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Feasibility and usefulness of an efficient anti-HBc screening programme in blood donors

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SUMMARY. Post-transfusion hepatitis B remains a risk for recipients of hepatitis B surface antigen (HBsAg) screened blood. Anti-hepatitis B core antibody (anti-HBc) screening may help reduce this risk. To evaluate its usefulness, 9,238 East Anglian blood donors were screened for anti-HBc. Those with isolated anti-HBc were identified with two confirmatory anti-HBc and anti-HB surface antibody (anti-HBs) assays. The prevalence of anti-HBc reactions in screening and confirmatory assays was 1·29% and 0·35%, respectively. The level of reactivity was significantly higher when two anti-HBc assays gave concordant results or, being concordant, were anti-HBs positive. All isolated anti-HBc-positive units (0·04%) were negative for additional HBV markers including DNA tested with nested polymerase chain reaction (PCR).

A 0·31% prevalence of past HBV infection was found in this population, all carrying both anti-HBc and anti-HBs antibody, most above the protective level (0·1 IU/ml).

The proposed screening schemes would limit the number of deferred donors and discarded units and keep the testing time within the remit of routine blood banking practices for an additional cost of approximately £1 per unit. However, no evidence was found in this donor population to suggest that anti-HBc screening would significantly reduce the incidence of post-transfusion hepatitis B.

Key words: anti-HBc, donor screening, hepatitis B, post-transfusion hepatitis

In the early 1980s, some countries introduced anti-HBc screening to further reduce the risk of non-A, non-B hepatitis in an HBs antigen and alanine amino transferase screened blood donor population (Aymard *et al.*, 1986; Koziol *et al.*, 1986). The relative efficacy of this surrogate test was largely overcome when specific screening assays for antibody to hepatitis C virus (HCV), the principal agent of non-A, non-B hepatitis, was introduced in 1990 (Kuo *et al.*, 1989). Anti-HBc testing was then retained to detect certain populations at high risk of HIV infection and to reduce the risk of hepatitis B transmission (Le Pont *et al.*, 1990). It has been known for many years that in addition to the 56-day window period preceding the detectability of HBs antigen, there is a second period later in the infection during which blood can be infectious but anti-HBc is the only marker of hepatitis B infection, prior to the development of neutralizing anti-HBs (Hoofnagle *et al.*, 1978). In addition, current HBs Ag assays lack sensitivity (Liang *et al.*, 1994), leaving the tail-end of HBV carriage detectable only by the presence of anti-HBc or HBV DNA. Some variants have been described which escape HBsAg screening assays but not anti-HBc detection (Lai *et al.*, 1989; Kojima *et al.*, 1991). In Europe, most of these variants are of Mediterranean origin.

The risk of HBV transmission by transfusion in countries such as the United Kingdom, where anti-HCV screening is implemented, but not anti-HBc, is estimated at 1 in 20–30,000 (Flanagan *et al.*, 1989; Martlew *et al.*, 1993). In East Anglia, two cases of transfusion-transmitted HBV were recorded over the past 5 years; both had received blood products from an anti-HBc-positive donor. Anti-HBc screening was considered a candidate assay to reduce this risk (Anderson *et al.*, 1992).

In Western countries where the prevalence of hepatitis B infection is low, it is expected that a large proportion of anti-HBc-positive blood donations

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would be false positive (Weare *et al.*, 1991). Another fraction of these reactive units are likely to correspond to past HBV infection and to contain neutralizing anti-HBs antibodies. Most of these anti-HBc/anti-HBs-positive units are safe for transfusion, provided the protective level of anti-HBs of 0·1 IU/ml is present (Aoki *et al.*, 1993). Donors who carry true anti-HBc as the only marker of HBV infection are susceptible to transmitting the virus and should be eliminated from the blood supply (Hoofnagle *et al.*, 1978; Larsen *et al.*, 1990; Lizuka *et al.*, 1992).

The study reported here was therefore designed to assess the potential efficacy of anti-HBc screening in East Anglian blood donors, with the following objectives:

1 to determine the prevalence of isolated anti-HBc;
2 to identify any HBV infectious units using PCR to detect HBV DNA;
3 to devise ways of limiting unnecessary discarding of blood units.

MATERIALS AND METHODS

During the last months of 1992, 9,238 consecutive new and repeat blood donors were tested at the East Anglian Blood Transfusion Centre in Cambridge. The donor panel at that time contained 12% new donors.

