 4% (1/28) of ELISA reactive, RIBA non-reactive samples had HCV RNA, it seems that the ELISA and RIBA C100 tests are specific for identifying individuals with HCV in a low-risk population. The number of samples shown to have HCV RNA may be an underestimate because current cDNA/PCR assays may not be as sensitive as PCR assays for DNA genomes and the methods of sample collection, storage, and freeze-thawing can influence the number of viral genomes available for cDNA analysis. PCR negative samples in this study should be viewed as ones which are less likely to transmit, but not necessarily incapable of transmitting, HCV. Our results indicate that both C100-3 ELISA and RIBA C100 reactive samples have a very high potential for transmitting HCV.

SUPPLEMENTAL HCV TESTS IN RELATION TO C100-3 ELISA OD VALUES

—	ELISA OD (mean of three readings):				
	≤0·55*	>0·55–0·8	>0·8–1·5	>1·5–2·4	2·5
RIBA C100					
Reactive	1	0	2	2	25
Indeterminate	3	1	2	2	1
Non-reactive	10	10	6	2	0
PCR positive	1†	0	3‡	2§	19

*Fluctuating borderline, including 1 RIBA non-reactive sample with an average OD of 0·69.
†RIBA indeterminate (5-1-1 reactive, C100-3 non-reactive).
‡1 RIBA indeterminate (5-1-1 non-reactive, C100-3 reactive), 1 RIBA non-reactive, and 1 RIBA reactive.
§1 RIBA indeterminate (5-1-1 non-reactive, C-100-3 reactive) and 1 RIBA reactive.

3 of 9 RIBA C100 indeterminate samples had detectable HCV RNA. Explanations for samples which are indeterminate in RIBA C100 are that the 5-1-1 antigen, which has only 43 of the 363 aminoacids in C100-3, has fewer immunoreactive epitopes; that C100-3 has a different immunogenicity on nitrocellulose than on the ELISA plate; or that since there were different extraction and purification methods for the 5-1-1 and C100-3 antigens synthesised in bacteria or in yeast, the purified antigens may be conformationally and immunologically distinct.

The usefulness of the C100-3 ELISA and RIBA C100 as specific tests for HCV is supported by the finding that only half the samples with the highest potential for transmitting HCV—ie, those which were both ELISA and RIBA reactive and had detectable viral RNA— would have been identified by surrogate markers.

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