Kawasaki Med. J. Vol. 4: No. 1, 53-56, 1977

BRIEF NOTE

A SIMPLE ISOELECTRIC FOCUSING PROCEDURE FOR SCREENING HUMAN HEMOGLOBIN COMPONENTS ON POLYACRYLAMIDE GEL

Accepted for Publication on March 8, 1978

Electrophoreses of various sorts, including the papers, the agar gel[1,2], the starch gel[3-5], the cellulose acetate membrane[6~8] and so forth, have hitherto been employed for the detection of normal and abnormal hemoglobin components, with exception of Hb F which is measured by alkali-denaturation and Hb A_2 which is quantified by DEAE cellulose chromategraphy. Recently an ingenious and elaborate electrophoretic procedure on polyacrylamide gel with rectilinear pH gradient, i. e. isoelectric focusing, was invented, but it was not useful in original manner for mass screening of abnormal hemoglobins, because the equipment was complicated, being composed of a glass column filled with the acrylamide gel. However, in our laboratory this has been improved and converted into an electrophoretic apparatus without using any column, which enables us to subject 50 hemolysates simultaneously to the acrylamide gel. Discrete separation of individual hemoglobin components is achieved within a short time (120 minutes). This is useful for mass screening. Our equipment and the result obtained by its use will be described briefly and illustrated in a picture (Figure 1) and a photograph (Figure 2).

A slight modification of the method described in the Application Note[9] of the LKB Co. was employed for the preparation of polyacrylamide gel with pH gradient from 6 to 9. Acrylamide-Bis solution (3.0 ml) which was made by dissolving acrylamide monomer (30 g) and N,N′-methylenebisacrylamide (0.8 g) in 100 ml of water, 20.8 percent sucrose solution (11 ml), ampholine of pH 3.5-10.0 (0.15 ml), ampholine of pH 7.0-9.0 (0.60 ml) and water (3.0 ml) are mixed completely and deaerated, followed by addition of 0.004 per cent aqueous riboflavin solution (0.05 ml), N,N,N′,N′-tetramethylethylenediamine (0.05 ml) and 10 percent aqueous ammonium persulfate solution (0.05 ml). The mixture thus prepared was introduced into the narrow space (1 mm in thickness) which is formed between the two sheets of glass plate (130 mm × 180 mm) that are held vertially and in parallel. A small amount of water is overlayed above the mixture, and the glass plate assembly is illuminated by ultra-

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violet light for 3 hours to polymerize the sandwiched mixture. At the end of the time one of the glass plate is removed. Thus a rectiangular acrylamide gel plate ($160 \times 110 \times 1$ mm) lying on another glass plate is obtained.

