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Population Frequencies of Forensically Important Genetic Markers: Phosphoglucomutase, Erythrocyte Acid Phosphatase, and Haptoglobin

Of the more than 500 antigenic systems theoretically available, relatively few (ABO, MN, and Rh) are routinely identified by criminalists. As a result of extensive blood bank typing throughout this country, comprehensive local population frequencies are available for the ABO and Rh systems.

In addition to these antigenic systems, there are numerous polymorphic protein systems (such as isoenzymes, albumin, group specific component, transferrin, hemoglobins, and haptoglobins) [*1*] that could be used, but only the isoenzymes phosphoglucomutase (PGM) [*2*], adenylate kinase (AK) [*2*], erythrocyte acid phosphatase (EAP) [*3*], esterase D [*4*], carbonic anhydrase,[5] and peptidase A [*5*] are being identified. Of the other available protein systems only hemoglobin (Hb), haptoglobin (Hp) [*2, 6, 7*], and Gm [*8*] are identified.

Presently, for all the basic systems that have been used forensically to individualize and compare blood samples, there is a paucity of statistical information pertinent to the United States for PGM, EAP, and Hp. The data being used are those primarily derived from the United Kingdom. It is imperative that these data be generated to establish or confirm the constancy from region to region. Also, the forensic scientist must be able to present to the court the distinctiveness for each of the system variants. The courts are especially interested in examining population values for the sector in which the crime occurred.

That these frequencies do change from one population to another has been illustrated in the Chinese and Korean populations for the immunoglobin allotypes [*9*] and in the German population for PGM [*10*].

Experimental Procedure

Phosphoglucomutase

Samples were obtained from either the central blood bank or the county jail, Pittsburgh,

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Pa. Each sample was classified as to age, sex, race, and date of collection. Several drops of each sample were placed onto cotton sheeting and air-dried.

For enzyme analysis the bloodstain samples were cut into 1- by 5-mm sections, placed in a porcelain spot plate, and moistened for 10 min with PGM gel buffer (1:10 diluted PGM tank buffer). The moistened threads were inserted into a 1-mm by 20-cm by 20-cm 10% starch gel. The gel preparation [*3*] consisted of an appropriate weight of starch mixed with a desired volume of gel buffer and heated to a boil. The gel buffer was a 1:10 dilution of PGM tank buffer containing tris(hydroxymethyl)aminomethane (Tris) (12.11 g/litre), maleic acid (11.62 g/litre), and magnesium chloride (2.03 g/litre), pH 7.4. The gel was degassed and poured quickly after it was boiled.

Approximately 500 ml of tank buffer was required for each plate in the electrophoresis tank, and the buffer was found to maintain integrity through two separate analyses.

Electrophoresis was performed for 22 to 24 h at 138 V in Kohn tanks with cooling platens at 4°C.

The position of the isoenzymes was visualized with an agar overlay. Overlay chemicals should be removed from storage 1 h before use to avoid condensation and subsequent deterioration.

The overlay reaction mixture was prepared as follows. Reaction buffer, 25 ml (3.64 g Tris/500 ml, pH = 8.0), was added to glucose-1 phosphate (Wessex Biochemicals) (87.5 mg), magnesium chloride (50 mg), nicotinamide-adenine dinucleotide phosphate (NADP) (7.5 mg), 3-(4,5-dimethyl thiazolyl-2)-2,5 (MTT) (2.5 mg), phenazine methosulfate (1.0 mg), and glucose-6-phosphate dehydrogenase (1.4 to 1.0 units). Agarose (0.5 g) was dissolved in 25 ml water by heating to 90°C. The heated solution was cooled to 65°C and immediately and rapidly mixed with the reaction mixture and then poured onto the starch gel; care was taken to avoid air bubbles. The plate was incubated at 37°C for approximately 2 h.

Erythrocyte Acid Phosphatase

The preparation of samples for electrophoresis was the same as for PGM except that EAP gel buffer containing Cleland's reagent was used to moisten the inserts and 2- by 5-mm inserts were put into a 2-mm thick, 10% starch gel. The gel buffer was a 1:100 dilution of the tank buffer, which was composed of sodium dihydrogen phosphate (33.8 g/litre) and trisodium citrate (32 g/litre), pH 5.9.

