The value of screening blood donors for antibody to hepatitis B core antigen

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SUMMARY Rapid counter-immunoelectrophoresis (CIE) and radioimmunoassay (RIA) methods for detecting antibody to hepatitis B core antigen (anti-HBc) were used to screen nearly 8000 blood donors, including 919 prisoners. The prevalence of anti-HBc in prisoner donors (3.4%) was significantly higher than that in other donors (0.7%). The three HBsAg positive donors in the series were all anti-HBc positive and, of the other 73 anti-HBc positive donors, 62 had antibody to HBsAg (anti-HBs).

Two panels of control sera, including 155 HBsAg positive samples, were tested by CIE for anti-HBc: 149 of the 155 were anti-HBc positive. Of the six negative samples, four were HBsAg positive only by RIA. One of the panels, containing 16 weakly HBsAg positive samples, was available for anti-HBc testing by RIA. Fifteen of the samples were positive and the other was slightly reactive. Donor sera that gave unconfirmable reactions in initial CIE tests were invariably negative when tested by RIA. The RIA was a more sensitive and specific test for anti-HBc than CIE.

The ways in which anti-HBc screening could meet the needs of blood transfusion centres are discussed. We suggest that, in areas of low prevalence, it has a role as a rapid confirmatory test of HBV infection and as a means of identifying those potentially infectious donations in which HBsAg cannot be detected.

Antibody to hepatitis B core antigen (anti-HBc) is a sensitive indicator of hepatitis B virus (HBV) infection. It develops soon after the surface antigen of hepatitis B (HBsAg) appears and persists, without interruption, through the phases of HBsAg loss and anti-HBs development. Some anti-HBc positive blood donations are infectious, and there have been several reports of post-transfusion hepatitis B due to HBsAg negative but anti-HBc positive blood. The extent of this risk from “anti-HBc only” positive blood is unclear.

Blood donations have not routinely been screened for anti-HBc in spite of the potential infectivity of anti-HBc positive blood. There have been several reasons for this. The antigen has been scarce, lack of funds has precluded an extra screening procedure, and it has been assumed that few anti-HBc positive donations that contain undetectable levels of HBsAg will be identified. Also, it has been shown that, in areas of high HBV prevalence, many transfusible donations will be rejected as a result of anti-HBc screening unless there is additional testing for anti-HBs.

To assess the value of screening tests for anti-HBc in an area of low prevalence, the occurrence of the antibody in donors in South-West England was investigated. Initially a counter-immunoelectrophoresis (CIE) method was used both for screening and to compare the sensitivity of the anti-HBc test with that of RPHA for HBsAg in detecting potential HBV infectivity in panels of control sera. Subsequently, the introduction of solid phase immunoassay for anti-HBc prompted an extension of the study. A competitive radioimmunoassay (RIA) with a short incubation time (one hour) and a microtitre format compatible with blood transfusion laboratory practice was adopted. This rapid RIA for anti-HBc was used to screen additional donors and to re-examine control sera.

Material and methods

COUNTER IMMUNOELECTROPHORESIS

From June to October 1979 blood donations
The value of screening blood donors for antibody to hepatitis B core antigen

![Graph showing range of results from four samples and 50% inhibition of 125I anti-HBc binding]

Radioimmunoassay for anti-HBc: effect of varying incubation time and temperature.

received at the South Western Regional Transfusion Centre were routinely screened for anti-HBc by CIE* and for HBsAg by a modified commercial reverse passive haemagglutination assay (RPHA).7 A total of 5392 donors was tested, including 919 prisoners. All were "new" donors—that is, not previously tested at the Transfusion Centre. Samples of donations found to be positive were sent to the Virus Reference Laboratory for confirmatory tests. These consisted of CIE for anti-HBc using HBCAg purified by isopycnic banding in caesium chloride, and RIA for HBsAg and anti-HBs (Auslab, Abbott Laboratories).

