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TECHNICAL NOTE

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Rapid Phenotyping of Esterase D by Starch Gel Electrophoresis

The intent of this paper is to report a time-saving modification of the system reported by Parkin and Adams [*1*] in conjunction with the developments of Streeter and Lovaas [*2*]. It has resulted in the development of a starch gel electrophoretic technique that can rapidly determine, with clear and reproducible results, the esterase D (EsD) phenotype of both fresh blood and bloodstains within 30 min, as compared with the 2 h required for previously reported starch gel techniques [*1*] and 60 min for bloodstains by cellulose acetate methods [*3*].

Experimental Procedure

The following buffers were used [*1*]: as gel buffer, 6.75 m*M* tris(hydroxymethyl)-aminomethane, 1.8 m*M* citric acid, 2.2 m*M* boric acid, and 0.2 m*M* lithium hydroxide, at pH 7.2, and as bridge buffer, 440 m*M* boric acid and 40 m*M* lithium hydroxide, at pH 7.2.

To support the gel, smaller-than-usual glass plates (8.5 by 11 cm, with a 1-mm-thick frame) were used [*2*]. This plate size accommodates up to eight samples. To facilitate simultaneous analysis of 16 samples, wider plates (8.5 by 15 cm) were used. A 9% (w/v) starch gel was prepared.

Fresh blood lysates were diluted 1:1 with 0.05*M* Cleland's reagent. The samples were absorbed onto 0.5-cm pieces of cotton thread for application to the gel. For bloodstains, whenever possible threads from bloodstained garments were applied directly to the gel after they were soaked in a minimal amount of Cleland's reagent for 10 to 15 min. When the substrate containing the stain could not be directly applied, the samples were extracted onto cotton thread with Cleland's reagent.

Slots 0.5 cm in length were cut into the gel at one third the distance from the cathode end of the plate. After the samples had been inserted, the wick conductors were placed on each end of the plate; approximately 1.5 cm of the gel surface was covered, and the gap between the wicks was 8.0 cm.

Electrophoresis was carried out on a 17- by 22-cm cooling platen for 30 min at a constant voltage of 350 V with an initial current of 12 mA.

Upon completion of the electrophoresis, visualization of the isozyme patterns was accomplished by using 5 mg of 4-methylumbelliferyl acetate dissolved in a drop or two of acetone and then adding 5 ml of a reaction buffer consisting of 0.05*M* sodium acetate

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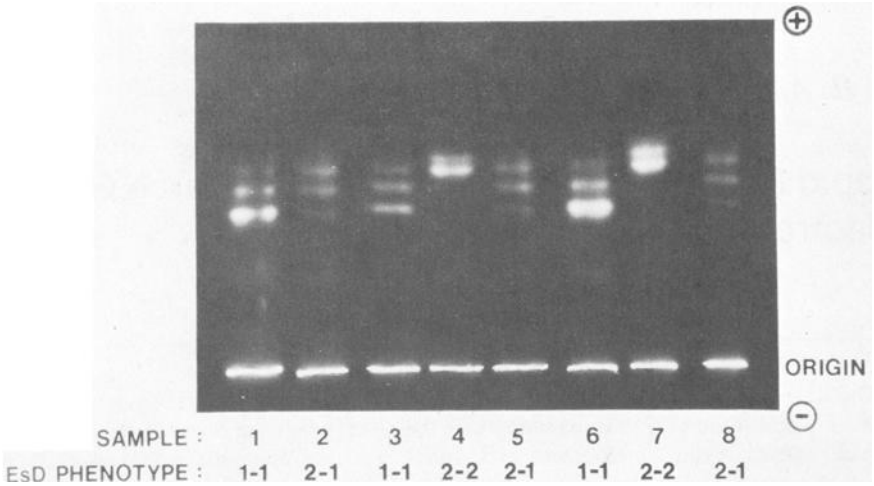

FIG. 1—*The electrophoretogram shows the three common EsD phenotype patterns. All eight samples are of fresh blood.*

at pH 6.5.[2] This solution was soaked into 3-mm Whatman filter paper, which was then placed on the gel area between the origin and anode. The reaction was allowed to progress at room temperature for 5 min, after which the isozyme patterns were observed under fluorescent light from an ultraviolet lamp at 365 nm.

Results and Discussion

Figure 1 shows the starch gel electrophoretogram of fresh blood with the method described. Equivalent results of the same quality are obtained with bloodstains with no additional preparation besides occasional sample extraction. The uniformly compact isozyme bands and the separation between them permit an easy identification of three common EsD phenotypes.

The reported starch gel procedure has the advantage of allowing the simultaneous analysis of up to 16 samples of fresh blood and bloodstains in as little as 30 min, with good reproducibility.

Acknowledgment

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References

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