TECHNICAL NOTE

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Phenotyping of Carbonic Anhydrase II in Fresh Blood and Bloodstains on Cellulose Acetate Membranes

REFERENCE: Andrus, R. H., **"Phenotyping of Carbonic Anhydrase II in Fresh Blood and Bloodstains on Cellulose Acetate Membranes,"** *Journal of Forensic Sciences,* JFSCA, Vol. 26, No. 1, Jan. 1981, pp. 181-183.

ABSTRACT: A simple and rapid procedure is presented for the identification of carbonic anhydrase II in fresh blood and bloodstains using cellulose acetate membranes. Identification of the phenotypes is simplified by the migration of the isozyme bands to one side and away from the origin.

KEYWORDS: pathology and biology, phenotyping, carbonic anhydrase, electrophoresis

A method is described for determining the carbonic anhydrase II (CA-II) polymorphism in fresh blood and bloodstains by electrophoresis. The procedure uses cellulose acetate membranes as the support medium and requires as little as 30 min of electrophoresis.

Identification of phenotypes is easily accomplished since both isozyme bands migrate to one side and away from the origin, an advantage over previously reported procedures [*1,2*].

Experimental Procedure

The electrophoresis was performed on the Sartophor-System using Sartorius cellulose acetate membranes (Sartorius Filters, Inc., Hayward, Calif.).

The tank and membrane buffers were a 1:14 dilution of a stock buffer consisting of 0.9*M* tris(hydroxymethyl)aminomethane, 0.5*M* boric acid, and 0.02*M* ethylenediaminetetraacetic acid at pH 8.6 [*3*].

Preparation of the samples consisted of diluting fresh blood lysates 1:2 with deionized water. Bloodstains and crusts were extracted in a minimal amount of water followed by a chloroform cleanup [*4*]. The prepared samples were then transferred to the sample holder.

A cellulose acetate membrane was wetted with the diluted stock buffer, blotted between clean blotter papers, and placed on the membrane bridge. The bridge and membrane

Received for publication 4 April 1980; revised manuscript received 25 June 1980; accepted for publication 27 June 1980.

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were then placed into the tank. The samples were applied to the membrane with an automatic ten-sample applicator and placed at sample position Number 8 on the tank cover. This places the samples approximately one third of the distance (25 mm) from the precut index holes on the anode side of the membrane. One or two applications, delivering approximately 0.25 μL per application, is sufficient for fresh blood samples. Three to five applications of the stain extracts were found adequate for most stains.

Electrophoresis was carried out at 300 V (1.0 to 1.6 mA) for 30 min at room temperature.

The developing gel was prepared by boiling 0.1 g of agarose in 9 mL of reaction buffer consisting of 0.1*M* phosphate at pH 6.5 [*3*]. Approximately 4 mg of fluorescein diacetate [*1*] was dissolved in a drop or two of acetone and this solution was added to 1 mL of the reaction buffer. The two mixtures were combined, stirred, poured into a square plastic petri dish, and then covered.

Upon completion of electrophoresis, the cellulose acetate membrane was cut at each end just outside the rows of index holes and placed facedown on the developing gel; care was taken to exclude all air bubbles.

The membrane was incubated at 37°C for 10 min or until the isozyme bands were clearly developed. Viewing a membrane under long-wave ultraviolet light revealed bright yellow fluorescent bands at the area of CA-II activity.

The developed membrane may be preserved for future review by removing it from the developing gel and air drying it.

Results

Figure 1 shows a cellulose acetate electrophoretogram of the results obtained by the described method.

The samples in Positions 3 and 8 are stains of Type 2 and 1, respectively. All other specimens are fresh blood lysates.

The Type 2 bloodstain depicted in Fig. 1 was 21 weeks old at the time of analysis. It was prepared in the laboratory and stored at room temperature until analyzed.

The zone of CA-I 1 activity does not pose a problem to the identification of the CA-II

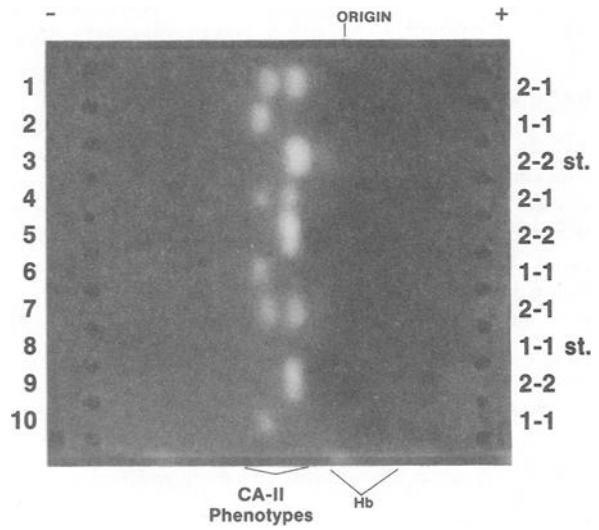

FIG. 1—*The electrophoretogram shows the three common CA-II phenotype patterns. Samples in Positions 3 and 8 are stain extracts, and all others are fresh blood lysates.*

phenotypes. The CA-I 1 isozyme migrates anodal to the CA-II Type 2 band and is only faintly apparent after prolonged development, well beyond the necessary 10 to 15 min required for adequate CA-II visualization.

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