The following testing scheme (Fig. 1) was used.

In *step 1*, all donors were tested for anti-HBc using the enzyme immunoassay (EIA) Corzyme generously provided by Abbott Laboratories. This assay in a competitive format was carried out according to the manufacturer's instruction with the Commander automated system (Weare *et al.*, 1991).

In *step 2*, all initially reactive samples were retested in duplicate with Corzyme and (*step 3*) an alternative anti-HBc screening assay (Ortho HBc ELISA, Ortho Diagnostics) which uses an indirect assay format. In this assay, recombinant HBV core antigen coated to the solid phase is used to capture anti-HBc whose presence is revealed by a conjugated anti-human immune globulin. In *step 4*, all initially reactive samples were retested once with a third assay (IMx core, Abbott Laboratories) using a format similar to Corzyme but with different solid phase and reagents (Eble *et al.*, 1991).

In *step 5*, serum samples positive with both Abbott and Ortho anti-HBc screening assays were considered positive for anti-HBc and further tested for the presence of anti-HBs using an anti-HBs assay (Abbott Ausab EIA). In this automated assay, recombinant hepatitis B surface antigen was used as capture for anti-HBs. The presence of antibodies was probed with biotinylated recombinant HBsAg. Absorptions of positive results were compared with a reference sample calibrated at 0·1 IU/ml of anti-HBs (Ostrow *et al.*, 1991). Samples with an optical density equal to or above the reference were considered immune; below that level, immunity to HBV was considered doubtful.

Anti-HBc-positive samples which were negative for anti-HBs were considered isolated anti-HBc and were subjected to a battery of additional HBV testing. This included an HBsAg assay more sensitive than the screening assay but impractical for donor screening (Murex HBsAg ELISA) using the long incubation mode; an IgM anti-HBc assay (Corzyme M, Abbott Laboratories) and HBV DNA using a nested PCR.

The HBsAg assay and the IgM anti-HBc assays were carried out according to the manufacturers' instructions. HBV DNA was performed using a previously described method (Harrison *et al.*, 1991). Briefly, DNA was extracted from 100 μl of serum by ethanol precipitation. The pellet was washed in 70% ethanol and resuspended in 20 μl of water. DNA from 10-μl aliquots was first amplified using an external primer pair (MD14/HCO3) flanking part of the 'a' region of the surface antigen gene. After 35 cycles of amplification (94°C, 1·5 min; 42°C, 1·5 min; 72°C,

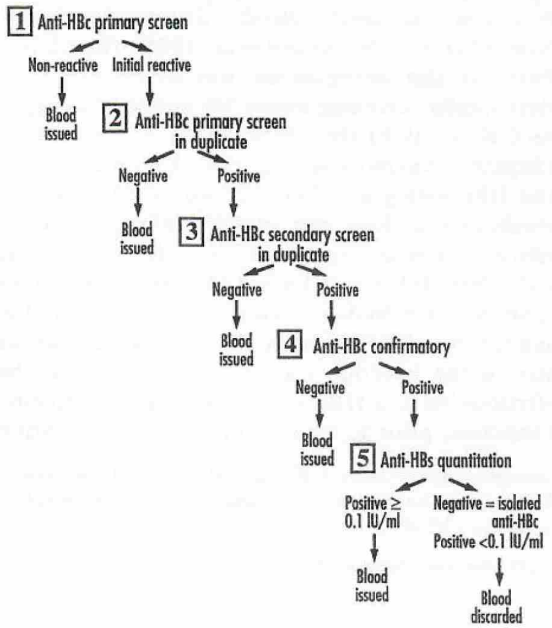

Fig. 1. Flow chart of the presented anti-HBc study. Each of the five steps is indicated by a boxed number.

Table 1. Anti-HBc screening in East Anglian blood donors

		Number tested	Number positive	% of total
Screening assay 1	initial	9,238	119	1·29
	repeat	119	99	1·07
Screening assay 2	repeat	119	52	0·56
Confirmatory anti-HBc		52	32	0·35
Anti-HBs	>0·1 IU/ml	32	24	
	<0·1 IU/ml		4	
Isolated anti-HBc			4	0·04

3 min) using a Techne PHC-2 thermal cycler, 2 μl of the 100 μl first round PCR preparation was used in a second PCR round. The ME15/HCO2 internal primer pair was used for 30 cycles of amplification according to the protocol used in the first round. PCR products were visualized by ethidium bromide staining in UV light after 2% agarose gel electropheresis. The sensitivity of the method was estimated at 60 copies per millilitre of serum.

