Development and use of a micro haemagglutination inhibition (HAI) technique, based on Hepatest, for the detection and quantitation of hepatitis B surface antibody (Anti-HB_s) in blood donors

I. C. WISEMAN¹

From the Regional Transfusion Centre, Longley Lane, Sheffield S5 7JN

SUMMARY A simple micro haemagglutination inhibition technique, based on Hepatest (Wellcome Reagents Limited), is described. Its application in the screening of blood donors is shown, and its sensitivity is discussed in two forms of usage and also when compared to the previous immunoelectrosmorphoresis technique used in this centre.

Previous workers have developed HA/HAI test systems for the detection of Anti-HB_s based on coated erythrocytes prepared in their own laboratories (Pert and Verch, 1975; Hopkins and Das, 1973). The use of these preparations is not always possible in other centres. This paper describes a simple micro haemagglutination inhibition (HAI) technique based on Hepatest (Wellcome Reagents Limited). The small volume of commercial reagent required for the test gives an inexpensive screening technique of high sensitivity, with an easily readable result available in 1½ hours.

Materials

Hepatest Kit (Wellcome Reagents Limited).
Terasaki plate, and inclined plate stand.
SMI Micropettor (Dynatech).
Hamilton repeating dispenser (V. A. Howe).
Pasteur dropping pipettes, calibrated to deliver 0.025 ml.
Serum from a known carrier of hepatitis B surface antigen (HB_sAg).
Working buffer.

Method

PRINCIPLE

To serum under test is added a measured amount of a previously standardised HB_sAg positive serum diluted in working buffer.

¹Present address: Department of Haematology, The Jessop Hospital for Women, Sheffield 3.

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This mixture is allowed to stand at room temperature for 30 minutes. It is then tested for reactivity against Hepatest test and control cells in the Terasaki plate test previously described (Wiseman, 1976).

Presumptive Anti-HB_s positive sera are further tested by titration for the inhibition of two antigens and confirmed by radioimmunoassay (RIA).

High-titre antibody-positive donor plasma is then separated and sent routinely to the Lister Institute for processing into hepatitis B antibody immunoglobulin (HBIG) for prophylaxis.

TECHNIQUE

Standardisation of antigen

A previously detected HB_sAg positive blood donor offered his cooperation and was first bled for testing purposes. His serum had previously been used as an IEOP screening reagent because of its availability and broad spectrum of reactivity against a panel of Anti-HB_s positive sera, when characterised by Dr Bradstreet at the Standards Laboratory, Colindale, London.

A full donation was collected from this donor into a dry bottle, and the serum was separated aseptically, dispensed into 1 ml aliquots, and stored in liquid nitrogen. This storage method was chosen after testing, to retain the full antigenic potency of the serum for as long a period of time as possible.

With the knowledge of the titre of this HB_sAg by Hepatest, and also of the dilution factors involved in the HAI technique, suitable dilutions were prepared and chequerboard titrations performed.

giving inhibition of several examples of Anti-HB_s, tests being performed against both test and control cells.

The examples of Anti-HB_s were selected to cover a range of IEOP titres from neat to 1 in 8. The final concentration of HB_sAg was then adjusted according to the titration results to give an inhibition titre of at least 1 in 16 against test cells in the case of the weakest Anti-HB_s positive serum, and a negative reaction against control cells.

Known Anti-HB_s positive and negative sera, including Colindale Quality Control panels B and C, were then tested neat and at dilutions of 1 in 10 and 1 in 20. This was done to confirm that the HB_sAg dilution selected was capable of detecting weak Anti-HB_s positive sera by their inhibition of the agglutination of test cells, yet producing an easily visible agglutination pattern with test cells in the case of an Anti-HB_s negative serum (see Fig. 1).

The HB_sAg used, 'OAT', had an optimum dilution of 1 in 500 in working buffer, and this was made up in 25 ml volumes from the preserved serum and stored at -40°C for up to one week before use.

Fig. 1 Thirty sera screened against test cells (rows A, B, and C) and against control cells (rows D, E, and F). Screen test Anti-HB_s positive sera are seen in wells A7, B2, and C6.

Screening methods

(a) The racks of 30 donor sera are pooled to produce three pools of 10 sera per rack.

From each well-mixed pool, 1 drop, ie, 0.025 ml, is pipetted into a Taylor tube (50 × 10 mm). To this is added 2 drops, ie, 0.050 ml, of the working HB_sAg dilution; the tube is mixed and allowed to stand at room temperature for 30 minutes.

