Bf TYPING IN BLOOD STAINS BY ISOELECTRIC FOCUSING

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INTRODUCTION

On account of the reliability of Bf testing and the stability of Bf proteins this marker system is very useful in paternity testing. Isoelectric focusing (IEF) methods for typing of Bf polymorphism increase the discriminative power of this system (GESERICK et al. 1983, WEIDINGER et al. 1984).
These properties of Bf system indicate a potential fitness for the analysis of blood stains. The common applicability of ultrathin layer IEF in the investigations of blood stains was demonstrated by some authors (BERGHAUS and STAAK 1982, SCHMITTER and KISSLING 1983).
We tested the usefulness of ultrathin layer IEF of Bf marker for characterization of blood stains.

MATERIALS AND METHODS

For the determination of Bf phenotypes by IEF we used the methods similar to those described by GESERICK et al. 1983.

Artificial blood stains

The samples were collected from 12 probands with known Bf phenotypes (Bf S, Bf FS, Bf F). We prepared, dried and stored stains on glass and cotton clothes one day - two months at $4^{o}C$ - $25^{o}C$.
The stains were applied on the gel in following way:
- soaked cotton cloth directly,
- eluated cotton cloth (50 ul physiologic saline, filter paper pieces Whatman No. 3),

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- dried blood drop directly,
- eluated dried blood drop (20 ul physiologic saline, filter paper pieces Whatman No. 3).

The filter paper pieces were used in weted and dried condition.

Isoelectric focusing

IEF was carried out in an ultrathin layer polyacrylamide gel (200 x 100 x 0,3 mm; T=4,5%, C=3%) with 12,5 % glycerine. The pH gradient was established with carrier ampholytes by mixing 3 % Servalyte pH range 5 - 7, 3 % Servalyte pH range 6 - 8, and 1,5 % Servalyte pH range 3 - 10. Polymerization was accomplished chemically with 0,03 % ammoniumpersulphate in the presence of 0,25 % TEMED. For electrode solution 0,1 M glutamic acid in 0,5 M H_3PO_4 was used at the anode and 0,1 M NaOH at the cathode. An electrode distance of 9 cm was chosen. Refrigeratory temperature: $8^{o}C$.

Electric values and focusing times:

- 7,5 W; 7,5 mA; 1 500 V (max.),
- prefocusing 45 min. (dried sample) or 75 min. (wet sample),
- separation with samples 60 min. (dried) or 30 min. (wet),
- separation without samples 195 min. (dried and wet).

We worked with a self made equipment.

Immunofication

Immunofication was performed with monospecific Bf antiserum from Atlantic Antibodies (Scarborough, U.S.A.). According to GESERICK et al. after IEF we applied directly antiserum 1 : 5 in destilled water on the gel surface. After immunoprecipitation the nonprecipitated components were washed out in physiologic saline overnight. After washing in destilled water for 2 h the gel was stained with Coomassie brilliant blue (Serva, Heidelberg).

RESULTS

Complete banding patterns of Bf from blood stains were well visualized. There are no differences between samples and normal serum of probands. No deviations were observed between the Bf patterns from blood drops on glass and blood soaked cotton clothes. On the other hand there were different results dependent on mode of application on gel surface:

- blood soaked cotton clothes directly: ++++,
- cotton clothes eluated with physiologic saline and application by filter paper pieces: ++++,
- dried blood drops directly: Ø,
- dried blood drops eluated with physiologic saline and applicated by filter paper pieces: ++++.

We achieved commonly the best results with only a little weted filter paper pieces.

The banding patterns staid steady within 2 weeks. Later the visualization of Bf types became problematically. We didn't observe any influence of storage temperature between $4^{o}C$ - $25^{o}C$.

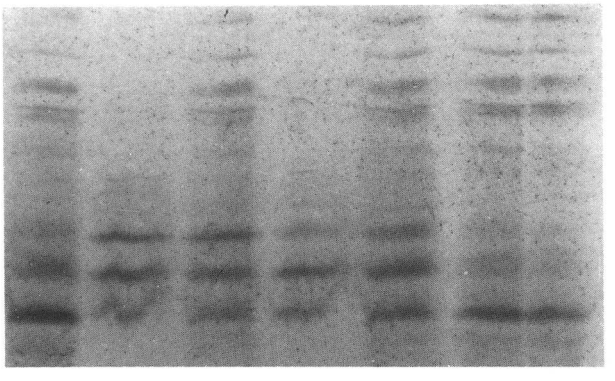

Fig. 1: Bf typing of blood staine

1 = Bf S; 1 day, $4^{o}C$
3,5 = Bf FS; 4 days, $4^{o}C$
6,7 = Bf S; 2 weeks, $25^{o}C$
2,4 = Bf FS; serum samples

CONCLUSIONS

- Bf phenotyping in blood stains by IEF is possible
- The alleles are demonstrable in like manner on glass and on cotton cloth
- The quantity of detectable blood substance amounts to below 10 ul
- Bf marker can be detected within 2 weeks at room temperature

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