HAPTOGLOBIN SUBTYPING IN BLOODSTAINS

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INTRODUCTION

Haptoglobin typing is routinely performed in numerous forensic laboratories for bloodstain investigation. Recently methods have been published of haptoglobin subtyping in fresh human serum using isoelectric focusing (1, 2). Compared to the conventional method, the discriminating power of haptoglobin subtyping using isoelectric focusing raises from 0.62 to 0.76 and therefore the new method becomes very promising in forensic bloodstain investigation. In this study we investigated the possibility of haptoglobin subtyping of bloodstains on a polyacrylamide gel by isoelectric focusing, using electroblotting and immunoperoxidisase visualization of the haptoglobin bands. Control bloodstains, stored at roomtemperature for up to eight months, and the validity of the system in casework were investigated.

MATERIALS AND METHODS

Preparation of the samples

Bloodstains

Cotton cloth was stained with blood from individuals with known haptoglobin types. The stains were stored at roomtemperature for up to eight months. Bloodstains from casework were kept at -20° C until investigation. A small piece of bloodstain (appr. 5 x 5 mm) was wetted with 15 µl neuraminidase (from Clostridium perfringens, Boehringer). After one hour at roomtemperature the wetted bloodstain was centrifuged. The extract was left overnight at 36° C. The next day 40 µl of 8 M urea solution containing 2% v/v ampholine pH 3.5 - 10 (LKB) and 10% v/v mercaptoethanol was added to the bloodstain extract. The samples were then frozen at -70° C for about two hours before applying on the gel.

Sera

5 µl serum was mixed with 5 µl of the neuraminidase solution. The procedure was then as described above.

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Electrophoresis

Gelpreparation

Polyacrylamidegels, 120 x 260 x 0.5 mm, were cast on the hydrophobic side of a GelBond PAG-film (LKB). Our gel contained 5.8 ml acrylamide/bisacrylamidesolution (T5C3), 8.4 ml aquadest, 40 µl TEMED (Merck), 580 µl persulphatesolution (100 mg/ml), 275 µl ampholine pH 3.5 - 5 (LKB), 275 µl ampholine pH 5 - 7 (LKB) and 100 µl ampholine pH 3.5 - 10 (LKB).

Isoelectric focusing

Isoelectric focusing was performed at 6° C. The electrode distance was 10 cm and the electrode solutions were 1M NaOH for the cathode and 1M H_3PO_4 for the anode. The samples were applied on sample application paper (Pharmacia), 3 x 10 mm for the stain samples and 5 x 2 mm for the serum samples, 1 cm from the cathode. Prefocusing was carried out for 1 hour (settings 500 V, 8 W). Focusing with the samples was carried out for 1 hour (settings 1500 V, 8 W) and without samples for 1½ hour (settings 2000 V, 8 W).

Blotting procedure

A nitrocellulose membrane filter (Schleicher & Schuell, West Germany) was presoaked in aquadest and put on top of the gel. (The nitrocellulose will adhere firmly to the gel.) The membrane and the peeled off gel are then subjected to electroblotting, using a Trans Blot Cell (BioRad) at 70 V for two hours. As blotbuffer was used: 6.06 g TRIS, 28.8 g glycine, in 400 ml methanol and 1600 ml aquadest. After blotting the nitrocellulose membrane was washed in a PBS-Tweenbuffer (13.6 g Na_2HPO_4 $2H_2O$, 4.9 g KH_2PO_4, 17.2 g NaCl and 3 ml Tween (Merck) in 2 l aquadest, pH 7.2) for 2 hours. The membrane is put in a 1:500 PBS-Tweenbuffer dilution of anti human haptoglobin, IgG fraction goat (Atlantic antibodies) to shake overnight. The next day the membrane was washed in several changes of PBS-Tweenbuffer for one hour before being subjected to a second antibody solution (125 µl peroxidase conjugated rabbit anti goat IgG (1g/ml, Behring) in 25 ml PBS-Tweenbuffer).
For the visualisation of the Hp-band the membrane is put in a solution of 30 mg 4-chloro-1-naphtol in 3 ml acetone, mixed with 50 ml of a 0.2 M NaCl, 59 mM TRIS-HCl buffer, pH 7.4 and 40 µl 30% H_2O_2 is added.

RESULTS AND DISCUSSION

Serumsamples from about 500 unrelated persons were investigated according to the described method. A result of a subtyping is shown in figure 1. The immunoblot shows a good distinction between the different subtypes, although it is sometimes difficult to distinguish between a 2FS and a 2FS-2SS subtype. The results of the subtyping by isoelectric focusing were compared

with the results of the ordinary haptoglobin typing using a polyacrylamide gradient gel. No discrepancies were observed. The subtyping of control bloodstains and bloodstains from casematerial gave immunoblots showing additional bands and more background staining, compared to the results of serum-samples (figures 2 and 3). The backgroundstaining sometimes masked haptoglobin bands of weak samples; treatment of the samples with solutions containing different amounts of neuraminidase in order to reduce the backgroundstaining gave no improvement. Sometimes the additional bands caused difficulties interpreting the results. In some instances ordinary haptoglobin typing was necessary to come to a conclusion. However in casematerial the quantity of bloodstain is limited. Therefore supplementary ordinary haptoglobin typing cannot always be done. In those cases it will not always be possible to give a reliable result of the haptoglobin subtyping. Further investigations will be necessary to overcome the described problems and then haptoglobin subtyping using isoelectric focusing can be a valuable tool in forensic bloodstain investigation.

