TECHNICAL NOTE

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A Simplified Method for Phenotyping Alpha-2-HS-glycoprotein

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ABSTRACT: Alpha-2-HS-glycoprotein is considered a reliable genetic marker for paternity testing and forensic investigation.

We describe a simple, semiautomated method for phenotyping this protein in serum samples, bloodstains and dental pulp using isoelectric focusing methods followed by immunofixation techniques. This method produces clear results even with very old biological evidence.

KEYWORDS: pathology and biology, phenotyping, forensic science, paternity testing, blood, dental pulp

Alpha-2-HS-glycoprotein (AHSG) is a serum protein composed of two polypeptide chains having a molecular weight of 49 000 daltons [*1*].

It has an isoelectric point of 5.5 and between 4.5–5.0 in its desialylated form. It is synthesized in the liver and its serum concentration ranges between 40–85 mg/dl.

Although its biological function is not well defined, its high concentration in bone tissue (above 300 times the concentration of other serum proteins) makes it probable that AHSG is somehow implicated in bone tissue synthesis [*2*].

Genetic polymorphism of AHSG was first described by Anderson in 1977 using a bidimensional method of electrophoresis [*3*].

Isoelectric focusing methods followed by immunofixation were first used in 1983 [*4*].

Today it is known that AHSG has two common alleles (AHSG*1 and AHSG*2) and 15 genetic variants numbered from 1 to 15 [*5*] and is considered a useful marker for paternity testing and bloodstain analysis. The population frequencies for the common alleles are: AHSG*1 = 0.6–0.7; AHSG*2 = 0.25–0.4.

Several isoelectric focusing techniques have been used with or without neuraminidase treatment of samples, with or without urea treatment of gels, immunoblotting techniques.

We here describe a very simple isoelectric focusing method for AHSG phenotyping using PhastSystem, a dedicated system for horizontal electrophoresis and isoelectric focusing in small gels with automated fixation, staining and destaining.

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TABLE 1—*Isoelectric focusing conditions for programming in the PhastSystem.*

Sample appl. down at 1.2					0 vh
Sample appl. up at 1.3					0 vh
Extra alarm to sound 1.1					73 vh
Sep. 1.1	2000 V	2.0 mA	2.5 W	15°C	75 vh
Sep. 1.2	200 V	2.0 mA	2.5 W	15°C	20 vh
Sep. 1.3	2000 V	10.0 mA	5.0 W	15°C	300 vh

Material and Methods

Sample Selection

We processed serum samples, bloodstains up to 2 years old and dental pulp of 4-year-old teeth. Serum samples were obtained from people coming to our Department for paternity testing; bloodstains were made from normal donors and all teeth were healthy (caries-free) obtained from orthodontic extractions. Bloodstains and teeth were stored at room temperature.

Sample Preparation

Serum was diluted 1:20 in a neuraminidase solution (2% w/v) and the same solution was used for extraction of dental pulp and bloodstains. Samples were incubated overnight at 4°C and then centrifuged at 2000 rpm for 5 minutes; the supernatant was used for assays.

Isoelectric Focusing Conditions

Samples were applied to PhastGel IEF 4–6.5 (Pharmacia) in cathodal position using Sample Applicator 8/1. Isoelectric focusing was performed in the PhastSystem (Pharmacia) according to the method given in Table 1.

After focusing (about 20 minutes), immunofixation was performed using specific immunoglobulins anti-human AHSG (ATAB) diluted 1:5 spread over the surface of the gels and incubated for 20 minutes at 37°C. After an overnight washing in buffered saline and conventional Coomassie staining in the development unit of the PhastSystem, a specific band pattern of AHSG was obtained.

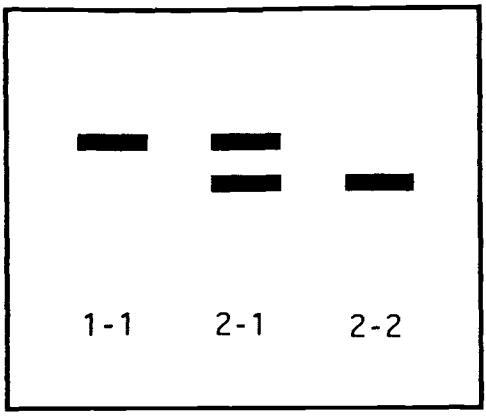
Results and Discussion

The band pattern obtained using the method we propose is very clear and easy to read. We avoid the extra bands that are so common when no neuraminidase pre-treatment is used. We obtained a single band for each allele and a considerable separation between phenotypes (Fig. 1).

There are some other advantages in the method proposed: the gels used are commercially available so there is no need for homemade gels which saves time and makes it easier to standardize.

We also find it unnecessary to use urea and other denaturing agents. Focusing time is about 20 minutes and the development process is automated.

We obtained very clear results with serum samples and in all cases with dental pulp. In the case of very old bloodstains we experienced some difficulty in clearly reading all phenotypes but this problem was rectified by using a conventional silver staining technique (also automated in the Phast System developing unit). Our experience has shown that good results may be obtained by this method with as little as 1 microlitre of sample.



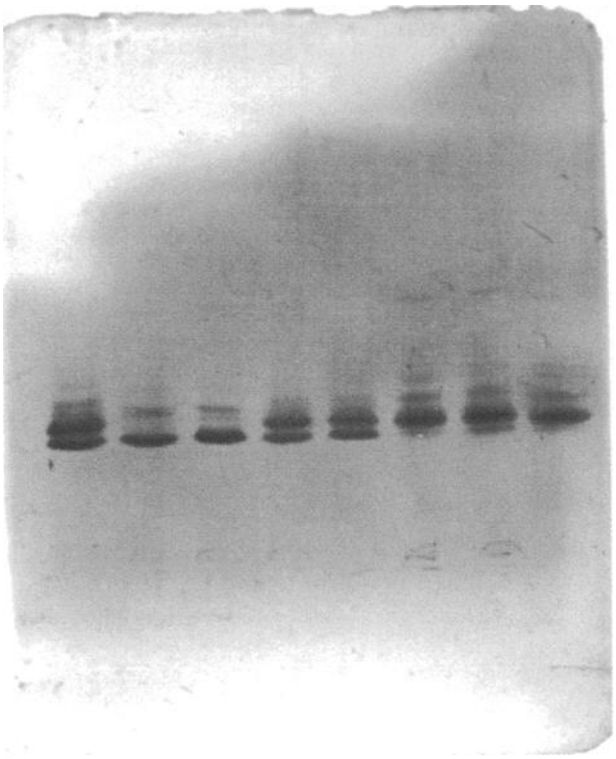


FIG. 1—*A/Band pattern of the common phenotypes of AHSG. B/AHSG phenotypes in PhastGel IEF 4–6.5. Anode is at the top. Phenotypes are from left to right: 2-1/2-2/2-2/2-1/2-1/1-1/1-1/1-1.*

Conclusions

We have developed a simple, reliable, rapid, and sensitive method for AHSG phenotyping of very old bloodstains and dental pulp. Using this method we think that AHSG could be a useful biological marker in paternity testing, investigation of very old bloodstains and for human identification purposes from a single tooth.

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