

# Immunoblotting and Immunofixation Techniques for Subtyping Gc in Old Bloodstains and Semen Stains

I. López-Abadía, A. Gremo, J.M. Ruiz de la Cuesta

Departamento Toxicología y Legislación Sanitaria. Sección  
Biología Forense. Universidad Complutense. 28040 Madrid, Spain

## INTRODUCTION

Group-specific component (Gc) is a polymorphic genetic marker used in blood characterization. A number of isoelectrofocusing methods have been described for separating common Gc subtypes - Gc 1F, Gc 1S and Gc 2- (Constans 1978, Lizana 1981, Cleve 1982)

Gc polymorphism has proved to be very useful in paternity testing, and, as stable protein, well suited for forensic casework.

We here compare immunofixation (Baxter 1982, Westwood 1985) and immunoblotting (Thomas 1989, Alonso 1988) techniques for subtyping Gc in old blood and semen stains.

## MATERIAL AND METHOD

The equipment used was FBE-2000 (Pharmacia) with power supply from Pharmacia ECPS 3000/150 with volthour integrator VH-1. Gels were made through the following mixture: PAGIEF T=6.2%, C=3.2%, sucrose=12%; ampholytes: 4-6=0.06%, 4-6.5=0.02%, 4.5-5.4=0.02%. Electrode gap was 95 mm. Samples consisted of bloodstains on 100% cotton cloth stored at room temperature and semen stains on 100% cotton cloth stored at 4°C. An urea 6M solution was used for extraction and samples were incubated overnight at 4°C. Samples were applied using 5×3 mm Whatman paper 1 cm from cathode. Isoelectric focusing conditions are shown in Table 1. Immunofixation was performed using antiGc serum + physiological serum 1:1 30 min at 37°C in moist chamber. Overnight washing in physiological serum. Development was carried out using silver staining techniques. Immunoblotting was carried out through a double antibody procedure according to Alonso (1988).

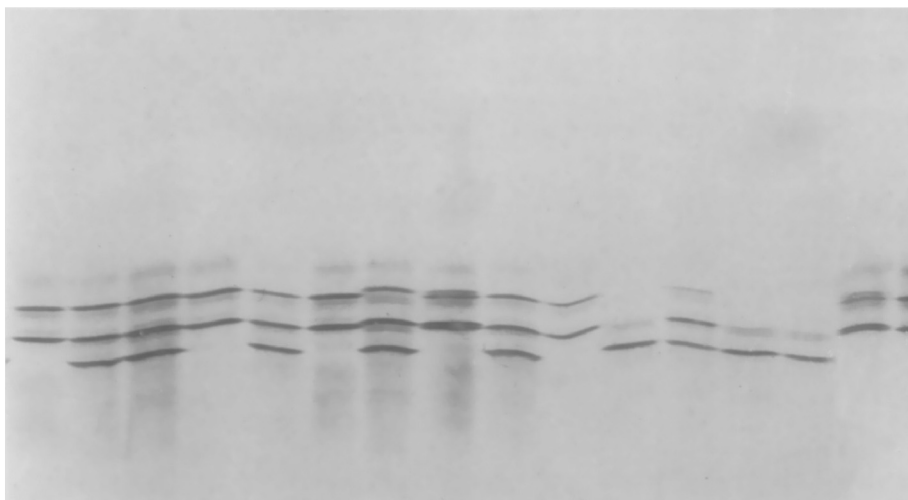


Fig 1. Gc protein. Immunofixation followed by silver staining. Anode is at the top. Phenotypes are: Lanes 1,4,6 and 10: 1s1s; Lanes 2,3,5,9 and 12: 2-1s; Lanes 11, 13 and 14: 2-2; Lanes 8 and 15: 1F-1S; Lane 7: 2-1F

