Fast Determination of TF Phenotypes Using Minigel Gradient 4-6.5 Modificated

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INTRODUCTION

Application of minigels and PhastSystem to obtain phenotyping results from blood samples in TF system was proposed by several authors recently. Miniaturized gels disposables commercially offer a number of advantages (no preparation casting gel), but no usually good resolution pattern bands. Microprcessor PhastSystem offer potential advantages in routine testing: faster separations are possible, 24 samples can be run simultaneously (two minigels), while two others minigels can be stained at the same time.

In this paper we propose a technique wich proved to be reproducible, fast and easy to perform in routine TF C testing (C_1, C_2, C_3). Advantages and disadvantages of the method are discussed.

MATERIALS AND METHODS

Conventional minigels gradient 4-6.5 (PhastGel 4-6.5, Pharmacia) and Microprocessor PhastSystem were used. After IEF procedures, a pretreatment of the minigel with a mixture of Servalyt 5-7 (Serva) and Nonidet P-40 (LKB) solution was done.

The sera samples were pretreated with Ferrous Ammonium Sulphate solution and incubated 3 h at 37ºC. TF phenotypes were carried out in Microprocessor PhastSystem using our IEF program method file. Visualization of results are not needing immunofixation and can be stained in th Development Unit with Coomassie Brilliant Blue (CBB R-250) staining technique.

Equipment: Microprocessor PhastSystem™

Minigel: PhastGel 4-6.5 (Pharmacia)

Pretreatment Minigel: Solution composed by:

Sacarose	0.6 g
Nonidet P-40 (20%)	0.25 ml
Servalyt 5-7 (Serva)	1 ml
Deionized water	5 ml

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

Pretreatment samples: sera ..... 10 µl (1:3)
Ferrous Ammonium Sulphate (0.3% w/v)..30 µl

in incubation 3h at 37ºC

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Samples Application: Applicator 8/1.0 or 12/1.0 (cathode).

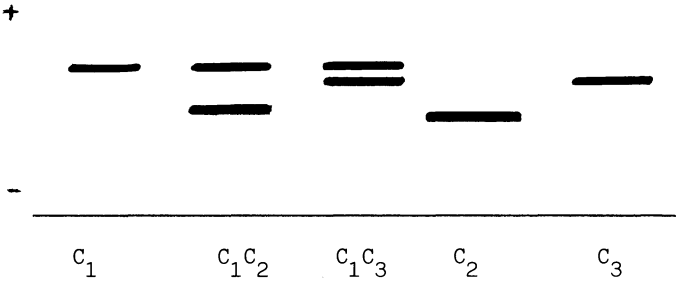
METHOD FOR IEF PROGRAM PHASTSYSTEM:

Sample	appl.	down at	1.2			0 vh
Sample	appl.	up at	1.3			0 vh
Extra	alarm	to sound at	1.4			73 vh
Sep.	1.1	2000V	2.0mA	3.5w	15ºC	75 vh
Sep.	1.2	2000V	2.0mA	3.5w	15ºC	20 vh
Sep.	1.3	2000V	2.0mA	3.5w	15ºC	700 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

Visualization Patterns: TF C (C_1, C_2, C_3)



DISCUSSION

Figure 1 shows the separation of several TF C phenotypes. Immerse minigel in a Solution composed with Servalyt 5-7 and Nonidet P-40 offer results comparable to those described using IEF gel with chemicals spacers. An increase in distance between the cathodal and anodal bands for the minigels using pretreatment solution was observed, specially for detection TF C_3 band.

The advantages of this technique being less expensive, fast procedure, good results and easy to work with make this improved method, highly desirable for routine paternity, population genetics, significant associations with diseases, and forensic practice.

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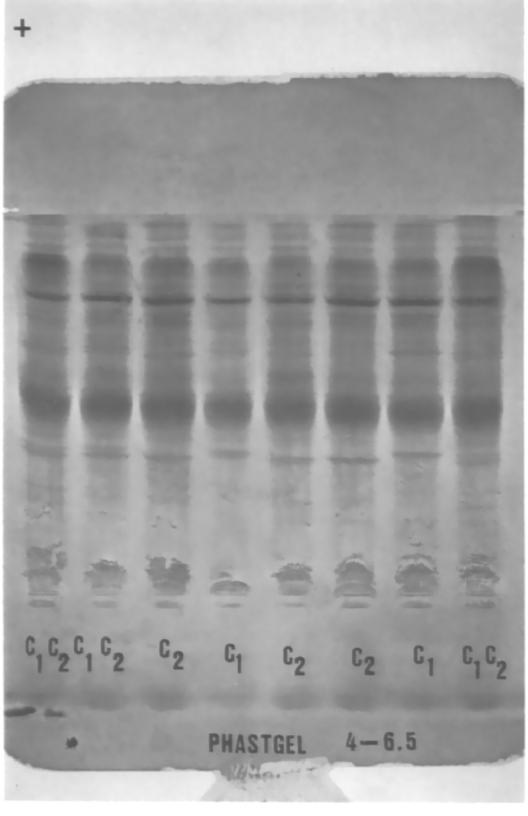

FIG. 1: TF C Patterns bands and PhastSystem