HAPTOGLOBIN SUBTYPING BY AGAROSE ISOELECTRIC FOCUSING

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INTRODUCTION

Some isoelectric focusing methods have been described for Haptoglobin subtyping on polyacrylamide gels [Olaisen et al. 1981, Shibata et al. 1982, Teige et al. 1985, Patzelt et al. 1985]. The aim of the present investigation was to develop an alternative method for Hp subtyping in a forensic laboratory.

We used an immunoprecipitation with subsequent separation of reduced and alkylated Hp [Patzelt et al. 1986, Teige et al. 1988] in agarose isoelectric focusing. We here described the band patterns for the common phenotypes and the gene frequencies of Hp-subtypes in a south-western Swiss population. Furthermore, we investigated the possibility of Hp determination in corpse plasma, in hemolyzed corpse blood and in stored hemolyzed blood samples containing between 50-300 mg/100 ml alcohol.

MATERIALS AND METHODS

Preparation of plasma samples

20 uL plasma
12 uL rabbit anti-human Hp (Dako)
80 uL polyethylenglycol solution (8% PEG 6000, 1.7% NaCl, 0.2% NaN3)
incubate overnight at 4 °C
centrifuge and wash with 1 ml NaCl
add 20 uL of a solution for reductive cleavage and mix with the immunoprecipitate [1 g urea and 22 mg dithiothreitol in 1.2 ml of a borate buffer pH 8.8 (0.1 M boric acid, 0.04 M sodium hydroxyde)]
incubate 45 min at 37 °C
add 8 uL of a iodacetamide solution (100 mg/mL)
incubate 15 min at room temperature
immediately freeze at -20 °C

Pretreatment of hemolyzed blood samples

60 uL hemolyzed blood
150 uL methylenchlorid
mix, centrifuge and collect the aqueous supernatant
then, prepare like plasma samples

Agarose gel 260x125x0.5 mm (16 mL)

0.2 g agarose Pharmacia
2.0 g sorbitol
1000 uL Ampholine pH 5-6.5 (LKB)
400 uL Ampholine pH 3.5-9.5 (LKB)

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Electrophoresis at 8 °C

Electrode solutions :	anode 1 M H_3PO_4 cathode 1 M NaOH
Samples :	20 uL on 10x5 mm filterpaper applied 0.5 cm from the cathode after drying 10 min
Prefocusing :	30 min at 1600 V, 10 mA, 5 W
Focusing with samples :	30 min at the same setting
Focusing without samples :	60 min at 1600 V, 10 mA, 10 W
Fixation and staining :	60 min with formaldehyde-ethanol and Serva Blue G solution according to Steck et al (1980)
Destaining :	according to need

RESULTS AND DISCUSSION

We have determined the Haptoglobin phenotypes in 500 fresh plasma samples and in 100 corpse plasma samples by agarose isoelectric focusing and by polyacrylamide electrophoresis [Heredero et al. 1974]. No discrepancies between the two methods were noticed.

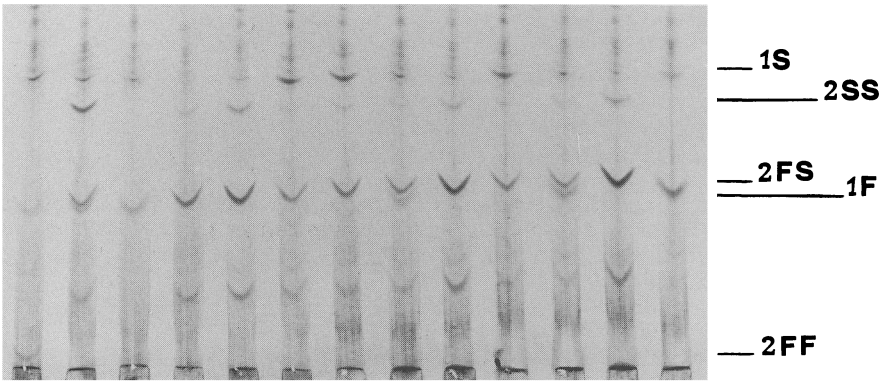

Fig. 1. Hp in plasma as obtained with IEF in agarose. From left to right : 1F-2FF;2FS-2SS;1F;2FS;2FS;1S-2FS;1S-2FS;1F-2FS;2FS;1S-2FS;1F-2FS;2FS;1F.

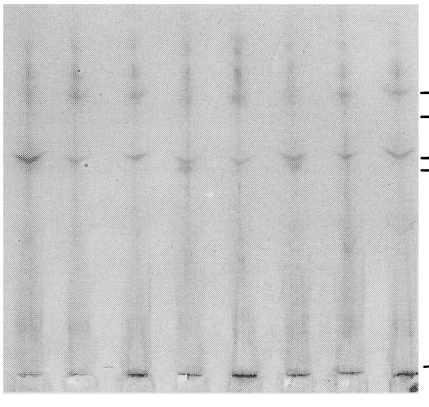

Fig. 2. Hp in hemolyzed corpse blood as obtained with IEF in agarose. From left to right : 2FS;1S-2FS; 1S-2FS;1F-2FS;1S-2FS; 1F-2FS;1S-2FS;1S-2FS.

Table 1. Distribution of Hp phenotypes among 500 unrelated blood donors from south western Switzerland.

phenotype	observed n°	%	expected n°		allele frequencies
1F	12	2.4	10.8	0.13	1F = 0.147
1F-1S	37	7.4	36.8	0.00	1S = 0.249
1S	28	5.6	31.3	0.34	2FF = 0.003
1F-2FF	0	0.0	0.3	0.30	2FS = 0.567
1S-2FF	0	0.0	0.5	0.50	2SS = 0.034
2FF-2FS	3	0.6	1.15	2.97	
2FF-2SS	0	0.0	0.05	0.05	
2FF	0	0.0	0.0	0.00	
1F-2FS	81	16.2	84.65	0.15	
1S-2FS	147	29.4	144.0	0.06	
2FS-2SS	17	3.4	14.45	0.45	
2FS	161	32.2	165.65	0.13	
1F-2SS	5	1.0	3.7	0.45	
1S-2SS	9	2.2	6.3	1.15	
2SS	0	0.0	0.3	0.30	

The common Hp subtype pattern obtained by agarose IEF of immunoprecipitated and cleaved Hp are shown in Fig. 1. The observed and expected phenotype frequencies and the corresponding gene frequencies among 500 unrelated blood donors from south western Switzerland are reported in table 1. Our gene frequencies are in good agreement with those established in other European countries [Thymann et al. 1977; Olaisen et al. 1981; Shibata et al. 1982; Patzelt and Schröder 1986, 1988; Teige at al. 1986, 1988; Zischler et al. 1987, 1988; Bertrams et al. 1988; Hjalmarson 1988]. Furthermore, we have determined the Hp subtypes in 100 corpse bloods and in 100 hemolyzed blood samples stored for five months and containing 50-300 mg/100 mL alcohol. The corpse blood samples were tested in two ways : first, we have determined the Hp subtypes in the plasma samples, secondly, the hemolyzed corpse blood were analyzed after freezing at -20 °C. The patterns of the hemolyzed corpse blood were the same as those obtained from the corresponding plasma samples.

Hp subtypes can be determined in hemolyzed blood samples as far as free hemoglobin is removed with methylenchlorid precipitation (Fig. 2). Without this pretreatment, the differentiation of Hp pattern becomes difficult as an additional band at 1S localisation appears.

In conclusion, the agarose IEF method presented here can be recommended for the determination of Hp subtypes in plasma and hemolyzed blood samples in forensic practice.

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