TECHNICAL NOTE

Bruce Budowle,[1] *Ph.D.*

An Agarose Gel Electrophoretic Method for Typing Phosphoglucomutase-1, Esterase D, or Glyoxalase I

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ABSTRACT: A conventional agarose gel electrophoretic method was described for typing phosphoglucomutase-1, esterase D, or glyoxalase I as single systems. Bloodstain extracts were absorbed into 1-mm-thick agarose gels via an application mask. The electrode wick distance was 12 cm and electrophoresis was carried out at 400 V at 6°C. The electrophoretic run times were 30 min for glyoxalase and 1 h for esterase D or phosphoglucomutase. This method is reliable and produces highly resolved band patterns. Additionally, the shorter separation times as a result of the increased voltage gradient permitted typing of more samples in a given time period compared with presently used methods. This technique requires little technical expertise and can be incorporated into the laboratory at a minimal cost.

KEYWORDS: forensic science, genetic typing, electrophoresis, phosphoglucomutase, esterase D, glyoxalase I, bloodstains, agarose gel electrophoresis, application mask

The genetic markers phosphoglucomutase-1 (PGM), glyoxalase I (GLO), and esterase D (EsD) are useful markers for the individualization of human bloodstains [*1-5*]. A commonly used and reliable method for simultaneously typing PGM, GLO, and EsD in human bloodstains is known as the Group I system [*6*]. When using the Group I system, this investigator has observed that the banding patterns for these markers can be diffused or smeared, particularly the GLO phenotypes. At times, band patterns of the various markers were not always linear across the gel. Often the EsD phenotypes were not detected unless freshly prepared bloodstains were analyzed. These problems lead to inconclusive results. While this author has extreme confidence in results obtained using the Group I system, there is always a need for alternative techniques that can improve resolution and detection of genetic markers. Therefore, this paper presents an agarose gel electrophoretic method which can be used to type either PGM, GLO, or EsD as single systems to overcome these problems.

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[1]Research chemist, Forensic Science Research Group, Laboratory Division, FBI Academy, Quantico, VA.

Materials and Methods

Blood samples were obtained by finger prick from donors at the FBI Academy. Bloodstains were prepared on washed cotton cloth, air-dried, and stored at −20°C until analyzed. Cuttings (2 by 5 mm) of the bloodstains were extracted for 30 min in 20 μL of 0.05*M* dithiothreitol for EsD and GLO and water for PGM.

The tank buffer was composed of 0.10*M* Tris, 0.10*M* maleic acid, 0.01*M* ethylenediaminetetraacetate (EDTA) (free base), and 0.01*M* $MgCl_2 \cdot 6H_20$, pH 7.4 [*6*]. The gel buffer (pH 7.4) was a 1 : 14 dilution of the tank buffer. A 1% agarose gel (Sigma Type V) (14 by 18 by 0.1 cm) was cast on a glass plate. The sample origin was 1.0 cm from the cathodal wick for either EsD or GLO. There can be two sample origin points for PGM at 1.0 cm and 6.0 cm from the cathodal wick. Ten microlitres of the extracts were then placed on an LKB application mask and allowed to absorb into the gel for ten minutes. Excess extract which had not absorbed into the gel after this period was removed by blotting with Whatmann No. 1 paper before removal of the application mask. Three layers of Whatmann No. 3 paper were used to connect the annodal and cathodal buffers to the gel. The distance between the anodal and cathodal wicks was 12 cm. Electrophoresis was performed at 400 V (approximately 50 mA) for 30 min at 6°C for GLO typing and 1 h at 6°C for either PGM or EsD typing.

After electrophoresis, the PGM, EsD, and GLO phenotypes were developed according to the methods of Sutton and Burgess [*4*], Hopkinson et al [*7*], and Kompf et al [*8*], respectively. When typing for PGM, the entire gel was overlayed with the enzyme development solution. The EsD overlay was placed at 2.5 to 9.5 cm from the cathodal wick. The GLO overlay was placed from 2.5 to 9.5 cm from the cathodal wick.

Results and Discussion

Figures 1, 2, and 3 show that the common phenotypes of PGM, EsD, and GLO, respectively, can be clearly resolved and are linear across the gel using the method described in this paper thus reducing the chances of obtaining inconclusive results. This method is one of the most rapid electrophoretic separations of GLO, taking only 30 min, and only 1 h for PGM or EsD. This single system technique that can be used for typing PGM, EsD, or GLO has given reproducible results on at least 50 separate gels for each marker.

