THE APPLICATION OF AGAROSE ISOELECTRIC FOCUSING IN PATERNITY TESTING

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Agarose as carrier medium for isoelectric focusing is a practicable alternative to polyacrylamid in routine application in parentage testing. Since the introduction of highly purified agarose 10 years ago, most of the new systems have been described with the agarose technique.

Agarose has the following advantages. In comparison to polyacrylamid it is non toxic. There is no limitation by gel pore size for larger proteins to penetrate the gel (for example native IgM-molecules with a molecular weight of 900,000). Immunological detection can be performed directly on the surface of the gel (immunofixation). Proteins with a low concentration in sera can be blotted with high transfer rates, an electroblot is not necessary.

The disadvantages of agarose are caused by its sensitivity to overloading, early cathodic drift and separation problems in narrow pH-gradients.

With a standardized agarose technique several proteins were examined regarding there usefulness in parentage testing. The gels were prepared with 0.8 % agarose, 10 % sorbitol and 2.5 % carrier ampholytes. Additional sulfonic acid derivates (ACES, MES, MOPS) were used in concentration of 0.8 %. Depending upon their concentration in serum or plasma immunofixation or capillary blotting were used with monospecific antibodies.

A genetically determined polymorphism of the coagulation factor XIII is known for the A- and B-subunit.
The enzymatic subunit (FXIIIA) is found in plasma. FXIIIA was first described by Board in 1979 with agarose gel electrophoresis.

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Three common phenotypes 1, 2-1 and 2 are observed. The allele frequencies for the two common allels are

FXIIIA*1 = 0.8021
FXIIIA*2 = 0.1959.

Variants are found in less than 0.2 %. For the theoretical exclusion rate we calculated a value of 14.8 %.

The B-subunit of coagulation factor XIII acts as a carrier molecule. After cleavage of the subunit A, factor XIIIB is detectable in serum. The genetic polymorphism was first described by Board in 1980. The common phenotypes FXIIIB 1, 2-1, 2, 3-2, 3-1 and 3 as well as products of the rare alleles FXIIIB*4 and B*6 are found in the southern German population. The allele frequencies for FXIIIB are

FXIIIB*1 = 0.7603
FXIIIB*2 = 0.0843
FXIIIB*3 = 0.1534
FXIIIB*V = 0.0019.

The theoretical exclusion rate for the FXIIIB system is calculated to be 22.7 %.

In agarose focusing coagulation factor XII (Hageman-factor) shows a heterogenous band pattern in the pH-range from 6.4 - 7.3. Four main bands and several minor bands are observed. FXII can be demonstrated with plasma and serum. Coagulation factor VII (proconvertin) shows a uniform two band pattern in the pH-range from 5.6 - 6.4. We examined 250 healthy unrelated persons and didn't find in factor XII as well as factor VII a polymorphism useful for parentage testing.

Alpha-2 antiplasmin shows a band pattern identical with plasminogen. Untreated as well as neuraminidase treated sera and the exa-

mination of three different antibodies shows Alpha-2 antiplasmin and PLG-bands in the same pI-positions. The interpretation of these results are causes in the high binding affinity of alpha-2 AP to plasmin or other complex formations. Alpha-2 AP shows 23 - 28 % homology to plasmin and to other protease inhibitors.

A polymorphism also correlated to plasminogen is observed in the Kallikrein system. The polymorphism is observed in the cathodic part of the band pattern. We excluded a cross reaction of the antibody with highly purified kallikrein and plasminogen. The genetic interpretation suggests a linkage of the genes of Plasminogen and Kallikrein on chromosome 6.

The polymorphism of plasminogen was first described by Hobart 1979 and Raum 1980. Besides the three common phenotypes A, AB and B, a number of variants are observed. The allelefrequences are

PLG*1 = 0.7150
PLG*2 = 0.2742
PLG*V = 0.0012.

The theoretical exclusion rate is calculated to be 18.7 %.

The genetically determined polymorphism of Orosomucoid (ORM) was also determined by agarose isoelectric focusing. We observed the following allele frequencies:

ORM1*F1 = 0.6463
ORM1*F2 = 0.0145
ORM1*S = 0.3387.

The theoretical chance for the exclusion of non- fathers was calculated to be 18.07 %.

In conclusion agarose isoelectric focusing offers many opportunities. Especially in the search for new systems which are applicable in parentage testing.

Board, P.G. (1979): Genetic Polymorphism of the A Subunit of Human Coagulation Factor XIII. Am J Hum Genet 31: 116.

Board, P.G. (1980): Genetic Polymorphism of the B Subunit of Human Coagulation Factor XIII. Am J Hum Genet 32: 348.

Dykes, D.D. (1983): Distribution of plasminogen allotypes in eight populations of the Western Hemisphere. Electrophoresis 4, 417.

Dykes, D.D., Polesky, H.F. (1985): FXIIIA Phenotyping by isoelectric focusing and immunoblotting: Gene frequencies in a population of US Whites and Blacks. Electrophoresis 6, 521.

Eiberg, H., Mohr, J., Nielsen (1981): Genetics and linkage relations of plasminogen. Clin Genet 19, 500.

