AGAROSE GEL ELECTROPHORESIS – APPLICATIONS IN CLINICAL CHEMISTRY

ELEKTROFOREZA NA AGAROZNOM GELU – PRIMENE U KLINIČKOJ HEMIJI

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Summary: Agarose gel electrophoresis is a well established technique routinely used in clinical laboratories for screening protein abnormalities in various biological fluids (serum, urine, CSF). It is based on the principles of zone electrophoresis. Electrophoretograms are evaluated visually for the presence of quantitatively or qualitatively abnormal protein bands. The technique is used for electrophoresis of serum, urine, CSF proteins, enzymes (ALP, LDH and CK), lipoproteins and hemoglobin. Serum protein electrophoresis (SPE) is a commonly used analytical method in clinical chemistry. Changes in the relative concentration of fractions allow easy recognition of pathological disorders associated with nephrotic syndrome, inflammatory reaction and hepatic diseases. SPE is a screening test for detecting the M component (MC). Immunofixation (IFE) with use of specific antisera allows detection of the type of MC. SPE is also a method for the quantification of MC and monitoring of disease that is essential for clinical evaluation and follow-up of patients with plasma cell disorders.

Keywords: agarose gel electrophoresis, serum protein electrophoresis, immunofixation, M component

Introduction

Agarose gel electrophoresis is a well established technique routinely used in clinical laboratories for screening protein abnormalities in various biological fluids (serum, urine, CSF). It is based on the principles of zone electrophoresis. Proteins as charged molecules migrate in a solid medium soaked with a buffer under the influence of an electrical field. Migration is dependent upon net electrical charge, isoelectric point and molecular mass of proteins. Protein zones are visualized by staining with a protein-specific stain. Electrophoretograms are evaluated visually for the presence of abnormal protein bands and/or quantitatively for the determination of the relative concentration of fractions by use of an appropriate optical device (densitometer or high resolution computer scanner). The technique is used for serum, urine, CSF protein electrophoresis as well as for electrophoresis of specific proteins such as enzymes (ALP, CK and LDH), lipoproteins and hemoglobin. With additional use of specific antisera (immunofixation) it can also be used for the identification of the monoclonal component in sera, urine and CSF or oligoclonal profiles in sera and in CSF. Although the technique of zone electrophoresis has been known since 1930 (1), it is still an essential aid to diagnosis and therapeutic follow-up of patients with plasma cell disorders (2).
**Materials and Methods**

*Serum protein electrophoresis (SPE)*

Agarose gel electrophoresis of fresh undiluted serum samples was performed in alkaline buffer, pH 9.2, using HYDRAGEL 15/30 PROTEIN(E) (Sebia, France), on an automated system HYDRASYS 2 SCAN (Sebia, France). The automated steps included: sample application, electrophoretic migration, drying, staining, destaining and final drying. Staining was performed with amido black. The tracks were evaluated visually for pattern abnormalities. Scanning of stained HYDRAGELS was performed on a HYDRASYS 2 SCAN (Sebia, France), and data were processed with the PHORESIS software (Sebia, France).

*Immunofixation (IFE)*

Agarose gel electrophoresis of fresh diluted serum samples (1/6 for G track, 1/3 for Elp, A, M K and L tracks in saline) was performed by using Hydragel IF (Sebia, France) on an automated system HYDRASYS 2 SCAN (Sebia, France). Upon migration in an alkaline buffer (pH 9.1), proteins were then incubated with individual antisera or with a chemical fixative solution to create an electrophoresis reference pattern for the specimen. Unreacted proteins were blotted and residual traces eliminated by a wash step, and gels were stained with acid violet to visualize the fixed proteins. The automated steps included: sample application, electrophoretic migration, incubation with fixative solution and antisera, drying, staining, destaining and final drying.

**Results**

The electrophoresis separation of human serum protein results in 5 to 6 clearly defined fractions, depending on the buffer used (3):

- the albumin fraction showing biochemical homogeneity;
- four groups of migrating globulins, α1, α2, β and γ globulins. Use of specific buffer, pH 8.5, allows separation of β fraction into two zones: β1 (mainly transferrin and LDL) and β2 (C3 component).

The interpretation of protein electrophoresis should be necessarily complemented by the quantification of total serum protein. The migration pattern of serum proteins is shown in Figure 1.

**Interpretation of the major abnormalities observed in serum protein electrophoresis**

1. **Changes in the albumin fraction**
   1.1. **Double band: bisalbuminemia**

   Bisalbuminemia is seen on the electrophoretogram as an albumin fraction split in two (Figure 2A and Figure 2B), and it can be of permanent or transient nature. It is a result of:

   ![Figure 2A](image1.png) Bisalbuminemia as seen on gels.

   ![Figure 2B](image2.png) Bisalbuminemia.

Abbreviations: N: normal pattern; P: pathological pattern; Alb: albumin; α1: α1 globulins; α2: α2 globulins; β: β globulins; γ: γ globulins.
• hereditary mutation: the double band is then a permanent sign of a genetic variant, generally without any observed pathological effect;
• acquired transient bisalbuminemia occurs: due to pancreatitis or a drug treatment such as high doses of beta-lactam in a patient with renal insufficiency, through binding of the antibiotic to albumin.