The increase in resolution and the shorter electrophoretic separation time for the PGM, EsD, and GLO variants compared with those obtained by the Group I system were a result of the differences in the physical parameters between the two systems. A reduction of the distance between the electrode wicks from 18 to 12 cm increased the voltage gradient (33.3 V/cm compared with 16.7 V/cm for the Group I system). The higher voltage gradient permitted faster migration of the proteins which resulted in more rapid electrophoretic separations, reduced diffusion, and greater resolution of the PGM, GLO, and EsD bands than is possible using the multisystem method.

Additionally, the 1-mm-thick gel has a greater surface-area-to-volume ratio than thicker gels and, thus, can more effectively dissipate heat [*9*]. With this greater efficiency of heat dissipation, there is less opportunity for deleterious thermal effects upon the proteins or the gel or both. Furthermore, when the same amount of protein is applied to both a thin gel and a thicker gel, the thin gel will contain more protein per unit gel volume. Thus a thinner gel also has the effect of presenting greater amounts of protein to the assay.

The commonly used method for sample application when typing PGM, EsD, or GLO in blood is to place cuttings of the stained material directly in the gel. This permits increased sample loading but reduces the resolution of the protein bands. Proteins do not evenly elute from a piece of cloth during electrophoresis which can lead to broad or distorted bands or both. This can be seen by observing hemoglobin as it migrates from the sample origin. Absorbing bloodstain extracts into the gel using an applicator mask reduced this problem. The proteins en-

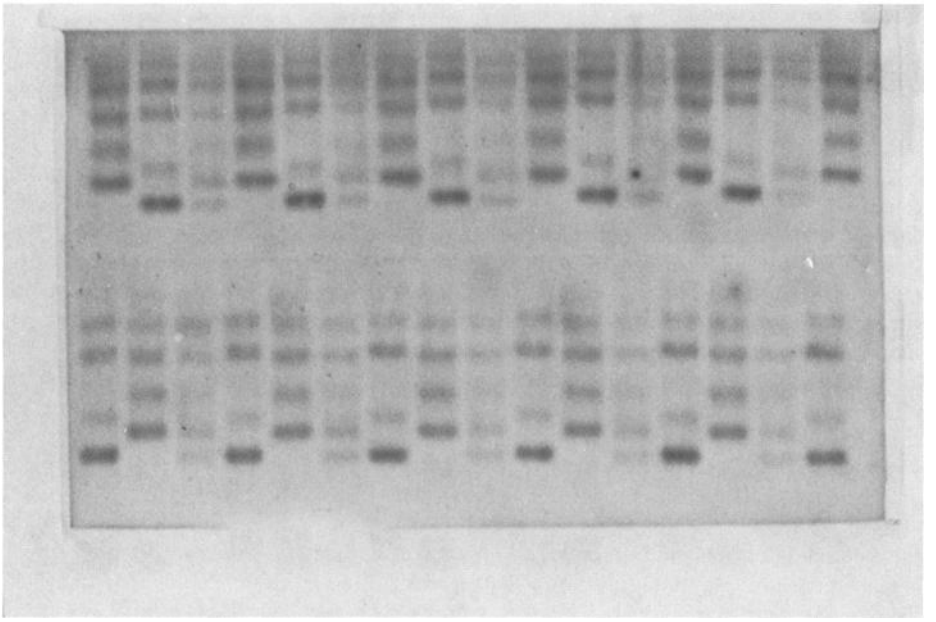

FIG. 1—*An agarose gel displaying PGM phenotypes. The phenotypes on the top row from left to right are: 2, 1, 2-1, 2, 1, 2-1, 2, 1, 2-1, 2, 1, 2-1, 2, 1, 2-1, and 2. The phenotypes on the bottom row from left to right are: 1, 2, 2-1, 1, 2, 2-1, 1, 2, 2-1, 1, 2, 2-1, 1, 2, 2-1, and 1. The anode is at the top.*

