Determination of ORM Phenotypes using gels PRECOTES 4-6® and PhastSystem™

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INTRODUCTION

Miniaturized IEF gels, run and stained with PhastSystem offer a number of advantages over conventional procedures. Two gels (24 samples) can be run simultaneously in the Separation Unit and two others gels can be stained in the Development Unit at same time. The system is better for proteins needing immuofixation, because only a minimal amount of expensiveantisera is needed.

In this paper we describel optimal program for separation of ORM patterns using miniaturized polyacrylamide gels PRECOTES 4-6 (Boheringer Ingelheim). This method has potential advantages not only in paternity cases but also in criminal investigations (e.g. typing a very minute bloodstains).

MATERIALS AND METHODS

Polyacrylamide gels PRECOTES 4-6 (Boheringer Ingelheim) were used. The gel is cut as a minigel size (5 x 5 cm) and pretreated before IEF with a Solution mixture of Servalyt 4-6 (Serva) and Nonidet P-40 (LKB).

The sera samples were diluted in Neuraminidase (type V, Sigma) and distilled water, and incubation 24 h at 4ºC (overnigth).

ORM phenotypes were carried out in Microprocessor PhastSystem (Pharmacia) using our IEF method file. Visualization of results was accomplished by Immuonofixation using anti-ORM (1:1 v/v), incubated 30 minutes at 37ºC in humid chamber and washing with distilled water for 24 h. The day after was staining into the Development Unit with CBB staining techinique.

Equipment: Microprocessor PhastSystem™

Minigel: PRECOTES 4-6 (Boheringer Ingelheim)
cutting slabs for aprppiate size (5 x 5 cm).

Pretreatment minigel: Solution:

Sacarose	0.6 g
Nonidet P-40 (20%)	0.25 ml
Servalyt 4-6 (Serva)	1 ml
Deionized water	5 ml

Place minigel in a chamber and immerse minigel in this solution 1 h at RT

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Pretreatment Samples: Plasme ..... 10 µl
Neuraminidase 2% ..... 80 µl
Distilled water ..... 80 µl
incubated 24 h at 4ºC.

Sample Application: Applicator 8/ 0.5 (cathode)

METHOD FOR IEF PROGRAM PHASTSYSTEM :

Sample appl.	down	at	1.2		0 vh
Sample appl.	up	at	1.2		20 vh
Extra alarm	to sound	at	1.1		70 vh

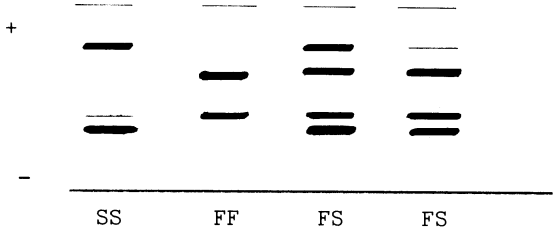
Sep. 1.1	2000V	10.0mA	2.0w	15ºC	75 vh
Sep. 1.2	2000V	5.0mA	1.5w	15ºC	20 vh
Sep. 1.3	2000V	25.0mA	4.0w	15ºC	600 vh

METHOD FOR COOMASSIE BLUE STAINING:

Dev. 1.1	IN=1	OUT=1	t=5 min.	T=20ºC
Dev. 1.2	IN=2	OUT=0	t=5 min.	T=50ºC
Dev. 1.3	IN=3	OUT=0	t=15 min.	T=50ºC
Dev. 1.4	IN=2	OUT=0	t=5 min.	T=30ºC

Immunofixation: anti-ORM diluted 1:1 with 0.9% saline
Directly on the minigel (2 cm anodal) and cover it
Place minigel in a humid chamber for 30 min at 37ºC
Rinse minigel in 0.9% saline for 1 day at RT
Staining into de Development Unit with CBB R-250

Visualization Patterns: ORM (FF, SS, FS)



DISCUSSION

Figure 1 shows the separation of patterns ORM clearly delineated on the minigel. An increase in distance between the cathodal and anodal ORM-bands using narrow ranfe (pH 4-6) and soaked it in solution of pretreatment was observed. The advantages of our improved method being good identification bands, less expensive, fast and easy to work with make this technique and desirable for routine paternity and forensic casework. The disadvantages are no differentiation between phenotypes F_1 and F_2. Further studies with our method migth solve this question.

REFERENCES

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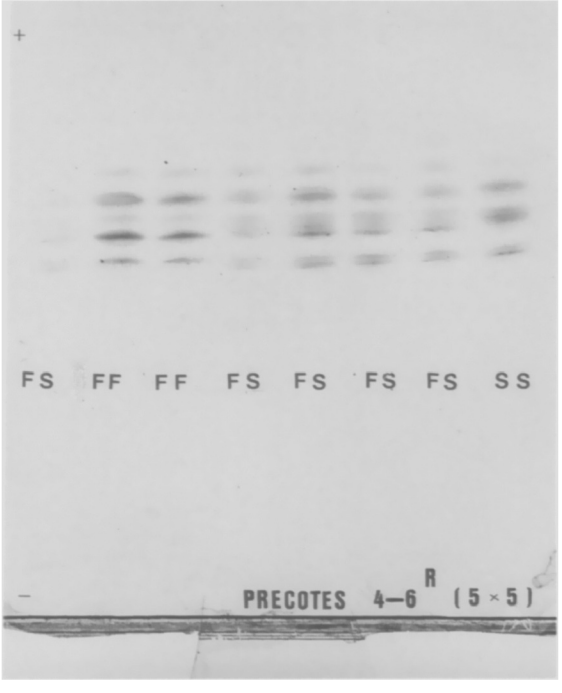

Fig. 1: ORM Patterns bands & PhastSystem