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Review article
Pregledni članak

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 very 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

Kratak sadržaj: Elektroforeza na agaroznom gelu je pouzdana tehnika koja se rutinski koristi u kliničkim laboratorijama za skrining abnormalnosti proteina u različitim biološkim tečnostima (serum, urin, cerebrospinalna tečnost). Zasniva se na principima zonske elektroforeze. Na elektroforetogramima se vizuelno može utvrditi prisustvo kvalitativno ili kvantitativno abnormalnih proteinskih nizova. Tehnika se koristi za elektroforezu seruma, urina, proteina u cerebrospinalnoj tečnosti, enzima (ALP, LDH i CK), lipoproteina i hemoglobina. Elektroforeza proteina u serumu (SPE) vrlo često se kao analitička metoda primenjuje u kliničkoj hemiji. Promene u relativnoj koncentraciji frakcija omogućavaju lako prepoznavanje patoloških poremećaja povezanih s nefrotskim sindromom, inflamatornom reakcijom i oboljenjima jetre. SPE predstavlja skrining test za otkrivanje M komponente (MC). Imunofiksacija uz upotrebu specifičnih antiseruma omogućava detekciju tipa MC. SPE je i metod za kvantifikaciju MC i praćenje toka bolesti što je neophodno za kliničku evaluaciju i praćenje pacijenata sa plazma-ćelijskim bolestima.

Ključne reči: elektroforeza na agaroznom gelu, elektroforeza proteina u serumu, imunofiksacija, M komponenta

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).

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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):

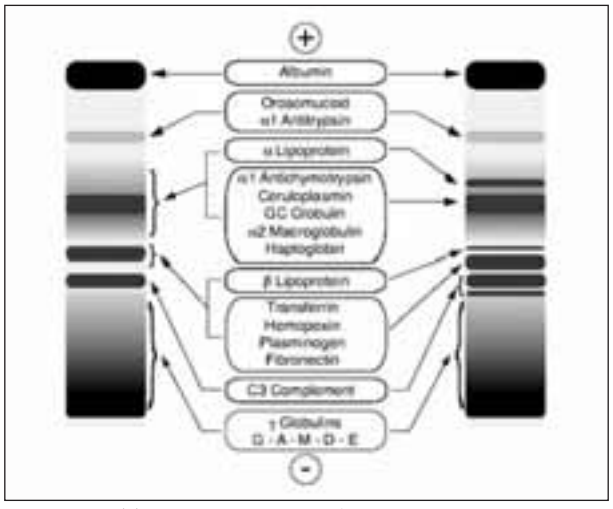

Figure 1 Migration pattern of serum proteins generated on HYDRAGEL Protein (left) and HYDRAGEL β1β2 (right).

- 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 transferin 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:

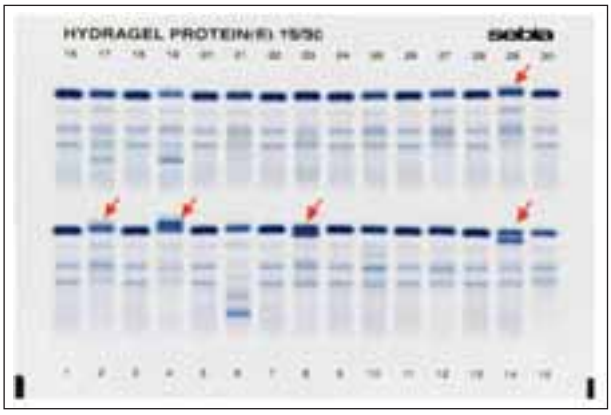

Figure 2A Bisalbuminemia as seen on gels.

