An evaluation of low voltage counterimmuno-electrophoresis for the detection of hepatitis-B antigen (HB Ag)

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SYNOPSIS A newly available low voltage counterimmuno-electrophoresis (CIEP) system for the detection of HB Ag (Hapindex, Ortho Diagnostics) was compared with a conventional CIEP method used at this laboratory. A total of 1216 sera were tested. The Hapindex system was found to be at least as sensitive as the conventional CIEP. No false positives were found in this series. The elimination of any preparative work makes the Hapindex system particularly suitable for laboratories not testing large numbers of sera for HB Ag. It also eliminates many of the contamination hazards inherent in the conventional method.

At the present time many methods are available for the detection of HB Ag. These include simple double diffusion (Blumberg, Alter, and Visnich, 1965), latex agglutination tests (Leach and Ruck, 1971), and the more commonly used or more complex tests such as haemagglutination (Reesink, Duimel, and Brummelhuis, 1973), counterimmuno-electrophoresis (CIEP) (Gocke and Howe, 1970), complement fixation (Shulman and Barker, 1969), immune electron microscopy (Almeida, Zuckerman, Taylor, and Waterson, 1969; Bayer, Blumberg, and Werner, 1968; Dane, Cameron, and Briggs, 1970), and radio-immunoassay (Prince, Brotman, Jass, and Ikram, 1973). Apart from the simple double-diffusion, latex agglutination, and the haemagglutination tests the remaining techniques require expensive equipment and/or technical expertise. It is generally accepted that of these, radioimmunoassay is the most sensitive test (Cossart, Boxall, and Flewett, 1973). The remaining methods vary in their sensitivity, the least and most sensitive being the double-diffusion and haemagglutination tests respectively.

Ortho Diagnostics have introduced a low voltage CIEP system (Hapindex) which because of its simplicity enables the routine pathological laboratories to carry out their own HB Ag screening tests. This paper describes and evaluates the Hapindex system in comparison with that of a conventional CIEP for the detection of HB Ag.

Materials

Between 1970 and 1973, 1216 sera were tested by conventional counterimmuno-electrophoresis methods used at this laboratory. All sera were subsequently stored at -30°C before testing by the Hapindex system. Of these sera, 41 were to be used as 'quality control' sera in a biochemistry laboratory. The remaining 1175 sera originated from the normal population and from patients with a variety of clinical diseases. These were divided into the following categories:

CATEGORY 1

Sera from blood donors were received from the National Blood Transfusion Service. These were not the normal blood donor population but were suspected on laboratory or epidemiological grounds of either carrying HB Ag or having HB antibody (HB Ab).

CATEGORY 2

Hospital staff.

CATEGORY 3

People with a past history of hepatitis, transplant donors, mothers of babies with cirrhosis, and children whose mothers had hepatitis.

CATEGORY 4

Patients with acute or chronic hepatitis, renal

failure, polyarteritis, leukaemia, Hodgkin's disease, and haemophilia.

CATEGORY 5

Pools of human sera which were to be used as 'quality control' sera in a biochemistry laboratory.

Low Voltage (17 Volt) Counterimmunoelectrophoresis (Hapindex)

The system consists of a cassette containing buffered agar with pre-cut rectangular wells and indwelling electrodes, a power unit and a separate viewer with a monochromatic light source. After the addition of the sera to the appropriate wells the cassette is plugged into the power unit and the current switched on. The power unit automatically switches off after one hour, this being indicated by the appearance of a light. The cassette is removed and examined in the viewer for lines of precipitation.

Twelve sera can be examined for HB Ag in each cassette including a known positive control. The HB Ab provided with the system is raised against 'ad' and 'ay' subtypes of HB Ag by Ortho Diagnostics and standardized to detect weak and strong concentrations of HB Ag in test sera. This system has been described elsewhere (McKee, Pollack, and Tripodi, 1973).

Conventional Counterimmunoelectrophoresis

The method of Milne and Barr (1971) with square petri dishes and barbitone buffer, pH 8.2, was modified.

HB ANTIBODY

The various batches of antibody used during this period (1970-1973) originated from human and animal sources. Working dilutions, previously standardized by titration, were stored at 4°C.

HB ANTIGEN (POSITIVE CONTROL)

Working dilutions were stored at 4°C. The dilution for use was obtained by titration against the HB Ab in current use.

TEST

Wells were cut in the agarose on the day of the test using a no. 1 cork borer (3.5 mm diameter) and a blood transfusion needle (2 mm diameter), using a template to give a distance of 5 mm between antibody and antigen wells. Each test serum was placed in one 2 mm and one 3.5 mm well and tested against two 2 mm antibody wells. This system was designed so that high and low concentrations of HB Ag could be detected in test sera.