During the trial, blood units confirmed positive for anti-HBc and with anti-HBs less than 0·1 IU/ml or with isolated anti-HBc were discarded.

RESULTS

Over a period of 5 weeks, 9,238 consecutive whole blood donors were screened for anti-HBc. As shown in Table 1, the repeatedly reactive rate was slightly above 1% of this previously unscreened population. The use of an alternative screening assay to perform the duplicate testing of initially reactive samples reduced by half the number of repeat reactive samples. This number was again nearly halved by the anti-HBc confirmatory assay to reach a 0·35% prevalence of anti-HBc. Eighty-eight per cent of these 32 donations contained antibody to hepatitis B virus surface antigen, most of them at a level considered immunoprotective.

Only four samples were left as isolated anti-HBc (0·04%). None of these four samples reacted with the sensitive HBs antigen assay, nor with the IgM anti-HBc assay. HBV DNA tested with a sensitive nested PCR was also negative.

In order to reduce the number of steps in our testing schemes, we explored the possibility of using either duplicate testing with an alternative screening assay (Ortho anti-HBc) or single testing with a confirmatory assay (Abbott IMx anti-HBc) to limit the number of false reactions obtained with the initial screening assay. As shown in Table 2, the alternative screening assay confirmed 46% and the confirmatory assay 31% of these reactive samples. Seventy-six per cent of samples (28/37) confirmed anti-HBc positive with the IMx confirmatory assay and 54% (29/54) confirmed with the alternative screening assay also contained anti-HBs antibodies (Table 3). All 30 samples negative for anti-HBs but positive for anti-HBc with both or either tests were negative for HBsAg, IgM anti-HBc and HBV DNA. These results suggest that 25 and 9 samples positive for anti-HBc with the alternative screening or the confirmatory assay, respectively, were false positives.

Since previous studies have emphasized the importance of anti-HBc levels to discriminate between true and false positive as well as infectious and

Table 2. Comparison of two anti-HBc assays for confirmation of 119 samples reactive with a screening assay

		Ortho screening anti-HBc		
		Pos	Neg	Total
Abbott IMx Anti-HBc	Positive	32	5	37
	Negative	22	60	82
	Total	54	65	119

Table 3. Detection of anti-HBs antibody in anti-HBc confirmed samples

Anti-HBc*		Anti-HBs (IU/ml)			
Test 1	Test 2	>0·1	<0·1	Negative	Total
+	+	24	4	4	32
+	−	1	0	21	22
−	+	0	0	5	5

* Test 1 is Ortho anti-HBc; Test 2 is Abbott IMx anti-HBc.

non-infectious blood units (Iizuka *et al.*, 1992; Douglas *et al.*, 1993) we conducted a similar analysis. The cut-off to sample ratio was calculated for each result obtained with the primary screening assay (Corzyme). These ratios were stratified according to reactivity with the alternative screening or the confirmatory assays (Fig. 2). Discordant samples between the two screening assays had a mean ratio of $1{\cdot}35 \pm 0{\cdot}579$ while concordant ones had a mean ratio of $5{\cdot}19 \pm 3{\cdot}82$. The difference was significant ($P < 0{\cdot}001$). Similarly, between the initial screening and confirmatory assays, the difference was significant ($1{\cdot}39 \pm 0{\cdot}366$ vs $6{\cdot}98 \pm 3{\cdot}52$; $P < 0{\cdot}001$).

Within the two groups of largely overlapping concordant results, we stratified the ratios according to the presence or absence of anti-HBs (Fig. 2). In samples concordant with the two screening assays, the mean ratios were $8{\cdot}02 \pm 2{\cdot}83$ vs $1{\cdot}87 \pm 1{\cdot}51$ and with the initial screening and the confirmatory assays $8{\cdot}22 \pm 2{\cdot}67$ vs $1{\cdot}66 \pm 2{\cdot}65$. There was a clear separation between low-level and high-level reactors, most of the latter being positive for anti-HBs.