Testing for anti-HBc by CIE was also carried out on two panels of hepatitis B sera intended for proficiency testing. The first was a Blood Transfusion Service (BTS) panel of 139 HBsAg positive samples which included 17 low titred samples provided by the West Scotland Transfusion Service and six low titred samples provided by the North London Blood Transfusion Centre. The second was the "Special Panel" for hepatitis B testing, containing only low titred HBsAg samples, and issued by the Public Health Laboratory Service (PHLS) Division for Microbiological Reagents and Quality Control. The special panel was also examined by RIA (see below).

RADIOIMMUNOASSAY

A competitive RIA method* was adapted to "Removawell" microtitre plates (Dynatech Laboratories Ltd). A mixture of 15 μl test sample and 85 μl 125I-labelled anti-HBc IgG was added to the solid phase which was coated with an optimal amount of HBCAg. The effect of various incubation times and temperatures was assessed by testing duplicate samples of strongly anti-HBc positive (M144 diluted 1/100) and weakly anti-HBc positive (M144 diluted 1/30 000) serum, and four anti-HBc negative sera. Though brief incubation at raised temperatures gave less 125I-anti-HBc binding than overnight incubation at room temperature, this did not impair detection even of low concentrations of anti-HBc (Figure). Subsequent tests were therefore incubated for one hour at 50°C. Inhibition of 125I-anti-HBc binding by >50% was regarded as a positive reaction.

Between August 1981 and February 1982 2586 donors, including 1210 new ones, were tested for anti-HBc by RIA and for HBsAg by the recently introduced Blood Products Laboratories (BPL) RIA.* As before, positive samples were sent to the Virus Reference Laboratory for confirmatory RIA tests for anti-HBc* and anti-HBs (Auslab).

Results

COUNTER-IMMUNOELECTROPHORESIS

In CIE screening, 129 of 5392 blood donors were anti-HBc positive. Sixty-one of these reactions were

| Table 1 | Prevalence of anti-HBc in blood donors measured (i) by CIE and (ii) by RIA |
|---------|-----------------|-----------------|-----------------|
|         | Donors          | Prisoner donors | Total           |
|         | Number tested   | Confirmed positive | Number tested   | Confirmed positive |
| CIE     | 4473            | 30              | 1210            | 8                |
|         |   |                 | 919             | 77               |
|         |   |                 | 31              | 2                |
|         |   |                 | 5392            | 2586             |
|         |   |                 | 61              | 15               |
confirmed using gradient-purified HBcAg (Table 1). The prevalence of anti-HBc was significantly higher (p<0.001) in prisoners (3.4%) than in other donors (0.7%). Three (5%) of the anti-HBc positive, but none of the anti-HBc negative, donations had HBsAg detectable by RPHA. Forty-nine (82%) of the anti-HBc positive donations were anti-HBs positive. Eight (13%) were neither HBsAg nor anti-HBs positive (Table 2).

One hundred and thirty-seven of the 139 specimens in the BTS panel of HBsAg positive samples contained anti-HBc detectable by CIE (Table 3). The two anti-HBc negative specimens had low levels of HBsAg. The CIE results on the PHLS special panel were similar (Table 4). Twelve of the 16 HBsAg weakly positive specimens were anti-HBc positive, and four negative. Two of these four specimens were positive for HBsAg by RIA alone.

**Table 2** HBsAg and anti-HBc status of anti-HBc positive donors

<table>
<thead>
<tr>
<th></th>
<th>HBsAg +</th>
<th>HBsAg -</th>
<th></th>
<th>HBsAg +</th>
<th>HBsAg -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HBc + (CIE)</td>
<td>61</td>
<td>3</td>
<td>49</td>
<td>8 (1*)</td>
<td></td>
</tr>
<tr>
<td>Anti-HBc + (RIA)</td>
<td>15</td>
<td>0</td>
<td>12</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

*Not available for anti-HBs testing.

