SERUM PROTEIN TYPING/-SUBTYPING by IEF in PAG

G. Geserick, H. Schröder, A. Correns, P. Otremba and H. Waltz

Institute for Forensic Medicine, Humboldt-University, Hannoversche Str. 06, O-1040 Berlin, FRG

INTRODUCTION

For several years we've been gathering experiences in the disengagement of serumprotein-polymorphisms using isoelectrofocusing (IEF). This is mainly due to the systems BF, GC, HP, PI, PLG and TF. During the recent years we raised the efficiency of several methods by modifying them, for instance for the BF-subtypes. Moreover we gathered during the recent years experiences in the forensic use of focusing A2HS, F13A, F13B and ORM1 too.
In the following the most important technical processes used for the optimal visualization of the systems are surveyed.

MATERIAL AND METHOD

With the exception of F13A, where plasma or thrombozyte-lysates are applied, in all systems serum has been used for the identification of the types.
Table 1 lists the necessary details concerning the ampholytes (all from LKB, only A2HS and ORM1 from PHARMACIA) and the electrolyte-solutions.
In table 2 the most important details of sample-application, focusing and immunofixation are enlistet.

The following methods are to be pointed out:
1. We prefer polyacrylamide gel (PAG) in all systems.
2. Besides PLG, F13B and ORM1 we suggest also for the BF-subtypes a pre-treatment with neuramidase. This results in an outstanding distinctiveness of the subtypes BF FA and FB (SIEMENS 1989).
3. As table 2 shows, the protein strings of the systems BF, PLG, F13B, ORM1, A2HS and F13A are very good detected through immunofixation.
In detail, a diluted (BF, ORM1, A2HS) or non-diluted (PLG, F13B, F13A) antiserum is directly put on the surface of the gel immediately after the separation and allowed to react for 15 to 60 minutes.
The figures 1 to 3 demonstrate the quality of the sucessful separations.
All the systems mentioned can be used efficiently for the descent identification. They provide the chance to exclude paternity between 14% (F13A) and more than 30% (GC-, PI-, HP-subtypes).

The frequencies of alleles had been observed:
A2HS: 1 = 0,6598, 2 = 0,3392, 3 = 0,0010 (N = 513)
ORM1: F1 = 0,5770, F2 = 0,0196, S = 0,4021, S1 = 0,0013 (n = 383)
F13B: 1 = 0,7750, 2 = 0,0872, 3 = 0,1339, 4 = 0,0027, 6 = 0,0009 (n = 560)
F13A: 1 = 0,7998, 2 = 0,1977, Var. = 0,0025 (n = 607)

Advances in Forensic Haemogenetics 4
Edited by Ch. Rittner and P. M. Schneider
© Springer-Verlag Berlin Heidelberg 1992
Advances in Forensic Haemogenetics 4 (c) Springer-Verlag Berlin Heidelberg

According to our experiences which coincide with them of other researchers, the following systems are especially appropriate to investigate blood stains: GC, A2HS, ORM1.
The other systems are also considered useful, though we look upon this from a critical point of view. That only does not apply to the HP-subtypes. Using preparation of haptoglobin-protein by immunoprecipitation good detection of types from blood stains is possible (DIMO-SIMONIN et al. 1990, SCHERZ et al. 1990, see also CORRENS et al. in this issue).

Table 1

IEF in PAG[1]

System	Ampholyte		Anolyt	Catholyt	Neuraminidase[2]
BF	4 - 6 5 - 8 3,5-10	0,5 ml 0,4 ml 0,1 ml	H_3PO_4 0,5 M Glutamic acid 0,1M	NaOH 0,2 M	+
PLG	3,5-10 6 - 8 7 - 9	0,4 ml 0,3 ml 0,3 ml	H_3PO_4 0,5 M	NaOH 0,5 M	+
F13B	3,5-10 4-6,5 5 - 7	0,1 ml 0,6 ml 0,8 ml	H_3PO_4 0,5 M	NaOH 0,5 M	+
ORM1	4,2-4,9 4,5-5,4	0,8 ml 0,2 ml	Glutamic- 0,04 M acid	NaOH 0,1 M	+
A2HS	4,2-4,9 4 -6,5	0,9 ml 0,1 ml	Glutamic- 0,04 M acid	NaOH 0,1 M	-
F13A	3,5-10 4 -6,5 5 - 7	0,1 ml 0,6 ml 0,8 ml	H_3PO_4 0,5 M	NaOH 0,5 M	-

------

[1] PAG T = 5 % C = 3 %

[2] SIGMA V 50 µl Serum + 10 µl NANA'se (= 0,1 U), 3 h, 37° C

Table 2

System	Sample-Application	Focusing	Immunofixation
BF	3x5 mm cathod. 30 min.	1 h prefoc. 4 h focusing 1600 V, 10 mA, 10W	Anti-BF ATAB 1:2 dil. 30 min
PLG	3x5 mm anod. 30 min.	1 h prefoc. 4 h focusing 1600 V, 10 mA, 10W	Anti-PLG goat self made undiluted, 15 min
F13B	13x5 mm anod. 60 min.	1 h prefoc. 4 h focusing 1600 V, 10 mA, 10W	Anti-F13B-S BEHRING undiluted 60 min.
ORM1	3x5 mm cathod. 30min/Se+NANAse 1:15 dil.	3 h focusing 2000 V, 15 mA, 20W	Anti-ORM ATAB 1 : 15 dil. 15 min.
A2HS	3x5 mm cathod. 30 min. Serum 1:4 diluted	1 h prefoc. 4 h foc. 1600 V, 10 mA, 10W	Anti-A2HS ATAB 1 : 4 dil. 30 min.
F13A	5x5 mm cathod. 60 min. Thromobzyte-Lysat or Plasma	1 h prefoc. 4 h foc. 1600 V, 10 mA, 10 W	Anti-F13A BEHRING undiluted 60 min.