1.2 Congenital analbuminemia
For rarely occurring cases of analbuminemia, the electrophoretic pattern is unusual (very low albumin band). The four globulin fractions increase in order to keep the osmotic pressure as high as possible. However, clinical symptoms are usually limited to discrete oedema.

1.3 Hypoalbuminemia
Since albumin is exclusively of hepatic origin, any decrease in the percentage of albumin is the result of one of the following mechanisms:
• severe chronic malnutrition;
• a decrease in synthesis: lymphoproliferative disorder, hepatocellular insufficiency (cirrhosis, hepatitis), inflammation;
• increased losses: urinary (nephrotic syndrome), digestive (exudative gastroenteropathy) or cutaneous (widespread burns);
• hypercatabolism: acquired endocrine disorders (thyrotoxicosis, Cushing’s disease), severe inflammatory syndromes.

1.4 Hyperalbuminemia
In healthy individuals, the presence of hyperalbuminemia does not necessarily have any pathological meaning; it can be seen usually in hospitalized patients, due to hemoconcentration (by dehydration) or albumin administration.

2. Changes in the α1 globulin zone
2.1 Decrease
• Brought about by hepatocellular insufficiency, malnutrition or protein loss, generally with concomitant decrease of albumin, α2 and β globulins.
• Caused by congenital deficiency of α1 antitrypsin, the predominant protein in the α1 zone. Such deficiencies are due to specific alleles of the α1 antitrypsin gene: Pi*S, Pi*Z or Pi*null that may be partially compensated when associated in a heterozygous state with an allele expressed at a normal level. This abnormality is sometimes associated with liver and lung diseases (emphysema).

2.2. Increase
It is mainly seen in inflammatory disorders associated with a notable increase in the α2 zone due to the electrophoretic localization of acute phase proteins: orosomucoid and α1 antitrypsin in the α1 zone and haptoglobin in the α2 zone (Figure 3).

3. Changes in the α2 globulin zone
3.1. Double band
Double α2 globulin band can occur in the following cases:
• in vitro hemolysis: hemoglobin if present in the sample is migrating in the α2 zone (complexed to haptoglobin);
• the presence of specific phenotypes of haptoglobin: Hp 1-1 shows a different electrophoretic mobility than Hp 1-2 or 2-2;
• more rarely: the presence of β lipoprotein (LDL) of α2 abnormal electrophoretic mobility, or
• presence of a monoclonal free light chain migrating in this area.

3.2. Decrease
• Brought about by hepatocellular insufficiency, malnutrition or protein loss;
• by intravascular hemolysis: the fall in haptoglobin will be even more visible in the protein electrophoresis if an associated inflammatory
syndrome exists (discrepancy between the increase in the $\alpha_1$ zone and the decrease in the $\alpha_2$ zone).

3.3. Increase

This increase is mainly seen in two types of syndromes and is related to the variable level of the two main proteins migrating into the $\alpha_2$ globulin zone:

- the inflammatory syndrome, by an increase of haptoglobin (the $\alpha_2$ fraction is then greater than 15%), associated with hyper $\alpha_1$ globulinemia;
- the nephrotic syndrome, by an often substantial increase of $\alpha_2$ macroglobulin associated with hypoalbuminemia (due to urinary loss), hyper $\beta$ globulinemia (in particular lipoid nephrosis) and with proteinuria exceeding 3 g/L (Figure 4).

4. Changes in the $\beta$ globulin zone

4.1. Decrease

- Induced by hepatocellular insufficiency, malnutrition or protein loss related to a decrease in transferrin migrating into the $\beta_1$ zone;
- induced by C3 consumption associated with a decrease in the $\beta_2$ zone; the decrease of $\beta_2$ can be due to ageing of the serum sample.

4.2. Increase

The causes may vary according to the extent of the increase:

- Non-monoclonal causes (usually limited increase):
  - hyper $\beta$ globulinemia by hypertransferrinemia in anemia or by increased $\beta$ lipoprotein;
  - hyper $\beta_2$ globulinemia by increased C3: inflammatory or secondary hyper $\beta_2$ globulinemia due to intra- or extrahepatic biliary obstruction;
  - $\beta$ zone elevated as a whole and associated with a $\beta$–$\gamma$ bridge, thus revealing the polyclonal hyper immunoglobulin A observed in alcoholic cirrhosis (Figure 5).

- Monoclonal proteins:
  - monoclonal immunoglobulin G or A (the most frequent);
  - monoclonal immunoglobulin M (Waldenström’s disease);
  - kappa or lambda monoclonal free light chains, seen in light chain myeloma or amyloidosis (Figure 6).