**Table 3** Results of anti-HBc tests on the Blood Transfusion Service panel of HBsAg positive specimens

<table>
<thead>
<tr>
<th>HBsAg</th>
<th>CIE</th>
<th>RPHA</th>
<th>CIE</th>
<th>RPHA</th>
<th>CIE</th>
<th>RPHA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HBc +</td>
<td>96</td>
<td>37</td>
<td>4</td>
<td>137</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HBc -</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 4** Results of tests on the Public Health Laboratory Service 'Special Panel' of sera for hepatitis testing

<table>
<thead>
<tr>
<th>Panel No</th>
<th>HBsAg</th>
<th>Anti-HBc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RPHA</td>
<td>BPL-RIA* CIE</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>&lt;2</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>&lt;2</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>71</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>155</td>
<td>+</td>
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<tr>
<td>8</td>
<td>147</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>72</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>135</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>20</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>177</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>25</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>134</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
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<td>16</td>
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<td>+</td>
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<tr>
<td>17</td>
<td>&lt;2</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>&lt;2</td>
<td>±</td>
</tr>
</tbody>
</table>

*Ratio of test/negative control count.
†Percentage inhibition of 131I-labelled anti-HBc (≥50% binding is considered positive).

Radioimmunoassay

Screening of 2586 blood donors yielded 32 anti-HBc positive reactions. Seventeen of these reactions were non-repeatable and negative in confirmatory tests, and 15 (0.6%) were reproducible and could be confirmed (Table 1). Of the new donors 0.7%, and of the known donors 0.4% had anti-HBc. There were no HBsAg positive donors in the series. Twelve of the 15 anti-HBc positive donors were anti-HBs positive and three anti-HBs negative (Table 2).

On examination of the PHLS special panel, all but one HBsAg positive sample, No 6, were anti-HBc positive by RIA. This sample, which had the lowest HBsAg activity in the BPL-RIA test and was RPHA negative, was slightly reactive in the anti-HBc assay (Table 4).

Seventy-four donations which had initially given an anti-HBc positive reaction on CIE screening were retested by RIA. None of the 19 giving unconfirmable CIE reactions was positive in the RIA test for anti-HBc, whereas all but two of the 53 confirmable CIE reactions were. There were no other markers of HBV infection in the two sera with discrepant results.

**Discussion**

The prevalence of anti-HBc in new blood donors, measured by both CIE and by RIA, was 0-7%. In a selected group of donors, those previously screened at this centre, the prevalence was lower, 0.4%, whereas in prisoner donors anti-HBc was much more common (3.4%). An increased occurrence of HBV infection in penal institutions has previously been reported. The prevalence of anti-HBc in blood donors was less than half that in another British survey of new donors. Tedder and colleagues apparently found 37 (1.85%) out of 2000 North London donors anti-HBc positive. Since the RIA used by them is known to be of similar sensitivity to that employed here, the difference probably reflects the more cosmopolitan background and varied socioeconomic habits of Londoners, and not a difference between the assays.

The results for the panels of control sera confirmed that the anti-HBc test was effective in identifying HBsAg positive specimens. All but two
of the panel of 139 HBsAg positive sera, as well as
the HBsAg positive donors, were anti-HBc positive
by CIE. The two negative anti-HBc results were on
specimens with very low levels of HBsAg. Four
other HBsAg weakly positive specimens, in the
PHLS special panel, were missed by the CIE test for
anti-HBc. When RIA for anti-HBc was applied to
the special panel one HBsAg specimen was not
clearly identified, though it did partially inhibit
anti-HBc binding. These results do not justify using
anti-HBc testing alone to screen blood donations,
but they indicate that it can usually confirm HBsAg
positivity in donors. Only blood collected in the
incubation period of HBV infection is likely to be
HBsAg positive but anti-HBc negative.

