

AN IMPROVED APPLICATOR SYSTEM FOR CELLULOSE ACETATE ELECTROPHORESIS¹

DESMOND H. FOLEY

Department of Entomology, University of Queensland, St Lucia, 4067, Queensland, Australia

ABSTRACT. An improved sample application system for cellulose acetate electrophoresis is described. This system features 30 interchangeable wells allowing sample reordering for side-by-side comparison of closely spaced electromorphs.

Electrophoresis is a powerful tool in studies on the population genetics, phylogeny and systematics of medically important arthropods. New or closely spaced electromorphs can be a problem in such studies since both genetic and nongenetic factors may influence the mobility of proteins in the gel. The ideal situation of exposing all samples to identical electrophoretic conditions is most closely approached by running samples in adjacent slots. Thus, reordering samples for side-by-side comparisons of bands may be necessary (Richardson et al. 1986). This paper reports modifications to a cellulose acetate electrophoresis system designed to improve efficiency and to offer greater convenience for side-by-side comparison of electromorphs with minor mobility differences.

Richardson et al. (1986) in their handbook on electrophoretic methodology describe a method of applying samples to cellulose acetate ("Cellogel," Chemetron, Milan, Italy). Thirty-five or more sample homogenates are drawn into capillary tubes and applied one at a time to a 30 cm gel with a ruler and draftsman's pen. One advantage of this method is that tubes can be reordered for subsequent gels. Unfortunately, loading one gel by draftsman's pen takes 20–40 min (Richardson et al. 1986), which necessitates working in a cold room or in a saturated atmosphere to minimize evaporative drift. Mistakes may also be made in the loading order by misordering the tubes, by applying a sample twice or by missing a sample. A device was needed which could deliver samples more quickly with greater surety in the ordering of samples.

The Helena Laboratories' Titan Zip Zone cellulose acetate electrophoresis system (Helena Laboratories, Beaumont, TX) is especially suited for the study of small arthropods (Easteal and Boussy 1987), including mosquitoes (Kreutzer et al. 1977). The Titan applicator (Super Z-12) uses independently suspended capillary tips which fit into a row of sample wells molded into a loading base. This system has the

advantage of delivering samples simultaneously to cellulose acetate gels but is inflexible in the order of applications and is limited to 12 samples. Commonly, more samples need to be compared on the one gel. Consequently, an extended applicator and sample well plate based on the Titan system but with interchangeable sample wells was built.

Thirty Titan applicator tips were incorporated into the applicator (Fig. 1). This number of tips fitted the largest Titan cellulose acetate gels (203 × 203 mm) while still allowing 8 mm clearance at each end. The gel was cut into 3 equal pieces (67.7 × 203 mm) and run in a Titan gel chamber that was modified by insertion of Perspex® (polymethyl methacrylate) spacer to reduce the bridge distance. The applicator bridge and caddy were constructed from 3 mm Perspex. Two spring loaded plungers were used to ensure the applicator ran smoothly in its tracks.

Thirty movable sample well palettes were constructed from Perspex; 3/16 in. (4.76 mm) thick × 0.246 in. (6.25 mm) wide × 1 5/8 in. (41.28 mm) long. A hole was drilled at each end and a sample well of approximately 35 μl capacity (3 mm wide × 2 mm deep × 5.5 mm long) milled in the center. Palettes were located on a baseboard (1/4 in. (6.35 mm) × 2 1/2 in. (63.50 mm) × 11 in. (279.40 mm)) by steel pins with 0.003–0.004 in. (0.076–0.102 mm) clearance in the holes. Pins may be roll pins but bearing needle rollers of at least 1/16 in. (1.59 mm) × 3/8 in. (9.53 mm) are preferred. The size of pins and their spacing is optional; in the present applicator they were 1.125 in. (28.58 mm) apart. An accuracy of 0.001 in. (0.025 mm) was maintained in the milling of palettes and placing of pins to ensure interchangeability. Matching numbers were engraved on the palettes and baseboard.

Samples can either be homogenized in a grinding block and transferred to the sample wells via pipette or, for small samples, ground directly in the sample wells with a power drill and Teflon® bit. Figure 2 shows 2 gels which were run using this applicator system. Due to the short application time neither an expensive cold room nor a saturated atmosphere were needed and as

¹ The use of trade names does not constitute endorsement of one product to the exclusion of others.

