Prediction of Hepatitis C Virus Infectivity in Seropositive Australian Blood Donors by Supplemental Immunoassays and Detection of Viral RNA


The prevalence of anti-hepatitis C virus (HCV) enzyme immunoassay (EIA)-positive in 167,511 Australian volunteer blood donors from Adelaide, Melbourne, Perth, and Sydney was 0.78%. One thousand two-hundred and eighteen EIA-positive serum samples were assessed by supplemental tests including a blocking EIA and two peptide EIA corresponding to major epitopes of the HCV C-100-3 antigen. Seven hundred and eighty samples (59%) were negative by supplemental testing; no evidence of reactivity with other HCV gene products or HCV RNA detected by cDNA polymerase chain reaction was found in selected samples from this group. In contrast, of 43 samples randomly selected from 400 samples (32.8%) positive by supplemental testing, 88% were reactive with HCV 33-C or core antigens and 52% contained HCV RNA, suggesting contact with HCV and infectivity of most donors in this group. Most samples equivocal by supplemental testing reacted only with C-100 and not with other HCV antigens when tested by dot immunoblot assay. Only 21% had detectable HCV RNA. The battery of assays used in this study indicated that approximately 32% of HCV EIA repeatedly reactive serum samples were serologically related to HCV, corresponding to a 0.25% prevalence of potentially infectious donors. © 1991 by The American Society of Hematology.

ANTIBODY to a nonstructural protein of the hepatitis C virus (HCV) is routinely screened in blood donors from several countries using enzyme immunoassays (EIAs). The recombinant antigen (C-100-3) that captures anti-HCV antibody is expressed in yeast with a super oxide dismutase promoter (SOD). In low-risk populations, such as volunteer blood donors from western Europe, the United States, or Japan, anti-HCV prevalence ranges from 0.2% to 1.2%. Epidemiologic data have shown that seropositivity is not necessarily associated with HCV infection, stressing the urgent need for confirmatory assays capable of distinguishing true from false positivity. False reactions have been shown to be related to a number of causes such as sample heat inactivation, elevated IgG level, antibody to SOD, or yeast antigens.

The use of the recombinant immunoblot assay (RIBA) represents a preliminary approach to confirmation. This assay differentiates between antibody directed against the C-100-3 antigen and SOD but does not provide definitive confirmation of HCV infection. A second approach to confirmation is the detection of circulating HCV RNA with the polymerase chain reaction (PCR) applied to HCV cDNA obtained by reverse transcription. This method is technically difficult, time consuming, and involves several critical steps, but represents the most direct indicator of infectivity. On the basis of limited data, a relatively poor correlation has been found between RIBA and PCR. Current data suggest that a majority of anti-HCV seropositive blood donors may not be infected with HCV, may not need counseling, and may not justify permanent deferral from the donor pool. To identify donors with HCV EIA nonspecific reactivity, we applied a battery of supplemental HCV antibody tests and PCR to a group of anti-HCV repeatedly reactive serum samples derived from 167,511 whole blood donations collected in Australia.

MATERIALS AND METHODS

Population

Volunteer blood donors attending four Australian Red Cross Blood Centers in Adelaide (South Australia), Melbourne (Victoria), Perth (West Australia), and Sydney (New South Wales) between February and June 1990 were systematically screened for antibody to HCV. Each center contributed 24.8%, 30.2%, 17.2%, and 27.9%, respectively, of the 167,511 donations tested. In Perth, a small number of donors repeated donations during the study period. The second donation was excluded from the study. This population had a gender ratio of 1.1 (0.96 to 1.5) and ranged in age from 18 to 65 years. The age distribution varied from site to site, with a younger population in Sydney and Perth, an older population in Adelaide, and an equal distribution in Melbourne. Donors found to be anti-HCV repeatedly reactive were tested for alanine aminotransferase (ALT) levels at all sites except Perth. All repeatedly reactive donations were excluded from the blood supply and donors were deferred.

Methods

Anti-HCV screening. Serum samples were tested with an anti-HCV EIA according to the manufacturer’s instructions (Abbott Laboratories, N. Chicago, IL). A recombinant nonstructural antigen (C-100-3) of the HCV genome was expressed in yeast, purified, and obtained from Chiron Corporation (Emeryville, CA). This antigen was coated on a 1/4-inch polystyrene bead. Two hundred microliters of a 1:40 dilution of each serum sample was added to the coated bead and incubated for 60 minutes at 40°C. After washing, 200 μL of horseradish peroxidase (HRPO)-labeled goat antihuman IgG antibody was added and incubated for 30 minutes at 40°C. After washing, the orthophenylenediamine substrate (OPD) was added, incubated for 30 minutes at room temperature, and color development stopped by the addition of 1 N sulfuric acid.

