A Simple Dilution Technique for Assessing Red Cell Enzyme Activity in Cases of Single System Incompatibilities

C.P.Phillips and P.J.Lincoln

The Department of Haematology, The London Hospital Medical College
Turner Street, London E1 2AD, England

INTRODUCTION

Laboratories using a large battery of blood group polymorphisms for paternity investigations will find a very small proportion of families give a single indirect exclusion which is inconsistent with the results obtained in the other grouping systems; sometimes these even appear to exclude the mother. It is important in such cases to distinguish between true exclusions and the presence of null alleles or activity variants which could provide additional evidence of parentage.

Single indirect exclusions occuring in red cell enzyme polymorphisms can be analysed by scanning the electrophoresis or isoelectric focusing (IEF) gel with a densitometer, to produce objective values for the relative band strength in each sample. Alternatively, enzyme activity can be assayed. Unfortunately certain red cell enzymes share activity with a wide range of non-polymorphic enzymes so that the reduced/altered-activity is masked and becomes undetectable.

This presentation details a very simple dilution technique which, when applied with IEF, shows reduced enzyme activity not detectable by enzyme assay.

MATERIALS AND METHODS

Samples of 3x saline washed, packed cells from each of the family members and the appropriate control homozygotes were diluted with distilled water to the following concentrations:

1/2, 1/4, 1/8, 1/16, 1/24, 1/36, 1/60

Standard IEF techniques were used to phenotype the samples (Divall & Ismail 1983, Divall 1984). The strength of the primary isozyme band of each sample in the dilution series was then visually assessed and scored on a ++++ to (+) scale. The dilution concentrations used give the optimum contrast in band strength between normal and reduced activity phenotypes following IEF.

Advances in Forensic Haemogenetics 3
Edited by H. F. Polesky and W. R. Mayr

RESULTS

Two widely used polymorphic enzyme systems which, when assayed, are masked by non-polymorphic enzyme activity are phosphoglucomutase (PGM) and esterase D (EsD). Examples are given of the dilution method for both of these grouping systems.

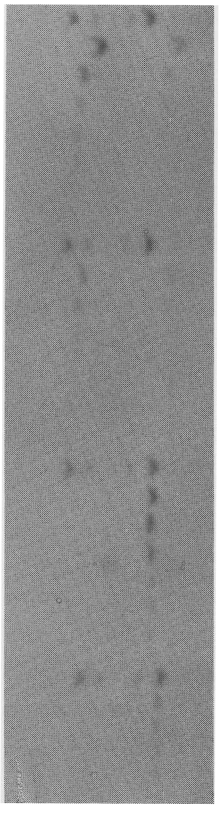
Family 1 - single indirect PGM exclusion

Father 2+
Mother 1+1-
Child 1+

Paternity index of 19 other polymorphisms including HLA = 5,000 times (relative chance of paternity 99.98%)

The isozyme pattern of the dilution series obtained after IEF is shown in Figure 1. Visual assessment of the relative band strengths was as follows:

	1/2	1/4	1/8	1/16	1/24	1/36	1/60
Homozygous 2+ control	++++	+++	++	+	(+)	-	-
Sample of father	+/++	+	(+)/+	(+)	-	-	-
Homozygous 1+ control	++++	+++	+/++	+	(+)	-	-
Sample of child	++	+	(+)	(+)	-	-	-



control
1-2
1-4
1-8
1-16
1-24
1-36
1-60
control
1-2
1-4
1-8
1-16
1-24
1-36
1-60
control
1-2
1-4
1-8
1-16
1-24
1-36
1-60
control
1-2
1-4
1-8
1-16
1-24

PGM 1+ control

1+ child

PGM 2+ control

2+ father

◄ Figure 1

EsD 2 control 1/2
1/4
1/8
1/16
1/24
1/36
Child 1/2
1/4
1/8
1/16
1/24
1/36
EsD 1 control 1/2
1/4
1/8
1/16
1/24
1/36
Father 1/2
1/4
1/8
1/16
1/24
1/36

Figure 2 ►

Family 2 - single indirect EsD exclusion

Father 1	Paternity index on 21 other polymorphisms
Mother 2.1	including HLA = 20,000 times
Child 2	(relative chance of paternity 99.995%)

The isozyme pattern of the dilution series obtained after IEF is shown in Figure 2. Visual assessment of the relative band strengths was as follows:

	1/2	1/4	1/8	1/16	1/24	1/36
Homozygous 1 control	++++	+++	++/+++	++	+/++	+
Sample of father	+++	++	+/++	+	+	(+)
Homozygous 2 control	++++	+++	++	+	(+)	(+)
Sample of child	+++	++	+	(+)	-	-

DISCUSSION

The example results from two single exclusion families demonstrate that this method is efficient at showing reduced enzyme activity in PGM and EsD. Enzyme activity is visibly lower at each stage of the dilution series in the family samples compared to the nOrmal activity homozygous controls. Using IEF to focus the polymorphic enzymes before assessing relative activity ensures the non-polymorphic enzymes are seperated out and do not obscure the true activity levels of interest. In laboratories lacking the resources for enzyme assay or scanning densitometry this method could provide the means to objectively assess reduced activity in any of the routinely used enzyme grouping systems.

Single-exclusion families occur with a regular although very low frequency; the average incidence of silent alleles in enzyme systems is estimated to be 0.001 (Polesky et.al. 1983). PGM and EsD are both close to this general figure with an incidence of 0.001 and 0.0007 respectively. Where it is not possible to investigate a single indirect exclusion with an enzyme assay the method outlined here is a straightforward but effective approach for highlighting the presence of null alleles or activity variants.

REFERENCES

Divall G, Ismail M (1983)
Forensic Science International 22:253-263.
Divall G. (1984)
Forensic Science International 26:255-267.
Polensky H, Souhrada J, Dykes D (1983)
10th Int. Congress of the Society for Forensic Haemogenetics, Munich p 161-166.