A sensitive single reverse passive haemagglutination test for detecting both HBsAg and anti-HBs

J. A. J. BARBARA, P. J. HARRISON, D. R. HOWELL, T. E. CLEGHORN,
D. S. DANE, MOYA BRIGGS, AND C. H. CAMERON

From the North London Blood Transfusion Centre, Edgware, Middlesex, and the Department of Virology, The Middlesex Hospital, London, UK

SUMMARY A trial of a modified reverse passive haemagglutination test for HBsAg using a 0.1 % cell suspension instead of the recommended 1 % showed an approximately eight-fold increase in detection sensitivity. The test can be performed within 30 minutes and lends itself to mass screening techniques. Confirmation tests can be done using the 0.1 % method. In addition, the same serological plates and cells used for HBsAg screening can then be used to screen for high-titre anti-HBs. This makes the overall screening for both HBsAg and high-titre anti-HBs donors cheap and convenient.

In a previous communication (Barbara *et al.*, 1977), we compared passive haemagglutination (RPHA)¹ with radioimmunoassay (RIA) for HBsAg screening. We have now made an extensive trial of a modified RPHA based on the method of Archer (1977). The modification involves the use of 0.1 % test cells (Archer used 0.2 % cells), instead of the usual 1 %, and is more sensitive than ordinary Hepatest. We shall refer to the modified test as '0.1 % Hepatest' and the standard test as '1 % Hepatest' throughout. It can be performed within 30 minutes, and unconfirmable positive screen tests are no more common than with 1 % Hepatest. It is slightly more complicated to perform but lends itself well to semi-automated sampling (Cameron and Barbara, 1978) and has the added advantage that plates used for HBsAg screening can also be used afterwards for anti-HBs screening without the need for further sampling or further reagents other than a diluted HBsAg positive serum.

Before the present trial, which was performed in stages, we had been screening all donations for HBsAg by 1 % Hepatest. Those from previously untested donors were also tested by RIA (Barbara *et al.*, 1977). In the first stage of the trial, 0.1 % Hepatest was added as a third test for new donors. After three months we were satisfied that it was reliable and used it as the only RPHA method for new donor screening. After a further three months we changed from 1 % Hepatest to 0.1 % Hepatest for

screening previously tested donors. We are now able to report 13 months' experience of screening new donors by 0.1 % Hepatest in parallel with RIA, and 7 months' experience of screening all donations by 0.1 % Hepatest.

Material and methods

BLOOD DONORS

Donors were classified as *old* (previously screened) or *new*, as described previously (Barbara *et al.*, 1977).

RIA for HBsAg

The Middlesex Hospital technique was used (Heathcote *et al.*, 1974; Barbara *et al.*, 1977). Detection sensitivity was 1 ng/ml HBsAg.

RPHA

Donations were screened against test cells at a 1 in 8 serum dilution (Cameron and Barbara, 1978) by 1 % Hepatest (Cayzer *et al.*, 1974) and/or by 0.1 % Hepatest.

For 0.1 % Hepatest the test and control cells were reconstituted to a 1 % concentration in distilled water containing 0.1 % sodium azide. These were stored frozen in 1 ml aliquots, thawed when needed, and diluted to 0.1 % in buffer. Hepatest buffer (Cayzer *et al.*, 1974) with 2 % turkey serum added was dispensed into 96-well V-bottomed microtitre plates (Sterilin Limited, Teddington, Middlesex, UK). Serum from donors was screened with test cells at a 1 in 8 dilution using a semiautomatic sampler (Cameron and Barbara, 1978). After the addition of

¹Hepatest, Wellcome Reagents Limited, Beckenham, UK

Received for publication 18 April 1979

Fig. 1 One corner of Microtitre plate showing the four positive controls (tight buttons) and the negative screen tests (teardrop streaks) 8 minutes after inclining the centrifuged plate at 70° .