From Abbott Laboratories, Diagnostics Division, Department of Medical Research, North Chicago, Ill; Australian Red Cross Blood Bank, Victoria Division, Melbourne; Australian Red Cross Blood Transfusion Service, NSW Division, Sydney; Australian Red Cross Blood Transfusion Service, SA Division, Adelaide; and Australian Red Cross Blood Transfusion Service, WA Division, Perth, Australia.


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Control and test tubes were read in a spectrophotometer at 492 nm.

The cut-off was defined as the mean negative control absorbance plus 0.25 times the positive control absorbance. All specimens with a sample to cut-off ratio greater than or equal to 1.0 were considered reactive and retested in duplicate. At least two of the three replicates above the cut-off defined a repeatably reactive result. All four sites included in this study used an automated pipetting and assay processing system with barcode reading.

Supplemental testing. Each repeatedly reactive serum sample was aliquoted, stored below -30°C, and shipped in large batches to the Abbott Virology Reference Laboratory for supplemental testing. The supplemental testing was designed to distinguish between true HCV-specific antibody binding to the C-100-3 antigen and false reactions. Three levels of assays were used, each being designed to answer a different question. Level one (supplemental testing) determined whether EIA reactivity was specific for the C-100-3 protein or yeast contaminants. Level 2 (dot immunoblot assay) evaluated the presence of antibodies to multiple highly immunogenic HCV gene products providing additional serologic evidence of an immune response to HCV. Level 3 (detection of HCV RNA with PCR) assessed potential infectivity in various serologic groups.

C-100-3 antibody blocking EIA. A soluble, purified fragment of C-100-3 spanning the C-terminal ¾ of the molecule was expressed in Escherichia coli. This fragment was used to block the reactivity of the repeatedly reactive samples from the HCV antibody EIA based on yeast-expressed C-100-3 antigen. Samples were tested in duplicate with the HCV EIA procedure using either the specimen diluent or a diluent containing the blocking antigen. After 30 minutes of preincubation at 40°C, assays were run as described with the HCV antibody EIA. Mean specimen absorbance values of duplicates of the standard or the blocked assay were used to calculate percent blocking according to the following formula:

\[
\%\text{ Blocking} = \frac{\text{Absorbance Standard Assay} - \text{Absorbance Blocked Assay}}{\text{Absorbance Standard Assay} - \text{Absorbance Negative Control}} \times 100
\]

Blocking greater than or equal to 50% was considered positive. Occasionally, the absorbance of the standard assay was above 2.2 optical density (OD) units and the percent blocking below 50%. Such samples were then prediluted with normal human plasma negative for anti-HCV until the lowest dilution yielding an OD below 2.2 was reached and the blocking assay performed as described with that dilution.

C-100-3 peptide 1 assay. A peptide of 65 amino acids corresponding to a major epitope of the mid portion of C-100-3 was synthesized and purified. Peptide 1 was coated on ¼-inch polystyrene beads, washed extensively, blocked, and dried. The assay reagents and procedure were identical to the HCV antibody EIA except for a different formulation of the specimen diluent. Each bead lot was monitored for consistency with a 50-member antibody-negative population and a 6-member sensitivity panel consisting of dilutions of a positive specimen. Positive and negative controls incorporated in each run were those used with the HCV screening antibody EIA. Each sample was tested in duplicate and a mean sample to cut-off ratio equal to or above 1.0 was considered positive. A gray zone corresponding to 20% above or below the cut-off was used in assay interpretation and to select samples for additional testing.

C-100-3 peptide 2 assay. A second 67 amino acid synthetic peptide corresponding to a secondary epitope of C-100-3 located at the C terminus of the molecule was also used as antigen in the supplemental testing algorithm. The bead coating was at pH 8.5. Otherwise, all other reagents and procedures were identical to the peptide 1 EIA. The sensitivity quality control was performed with a 3-member dilution panel.

