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Fast Isoelectric Focusing of Some Polymorphic Proteins and Enzymes in Miniaturized Gels Using an Automated System

REFERENCE: Carracedo, A., Andrade-Vide, C., Rodriguez-Calvo, M. S., Montiel, M. D., and Lareu, M. V., **"Fast Isoelectric Focusing of Some Polymorphic Proteins and Enzymes in Miniaturized Gels Using an Automated System,"** *Journal of Forensic Sciences*, JFSCA, Vol. 33, No. 6, Nov. 1988, pp. 1379-1384.

ABSTRACT: Optimal programs for the separation of polymorphic proteins and enzymes in miniaturized polyacrylamide gels using an automated system (PhastSystem) are described. The potential advantages and disadvantages of the method and its application to forensic science laboratories are discussed.

KEYWORDS: forensic science, isoelectric focusing, miniaturized gels, automated systems, bloodstain analysis

Although the use of miniaturized gels in isoelectric focusing (IEF) was proposed many years ago [*1-3*], such use did not become widespread until the recent introduction of automated systems.

PhastSystem (Pharmacia Fine Chemicals, Uppsala, Sweden) is an automated system which consists of one separator and one development unit and a control unit containing the microprocessor, which controls and monitors both separation and development processes according to programmed methods.

Methods are programmed using the keyboard. When separation and development methods start functioning, the display shows the actual running conditions, thus permitting the monitoring of the progress of the method.

The separation and control units also contain the separation compartment and the power supply.

IEF, polyacrylamide electrophoresis (PAGE), sodium dodecyl sulfate (SDS)-PAGE, and titration curves can be carried out with the system in miniaturized gels of 5 by 5 cm.

Automated IEF in miniaturized gels and automated systems is being used increasingly in various scientific fields [*4*], including forensic science recently [*5,6*].

This work has been supported by Grant 2654/83 from the "Comisión Asesora de Investigación Cientifica y Técnica (Ministerio de Educación y Ciencia). Received for publication 19 Jan. 1988; accepted for publication 29 Feb. 1988.

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In this paper we describe optimal programs for the separation of polymorphic proteins and enzymes using IEF in miniaturized polyacrylamide gels, and discuss the potential advantages and disadvantages of the method.

Material and Methods

Polyacrylamide gels were cast over Gel Bond PAG films, with a polymer concentration of $T = 5\%$ (T = acrylamide + bis/100) and $C = 3\%$ (C = bis/acrylamide + bis), containing Ampholine (LKB) and Pharmalyte (Pharmacia) carrier ampholytes in a concentration of 22 μM/mL-pH unit. Photopolymerization was carried out with 2% (v/v) riboflavin (BioRad) solution (20 mg/100 mL of distilled water) under ultraviolet light (360 nm).

The pH ranges for the typing of the genetic markers used in this paper can be seen in Table 1.

Optimal programs to feed into the separation method file can be seen in Table 2.

Of the sample (serum or hemolysate), 0.5 μL were applied in the anodal position for phosphoglucomutase (PGM), acid phosphatase (AcP), and transferrin (Tf) and in cathodal position for orosomucoid (ORM), α_1-antitrypsin (Pi), group specific component (Gc), and esterase D (EsD).

After running, gels were placed in the development chamber of the development unit and stained according to the method described in Table 3. For protein polymorphisms investigation in minute bloodstains, gels were stained with the silver staining method described in Table 4. After the silver nitrate step (Step 7), the gels were stained outside in a petri dish.

TABLE 1—*pH ranges and ampholyte composition.*

Marker	Ampholyte Composition
α_1-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 (PGM_1)	Ampholine pH 5–7
Acid phosphatase (AcP)	Ampholine pH 4–6; Ampholine pH 6–8 (1 : 1)
Esterase D (EsD)	Ampholine pH 4–6; Ampholine pH 5–7 (1 : 1)

TABLE 2—*Optimized method for IEF to program into the separation method file of PhastSystem.*

Sample application down at			1.2		0 V/h
Sample application up at			1.3		0 V/h
Extra alarmed to sound at			1.1		70 V/h
SEP 1.1	2000 V	2.0 mA	3.5 W	15°C	15 V/h
SEP 1.2	200 V	2.0 mA	3.5 W	15°C	15 V/h
		ALL MARKERS EXCEPT ACP AND PI			
SEP 1.3	2000 V	5.0 mA	3.5 W	15°C	450 V/h
		FOR ACP			
SEP 1.3	2000 V	5.0 mA	3.5 W	15°C	350 V/h
		FOR PI			
SEP 1.3	2000 V	5.0 mA	3.5 W	15°C	500 V/h

TABLE 3—*Coomassie staining method to program into the development method file.*

Step	Solution	IN Port	OUT Port	Time, min	Temperature, °C
1	Trichloroacetic acid 12%	1	1	5	20
2	30% methanol, 10% acetic acid	2	2	2	20
3	0.02 Coomassie blue R-250 in 30% methanol and 10% acetic acid and 0.1% (w/v) $CuSO_4$	3	0	10	50
4	30% methanol and 10% acetic acid in distilled water	4	0	10	50

TABLE 4—*Silver staining method to program into the development method file.*

Step	Solution	IN Port	OUT Port	Time, min	Temperature, °C
1	10% trichloroacetic acid	1	1	5	20
2	50% ethanol, 10% acetic acid	2	0	5	50
3	7% ethanol, 5% acetic acid	2	0	10	50
4	10% glutardialdehyde	3	0	5	50
5	distilled water	4	0	15	50
6	0.05% (w/v) dithiothreitol	5	0	5	20
7	0.1% silver nitrate	6	0	10	20
8	development and stopping (outside)				

First, they were given two rinses, one in 10-mL distilled water and then with a small amount of developer. The gels were then soaked in the rest of the developer (10 μL of 37% formaldehyde in 10 mL of 3% sodium carbonate) until enough contrast in the bands was obtained. The staining was stopped by adding 10 mL of 2*M* citric acid and shaking for 5 min.

