# Subtyping of haptoglobin

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The haptoglobin molecule consists of α- and β-chains linked with disulfide bridges. The isoelectric heterogeneity observed in the β-chain is due to its content of sialic acid. The α-chain shows genetically determined structural polymorphism, and the most common subtypes 1S, 1F, 2FS, 2SS, 2FF and Johnson may be separated by isoelectric focusing (1,2). A subtyping method is developed which is well suited for large scale haptoglobin subtyping, and which requires no purification of the haptoglobin molecule prior to isofocusing (3): Five µl serum is treated with neuraminidase and reduced. Approximately one third of this mixture is subjected to polyacrylamide gel isoelectric focusing, and the remaining sample may be frozen and applied on a new gel if necessary. The neuraminidase treatment is included in order to diminish background staining, and to avoid interference between the isoelectric band patterns of the α- and β-chains. The Hp band pattern is visualized with an immunoblotting procedure using anti human haptoglobin as the first antibody and a peroxidase conjugated second antibody. In figure 1 is presented an immunoblot showing Hp-subtypes in serum samples from routine cases of disputed paternity.

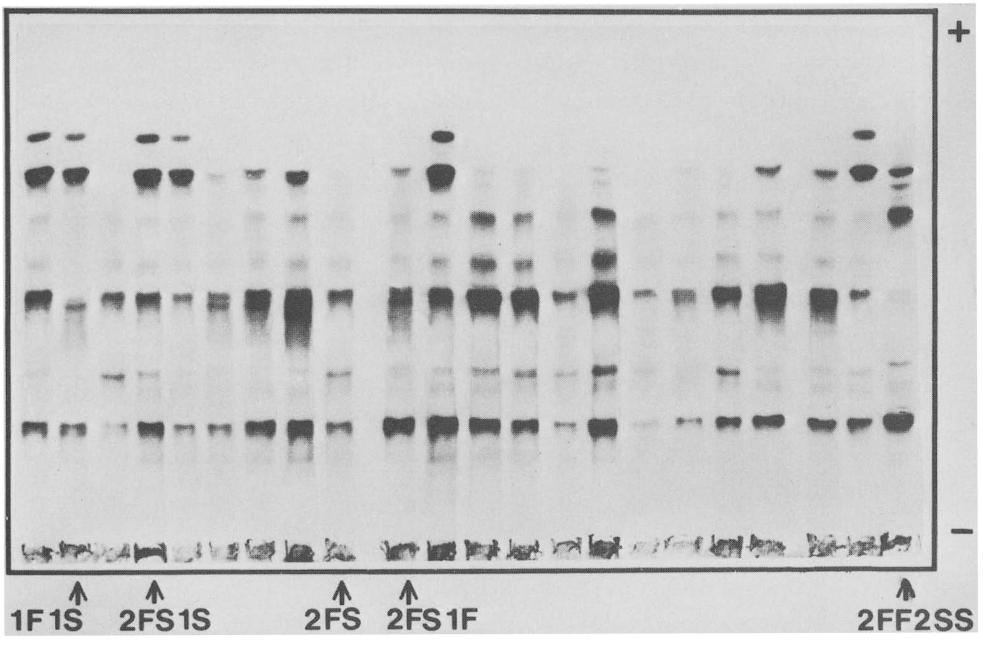


*Figure 1. Haptoglobin isofocusing band patterns of neuraminidase treated serum samples. The method is described in the text.*



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Neuraminidase treated β-chains with different isoelectric points, can be detected by the described subtyping method. One such β-chain variant is haptoglobin Marburg (4). The difference in isoelectric points between normal neuraminidase treated β-chain and neuraminidase treated Marburg β-chain has been demonstrated earlier by two-dimensional electrophoresis (5).

A new β-chain variant has been detected with the described method during routine Hp-subtyping of serum in cases of disputed paternity. After neuraminidase treatment this β-chain variant has a more basic isoelectric point than both normal and Marburg neuraminidase treated β-chains. The new β-chain variant and Marburg are demonstrated in figure 2. Two-dimensional electrophoresis has verified that the observed variant is due to a variation in the β-chain; figure 3. The two-dimensional pattern shows that the new β-chain variant has the same molecular weight as normal β-chain. It further confirms the one-dimensional isofocusing observation; that the two β-chains have different isoelectric points. The starch gel electrophoretic Hp-pattern of serum containing the variant, was that of a normal Hp2.