----

* Antiserum was poured directly on the gel

Advances in Forensic Haemogenetics 4 (c) Springer-Verlag Berlin Heidelberg

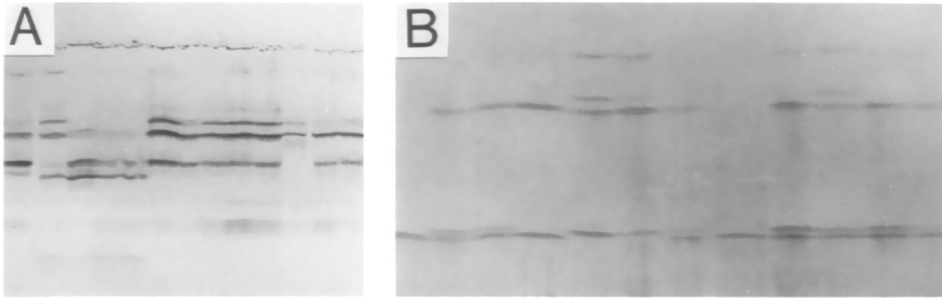

Fig. 1A. BF-phenotypes (nativ). From left to right: S; FASO,7; SSO,7; SSO,7; SSO,7; FAS; FBS; FAS; FAS; FAS; FA; FBS; FBS;
Fig. 1B. BF-phenotypes (NANA'se). From left to right: S; FBS; FBS; FBS; FA; FA; FBS; S; FAS; FAS; FBS; FBS. The anode is at the top

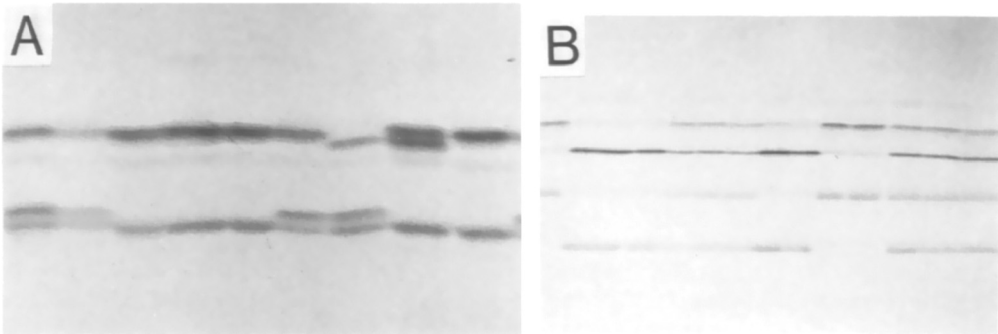

Fig. 2A. ORM1-phenotypes. From left to right: F1S; F1S; F1; F1; F1; F1S; F2S; F1F2; F1.
Fig. 2B. PLG-phenotypes. From left to right: A; B; B; B; AB; AB; AB; B; B; A; A; AB; AB; AB. The anode is at the top

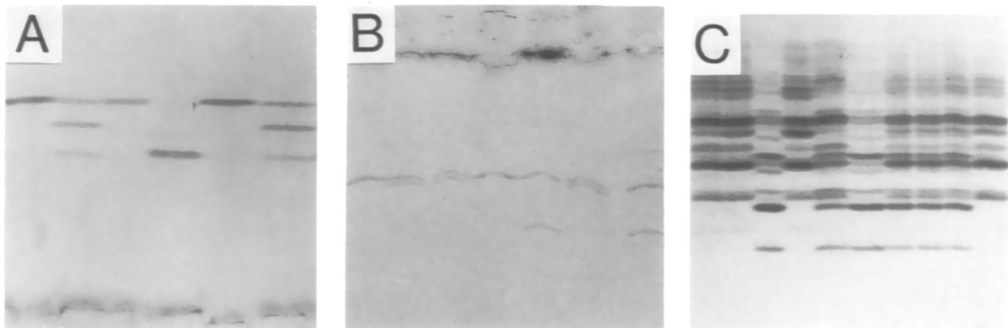

Fig. 3A. F13A-phenotypes. From left to right: 1; 1-2; 1; 2; 1; 1-2.
Fig. 3B. F13B-phenotypes. From left to right: 1; 1-2; 1-2; 1; 1-3; 1-2; 1-3.
Fig. 3C. A2HS-phenotypes. From left to right: 1; 1; 2; 1; 1-2; 2; 1-2; 1-2; 1-2; 1. The anode is at the top

Advances in Forensic Haemogenetics 4 (c) Springer-Verlag Berlin Heidelberg