TECHNICAL NOTE

Ted A. Smith[1] *and H. Brent Myers*[1]

The Detection of Group Specific Component (Gc) in the General Population of West Virginia

REFERENCE: Smith, T. A. and Myers, H. B., **"The Detection of Group Specific Component (Gc) in the General Population of West Virginia,"** *Journal of Forensic Sciences*, JFSCA, Vol. 35, No. 6, Nov. 1990, pp. 1436–1440.

ABSTRACT: An isoelectric focusing method is described for the detection of group specific component (Gc) in forensic casework. Gc can be subtyped in one day using this reliable and reproducible method. The gene frequency data collected indicate that the occurrence of Gc phenotypes in the population of West Virginia is consistent with established frequencies for the system.

KEYWORDS: pathology and biology, genetic typing, blood, group specific component, isoelectric focusing, phenotypic frequencies

The isoelectric focusing of group specific component (Gc) has become a routine matter for many forensic science laboratories. To date there has been a wide array of methods published [*1–14*]. Unfortunately, the practical aspects of many of these methods did not fit readily into the daily operation of the authors' laboratory. Consequently, we developed the following method for isoelectric focusing of Gc. The method presented here is a labor-saving one-day method which was easily incorporated into the routine of our laboratory. It utilizes a thin polyacrylamide gel, a mixture of separators, narrow-range ampholytes, and immunoblotting techniques to separate and visualize the Gc phenotypes.

The compilation of population data is always a concern for the testifying forensic serologist. This method has proved to be a valuable tool in that regard for our laboratory. For court purposes we have been collecting data for two years on the Gc subtypes present in the general population of West Virginia. The data collected have been processed and are included in this paper.

Materials and Methods

Gel Preparation

To prepare the stock acrylamide solution, 10 g of acrylamide and 0.3 g of *N,N*-methylenebisacrylamide (BIS) were dissolved in 25 mL of distilled water. The acrylamide solution was prepared every three days.

Received for publication 31 Aug. 1989; revised manuscript received 6 Nov. 1989; accepted for publication 7 Nov. 1989.

[1]Section head of serology and forensic scientist, respectively, West Virginia State Police Crime Laboratory, South Charleston, WV.

Initially, we were following the guidelines established by Budowle concerning the life span of stock acrylamide [*15*]. However, through routine use, we soon discovered that gels made with older stock acrylamide produced increasingly diffuse bands. Our suspicions that poor grade reagents were responsible proved to be unfounded. Budowle indicates that the polymerization of a gel is affected by the age of the stock solution [*15*]. In our method, it appears that older acrylamide does not polymerize fully within the time limits we have established, but, to date, we have not confirmed incomplete polymerization as the specific cause of this diffusion.

Following the recommendations of Westwood and Werret [*2*], the gel was prepared by adding 7 mL of stock solution, 50 μL of *N*-tetramethylethylenediamine (TEMED), 6 mg of ammonium persulfate, 0.8 mL of 4.5 to 5.4 pharmalytes (Pharmacia), 0.27 g of 3-*N*-morpholinopropanesulfonic acid (MOPS), and 0.03 g of *N*-2-hydroxyethylpiperazineethanesulfonic acid (HEPES) to 12 mL of distilled water. The 0.2 by 115 by 220-mm gel was cast onto a sheet of Gel Fix (Ephortec), using the flap technique.

The gel was allowed to polymerize for a minimum of 1 h before its use. The gel had to be cast on the day it was used and the amount of ammonium persulfate had to be strictly regulated, otherwise excessive waviness resulted in the Gc banding patterns.

Sample Preparation

Whole serum (5 μL) less than one month old was diluted 1:10 with distilled water. Serum more than one month old was diluted 1:5. These dilutions can be increased if the Gc bands produced are excessive. The samples were absorbed onto 0.5 by 0.3-mm paper applicator tabs for gel application.

Bloodstain cuttings (0.5 to 1.0 cm) were extracted with 100 μL of freshly prepared 6*M* urea for a minimum of 30 min. Extracts were ultracentrifuged (15 000 rpm for 2 min), and the supernatant was absorbed onto 0.5 by 0.3-mm paper applicator tabs. All samples were blotted to remove excess amounts before they were placed on the gel.

