Group Specific Component Phenotyping by Ultrathin-layer Agarose Isoelectric Focusing Using Chemical Spacers

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INTRODUCTION

Polyacrylamide gel isoelectric focusing has become the method of choice for classification of the common GC phenotypes, primarily because of improved resolution. Isoelectric focusing in immobilized pH gradients provided far superior results in delineating the phenotypes but has not proven practical for routine testing (Cleve et al 1982). With the introduction of narrow pH range carrier ampholytes by Pharmacia, there was improved resolution of the common GC phenotypes for both polyacrylamide and agarose IEF using a pH range of 4.5-5.4 (Lizana and Olsson 1984). Since the common GC phenotypes fall within a pH range of 4.8-5.1, further methods have been used to flatten the pH gradient curve in this region to improve resolving power. Edwards (1986) used ultrathin-layer IEF in polyacrylamide gel containing a combination of chemical spacers with narrow range Pharmalytes (pH 4.5-5.4) to improve the separation of the GC 1F and GC 1S bands. The results compared favorably with those obtained in immobilized pH gradient gels.

A problem associated with using polyacrylamide is that it is a potential health hazard due to it toxic properties. Agarose, on the other hand, is virtually non-toxic and improvements in resolution with this media should be considered. The method described here utilizes agarose as the media and employs a modification of the method described by Edwards (1986).

MATERIALS AND METHODS

Reagents

Agarose IEF, Pharmalyte 4.5-5.4, and Repel-Silane were from LKB-Pharmacia. Acrylamide, Bis-acrylamide, Sucrose, MOPS, HEPES, and Coomassie Brilliant Blue R-250 were obtained from SIGMA. Rabbit Anti-GC-globulin was obtained from Atlantic Antibodies. All other chemicals used were of analytical grade.

Serum samples were obtained from healthy laboratory personnel at Rush Medical Center and from the AABB's Paternity Specimen Program (PSP). Included in these samples were 8 known rare variants (see Table 1). The samples were aliquoted and stored frozen at either -20°C or -70°C until tested.

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Table 1. Known Rare GC Variants Used

GC 1 Variants	GC 2 Variants
1F-1C10	1S-2C14
1S-1C6	1F-2A3
2-1A10	
1S-1C32	
1S-1C33	
1F-1C16	

Gel Preparation

Agarose IEF gels (225x114x1 mm) were cast on GelBond^R film (FMC). Add 0.2g Agarose IEF, 2.5g sucrose, 0.16g HEPES, 0.5g MOPS to 20 ml distilled water and dissolve by boiling. (This is sufficient for two gels.) Cool to 75°C and add 1.5 ml Pharmalyte 4.5-5.4. Aspirate molten agarose solution into a 25 ml syringe and quickly dispense into a preheated LKB capillary gel casting kit with constant pressure on the syringe barrel and the glass plates at about a 45° angle. Permit to gel for 20 minutes. Store at 4°C for a minimum of 1 hour in a humidity chamber. Gels may be stored for up to 1 month. Agarose gels were also made in the same manner without the MOPS and HEPES. Acrylamide gels were made according to Edwards (1986).

Sample Preparation

All serum samples were treated with 6 M Urea in a 1:5 dilution to dissociate any GC that may be bound to actin. The GC-actin complexes alter the pI; therefore, incomplete dissociation may produce additional banding making it more difficult to interpret the banding pattern (Emerson et al 1984). To the gel at a position 3 mm from the cathode, approximately 5 ul of sample solution with sample application pieces (3 x 3 mm).

Running Conditions

An LKB Multiphor 2117 tank was used having the electrodes separated by 9.5 cm. A 0.5 N NaOH solution was used on the cathodal wick and a 1 M H_2PO_4 solution was used on the anodal wick. Agarose IEF gels were focused at a maximum of 1650 V, unlimited W, and unlimited mA for 90 minutes at a temperature of 4°C. Acrylamide IEF gels were focused according to Edwards (1986).

