Haptoglobin Subtypes in the Swiss Population: Phenotype and Gene Frequencies - Description of an Easy Method for Routine Typing

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INTRODUCTION

We describe a technique which proved to be reproducible and easy to perform in routine testing.

In principle:
1. Isolation of haptoglobin (Hp) by immunoprecipitation
2. Reduction and alkylation in presence of urea
3. Isoelectric focusing
4. Protein staining

MATERIAL AND METHODS

Serum of 699 blood donors and EDTA-plasma of 567 unrelated individuals and 248 children engaged in cases of disputed paternity

Reagents:
- Acrylamide / Bis Solution: 14.55 g Acrylamide / 0.45 g N,N'Methylenediacrylamide dissolved in distilled water ad 100 ml
- Anolyte: 1 M H_3PO_4 - Catholyte: 1 M NaOH
- Staining Solution: 0.5 g Serva Blue R dissolved in 90 ml Ethanol / 50 ml Formaldehyde 36.5 % and 210 ml distilled water added
- Destaining solution: 250 ml Ethanol / 10 ml Formaldehyde 36.5 % and 750 ml distilled water added
- Anti-Haptoglobin: Rabbit-Anti-Human Haptoglobin, IgG fraction, Dakopatts, Code Q 330
- Precipitating aid: 8 g Polyethyleneglycol 6000 / 1.7 g NaCl / 0.2 g NaN_3 dissolved ad 100 ml in distilled water
- Reducing agent (freshly prepared): 1 g urea dissolved in 1.2 ml Borate Buffer (0.1 M Boric acid adjusted with 0.04 NaOH to pH 8.8) / 20 µl 2-mercaptoethanol or alternatively 22 mg DL-Dithiothreitol added
- Alkylating agent: 100 mg Iodoacetamide dissolved in 1 ml distilled water

Gel:
- Cassette: 260 x 125 x 0.5 mm / support: Gelfix for PAGE membrane (Serva) / cover: glass plate treated with Repel Silane
- Gel: Acrylamide / Bis solution 5.6 ml / Glycerol 87 % 2.25 ml / LKB Ampholine pH 5 - 7 675 µl / pH 6 - 8 225 µl / pH 3.5 - 5 225 µl / pH 3.5 - 9,5 113 µl / Servalyte pH 5 - 6 225 µl / distilled water 8.1 ml / degas in vacuum, then add TMED 56 µl / 1% Ammoniumpersulfate, freshly prep. 560 µl / pour mixture into precooled (4° C) cassette / polymerize at room temperature for at least 3 hours.
- Sample preparation: to Eppendorf centrifuge tube successively add precipitating aid 80 µl / EDTA plasma or serum 20 µl (if Hp conc. is low, add more sample, max. 160 µl) / anti-Hp antiserum 12 µl / vortex and keep at 4° C over night / centrifuge (8000 g) for 4 minutes / discard supernatant / wash residue with 500 µl saline / remove washing fluid completely / dissolve residue in 20 µl of reducing agent and mix / incubate at 37° C for 45 minutes / add 8 µl of alkylating agent and mix / after 15 min. samples may be applied on IEF gel or they may be frozen at -20° C for later separation

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IEF:
- Settings of power supply (max.): 1600 V, 10 mA, 15 W, constant throughout the whole procedure, temperature of cooling plate: 10^{o} C
- Prefocusing: 30 min.
- Sample application: transfer the total of prepared samples on pieces of Whatman No 1 filter paper 5 x 17 mm which have been prealigned on application tape / apply 1.5 cm from cathode
- Focusing: use same setting of power supply / focus for 45 min. / remove application tape with filter paper pieces / continue focusing under same conditions for another 90 min.
- Staining: immerse gel for 90 min. in staining solution at room temperature on a rocking table / destain with changes of destaining solution until a colourless background is achieved / for preservation the gel may be dried at room temperature and covered with a transparent membrane.

RESULTS AND DISCUSSION

The Hp subtype pattern is shown in figure 1. Phenotype and gene frequencies are presented in table 1. They are in good agreement with the expected ones according to the Hardy-Weinberg law.

Gene frequencies correspond rather well with observations in other European populations (1-5). In a total of 248 mother/child pairs from cases of disputed paternities no "impossible" phenotype combinations were observed.

The isolated chance of exclusion of a falsely accused father almost doubles (from about 18% to 33%) by subtyping compared to the classical typing. This is also illustrated by our observation of 22 exclusions in the Hp subtypes during the last few months among our routine paternity cases, where only 10 among them were also excluded by the classical Hp typing. All of these 22 exclusions were corroborated in one or more other systems.

Hypohaptoglobinemia due to clinical reasons occur quite often and are usually not readily typable. With immunoprecipitation such samples can be typed by simply increasing the amount of sample volume in the sample preparation step.

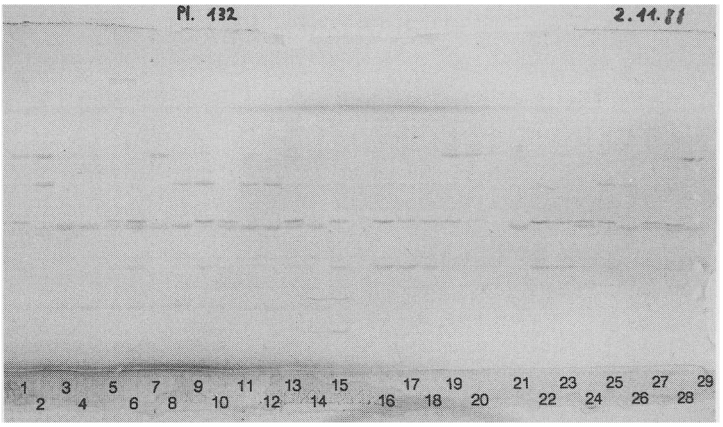

Figure 1. IEF gel of Hp subtypes. 1S-2FS (1, 19, 20); 1S-2SS (2); 1F (3, 4, 21); 1F-2FS (5, 6, 10, 13, 24, 27, 28); 1F-1S (7, 29); 1F-2SS (8, 11, 12, 26); 2FS-2SS (9, 25); 1F-2FF (14); 2FF-2FS (15); 2FS (16, 17, 18, 22, 23)

Table 1. Phenotype and Gene Frequencies of Haptoglobin in the Swiss Population

Hapto Subtypes	observed N	%	expected N	%	chi-sq.	Hapto Genefreq.
1F	21	1.66	20.10	1.59	0.0403	0.1260
1F-1S	70	5.53	76.22	6.02	0.5076	
1F-2FF	2	0.16	3.15	0.25	0.4198	
1F-2FS	192	15.16	185.96	14.69	0.1962	
1F-2SS	13	1.03	13.48	1.06	0.0171	
1S	64	5.05	72.28	5.71	0.9485	0.2389
1S-2FF	2	0.16	5.97	0.47	2.6400	
1S-2FS	386	30.49	352.68	27.86	3.1480	
1S-2SS	19	1.50	25.57	2.02	1.6881	
2FF	0	0	0.12	0.01	0.1200	0.0099
2FF-2FS	18	1.42	14.57	1.15	0.8075	
2FF-2SS	3	0.24	1.06	0.08	3.5506	
2FS	407	32.15	430.21	33.98	1.2522	0.5829
2FS-2SS	66	5.21	62.37	4.93	0.2113	
2SS	3	0.24	2.26	0.18	0.2423	0.0423
Total	1266	100	1266	100	15.7894	1
d.f. = 10	$0.10 < p < 0.20$					

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