SIMPLE METHOD FOR HAPTOGLOBIN SUBTYPING

D. Patzelt and H. Schröder
Institute of Forensic Medicine, Humboldt-Universität,
GDR - 1040 Berlin

Hardly any forensic use is being made of the informative Hp subtype polymorphism because of the need for Hp purification which so far has been considered time-consuming. The introduction to forensic serology of highly sensitive iso-electric focusing encouraged some rethinking of the problem of Hp preparation. Proceeding from existing experience, we have succeeded in developing a practicable method which meets the demands on paternity assessment.

Material and methods

The technique described is primarily based on Smithies' specifications (Smithies et al., 1962) which were modified in line with suggestions made by Schössler et al. (1979) and Shibata et al. (1982). Serum haptoglobin is linked through a batch technique to a DEAE cellulose ion-exchanger which can be re-separated from the serum by centrifugation and subsequently washed. The amount of haptoglobin eluated from the ion-exchanger by addition of ammonium acetate solution (between ten and 15 per cent of the original quantity) is obtained from supernatant again by centrifugation and is then reductively decomposed by means of urea-mercapto-ethanol solution and carboxymethylated by addition of iodine acetamide. The preparations can then be cracked by iso-electric focusing either immediately or on the next day.

A diagram of the steps of Hp preparation is depicted in Fig. 1.

Focusing data may be seen from Table 1. More than 1,000 sera have so far been tested from clinically intact blood donors or persons involved in paternity assessment procedures in Berlin.

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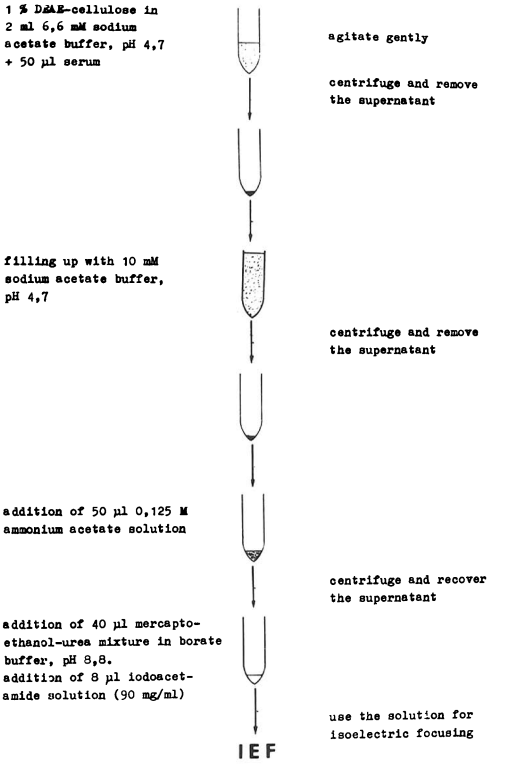

Fig. 1: Steps of haptoglobin preparation

Hp - Subtyping

Gel	polyacrylamide gel, T=5,5%, C=3% 260 x 125 x 0,5 mm (16 ml)
Carrier ampholytes (LKB ampholine)	2,2% (0,4 ml pH 3,5-10, 0,4 ml pH 5-7, 0,2 ml pH 6-8)
Electrode solutions	
anode	0,5 M H_3PO_4
cathode	0,5 M NaOH
Samples	ca. 10 µl purified and reductive cleavaged serum haptoglobin (paper pieces 10 x 5 mm)
Application area	at cathodic end of the gel
Maximum electric values	P = 10 W, U = 1600 V, I = 10 mA
Cooling temperature	10 °C
Prefocusing	30 min
Focusing with samples	30 min
Focusing without samples	2 h
Total time of focusing	3 h
Fixation and staining	simultaneously by formaldehyde-ethanol and Coomassie R 250 according Steck et al. (1980)
Destaining	as required

Table 1: Data for isoelectric focusing of Hp-α peptides

Results

The results obtained from iso-electric focusing of haptoglobin-α peptides, as have been prepared by our method, are depicted as diagram in Fig. 2 and in original in Fig. 3. The following allelic frequencies were recorded from the persons mentioned in the area of Berlin/GDR:
Hp *1F = 0.1472; *1S = 0.2500; *2FF = 0.0020; *2FS = 0.5757; *2SS = 0.0243.

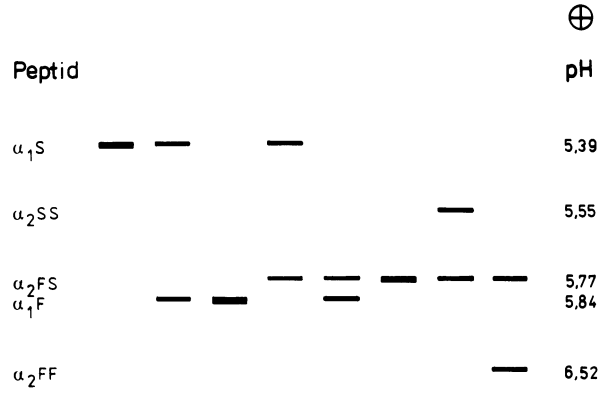

Fig. 2: Diagram of Hp-α peptide mobility in response to isoelectric focusing

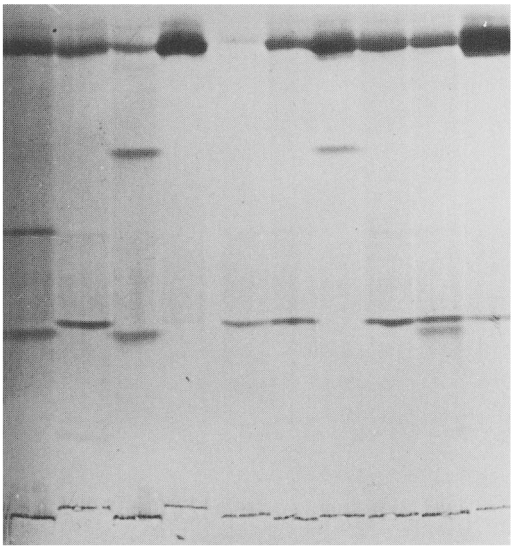

Fig. 3: Representation of Hp subtypes; types, from left to right, are: Hp 1F-2SS, 2FS, 1F-1S, 2FS? 2FS, 2FS, 1S, 2FS, 1F-2FS, 2 FS

Discussion

This method can be used in routine representation of haptoglobin subtypes for both paternity assessment and studies into population genetics. Serum preparation of about 40 serum samples, as can be separated in one operation with LKB multiphor hardware, takes about one hour. We feel that the favourable distribution of types, which resulted in a paternity exclusion chance of about 33 per cent in the region reviewed, is likely to justify the slightly increased consumption of time which proved to be absolutely comparable, for example, to the preparation of stroma-free haemolysates or thrombolysates. A comparison of results obtained from phenotyping by means of starch gel electrophoresis (three phenotypes) with those recorded from isoelectric focusing (15 phenotypes) revealed compatibility in all cases. The limitation must be added that this method is applicable only to typing of haemolysis-free sera, since, with the pH chosen for the ion-exchanger, the Hp-Hb complex,with its higher iso-electric point in comparison to free Hp, cannot be separated. This would eliminate the tracing function of haptoglobin heterogeneity.

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