

ELECTROPHORETIC "SUBTYPING" OF RARE ESD VARIANTS

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1. Introduction

The common allozymes 1,2 and 5 of red cell esterase D can be determined in a clear-cut manner by agarose gel electrophoresis using a continuous buffer system at pH 5.35.-5.45 composed of malic acid (1). At acidic pH values the allozyme 5 migrates definitely more anodically than the allozyme 2; at neutral pH this distinction is ambiguous. With this new electrophoretic method, isoelectric focusing of ESD 2-1 and 2 is not necessary anymore to detect the gene product of ESD 5. To show the usefulness of the electrophoretic method to detect rare variants was the aim of this investigation.

2. Methods

2.1. Sample Preparation

Blood was collected by venepuncture and hemolysates were made from washed and packed red cells by dilution 1:1 with redistilled water followed by freezing. Destromatisation of the hemolysates was effected by CCl₄.

2.2. Electrophoresis

Gel buffer: 12 mM malic acid
Bridge buffer: 100 mM malic acid
pH-value: 5.35 - 5.45 (adjust pH definitely after 24 h)
Agarose: Seakem LE50002 FMC Corp.
Sample Application: 6 cm from the cathodical edge with a
sample applicator(Code No. 19-29411-11 Pharma.)
Electrophoresis: 24 v/cm, 3,5 - 4 hrs, 5 C
Paper wicks: 4 layers of Whatmann No. 1
Sample Staining: 1 mg umbelliferyl acetate dissolved in acetone
and buffered by a phosphate buffer og pH 6.9. Filter paper over-
lay placed both anodic- and cathodically

3. Results and Discussion

Different red cell ESD variants can be detected by electrophoresis at acidic pH values. The split of ESD 1 in ESD 7-1 described by Nishigaki (2) for isofocusing is also detectable with our method (3). Furthermore, when using the electrophoretic method, ESD 1 is

always "subtyped" in contrast to isofocusing where in practice only ESD 2-1 and 2 are retyped to detect ESD 5. ESD variants are very rare in our material. ESD 4-1 is situated very cathodically. Staining of this part of the gel is imperative to detect these variants. The most frequent variant encountered was ESD 7-1.

4. References:

- 1) Bär W, Häni M, Biedermann V (1984) Esterase D: Simultaneous electrophoretic determination of the three common allozymes (ESD 1,2,5). *Electrophoresis* 5: 280-281
- 2) Nishigaki I, Itoh T (1984) Isoelectric focusing studies of human red cell esterase D: Evidence for polymorphic occurrence of a new allele ESD7 in Japanese. *Hum Genet* 66:92-95
- 3) Henke J, Schweitzer H, Bär W (1985) Elektrophoretische Darstellung der Produkte der Esterase-D-Allele ESD*1,ESD*2,ESD*5 und ESD*7. *Aerztl Lab* 31:123-124

