EVALUATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DETECTION OF HBsAg

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Summary

An ELISA (enzyme-linked immunosorbent assay) method for detecting HBsAg was compared with the RPHA (reverse passive haemagglutination) method. A total of 90 specimens were tested and it was found that the ELISA method was equal in sensitivity to the RPHA method; 45 out of 90 samples were positive by both ELISA and RPHA and 42 out of 90 negative. In addition, 3 samples weakly positive by RPHA were positive by the ELISA method but could not be confirmed by the RPHA confirmatory test for HBsAg and may thus represent false positives.

The discovery by Blumberg et al. (1) of the Australia antigen (now known as HBsAg - hepatitis B surface antigen) has been followed by the development of a variety of laboratory tests to detect the presence of this antigen in human blood. It was first detected by agar gel immunodiffusion (1) followed by counter-immunoelectrophoresis (CIEP) (2), complement fixation (3) and later by the more sensitive reverse passive haemagglutination (RPHA) (4, 5) and radioimmunoassay (RIA) methods (6). The development of enzyme-linked immunosorbent assays (ELISA) (7, 8) has also resulted in this technique being adapted for detecting HBsAg (9, 10). ELISA appears to have the advantage of giving objective results, high sensitivity and simplicity of procedure but, in view of its potentially wide application, needs to be carefully evaluated with respect to its sensitivity and specificity. This communication reports the evaluation of the ELISA method for detecting HBsAg compared to the RPHA method currently in use in this laboratory.

MATERIALS AND METHODS

Source of specimens

Serum specimens were obtained from 90 patients with suspected hepatitis B infection from the wards of the University Hospital, Kuala Lumpur.

RPHA Tests

All specimens were tested by the RPHA method (‘Auscell’, Abbot Laboratories, North Chicago, Ill., U.S.A.) based on the agglutination of human erythrocytes coated with anti-HBsAg. Positive and presumptive positive samples were confirmed using the ‘Auscell’ confirmatory kit according to the manufacturer’s instructions.

ELISA Tests

All the specimens were also tested by the ELISA method using a commercial kit (Enzyme Immunoassay HBsAg, Behringwerke, W. Germany). The test is based on the double antibody ‘sandwich’ principle where HBsAg in the patient’s sample is first bound to anti-HBsAg immobilized on the surface of plastic tubes. After washing, peroxidase-conjugated antibodies to HBsAg are reacted with the remaining antigenic determinants. The unbound enzyme-linked antibodies are then removed by washing and the enzyme activity on the solid phase determined by addition of enzyme substrate (hydrogen peroxide) and a chromogenic compound (o-phenylenediamine dihydrochloride, OPD) to detect the colour change. The reaction is stopped by the addition of diluted H2SO4 and colour intensity determined spectrophotometrically at 492 nm (Elisa Reader, Dynatech Laboratories Inc., Virginia, U.S.A.). The method is illustrated in Fig. 1.

RESULTS

A total of 90 serum specimens were tested by the two methods. Of this group, 42 proved to be negative by the ‘Auscell’ RPHA method. These were also negative by the ELISA method, both visually and spectrophotometrically (Table 1). A further 45 samples were shown to

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be positive by RPHA and were positive visually and spectrophotometrically by ELISA with a mean absorbance value of 5.0 (range = 1.4–8.1) which was well above the 'cut-off' value of 0.10 (Table 1). Finally, 3 specimens which were doubtful or weakly positive by RPHA were shown to be positive by the ELISA method (Table 1). These three positive results, however, could not be confirmed by the RPHA confirmatory test for HBsAg. It should also be noted that the absorbance values for these 3 specimens were above the 'cut-off' value and within the range for the definitely positive samples (Table 1).

DISCUSSION
Following the initial detection of HBsAg by agar gel immunodiffusion(1), the methods later developed and used have become increasingly more sensitive, culminating in the 'third generation' tests for detection of HBsAg e.g. RPHA and RIA. RIA, however, involves the use of radioisotopes of short half-lives, complex equipment and can be a medical hazard. Thus, the introduction of enzyme immunoassays for HBsAg(9,10) has offered an alternative method of equal sensitivity. ELISA provides objective results and is reported to be extremely sensitive. Reagents used present no health hazard, are stable for long periods and results can be estimated visually or photometrically with a simple spectrophotometer.