5. Modifications in the $\gamma$ globulin zone

The $\gamma$ fraction is the migration zone of immunoglobulins (immunoglobulin G, A, M, D, E). Clinical data, together with age of the patient, should be considered when interpreting this fraction.
5.1. Hypoglobulinemia

- Physiological hypoglobulinemia in babies;
- isolated or total primary immunodeficiency (involving one or more immunoglobulin classes), in children and adults;
- secondary hypoglobulinemia: associated with myeloma or due to corticosteroids and immunosuppressive treatments, chemotherapy or radiotherapy;
- light-chain myeloma hypoglobulinemia: the diagnosis will be confirmed by the detection of Bence Jones protein in the urine.

5.2. Hyperγglobulinemia

- Polyclonal hyperγglobulinemia (diffuse increase) (Figure 7) mainly observed in viral or bacterial infections, AIDS or autoimmune diseases;
- monoclonal hyperγglobulinemia: sharp, narrow and homogeneous electrophoretic band, or bands if present under different polymerization forms, as a result of the presence of a monoclonal component (MC) (Figure 8A and 8B);
- oligoclonal hyperγglobulinemia (several narrow and homogeneous bands) (3). In specific cases, hyperγglobulinemia arises from an increase in some subclasses resulting in a particular oligoclonal pattern (Figure 9).

These immunoglobulins correspond either to:

- autoantibodies seen in some autoimmune diseases: rheumatoid arthritis, Sjögren’s syndrome, lupus erythematosus, progressive systemic sclerosis;
- antibodies directed against viral proteins: seropositive individuals with HIV, viral hepatitis, meningitis, cytomegalovirus infections;

![Figure 7](image7.png) Polyclonal hyper γ globulinemia.

![Figure 8A](image8A.png) Weak monoclonal component.

![Figure 8B](image8B.png) Strong monoclonal component.

![Figure 9](image9.png) Oligoclonal pattern.
• autoimmune responses in transplanted patients on immunosuppressive therapy;
• immune responses in normal individuals: 1 to 5% of normal individuals may show an oligo-
clonal pattern of no clinical value. These monoclonal-type bands are often present in low
concentration, and usually transient.

Monoclonal gammopathy

MC is usually associated with monoclonal neo-
plasms known as plasma cell disorders. Multiple mye-
loma, Waldenström’s macroglobulinemia, primary amyloidosis, and the heavy chain disease comprise a group of plasma cell disorders known as monoclonal gammopathies, paraproteinemias, plasma cell dys-
crasias, and dysproteinemias. M components may be
also detected in other lymphoid neoplasms such as chronic lymphocytic leukemia and lymphomas of B or T cell origin; nonlymphoid neoplasms such as chronic myeloid leukemia, breast cancer, and colon cancer; a variety of nonneoplastic conditions such as cirrhosis, sarcoidosis, parasitic diseases, Gaucher disease, and pyoderma gangrenosum; and a number of auto-
immune conditions, including rheumatoid arthritis, myasthenia gravis, and cold agglutinin disease. At
least two very rare skin diseases, lichen myxedematosi-
sus, or papular mucinosis, and necrobiotic xanthogran-
uloma, are associated with a monoclonal gammopa-
thy (2).

Monoclonal gammopathy of undetermined sig-
nificance (MGUS) and smoldering multiple myeloma
are asymptomatic disorders characterized by mono-
clonal plasma cell proliferation in the bone marrow in
the absence of end-organ damage. MGUS are more
common than myeloma, occurring in 1% of the pop-
ulation over the age of 50 and in up to 10% individu-
als over the age of 75. With long-term follow-up,
—1% per year of patients with MGUS go on to deve-
lomp myeloma (2).

Electrophoresis laboratory investigation of mono-
clonal gammopathy includes:
• serum protein electrophoresis:
  – as a screening test for detecting M component
    (MC);
  – as a method for quantification of MC and dis-
    ease monitoring. The concentration of MC can
    be calculated based on the MC in the serum
    protein electrophoresis and total protein. For
    quantitative determinations of the M compo-
    nent, serum protein electrophoresis is more reli-
    able than immunonephelometric or immuno-
turbidimetric methods since MC are quantified
    independently from antigen-antibody binding
    by means of a reaction with dyes (4). The low-
    est detected concentration of a monoclonal
    protein was 0.17 g/L (5). According to the
    immunoglobulin type, its position and the poly-
clonal background, the detection limit may vary.
• Serum or urine immunoelectrophoresis or
  immunofixation for the detection of the MC
type.

Immunofixation electrophoresis (IFE) is gradual-
ly replacing immunoelectrophoresis (IEP) because of
its rapidity and ease of interpretation. It is also 50
times more sensitive that IEP (4). Analytical sensitivi-
ty is 60–250 mg/L (5).

Conclusion

SPE is a very commonly used analytical method
in clinical chemistry. Changes in the relative concen-
tration of fractions allow easy recognition of patholo-
gical disorders associated with nephrotic syndrome,
inflammatory reaction and hepatic diseases. SPE is a
screening test for detecting the M component (MC).
IFE with the use of specific antisera allows the detec-
tion of the type of MC in serum and urine. SPE is also
a method for quantification of MC and monitoring of
disease that is essential for clinical evaluation and fol-
low-up of patients with plasma cell disorders (6, 7).

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