Technical methods

A modification of Hepatest, using the Terasaki plate, for the detection of HB_sAg in blood donors

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A simple modification of Hepatest (Wellcome Reagents) is described, which lends itself to the rapid large-scale screening of blood donors. The technique uses 4 μ l of HB_sAb coated turkey cells (Cayzer *et al.*, 1974), and Terasaki plates (Hopkins and Das, 1973), in contrast to the 25 μ l used in the microtitre tray technique.

This results in a considerable saving on the cost of reagents, the cost per test being reduced to 16% of the cost of the recommended method. In our hands, this modification is as convenient to use as the manual microtitre tray technique.

Positive sera from the screen test are also tested for non-specificity using horse IgG coated turkey control cells. The false positive screen test rate has been reduced by the adoption of a modified buffer containing turkey serum.

Materials

Hepatest kit (Wellcome Reagents)
Terasaki plates and inclined plate stand
SMI Micropettor (Dynatech)
Oxford dispenser
Hamilton repeating dispenser (V. A. Howe)
Calibrated Pasteur dropping pipettes—0.025 ml

(a) Stock buffer

0.1 M Na₂HPO₄ 12H₂O 35.8 g/l (solution A)

0.1 M NaH₂PO₄ 2H₂O 15.6 g/l (solution B)

Solutions A and B are mixed together in proportions to produce a solution of pH 7.0 (100 ml of solution A + 92 ml of solution B). To this is added an equal volume of 0.15 M NaCl.

(b) Buffers for use—prepared fresh daily

- i Original formula—stock buffer + 5% human group AB serum (HB_sAg free), and 2% horse serum (HSO2 Wellcome Reagents).
- ii Modified formula—stock buffer + 4% human group AB serum (HB_sAg free), 2% horse serum (HSO2 Wellcome Reagents), and 2% pooled turkey serum.

Method

PRINCIPLE

Hepatest is a passive haemagglutination technique in which HB_sAg in the serum under test causes agglutination of tanned, fixed turkey erythrocytes coated with affinity purified horse HB_sAb (test cells). The specificity of this reaction is confirmed by the use of tanned, fixed turkey erythrocytes coated with horse IgG (control cells).

SCREENING TEST

Serum samples are received from the Blood Donor Laboratory in precipitin (50 × 7 mm) tubes in numbered perspex 50 hole racks. These are centrifuged to free the serum of red cells using the Griffin and Christ bench centrifuge with serological head.

0.225 ml of working buffer is dispensed into duplicate precipitin tubes, one drop (0.025 ml) of serum from a Pasteur dropping pipette is added to this tube from each serum sample, and these dilutions are well mixed. Using the Micropettor, 8 μ l of each serum dilution is pipetted into a well of a Terasaki plate. When all the sera have been pipetted, 4 μ l of well mixed Hepatest cells is dispensed into each well using the Hamilton dispenser.

The lids are replaced, and the plates are stood in the inclined plate stand for 30–60 minutes, on a vibration free surface, at an angle of 25° to 30° from the horizontal (fig 1).

CONTROLS

With each batch of samples, a titration of Hepatest positive control serum is incorporated. If the titre obtained with test cells is less than 1:32, the tests must be considered invalid and repeated using fresh reagents.

Results

The results are read macroscopically by placing the complete plate and stand over a diffused light source.

A negative reaction is seen as a crescent of settled cells, and a positive as a poorly formed or absent crescent, the agglutinated cells forming a carpet on the lower lip of the well (fig 2).

CONFIRMATORY TESTING

Doubling dilutions of any screen test positive sera are prepared from 1:4 to 1:64 in working buffer, and tested simultaneously with test and control cells. Any sample with a titre of 1:64 or above with either

Fig 1 Terasaki plate in inclined stand.

Fig 2 Positive sera in wells B9, C6, D2, E7, F3, and F10.

cell, or having a test cell titre at least two doubling dilutions greater than that with control cells, is further tested in a 10 tube titre 1:4 to 1:2048 with both test and control cells.

For a positive sample, a duplicate titre is also performed taking serum direct from the 'Master' clotted sample to ensure correct sample identity.

A confirmed Hepatest positive sample, therefore, has a titre (a) greater than 1:4 with test cells, and

(b) at least two doubling dilutions greater with test cells than with control cells.

Discussion

A total of 3310 samples were tested by both the microtitre tray method and this modification. Fifty-one samples were screen test positive by both techniques. Subsequent confirmatory testing showed

that 50 were false positives, and that one sample was genuinely HB_sAg positive.

The Quality Control panel B (Standards Laboratory, Central Public Health Laboratory, Colindale) and 25 other known HB_sAg positive sera were tested while the original buffer was in use. Forty-four of the 45 were positive by this technique, the one exception being a sample from a donor implicated in post-transfusion jaundice, which had previously been found positive for HB_sAg by radioimmunoassay (RIA) only (Dane, 1974). All of the 45 sera were positive by RIA, and only 40 were detectable by immunoelectro-osmophoresis (IEOP) at this centre.

Titration of six known HB_sAg positive sera were also performed, and parallel testing showed that the Terasaki plate technique is at least as sensitive as the recommended method, and, as previously described, passive haemagglutination is much more sensitive than IEOP and closely approaches the sensitivity of RIA (Christie *et al*, 1974).

The angle of the plate on the inclined plate stand was varied from 15° to 50° from the horizontal. Interpretation of the result, with a clear distinction between positive and negative reactions, was found to be easiest at 25° to 30°.

As in the microtitre tray technique, the results may be read after 30 minutes, but the interpretation is easier after 60 minutes.

Studies on the constituents of the original buffer have shown that alterations in the amounts of horse serum and human group AB serum have little

Donations screened using original buffer, 1-31 August 1975	8418
Total number of screen test false positives	111
% Frequency	1.3%
Donations screened in parallel using (a) original buffer, (b) turkey serum buffer	3213
Number of screen test false positives in original buffer	32
% Frequency	1.0%
Number of screen test false positives in turkey serum buffer	5
% Frequency	0.15%

Table I False positive screen test rate for Hepatest

Serum No.	Reciprocal of Titre in Original Buffer	Reciprocal of Titre in Turkey Serum Buffer
1	40	80
2	2560	2560
3	2560	1280
4	2560	2560
5	2560	2560
6	2560	2560

Table II Known HB_sAg positive sera titrated in both buffers

Total number of HB _s Ag positive sera tested	30
Number found HB _s Ag positive in original buffer	30
Number found HB _s Ag positive in turkey serum buffer	30
Number giving a positive reaction against control cells at a dilution of 1:10	
(a) using original buffer	2
(b) using turkey serum buffer	Nil

Table III Known HB_sAg positive sera tested in both buffers

beneficial effect, but the introduction of pooled turkey serum produces a marked reduction in the false positive screen test rate (table I). From tables II and III it is also apparent that this buffer does not cause any loss of sensitivity of the Hepatest reagents.

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