TECHNICAL NOTE

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Discontinuous Polyacrylamide Gel Electrophoresis for Typing Haptoglobin in Bloodstains

REFERENCE: Budowle, B. and Chow, G. H., **"Discontinuous Polyacrylamide Gel Electrophoresis for Typing Haptoglobin in Bloodstains,"** *Journal of Forensic Sciences*, JFSCA, Vol. 30, No. 3, July 1985, pp. 893-897.

ABSTRACT: A routine method is described for obtaining reproducible haptoglobin patterns from bloodstains by discontinuous polyacrylamide gel electrophoresis. By employing a stacking gel, proteins from bloodstain extracts are concentrated into narrow zones, before entering the resolving gel. This effect yields highly resolved haptoglobin patterns. Therefore, laboratories without the specialized equipment and expertise for polyacrylamide gradient gel preparation can still obtain highly resolved haptoglobin patterns from bloodstains.

KEYWORDS: pathology and biology, haptoglobin, blood, bloodstains, discontinuous polyacrylamide gel, electrophoresis

Haptoglobin (Hp) is a serum glycoprotein of the alpha 2-globulin class that binds hemoglobin (Hb) released into the vascular circulation. Haptoglobin is a valuable forensic science marker, because it is polymorphic [*1,2*], its phenotypes are readily distinguishable, and it is very stable in bloodstains [*3*]. But despite these favorable characteristics, there are laboratories that do not perform Hp analysis on bloodstains. Part of the reluctance results because one of the most widely used methods for typing Hp in the forensic science arena, continuous polyacrylamide gradient gel electrophoresis [*4*], is an obstacle for many laboratories because of requirements for specialized gel-casting equipment, the difficulty of preparing thin gels (less than 1 mm), and the high cost of commercially prepared gels. This paper describes an alternative method for Hp typing using a discontinuous polyacrylamide gel electrophoretic technique that is easily implemented, requires no gradient gels, and yet clearly resolves the Hp variants.

Materials and Methods

Bloodstains from 74 individuals were prepared as previously described [*5*]. There were 11, 37, 25, and 1 individuals expressing the Hp 1, Hp 2-1, Hp 2, and Hp 2-1M phenotypes, respectively. These samples were used to test the efficacy of the following procedure. Bloodstain cut-

This is publication 84-20 of the Laboratory Division of the Federal Bureau of Investigation. Names of commercial manufacturers are provided for identification only and inclusion does not imply endorsement by the Federal Bureau of Investigation. Received for publication 22 Sept. 1984; revised manuscript received 8 Nov. 1984; accepted for publication 9 Nov. 1984.

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tings (5 by 5 mm) were extracted in 60 μL of sample buffer (Table 1) for 30 min. Methylene chloride or chloroform (200 μL) were added to each extract to precipitate degraded Hb [3]. Each sample was vortexed several times over a 5-min period. The aqueous layer (40-μL/sample for a gel with ten sample wells and 30-μL/sample for a gel with twenty sample wells) was removed and applied to the cathodal end of the gel.

The LKB 2001 vertical electrophoretic unit and LKB 2103 power supply were used according to the instructions supplied with the vertical electrophoretic unit.

The discontinuous polyacrylamide gel electrophoretic technique described by Ornstein [6] and Davis [7] was used. The resolving gel mixture (Tables 1 and 2) except for the Temed was prepared in a side-arm flask. The solution was degassed for 1 to 2 min under vacuum, and then the Temed was added with gentle stirring. This solution was poured between the glass plates (160 by 180 mm) leaving a space of 3 cm from the top of the plates. The recipe in Table 2 was sufficient to pour two gels (130 by 140 by 0.75 mm). Water was gently layered over the top of the resolving gel solution surface. Polymerization of the resolving gel occurred within 30 min, which was evidenced by a sharp interface between the polymerized gel and the water overlay. The overlay was then decanted and the well-former "comb" was placed between the glass plates, leaving a 1-cm space between the base of each well and the resolving gel. The stacking gel was prepared according to Tables 1 and 2 and was poured around the well-former to the top of the plates. Polymerization was allowed to proceed for approximately 30 min. After polymerization, the well-former was gently removed leaving wells for samples to be applied within the stacking gel. A diagram of the discontinuous gel setup is displayed in Fig. 1.

