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# Micromodification of Serodia HBe haemagglutination kit and modification of kit to test for anti-HBe

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SUMMARY Modification of the Serodia HBe haemagglutination kit produced by Fujirebio Incorporated, Tokyo, Japan, gave a rapid, economical, and easily performed test for hepatitis Be antigen (HBeAg). The use of this method to test serum samples for HBeAg is described. A modification of the kit to screen for anti-HBe is also described. The results obtained showed that this kit, when used by the method described, gave results comparable with those of a sensitive, commercial enzyme immunoassay method.

The presence of hepatitis Be antigen (HBeAg) in the serum of patients suffering from hepatitis B indicates that such patients have a high risk of transmitting the hepatitis B virus,[1] that their infection may follow an aggressive course,[2] or that the infection is in an acute phase.[3] The presence of HBeAg in the maternal circulation is also widely accepted as representing the need for administration of hepatitis B immunoglobulin and active immunisation with hepatitis B vaccine to babies of HBsAg positive mothers.[4 5] After accidental needlestick injury hepatitis B immunoglobulin should be given to the subject at risk if the patient is shown to be HBeAg positive.[6] No protection is necessary if the accidentally injected material contains anti-HBe. Existing methods are either slow and insensitive (gel diffusion), or time consuming, expensive, and suitable only for reference laboratories with access to complex equipment (enzyme linked immunosorbant assay and radioimmunoassay). These problems have often led to expensive treatments being given unnecessarily or, worse still, no treatment being offered to subjects at risk.[7] It is also often clinically desirable for an HBe result to be made available rapidly so that suitable levels of patient care and containment can be instigated. With the introduction of the HBe haemagglutination (RPHA) kit reliable HBeAg results can be given in less than one hour from receipt of specimen; anti-HBe results take a slightly longer time.

## Material and methods

The Serodia HBe kit is a reverse passive haemagglutination (RPHA) kit that uses fixed chicken cells coated with human anti-HBe antigen. These cells are specifically agglutinated by the presence of HBe antigen in the serum. The kit also includes chicken cells coated with normal human IgG antigen (control cells). The kit as supplied contains positive control serum, diluent liquid, and tablets to absorb non-specifically reacting serum samples, plates, and pipettes. The micromodification allows 300 tests to be performed with the 50 test kit or 1200 tests from the 200 test kit.

A total of 341 serum samples were tested for HBeAg by the micromodified Seroda method. The samples tested were from 112 HBsAg positive antenatal patients; seven HBsAg positive cord samples from babies with HBsAg positive mothers, 20 from HBsAg positive blood donors, and 15 from HBsAg positive genitourinary and haemophiliac patients. The method was also used to test 187 serum samples from blood donors who were negative for HBsAg and anti-HBs antigen when tested by the radioimmunoassay test kit supplied by the Blood Products Laboratory, Elstree (BPL-RIA). This is a solid phase "sandwich" immunoradiometric assay, which uses antibody coated styrene wells and anti-HBs labelled with iodine-125.[8] The samples from the HBsAg positive cases were also tested for HBeAg using the Abbott HBe enzyme immunoassay system (Abbott-EIA), in which the test serum is incubated with beads coated with human anti-HBe antigen. If HBe antigen is present in the test samples specific coupling to the antibody coated beads takes place. During a second incubation anti-HBe conjugated with horseradish peroxidase is bound to any HBe antigen on the bead. After washing, o-phenylene diamine solution containing hydrogen peroxide is added to the bead, and yellow colour develops proportional to the amount of HBeAg in the test serum.[9]

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The micromodification of the kit for HBeAg screening uses the now well established Terasaki plate method.[10] A volume of 4 $\mu$l of a preprepared 1 in 10 dilution of serum is placed into two adjacent wells of a Terasaki plate. All serum dilutions are prepared in phosphate buffered saline, pH 7, containing 5% human serum known to be negative for all hepatitis B markers. HBe antibody coated cells (4 $\mu$l) are added to well 1 and normal immunoglobulin coated cells (4 $\mu$l) to well 2. The cells are used at the manufacturers' recommended strength and must be reconstituted using the buffer supplied with the kit. The plates are placed at a slope of 30° at room temperature and settling patterns given by the cells are read after 30 minutes. Serum samples that do not contain HBeAg give a crescent of unagglutinated cells at the bottom of the well. Positive samples give a mat of agglutinated cells throughout the well. All results are read with reference to the control serum provided in the kit. Titration of samples positive for the HBeAg screen test can be performed by preparing dilutions of serum from 1 in 10 to 1 in 5120 and testing each dilution as before.