Immunofixation

At the completion of the run, the GC proteins were identified by immunofixation using anti-GC globulin. The acrylamide gels were immunofixed according to Edwards (1986). For the agarose gels, anti-GC was diluted 1:6 with 0.9% saline and poured directly on the gel 2 cm anodal to the sample application pieces. Cover gel with a saline moistened filter paper. Place gel in a humid chamber for 1 hour at room temperature. Remove filter paper, then press and dry gel with layers of paper towels, a glass plate, and a 5 kg weight. Rinse gel in 0.9% saline for

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at least 1 hour. Gels are then rinsed in distilled water for 5 minutes before staining in Coomassie Brilliant blue R-250. (Staining is the same as with cellulose acetate strip for the polyacrylamide gel according to Edwards).

RESULTS AND DISCUSSION

Narrow range (pH 4.5-5.4) ultrathin-layer agarose IEF with MOPS and HEPES proved to differentiate the common and rare GC phenotypes tested. Results were comparable to those described by Edwards (1986) using narrow range ultrathin-layer polyacrylamide gel IEF with chemical spacers. Both of these methods separated the GC 1S and 1F bands by approximately 2 mm, while the narrow range agarose IEF gel without the chemical spacers separated these bands by only about 1 mm. An increase in distance between the cathodal and anodal bands by 1-2 mm for the gels using the MOPS and HEPES was also observed. Figure 1 shows the separation of several GC variants by all three methods. The improvement in separation can be emphasized by looking at the rare GC variants GC 1S-1C6, GC 1S-1C32, and GC 1S-1C33 on each gel. These variants appear identical on the agarose gel without the chemical spacers, but are clearly delineated on the other gels.

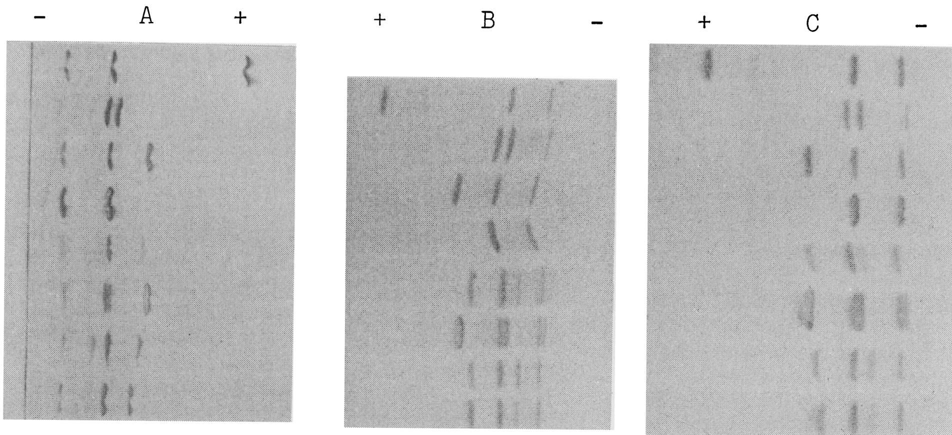

Figure 1. From top: 1S-2C14;1F-2A3;2-1S;1S;1S-1C6;2-1S1F control; 1S-1C32;1S-1C33. A)Polyacrylamide gel with MOPS and HEPES, B)Agarose gel, C)Agarose gel with MOPS and HEPES.

As a blind trial, 71 laboratory personnel of Rush Medical Center were tested by all three methods and found to be easily subtyped. Identical phenotypes were obtained for all specimens by each method.

A new improved method using agarose IEF has been presented. Results by this method compared favorably with those obtained by polyacrylamide gel IEF. The advantages of agarose being non-toxic, less expensive, and easy to work with make this technique highly desirable for routine paternity and forensic casework.

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REFERENCES

Cleve H, Patutschnick W, Postel W, Weser J, Gorg A (1982) Analysis of the genetic variants of the human GC system (VDBP) by isoelectric focusing in immobilized pH gradients. Electrophoresis 3:342-345

Edwards JF (1986) Group Specific component subtyping by isoelectric focusing in narrow range ultrathin-layer polyacrylamide gels containing a mixture of separators. J. For. Sci. Soc. 26:441-447

Emerson DL, Galbraith RM, Arnaud P (1984) Electrophoretic demonstration of interactions between group specific component (vitamin D binding protein), actin, and 25-hydroxycholecalciferol. Electrophoresis 5:22-26

Lizana J, Olsson I (1984) High resolution isoelectric focusing in a narrow pH interval for the phenotyping of Vitamin D-binding protein (GC-globulin). J. Clin. Chem. Clin. Biochem. 22:545-549

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