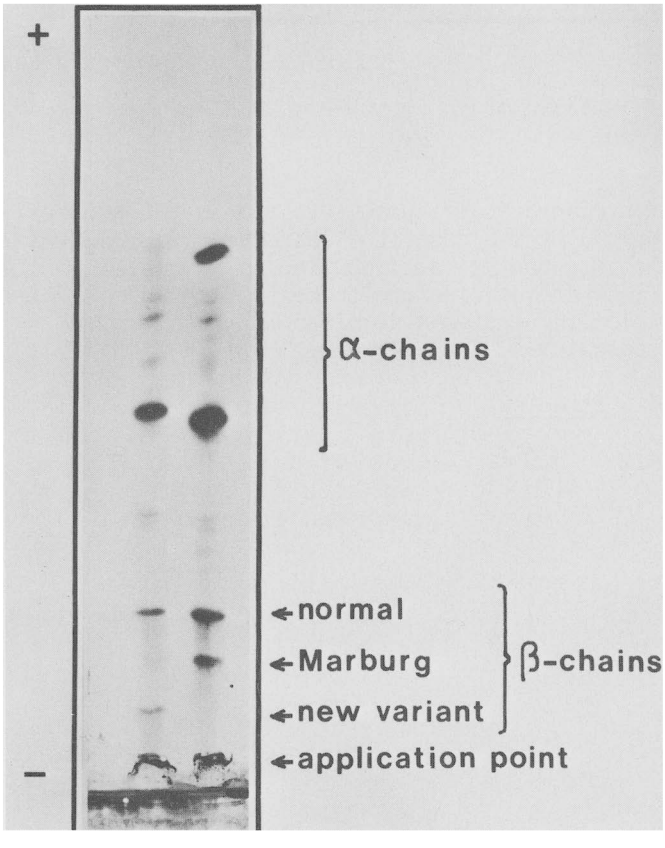


*Figure 2. Haptoglobin isofocusing band patterns of two neuraminidase treated serum samples demonstrating β-chain variants.*

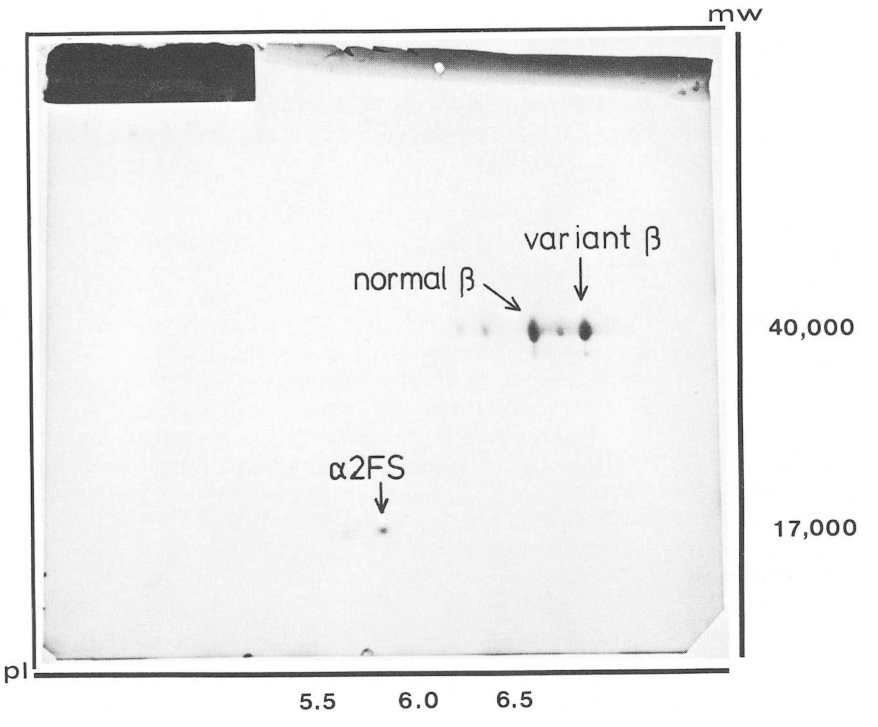


*Figure 3. Two-dimensional blot demonstrating the new Hp β-chain variant.*

Sixhundred and six unrelated individuals involved in paternity cases have been subtyped by the described isofocusing/immunoblot method. The haptoglobin phenotype distribution is presented in table 1, and the allele frequencies are presented in table 2. For comparison, the allele frequencies determined in three earlier works (1,2,6) have been included in table 2.