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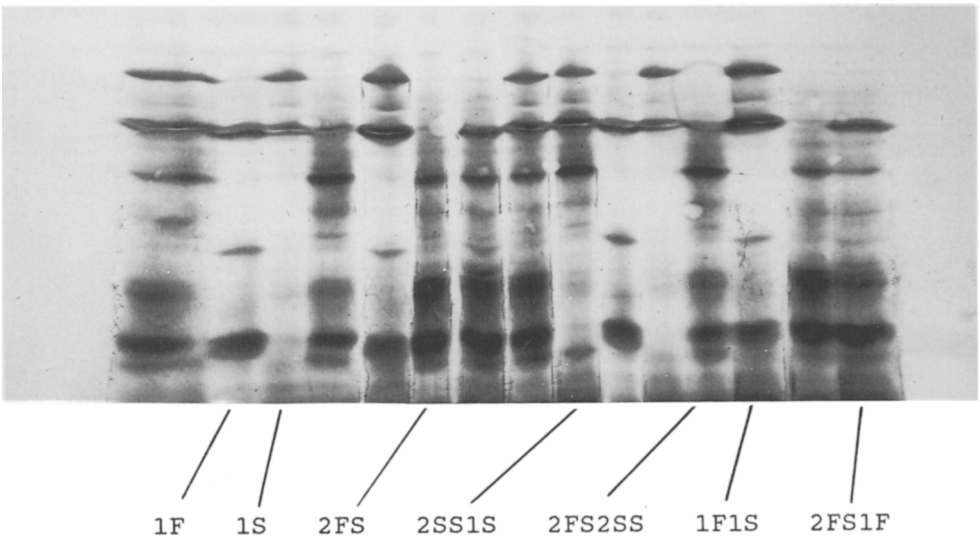

Figure 1. Haptoglobin subtyping in sera.

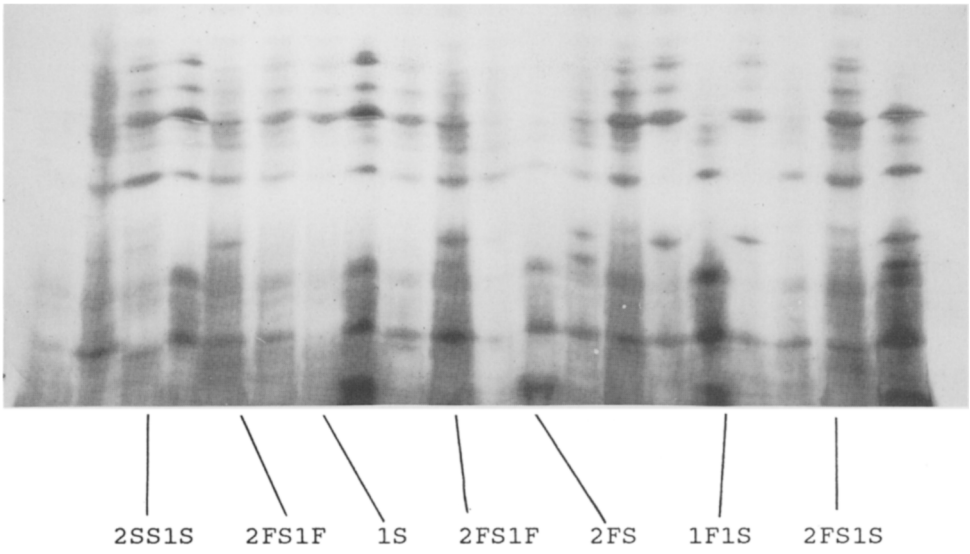

Figure 2. Haptoglobin subtyping of 8 months old control bloodstains.

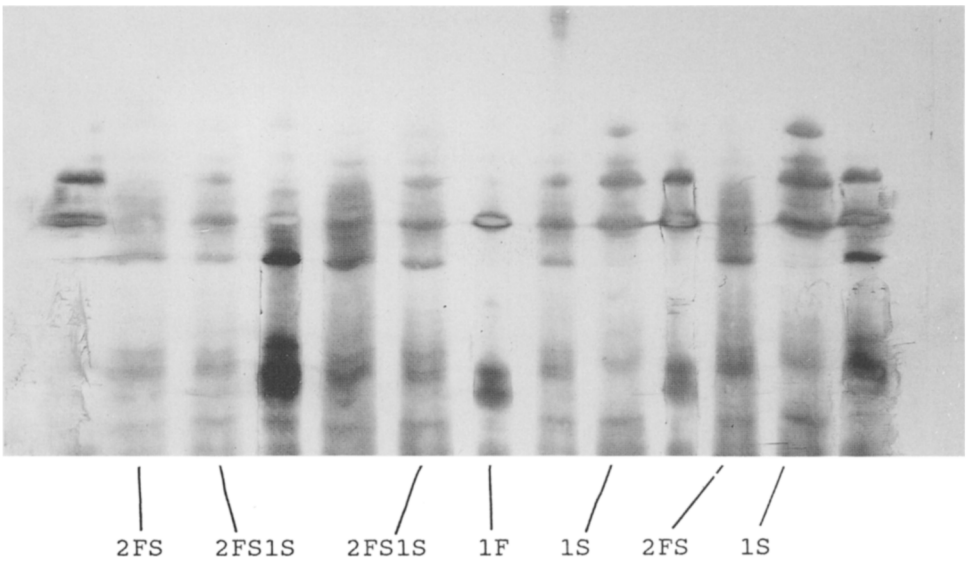

Figure 3. Haptoglobin subtyping of bloodstains from case-material.