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TECHNICAL NOTE

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Simultaneous Electrophoresis of Peptidase A, Phosphoglucomutase, and Adenylate Kinase

Peptidase A variant determinations may potentially be valuable tools for determining the racial origin of bloodstains and seminal stains. The Caucasian population is reported as predominantly the Type 1 polymorph (99.8%); however, variances are recorded within the Negroid population of Type 1 (85.2%), Type 2–1 (13.1%), Type 2 (1.5%), and Type 3–1 (0.2%) [*1,2*]. Table 1 contains the percentages of the phenotypes of peptidase A observed in a tested Negroid population.

Forensic serologists have routinely determined phosphoglucomutase (PGM) types in "case" blood because of the advantageous population frequencies. Although the adenylate kinase (AK) population frequencies normally would not be significant to warrant independent electrophoretic analysis, Marone's[4] modification of Culliford's [*3*] combined PGM and AK analysis permits the simultaneous determination of both. By combining and adapting the electrophoretic methods of Lewis and Harris [*1*] for peptidase A and Marone for PGM and AK, a single plate can be developed for all three isoenzyme types.

Experimental

Preparation of Buffers and Gels

The bridge buffer is a 0.1*M* tris(hydroxymethyl)aminomethane(tris base), 0.1*M* maleic acid, and 0.01*M* magnesium chloride brought to a pH of 7.4 by using sodium hydroxide (prepared with deionized distilled water). A 1:10 dilution of the tank buffer is used for preparing a 1-mm 10% starch gel.

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TABLE 1—*Percentages of phenotypes of peptidase A observed in a Negroid population of 157 persons.*

Phenotype	Number	Percentage
1–1	139	88.5
2–1	13	8.3
2–2	4	2.5
3–1	1	0.6

Preparation of Stains and Electrophoretic Conditions

Dried Bloodstains—Threads were cut approximately 9 by 1 mm and moistened for 2 to 3 min in gel buffer. The threads were inserted in the gel near the cathode end and electrophoresis was carried out on cooling plates (Shandon-Kohn Mark II electrophoretic chamber SAE-3230) for approximately 20 h at 6 V/cm.

Seminal Stains—Peptidase A, like PGM [3], can be demonstrated in seminal stains. Preparation of the dried stain is the same as described for bloodstains.

Development

Peptidase A—The procedure developed by Lewis and Harris [1] was used. The overlay mixture was composed of 20 ml 0.2*M* Na_2HPO_4, pH 7.4, 0.5 ml 0.1*M* $MnCl_2$, 0.2 ml dianisidine dihydrochloride (500 mg in 70 ml H_2O), 2.5 mg amino acid oxidase, 5 mg peroxidase, and 10 mg L-valyl-L-leucine.

The agar, 0.3 g Ionagar® in 15 ml deionized distilled water, was prepared in a separate flask, heated to a boil, cooled to 56°C, combined with the above solution, and poured over the surface of the gel.

Phosphoglucomutase—Marone's modification of Culliford's [3] system was employed for the PGM overlay system. This consists of 87.5 mg glucose-1-phosphate containing 1% glucose-1:6-diphosphate, 50.0 mg magnesium chloride, 7.5 mg nicotinamide-adenine dinucleotide phosphate (NADP), 2.5 mg 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT tetrazolium), 2.5 mg phenazine methosulfate (PMS), 0.4 to 1.0 unit glucose-6-phosphate dehydrogenase, and 20 ml of PGM reaction buffer [0.03*M* tris, 6 m*M* ethylenediaminetetraacetic acid (EDTA), pH 8.0, prepared using deionized distilled water].

An agar mixture composed of 0.3 g Ionogar® in 15 ml of deionized distilled water was prepared, heated to a boil, cooled to 56°C, combined with the above PGM reaction solution, and poured.

Adenylate Kinase—Marone's modification of Culliford's system [3] was used to develop AK. This system is composed of 5.0 mg adenosine diphosphate, 18.0 mg glucose, 40.0 mg magnesium chloride, 7.0 mg NADP, 1.0 mg MTT tetrazolium, 1.0 mg PMS, 0.8 units hexokinase and glucose-6-phosphate dehydrogenase (Sigma No. H-8629), and 20 ml AK reaction buffer (0.1*M* tris, pH 8.0, prepared using deionized distilled water). An agar mixture composed of 0.3 g Ionogar® in 15 ml deionized distilled water was prepared, combined with the AK reaction solution, and poured.

Results and Discussion

Upon completion of the electrophoretic run, the plate was divided into three areas by placing glass strips on end into the starch (Fig. 1). These figures illustrate the results obtained with the tri-enzyme system, which are diagrammed in Fig. 2. Figure 2 demonstrates the position of the overlay, which consists of 3.0 cm (1.5 cm on each side of the origin for AK development), 5.0 cm above this for PGM development, and 5.0 cm atop the PGM area for peptidase A development. The overlays were poured and the

