TWO-STAGE ROCKET-ELECTROPHORESIS ON CELLULOSE ACETATE FILMS

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The method of two-stage rocket electrophoresis on gelatinized cellulose acetate film (Cellogel) was developed. The method is based on electroimmunodiffusion detection of antigen on cellulose acetate films containing monospecific antiserum of a test system, followed by detection of precipitation bands with the appearance of stained "rockets" if the reaction takes place in the visible zone, or with further treatment of cellulose acetate strips containing invisible precipitates with antiglobulin antibodies, complement, or a combination of both. The sensitivity of the method is increased to 30-60 ng/ml in the visible zone of reaction by simultaneously reducing the concentration of antibodies and increasing the absolute amount of antigen for electrophoresis to 50-100 μl. Details of the method were worked out for human α-fetoprotein.

KEY WORDS: α-fetoprotein; cellulose acetate; immunoelectrodiffusion; rocket electrophoresis.

One of the simplest and most widespread methods of quantitative determination of antigens is what is called rocket electrophoresis [3]. In this method the test antigen is subjected to electrophoresis in a plate of agarose gel mixed with the corresponding antiserum. The rocket-shaped peak of precipitation thus formed has an area which is proportional to the quantity of antigen in the sample or, more accurately, to the ratio between the antigen concentration in the sample and the antibody concentration in the gel.

The sensitivity of the method can be increased by reducing the antibody concentration in the gel or increasing the absolute amount of antigen for electrophoresis through a unit of section of the gel plate. The first method is used in conjunction with autoradiographic detection of invisible precipitates [4]. For the second method a special "bottle-shaped" reservoir has been suggested, so that larger volumes of antigen in low concentrations can be subjected to electrophoresis [2].

In the present study this variant was modified for use during electrophoresis on cellulose acetate films, which has many advantages over electrophoresis in gels.

Gelatinized cellulose acetate (Cellogel) films (from Chemetron, Italy) were used. Human α-fetoprotein (α-FP) and monospecific rabbit antiserum against it (anti-α FP; diagnostic immune serum for primary carcinoma of the liver and teratoblastoma, prepared by the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) were used as the test system. The antigens of this diagnostic serum contains αFP in a concentration of 50-55 μg/ml in a solution of human albumin.

The principle of the method is shown in Fig. 1. A Cellogel film saturated with antiserum, made up in Tris-HCl buffer (0.06M Tris, HCl to pH 8.6; B-III), was placed in the electrophoresis apparatus. A ball of agarose 3 μl in volume, made up in the same buffer with the addition of bromphenol blue (BPB) was placed on the film in its cathodal part, and a second film, carrying the antigen, was placed on the ball. The two films were in contact with the cathode. During electrophoresis the antigen migrated from the top film through the point contact to the bottom film, saturated with antiserum (Fig. 1B). The volume of the sample applied to the top film was 10-30 times greater than the volume of the agarose drop through which the antigens migrated. In this way the capacity of the drops was increased without increasing the area of its contact with the film containing antiserum. The area of the rocket was increased proportionally to the increase in volume of the sample on the top film, i.e., the sensitivity of the method was increased.

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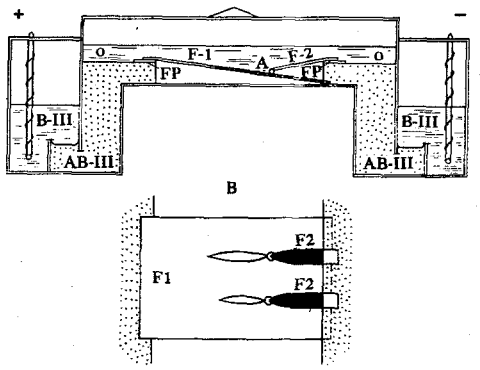

Fig. 1

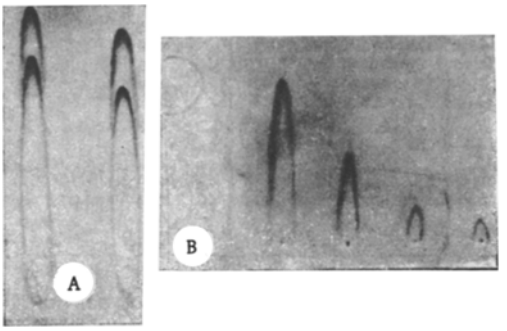

Fig. 2

Fig. 1. Principle of the method. A. F1) Cellulose acetate film saturated with antiserum; F2) cellulose acetate film carrying antigen; A) agarose drop; FP) filter paper saturated with buffer III; O) mineral oil; B-III) electrode dish Tris HCl buffer (0.06M Tris, HCl to pH 8.6): all reagents, antigen and antiserum were diluted in the same buffer; AB-III) 1% agarose in B-III. B. Antigen in a volume of 50-100 μl migrates by electrophoresis from top film (F2) through drop (3 μl) of agarose gel into cellulose acetate film (F1) saturated with antiserum in B-III, i.e., volume of sample applied to top film is 10-30 times greater than volume of agarose drop through which antigen migrates.