Eight microlitres of each dilution is then pipetted

in duplicate into two wells of a Terasaki plate. After the 30 serum dilutions have been pipetted out, 4 µl of test cells is added to one set of wells, and 4 µl of control cells to the other.

The plates are then inclined as previously described, and the results are read after one hour (see Fig. 1).

(b) All donor sera are tested singly instead of in pools, otherwise the technique is identical.

CONTROLS

With each batch of tests, a known Anti-HB_s positive serum is tested neat, and at dilutions of 1 in 10 and 1 in 20. For the tests to be valid, there must be no reaction against either test cells or control cells in any of these six wells.

The potency of the test cells is checked daily by using the Wellcome HB_sAg positive control serum supplied with the Test Kit in the HB_sAg screening test as previously described.

Results

The results are read macroscopically over a diffused light source. A serum, or pool of sera, is presumed to be Anti-HB_s positive if there is no agglutination of test cells or control cells. A negative result for Anti-HB_s is shown by agglutination of the test cells and no agglutination of the control cells.

If any serum, or pool of sera, agglutinates the control cells, then the test is invalid and must be repeated after the serum has been absorbed with control cells.

Confirmatory testing and quantitation

If a pool of serum gives a presumptive Anti-HB_s positive reaction in the screening test, the component sera in that pool are further tested singly to establish which one or more sera are Anti-HB_s positive.

The next stage of the confirmatory testing is then performed on serum taken direct from the master clotted sample to ensure correct sample identity.

Doubling dilutions from neat to 1 in 512 are prepared in group AB serum, negative for HB_sAg and Anti-HB_s.

To duplicate sets of these dilutions are added two different HB_sAg positive sera at their respective working dilutions.

Each of these dilutions is then tested with test and control cells in Terasaki plates, and the titre of the Anti-HB_s positive serum is that greatest dilution showing inhibition of agglutination of the test cells, the control cells not being agglutinated in any dilution (see Fig. 2).

Thus an inhibition titre against two different

Fig. 2 Titration of a screen test Anti-HB_s positive serum. Rows B and C are titrations against HB_sAg (I), row B test cells, row C control cells. Inhibition titre 1:32.

Rows D and E are titrations against HB_sAg (II), row D test cells, row E control cells. Inhibition titre 1:128.

Table Blood donor screening for Anti-HB_s

Period	Type of testing	Number tested	Anti-HB _s frequency
1 Jan '73-31 Dec '74	Single donation testing by IEOP	229 856	1 × 4721
1 Jan '75-31 Mar '75	Single donation testing by IEOP	29 740	1 × 4248
3 Nov '75-5 Apr '76	Micro HAI testing of pools	54 260	1 × 2583
26 Jan '76-5 Apr '76	Micro HAI testing of single donations	2480	1 × 413

examples of HB_sAg can be obtained in the case of a genuine Anti-HB_s positive donor.

A standard Anti-HB_s positive serum of known potency, previously tested by the Lister Institute, is also tested as a control. If the Anti-HB_s titre in the donor serum is as great as or greater than the control, then the donor plasma is judged to be suitable for HBIG production. The plasma is then separated and sent to the Lister Institute for processing.

Anti-HB_s positive sera, thus confirmed by double titration, are then sent for final confirmation by RIA testing to Dr Dane, Middlesex Hospital Medical School, London.

Discussion

From the Table it is apparent that this method of HAI is, at best, about 10 times as sensitive as

IEOP for the detection of Anti-HB_s in the Trent Regional Health Authority area.

It is interesting to note that in the series of 2480 donations tested, none of the six Anti-HB_s positive sera detected was of sufficient titre to be positive in the pool test.

However, while in the past it has been suggested that Anti-HB_s positive donors be excluded (Lehmann and Schlaak, 1975; Renton and Wadsworth, 1975a and b), there has more recently been an increasing weight of opinion to suggest that such action is unjustified (De La Concha *et al.*, 1974; Aach *et al.*, 1974; Fiedler, 1975; International Forum 1975).

The prophylactic value of HBIG is now well recognised (Szmuness *et al.*, 1974; Seef *et al.*, 1975; Grady and Lee, 1975; Prince *et al.*, 1975; Redeker *et al.*, 1975; Alter *et al.*, 1975; British Medical Journal, 1976) so until the development of a safe and effective vaccine there will be a demand for this form of prophylaxis, and already broader use of HBIG is envisaged to protect such persons as the spouses of HB_sAg carriers (Redeker *et al.*, 1975).

There is, therefore, a place for a simple procedure to detect and quantitate Anti-HB_s, and the advantage of this technique is that its sensitivity can be set to detect only levels of Anti-HB_s suitable for HBIG production.

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