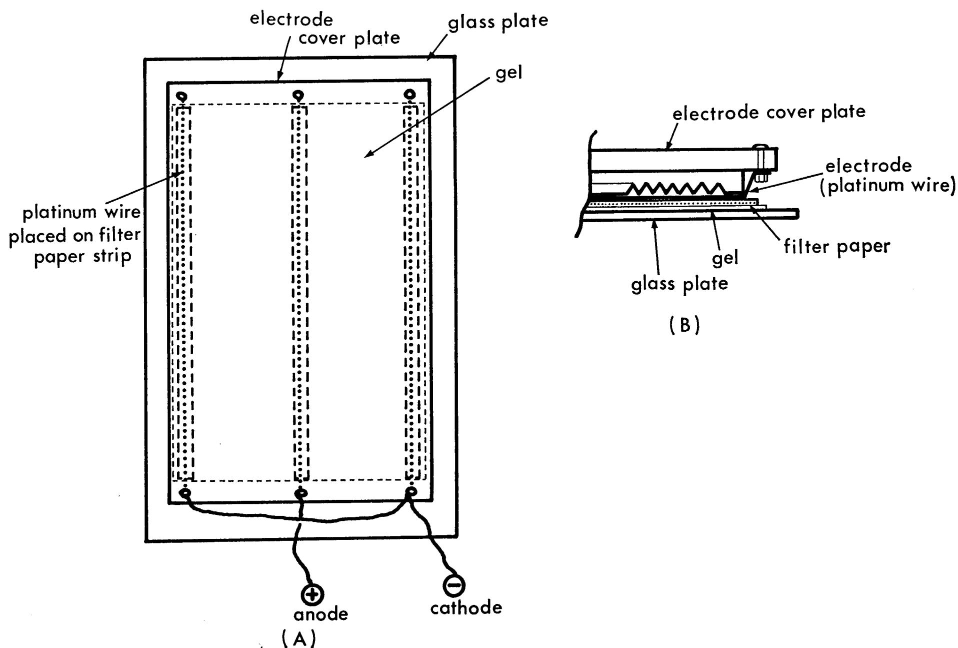

Fig. 1. Electrophoresis assembly (A) Plan; (B) Elevation

As depicted in Figure 1, a long rectiangular strip ($6 \times 160 \times 0.75$ mm) of a thick filter paper was laied on the middle line of the gel surface to make it serve as the mat of the anode of electrophoresis, while at the bilateral edges of the same gel are placed the filter paper strips of the same shape and size for the purpose of the cathodes. The filter paper strip of the anode is moistened with 0.02 M aqueous solution of phosphoric acid while those of the cathodes are imbibed with a sufficient amount of 20 percent aqueous ethylenediamine solution. Hemolysates (1 μl) are individually spotted on the gel plate with 5 mm interspaces along the rectilinear line 1 cm apart from the cathode lines. The gel plate is placed in a cooling chamber at 8°C. The anode and cathodes are connected to the electric source (Figure 1), and an electric current of 200 volt/32 cm is run for an hour (80 mA) and then the current is raised to 300 volt/32 cm (5 mA) and stabilized at this level for the next one hour. The pH gradient is rectilinear from 6 to 9. At the end of the time hemoglo-

bin constituents are separated from the cathode to the anode, Hb A_2, Half-Met Hb A, Hb F and Hb A, in the order mentioned. This is shown in Figure 2.

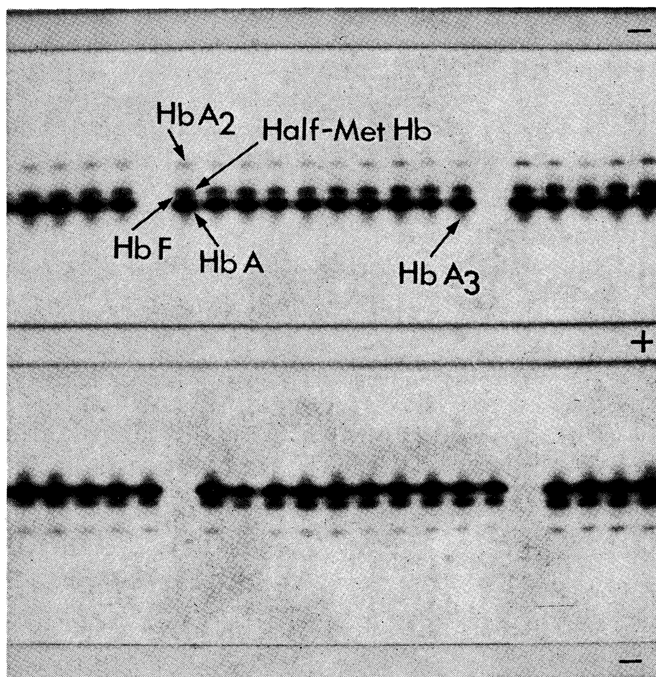

Fig. 2. An example of mass screening of hemolysates by our method. Arrows indicate hemoglobin components. +: Anode, —: Cathode

The stripe of Half-Met Hb A is sometimes seen when an old hemolysate is treated, and may be confused with Hb F. The hemoglobin stripes are discrete. If electrophoretically abnormal hemoglobin is present, it is easily detected by this procedure. This is particularly suitable for the mass screening for the hemoglobin survey of a large population. It is not so expensive (20 yen per one hemolysate), being simple and convenient in manipulation.

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