Isoelectric Focusing

Isoelectric focusing was performed on a LKB Multiphor II using a 2297 Microdrive 5 power supply. The cooling plate was blotted completely dry before the gel was placed on it. The electrode solutions, 0.1*M* sodium hydroxide (cathode) and 0.05*M* phosphoric acid (anode), were absorbed onto 0.7 by 22-cc paper wicks. The wicks were blotted dry to the touch to avoid the waviness that excessive solution causes and were placed on the gel 9 cm apart.

Following general guidelines proposed by Johnson [*16*], the gel was prefocused for 30 min at 500 V, 25 mA, and 15 W. After the prefocus, the samples were placed on the gel 1.5 cm from the cathodic wick and focused for 30 min at 600 V, 25 mA, and 15 W. The final focusing stage was for 180 min at 1200 V, 25 mA, and 15 W. The samples were allowed to remain on the gel during some runs and removed from the gel during others. No difference in results was observed.

Immunoblotting

"Atlantic antibodies" anti-human Gc (goat) was diluted 1:5 with 0.9% saline and absorbed onto a cellulose acetate membrane (Sartorius). The wet membrane was centered on the gel between the origin and the anodic wick. After the membrane was incubated at room temperature for 30 min, it was removed from the gel and washed in 0.9% saline for 1 to 1.5 h. The membrane was stained with Coomassie blue for 5 to 10 min and destained to visualize the Gc bands. The destain was a 5:5:1 solution of methanol,

distilled water, and glacial acetic acid. The membrane was immersed in the solution and rotated for 5 to 10 min. At this point in the process, if the bands were faint, silver staining techniques were employed [*11,15,17*], but generally that was not necessary.

Results and Discussion

Six subtypes (1F, 1S, 1F1S, 2-1F, 2-1S, 2) and several proficiency variants were detected using this method. Figure 1 demonstrates the banding patterns that resulted when this method was used.

As Fig. 1 demonstrates, there is a distinct separation (1 mm) between the 1F and 1S bands. The streaked appearance of some of the samples is due to an uneven destaining of the membrane. The photograph tends to enhance the appearance of this background interference.

Our experiments with this procedure indicate that MOPS improved the separation distance between the bands to a greater extent than did HEPES, but HEPES tended to produce visibly tighter bands. The concentrations and ratios of MOPS and HEPES were varied from 0 to 100% in order to ascertain the optimal separation and resolution for the method. The average separation between the fastest and slowest bands was approximately 5 mm when no MOPS or HEPES was added, 15 mm when only MOPS was added, and 10 mm when only HEPES was added. The working ratio that yielded the optimal visible results, in our opinion, was a 9:1 of MOPS to HEPES. This ratio made all of the subtypes readily identifiable.

A major area of concern for this method, as in all isoelectric focusing methods, was excessive waviness. If waviness occurred it was very difficult, if not impossible, to distinguish between the fast and slow bands. As Budowle [*15*] and Pharmacia [*10*] point out, waviness is generally the product of several sources: incomplete and uneven polymerization of the gel, protein loading tolerances, and wick solution and concentration. In practical terms, we found the waviness to be the product of surplus ammonium persulfate, old gels, overloaded and underdiluted samples, uneven wick saturation and blotting, and excessive molar concentrations in wick solution. Our experimentation with the parameters of this method led us to the following conclusions: ammonium persulfate in excess of 7 mg per gel and fresh gels that polymerized longer than 3 h produced waviness; sample volumes greater than 10 μL, diluted at a ratio of less than 1:5, resulted in waviness and blurred fast-slow distinctions; and wicks with solution concentrations greater than 0.1*M* sodium hydroxide (NaOH) and 0.05 phosphoric acid (H_3PO_4) created uneven band lines. If these variables were strictly controlled, this method produced very reliable and reproducible results.