For ORM typing in neuraminidase treated serum, gels were previously immunofixed with anti-ORM (Atlantic Antibodies) diluted 1:2 with saline. Over the gel surface 20 μL of diluted antiserum were applied, and the plates were then incubated in a moistened chamber for 20 min at 37°C. Gels were washed 5 h with saline and then stained with Coomassie blue R-250 as described in Table 3.

The enzymes were stained outside the development unit by laying on the gel 2 mL of 1% agarose containing the appropriate substrate. AcP was stained with 4-methylumbelliferyl-phosphate according to Burdett and Whitehead [7], EsD was stained with 4-methylumbelliferyl-acetate according to Hopkinson et al. [8], and PGM isoenzymes were detected according to Sutton and Burgess [9].

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 as a result of short separation distances and the high field strength applied. Second, the separations generally have a good resolution because of efficient cooling and the high number of volts per centimetres. In general, results are much more easily reproduced since all variables can be programmed (for example, number of volts per hour, temperature, staining).

Two gels (twenty-four 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.

The system is much more useful for proteins, especially those systems needing immunofixation because only a minimal amount of expensive antisera is needed (Fig. 1). Another advantage is the fast Coomassie and silver staining as a result of 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 provide a permanent record of the results. Gels can also be mounted in frames and projected directly as slides.

Conclusion

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 (for ex-

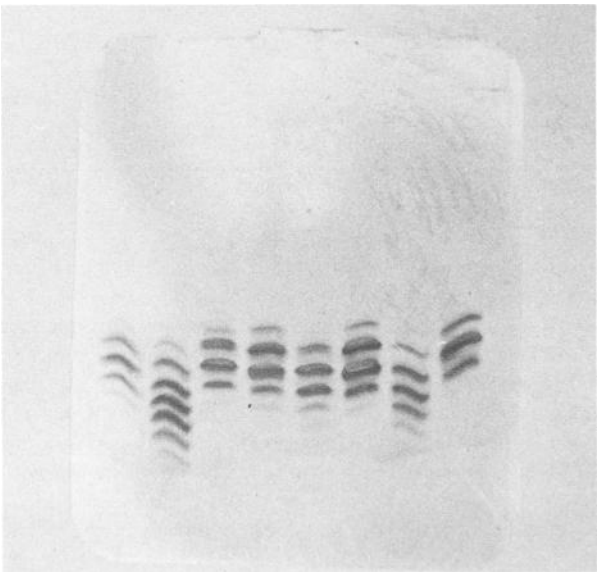

FIG. 1—*ORM phenotypes in miniaturized gels pH 4 to 6 followed by print immunofixation. From left to right, phenotypes: F1S, SS1, F2S, F1S, S, F1S, SS2, and F1. Anode is at the top.*

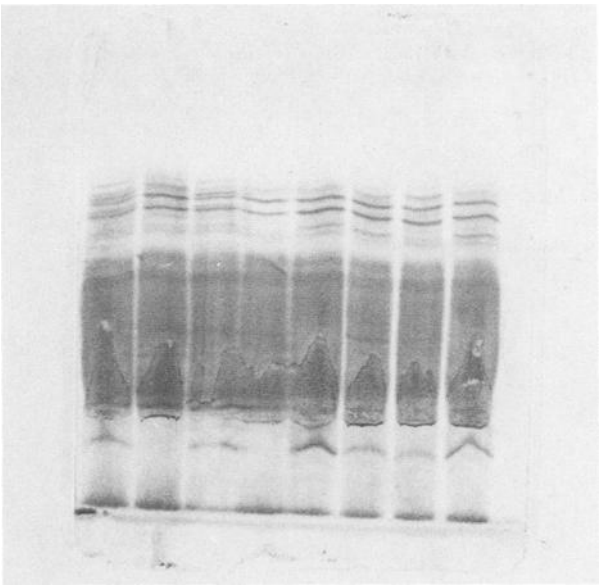

FIG. 2—*Pi phenotypes in minute bloodstains (10 μL of one-week-old bloodstains in 1 mL of distilled water) followed by silver staining. From left to right, phenotypes: M1F, M1, M1F, M1, M1S, M1S, M1M2, and M1S. Anode is at the top.*

ample, bloodstain analysis). Thus, only 0.3 μL of sample need be applied over the gel, and with silver staining methods as little as 1 ng/μL of protein can be detected, thus permitting typing of polymorphic proteins in very minute bloodstains (Fig. 2).

Acknowledgments

The authors thankfully acknowledge Prof. Luis Concheiro and Prof. Fernando Oliveira-Sá, who encouraged a collaboration of the two laboratory staffs, and wish to thank Dr. F. Barros for his help.

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