Technical methods

Quantitative estimation of protein by electroimmunodiffusion on Cellogel acetate membranes

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The Mancini technique, in which the antigen is allowed to diffuse radially into an agar gel containing precipitating antibodies, is widely used for the routine estimation of proteins in biological fluids. Unfortunately, this technique requires at least 24 hours for the determination, and its use is somewhat restricted to proteins at serum concentrations. In 1966 Laurell published details of a more rapid technique based on electrophoresis of the antigen in a gel containing the specific antibodies. Migration of the antigen and antibodies in the electric field causes the rapid formation of precipitation zones resembling comets. The position of the precipitation front at the end of the electrophoresis in a given antibody concentration is a function of antigen concentration. This technique was refined by Merrill, Hartley, and Claman (1967) for the quantitation of immunoglobulins in dilute biological fluids such as cerebrospinal fluid, tears, and saliva. These authors refer to the technique as electroimmunodiffusion. Further modifications of the Laurell technique in which agar is replaced by a cellulose acetate membrane simplify the procedure and significantly reduce the amount of antiserum needed (Krøll, 1968).

Recently a new type of acetate membrane, Cellogel, has been introduced by Chemetron, Milan (Italy). Its essential difference is that only one surface is penetrable by protein solutions and this leads to more sharply defined sample application and more sharply separated zones on electrophoresis. Cellogel is a suitable substrate for the Mancini technique and its manufacturers market a kit for this application. Unlike the acetate techniques described to date, in which the membrane is soaked in relatively large volumes of diluted antiserum, a small volume (200 λ) of diluted antiserum is applied uniformly to the absorbent surface with a simple volumetric spreader.

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This communication describes how the 'immunostrip' so prepared can be used in an electroimmunodiffusion technique for the quantitation of immunoglobulins and other serum proteins.

Procedure

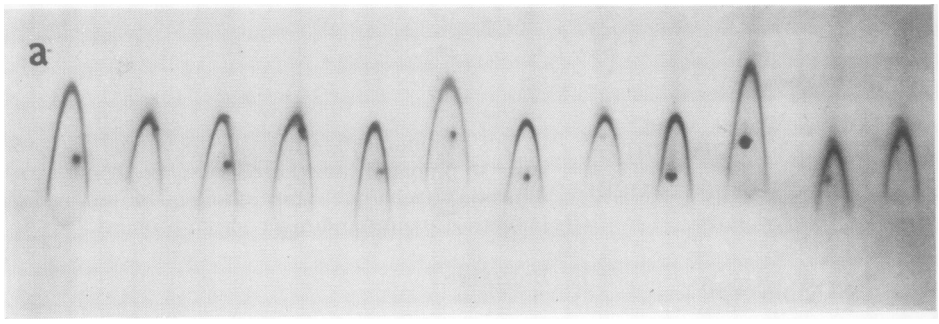
A 14 × 5·7 cm Cellogel strip is soaked in 0·036M tris-barbitone buffer, *p*H 8·8, for 10 to 30 minutes. After blotting off the excess of liquid with filter paper the strip is tensioned between the two clips in the Chemetron immunodiffusion tank (after Vergani, Stabilini, and Agostoni, 1967) with the absorbent surface uppermost.

The volume of specific antiserum required varies with the potency. For many of the antisera (Behringwerke AG) used in this laboratory 50-150 λ is satisfactory, the volume being adjusted to 200 λ with normal saline stained with a crystal of bromothymol blue. The antiserum load is applied to the paper with the spreader, producing a uniform blue-green colour. The immunodiffusion tank is then covered and set aside for an hour at 4° to increase the uniformity of the 'immunostrip'.

To construct a standard curve, suitable dilutions of either a standard serum or of the purified antigen fraction are made with saline stained with erythrosin, using Drummond microcaps. Many serum components may be quantitated at serum dilutions of 1:20 or 1:30, but 1:10 is normally satisfactory. Other biological fluids such as saliva should be applied without dilution. Samples (2 λ) of standards and samples are applied to the immunostrip with microcaps through the perforated lid of the immunodiffusion tank, applying the loads along the central long axis of the paper in a staggered row. The immunostrip will accommodate 13 samples in this manner.

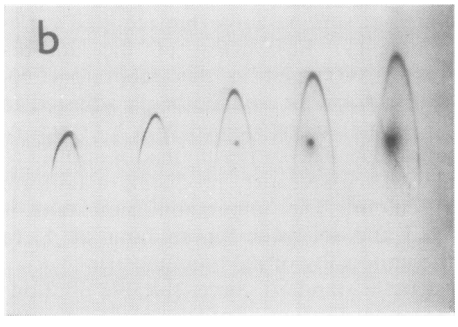
After loading the immunostrip is transferred to a suitable electrophoresis tank (eg, Shandon), adjusted to a 5 cm gap, and electrophoresis is performed in the direction of the short axis (one and a half hours; 9-10 MA; 80-100 v) using 0·036M trisbarbitone, *p*H 8·8, as the tank electrolyte. After electrophoresis the strip is washed with normal saline for one hour, changing the saline every 10 minutes, stained with Ponceau S (0·2% in 3% TCA), and finally destained with 5% acetic acid.

