Technical methods

Crossover electrophoresis for the rapid detection of serum hepatitis (Australia) antigen and antibody

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Crossover electrophoresis in agar gel has proved to be a useful technique for detecting serum hepatitis (Australia) antigen and antibody (Krassnitzky, Pesendorfer, and Wewalka, 1970; Pesendorfer, Krassnitzky, and Wewalka, 1970).

A cellulose acetate membrane was introduced by Kohn (1968) as a supporting medium and the gelatinized cellulose acetate sheet, Cellogel (Chemtron, Milan), is suitable and improves the results, producing sharp, clear-cut precipitation bands.

Patients' sera (3-5 μ l) are applied on the Cellogel sheet (10 $\times$ 20 cm), stretched on a Shandon electrophoresis apparatus, midway between cathode and

Received for publication 2 July 1970.

anode in the form of suitably spaced drops. One centimetre from the point of application in front of each spot a drop (3-5 μ l) of a known sample containing serum hepatitis antigen is applied toward the cathode and a drop of a known sample containing serum hepatitis antibody toward the anode. Our antiserum was obtained from a haemophilic patient who had had many transfusions and when tested by the micro-Ouchterlony-Elek technique showed an identical reaction with the reference anti serum anti-Au (1) CO 66720.

Using veronal buffer, pH 9, 2M 0.05 (sodium diethylbarbiturate 9.8 g, HCl 0.1 N 24 ml, distilled water to 1,000 ml) with a bridge gap of 8 cm the electrophoretic run is performed in 20 minutes by applying a constant voltage of 20 to 25 V/cm. The sheet is then put into saline for one hour to remove by shaking the proteins which did not take part in the precipitating reaction and is stained for five minutes in a 0.5% amido Schwarz solution in methanol/acetic acid/water (50/10/40). The stain is removed by several washings in methanol/acetic acid/water (5/3/45). Alternately the more

Fig. Crossover electrophoresis on Cellogel of 20 sera from patients with Down's syndrome (Pt). Serum containing serum hepatitis (Australia) antibody (extreme left) and serum containing antigen (extreme right) are included for comparison. Note that the six Down's syndrome patients' sera are positive for antigen.

Ab = known serum with serum hepatitis (Australia) antibody.

Ag = known serum with serum hepatitis (Australia) antigen.

sensitive Nigrosin stain may be used (Nigrosin 5 mg in 100 ml 2% acetic acid). When the bands are completely stained (the sheet is best kept in the staining bath overnight) a quick rinse in tap water is quite sufficient.

If the serum to be tested contains antigen a sharp precipitation band is visible toward the anode; if it contains antibody a band shows up towards the cathode.

The advantages of crossover electrophoresis on Cellogel are:

(1) With one electrophoretic run about 30 samples can be thoroughly examined after an hour and a half. This makes the technique particularly suitable for rapid checking of blood donors' sera.

(2) The technique is more sensitive than the micro-Ouchterlony-Elek double-diffusion method. This is because all the reactants in the samples move towards each other. Under these conditions it was possible to detect a precipitation band after staining with Nigrosin by applying 3 μ l of a sample containing serum hepatitis antigen diluted up to 1:30.

(3) The precipitation band is clearly visible leaving no doubt in interpretation (Fig.).

Compared with crossover electrophoresis in agar gel, our technique, which is easily handled, allows us to test more samples in a shorter time using smaller quantities of reagent. Cellogel staining is fairly simple and a permanent record of the results is obtained after clearing.

Dr B. S. Blumberg kindly supplied the reference antiserum.

References

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An economical way of measuring fibrin degradation products

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The measurement of fibrin degradation products is becoming increasingly used in the assessment of disorders of blood coagulation and fibrinolysis.

The kit supplied by Wellcome Research Laboratories is very useful for this purpose, but the high cost of the microtitre kit (£172) puts the test into the luxury class, and confines it to a few centres.

A modification of the test is described, using standard laboratory equipment. If it is less accurate than the micro-titre method, it must be minimally so, since the pipettes used are accurately calibrated.

The tests are done exactly as described in the instruction leaflet supplied with the fibrin degradation products kit, but the titrations are done in plastic rhesus grouping tubes, using haemoglobin pipettes (0.02 ml). If the volume of buffer is pipetted gently into the tube it will stay at the top of the tube, and the dilution mixtures can be made there before the resultant solution is shaken to the bottom of the tube. This is necessary because the haemoglobin pipettes are too large to fit into the tubes. A marked Pasteur pipette could be used instead, but the same pipette would have to be used throughout instead of a new pipette for each test. With a little practice, however, it is quite easy to use the haemoglobin pipettes.

Plastic ampoule holders with lids, having 10 divisions, are obtainable from any pharmacy, and these make admirable racks for this purpose.

The advantage of the tubes is that, owing to the small surface area, they can be left on the bench for long periods without detriment, if the lids are on the boxes.

If the sensitized cells are diluted with 5 ml of citrate buffer instead of 2.5 ml, and the tests are left overnight, the endpoints are very easy to read in a concave microscope mirror.

Received for publication 15 May 1970.