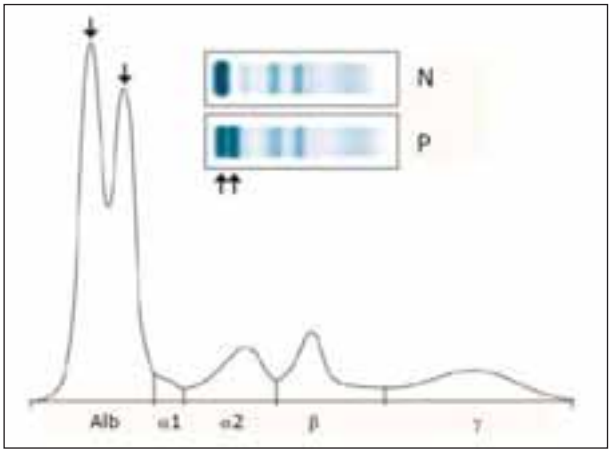

Figure 2B Bisalbuminemia.

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 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).

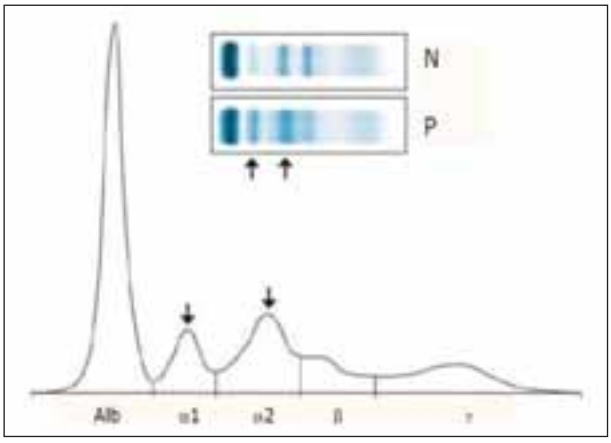

Figure 3 Inflammatory syndrome.

Abbreviations: N: normal pattern; P: pathological pattern; Alb: albumin; α1: α1 globulins; α2: α2 globulins; β: β globulins; γ : γ globulins.

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

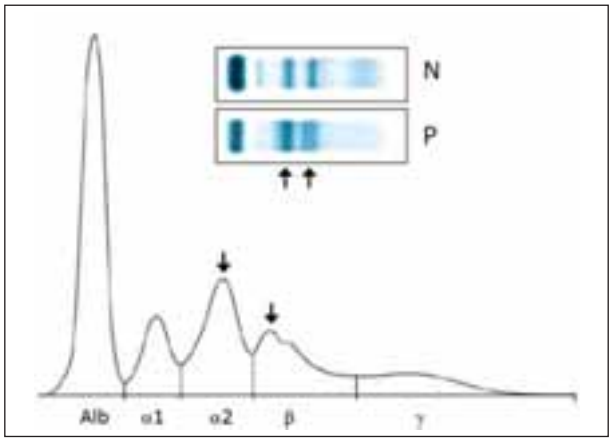

Figure 4 Nephrotic syndrome.

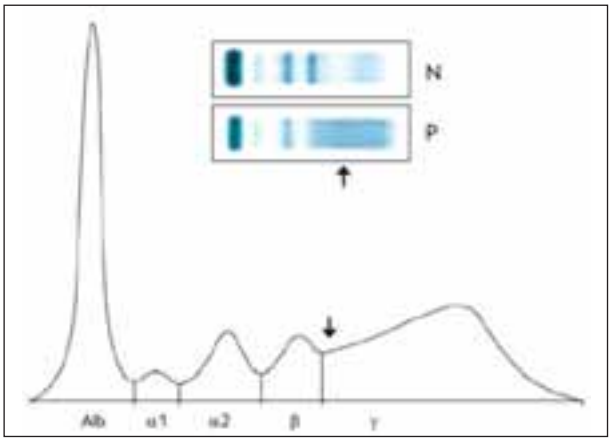

Figure 5 Beta gamma bridge.

syndrome exists (discrepancy between the increase in the α1 zone and the decrease in the α2 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 α2 globulin zone:

- the inflammatory syndrome, by an increase of haptoglobin (the α2 fraction is then greater than 15%), associated with hyper α1 globulinemia;
- the nephrotic syndrome, by an often substantial increase of α2 macroglobulin associated with hypoalbuminemia (due to urinary loss), hyper β globulinemia (in particular lipoid nephrosis) and with proteinuria exceeding 3 g/L (*Figure 4*).

