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

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Summary

An ELISA (enzyme-linked immunosorbent assay) method for detecting HB_sAg 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 HB_sAg and may thus represent false positives.

The discovery by Blumberg *et* al. (1) of the Australia antigen (now known as HB_sAg – 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 imrnunosorbent assays (ELISA)(7,8) has also resulted in this technique being adapted for detecting HB_sAg(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 HB_sAg 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-HB_s Ag. 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 HB_sAg, Behringwerke, W. Germany). The test is based on the double antibody 'sandwich' principle where HB_sAg in the patient's sample is first bound to anti-HB_sAg immobilized on the surface of plastic tubes. After washing, peroxidase-conjugated antibodies to HB_sAg 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-phenylendiamine dihydrochloride, OPD) to detect the colour change. The reaction is stopped by the addition of diluted H_2SO_4 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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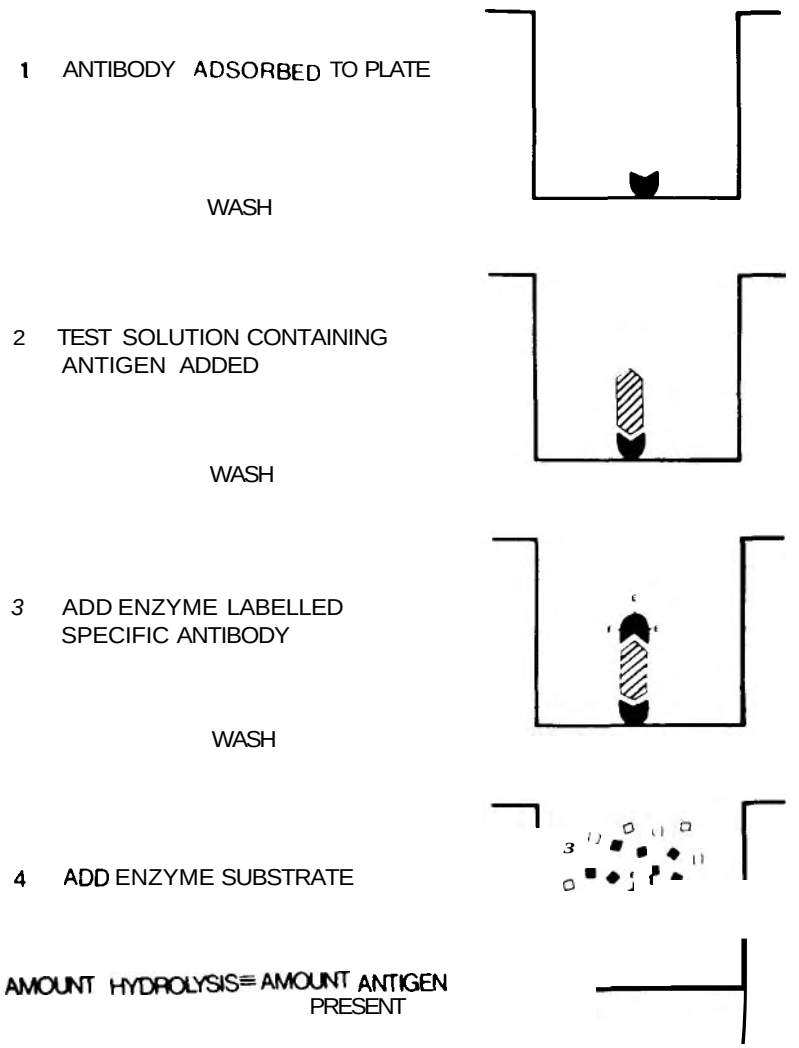

FIGURE 1.

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 I). 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 HB_sAg. 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 HB_sAg 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 HB_sAg 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 HB_sAg(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 HB_sAg was compared with the RPHA

TABLE I
COMPARISON OF ELISA AND RPHA METHODS FOR DETECTING HB_sAg

Sample	Number of specimens	'AUSCELL' reading*			ELISA: visual reading+		ELISA: Spectrophotometric reading** (absorbance at 492 nm)
		+	+	–	+	–	
Negative control	6	NT	NT	NT	–	6	0.05
Positive control	2	NT	NT	NT	2	–	1.65
Positive specimens	45	45	–	–	45	–	5.0 ± 1.6 (range 1.4–8.1)
Negative specimens	42	–	–	42	–	42	0.06 ± 0.02 (range 0.04–0.15)
Weakly positive specimens	3	–		3	– 3	–	2.1 ± 1.2 (range 1.2–3.5)

*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 HB_sAg.

test commonly used in this region(11). The results obtained showed that ELISA is equal in sensitivity to RPHA in detecting HB_sAg; 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 HB_sAg 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 HB_sAg 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 HB_sAg 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 HB_sAg(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 HB_sAg by the ELISA method. It is very likely that the cost per test by the ELISA method will be considerably more than the RPHA test. However, the preparation of the various ELISA reagents in the laboratory (e.g. coating of tubes with antigen or antibody, preparation of antibody – enzyme conjugate etc.), as opposed to purchasing 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.

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