one drop ($25\ \mu\text{l}$) of 0.1% test cells to each well the plate was covered, left on the bench for 10 minutes, and then centrifuged in an MSE mistral 6L (with an M/19 head) for 40 seconds at 260g, which is just sufficient to deposit the cells in tight buttons at the bottom of the V wells. The plate was then inclined at 70° over an illuminated background and read after 5 to 10 minutes. In samples containing HBsAg, the cells stayed as tight buttons in the bottom of the wells whereas with negative samples they ran down the side of the well, forming a smooth teardrop streak (Fig. 1). All screen test positives were titrated and confirmed by neutralisation, as previously described (Cayzer *et al.*, 1974; Barbara *et al.*, 1977). In the confirmation test using 0.1% Hepatest, dilutions were made using a multichannel Titertek pipette (Flow Laboratories Limited, Irvine, UK) with disposable plastic tips to avoid scratching the plastic wells.

Plasma samples often gave a false-positive screen test unless first absorbed with 10% control cells for half an hour or treated with kaolin (Light, BDH Limited, Poole, Dorset, UK) at 0.1 g/ml plasma. Serum samples should be stored at 4°C or frozen, as

bacterial growth can cause non-specific agglutination. Care must be taken to avoid carrying over red cells during serum sampling as these may cause a false-positive screen test.

The different RPHA and RIA tests were all read separately, and all HBsAg positives detected were confirmed and subtyped by RIA, as described previously (Heathcote *et al.*, 1974).

TESTING FOR ANTI-HBS

After the 0.1% Hepatest for HBsAg had been read the plates were centrifuged once more to deposit the test cells, and one drop ($25\ \mu\text{l}$) of diluted, heat-treated HBsAg positive serum was added to each well. Heat treatment was to reduce infectivity; after an initial 1 in 4 dilution in saline, the positive serum was heated in a waterbath at 60°C for 10 hours and then diluted to contain two complete haemagglutinating doses of HBsAg per $25\ \mu\text{l}$. The plates, with cell buttons undisturbed, were left overnight at 4°C . In the morning the cells were resuspended using an AM 60 Cooke Microshaker (Dynatech Laboratories Limited, Sussex, UK) and left at room temperature for 30 minutes. The test procedure was then as for the 0.1% Hepatest RPHA for HBsAg although the reading of positives was reversed; cells in the samples containing anti-HBs formed a teardrop streak whereas cells in samples negative for anti-HBs remained in tight buttons. The same principle was used for detecting anti-HBs after the 1% Hepatest for HBsAg; in this instance the plates were read after the resuspended cells had settled in the usual fashion.

HBsAg DETECTION RATES

The rates of HBsAg detection in new and old donors are shown in the Table. Eleven per cent more HBsAg positive new donors were found using RIA than were found using 1% Hepatest, which was the same as we had found previously (Barbara *et al.*, 1977). This increment was reduced to 6% using 0.1% Hepatest. About 5% of new donors were from countries with high HBsAg carrier rates, and 22 (42%) of the 52 HBsAg positive new donors were from these areas. During the 13-month period, 156 200 old donors were screened by one or other of the RPHA techniques, and four were found to be HBsAg positive, giving an incidence of 1 in 39 000.

FOLLOW-UP OF HBsAg POSITIVE DONORS

Of 56 HBsAg positive donors, the four who were old donors all had acute anicteric hepatitis B. We were able to resample 43 of the remaining 52, and of these, six had acute infections although only one was icteric. Two of the three HBsAg positive donors,

Fig. 2 Parallel titrations of HBsAg-positive samples by 1% Hepatest and 0.1% Hepatest.

who could be detected by RIA only, had acute infections; no follow-up sample was available from the other one.

DETECTION SENSITIVITY OF 0.1% HEPATEST COMPARED WITH 1% HEPATEST

Figure 2 illustrates the increased sensitivity of 0.1% Hepatest over 1% Hepatest when 48 samples with different titres of HBsAg were titrated by both methods in parallel. The graph was plotted on natural logarithmic scales to encompass the full range of titres, and the best straight line was found by regression. The intercept on the logarithmic ordinate converted to an approximately eightfold increment in sensitivity. The slope of the best straight line was 1.0. Half of the 48 samples were subtype *ad* and half were *ay*.

The increased sensitivity of 0.1% Hepatest over the 1% test resulted in two additional HBsAg positive new donors being found in 25 200 screened (see Table).