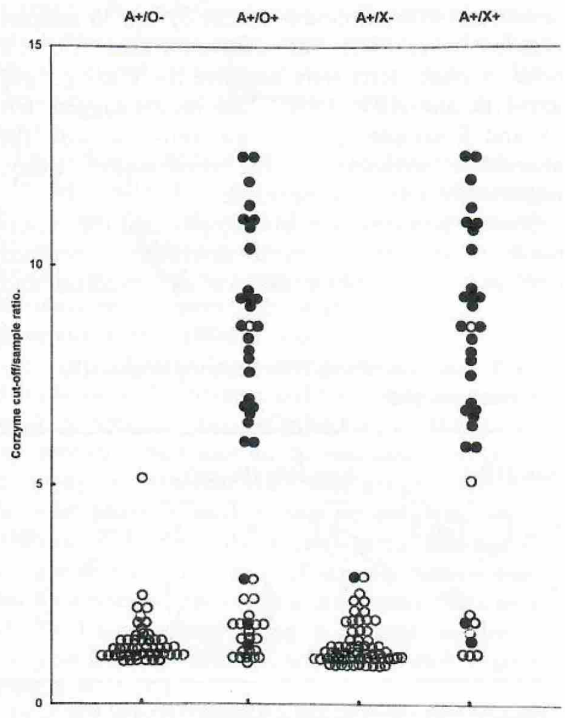

Fig. 2. Distribution of cut-off/sample ratio of Corzyme (A) mean results of initial and repeat testing according to discordance (+/−) or concordance (+/+) with anti-HBc tested with Ortho (O) or IMx (X) anti-HBc assays. Closed circles indicate the presence of anti-HBs.

The identification of potential plasmapheresis donors for anti-HBs hyperimmune plasma is often difficult when naturally immunized donors are desired. For this purpose, anti-HBs levels were determined for each of our anti-HBc-positive samples. Six (21%) had a titre above 1 IU/ml, 19 (66%) a titre between 0·1 and 1 IU/ml and only four had a titre below 0·1 IU/ml which was the National guideline level for discarding the unit. Using the study protocol, only eight whole blood units were discarded (0·09%).

On the basis of our results, we evaluated the potential cost of an anti-HBc screening programme per 10,000 donations using the two-step or one-step confirmatory protocols. To simplify this estimation, we took a rounded figure of 70p for screening tests, £2.50 for anti-HBc confirmatory and £2.70 for anti-HBs. Each blood unit was given a £90.00 value. As shown in Table 4, the scheme chosen for this study was the cheapest one, mostly because it allowed the lowest number of discarded units. Since the added confirmatory test required only 45 min to perform, it did not unduly delay the release of the concerned units. As carried out, the total testing time was 8·5 h calculated on the basis of test performance alone. Since 83% of the screen initially reactive samples are repeatedly reactive with the same assay, consideration could be given to proceed with an alternative assay for repeat testing, therefore saving 2·5 h and making the overall procedure compatible with routine blood bank testing. Under these conditions, testing time would be reduced to 6, 5·25 and 4·5 h with schemes 1, 2 and 3 respectively (Table 4).

DISCUSSION

This study provided data on the prevalence of anti-HBc-positive samples in East Anglian blood donors (Table 1). These results showed a considerably lower prevalence than previously found by a neighbouring Transfusion Centre in 1987 with the same screening assay (Kitchen *et al.*, 1988): the initial and repeat reactive rates were 67 and 58% less, respectively. This difference may be accounted for by an improvement in the specificity of the assay from the same manufacturer (Weare *et al.*, 1991). A more recent study in three Centres in the UK found an anti-HBc prevalence similar to ours using a different screening assay but a higher rate of confirmed positive, probably related to a less efficient confirmatory scheme and/or a difference in populations (Anderson *et al.*, 1992).

Prevalences of anti-HBc in blood donors greatly vary according to the background prevalence of HBV infection and, on a low prevalence area like the UK,

Table 4. Estimated cost of anti-HBc screening excluding equipment and overhead per 10,000 donations

	Estimated no. of samples to be tested	Cost (per £10,000 donations)		
		Scheme 1*	2	3
1. Initial screen	10,000	7,000	7,000	7,000
2. Repeat testing	129	181	181	181
3. Alternative screening	107	150	150	
4. Confirmatory	60	150		
	107			268
5. Anti-HBs	35	95		
	60		162	
	40			108
Discarded blood units	9	810		
	29		2,610	
	13			1,170
Personnel $\frac{1}{2}$ MLA Full-time equivalent		570	570	570
Total		8,956	10,673	9,297

