Tf and Alpha-1-AT typing in bloodstains by Isoelectric Focusing

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INTRODUCTION

Conventional staining procedure for serum protein have employed the use of Coomassie Brilliant Blue R-250, however, with this staining is impossible to detect these proteins when they are in small quantities, like in forensic samples, even if a highly sensitive method as IEF are used.

Silver staining, is recognized as a highly sensitive method for detect proteins in polyacrylamide gels. Infact, it was been demonstrated that for froensic purpose , silver staining is at least 100-fold more sensitive than Coomassie.

This report evaluated the use comining of PAGIF and silver staining as routine techniques for phenotyping Tf and Alpha-1-AT in bloodstains stored at various temperature.

MATERIAL AND METHODS

TRANSFERRIN:

Gel size: 250x125x0.2 mm

Gel composition:
T=5% C=3.2%
sucrose 12% (w/v)
Ampholine pH 5-7 3% (w/v)
Ammonium persulphate 0.05% (w/v)

Electrodic solutions:

Anolyte: 0.04 M Glutamic acid
Catholyte: 1M Ethanolamine

Bloodstains
Each bloodstains (made with 10 µl of blood) was soaked in 150 µl of 0.3% Ferrous ammonium sulphate solution for 3hours at room temperature. Eluates were applied by means small papers (W 3MM 7x4 mm) at 4 cm from the cathodal end.

Running conditions
1200 V , 20 mA, 3W, 6°C
Prefocusing 60 min
Focusing 150 min (papers were removed after 75 min)

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ALPHA-1-ANTITRYPSIN
Gel size: 250x125x0.5 mm

Gel composition:
T=5% C=3%
glycerol 13% (w/v)
Pharmalyte pH4.2-4.9 6% (w/v)
ACES 0.05% (w/v)
Ammonium persulphate 0.05% (w/v)
TEMED 0.03%

Electrodic solutions:
Anolyte: 0.04 M Glutamic acid
Catholyte: 1 M β-Alanine

Bloodstains:
Each bloodstains(made with 10 µl of blood) was soaked overnight in 150 µl of 0.05 MDithiotreitol .Eluates were applied at 2 cm from the cathode by means of small papers (W 3MM 7x4 mm)

Running conditions:
2000 V, 16W, 20mA, 8°C
Focusing was carried out for 240 min(paper was removed after 60 min)

STAINING

Conditions reported by Carracedo et al.(1)were adopted for silver staining of Tf and Alpha-1-AT with the difference that we used shorter steps.

RESULTS AND DISCUSSION

Our results show that IEF and silver staining are reliable for determining Tf and Alpha-1-AT in forensic investigations on bloodstains.
With bloodstains keep at room temperature and frozen, the staining with Coomassie, for the above mentioned markers, given unreliable results: the bands were well detectable, but the phenotype was uniform according to the storage conditions.
By using silver staining, it was possible not only typing Tf and Alpha-1-AT two months old, keep at room temperature, but also, in minute bloodstains. Infact, we have obtained good results with stains diluted until 1:32. Frozen bloodstains are well typed for a few months.

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