TECHNICAL NOTE

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A Simple Pattern Method for Alpha$_2$ HS-Glycoprotein Typing

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ABSTRACT: An improved method for the separation of the genetic variants of the human serum alpha$_2$ HS-glycoprotein using isoelectric focusing in ultrathin-layer polyacrylamide gel containing carrier ampholyte pH 4 to 6.5 gradient and neuraminidase pretreated samples is described. The pattern obtained is simple, easily interpretable, and reproducible.

KEYWORDS: forensic science, genetic typing, blood serum, isoelectric focusing, alpha$_2$ HS-glycoprotein, immunoblotting

The genetic variation of the human serum alpha$_2$ HS-glycoprotein (α_2 HSG) was first demonstrated by Anderson and Anderson [*1*] using two-dimensional electrophoresis for separation. Subsequently, isoelectric focusing revealed two common alleles in addition to several rare ones in the studied population samples [*2–9*]. The large number of the observed bands even after desialylation of the samples was the major problem in α_2 HSG typing and probable reason for misinterpretation of the pattern obtained.

This study provides a simple pattern with high resolution and reliable method for α_2 HSG typing.

Materials and Methods

Samples

Sera were treated with neuraminidase type V from *Clostridium perfringens* by adding 20 μL of 1 U/mL enzyme in 0.1 *M* phosphate buffer at pH 6.8, to 5 μL of serum and the mixture was incubated at 37°C for about 24 h before analysis.

Isoelectric focusing

Electrofocusing was carried out using ultrathin-layer polyacrylamide gels of 0.2-mm thickness, containing 3.5% pharmalyte pH range 4 to 6.5, 12.5% sucrose, gel concentration

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(T) = 5% and degree of cross-linkage (C) = 6%. Ammonium persulphate and N, N, N', N' tetramethylethylenediamine (TEMED) were used for polymerization, after which the gel was kept in the refrigerator overnight before use. The electrode solutions were 0.04M L-glutamic acid for the anode and 0.1M β-alanine for the cathode. The power unit was adjusted to supply an initial voltage of 300 V and maximum voltage of 1500 V. The gel was prefocused for 60 min, then 5 μL of neuraminidase treated samples were applied at a distance of 2 cm from the cathodal electrode strip using 5- by 7-mm paper strips (Toyo No. 2). The paper strips were removed after 30 min and the total time was 180 min. Circulating water at 2°C was used during the run. Untreated sera were typed as described above except for using pharmalyte pH range 4.2 to 4.9 and total time was increased to 240 min.

Immunoblotting

This was performed using a 1:250 dilution for the first antibody antihuman α_2 HSG (Behring) and 1:500 dilution for the second antibody peroxidase conjugated anti-rabbit immunoglobulin G (IgG) (cappel).

Results and Discussion

Results obtained for α_2 HSG typing are shown in Fig. 1. The demonstrated pattern is very simple and can be easily interpreted if compared with that obtained with native sera or plasma (Fig. 2). The homozygote α_2 HSG 1 phenotype is represented by one major anodal band and two minor components; one at the anodal and the second at the cathodal sides of the major band. The homozygote α_2 HSG 2 phenotype exhibits a single major cathodal component and a very faint minor anodal band, and the heterozygote phenotype α_2 HSG 2-1 shows combined pattern of all the components.

At the beginning of this study several batches of the commercially available carrier ampholytes were tested, pH ranges 4 to 6.5, 4 to 6, 3.5 to 5, and a mixture of 4 to 6 and 3.5 to 5. We observed that the number of the obtained bands is affected by the used pH range. The simplest pattern with minimal number of bands was obtained in the pharmalyte pH range 4

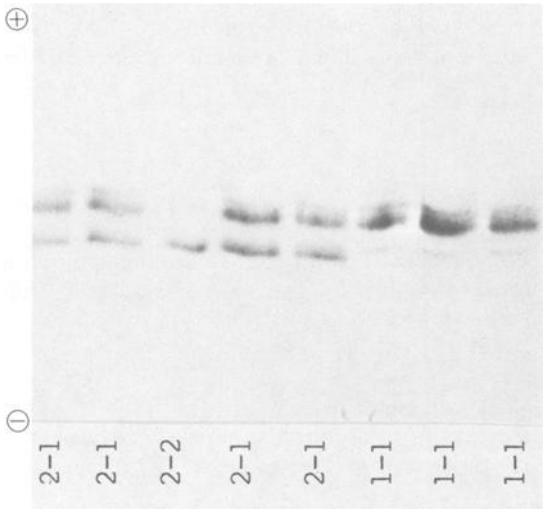

FIG. 1—α_2 *HSG common phenotypes after electrofocusing and immunoblotting of neuraminidase treated sera (pH 4 to 6.5).*

