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COMPARISON OF ENZYME IMMUNOASSAY WITH RADIOIMMUNOASSAY FOR THE DETECTION OF HEPATITIS B SURFACE ANTIGEN

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SUMMARY. A direct solid-phase enzyme immunoassay (Auszyme I) and a direct solid-phase radioimmunoassay (Ausria II) for detection of hepatitis B surface antigen (HBsAg) were compared in tests with a panel of 347 human sera. Compared with RIA, EIA showed a sensitivity of 98% with 153 HBsAg-positive sera and a specificity of 99% with 194 HBsAg-negative sera. Sera that gave false negative and false positive results by EIA were re-examined by both RIA and EIA to confirm the initial result. Use of less than the recommended volume of serum for EIA produced results inconsistent with RIA in four of 27 sera examined. Quantitative correlation between RIA and EIA was low ($r=0.691$). Positive controls used for EIA showed considerable variation from day to day, although intra-assay variation was much less. The sensitivity of the EIA method examined compares favourably with previously published EIA studies and with the RIA used in this study. Auszyme I EIA is a sensitive and specific third generation test for HBsAg that offers several advantages over currently used RIA techniques.

INTRODUCTION

Hepatitis B surface antigen (HBsAg) can be detected in human serum by several

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immunochemical methods. Counterimmunoelectrophoresis (Alter, Holland, and Purcell, 1971), reverse passive latex agglutination (Kachani and Gocke, 1973) and haemagglutination (Vyas and Shulman, 1970) lack the sensitivity needed for detection of small amounts (< 20 ng) of HBsAg. Solid phase radioimmunoassay (RIA) using ^{125}I -labelled antibody has been employed successfully for HBsAg detection (Hollinger, Vorndam, and Dreesman, 1971; Ling and Overby, 1972; Purcell *et al.*, 1973). RIA kits for HBsAg detection are available commercially and combine good sensitivity and specificity with ease of performance.

Enzyme immunoassay (EIA) techniques provide the sensitivity of RIA methods without the problems associated with radioisotope handling. EIA techniques have been applied successfully to the detection of various antibodies and antigens (Wisdom, 1976) including HBsAg (Wolters *et al.*, 1976; Halbert and Anken, 1977; Wei *et al.*, 1977). EIA kits for HBsAg testing have become available commercially and have been compared with RIA (Hopkins *et al.*, 1978; Kacaki *et al.*, 1978; Hyland *et al.*, 1979; Haas and Hotz, 1980). These studies compared a direct EIA, (Hepanostika®, Organon, Teknika), with RIA (Ausria II®, Abbott Laboratories) in tests with serum panels obtained in Europe and Australia. The studies reported in this paper compare a solid phase EIA (Auszyme I®, Abbott Laboratories) with Ausria II in tests with a serum panel obtained in the USA.

MATERIALS AND METHODS

Sera. Sera submitted for HBsAg testing were obtained from Bioran Medical Laboratories, Cambridge, MA 02139. After collection, the sera were held at 4°C for 12–24 h, assayed for HBsAg by RIA and stored at –20°C until assayed for HBsAg by EIA.

Radioimmunoassay. Solid-phase RIA of HBsAg was performed with Ausria II-125 kits (Abbott Laboratories, N. Chicago, IL, 60064). Sera were incubated with guinea-pig anti-HBsAg-coated beads for 2 h at 45°C, then incubated with ^{125}I -labelled human anti-HBsAg serum for 1 h at 45°C. Bound ^{125}I was measured by a Searle model 1285 automatic gamma spectrometer. A result was considered to be positive if the net count per minute (cpm) from bound ^{125}I -labelled anti-HBsAg was 2.1 times that of the mean negative control count in two separate assays.

Enzyme immunoassay. Solid-phase EIA of HBsAg was performed with Auszyme I kits (Abbott Laboratories). Sera were incubated with guinea-pig anti-HBsAg-coated beads for 2 h at 40°C, then incubated with horseradish-peroxidase-labelled goat anti-HBsAg serum for 1 h at 40°C. *O*-phenylenediamine substrate was added and colour was allowed to develop for 0.5 h in the dark before the reaction was ended with 1N HCl. Absorbance at 492nm was measured by an Abbott Quantum I split beam spectrophotometer. A result was considered to be positive if the A_{492} was at least 0.05 units greater than the mean of the negative controls in two separate assays. When the EIA results were inconsistent with those obtained previously by RIA, the tests

TABLE I
Comparison of EIA with RIA for detection of HBsAg in serum

Result with EIA	Number of sera for which RIA result was	
	positive	negative
positive	150	2
negative	3	192
Total	153	194

were repeated. Specimens that gave negative results by both assays were not retested. Initial EIA results were obtained without prior knowledge of the corresponding RIA results.

RESULTS

A total of 352 sera were tested by both EIA and RIA. Five of these gave inconsistent results and will be discussed below, 347 sera gave consistent results that are summarised in table I; 192 sera gave negative results by both methods and 150 sera gave repeatedly positive results by both EIA and RIA. Three sera gave negative results by EIA and positive results by RIA, whereas two sera gave positive results by EIA and negative results by RIA. Accepting RIA as the standard method, the sensitivity of EIA was 98% and the specificity 99%. Of the 150 sera that gave positive results by EIA and RIA, 147 (99%) had absorbances of > 0.150 and were considered positive by visual inspection.

