A Rapid High Resolution Seperator IEF Technique for Phenotyping Alpha-1 Antitrypsin

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INTRODUCTION

1 antitrypsin (Pi) ranks amongst the most informative of the Polymorphic serum proteins used in forensic science (exclusion rate 31% in parentage testing, discriminatory index 74% in criminal investigations). While complete Pi phenotyping usually requires the use of intricate immobilized pH gradients, this presentation outlines a straightforward, rapid, high-resolution separator isoelectric focusing (IEF) technique. Focused bands are often sharp enough to distinguish Pi M subtypes with similar mobilities such as M3 and M4.

With the incorporation of ACES into an ultrathin gel to produce a pH 4.2 - 4.9 gradient flattened in the middle, and by pretreating serum samples with both a reducing and an alkylating agent, Pi M 1, 2 and 3 are fully resolved. The Pi bands are differentiated clearly in the middle of the gel allowing confident phenotyping of the reduced concentration alleles Pi Z and Pi S, even though a general protein stain is used to visualize the bands.

MATERIALS AND METHODS

Gel Composition

The following mixture produces 6 gels with a polyacrylamide matrix of T 5.3%, C 3.8%.

30ml acrylamide premix (26.7g acrylamide, 40.0g Acrylogel (BDH) in 1L H_2O)
5.2ml glycerol (BDH)
2.5ml Pharmalyte 4.2 - 4.9 (Pharmacia)
60mg ACES* (Sigma)
0.3ml 10mg% riboflavin (Sigma)

38ml

Gels measure 230 x 115 x 0.25mm^3 using one layer of Dymo tape to set gel thickness.

* ACES (N-[2-Acetamidol]-2-aminoethanesulfonic acid)

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Serum Pretreatment

30ul serum
10ul 7.5mg/ml dithiothreitol (Sigma)

20 minutes at room temperature, followed by

10ul 9mg/ml iodoacetic acid (BDH)

30 minutes at room temperature (during prefocus)

IEF Details

Samples inserted on 5 x 5mm^2 Whatman No. 1 filter paper, 1cm from cathode edge.

Whatman No. 17 electrode strips soaked in 1M sodium hydroxide (cathode), 1M orthophosphoric acid (anode). Gels run at 10^{o}C.

Stepwise voltages applied as follows:

550 volts	40 minutes
1,100 volts	60 minutes
2,000 volts	30 minutes
2,500 volts	15 minutes
3,000 volts	5 minutes

Visualization

Gel fixed for 5 minutes with 12.5% trichloroacetic acid, then washed briefly in tap water.

Gel stained with 0.04% Serva Blue W (Serva) at 50^{o}C for 10 minutes and destained thoroughly under running tap water.

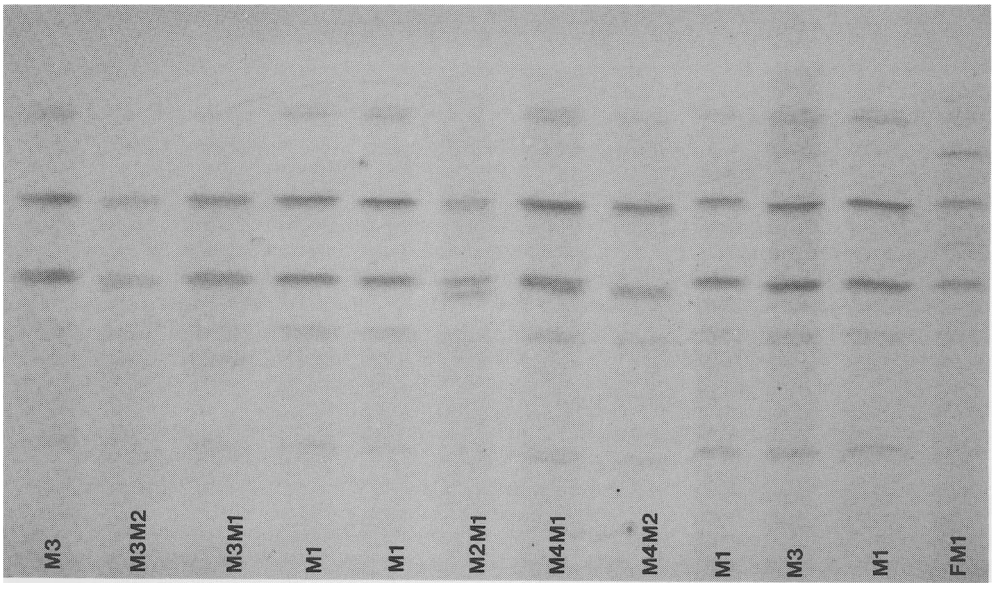

Figure 1

Table 1

Phenotype	Obs.	Exp.
M1	1,018	1,022.154
M2M1	407	426.563
M2	53	44.503
M3M1	403	381.694
M3M2	78	79.644
M3	28	35.633
M1S	143	142.290
M2S	33	29.690
M3S	26	26.567
SZ	4	3.893
FS	1	1.241
SS	3	4.952
M1Z	57	55.933
M2Z	14	11.671
M3Z	9	10.443
FZ	0	0.488
ZZ	0	0.765
FM1	23	17.825
FM2	2	3.719
FM3	1	3.328
FF	0	0.078
M1Mvar	4	4.610
M2Mvar	1	0.962
M3Mvar	1	0.861
MvarS	1	0.321
MvarZ	0	0.126
FMvar	0	0.040
MvarMvar	0	0.005
TOTAL	2,310	2,309.999

Gene Frequencies

M1	=	0.6652
M2	=	0.1388
M3	=	0.1242
S	=	0.0463
Z	=	0.0183
F	=	0.0058
Mvar	=	0.0014

DISCUSSION

Figure 1 shows the focusing patterns of common and rare Pi phenotypes.

The clarity of resolution compared to other IEF methods is due largely to the thorough reduction/alkylation of the Pi molecule. Incorporation of ACES into the gel mixture flattens the pH gradient, particularly in the M8 region where Pi M subtypes are most readily distinguished. This also helps keep the middle of the gel clear of proteins with similar isoelectric points such as haptoglobin and group specific component.

Table 1 summarizes the population analysis of over 2,000 unrelated caucasians received at the London Hospital Medical College in the course of paternity investigations. In such cases no distinction is made between Pi M3 and M4 subtypes.

This method demonstrates that a simple, rapid procedure can produce high resolution focusing.