The samples were applied to the wells (cathodal end of gel) using a syringe. The remaining space within each well was layered with tank buffer (Table 1) to minimize disturbance of each

TABLE 1—*Recipes for preparing various solutions for discontinuous polyacrylamide gel electrophoresis.*

Resolving gel acrylamide solution	28.8-g acrylamide and 1.2-g bisacrylamide are dissolved in 50-mL water, filtered, and water is added to a final volume of 100 mL
Stacking gel acrylamide solution	29.1-g acrylamide and 0.9-g bisacrylamide are dissolved in 50-mL water, filtered, and water is added to a final volume of 100 mL
Resolving gel buffer	1.5*M* Tris-HCl, pH 8.9: 181.8-g-Tris brought to a volume of 750 mL with water, adjust pH with HCl, and add water to a final volume of 1000 mL
Stacking gel buffer	0.5*M* Tris-HCl, pH 6.8: 60.6-g Tris brought to a volume of 750 mL with water, adjust pH with HCl, and add water to a final volume of 1000 mL
Tank or reservoir buffer	Tris-glycine, pH 8.3: 6-g-Tris and 28.8-g glycine, add water to a final volume of 1000 mL. Dilute 1:5 with water when used
Sample buffer	8.75 mL of 0.5*M* Tris HCl, pH 6.8, 13.75-mL water, 10-mL glycerol, and 2-mL 0.01% bromophenol blue

TABLE 2—*Recipes for gel preparation.*

Solutions/Reagents	Resolving Gel[b] (7% T, 4% C)	Stacking Gel[b] (5% T, 3% C)
Solution A[a]	7.89 mL	...
Solution B[a]	...	1.64 mL
Solution C[a]	8.45 mL	...
Solution D[a]	...	2.50 mL
Water	17.30 mL	5.86 mL
Ammonium persulfate	14 mg	6mg
Degas	1 to 2 min	1 to 2 min
Temed	17 μL	10μL

[a] From Table 1.
[b] Volumes enough to pour two gels.

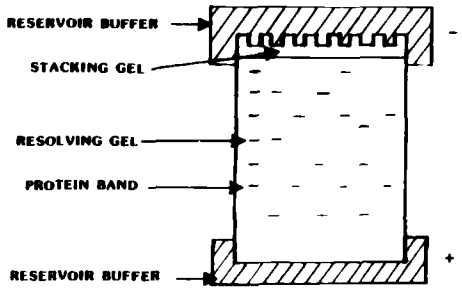

FIG. 1—*A diagrammatic representation of the discontinuous polyacrylamide gel system.*

sample. The gels, housed within their glass plate sandwiches, were attached to the vertical apparatus and tank buffer (Table 1) was added to the reservoirs.

Electrophoresis was at a constant current of 20 mA at 10°C. When the dye front (bromophenol blue) entered the resolving gel (approximately 75 min), the constant current mode was increased to 60 mA. Electrophoresis was terminated when the dye front was 3.5 cm from the anode (an additional 75 min). The gels were then carefully removed from the glass plates.

The Hp markers were stained according to a modification of the method of Compton et al [*8*]. Of *o*-dianisidine dihydrochloric acid 100 mg was dissolved in 180 mL of heated water (50°C). Then 20 mL of 1.5*M* sodium acetate buffer, pH 4.7, and 150 μL of 30% hydrogen peroxide were added. This solution was poured over the gels.

Typing of Hp was also performed using the continuous polyacrylamide gradient gel method of Culliford [*4*] for phenotype comparison purposes.

Results and Discussion

Figure 2 illustrates the separation of Hp variants in bloodstains using discontinuous polyacrylamide gel electrophoresis. The Hp patterns were sharp, distinct, and clearly resolved. As many as 20 individual samples could be run at 1 time per gel. Thus, a method using a simplified polyacrylamide gel preparation for Hp typing has been developed. This procedure has been reproduced on at least 40 separate occasions.