| Hp-types | Observed | Expected |
|---|---|---|
| 1S | 23 | 26.5 |
| 1F | 10 | 15.9 |
| 1F1S | 43 | 41.0 |
| 2FS | 207 | 209.5 |
| 2FS2SS | 23 | 27.1 |
| 2FS2FF | 2 | 2.1 |
| 2SS2FF | 1 | 0.1 |
| 2SS | 0 | 0.9 |
| 2FF | 0 | 0.0 |
| 2FS1S | 150 | 148.9 |
| 2FS1F | 124 | 115.5 |
| 2SS1S | 13 | 9.6 |
| 2SS1F | 9 | 7.5 |
| 2FF1S | 1 | 0.8 |
| 2FF1F | 0 | 0.6 |
| N= | 606 | 606.0 |

*Table 1. Haptoglobin phenotype distribution in 606 unrelated individuals involved in cases of disputed paternity.*

*Table 2. Haptoglobin allele frequencies in 606 unrelated individuals involved in cases of disputed paternity.*

HP ALLELE FREQUENCIES

| | 1F | 1S | 2FS | 2SS | 2FF | |
|---|---|---|---|---|---|---|
| Olaisen et al (Norway) | 0.13 | 0.21 | 0.63 | 0.03 | | (N=52) |
| Shibata et al (France) | 0.139 | 0.245 | 0.547 | 0.045 | 0.012 | (N=202) |
| Thymann (Denmark) | 0.16 | 0.27 | 0.54 | 0.03 | | (N=208) |
| This material (Norway) | 0.162 | 0.209 | 0.588 | 0.038 | 0.003 | (N=606) |

By looking at men excluded in other systems than Hp, it is possible to evaluate the exclusion efficiency of the Hp-system in a material of non-fathers. So far our material consists of 85 non-fathers. As seen from table 3, Hp excluded 11 of these men when typed by starch gel electrophoresis and 26 when subtyped by the isofocusing/immunoblot technique.(During a test period all serum samples arriving at the Institute in connection with cases of disputed paternity are Hp-typed with conventional starch gel electophoresis and with the new isofocusing/immunoblot method.) The results from this small preliminary study look promising regarding the usefulness of the new Hp-subtyping system in cases of disputed paternity.

*Table 3. Haptoglobin paternity exclusion efficiency in a material of 85 non-fathers*[1]

| | Hp (starch-gel) | | Hp (new method) | |
|---|---|---|---|---|
| | observed | (expected) | observed | (expected) |
| Number of Hp exclusions | 11 | (15.3) | 26 | (27.2) |
| % Hp exclusions | 12.9 | (18) | 30.6 | (32) |

[1] *excluded in other systems*

One advantage exhibited by the new subtyping method is the small amount of serum necessary. Five µl serum is enough for at least 3 gel applications. Another advantage seems to be its ability to detect Hp in sera with rather low Hp content. Serum samples from infants are often small and low in Hp. So far only 42 infants less than 4 months old have been typed by both methods. In this small material, the isofocusing/immunoblot method typed 93% (n=39), and the starch-gel method 83% (n=35).

Conclusion

The isofocusing/immunoblot method

1) detect isoelectric α- and β-chain variants

2) is well suited for large scale routine Hp-sybtyping

3) indicates approx. 30% paternity exclusion efficiency

4) indicates increased sensitivity compared with conventional starch gel electrophoresis

References

1. Olaisen B,Mevaag B,Teisberg P,Gedde-Dahl T Jr,Helland R,Siverts A,Jonassen R,Hjalmarsson K,Swan T. Plasma protein polymorphisms in two-dimensional electrofocusing/SDS electrophoresis. Proceedings. 9.International Congress of the Society for Forensic Haemogenetics, Bern 29.9.-3.10 1981 pp 543-555

2. Shibata K,Constans J,Vian M,Matsumoto H. Polymorphism of the haptoglobin peptides by isoelectric focusing electrophoresis and isoelectric point determinations. Hum Genet 61:210-214(1982).

3. Teige B,Olaisen B,Pedersen L. Subtyping of haptoglobin - Presentation of a new method. Hum Genet 70:163-167(1985).

4. Von Weerts G,Nix W,Deicher H. Isolierung und nähere Charakterisierung eines neuen Haptoglobins:Hp-Marburg. Blut 12:65-77(1966).

5. Teige B,Mevaag B,Olaisen B,Pedersen L. Haptoglobin subtyping in routine paternity cases - presentation of a promising new metod. Proceedings, 10.International Congress of the Society for Forensic Haemogenetics, München,11-15.10 1983 pp 373-379.

6. Thymann M. Improved haptoglobin subtyping demonstrating polymorphism in the Danish population. Proceedings. 7.International Congress of the Society for Forensic Haemogenetics, Hamburg 25.-29.9 1977 pp 187-192.