Comparison of an enzyme-immunoassay with a radio-immunoassay method for the detection of the hepatitis markers anti-HBs, anti-HBc and HBsAg

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Summary

A comparative study was carried out on a radio-immunoassay (RIA) and enzyme-immunoassay (EIA) method for detecting the hepatitis markers anti-HBs, anti-HBc and HBsAg. The results indicated that the RIA and EIA were comparable for the HBsAg marker but that the RIA test was more sensitive for anti-HBs and more specific for anti-HBc. The conclusion was that if the EIA test is used for these markers, the laboratory and clinician must be aware of these limitations.

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Enzyme-immunoassay (EIA) tests have recently become available as commercial kits and are rapidly gaining popularity because they are easy to use, avoid the need for radioactive isotopes and have a long shelf-life. A number of studies[1,2] have, however, revealed significant limitations of EIA compared with radio-immunoassay (RIA), which has been the major test for hepatitis markers.

Data from a local evaluation of one commercially available EIA kit compared with the routine RIA test carried out at the National Institute for Virology are presented.

Materials and methods

Sera

Sera routinely submitted to the laboratory for testing for one or more hepatitis markers were investigated by an RIA and EIA enzyme-linked immunosorbent assay procedure within 48 hours of receipt. The number of tests performed for the three markers included in the study were: 259 anti-HBc; 196 anti-HBs; and 358 HBsAg.

RIA

The methods used were as set down by Abbott Laboratories for CORAB anti-HBc, AUSAB anti-HBs and AUSRIA HBsAg (Abbott Laboratories, Abbott Park, North Chicago, USA).

The anti-HBs test uses a 'sandwich' method in which the patient's serum is added to beads coated with HBsAg and, after a period of incubation, HBsAg conjugated with iodine-125 which binds to any anti-HBs on the bead, is added.

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The anti-HBc test is a competitive RIA in which non-radioactive anti-HBc in the serum competes with human anti-HBc ^{125}I for binding sites on beads coated with HBcAg. Therefore the lower the count the greater the amount of anti-HBc in the test serum.

The test for HBsAg is a 'sandwich' RIA with a principle similar to that used for anti-HBs. It is recommended by the manufacturer that all positives be confirmed.

All readings were carried out on an automated gamma counter (Abbott model A.N.S.R.).

EIA

A commercial kit was used and the methods for anti-HBc, anti-HBs and HBsAg were as set down by the manufacturer (F. Hoffman, La Roche & Co. Ltd Diagnostica, Basle, Switzerland). All tests were by the short incubation method.

The anti-HBs test is a competitive EIA in which the test serum is mixed with an HBsAg reagent. After the prescribed period of incubation peroxidase-labelled anti-HBs solution and a bead coated with anti-HBs are added to detect and quantitate non-bound HBsAg.

A substrate O-phenylenediamine is then added for colour development proportional to the amount of bound anti-HBs peroxidase. The absorbance is read in a photometer at 492 nm.

The test for anti-HBc is also a competitive EIA. The cut-off value and interpretation is the same as for the anti-HBs test.

The test for HBsAg is based on the 'sandwich' principle. The test serum is incubated with peroxidase-labelled anti-HBs and a bead coated with anti-HBs. The anti-HBs peroxidase conjugate binds to the HBsAg attached to the bead. After the addition of substrate and stopping solution, the intensity of colour development is read on a photometer. It is recommended by the manufacturer that all positives be confirmed.

All readings were carried out on an automated photometer (Roche EIA photometer).

Results

The results are summarised in Table I. The low-positive (±) results presented in Table I follow the same trend as the positives (+), but since the calculated low-positive range was so close to the cut-off value these results are not included in the discussion.

The anti-HBs results showed that 16,8% (16/95) of all positives by RIA were negative by EIA representing 8,2% (16/195) of all sera tested for this marker. Thirteen of the 16 anti-HBs EIA−/RIA+ sera were also treated for anti-HBc, 12 of which were positive by both methods.