Visualization of the isoenzyme was accomplished by pouring 10 ml of reaction buffer containing 4 mg of 4-methylumbelliferyl phosphate (Sigma) in 0.05*M* citrate buffer, pH 5.0, onto precut filter paper. The moistened filter paper was placed onto the starch gel and the plate incubated for 2 h at 37°C. The isoenzymes appeared as fluorescent bands on a dark background under long-wave ultraviolet light.

Haptoglobin

For population screening purposes, serum samples were used to eliminate the need for a gradient gel system, which has been found to be necessary for use with dried stains [*2*].

The sera were obtained from those samples used for the preparation of the stains mentioned under the PGM section of this report. The sera were separated from the red cells and four drops placed into disposable glass tubes (10 by 75 mm). To each tube was added one drop of a red cell hemolysate. This hemolysate was prepared by incubating red cells and toluene (3:1) for 30 min, followed by centrifugation. The red, lower layer was removed for use with the serum. Hemolysates were also prepared by freezing and thawing of packed cells (×3). One drop of hemolysate and three drops of serum constituted the serum hemolysate solution.

Filter paper wicks (1 by 3 mm) were soaked in the serum hemolysate solution and inserted into slots which had been cut in a 1-mm thick, 5% ammonium persulfate polymerized acrylamide gel (Cyanogum). The gel buffer was composed of Tris (9.2 g/litre) and citric acid (1.05 g/litre), pH 8.6.

The visualization of the haptoglobin banding patterns was accomplished as follows. A 67% acetic acid solution (7.5 ml) containing 0.24 g sodium perborate and either 0.075 g malachite green, 0.1 g benzidine, or 0.1 g tetramethylbenzidine was poured onto precut filter paper. The paper was laid onto the gel surface at room temperature until staining began (approximately 10 min) and then removed. This procedure insured that discernible, not too heavily stained bands were developed. Of the three stains, benzidine proved to be the most sensitive.

Results and Discussion

The statistical results of this study are presented in Tables 1, 2, and 3 for PGM, EAP, and Hp, respectively. These results, plus the experimental section for each of these systems, provide a practical approach to the determination of genetic markers for forensic serologists. These data present the forensic serologist with a reliable method for identifying the type for each of these polymorphic systems and can be used for calculating the distinctiveness of a particular blood specimen in the population sampled.

The samples for this study were obtained from both the Pittsburgh Central Blood Bank and the Allegheny County Jail. The race, age, and sex of the donor and the drawing date were recorded for each sample. Care was taken to insure that no duplicate samples were taken and that only samples representative of the population were obtained. The jail samples included a significant number of black persons in the study. Samples were taken from all persons admitted to the jail whether they were released or held.

Table 1 shows the results obtained for the PGM system. It should be noted that the values for the black and white populations are not the same. This discrepancy must be taken into consideration by the forensic serologist. For example, a stain may be analyzed as being PGM 1-1, but the percentage of this phenotype in the population will differ depending on the racial origin of the stain. If the origin of the stain is not known, the statistics for the general population of that area could be used. These data must be accurately portrayed. However, if it is known that the stain originates from a black person, the value for the black population, that is, 67.4%, must be used.

Table 2 shows the statistics for the EAP system. Again, it must be noted that there is a difference between the black and white population frequencies and that the same precautions must be taken in calculating the distinctiveness of a particular sample. With this system the identification of the different isoenzyme types is dependent on the different intensity of certain bands in the zymogram. The interpretation of these bands can become very difficult for certain of the banding patterns, especially for B, CB, and C. There are no problems in the identification of BA, A, or CA, but it is possible that mistyping could occur for the C, CB, or B isoenzymes [*4*]. Considerable care in the preparation of samples and considerable experience are necessary before an attempt can be made to utilize this system in case work.

Table 3 shows the statistics compiled for the Hp system. Again, as with the PGM and EAP systems, there are differences in the values for the black and white populations. One facet of Hp analysis which must be of concern to the forensic serologist is the possibility of an Hp0 result (no haptoglobin present in the individual and therefore none in the sample). Should this situation occur it must be interpreted with caution since the inability to demonstrate haptoglobins by electrophoretic technique does not necessarily indicate the presence of the *Hp0* genotype but may instead represent degradation of haptoglobins in that particular sample. If it is known that the victim's blood is of the Hp0 type, then the