Table II shows the quantitative results of the three false negative and two false positive sera. When sufficient volumes were available, sera were sent to Abbott Laboratories for repeat testing with Auszyme II and Ausria II. With the three sera examined, both EIA and RIA confirmed the initial RIA results. One serum (no. 12925) was found to be negative initially by Auszyme I but positive on subsequent EIAs by Auszyme II, confirming the initial RIA result. The chromogen solution used in Auszyme II is more concentrated than that used in Auszyme I and produces slightly increased absorbance with weakly positive specimens but little or no increase with negative controls. In a clinical situation, this serum would not have been retested. Therefore we have considered it to be a false negative.

There were five sera that gave inconsistent results by EIA. Four were negative by RIA and positive in the first EIA, then negative repeatedly by EIA, suggesting contamination from adjacent positive wells in the first assay. The other serum was negative by RIA, but positive twice by EIA and was repeatably positive by EIA and RIA when tested at Abbott Laboratories. This serum represents an RIA false negative.

Additionally, 27 sera were tested using less than the 0.2 ml of serum recommended by the manufacturers because of inadequate sample size. In 23 cases, the EIA result

TABLE II
Results of assays with sera for which RIA and EIA results are discordant

Serum no.	Results obtained with				
	EIA*			RIA†	
<i>(EIA -, RIA +)</i>					
77828	0.008		0.009	2.87	2.46
77797	0.013			2.33	2.73
12925	0.043	0.123	0.056	3.42	3.76
<i>(EIA +, RIA -)</i>					
43788	0.086	0.060	0.074	0.82	
96116	> 2.0	0.058	0.028	1.07	

* $\Delta A_{492} > 0.050$ indicates positive result.

† Sample $\text{cpm}/\bar{x}_{\text{neg}} \text{cpm} > 2.1$ indicates positive result.

FIGURE—Quantitative correlation between EIA and RIA for HBsAg-positive sera.

with <0.2ml of serum confirmed the previous RIA result (7 negative, 16 positive). Of the remaining four sera, three were RIA negative EIA positive and one was RIA positive EIA negative when <0.2ml serum was used for EIA.

Quantitative correlation between EIA A_{492} and RIA (bound cpm) for 46 HBsAg-positive sera is shown in the figure. Because the spectrophotometer used for EIA produced quantitative data only within the range 0–2.0 absorbance units, the majority of positive specimens with $A_{492} > 2.0$ could not be included in the analysis. For the sera shown in the figure a correlation coefficient of $r = 0.691$ was obtained.

Interassay variation of EIA-positive and -negative controls was evaluated. The negative controls represent the mean of triplicate samples of recalcified human plasma unreactive for HBsAg and anti-HBsAg. The positive controls represent duplicate samples of human HBsAg at a concentration of 20 ± 5 ng/ml. The negative controls gave reproducible results from run to run, with a mean absorbance of 0.010 and standard deviation of 0.011. The reactivity of the positive controls varied considerably from run to run with a mean absorbance of 0.702 and a standard deviation of 0.189.

DISCUSSION

The results show that the Auszyme I EIA is a sensitive and specific third generation test for the detection of HBsAg in human sera. When compared with Ausria II RIA, sensitivity was 98% and specificity was 99% with a false positive frequency of 1%. Previous studies have compared the Hepanostika EIA with the Ausria II RIA. In one such study, EIA was found to have a sensitivity comparable with RIA and a false positive rate of 2.2–3.7% which could be reduced to 0.3–1.2% by confirmatory tests for positive sera (Kacaki *et al.*, 1978). In another study, Hepanostika EIA was 2–20 times less sensitive than Ausria II RIA and gave a false negative rate of 4% (Hyland *et al.*, 1979). A similar study found comparable sensitivity between the two methods and a false positive rate of 0.48% (Hopkins *et al.*, 1978). False negative results with HBsAg assays constitute a far greater risk than false positives. Using Auszyme I, we found 2%

false negatives. The incidence of false negatives with the Hepanostika system was 0% on initial small scale testing (Wolters *et al.*, 1976) but on subsequent large scale clinical trials an incidence of 3·8% was found (Kacaki *et al.*, 1978). In comparative studies with sera selected for low HBsAg content, Hyland *et al.* (1979) found a false negative rate of 4·7% and Vandervelde (1978) has reported a false negative rate of 10·6% using Hepanostika reagents.

All sera falsely negative by EIA were weakly positive by RIA, with sample cpm/negative control cpm ratios of 2–3. For the three sera that were initially EIA-positive then repeatedly negative, cross contamination from adjacent positive wells appears to be responsible for the non-specific results and has been reported previously (Kacaki *et al.*, 1978; Farr, 1979). This problem can be eliminated by requiring two separate positive assays or a confirmatory blocking test or both before considering a serum positive for HBsAg. Although we obtained unreliable results when using <0·2 ml of serum, it is unlikely that the volume of serum required for each assay would present a problem clinically because only 0·2 ml of serum is required for a valid test.

The Auszyme I EIA cannot be used as a quantitative assay on a single serum dilution containing HBsAg > 20 ng/ml. Although, in RIA, the net cpm-bound is directly proportional to the concentration of bound antibody over a wide range, the final net change in absorbance measured by EIA is logarithmically proportional to bound label over a narrow range (1–20 ng/ml). Mean negative control absorbance values remained low and varied slightly, but mean positive control absorbance values from single lots varied considerably on successive days. These factors may contribute to the low quantitative correlation observed between RIA and EIA ($r=0\cdot691$).

The majority of sera positive by EIA were readily detected by visual inspection. Although most false negative and false positive sera would not be detected visually, three true positive sera would also not have been detected visually. The assay can be performed in 4 h and we observed no deterioration of reagents over a 6-month period using a single lot. We have found the Auszyme I EIA to be a rapid and convenient means of HBsAg testing with a sensitivity equivalent to RIA techniques.

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