To precipitate degraded Hb in bloodstains that might mask the Hp patterns, a chloroform extraction of the aqueous bloodstain extract has been suggested [*3*]. In the present study, methylene chloride was also used. The methylene chloride-treated samples presented slightly more intense Hp patterns compared with samples treated with chloroform (Fig. 3). No quantitative comparison was made since a densitometer was not available. The increased Hp pattern intensity, using the methylene chloride extraction, suggests that consideration should be given to using this method for Hp analysis of forensic science samples especially since the quality of case samples is often uncertain and frequently minute amounts must be detected.

An additional advantage of this discontinuous electrophoretic method is that proteins enter the resolving gel in a narrow zone which yields highly resolved patterns [*6, 7*]. This concentration of proteins at the interface of the stacking and resolving gels permits the loading of larger sample volumes on the gel without increased diffusion effects. Dilute, weak, or older samples may be typeable for Hp where previously impossible.

This discontinuous polyacrylamide gel electrophoretic method for typing Hp is a rapid and reliable procedure for bloodstain analysis that can be easily incorporated into the laboratory. Less technical expertise is needed for gel preparation, since gradient gels are not used. Moreover, larger sample volumes can be applied to the gel without loss of resolution, thus increasing the chances of obtaining a positive result. Forty samples (two gels) can be typed in less than half

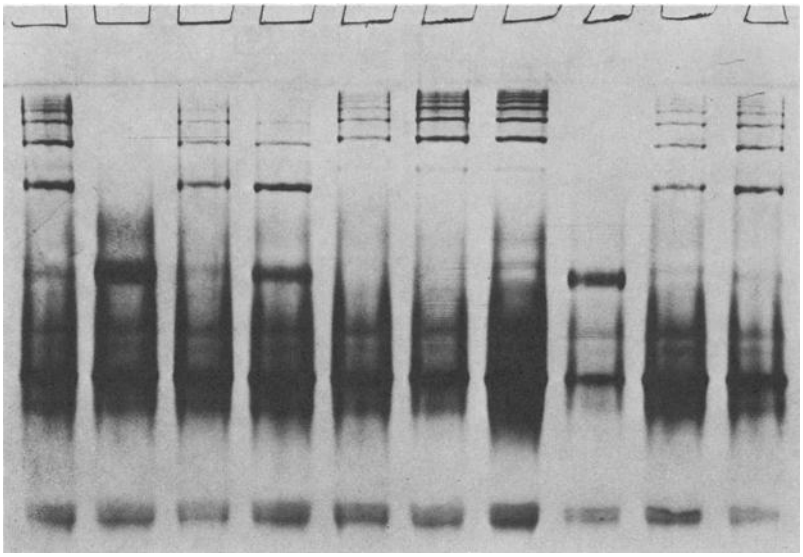

FIG. 2—*A disconotinuous polyacrylamide gel displaying haptoglobin phenotypes from bloodstains. The phenotypes from left to right are: 2-1, 1, 2-1, 2-1M, 2, 2, 2, 1, 2-1, and 2-1. The cathode is at the top.*

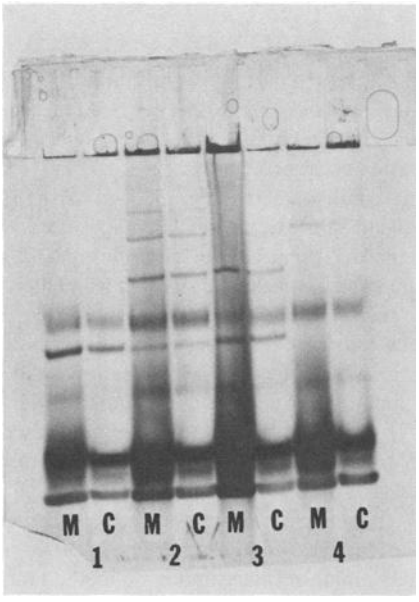

FIG. 3—*A gradient gel displaying haptoglobin phenotypes from bloodstains which have been extracted by methylene chloride* (m) *or chloroform* (c).

a working day. These advantages can make this method for typing Hp valuable to the forensic science laboratory.

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