HAPTOGLOBIN SUBTYPE DETERMINATION OF BLOODSTAINS

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INTRODUCTION

The high discriminating power (78%) of the haptoglobin subtypes makes it desirable to include this system in the panel used for analysis of bloodstains in criminal cases. Hp subtyping by isoelectric focusing of neuraminidase treated serum was introduced by Teige et al. (1985, 1988) and appeared to be a very suitable procedure for Hp subtyping in paternity investigations. The procedure was modified to make it applicable to analysis of blood stains. Urea was added to the gel, and electroblotting was used for the transfer of protein to the membrane.

MATERIAL AND METHODS

Bloodstains and extraction

Bloodstains from individuals with known Hp subtypes were made on cotton cloth and stored at room temperature for up to thirty months. Case work material was kept at room temperature until examined. Bloodstains (2.5 x 5 mm) were cut into small pieces and extracted at room temperature over night with 30 µl neuraminidase (Clostridium perfringens, Boehringer 0.5 U/ml 0.1 M sodiumacetate pH 5.5). The stains were centrifuged, 25 µl of urea-mercaptoethanol-ampholine (Teige et al. 1988) was added and the extracts frozen at -20°C for 18 h.

Polyacrylamide gel

The gel (120x250x0.5 mm) contained 3.0 ml acrylamide/bisacrylamide (BioRad T=30, C=3.3), 9.5 ml dest. water, 1.6 ml glycerol, 1.0 g urea, 300 µl Resolyt pH 4-8 (BDH), 250 µl Ampholine pH 5-7, 550 µl Ampholine pH 4-6 (LKB), 40 µl TEMED and 110 µl 10 % ammoniumpersulphate. The gel was cast on GelBond PAG film.

Isoelectric focusing

This was performed at 5°C with the electrodes placed directly on the gel surface (Multiphor II, LKB). Prefocusing was carried out for 1 h (max. 500 V, 15 mA, 10 W). The samples were applied 10 mm from the cathode on 4x10 mm filter paper (Whatman nr. 17), and focusing was performed for 15 min. The filter paper was removed and the focusing continued for 1.5 h (max. 2000 V, 15 mA, 15W).

Electroblotting

The membrane (Immobilon-P, 45 µm, Millipore) was soaked in ethanol and subsequently in dest. water. The gel was removed from the Gel Bond-film by means of a Film Remover (LKB) and the membrane was placed on the gel. The gel/membrane sandwich was covered with dry filterpaper (AGF 138 15 x 25 cm) and electro-

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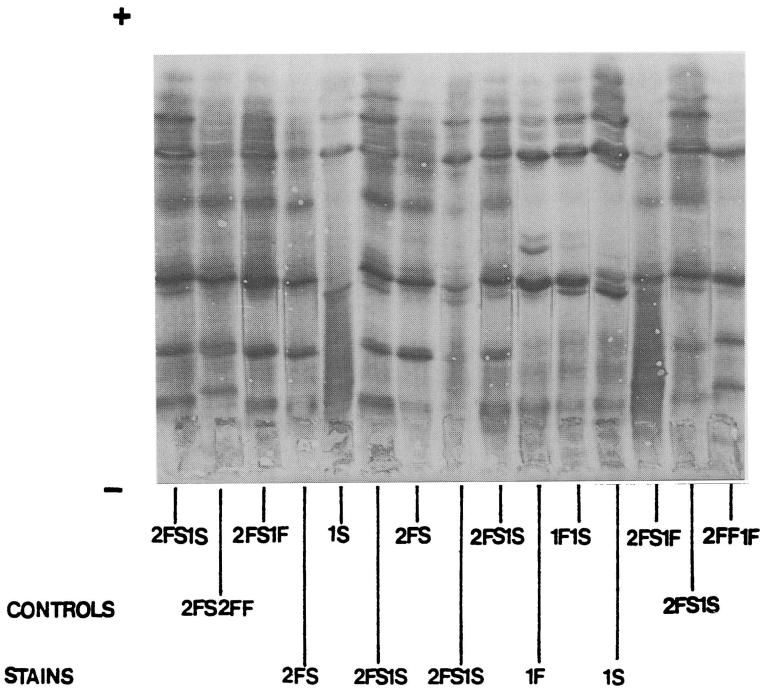
blotting performed for 1.5 h at 130 mA using a Semi-Dry Electroblotter (JKA-Biotech). The anode buffer was 0.3 M tris, 20% ethanol, pH 10.4, and the cathode buffer 0.04 M 6-amino-n-hexanoic acid, pH 9.4.

Visualization

The technique was as described for the detection of orosomucoid (Thymann and Weidinger 1988) using the following concentrations and incubation times: rabbit anti human Hp 1/2000, over night; biotinylated swine anti-rabbit IgG 1/2000, 2 h; alkaline phosphatase conjugated avidin 1/6000, 2 h.

RESULTS AND DISCUSSION

The Hp subtype patterns obtained with bloodstains from case work are shown in fig.1. The common subtypes were easily recognizable. The 2FF bands were more distinct than by agarose gel electrophoresis (Thymann et al. 1989), but it was difficult to distinguish the type 2SS-2FS from the type 2FS. This has to be taken into consideration when type 2FS stains are encountered. The method is more sensitive than Hp subtyping by agarose gel electrophoresis and therefore better suited for the analysis of stains. The technique is also useful for reexamination of weak or doubtful Hp patterns found by our routine agarose method for Hp subtyping of serum samples. Normal serum samples could be subtyped in dilutions of 1 in 64. Hp subtyping of 26 control stains of various age was compared to Hp typing by polyacrylamide gradient gel electrophoresis. No discrepancies were found. Stains up to 24 months old were succesfully subtyped. In a blind trial comprising 14 one month old bloodstains correct results were obtained for 13 stains, for the last one the type (disclosed as 2SS-2FS) could not be determined. 30 stains from case work were examined. Subtyping and grouping by conventional Hp typing was possible with 22 stains and the results were in agreement. 4 stains could be classified within the main groups whearas subtyping was doubtful. 4 stains could not be typed by any of the methods



Hp subtype pattern of case work stains

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