SIMULTANEOUS FOCUSING OF PGM1 AND ACP PHENOTYPES USING MINIATURIZED GELS AND 3-ELECTRODE TECHNIQUE

W. Kuchheuser, D. Krause

Institute for Forensic Medicine, University of Magdeburg
Leipziger Str. 44, D-39120 Magdeburg/FRG

Phosphoglucomutase (PGM1) and acid erythrocyte phosphatase (ACP) have been suitable erythrocyte enzyme markers in forensic paternity testing since their first description (Hopkinson, Spencer, Harris 1963; Spencer, Hopkinson, Harris, 1964). After the discovery of PGM_1-subalIeles, IEF has been established as the method of choice (Bark, Harris, Firth 1976; Kühnl, Schmidtmann, Spielmann 1977; Sutton, Burgess 1978).
In the present paper, we describe a method for simultaneous PGM1-subtyping and ACP-typing using ultrathin-layer gels and a reduced interelectrode distance.

We used ready-to-use precast gels SERVALYT^R PRECOTES^R PGM Kit (Cat. No. 42888; Serva, Heidelberg, FRG). For technical details see producers instructions for use.

Deviating from the usual procedure, the gels are horizontally divided by placing the cathode strip in the central position and the anode strips on the outer edges of the gel resulting in two gel halves with a common cathode and a distance of 5 cm to each anode.
Electrical parameters and running conditions have been adapted to the changed gel configuration: I= 10 mA, U= 2000 V, P= 10 W; 30 Vh (appr. 30 min.) prefocusing, up to 90 Vh (appr. 30 min.) focusing with samples, and up to 1200 Vh (appr. 1:30 h) focusing without samples. Cooling temperature is 8 °C.
Fresh or stored (-70 °C) stroma-free hemolysates are placed with 4 x 3 mm filter paper pieces (Desaga/FRG, No. 121231) 1 cm apart from the anodal strips.

After the focusing run, the gel is cut into two halves along the cathode strip. The detection steps take place for PGM1 and ACP in the upper, and the lower gel half, respectively. In this way, disturbancies caused by e. g. the considerable difference of the pH-optimum of the enzymes are avoided. The one half of the gel is covered with filter paper soaked in a solution of methylumbelliferylphosphate (solved in citrate buffer, pH 4.8), the other one with an agarose overlay (Sutton and Burgess 1978).
After 15 minutes at, 37 °C, the ACP-spots are visible under ultraviolet light. The PGM-spots appear as violet bands after about 30 minutes at 37 °C in a dark place.

The band configuration of the ACP phenotypes differs from that after conventional electrophoresis as described by other authors (Budowle 1984; Burdett and Whitehead 1977; Divall 1983; Dorrill and Sutton 1983). The A-band is located close to the focused hemoglobin near the cathode, whereas the B-band is

near to the anodal side, and the C-band more in the middle. Fig. 1 shows the band configuration of both markers schematically.
There is a marked difference between the PGM1 1A, 1B, 2B and 2A band concerning their position. Despite the reduced resolving power using 5 cm interelectrode distance the phenotypes of both markers are easy to differentiate without any risk of mistyping. There is no need to use separators to flatten the pH gradient (Gill and Sutton 1985).

The optimized method described here allows the typing of more than 40 hemolysates per run for each marker or the double number for one marker. The costs and the desired time are reduced considerably.

After more than 1 year of use the three-electrode-technique has turned out to be an economical and reliable procedure for routine work. It is possible to use the 3-electrode-technique for other markers, e. g. group specific component, transferrin (Kuchheuser and Krause 1993), and coagulation factor 13B, too.

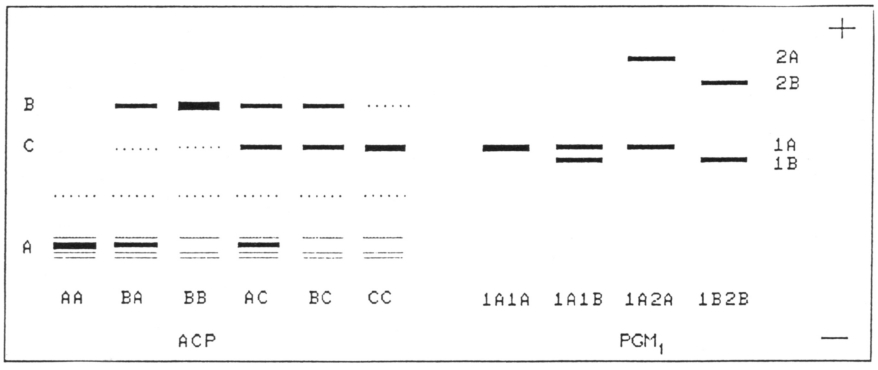

Fig 1: Schematic representation of ACP and PGM bands

References

Bark JE, Harris MJ, Firth MJ (1976) Typing of the common PGM1 variants using isoelectric focusing - a new interpretation of the PGM_1 system. Forens Sci Soc 16:115-120

Budowle B (1984) Rapid electrofocusing of erythrocyte acid phosphatase. Electrophoresis 5:254-255

Burdett PE, Whitehead PH (1977) The separation of the phenotypes of phosphoglucomutase, erythrocyte acid phosphatase, and some hemoglobin variants by isoelectric focusing. Analyt Biochem 77:419-428

Divall GB (1981) Studies on use of isoelectric focusing as a method of phenotyping erythrocyte acid phosphatase. For Sc Int 18:67-78

Dorrill MJ, Sutton JG (1983) Simultaneous isoelectric focusing of EAP and PGM_1 on 0.15 mm polyacrylamide gels. J For Sc Soc 23:131-134

Gill P, Sutton JG (1985) Enhanced resolution (PGM_1) by the addition of a separator to ultrathin isoelectric focusing gels. Electrophoresis 6:23-26

Hopkinson DA, Spencer N, Harris H (1963) Red cell acid phosphatase variants: A new human polymorphism. Nature (Lond) 199:969-971

Kuchheuser W, Krause D (1993) Possibilities for optimized representation of protein markers by isoelectric focusing. IAFS Düsseldorf, 22.-28.8.1993, 121

Kühnl P, Schmidtmann U, Spielmann W (1977) Evidence for two additional common allels at the PGM1 locus (Phosphoglucomutase-E.C.2.7.5.1). Hum Genet 35:219-223

Spencer N, Hopkinson DA, Harris H (1964) Phosphoglucomutase polymorphism in man. Nature 204:742-745

Sutton JG, Burgess R (1978) Genetic evidence for four common alleles at the phosphoglucomutase-1 locus (PGM_1) detectable by isoelectric focusing. Vox Sang 34:97-103