Supplemental testing algorithm. All HCV antibody EIA repeatedly reactive specimens were tested with the HCV antibody blocking assay and the peptide 1 EIA (Fig 1). When peptide 1 EIA was negative, peptide 2 EIA was performed. Occasionally, specimens identified as HCV antibody EIA repeatedly reactive specimens in Australia did not reproduce when tested with the control portion of the antibody blocking EIA. In most cases, the absorbance was above a sample/cut-off ratio (S/CO) of 0.5. In such samples, the antibody blocking EIA was not interpretable and both peptide 1 and peptide 2 EIAs were performed. After supplemental testing, samples were classified as positive, equivocal, or negative. Positive was defined as an antibody blocking EIA positive and one peptide EIA positive. Equivocal was defined as either blocking antibody EIA only positive or peptide 1 and/or peptide 2 EIA only positive. A negative result was obtained when all these assays were negative.

All available samples equivocal by supplemental testing as well as randomly selected positive and negative samples were also tested with a semi-automated dot blot assay and a genomic HCV RNA detection system using reverse transcription (RT) and PCR.

Semi-automated dot blot assay. Antibodies to C-100-3 and additional HCV proteins were assayed with an HCV semi-automated dot blot immunoassay (MATRIX; Abbott Laboratories) that uses an array of purified recombinant HCV antigens spotted on nitrocellulose. This technology has been described in detail. Antigen test panels consisted of the SOD-C-100-3 fusion polypeptide expressed in yeast plus three recombinant polypeptides expressed in E. coli as fusions with CMP-KDO synthetase (CKS) derived from C-100-3 and the HCV NS-3 (C-33) and S'-structural regions; in addition, each array contained controls (antihuman IgG and human IgG) to verify sample and reagent addition.

The prepared solid phase was incubated in a reaction cell with the test sample (diluted 1:100) for 1 hour at 35°C followed by sequential 30-minute incubations at 35°C with biotin-labeled goat antihuman IgG, alkaline phosphatase-labeled rabbit antibiotin, and bromochloroindolyl phosphate. All incubations and washes were automated. After completing the final incubation, the analyzer dried the nitrocellulose surface and determined the reac-

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**Fig 1.** HCV supplemental testing algorithm. Interpretation of results is discussed in Materials and Methods.
ance at defined locations within the array, thereby providing an objective measure of the extent of the individual reactions. Cut-off values were calculated for each antigen based on a 50-member antibody-negative population; S/CO values of ≥1.0 were considered reactive.

Detection of HCV sequences. Twenty-five microliters of serum was digested with proteinase K (1 mg/mL) and sodium dodecyl sulfate (SDS) (0.5%) for 60 minutes at 37°C. RNA was extracted using phenol/phenolchloroform and precipitated overnight at −20°C with 2.5 vol 100% ethanol in the presence of 0.2 mol/L sodium acetate. The pellet was resuspended in diethylpyrocarbonate-treated water containing RNasin. cDNA was synthesized from extracted RNA using Avian myeloblastosis virus RT. Reactions were performed according to the manufacturer's instructions (Bethesda Research Laboratory cDNA kit and Perkin Elmer Cetus [Emeryville, CA] PCR kit). The primer sequences for cDNA synthesis and PCR were 5'-GCATGTCATGATGTAT-3' and 5'-ACAATACGTGTGTCAC-3'. Thirty-five to 50 cycles of PCR were performed as follows: denaturation for 1 minute at 94°C, annealing for 2 minutes at 37°C, and extension for 3 minutes at 72°C. Additional amplification was achieved by double PCR (PCR/PCR technique) or nested PCR. Double PCR was performed under the same reaction conditions using an aliquot of the round one PCR mixture. Inner primer pairs for nested PCR were 5'-CAGTCTGTATAGCAGG-3' and 5'-CA'ITGAGACAATCAC-3'.

Control samples included normal human sera, a dilution series of chimpanzee plasma of known HCV infectious titer, and human sera positive for HCV RNA. Negative and positive controls were included in each independent extraction series. Reagents were tested in each PCR run for possible contamination. Precautions for avoiding PCR contamination were strictly adhered to. PCR products were analyzed by a quantitative solution-phase hybridization method using a high specific-activity single-stranded 32P DNA probe and by spot hybridization with a single-stranded 32P DNA probe. Both probes were at least 250 bases long and specific for the region between the primer pairs. Hybridization conditions were at high stringency. Specificity of the PCR product detection methods was confirmed by electrophoretic and Southern blot analysis. Clinical specimens positive for HCV RNA were retested at least once starting from the RNA extraction step of the procedure. DNA preparations from the initial amplification of all clinical specimens were subsequently tested with the nested primer pair.