Restaining in nigrosin (0·01% in 2% acetic acid) is usually necessary to define clearly the precipitin comets. The latter can be measured directly on the

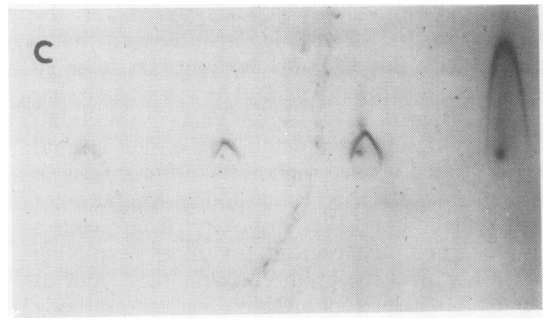


(a) *Normal and pathological sera diluted 1:10*

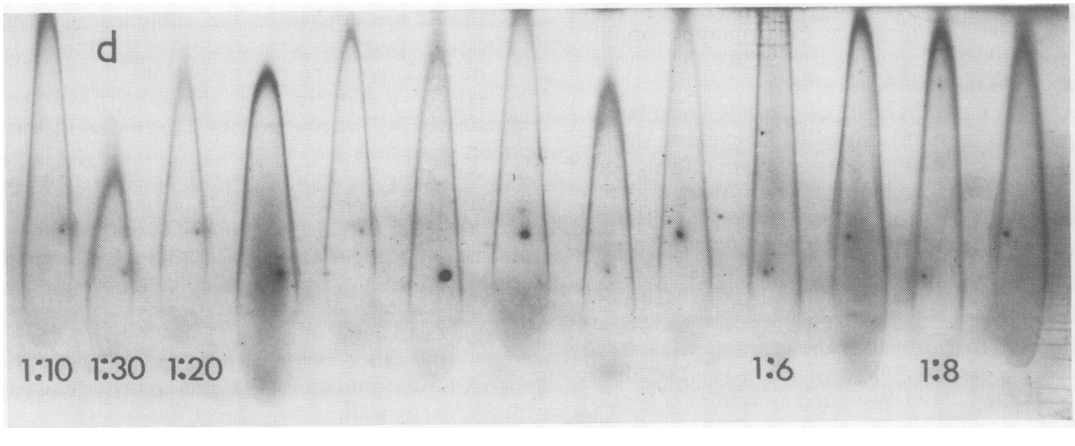
Fig. 1 *Electroimmunodiffusion determinations of immunoglobulins.*
a, b, c *Immunostrip prepared with 100λ goat antihuman γ A antiserum (Behringwerke AG)*



(b) *Dilutions of a standard normal serum, reading from left to right, 1:30; 1:20; 1:10; 1:6; 1:2*



(c) *Dilutions of human saliva together with serum standard, reading from left to right, saliva diluted 1:2, 1:1, and undiluted saliva; serum diluted 1:2.*



(d) *Immunostrip prepared with 50λ goat antihuman γG antiserum (Hyland). Antigen samples, various dilutions of a standard normal serum and pathological sera diluted 1:10; the standard dilutions are indicated.*

wet strip using a pair of dividers and a suitable millimetre scale. The antigen concentrations in the unknowns can then be read off from a graph correlating the reference standards with peak heights.

Comment

Figure 1 shows typical electroimmunodiffusion quantitations of immunoglobulins on Cellogel. The technique is also satisfactory for estimating other serum proteins, eg, β-lipoproteins. It has the advantages of speed and economy of antiserum: the whole procedure from preparing the immunostrip to estimating the antigen can be carried out within five hours and it consumes only very small measured amounts of antiserum. It appears to be especially applicable to measurement of 'minor' serum components and immunoglobulins present at low dilutions in other biological fluids.

Cellogel also achieves economy in the Mancini technique, since a single immunostrip will accommodate up to 59 determinations.

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A simple capillary tube method for the determination of the specific gravity of 25 and 50 μl quantities of urine

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When large amounts of urine are available in the laboratory, measuring specific gravity is one of the simplest procedures. Methods for small quantities, however, usually entail more elaborate techniques such as placing a drop of urine in a mixture of two solvents, one of high and the other of low specific gravity, and adjusting the proportions by titration (Bowler, 1951) or suspending a drop of urine in a specially calibrated gradient column (Brown, 1958). The titration method is laborious and time consuming. The gradient column method is quicker, but troublesome to assemble and the column reagent has a life limited to a few months.

The weighed specific gravity bottle principle is much simpler, and a constant volume capillary tube of small capacity can be substituted for the larger volume pycnometer. The method was employed in the investigation of a strain of mice suffering from an inherited kidney disease, the determining gene for which has been given the name and genetic symbol, *kidney disease, kd* (Lyon and Hulse, 1971). It is suggested that the method may be applicable clinically, eg, to the small urine samples derived from infants or from ureteric catheterization in man.

Method

Disposable microcap tubes of 25 or 50 μl capacity (Drummond Scientific Company) are selected according to the available volume of fluid. A small number of trials made with plain microhaematocrit tubes (Hawksley) having a volume of around 45 μl indicated that any other small capillary tube of uniform bore and standard size can be used. Urine samples are permitted to reach room temperature (22°C) before weighing. Weighings are made to 0·1 mg on an automatic analytical balance immediately after the introduction of the liquid into a tube.

A dry microcap tube filled by capillary attraction with glass-distilled water is dried on the outside by

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