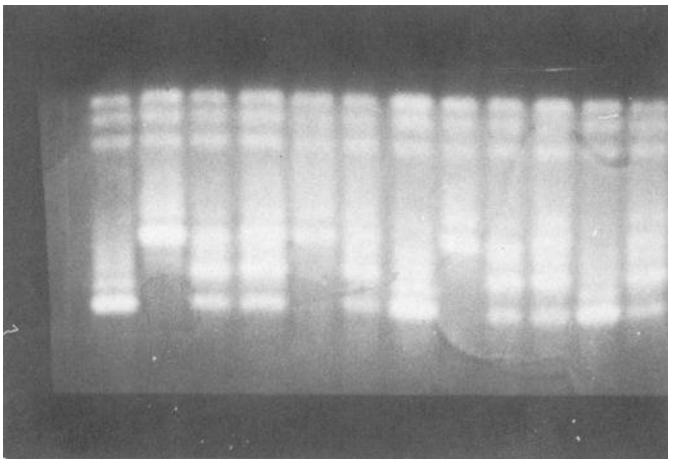

FIG. 2—*An agarose gel displaying EsD phenotypes. The phenotypes from left to right are: 1, 2, 2-1, 2-1, 2, 2-1, 1, 2, 2-1, 2-1, 1, and 2-1. The anode is at the top.*

counter less resistance during initial migration than proteins eluting from cloth. Additional advantages of using the mask included little, if any, damage to the gel and fewer technique related problems in applying a bloodstain cutting to the gel. These changes yielded superior resolution of the GLO variants (Fig. 3). Discrete band patterns were observed for GLO, as opposed to the more diffused patterns typically produced by the Group I system.

The assay parameters for EsD were an additional improvement. Nishigaki et al [*10*] have

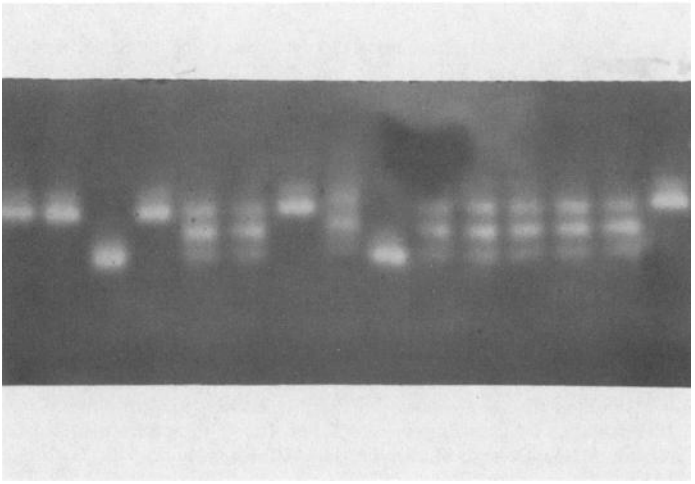

FIG. 3—*An agarose gel displaying GLO phenotypes. The phenotypes from left to right are: 2, 1, 2, 2-1, 2-1, 2, 2-1, 1, 2-1, 2-1, 2-1, 2-1, 2-1, and 2. The anode is at the top.*

TABLE 1—*A comparison of incubation*[a] *time on the detection of EsD.*[b]

Phenotype	Number Tested	5 min[c]	15 min[c]
1	17	12	17
2-1	7	4	7
2	1	0	1

[a]The incubation temperature was 37°C.

[b]Bloodstain samples were maintained at room temperature for two weeks before analysis.

[c]The numbers reflect the positive results at the particular incubation time.

shown that lengthening the incubation time for the EsD assay resulted in increased activity of the EsD bands. The substrate overlay for EsD must incubate for at least 15 min at 37°C, not 5 min at room temperature as suggested for the Group I system. This increase in incubation time and temperature permits successful detection of EsD by this method as well as for the Group I system (Table 1). The change in incubation time and temperature increased the ability to detect EsD in bloodstains.

Phosphoglucomutase was also typed using the system described above. The advantage of using this system for PGM typing is that two points of sample application can be used. This permits the typing of twice the number of samples for PGM during one run. Therefore, PGM typing of a large number of case samples can be handled in a short time.

This single system agarose gel electrophoretic method for typing PGM, EsD, or GLO is a rapid and reliable procedure for bloodstain analysis that can be easily incorporated into the laboratory. The amount of sample required for analysis of these three markers by the single systems is similar to that used for analysis with the Group I system; thus, there was no compromise of sample size between the two methods. The technique described here can increase the resolution of genetic markers, particularly GLO, can increase the confidence of the forensic science analyst, as well as reduce the number of inconclusive results from forensic science samples.

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Address requests for reprints or additional information to
Bruce Budowle, Ph.D.
Forensic Science Research Group
Laboratory Division
FBI Academy
Quantico, VA 22135