Statistical Analysis

For the comparison of groups two-tailed Fisher's exact test was used.

RESULTS

A total of 1,310 donor samples from 167,511 blood donations were found repeatedly reactive with HCV antibody EIA, corresponding to a positive rate of 0.78%. An aliquot of 1,218 of these samples (93%) was sent to the reference laboratory for supplemental testing. As shown in Table 1, when retested at the reference laboratory, 16% of these EIA-positive samples were below the cut-off, most with an S/CO greater than 0.5. This percentage varied substantially among sites, ranging from 8% to 24%. The HCV blocking antibody assay was therefore applicable to 1,023 samples and 480 (46.9%) were positive. In only 25 samples, a predilution of 1:10 or 1:20 was necessary to adequately perform the assay, suggesting a small number with high levels of antibody.

The peptide 1 assay was performed on all 1,218 samples and 410 (33.7%) were positive. Among the 195 samples below the cut-off of the screening assay at the reference laboratory, 12 (6.2%) were positive with peptide 1 EIA. There were 387 samples positive for both peptide 1 and the blocking assay, corresponding to 80.6% overlap of the two assays. Eleven (2.0%) of 543 samples negative with the blocking assay were positive with peptide 1 EIA and nine additional samples (1.7%) were positive with peptide 2 EIA only.

Overall, from 1,218 samples repeatedly reactive for HCV antibody EIA in Australia, 400 (32.8%) were positive, 100 (8.2%) were equivocal, and 718 (59.0%) were negative by the three-assay supplemental testing procedure. When ranked according to S/CO values, the distribution of samples in each of these three groups varied considerably (Fig 2). In samples below the assay cut-off when retested at the reference laboratory, 97% were supplemental testing negative. This percentage progressively decreased with increasing S/CO, while the percentage of supplemental testing-positive results progressively increased, reaching 87% with S/CO above 4. Seventy-five percent of equivocal results were observed in samples with S/CO ranging from 0.8 to 2.0. In the 1 to 2 S/CO range, 94% of equivocal classification were related to a blocking assay-positive result only.

The HCV dot immunoblot assay was used to assess samples with various patterns of reactivity in the supplemen-

Table 1. HCV Supplemental Testing on 1,218 Australian Blood Donors

<table>
<thead>
<tr>
<th>Origin</th>
<th>No. of Samples Tested</th>
<th>HCV EIA</th>
<th>Blocking Assay</th>
<th>Peptide EIA Pos</th>
<th>Final Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
<td>Pept 1</td>
</tr>
<tr>
<td>Adelaide</td>
<td>176</td>
<td>133</td>
<td>43</td>
<td>59</td>
<td>74</td>
</tr>
<tr>
<td>Melbourne</td>
<td>399</td>
<td>318</td>
<td>81</td>
<td>133</td>
<td>185</td>
</tr>
<tr>
<td>Perth</td>
<td>153</td>
<td>122</td>
<td>31</td>
<td>68</td>
<td>54</td>
</tr>
<tr>
<td>Sydney</td>
<td>490</td>
<td>450</td>
<td>40</td>
<td>220</td>
<td>230</td>
</tr>
<tr>
<td>Total (%)</td>
<td>1,218</td>
<td>1,023</td>
<td>195</td>
<td>480</td>
<td>543</td>
</tr>
</tbody>
</table>

Abbreviations: Pos, positive; Neg, negative; Eq, equivocal.
*Percentage calculated from HCV antibody EIA-positive samples.
†Twelve samples were peptide 1 EIA positive but anti-HCV EIA negative (five in Adelaide, four in Melbourne, two in Perth, and one in Sydney), representing 8.2% of HCV EIA-negative samples at the reference laboratory.
‡Percentage calculated from total number of samples tested (1,218).
were tested in parallel as a negative control population. As shown in Table 3, all 34 members of the normal human serum population were negative for HCV RNA. No HCV RNA-positive results were found among the 23 samples negative by supplemental testing (ie, negative with both the blocking EIA and peptide EIA). In contrast, 52% of samples positive by supplemental testing were HCV RNA positive. Of 72 available equivocal specimens, 21% were HCV RNA positive with a higher proportion of HCV RNA positives observed among samples negative in the EIA blocking assay and positive in the peptide EIA. All PCR-amplified samples were also tested with the nested pair of primers; no additional positives were found.

