Sensitivity of a micromethod for Haptoglobin typing

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INTRODUCTION

The Haptoglobin phenotypes can be quickly and easily determined in bloodstains by an electrophoretic micromethod (Dimo et al. 1987), using a discontinuous buffer system according to Heredero (1974) with some modifications.
This new electrophoretic method presents some advantages : the gel preparation is simple and the precipitation of hemoglobin before electrophoresis is not necessary. Furthermore, this technique seems to be very sensitive. The determination of its sensitivity limit is presented here.

MATERIAL AND METHODS

Sample preparation

5 uL of sera samples of three Hp phenotypes at various concentration levels with hemoglobin traces were prepared.

Gel casting

The stacking gel (7.41% acrylamide, 0.19% bisacrylamide was immediately poured on the resolving gel (10.92% acrylamide, 0.28% bisacrylamide, 16.35% glycerol) in the Midget Twincast gel (LKB Instructions) prepared for two 0.75 mm thin gels with 15 well-former Combs.

Polymerization

One hour at room temperature

Electrophoresis

Discontinuous buffer system (Tris/Glycine) proposed by Heredero et al. (1974). Running at 40 mA constant current with two 0.75 mm thin gels.

Staining

O-Tolidine and hydrogen peroxide in acidic medium

Quantitative assay

Determination of the amount of Hp in three phenotypes at increasing dilutions of sera was carried out by rate nephelometric Immunochemistry System (Beckman Instrument) according to Sternberg (1977) and by radial Immunodiffusion (Mancini et al. 1975)

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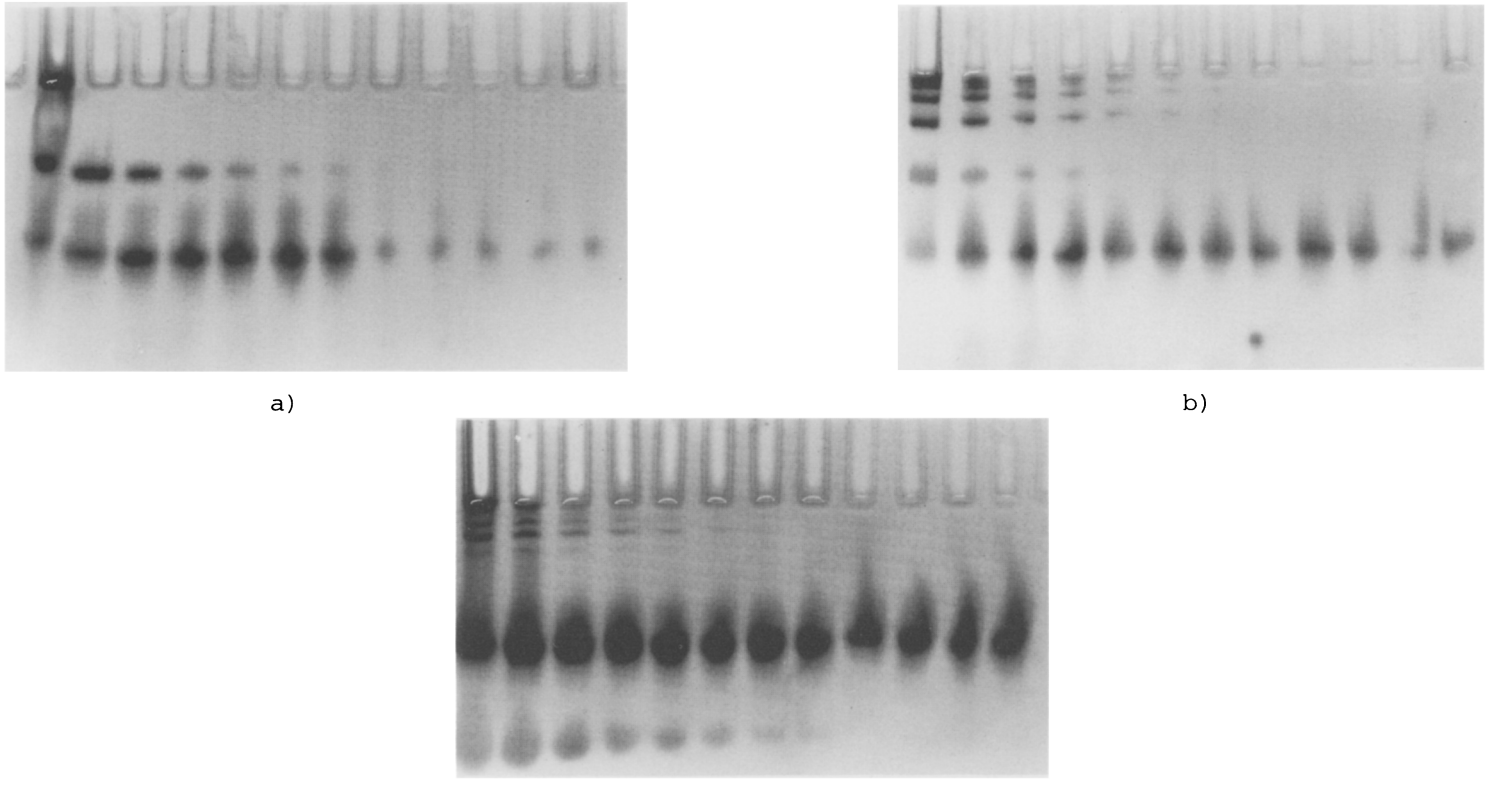

Fig. 1 Electrophoretic pattern of Hp phenotypes at increasing dilutions of serum
a) Hp 1 b) Hp 2-1 c) Hp 2

Table 1. Values obtained by nephelometric rate and Mancini test for the three Hp phenotypes at increasing dilutions of serum.

	NEPHELOMETRY mg/dL			MANCINI mg/dL		
phenotypes :	1	2-1	2	1	2-1	2
serum dilutions						
1/2	31.4	41.3	13.5	91.5	106.0	57.3
1/4	16.6	23.5	6.43	50.5	63.5	35.0
1/8	8.4	11.1	3.47	-	-	-
1/16	4.26	5.28	1.68	-	-	-
1/32	2.24	2.9	0.86	-	-	-
1/64	1.16	1.36	*	-	-	-
1/128	*	*	*	-	-	-
1/256	*	*	*	-	-	-

*) < 0.833 mg/dL

-) not detectable

Reference values : 27 - 139 mg/dL 70 - 380 mg/dL

RESULTS AND DISCUSSION

The interpretation of the electrophoretic pattern for Hp 1, 2-1 and 2 phenotypes is possible in 5 µL of serum at a dilution of 1/512, 1/256 and 1/512 respectively (fig. 1).

The quantitative analysis was carried out by Immunochemistry System (nephelometric rate) and Mancini test. Table 1 shows the values obtained by these two methods. The electrophoretic determination is possible with serum containing 0.833 g/dL of Haptoglobin.

This micromethod is extremely sensitive compared to other electrophoretic techniques using the classical "pseudo-peroxidasic" stain (Hoste 1986). This author has detected the Hp phenotype 2-1 in 10 µL of serum at a dilution of 1/8. To obtain the same sensitivity limit achieved by our method, he has used the enzyme immuno-assay after blotting with three different antibodies.

In conclusion, this procedure is simple and sensitive and can determine Haptoglobin in bloodstains : we have found that 0.02 µL of blood was necessary with experimentally produced stains.

This technique is used as a routine method in our laboratory and has been proved to be successful in forensic case-work.

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