TABLE 1—*Results for PGM.*

	Phenotypes[a]			Gene Frequency			
	1-1	2-1	2-2	PGM_1	PGM_2	χ^2	Probability
			For 1253 Whites				
Observed number	698	487	67	0.751	0.248	...	...
Phenotypic frequency	0.577	0.389	0.053	...	...	...	...
Expected proportion	0.564	0.373	0.062	...	...	...	...
Expected number	706.7	466.4	76.9	...	...	...	...
$(O - E)^2/E$	0.107	0.910	1.285	...	...	2.302	0.13
			For 714 Blacks				
Observed number	481	209	24	0.821	0.181	...	...
Phenotypic frequency	0.674	0.293	0.034	...	...	...	...
Expected proportion	0.674	0.297	0.033	...	...	...	...
Expected number	480.7	211.5	23.3	...	...	...	...
$(O - E)^2/E$	0.0002	0.0295	0.0210	...	...	0.0507	0.87

[a]One sample showed the 6-2 phenotype.

TABLE 2—*Results for EAP.*

	Phenotypes							Gene Frequency				
	A	BA	B	CA	CB	C	RA	p_A	p_B	p_C	χ^2	Probability
					For 1239 Whites							
Observed number	145	526	491	20	57	...	...	...	...	...	...	...
Phenotypic frequency	0.117	0.425	0.396	0.016	0.046	...	...	0.337	0.632	0.031	...	...
Expected proportion	0.114	0.426	0.399	0.021	0.039	0.001	...	...	...	...	...	...
Expected number	140.7	527.8	494.9	25.9	48.5	1.2	...	...	...	...	...	...
$(O - E)^2/E$	0.131	0.006	0.031	1.34	1.490	1.2	...	...	...	...	4.198	0.38
					For 718 Blacks							
Observed number	39	239	426	2	11	0	1	...	...	...	...	...
Phenotypic frequency	0.054	0.333	0.593	0.003	0.015	0	0.001	0.222	0.767	0.009	...	...
Expected proportion	0.049	0.341	0.588	0.004	0.014	0.0001	...	...	...	...	...	...
Expected number	35.4	244.5	422.4	2.9	9.9	0.1	...	...	...	...	...	...
$(O - E)^2/E$	0.366	0.124	0.031	0.279	0.122	0.1	...	...	...	...	1.022	0.91

TABLE 3—*Results for Hp.*

	Phenotypes					Gene Frequency			
	1-1	2-1	2-1M	2-2	0	Hp_1	Hp_2	χ^2	Probability
				For 1263 Whites					
Observed number	185	555	26	495	2	0.38	0.62	...	...
Phenotypic frequency	0.146	0.460	...	0.392	...	...	...	...	...
(2-1) + (2-1M)	...	581	...	...	...	...	...	...	...
Expected proportion	0.144	0.471	...	0.394	...	...	...	...	...
Expected number	182.4	595.1	...	485.5	...	...	...	..	...
$(O - E)^2/E$	0.038	0.335	...	0.186	...	...	...	0.559	0.46
				For 721 Blacks					
Observed number	206	293	66	148	8	0.535	0.454	...	...
Phenotypic frequency	0.286	0.498	...	0.205	...	...	...	...	...
(2-1) + (2-1M)	...	359	...	...	...	...	...	...	...
Expected proportion	0.286	0.486	...	0.206	...	...	...	...	...
Expected number	206.4	350.2	...	148.6	...	...	...	...	...
$(O - E)^2/E$	0.001	0.221	...	0.002	...	...	...	0.224	0.65

corresponding stain would not be expected to give a discernible banding pattern. The same caution in interpreting Hp 2-1M types must be exercised. This is true since the identification of this Hp type is accomplished by recognizing that certain 2-1 bands are not present. It would be difficult to determine whether their absence is genetic or results from degradation. Also, the more concentrated bands in a degrading Hp 2-1 type may be the only ones visualized by a particular staining procedure, but the sample may appear as a Hp 2-1M, thus changing drastically the population frequency. One should not positively identify 2-1M or Hp0 phenotypes in stains. Where possible, serum samples should be run for comparison. If a serum sample of the victim or suspect is available, it should be used for a type comparison. The determination of the Hp type of the stain should then be compared to this to determine the viability of the haptoglobins in that stain.

The data obtained during this study were from the western Pennsylvania region of the country and, until data are available to support it, they should not be extrapolated to other local regions.

Proficiency testing results have shown that errors are being made during the phenotyping of isoenzymes. It is difficult to determine the cause of the errors a priori. Any that do occur because of misinterpretation of zymograms can be minimized by having more than one member of the laboratory record the results on a blind basis.

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