VARYING TEST CONDITIONS FOR 0.1% HEPATEST

When test cells were added to the test serum dilutions, maximum sensitivity was reached after 10 minutes' incubation on the bench. Shaking the plate during

Table Blood donor screening January 1977 to January 1978

Donors	Period	No. tested	No. HBsAg positive by different tests	Incidence of HBsAg positive donors
New	Jan 1977 to Jan 1978	25 200	1% Hepatest: 47 0.1% Hepatest: 49 RIA: 52	1 in 536 1 in 514 1 in 485
Old	Jan 1977 to Jun 1977 Jul 1977 to Jan 1978	72 000 84 200	1% Hepatest: 2 0.1% Hepatest: 2*	1 in 36 000 1 in 42 100

*One of these could be detected by 1% RPHA.

this period did not increase sensitivity, nor did incubation at 37°C.

The speed and time of centrifugation of plates and the angle of slope of the plates can be adjusted to suit the operator. The conditions stated in Material and methods provide a reading within 5 to 10 minutes with most batches of cells tested. This means a total test time of 20 minutes. The 2% turkey serum added to the Hepatest buffer is necessary in the 0.1% method to keep the number of false-positive screen tests down to approximately 1%.

OTHER CONSIDERATIONS IN THE USE OF 0.1% HEPATEST

Bacterial contamination of serum samples causes diffuse non-specific agglutination, and neither streaks nor buttons are seen.

The false-positive effect caused by plasma in 1% Hepatest is enhanced using the 0.1% method but can be absorbed out.

Screen test false-positives occurred at the same rate as with 1% Hepatest (approximately 1 per 96-well microtitre plate) and could be identified as such by confirmation testing.

No prozoning was observed when very high titre subtype *ad* and *ay* samples were titrated by 0.1% Hepatest.

ANTI-HBS SCREENING

Of 10 250 new and old donors randomly tested by the 0.1% Hepatest HAI, 78 screen test positives were picked out; these included possible weak positive reactions as well as clearcut positives. Thirty-six were found to be known anti-HBs positive donors, and of the remaining 42, 32 were confirmed positive by an RIA blocking test for anti-HBs, and half of these were detectable using the less sensitive 1% Hepatest HAI.

Discussion

Hepatest using 0.1% cells has proved a convenient,

sensitive, and economical test and is now the routine method of screening for HBsAg at NLBTC although new donors are also tested by RIA. The approximately eightfold increase in detection sensitivity resulted in a modest 4% increment in the number of HBsAg positive donors discovered.

The use of the 0.1% Hepatest HBsAg screening plates for anti-HBs testing was also economical and convenient, as the serum sampling had already been done and no further cells were required. We now use it routinely to maintain a panel of anti-HBs positive donors for anti-HBs immunoglobulin production. Anti-HBs of sufficient strength to be detected by immunodiffusion could be found readily by the 0.1% HAI test. The test can be made less sensitive by altering the concentration of HBsAg added to the wells. The donors with higher titre anti-HBs could be detected by the 1% HAI test.

Buffer suitable for 1% Hepatest is not necessarily suitable for the 0.1% test using the same batch of cells and must be checked for suitability with the latter test. This variability has been due to so far undefined qualities of the human, horse, and turkey serum used in the buffer. Also some batches of cells may not lend themselves as well as others to the 0.1% test.

The finding of only four HBsAg positive donations, all of which came from donors with acute hepatitis B infections, among 156 200 previously screened donors indicates that these donors have a very low incidence of acute hepatitis B infection and that our screening of new donors is efficient. Anicteric acute infections

were considerably more common than icteric infections. In a total of 47 HBsAg positive donors from whom we had follow-up samples of blood, 10 had acute infections but only one was jaundiced. Donors who had recently had jaundice would not qualify to donate even if they felt well enough, and this may have increased the proportion of anicteric infections found.

We thank Mr P Mochnaty for Figure 1.

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Requests for reprints to: Dr J. A. J. Barbara, National Blood Transfusion Service, North London Blood Transfusion Centre, Deansbrook Road, Edgware, Middlesex HA8 9BD, UK.