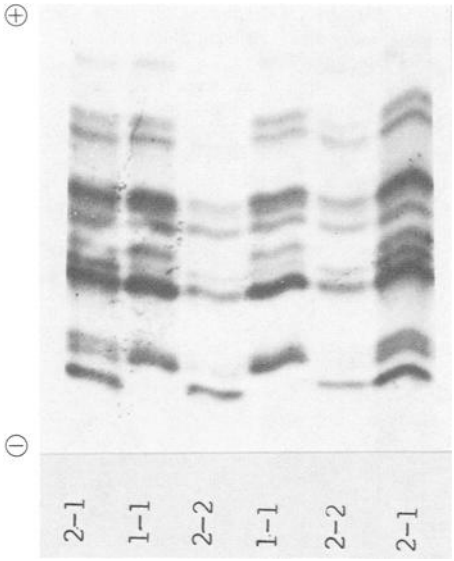

FIG. 2—α_2 *HSG common phenotypes after electrofocusing and immunoblotting of native sera (pH 4.2 to 4.9).*

to 6.5. These findings may explain the pattern differences which are described in the previous publications [*10, 11*]. Cox et al. [*10*], using a mixture of pH ranges 4 to 6 and 5 to 7 with neuraminidase treated samples, reported that the number of α_2 HSG bands was not reduced and attributed the extensive heterogeneity that remained after the removal of sialic acid to posttranslation modifications of amino acids. Subsequently, Umetsu et al. [*11*] observed fewer bands than described by Cox et al. [*10*], after using isoelectric focusing in 2.5*M* urea, pH 5 to 6, and desialyzed α_2 HSG, but much more than described in this study. To test for reliability of this method, more than 300 samples were analyzed using both neuraminidase treated and native sera. The results were consistent. The presented method offers the simplest pattern with high resolution and reliability for α_2 HSG typing.

References

[*1*] Anderson, L. and Anderson N. G., "High Resolution Two-Dimensional Electrophoresis of Human Plasma Proteins," *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 74, 1977, pp. 5421-5425.

[*2*] Cox, D. W. and Andrews, B. J., "Silver Stain Immunofixation for α_2 HS-Glycoprotein: A New Method for Detection of Protein Heterogeneity," in *Electrophoresis '82,* D. Stathakos, Ed., Walter de Gruyter, Berlin, New York, 1983, pp. 243-247.

[*3*] Umetsu, K., Kashimura, S., Ikeda, N., and Suzuki, T., "Classification of α_2 HS-Glycoprotein (α_2 HS) Types by Isoelectric Focusing," *Zeitschrift für Rechtsmedizin,* Vol. 91, 1983, pp. 33-35.

[*4*] Weidinger, S., Schwarzfischer, F., Burgemeister, R., and Cleve, H., "Genetic Alpha-2-HS Glycoprotein Phenotypes Demonstrated by Isoelectric Focusing and Immunofixation," in *Electrophoresis '84,* V. Neuhoff, Ed., Verlag Chemie, Weinheim, 1984, pp. 487-490.

[*5*] Yuasa, I., Taira, T., Suenaga, K., Ito, K., and Okada, K., "Determination of α_2 HS-Glycoprotein Phenotypes by Isoelectric Focusing and Immunoblotting: Polymorphic Occurrence of HSGA*5 in Okinawa," *Human Genetics,* Vol. 70, 1985, pp. 32-34.

[*6*] Ohya, I., Iwasa, M., Komoriya, H., and Bunai, Y., "α_2 HS-Glycoprotein Phenotypes in Gifu Prefecture," *The Research and Practice in Forensic Medicine,* Vol. 28, 1985, pp. 5-8.

[7] Boutin, B., Feng, S. H., and Arnaud, P., "The Genetic Polymorphism of Alpha$_2$-HS Glycoprotein: Study by Ultrathin-Layer Isoelectric Focusing and Immunoblot," *American Journal of Human Genetics*, Vol. 37, 1985, pp. 1098–1105.

[8] Weidinger, S., "Isoelektrische fokussierung und printimmunfixation: ein schnelles verfahren zur typisierung des alpha-2-HS-glykoproteins," in *Elektrophorese Forum '86*, B. J. Radola, Ed., Technische Universität München, Freising-Weihenstephan, 1986, pp. 156–162.

[9] Abe, S., Hiraiwa, K., and Sebetan, I. M., "Distribution of α_2 HS-Glycoprotein Types in Egyptian," *Journal of the Anthropological Society Nippon*, Vol. 95, 1987, pp. 137–139.

[10] Cox, D. W., Andrews, B. J., and Wills, D. E., "Genetic Polymorphism of α_2 HS-Glycoprotein," *American Journal of Human Genetics*, Vol. 38, 1986, pp. 699–706.

[11] Umetsu, K., Yuasa, I., and Suzuki, T., "The Polymorphism of Desialyzed α_2 HS-Glycoprotein (AHS): Isoelectric Focusing in 2.5*M* Urea as a Method for Identification of Genetic Variants," *Human Genetics*, Vol. 73, 1986, pp. 372–373.

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