In the tests for anti-HBc, 32,3% (32/99) of all positives by EIA were negative by the RIA, representing 12,4% (32/259) of all sera tested for this marker. Twenty-eight of the 32 anti-HBc EIA+/RIA− sera were tested for the anti-HBs marker, 26 of which were negative by both methods.

TABLE I. RESULTS OF COMPARATIVE TESTS ON HUMAN SERA FOR THREE HEPATITIS MARKERS USING RIA AND EIA

	EIA−/RIA−*	EIA+/RIA+†	EIA+/RIA−‡	EIA±/RIA−§	EIA−/RIA+‡	EIA−/RIA±§	Total
Anti-HBc	**144**	**67**	**32**	**12**	**2**	**2**	**259**
HBsAg	**326**	**27**	**3**	**0**	**1**	**1**	**358**
Anti-HBs	**86**	**79**	**0**	**0**	**16**	**15**	**196**

* Negative by both methods.
† Positive by both methods.
‡ Positive by one method only.
§ Low positive by one method only.

The results of tests for HBsAg marker were similar by both methods with only 0,92% (3/326) EIA+/RIA− and 0,3% (1/326) EIA−/RIA+, although 6 of the EIA negatives were positive on first testing.

Discussion

The EIA method used in the study has several advantages over the RIA system in that it offers a 1-day test, no exposure to radioactivity, the possibility of cross-contamination is reduced to a minimum, and the kits have a longer shelf-life, but the most important criter ia when comparing serological methods must be sensitivity and specificity.

In the test of anti-HBs 8,2% of the total sera tested or 16,8% of the total positives by RIA were negative by the EIA test. Kruining *et al.*[1] tested vaccinees and convalescent hepatitis B patients for anti-HBs and also found a higher percentage were positive by RIA (Abbott Laboratories) than EIA (Organon Teknika). The hepatitis B sera from convalescent subjects were all positive for anti-HBc indicating that the RIA results were more likely to be correct. In the present study 12 out of 13 of the RIA+/EIA− sera tested were positive for anti-HBc and it was thus concluded that the RIA test was more sensitive for anti-HBs.

In the tests for anti-HBc, 12,4% of the sera tested were positive by EIA and negative by RIA, this number represented 32,2% of all positives. Similar results were obtained by Hanson and Polesky[2] who tested 22 346 sera from voluntary blood donors for anti-HBc. Of these 482 were positive by EIA (Abbott Laboratories) but only 221/482 were positive by RIA (Abbott Laboratories). The 261 anti-HBc RIA− and EIA+ sera were all negative for anti-HBs, indicating that the RIA results were more likely to be correct. In this present study 28 of the 32 RIA−/EIA+ sera were tested for anti-HBs and 26 were negative. The conclusion was that the RIA test is more specific for anti-HBc.

The test results for HBsAg by RIA and EIA were comparable; the EIA method gave a few positives that became negative on retesting, but the test protocol requires that all HBsAg positives be confirmed.

The EIA test is a well established and commonly used diagnostic test for a wide variety of infectious agents, and it is difficult to understand why this system tends to give questionable results for anti-HBc and anti-HBs. Ratnam and Tobin[3] reported variation in results with the EIA kits from different manufacturers, and used the RIA to confirm the results. Kruining *et al.*[1] made a strong case that the reference sera used in the EIA could be the problem, noting that the RIA test results were least affected by different reference serum panels. These workers[1] suggested that manufacturers should get together and use a common reference serum panel. Nelles *et al.*[4] suggested that the competition assays were a difficulty in EIA but not in the RIA. They tested 1 150 blood-donor samples and 30 were repeatedly positive for anti-HBc by a competition EIA (Organon Teknika) but only 21 by RIA (Abbott Laboratories). The 9 sera which were anti-HBc EIA+ and RIA− were retested by two variations of a direct EIA method, 7 became negative by one method and all 9 were negative by the second.

In our hands and those of some other workers the EIA test for hepatitis anti-HBc and anti-HBs would appear to have limitations that are intrinsic to the test and not peculiar to one manufacturer. If the EIA is the only practical test available, then it is important for the laboratory and the clinician to be aware of the extent of these limitations.

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