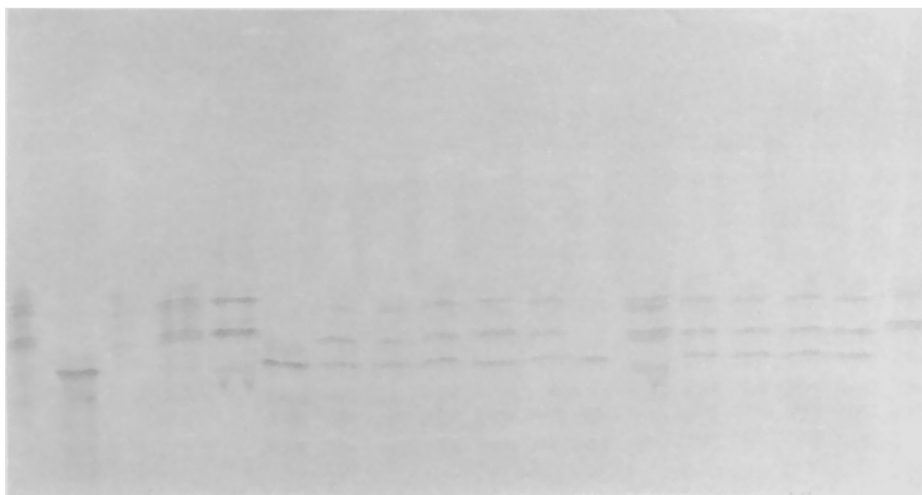


Fig 2. Gc protein. Immunoblotting. Anode is at the top. Phenotypes are: Lanes 1,4,13 and 18: 1F1S; Lanes 2,6 and 12: 2-2; Lanes 3: 1S1S; Lane 5: 1F-1F; Lanes 7,8,9,11,14,15,16 and 17: 2-1S; Lane 10: 2-1F

Table 1. Isoelectric focusing conditions

Prefocusing	1500V	150mA	8W	8°C	500vh
Samples	1500V	150mA	5W	8°C	700vh
Focusing	1500V	150mA	6W	8°C	5000vh

## RESULTS AND CONCLUSIONS

Both methods showed a high reliability for Gc characterization even with very old bloodstains; Gc subtyping was possible in at least 10 months-old bloodstains maintained at room temperature. Both techniques were very sensitive and we did not find any additional band.

We consider immunofixation easier to carry out, quicker and cheaper than immunoblotting and as we did not find differences in the results obtained we advise the use of the former for routine casework involving bloodstains or semen stains.

## REFERENCES

- Alonso A (1988) Group specific component subtyping in bloodstains by separator isoelectric focusing in micro-ultrathin polyacrilamide gels followed by immunoblotting. *J For Sci* 33:1277-1272
- Baxter M, Randall TW, Thorpe JW (1982) A method for phenotyping group specific component protein from dried bloodstains by immunofixation thin-layer polyacrilamide gel isoelectric focusing. *J For Sci* 22:367-371
- Cleve H et al (1982) Analysis of the genetic variants of the human Gc system (VDBP) by isoelectric focusing in immobilized pH gradients. *Electrophoresis* 3:342-345
- Constans J et al (1978) Analysis of the Gc polymorphism in human populations by isoelectric focusing on polyacrilamide gels. Demonstration of sub-types of Gc 1 allele and of additional Gc variants. *Hum Genet* 41:53-60
- Lizana J, Savill B, Olsson I (1981) Agarose isoelectric focusing and related immunochemical techniques in the analysis of serum Gc globulin. In: Allen RC and Arnaud P (eds) *Electrophoresis* 81, Walter de Gruyter, Berlin (pub) p 549
- Thomas AS, Ansford AJ, Aaskov JG (1989) The application of immunoblotting to the phenotyping of Group-specific component. *J For Sci* 29:197-205
- Westwood SA (1985) Silver staining of immunofixed group specific component on cellulose acetate membranes after isoelectric focusing in narrow pH interval gels. *Electrophoresis* 6:498-503