Screening for anti-HBe antigen is performed using a pool of strong HBeAg positive samples, which have been inactivated at 60°C for 15 hours. One volume of test serum is added to one volume of the HBeAg positive pool, mixed, and allowed to stand at room temperature for one hour. A 1 in 10 dilution of this mixture is prepared in phosphate buffered saline with 5% serum. This dilution is then tested using both antibody coated and control cells by the method previously described. Serum containing anti-HBe antigen neutralises the added HBeAg and inhibits agglutination of the cells. Serum that does not contain anti-HBe allows the HBe antigen in the pool to cause agglutination of the antibody coated cells. Antibody titration can be performed by prediluting the test serum from neat to 1 in 256 before mixing with the HBe antigen positive pool, and testing as before.

## Results

Table 1 summarises the results. Twenty four serum samples were HBe antigen positive by both the Serodia micromethod and the Abbott enzyme immunoassay method: two were HBeAg positive by the micromethod and negative by the Abbott method, and two were positive by the Abbott but negative by the Serodia micromethod. The four samples that gave discrepant results were anti-HBe negative when tested by both the modified haemagglutination inhibition and Abbott methods. One hundred and twenty six serum samples from HBsAg positive patients were negative for HBeAg by both methods. The 187 blood donors negative for HBsAg and anti-HBs were negative for HBeAg by the Serodia micromethod.

Ninety five serum samples were tested for anti-HBe using the micromodified reverse passive haemagglutination inhibition method described. Thirty three of 47 samples from HBsAg positive antenatal patients were positive for anti-HBe by this method, and anti-HBe was not detected in 14 samples; 36 of these were anti-HBe positive by inhibition of Abbott enzyme immunoassay and anti-HBe was not detected in 11 samples by either method. Twelve out of 16 samples from HBsAg positive blood donors contained anti-HBe by both methods. Anti-HBe was not detected in 32 samples from blood donors negative for HBsAg and anti-HBs when tested for anti HBe by the reverse passive haemagglutination inhibition method. Table 2 summarises the results.

Table 1 *Comparison of RPHA micromethod and Abbott HBe enzyme immunoassay (EIA)*

| | *RPHA positive EIA positive* | *RPHA positive EIA negative* | *RPHA negative EIA positive* | *RPHA negative EIA negative* |
|---|---|---|---|---|
| HBsAg positive antenatal patients | 16 | 1 | 2 | 93 |
| Cord blood from babies with HBsAg positive mothers | 1 | 0 | 0 | 6 |
| HBsAg positive blood donors | 1 | 0 | 0 | 19 |
| Other HBsAg positive patients | 6 | 1 | 0 | 8 |
| Total | 24 | 2 | 2 | 126 |

Table 2 *Anti-HBe detection by (RPHA-I) and enzyme immunoassay (EIA-I)*

| | *Anti-HBe detected by RPHAI and EIA-I* | *Anti-HBE detected by EIA-I only* | *Anti-HBe not detected by RPHAI or EIA-I* |
|---|---|---|---|
| HBsAg positive blood donors | 12 | 0 | 4 |
| HbsAg positive antenatal patients | 33 | 3 | 11 |
| Total | 45 | 3 | 15 |

RPHA-1 = RPHA inhibition; EIA-I = enzyme immunoassay inhibition.

## Discussion

The micromodification of the Serodia HBe reverse passive haemagglutination kit was found to be of comparable sensitivity with one of the most sensitive techniques (Abbott enzyme immunoassay) for the detection of HBe antigen. In addition, the further modification described allows sensitive and rapid screening for anti-HBe. Using the micromodification of the Serodia HBe kit, 26 (16%) of 154 HBsAg positive serum samples were found to contain HBe antigen, and 45 (71%) of 63 HBsAg positive samples were found to contain anti-HBe. No non-specific reactions were observed in the HBs antigen negative blood donors. The test as described is rapid and far less complex to perform than tests of equal sensitivity. It is extremely suitable for laboratories without access to complex equipment needed for many of the other tests.

### References

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