As well as identifying more weak HBsAg positive
specimens, RIA for anti-HBc proved to be more
specific than CIE. It gave negative results for all the
19 donors whose sera produced unconfirmable re-
tions on initial CIE screening. It gave only three
positive reactions, out of 2586 donors tested, that
were not corroborated by a positive anti-HBs result.
These three may have been false-positive reactions
or they may belong in a true “anti-HBc only” categ-
ory. The eight donor sera that were “anti-HBc only”
when tested by CIE may fit into the same category.
Donor blood with this pattern of reactivity has given
rise to both type B and non-A, non-B post-
transfusion hepatitis. It is therefore un-
desirable that it should be transfused.

The donations found by us to be anti-HBc posi-
tive were dealt with as follows: those known to be
HBsAg positive were discarded; those anti-HBs
positive were sent for inclusion in pools for the pro-
duction of HBV immune globulin (HBIG); and
those in the “anti-HBc only” category were set aside
for possible use in blood product manufacture
involving processes that inactivate HBV. In our
donor population the prevalence of anti-HBc was
low enough for all anti-HBc positive blood to be
withheld from transfusion. As most anti-HBs posi-
tive donations also have anti-HBc (Tedder and
coworkers found 27 with and 15 without anti-HBc
in 2000 donors) considerably fewer donations with
any marker of HBV infection can have been trans-
fused while anti-HBc screening was being applied.

A striking advantage of the anti-HBc test was its
rapidity. Both methods described give a result
within two hours, and allow quick confirmation of
HBsAg screening results and fast release of urgently
required fresh blood and blood constituents. This
may be particularly useful in providing for those
needing multiple transfusions—for example, renal
dialysis and immunosuppressed patients. The main
disadvantage of anti-HBc testing was the high inci-
dence of apparently non-specific reactions. Many
results could not be reproduced in the more experi-
enced, reference laboratory. Another drawback at
the time of the study was the short supply of antigen,
though this obstacle has now been overcome by the
cloning of HBV DNA sequences in E. coli. E coli-
derived HBeAg is suitable for an RIA of the kind
used here and may give rise to fewer false-positive
reactions.

Other disincentives to anti-HBc screening are the
potential loss of transfusable blood, and the cost,
both of the test itself and of anti-HBs tests that must
be done if anti-HBc positive, HBsAg negative units
are to be used. These factors will certainly prevent
anti-HBc tests being used by poorly-funded laborato-
ries in areas of high prevalence. Elsewhere the
benefits from anti-HBc testing, of confirming
HBsAg reactions, of identifying donations suitable
for HBIG manufacture and of lessening the risk of
post-transfusion hepatitis by excluding “anti-HBc
only” blood, ought to be studied. Several authors
have argued that an anti-HBc test is not useful in
blood donor screening. They cite the small
number of cases of post-transfusion hepatitis due
to anti-HBc positive units brought to the notice
of transfusion centres. However they ignore the larger
numbers of sub-clinical infections, with HBV and
possibly non-A, non-B hepatitis viruses, that may
follow transfusion of anti-HBc positive blood. They
also overlook several practical advantages of having
an anti-HBc test available in transfusion
laboratories. In the form of a rapid, one-step RIA
the test is well-suited to screening large batches of
sera. With an abundant supply of HBeAg from E
coli becoming available, it should be reconsidered
as an adjunct to HBsAg screening of blood donors.

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References

1 Hoofnagle JH, Gerety RJ, Ni LY, Barker LF. Antibody to
hepatitis B core antigen: a sensitive indicator of hepatitis B
2 Lander JT, Gitnick GL, Gelb LH, Aach RD. Anti-core antibody
3 Hoofnagle JH, Sceff LB, Bales ZB, Simmerman HJ, and the
Veterans Administrative hepatitis co-operative study. Type B
hepatitis after transfusion with blood containing antibody to
4 Katchaki JN, Siem TH, Brouwer R. Serological evidence of pres-
ence of HBsAg undetectable by conventional radio-
imunosassay in anti-HBc positive blood donors. J Clin Pathol
5 Wilkinson R. The impact of anti-HBc screening on a Black blood
6 Cohen BJ, Cossart YE. Application of a screening test for anti-


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