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TECHNICAL NOTE

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Problems of Reliability in the Phenotyping of Erythrocyte Acid Phosphatase in Bloodstains

Erythrocyte acid phosphatase (EAP) phenotypes have been shown to have a good frequency distribution [*1*] and EAP is therefore a useful discriminator in forensic investigations. The five common phenotypes, namely AA, BA, BB, CA, and CB, can now be readily and accurately determined with fresh blood [*2*]. Bloodstains aged for 30 days on white cotton cloth under laboratory conditions were also accurately typed [*2*].

However, inherent characteristics of the EAP system give rise to the possibility of very serious errors in phenotyping on other than fresh blood, especially if the history of the sample is not fully known. McWright et al [*3*] have reported mistyping instances in aged specimens and have suggested that EAP bands may be affected by neuraminidase produced by putrefying bacteria.

Wraxall and Emes [*4*] report no mistyping on 387 bloodstains aged from two days to six weeks. Twenty-five of these stains were indeterminate because of weak patterns. Apparently the bloodstains they used were prepared under laboratory conditions and the history of drying and preservation were well known. They caution against allowing overheating during electrophoresis. However, blood or bloodstains collected for forensic investigation may already have been subjected to excessive heat and, therefore, may have undergone irreversible changes prior to submission to the laboratory.

The experimentation described below was conducted to test the reliability and suggest the limitations of EAP phenotyping in the forensic laboratory. This study was not concerned with degradation of the blood samples to the point of total loss of activity; rather, it dealt with partial degradation to observe changes in the isoenzyme band patterns.

Experiment

Blood samples of known EAP phenotypes, CB, BA, BB, and CA, were drawn by venipuncture from employees of this crime laboratory.[2] These individuals had previously been phenotyped for EAP on both cellulose acetate membranes and starch gel by several independent laboratories.

Stains were prepared by applying the fresh whole blood to clean cotton cloth and air-

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drying at ambient conditions. Standard hemolysates were prepared by using a portion of each blood sample.

A portion of each liquid blood sample was maintained at 37°C up to 48 h. After heat treatment, the liquid samples were frozen for storage to preserve the samples and prevent further degradation until electrophoresis. The blood samples were prepared in this manner to simulate the adverse conditions which may take place in actual casework submitted to the crime laboratory. It is not uncommon to have dead bodies and bloodstained clothing or objects subjected to heat and humidity for several days prior to discovery and collection. Air temperatures are frequently near or above body temperature (37°C) and can be considerably higher in desert or tropical climates, in closed rooms, closed automobiles, or in direct sunlight.

The reducing agent, Cleland's reagent (dithiothreitol), was applied to all samples prior to electrophoresis. Theoretically, Cleland's reagent reconstitutes the sulfhydryl bonds, thus restoring the integral native enzyme molecule. Actually, there is no way of knowing whether this is a partial or complete restoration [5].

Experimental samples were phenotyped with great care by using recently improved methods for cellulose acetate membrane [2] and starch gel.[3] The results were read independently by four individuals having extensive experience with both methods of EAP phenotyping, in research and casework situations. The readers had no knowledge of the phenotypes, the age of the samples, or the way in which they were prepared prior to analysis. Their readings are given in Table 1.

TABLE 1—*Results of phenotyping of experimental samples.*[a]

Samples	Starch Gel Readers				Cellulose Acetate Readers			
	1	2	3	4	1	2	3	4
1. BA, standard hemolysate	BA	BA	BA	BA	BA	BA	BA	BA
2. BA, stain	BA	BA	BA	BA	inc	BA	BA	BA
3. BA, liquid, heat degraded	BA	CA	CA	CA	inc	CB?	B?	inc (CB?)
4. B, stain	B	B	B	B	B	B	B	B
5. B, liquid, heat degraded	CB	CB	CB	CB	CB	CB	CB	inc (CB?)
6. CB, stain	CB	B	B	B	inc	CB?	B?	inc (CB?)
7. CB, liquid, heat degraded	CB	CB	CB	CB	CB	CB	CB	CB
8. CA, stain	...	...	...	...	CA	CA	CA	CA
9. CA, liquid, heat degraded	...	...	...	...	CA?	CA	CA	inc (CA?)
10. CA, stain	...	...	...	...	CA	CA	CA	CA
11. CA, liquid, heat degraded	...	...	...	...	CA	CA	CA	CA?
12. A, standard hemolysate	...	...	...	...	A	A	A	A

[a] Notes:

inc = inconclusive result.

? = indicated inconclusive, band patterns questionable but similar to the phenotype named.

BA and CB stains and liquids were 19 days old when electrophoresed (Samples 2, 3, 6, 7); B stain and liquid were 5 days old when electrophoresed (Samples 4 and 5); CA stains and liquids were 26 days old when electrophoresed (Samples 8, 9, 10, 11).

The CA and A samples were run on cellulose acetate and not starch because of additional positions available on the membrane during the set of runs (Samples 8 through 12).

The stains and liquid samples were chosen at random from those prepared and run as though they were forensic casework.

[3]The starch gel procedure is essentially that described by Wraxall and Emes [4] with minor modifications and improvements developed at the University of California's White Mountain Research Station.

Results and Discussion

Although only a limited number of samples were used in this initial experiment, the results shown in Table 1 clearly indicate that there can be a definite problem with the EAP phenotyping no matter which electrophoretic supporting medium is used.

Unlike other enzyme systems, EAP phenotyping depends not only on a pattern of relative distribution of bands but also on the relative intensities of the bands [*6*]. When blood is aged, the individual isoenzymes tend to degrade at different rates [*7*], further exacerbating the difficulties of true phenotype identification.

The problem with misidentification of the samples in this study was not due to weak or indistinct band patterns. Rather, discrete bands were present and readable, but they had been altered to indicate erroneous phenotypes. For example, in Specimen 3 on the starch gel electrophoretogram, the BA sample showed three bands of equal intensities in the positions *c*, *a'*, and *b*, instead of the normal BA pattern with the more intense *b* band and presence of the *a* band. Consequently, three of the readers called this sample CA. This same sample run on cellulose acetate membrane showed a band pattern sufficiently questionable that all readers gave an inconclusive result.

In the case of the BB phenotype, Specimen 5, the resulting pattern was two bands of equal intensities in the *c* and *b* positions, rather than the true BB pattern with a much more intense *b* band.

Other errors and inconclusive readings of a similar nature are also indicated in Table 1. Certainly such determinations based on partial or altered patterns are questionable and unreliable and could, and should, be challenged in a court of law.

Summary

Erythrocyte acid phosphatase is a useful system for the crime laboratory for both fresh and degraded blood and bloodstains, provided the inherent problems of phenotyping this particular enzyme system are recognized. Because of the great number of variables affecting this enzyme system in vitro, phenotyping should not be attempted unless the complete history of origin and handling of the sample is known.

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