Fast IEF of some polymorphic proteins and enzymes in bloodstains using the PhastSystem

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INTRODUCTION

Although the use of miniaturized gels in electrophoresis was proposed many years ago (Radola 1980), such use did not become widespread until the recente introduction of automated systems. PhastSystem (Pharmacia Fine Chemicals, Uppsala, Sweden) is an automated system wich consists of one separator and one development unit, an a control unit containing the microprocessor, wich controls and monitors both separation and development processers according to programmed methods.
In this paper we describe optimal programs for the separation of polymorphic proteins and enzymes using miniaturized polyacrylamide gels, and discuss the potential advantages and disadvantages of the method.

MATERIAL AND METHODS

Polyacrylamide gels were cast over GelBond PAG films and cut from larger slabs to an appropriate size (5x5 cm).

Gel characteristics

T = 5%
C = 3%
Ampholyte concentration = 2% w/v
Photopolymerization with riboflavin and UV light

pH ranges and ampholyte composition

Marker	Ampholyte composition
α1-antitrypsin (Pi)	Pharmalyte pH 2.5-5; Ampholine pH 3.5-5 (1:1)
Group-specific component (Gc)	Pharmalyte pH 4-6.5; Ampholine pH 4-6 (1:1)
Transferrin (Tf)	Ampholine pH 5-7
Orosomucoid (ORM)	Ampholine pH 4-6
Phosphoglucomutase (PGM1)	Ampholine pH 5-7
Acid phosphatase (ACP)	Ampholine pH 4-6;

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	Ampholine pH 6-8 (1:1)
Esterase D (ESD)	Ampholine pH 4-6;
	Ampholine pH 5-7 (1:1)
Plasminogen (PLG)	Ampholine pH 3-9
Properdin Factor B (Bf)	Ampholine pH 5-7;
	Pharmalyte pH 4-6.5 (1:1)
Factor XIII A (FXIIIA)	Pharmalyte pH 4-6.5;
	Ampholine pH 5-7 (2:1)
Factor XIII B (FXIIIB)	Pharmalyte pH 4-6.5;
	Ampholine pH 5-7 (1:1)
Complement C3 (C3)	Ampholine pH 4-6

Sample application (0.5 µL)

+ : PGM, ACP, Tf
- : ORM, Pi, Gc, ESD, Bf, PLG, FXIII A,FXIII B, C3

Optimized method for IEF program into the separation method file of PhastSystem.

Sample application down at			1.2			0 Vh
Sample application up at			1.3			0 Vh
Extra alarmed to sound at			1.1			70 Vh
SEP 1.1	2000 V	2.0 mA		3.5 W	15 °C	15 Vh
SEP 1.2	200 V	2.0 mA		3.5 W	15 °C	15 Vh
All markers except ACP and Pi						
SEP 1.3	2000 V	5 mA		3.5 W	15 °C	450 Vh
For ACP						
SEP 1.3	2000 V	5 mA		3.5 W	15 °C	350 Vh
For Pi						
SEP 1.3	2000 V	5 mA		3.5 W	15 °C	500 Vh

For ORM, Bf, PLG, FXIIIA, FXIIIB and C3 the gels were immunofixed according to (2) or transfer to Immobilon membranes, according to (3), washed 5h with saline and then stained with CBB.
The enzymes were stained outside the development unit by laying on the gel 2 mL of 1% agarose containing the appropriate substrate. CBB and silver staining were carried out as described in the Phastsystem Manual (Pharmacia. Uppsale. Sweden)

Native PAGE for Hp typing

	Stacking	Separation gel zone
Gel characteristics	T = 5%	T = 7%
	C = 3%	C = 2%

Buffer in gels 0.112 Acetate, 0.112 Tris, pH 0.5
Buffer in strips (0.2% agarose) 0.88 M L-alanine,
0.25 M Tris; pH 8.8

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Separator method for Native-PAGE

Sample application down at			1.2		0 Vh
Sample application up at			1.3		0 Vh
SEP 1.1	600 V	12.5 mA	3.5 W	15 °C	60 Vh
SEP 1.2	600 V	1.0 mA	3.5 W	15 °C	2 Vh
SEP 1.2	600 V	12.5 mA	3.5 W	15 °C	60 Vh

Staining with o-dianisidine (0.2% w/v in 5% acetic acid and 5% H_2O)

DISCUSSION

Miniaturized IEF gels, run and stained with the PhastSystem, offer a number of advantages over conventional IEF in normal size gels. First, faster separations are possible due to short separation distances and the high field strength applied. Second, the separations generally have a good resolution due to efficient cooling and the high number of V/cm. In general, results are much more easily reproduced since all variables can be programmed (e.g. number of V/h, temperature, staining).
Two gels (24 samples) can be run simultaneously in the separation unit while at the same time two other gels can be stained in the development unit.
Although PGM, ACP and ESD phenotypes can be clearly distinguished, the resolution for enzymes is not as good as that for proteins, because diffusion is proportionally higher than in gels of normal size and bands which are close together can appear as a single, broader band. Only those enzymes with fast detection methods and well-separated isoenzymes can be typed with this method. However, the small amount of substrate needed for staining should be kept in mind. Nevertheless IPGs gels or HIEF can be used and in some cases this problem solved.
The system is much more useful for proteins, especially those systems needing immunofixation because only a minimal amount of expensive antisera is needed. Transfer to nitrocellulose is also possible with good results . An other advantage is the fast Coomassie and silver staining due to automation and elevated temperatures. Nevertheless, we recommend developing the last step of silver staining outside the development unit for better control of band intensity. After staining, gels can be dried with a hair-dryer and so provided a permanent record of the results. Gels can also be mounted in frames and projected directly as slides.
Native-PAGE for Hp typing with the PhastSystem gave good and reproducilble results, being an advantageous method over conventional normal size gels.
This system has potential advantages not only for the study of polymorphic proteins in paternity cases and population genetics research, but also in criminal investigations (e.g. bloodstains analysis). Thus, only 0,3 fL of sample need be applied over the gel, and with silver staining methods as little as 1 ng/fL of protein can be detected, thus permitting typing of polymorphic proteins in very minute bloodstains

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