Ninety-seven specimens were tested both by cDNA-PCR and the HCV antigen dot immunoblot assay (Table 4). Among 20 HCV RNA-positive samples, 11 (55%) were reactive with antigens from multiple HCV gene regions while five (25%) were reactive only with C-100-3 or its truncated form. Eighty-nine percent of the dot immunoblot-negative samples were HCV RNA negative. Evaluation of ALT levels in 107 of the samples tested by RT-PCR showed that six (35%) of 17 samples with elevated ALT were HCV RNA positive.

Depending on the site, age distribution of blood donor populations varied considerably, ranging from 0.96 in Perth to 1.5 in Adelaide (Fig 3). The age distribution of anti-HCV EIA repeatedly reactive donors was not significantly different from the whole population. However, when stratified according to positivity or negativity of supplemental testing, positives were in significant excess in the 30 to 39 years of age group (53%, P < .01). The percentage of samples negative by supplemental testing progressively increased with donor’s age, reaching 31% in the donors beyond age 30 (P < .01). HCV EIA repeatedly reactive samples were found mostly in males (66.0%), but the distribution of age and supplemental testing results was, however, similar between genders.

In representative donor populations at the three sites where it was available, the prevalence of ALT levels above upper normal level ranged from 2.8% to 6%. Upper normal ALT levels were 45 IU/L in Melbourne and Sydney and 50 IU/L in Adelaide, which may explain in part such a wide range. At each center, however, ALT levels above two times upper normal level ranged from 2.8% to 6%. Upper normal ALT levels above two times upper normal level accounted for less than 10% of the elevated values. In contrast, 19.4% of anti-HCV EIA-positive donors had elevated ALT and 7.3% were above

![Fig 2. Distribution of supplemental testing results according to anti-HCV EIA S/CO](image)

**Table 2. Correlation Between HCV Antibody Supplemental Testing and the Dot Immunoblot Assay**

<table>
<thead>
<tr>
<th>Supplemental Testing Results</th>
<th>Dot-Blot Immunoblot Result (N, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 or 3 Antigens Negative</td>
<td>39 (90.7)</td>
</tr>
<tr>
<td>2 or 3 Antigens Positive</td>
<td>3 (7.0)</td>
</tr>
<tr>
<td>C-100 and/or Truncated Antigen Positive</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>C-100 and/or Truncated Antigen Negative</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>All Antigens Negative</td>
<td>34 (60.7)</td>
</tr>
<tr>
<td>All Antigens Positive</td>
<td>12 (21.5)</td>
</tr>
<tr>
<td>Other</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td>Positive</td>
<td>42 (89.5)</td>
</tr>
<tr>
<td>Negative</td>
<td>3 (6.5)</td>
</tr>
</tbody>
</table>

*For the purpose of this table, samples in which the blocking assay was negative or not applicable were grouped.

†All samples were weakly reactive only with the 5' antigen (ratio < 2).
two times upper normal level. Among 282 donors with a positive supplemental test result, 131 (46.5%) had elevated ALT and 56 (19.9%) were above twice upper normal levels; only 42 of 583 negative results (7.2%) had elevated ALT level and eight (1.4%) were above twice normal levels. This difference was highly significant \(P < .001\). Fifty percent of elevated ALT levels were found in the 30 to 39 years of age group (Fig 3).

**DISCUSSION**

The prevalence of positive anti-HCV EIA was 0.78%. This population was previously unscreened and of sufficient size and geographic distribution to be representative of Australian blood donors. No significant variation in prevalence was seen among sites. Similar prevalences were previously reported from western Europe and the United States.\(^1\)\(^3\)\(^5\)\(^7\) As previously described, anti-HCV seropositivity was significantly associated with elevated ALT when elevation was defined as above upper normal level or above twice normal level.\(^1\)\(^3\)\(^7\)

The supplemental testing algorithm used in this study (Fig 1) was based on two separate assays that detected antibodies to HCV C-100 antigen but not to the contaminants of the yeast-expressed C-100-3 known to cause false reactions. The blocking reagent was expressed in *E. coli* and peptides 1 and 2 were synthetically obtained. As shown in Table 1 and Fig 2, nearly 60% of anti-HCV EIA repetitively reactive samples did not appear to contain anti-C-100-3 antibodies. This false positivity was further supported by its decreasing prevalence as the S/CO increased. In addition, only 7% of donors in the supplemental testing negative group had ALT levels above normal and 1.4% above twice upper normal level. In a randomly selected number of supplemental testing negative samples, none were reactive with multiple HCV antigens or positive for HCV RNA (Tables 2 and 3). Thus, in this large number of donors positive by the HCV screening assay but negative by HCV supplemental testing, the likelihood of current or past infection with HCV appears lower than 10% in this limited number of negative samples.