Gathof, B., Hesse, R., Howe, J. (1988): Orosomucoid Subtyping by Isoelectric Focusing on Agarose Gels. Abstracts Int Cong ISBT-BBTS, London, 294.

Hobart, M.J. (1979): Genetic polymorphism of human plasminogen. Ann Hum Genet 42, 419.

Hummel, K., Jäger, H., Krüger H.J. (1983): Bestimmung der Phänotypenhäufigkeiten im Plasminogen (PLG)-System bei Einwohnern der Stadt Mexiko. Ärztl Lab 29, 198.

Kera, Y., Nishimukai, H. (1982): Genetic polymorphism of the A subunit of human coagulation factor XIII in Japanese. Hum Hered 32: 216.

Kreckel, P., Kühnl, P. and Spielmann, W. (1982): Human Coagulation Factor XIIIA (FXIIIA) Phenotyping by Immunofixation Agarose Gel Electrophoresis (IAGE). Blut 44: 309.

Kreckel P. and Kühnl, P. (1982): Improved Coagulation Factor XIIIB (FXIIIB) Phenotyping after Neuraminidase Treatment of Plasma and First Description of the FXIIIB 2 Phenotype. Blut 45, 337.

Leifheit, H.-J., Gathof, A.G. and Weidinger, S. (1985): Klassifizierung der Faktor XIII B Allotypen mit der isoelektrischen Fokussierung. Ärztl Lab 31: 159.

Leifheit, H.-J., Gathof, A.G. and Cleve, H. (1886): Genetic FXIIIB-Variants demonstrated by Isoelectric Focusing on Agarose Gels. Advances in Forensic Haemogenetics 1, Ed. by B. Brinkmann and K. Henningsen, Springer Verlag, Berlin, 146.

Leifheit, H.-J., Gathof, A.G. and Cleve, H. (1987): Plasminogen (PLG)-Typisierung mittels isoelektrischer Fokussierung auf Agarose-Gelen und Immunfixation. Ärztl Lab 33: 10.

Leifheit, H.-J., Howe, J. and Gathof, A.G. (1988): Agarose Isoelectric Focusing for Classification of Plasminogen-Variants. Advances in Forensic Haemogenetics 2, ed. by W.R. Mayr, Springer Verlag, Berlin, 202.

Leifheit, H.-J., Gathof, A.G. and Cleve, H. (1988): Improved FXIIIB-Phenotyping by Isoelectric Focusing with Immobilized pH-Gradients. Advances in Forensic Haemogenetics 2, ed. by W.R. Mayr, Springer Verlag, Berlin, 207.

Leifheit, H.-J. and Cleve, H. (1988): Analysis of the genetic polymorphism of coagulation factor XIIIB (FXIIIB) by isoelectric focusing. Electrophoresis 9, 426.

Leifheit, H.-J., Gathof, A.G. and Cleve, H. (1988): Isoelectric Focusing of Coagulation Factor XII (Hageman Factor). Abstracts Int Cong ISBT-BBTS, London, 294.

Nakamura, S. and Abe, K. (1982): Genetic polymorphism of coagulation factor XIIIB subunit in Japanese. Ann Hum Genet 46: 203.

Nakamura, S., Ohue, O. and Abe K. (1986): Genetic polymorphism of coagulation factor XIIIB subunit in the Japanese population: description of three new rare alleles. Hum Genet 73, 183.

Raum, D., Marcus D., Alper, C.A. (1979): Genetic control of human plasminogen (PLG). Clin Res 27, 458.

Schmid, K. (1961): Polymorphism of alphal-acid glycoprotein. Nature 190, 630.

Skoda, U., Bertrams, J., Dykes, D., Eiberg, H., Hobart, M., Hummel, K., Kühnl, P., Mauff, C., Nakamura, S., Nishimukai, H., Raum, D., Tokunaga, K., Weidinger, S. (1986): Proposal for the Nomenclature of Human Plasminogen (PLG) Polymorphism. Vox Sang 51, 244.

Spielmann, W. and Kühnl, P. (1982): Erfahrungen mit neueren Blutgruppensystemen für die Vaterschaftsbegutachtung: FXIIIA and FXIIIB, PLGN, AMY2, GAA, GDH, PGM1-Thermostabilitätsvarianten. Ärztl Lab 28: 367.

Thymann, M., Eiberg, H. (1986): Orosomucoid polymorphism: determination by separator isoelectric focusing and demonstration of ORMF subtypes. Advances in Forensic Haemogenetics 1, Ed. by B. Brinkmann and K. Henningsen, Springer Verlag, Berlin, 162.

Weidinger, S., Schwarzfischer, F., Leifheit, H.-J. und Cleve H. (1983): Improved Method for Phenotyping of Human Coagulation Factor XIIIA. Lecture 10th Int Cong Forensic Haemo, 447.

Weidinger, S., Schwarzfischer, F., Müller, H., Cleve, H. (1985): Plasminogen (PLG): A Useful Genetic Marker for Paternity Examinations. Z Rechtsmed 94, 165.