Test sera and antibody were placed in their respective wells, filter wicks were introduced (100 mm × 80 mm) and the plate was placed in the electrophoresis tank. The current was switched on, adjusted to 30 milliamps, and the apparatus was left for 75 minutes. At the end of this time the plate was removed and examined for lines of precipitation using a bright indirect light source with a black background. Results were recorded and the agarose was then flooded with normal saline and left at room temperature overnight. The following day the plate was re-examined for any additional lines of precipitation which may have developed.

Immune Electron Microscopy

Test serum (0.2 ml) was added to 0.1 ml of HB Ab (previously standardized by chessboard titration) and left at room temperature for 30 minutes. The mixtures were then centrifuged at 49 000 *g* in the SM 24 rotor of a Sorvall RC2-B centrifuge for 75 minutes. After discarding the supernatant, the deposit was resuspended in one drop of glass-distilled water. A formvar-coated 400 mesh Athene grid was inoculated with the deposit, and, after washing in glass-distilled water, stained with 1.5% (w/v) sodium phosphotungstate, pH 6.5. Grids were examined in an AEI 801 electron microscope at a magnification of × 40 000 for 15 minutes before they were considered negative. A known positive serum containing HB Ag was included in each batch of tests.

Results

A total of 1216 sera were tested by the Hapindex and conventional CIEP methods (see table). Of these, 133 sera were positive by Hapindex whereas 131 were positive by CIEP. Thus Hapindex detected two additional HB Ag-positive sera. Both of these were confirmed by immune electron microscopy. Immune electron microscopy was also carried out on all the sera in 'category 5' and HB Ag was detected in 2/41 sera; neither were detected by the two counterimmunoelectrophoresis systems used.

QUALITY CONTROL

In order to determine the reproducibility of the Hapindex system, two HB Ag-positive sera were tested against three different batches of HB Ab in six different batches of cassettes. One of the HB Ag-positive sera was known to give a very weak precipitation line whilst the second serum gave a strong prozone reaction. There was no variation between the different batches of cassettes or antibodies in the

type of precipitation lines produced by the two antigens.

Discussion

The Hapindex low-voltage counterimmunoelectrophoresis is a simple test requiring little technical expertise. It has certain advantages over the conventional CIEP in that the cassette is ready for use and requires no preparative work. We found the system completely reliable during the course of this evaluation. The use of monochromatic light in the viewer was considered to be particularly good for the recognition of very weak precipitation lines. This was impressively demonstrated when lines of precipitation were examined on the two systems by totally inexperienced staff who found the Hapindex monochromatic light system superior.

Using this system, contamination of the operator is reduced to a minimum by removing the necessity of handling possibly contaminated wicks and buffer. Also the cassette is provided with a lid which is kept in place after the reagents have been added and during the test period. When the test has been completed the entire cassette is disposable. The use of low voltage also reduces the risk to the operator.

The detection of two additional positive sera by Hapindex (see table) may have been due to greater

Two sera were positive for HB Ag by immune electron microscopy (see table) but were not detected by the two systems described here. These were pools of human sera, so that any HB Ag present in one serum would be diluted by the other sera in the pool. Thus the two CIEP systems described were not sufficiently sensitive to detect the low concentration of antigen present.

On no occasion did we obtain a false positive result using the Hapindex system. This is an advantage over the latex agglutination test (Leach and Ruck, 1971; Cossart, Field, March, and Porter, 1972; Klossner and Willman, 1973) and some haemagglutination tests (Reesink *et al*, 1973).

We considered that Hapindex suffered from the disadvantage of having only sufficient wells to test 12 sera (including one positive control) on each cassette as compared with 100 tests on our conventional counterimmunoelectrophoresis.

Although we think that Hapindex is not suitable for mass screening of sera for HB Ag it is an ideal system for smaller laboratories which receive relatively few requests for HB Ag detection. It may also be useful to biochemistry departments for screening sera before autoanalysis and to laboratories involved in venereal disease serology.

We wish to acknowledge Ortho Diagnostics for supplying the Hapindex system with which this work was carried out. We are indebted to Dr S. K. R. Clarke for her advice and criticism in the preparation of this paper.

Category	Total Sera Tested	Hapindex		CIEP	
1	183	77	106	77	106
2	136	4	132	4	132
3	96	4	92	4	92
4	760	48 ¹	712	46	714
5	41 ²	0	41	0	41
Total	1216	133	1083	131	1085

Table Comparative results obtained by Hapindex and conventional CIEP

¹The two additional positives detected by Hapindex were confirmed by electron microscopy.

²Two of these sera were positive by electron microscopy.

sensitivity of the Hapindex system, which uses a broad-spectrum antibody and is balanced to detect both weak and strong concentrations of antigen (McKee *et al*, 1973). A further possibility is that this system uses rectangular rather than the conventional circular wells. This possibly results in a higher concentration of antigen at the point of precipitation. The antibodies used in the conventional test originated from either a human or animal source and did not have a broad spectrum. The Hapindex system has also been found to be more sensitive than the conventional CIEP by other workers (McKee *et al*, 1973) but it is unlikely to be as sensitive as haemagglutination or radioimmunoassay.

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