* Scheme 1 includes steps 1–5 as in Fig. 1. Scheme 2 includes steps 1, 2, 3, 5. Scheme 3 includes steps 1, 2, 4, 5.

according to assay specificity. Data collected in Japan (Iizuka *et al.*, 1992) and in the USA (Douglas *et al.*, 1993) suggest that low-titre anti-HBc corresponds to false positives and that only high titre, i.e. $\geq 1:16$, should be taken into consideration. Our results confirm this observation as anti-HBs levels estimated by cut-off to sample ratios were significantly lower in discordant than in concordant samples with either pair of assays. However, some true anti-HBc, i.e. also anti-HBs-positive samples, were clearly in the presumably false positive population (Fig. 2). This may also be the case for a minority of isolated anti-HBc. Two samples with high anti-HBc ratio, negative for anti-HBs, might well however correspond to isolated anti-HBc.

In the East Anglian population the prevalence of HBV markers, after exclusion of anti-HBc false positive, can be calculated at 0·31% on the basis of the presence of both anti-HBc and anti-HBs, since no donors were found positive for HBsAg during the trial. This prevalence should genuinely represent naturally occurring HBV infection as HBV vaccines in England are almost exclusively recombinant which raises isolated anti-HBs antibody. In a 10,000 blood donor population from an endemic area like Japan, a rate of 10·85% of genuine HBV infection markers was found (Iizuka *et al.*, 1992). While the prevalence of isolated anti-HBc donors was high (2·9%) only 4% contained HBV DNA, suggesting a poor specificity of the anti-HBc screening assay. However, isolated anti-HBc-positive/DNA-positive individuals comprise only 1 in 90 of people showing any markers of HBV infection (HBsAg, anti-HBc + DNA positivity, or anti-HBsAg + anti-HBc). Applying this ratio to our prevalence of HBV infection, the expected rate of HBV DNA-positive/anti-HBc-positive donors is 1 in 30,000. Our negative result is therefore compatible with that prediction. Data supporting these calculations were collected in the USA where no anti-HBc-positive/HBV DNA-positive donors were found in a 26,000 donor study (Douglas *et al.*, 1993). Another American study reported that 30% of donors with isolated anti-HBc carried PCR detectable HBV DNA (Liang *et al.*, 1994). It is possible that the method we used for HBV DNA detection was insufficiently sensitive to screen out all potentially infectious donors. Its sensitivity of 60 virions per millilitre of serum is comparable with the results of other investigations (Keneko *et al.*, 1989; Yang *et al.*, 1993) but still too low to match the 100 particle threshold of infectivity (Prince *et al.*, 1983). Even with a sensitive method, a small number of virions below PCR detection threshold may still be infectious in a transfused blood product. As the gap between the detection limit of HBsAg assays used in this study and HBV DNA is several orders of magnitude, it would be surprising if all of the isolated anti-HBc samples tested were negative for HBV DNA.

The testing scheme used in this study has several advantages over previous approaches to anti-HBc testing. Alternative screening and/or confirmatory assays considerably reduce the number of false positives (Tables 2 and 3). The addition of an anti-HBs quantitative and rapid assay reduces the number of isolated anti-HBc to 4/10,000, identified HBV immune donors and limits the number of discarded units to a minimum. In doing so, a substantial number of donors can be kept on the panel, the stressing situation of undue removal from blood donor status can be spared and potential anti-HBs hyperimmune plasma donors can be identified. The time required to perform these various assays within a time frame compatible with routine blood bank practice can be considerably shortened if initially reactive samples are retested with different assays, particularly when rapid and highly specific. The whole process can be carried out comfortably in 5 h with scheme 3 (Table 4) minus the repeat testing with the screening assay. However, should anti-HBc screening be implemented in England, the actual testing scheme adopted would require national endorsement.

While the potential benefit of anti-HBc screening to reduce the risk of transfusion-transmitted HBV infection remains doubtful, marginal benefits in terms of reduction of HIV high-risk population and collection of anti-HBs plasma donors are anticipated. As shown in Table 4, such screening scheme could be implemented at an extra cost of £1 per unit of blood. As it is unsatisfactory to base policy on negative results, a larger study of 50 or 100,000 blood donors would provide a more definite answer to the question of how to reduce the risk of HBV infection in recipients of blood products.

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