At the outset of this work, our principle goal was the formulation of a reliable Gc

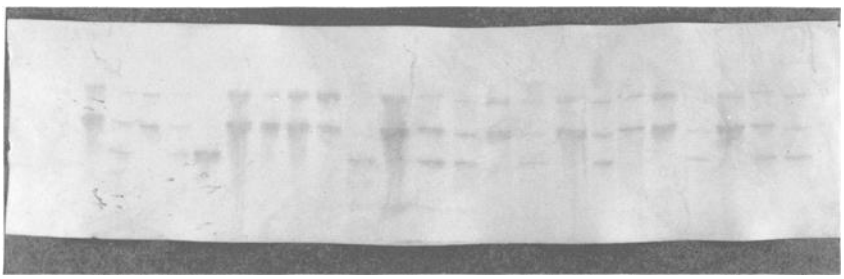

FIG. 1—*Gc phenotypes* (left to right): *Samples 1, 6–9, 11, 14, 16, 19, and 21 are Gc 1F1S; Samples 2, 12, and 22 are Gc 2-1F; Sample 3 is Gc 1S; Samples 4, 13, 15, 17, 20, and 23 are Gc 2-1S; Samples 5 and 10 are Gc 2; and Sample 18 is Gc 1F.*

TABLE 1—*Gc phenotypes.*[a]

Type	Observed Number	Expected Number	Observed Frequencies
1F	15	12	0.03
S	160	159	0.32
1F1S	83	87	0.16
2-1F	42	44	0.08
2-1S	164	161	0.33
2	40	41	0.08

[a]Sigma $\chi^2 = 1.022$; degrees of freedom = 3; $0.90 < P < 0.70$.

subtyping method that was not labor-intensive. Having used this method routinely for the past couple of years, we have found it to be both reliable and labor-saving. The most time-consuming aspect of the method was sample preparation. Generally, we focused 30 samples per run. This volume alone made sample preparation time-consuming. Once the samples were prepared and applied to the gel, the process required only occasional activity.

The whole method was completed easily in one day. The gel was cast and allowed to polymerize while the sample extraction was occurring. The focusing aspect of the method required about 4 h, whereas the four-step development process consumed about 2 h. The total time expenditure, from beginning to end, was generally less than 8 h.

With this method we were attempting to eliminate some of the more time-consuming elements utilized in other methods. As a result, a method was produced that did not require repeated voltage adjustments [*7*], overnight membrane incubation [*1*], extensive prefocusing [*8*], or even overnight polymerization of gels [*15*]. At its best, this method allowed us freedom for other pursuits and required only periodic attention.

For the past two years, 504 serums and bloodstains from forensic casework were typed using this method. The results are given in Table 1.

The chi-square value of 1.022 indicates that the phenotypic distribution of this system is in Hardy-Weinberg equilibrium. Consequently, the discrepancy between the observed and expected values appearing in the results is consistent with the discrepancy that would occur in any random sampling of the population.

Of the six common phenotypes identified, the 1S and 2-1S patterns were the most common. The 1F phenotype was the least common. These results are in agreement with those from other geographic areas [*8,9,16,18,19*]. Because our population is 96% Caucasian, a high occurrence of 1S and 2-1S phenotypes and a low occurrence of 1F phenotypes are to be expected [*8,9,16*].

Analysis of these data indicates that there is no evidence that our population is atypical in regard to this blood marker system. Generally speaking, it is safe to say, on the basis of the markers identified using this method, that the Caucasian population of West Virginia falls well within the accepted frequency distributions for the Gc system.

References

[*1*] Alonso, A., "Group Specific Component Subtyping in Bloodstains by Separator Isoelectric Focusing in Micro-Ultrathin Polyacrylamide Gels Followed by Immunoblotting," *Journal of Forensic Sciences*, Vol. 33, No. 5, Sept. 1988, pp. 1267–1272.

[*2*] Westwood, S. A. and Werret, D. J., "Group-Specific Component: A Review of the Isoelectric Focusing Methods and Auxiliary Methods Available for the Separation of its Phenotypes," *Forensic Science International*, Vol. 32, No. 1, 1986, pp. 135–150.

[*3*] Baxter, M., Randall, T. W., and Thorpe, J. W., "A Method for Phenotyping Group-Specific

Component Protein from Dried Bloodstains by Immunofixation Thin-Layer Polyacrylamide Gel Isoelectric Focusing," *Journal of the Forensic Science Society*, Vol. 22, 1982, pp. 367–371.

