Technical methods

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

I. C. WISEMAN  From the Regional Blood Transfusion Centre, Longley Lane, Sheffield S5 7JN

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 μl of HBsAb coated turkey cells (Cayzer et al., 1974) and Terasaki plates (Hopkins and Das, 1973), in contrast to the 25 μl 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 Micropetttor (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 (HBsAg free), and 2% horse serum (HSO2 Wellcome Reagents).
ii Modified formula—stock buffer + 4% human group AB serum (HBsAg free), 2% horse serum (HSO2 Wellcome Reagents), and 2% pooled turkey serum.

Received for publication 31 October 1975
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 HBsAg positive.

The Quality Control panel B (Standards Laboratory, Central Public Health Laboratory, Colindale) and 25 other known HBsAg 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 HBsAg 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.

Titrations of six known HBsAg 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 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 Hepatost reagents.

I wish to thank the Director and staff of this Centre for their encouragement and advice, and Dr Bradstreet for supplying the Quality Control panel.

References