4. Changes in the β globulin zone

4.1. Decrease

• Induced by hepatocellular insufficiency, malnutrition or protein loss related to a decrease in transferrin migrating into the β1 zone;

• induced by C3 consumption associated with a decrease in the β2 zone; the decrease of β2 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 β globulinemia by hypertransferrinemia in anemia or by increased β lipoprotein;

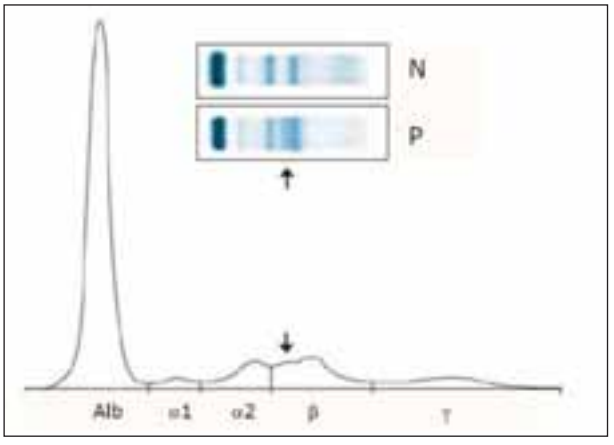

Figure 6 Free light chain disease.

– hyper β2 globulinemia by increased C3: inflammatory or secondary hyper β2 globulinemia due to intra- or extrahepatic biliary obstruction;

– β zone elevated as a whole and associated with a β–γ 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 γ globulin zone

The γ 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. Hypo γ globulinemia

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

5.2. Hyper γ globulinemia

- Polyclonal hyper γ globulinemia (diffuse increase) *(Figure 7*) mainly observed in viral or bacterial infections, AIDS or autoimmune diseases;
- monoclonal hyper γ globulinemia: 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 hyper γ globulinemia (several narrow and homogeneous bands) (3). In specific cases, hyper γ globulinemia 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 erythematosis, progressive systemic sclerosis;
- antibodies directed against viral proteins: seropositive individuals with HIV, viral hepatitis, meningitis, cytomegalovirus infections;

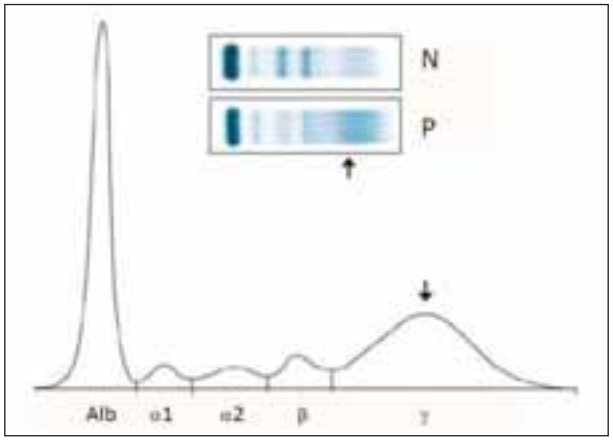

Figure 7 Polyclonal hyper γ globulinemia.

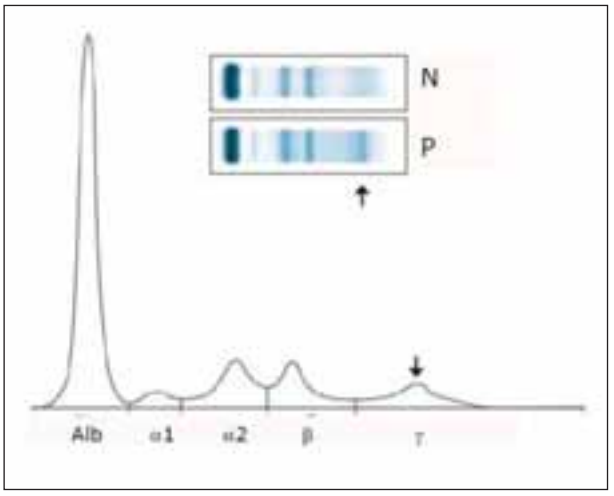

Figure 8A Weak monoclonal component.

