

Modification of the Starch Block Electrophoresis Method for the Preparation of Immunoglobulin A from Multiple Myeloma Sera on a Large Scale

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Separation of immunoglobulin A from a large sample of human serum from patients with multiple myeloma was done by utilizing a large zone electrophoresis apparatus.

Starch block electrophoresis, a variant of zone electrophoresis, is a convenient method for the separation of various components of serum (7). In contrast to column electrophoresis, the surface of the stabilizing medium is directly accessible, and contact photographic prints can be made of the staining reactions used to detect separated proteins. One of the limitations of the method as originally described by Kunkel (2, 3) is that only a relatively small volume of serum can be processed (15–20 ml) since, among other reasons, the thickness of the block and the time the current is applied may have a direct effect on the temperature that is produced (1).

This technique has been modified for separation in a high yield of immunoglobulin A (IgA) from multiple myeloma sera by utilizing 112 ml of a serum sample, the maximum amount used in one block in this laboratory. Pevikon (Pevikon, C-870, Mercer Chemical Corp.), a copolymer of vinyl chloride and vinyl acetate, was utilized instead of starch for the separation procedure, as described by Muller (5). Pevikon is a substance composed of very fine white granules that do not swell when hydrated (5). A relatively thick and wide (62 by 52 by 2.5 cm) block was used at 4 C, without the use of a water-cooling jacket. The general layout of the system was similar to the one described by Smithies (6), with some modifications to suit our particular purposes. A larger block system was utilized in which two serum samples could be applied and the electric current maintained for a longer period of time (see Fig. 1). Pevikon was washed three times in demineralized water and twice in barbital buffer (0.06 M diethyl barbituric acid, Fisher; pH adjusted to 8.6 with NaOH) which was removed by filtration. It was

then mixed with the same buffer until it was slightly viscous. This viscosity was obtained by using approximately 4,950 g of dry Pevikon and mixing with approximately 4 liters of the buffer. An amount sufficient to make a thickness of 2.5 cm was poured on a fiber glass tray (58 by 68 by 7.5 cm) that contained two folded thick filter papers on each side of the tray. Excess moisture was removed with absorbent paper. Two parallel canals or troughs 31 cm apart, 2 mm wide by 50 cm long, were cut into the block (see Fig. 1). The serum was divided into two equal portions (56 ml each) and applied separately with a dropper in 3-ml samples into each corresponding trough. The block was supported on the edges of two plastic containers of barbital buffer, pH 8.6, as described by Kunkel and Trautman (3). A connecting bridge was made on both sides, between the tray of barbital buffer and a beaker which contained phosphate buffer (which contained 0.038 M sodium phosphate buffer, pH 7.5). Carbon rods were immersed in both beakers (positive and negative

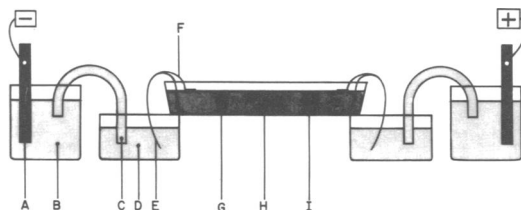


FIG. 1. Block system. A, electrode; B, phosphate buffer (pH 7.5); C, glass connecting bridge; D, barbital buffer (pH 8.6); E, thick filter paper connecting buffer and bottom of block; F, paper towel connecting filter paper and top surface of block; G, serum sample no. 1; H, pevikon surface; I, serum sample no. 2.

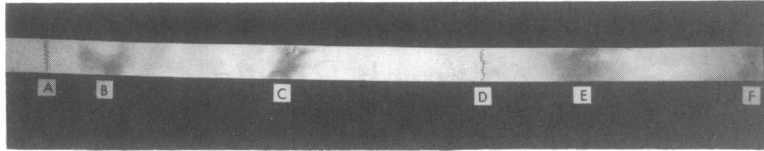


FIG. 2. Contact prints showing migration of the two serum samples. Serum sample no. 1, applied at position A, showing migration of IgA (B) and albumin (C); and serum sample no. 2, applied at position D, showing migration of IgA (E) and albumin (F). Staining was performed with bromophenol blue.

poles). An electrical contact was produced on the surface by using paper towels and through the block by the filter paper that was left in the Pevikon (on both ends). The block was covered with a plastic sheet. The system was run at 450 V and generated 130 mA for 47 to 48 h.

Paper strips were stained from contact prints placed on the Pevikon block. The strips were stained with bromophenol blue, dissolved in ethanol, and saturated with mercuric chloride. Acetic acid (25%) was used to destain the paper strips. The strips were exposed to fumes of ammonium bicarbonate to intensify the protein bands. The migration of IgA and albumin was clearly observed on the paper strips (see Fig. 2). The developed paper strips were placed on the block, previously covered with transparent plastic, to avoid contamination; using the stained bands as guides, sections of the block were removed and eluted with phosphate-buffered saline to remove the separated IgA. The sectioning of Pevikon containing the separated material is one of the most crucial steps in block electrophoresis. The IgA obtained was tested for purity by using Ouchterlony analysis and was found to be free of other substances. The original serum myeloma sample was assayed by the technique of Mancini (4) and found to contain 2,419 mg of IgA. The IgA fraction isolated by our electrophoresis method contained 1,904 mg of IgA, as assayed by the method of Mancini. This represented a recoverable yield of 78%.

This block electrophoresis procedure should be of value for the separation of high yields from large quantities of multiple myeloma sera. It could also be applied for the initial or preparative separation of IgA from other nonmyeloma sera in which the purification of IgA is a particularly difficult procedure and large quantities are involved, although further purification such as diethylaminoethyl column separation may be required.

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