[4] Baxter, M. and White, I., "A Method for the Identification and Typing of the Sub-types of the Gc Allele from Dried Bloodstains," *Journal of the Forensic Science Society*, Vol. 24, 1984, pp. 483–488.

[5] Westwood, S. A. and Fryer, K., "The Typing of Group-Specific Component in Case Bloodstains Using Narrow pH Interval Isoelectric Focusing Gels," *Journal of the Forensic Science Society*, Vol. 26, 1986, pp. 267–274.

[6] Edwards, J., "Gc Subtyping by Isoelectric Focusing in Ultra-Thin Polyacrylamide Gels Containing a Mixture of Separators 3-(*N*-Morpholine) Propanesulphonic Acid (MOPS) and *N*-2-Hydroxyethylpeperazine *N*-2-Ethanesulphonic Acid (HEPES)," *Journal of the Forensic Science Society*, Vol. 26, 1986, pp. 441–447.

[7] Budowle, B., "A Method for Subtyping Group-Specific Component in Bloodstains," *Forensic Science International*, Vol. 33, 1987, pp. 187–196.

[8] Dykes, D., Polesky, H., and Cox, E., "Isoelectric Focusing of Gc (Vitamin D Binding Globulin) in Parentage Testing," *Human Genetics*, Vol. 58, 1981, pp. 174–175.

[9] Westwood, S. A. et al., "The Phenotypic Frequencies of Group-Specific Component and a-2-HS Glycoprotein in Three Ethnic Groups: The Use of Proteins as Racial Markers in Forensic Biology," *Forensic Science International*, Vol. 35, 1987, pp. 197–207.

[10] *Isoelectric Focusing: Principles and Methods*, Pharmacia, Sweden, 1982.

[11] Westwood, S. A., "Silverstaining of Immunofixed Group Specific Component on Cellulose Acetate Membrane After Isoelectric Focusing in Narrow pH Interval Gels," *Electrophoresis*, Vol. 6, 1985, pp. 498–503.

[12] Westwood, S. A. et al., "Analysis of the Common Genetic Variants of the Human Gc System in Plasma and Bloodstains by Isoelectric Focusing in Immobilized pH Gradients," *Journal of the Forensic Science Society*, Vol. 24, 1984, pp. 519–528.

[13] Kimura, H., Shinomiya, K., Yoshida, K., and Shinomiya, T., "The Typing of Group Specific Component (Gc Protein) in Human Bloodstains," *Forensic Science International*, Vol. 22, 1983, pp. 49–55.

[14] Westwood, S. A., "A Method for Dissociating Group Specific Component from Protein Complexes in Human Bloodstains and Its Significance in Forensic Biology," *Electrophoresis*, Vol. 5, 1984, pp. 316–318.

[15] Budowle, B., "Making Ultrathin-Layer Polyacrylamide Gel Isoelectric Focusing a Reproducible Method," *Proceedings of the 1986 Meeting of the America's Branch of the Electrophoresis Society*, Gaithersburg, MD, March 1986.

[16] Johnson, M. A., "Group Specific Component," *Protein Phenotyping Techniques*, Atlantic Antibodies Publication, Stillwater, MN.

[17] Budowle, B., "Increasing the Sensitivity of Detection of the Electrophoretic Marker Group Specific Component in Agarose Gels by Double Staining with Coomassie Brilliant Blue R250 and Silver," *Journal of Forensic Sciences*, Vol. 29, No. 4, Oct. 1984, pp. 1183–1186.

[18] Dykes, D. and Polesky, H., "Review of Isoelectric Focusing for Gc, PGM, Tf, and Pi Subtypes: Population Distributions," *Critical Review Clinical Laboratory Science*, Vol. 20, 1984, pp. 115–151.

[19] Gaensslen, R. E., Bell, S. C., and Lee, H. C., "Distributions of Genetic Markers in United States Populations: III. Serum Group Systems and Hemoglobin Variants," *Journal of Forensic Sciences*, Vol. 32, No. 6, Nov. 1987, pp. 1754–1774.

Address requests for reprints or additional information to
Ted A. Smith, Section Head—Serology
State Police Crime Laboratory
725 Jefferson Rd.
South Charleston, WV 25309