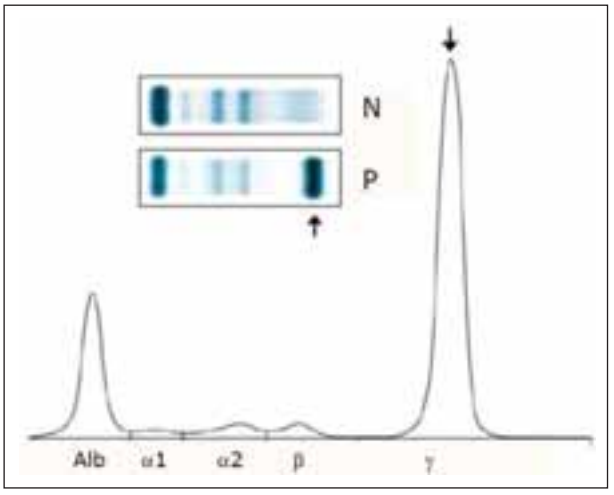

Figure 8B Strong monoclonal component.

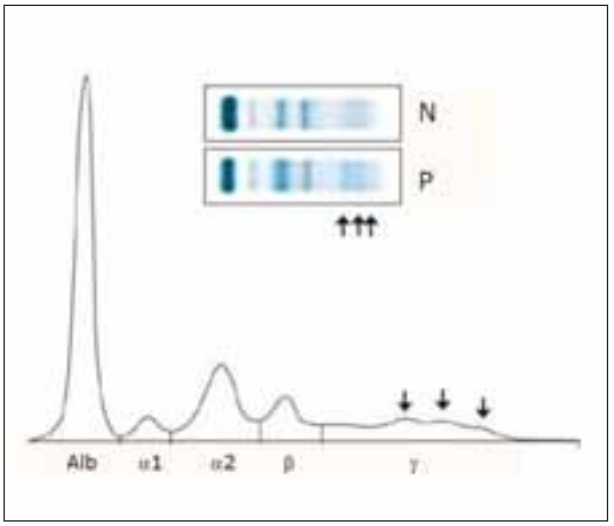

Figure 9 Oligoclonal pattern.

- autoimmune responses in transplanted patients on immunosuppressive therapy;
- immune responses in normal individuals: 1 to 5% of normal individuals may show an oligoclonal 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 neoplasms known as plasma cell disorders. Multiple myeloma, Waldenström's macroglobulinemia, primary amyloidosis, and the heavy chain disease comprise a group of plasma cell disorders known as monoclonal gammopathies, paraproteinemias, plasma cell dyscrasias, 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 autoimmune conditions, including rheumatoid arthritis, myasthenia gravis, and cold agglutinin disease. At least two very rare skin diseases, lichen myxedematosus, or papular mucinosis, and necrobiotic xanthogranuloma, are associated with a monoclonal gammopathy (2).

Monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma are asymptomatic disorders characterized by monoclonal 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 population over the age of 50 and in up to 10% individuals over the age of 75. With long-term follow-up, ~1% per year of patients with MGUS go on to develop myeloma (2).

Electrophoresis laboratory investigation of monoclonal gammopathy includes:

- serum protein electrophoresis:
  - as a screening test for detecting M component (MC);
  - as a method for quantification of MC and disease 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 component, serum protein electrophoresis is more reliable than immunonephelometric or immunoturbidimetric methods since MC are quantified independently from antigen-antibody binding by means of a reaction with dyes (4). The lowest detected concentration of a monoclonal protein was 0.17 g/L (5). According to the immunoglobulin type, its position and the polyclonal background, the detection limit may vary.
- Serum or urine immunoelectrophoresis or immunofixation for the detection of the MC type.

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

Conclusion

SPE is a very 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). IFE with the use of specific antisera allows the detection 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 follow-up of patients with plasma cell disorders (6, 7).

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