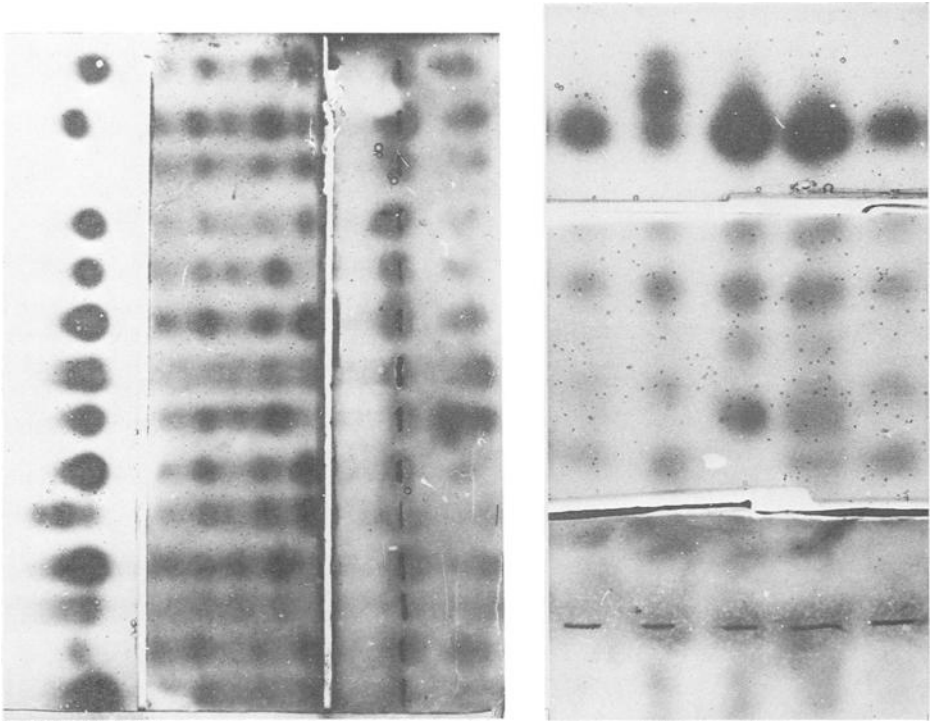

FIG. 1—*Photographs of a starch gel stained for the tri-enzyme system AK, PGM, and peptidase A.*

plate incubated at 37°C. The AK and peptidase A isoenzymes were visible within 1 h, whereas the PGM bands took from 1½ to 2 h to develop.

The EDTA present in Marone's and Culliford's PGM-AK tank and gel buffers was eliminated because it causes inhibition of the peptidase A activity. When EDTA is present, peptidase A takes up to 12 h to develop. To compensate for the removal of the EDTA, deionized distilled water was used to avoid PGM cation inhibitions and EDTA was incorporated into the PGM reaction buffer.

There are several precautions which should be stated when doing multiple enzyme systems. First, there are the considerations of pH and ionic strength which are not necessarily optimal for each enzyme studied. Second, in any multiple enzyme system, the rarer variant banding pattern of any one of the enzymes that overlaps into the developmental region of a second enzyme will not be visualized. Thus, multiple enzyme systems might best be used as a rapid screening test to observe basic enzyme variants.

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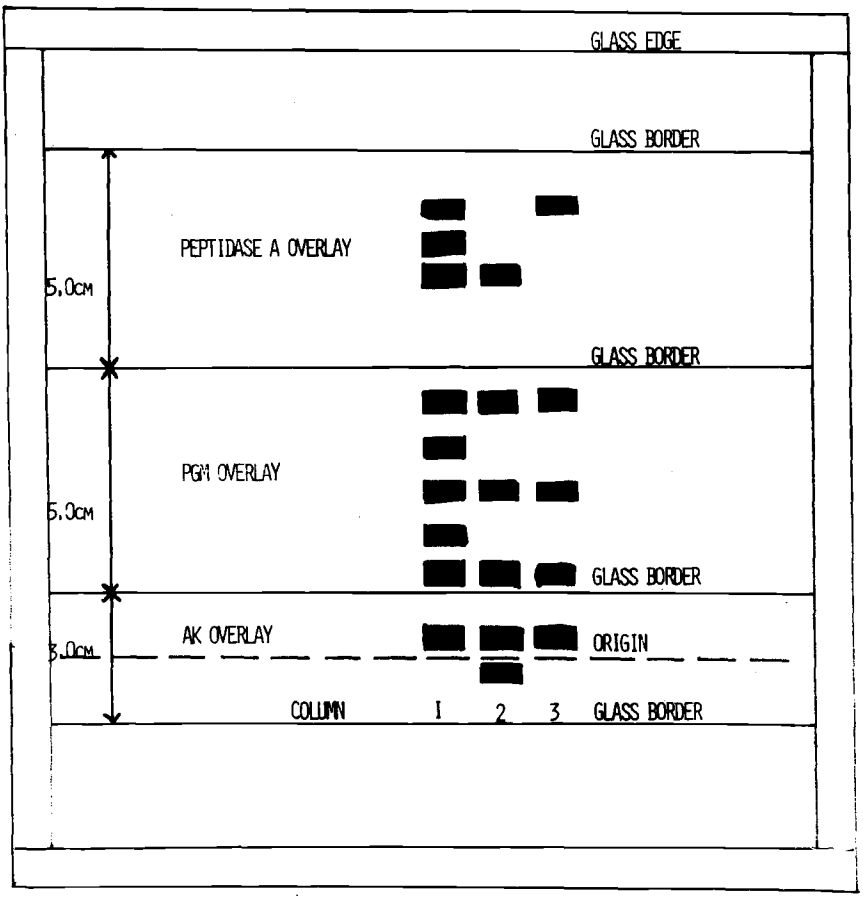

FIG. 2—*Diagram of the positioning of the triple overlays and approximate placement of the isoenzyme bands. Column 1 represents AK 1-1, PGM 2-1, and peptidase A 2-1; Column 2 represents AK 2-1, PGM 1-1, and peptidase A 1-1; and Column 3 represents AK 1-1, PGM 1-1, and peptidase A 2-2.*

References

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