In the present study, the ELISA method for detecting HBsAg was compared with the RPHA
TABLE I
COMPARISON OF ELISA AND RPHA METHODS FOR DETECTING HBsAg

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of specimens</th>
<th>'AUSCE LL' reading*</th>
<th>ELISA: visual reading†</th>
<th>ELISA: Spectrophotometric reading** (absorbance at 492 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>6</td>
<td>NT</td>
<td>NT</td>
<td>6</td>
</tr>
<tr>
<td>Positive control</td>
<td>2</td>
<td>NT</td>
<td>NT</td>
<td>2</td>
</tr>
<tr>
<td>Positive specimens</td>
<td>45</td>
<td>45</td>
<td>-</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(range 1.4–8.1)</td>
</tr>
<tr>
<td>Negative specimens</td>
<td>42</td>
<td>-</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(range 0.04–0.15)</td>
</tr>
<tr>
<td>Weakly positive specimens</td>
<td>3</td>
<td>-</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(range 1.2–3.5)</td>
</tr>
</tbody>
</table>

* NT = Not tested
† Visual reading is based on the appearance of orang-yellow colour for the positive control compared to the almost colourless negative controls.

** Mean ± S.D. (where applicable). A 'cut-off' absorbance value is calculated by adding 0.05 absorbance units to the mean absorbance of the negative controls (ie. 'cut-off' value = 0.10). Samples with absorbances less than this value are considered negative for HBsAg.

test commonly used in this region(11). The results obtained showed that ELISA is equal in sensitivity to RPHA in detecting HBsAg; all 45 samples positive by the RPHA test were also positive by ELISA. In addition, three samples which were weakly positive by RPHA proved to be positive by ELISA. However, these three specimens could not be confirmed as positives by the RPHA confirmatory test for HBsAg and may thus represent false positives. The results also indicate that positive specimens by the ELISA test exhibit a range of absorbance values from 1.4 to 8.1 (mean = 5.0). Although these absorbance values are directly proportional to the amount of HBsAg present(7,8), the clinical significance is not known and will be the subject of further investigations. It is also clear that a larger number of specimens are needed for the comparison before any firm conclusions are made regarding the two tests.

Other studies(12,13) indicate that ELISA has a significantly higher sensitivity compared to RPHA for detecting HBsAg but a slightly lower specificity ie. more false positives were detected by ELISA. A false positive pick-up rate of 2.2% was found with the ELISA method(12) which appears to be borne out in the present study (3 out of 90 specimens or 3.3%). The ELISA method has been shown to possess a sensitivity similar to RIA for detecting HBsAg(9,10,14) although minor sensitivity differences were noted with the detection of hepatitis B subtypes ad and ay where ELISA appears to be slightly less sensitive(9,14). The ELISA method has also been used for other viral infections e.g. rubella(15,16), measles(16), cytomegalovirus(16), hepatitis A(17), herpes simplex virus type 2(18), RSV (respiratory syncytial virus)(19) and influenza(20) as well as bacterial(21), parasitic(22) and fungal
infections(23). It has also been used in the
diagnosis of immunological disorders(24) and
haematological disease states(25).

One important consideration, especially if
ELISA is to be used routinely and on a large
scale, is the cost of testing for HBsAg by the
ELISA method. It is very likely that the cost
per test by the ELISA method will be consid-
ervably more than the RPHA test. However, the
preparation of the various ELISA reagents in
the laboratory (e.g. coating of tubes with anti-
gen or antibody, preparation of antibody –
enzyme conjugate etc.), as opposed to pur-
chasing complete kits, could conceivably lower
the cost per test to comparable levels. High
costs aside, the main appeal of ELISA methods
still lies in its high sensitivity, specificity,
simplicity of procedures and most important,
its potentially wide application not just to
infectious disease but to other clinical
conditions.

ACKNOWLEDGEMENT
We would like to thank Dr Peter Simmons,
Department of Pathology, Faculty of Medicine,
University of Malaya, for the gift of the ELISA
kit and Mr TL Saw for technical assistance.

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