The population of 400 samples positive by the anti-HCV EIA, both at the site and at the reference laboratory and by supplemental testing, represented 33% of the total screening positive samples and had the following characteristics. Most had an S/CO value above or equal to 4 (69%) by the screening assay and close to 50% had elevated ALT levels. Reactivity with C-100-3 of HCV was confirmed by binding to both the *E. coli*-expressed protein and its main epitope (synthetic peptide 1). In addition, over 90% were reactive with two other gene products of HCV (Table 2) in the dot immunoblot assay. Finally, 39% of these samples contained detectable HCV RNA, consistent with the presence of infectious virus. Thus, multiple lines of evidence suggest that this group of blood donors has been in contact with HCV, that at least half of them have chronic liver disease, as indicated by elevated ALT, and/or are able to transmit HCV because viral RNA is present in their serum. These results are similar to data obtained on a limited number of anti-HCV EIA-positive blood donor samples tested with the RIBA and by PCR.\(^8\) Higher rates of positivity for HCV RNA have been obtained with primer pairs from the highly conserved 5' noncoding region of the genome, suggesting that NS4 primer pairs have limited sensitivity, probably related to sequence variations.\(^9\) It is therefore likely that more donors positive with the supplemental testing procedure have HCV RNA and are therefore potentially infectious.

The observation of a maximum frequency of supplemen-
HCV Antibody in Australian Blood Donors

The group called “equivocal” by supplemental testing included a heterogeneous population of samples discordant between the blocking and the peptide assays. The first subgroup included 79% of equivocal samples positive only with the blocking assay. The characteristics of this subgroup included an age and gender distribution similar to the overall donor population and a 12% frequency of elevated ALT. A few samples reacted with other HCV gene products and only one sample tested by both dot immunoblot and PCR was positive by both assays. In contrast, four samples reacting only with C-100 antigens in the dot immunoblot were positive with PCR (Table 4). These results suggest that, although most samples in this group are probably false reactions with the C-100 protein, some clearly contain serologic markers of HCV and/or viral RNA.

The second subgroup of equivocal samples are reactive only with the peptide assay. Most correspond to donors of both genders over 30 years of age with normal ALT level. A majority contains antibody to one or two HCV proteins in addition to C-100 and 25% are positive for HCV RNA (Table 3), suggesting that these donors have been in contact with HCV.

There is evidence from our data that donors with an equivocal supplemental testing result have a clear risk of being infected by and transmitting HCV. This risk appears clearly higher in samples positive only with the peptide EIA than in those positive only with the blocking assay. Similar results were reported with the RIBA indeterminate subgroup.

From the data collected in a large number of Australian blood donors whose serum was reactive with anti-HCV EIA, the various confirmatory procedures used in this study strongly suggest that 59% do not have evidence of contact or infection with HCV. Our data indicate that the blocking assay provides interesting confirmatory information but seems to lack specificity. In contrast, the peptide 1 EIA appears more specific, applicable to all repeatedly reactive samples, and more closely correlated with the dot immunoblot and PCR results. Peptide 1 EIA is therefore suggested as a simple and reliable way to confirm results of the C-100-based HCV screening assay. Supplemental testing negative donors might be notified and counselling may suggest that EIA results were an inconsequential laboratory anomaly. They might also be candidates for potential reinstatement provided that an extensive look-back study of recipients of previous donations fails to detect transmission of HCV.

In contrast, donors anti-HCV EIA-reactive and -positive with the blocking assay carry a risk of transmitting HCV infection according to supplemental testing result: low for donors positive with the blocking assay only, greater when positive with the peptide EIA only, and maximum when both assays are positive.

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Prediction of hepatitis C virus infectivity in seropositive Australian blood donors by supplemental immunoassays and detection of viral RNA

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