Fig. 2. Quantitative determination of antigen (human αFP) by two-stage rocket-electrophoresis on gelatinized cellulose acetate membranes. A) Rockets Nos. 1 and 2 in parallel test, with human αFP as antigen, 50 ng per sample (inner rockets) and mouse αFP as control antigen, 70 ng per sample (outer rockets). Rabbit monospecific antiserum against human αFP in dilution of 1:25; rabbit monospecific antiserum against mouse αFP in dilution of 1:25. Ratios between test and control antigens in parallel tests kept constant for comparison of areas occupied by their precipitation peaks. B) Human αFP from 30 to 3 ng per sample. Volume of sample tested 50 μl. Rabbit monospecific antiserum against human αFP in dilution of 1:25.

Electrophoresis was carried out under a layer of mineral oil in the apparatus described previously [1]. Agarose blocks, to ensure contact between the films and electrode dishes, were made from 1% agarose (from Serva, West Germany) in Tris-HCl buffer (B-III). The electrode dishes were filled with the same buffer and all reagents – antigen and antiserum – were diluted with it. The antigen was mixed at 45°C with 1% or 2% agarose in the same buffer, so that the final concentration was 0.4-0.5%. The sample was stained beforehand with BPB. The mixture was applied to a strip of Cellogel which tapered to a point (width of strip 3-4 mm, length 20-25 mm). The strip was first saturated with B-III and the excess of fluid removed by soaking on wet filter paper. The bottom film, measuring 8 × 4 cm, was immersed in a solution of antiserum made up in B-III for not less than 30 min. After saturation with antiserum it was transferred to a histology container with mineral oil and placed vertically to allow the excess liquid to drain away. Four hollows for agar drops were made on the film nearer to its cathodal end. The agarose drops in buffer, 3 μl in volume, were made beforehand and kept in the cold in a dish containing mineral oil. An automatic 3-μl micropipet can conveniently be used for making the drops.

The film with antiserum was placed with its anodal edge on the top of the agarose block and its cathodal edge was placed beneath the agarose block in the cathodal part of the instrument. The film with antigen was placed with its wide edge on the cathodal agarose block and its pointed end on the drop (Fig. 1A). Electrophoresis was carried out for 15-18 h with a voltage gradient of 2-2.5 V/cm. After the end of electrophoresis the bottom film was washed to remove antiserum and stained with 0.25% Coomassie R-250 in 40% methanol and 5% acetic acid.

Clearly visible precipitation peaks were obtained with antiserum in a dilution of 1:25. By this method 3-5 ng of αFP could be detected in the visible zone of the reaction regardless whether the volume of the sample was 50 or 100 μl, equivalent to 30-60 ng/ml (Fig. 2). A further increase in sensitivity was obtained by diluting the antiserum and detecting invisible precipitates (Table 1). The precipitation bands were intensified by treatment with donkey antiserum against rabbit immunoglobulins or with complement, or with a combination of both.

TABLE 1. Sensitivity of Different Variants of Two-Stage Electrophoresis on Cellogel Cellulose Acetate Films

Two-stage rocket electrophoresis	Maximal sensitivity of determination of αFP, ng/ml	
	dilution of antiserum 1:25, staining to detect precipitation bands	dilution of antiserum 1:50, antiglobulin antibodies or complement + staining to detect precipitation
From 5 μl	600	300
From 50 μl	60	30
From 100 μl	30	15

The variant of rocket electrophoresis described above increases the sensitivity of this method greatly and makes it comparable with radioimmunological tests. However, in the form described it can be used only to detect antigens with high electrophoretic mobility, such as serum albumin and α globulins. Further modifications of the method are necessary before it can be used for work with "slow" antigens.

LITERATURE CITED

1. G. I. Abelev and A. A. Sokolenko, Byull. Éksp. Biol. Med., No. 7, 121 (1977).
2. J. Kroll, J. Immunol. Methods, 13, 333 (1976).
3. C. B. Laurell, Anal. Biochem., 15, 45 (1966).
4. B. Norgaard-Pedersen, Clin. Chim. Acta, 71, 343 (1976).