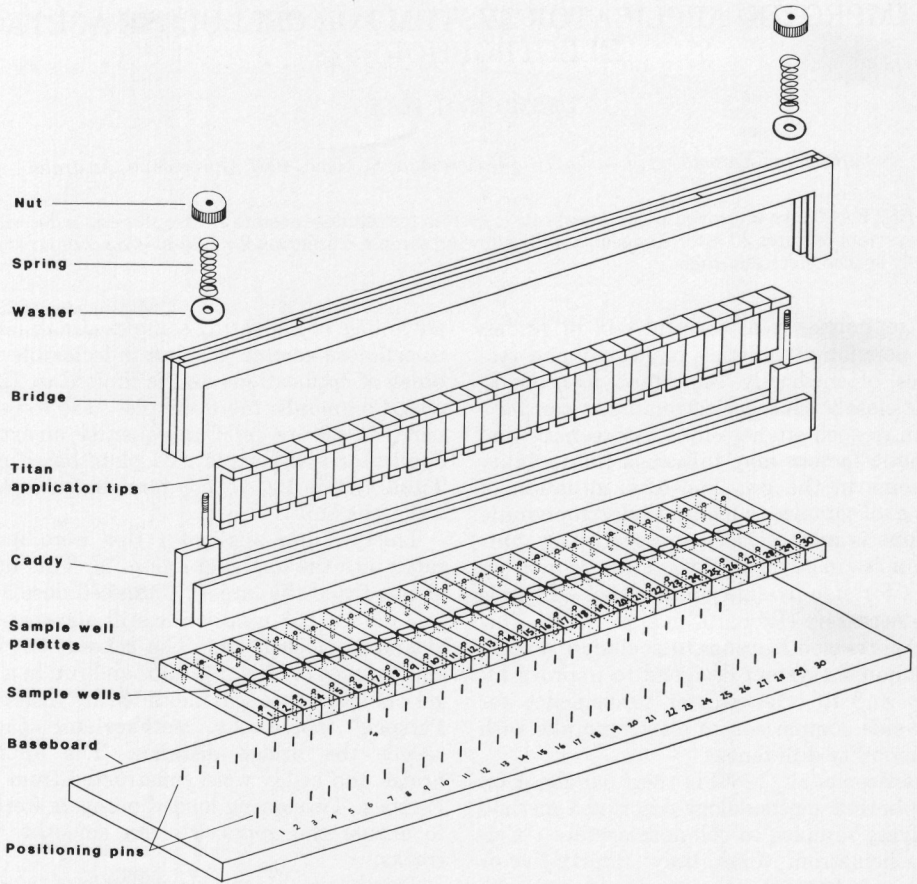


Fig. 1. Exploded view of extended cellulose acetate electrophoresis applicator featuring interchangeable sample well palettes.

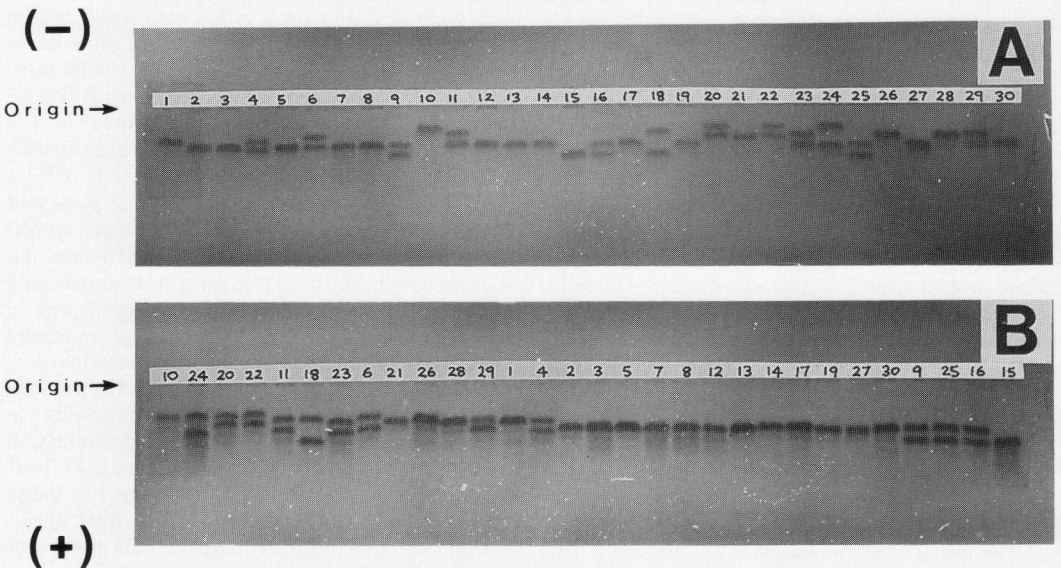


Fig. 2. Two Titan cellulose acetate gels stained for PGM following electrophoresis of *Anopheles annulipes s.l.* A) Unordered gel with samples numbered 1-30. B) Second gel demonstrating sample reordering facility of the improved applicator system.

evaporative drift was minimized, bands were sharper. The first gel is of unordered *Anopheles annulipes* s.l. and was stained for phosphoglucomutase (PGM) according to the methods of Easteal and Boussy (1987). The shorter run time (15–45 min) of the Titan cellulose acetate system compared to other electrophoresis systems meant that excess homogenate in the sample wells could be covered and stored in the refrigerator for later use. The results from the first gel were analyzed and samples reordered for the second gel by simply changing the positions of the palettes. The provision of clearly numbered palettes and simultaneous application minimizes the chance of mistakes occurring in the loading order.

Reordering took less than 3 min and application less than 0.5 min. This device should be suitable for other cellulose acetate support media such as Cellogel. A full 30-cm Cellogel gel would accommodate 45 samples delivered by

Titan tips from an extended version of the applicator. When many gels are to be loaded, this new method is considerably more time efficient than the draftsman's pen method.

I am grateful to Rex Williams of Timeless Engineers (Wooloowin, Qld, Australia) for manufacturing the palettes and baseboard.

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