

# Serum Proteins Electrophoresis by Agarose Gel – M-Spike Screening and Beyond - Review

Julieta Hristova<sup>1</sup>, Mariana Genova<sup>2</sup>

<sup>1,2</sup>Medical University of Sofia, 15 Acad. Ivan Geshov Blvd, Sofia 1431, Bulgaria

**Abstract:** Serum protein electrophoresis (SPE) is a laboratory technique used as a screening test for dysproteinemia. One of the most commonly used media is agarose presenting excellent visualization and densitometric quantification. Clinical presentation of diseases related to monoclonal protein is often obscure at the onset and the complications are the first to come forward in an advanced disease. SPE is also included in different diagnostic panels as a helpful diagnostic tool in the context of liver diseases, nephrotic syndrome, acute and chronic inflammation, hemolytic anemia, iron-deficiency anemia, hyperlipidemia, and polyclonal gammopathies.

**Keywords:** serum protein electrophoresis, agarose gel, dysproteinemia, monoclonal gammopathy.

## 1. Introduction

Electrophoresis is a technique used for separation of charged biomolecules based on the rates of their migration in an applied electrical field with specified parameters. The first electrophoretic method for studying proteins was the moving boundary method invented by Tiselius in 1937. The apparatus was able to resolve serum proteins in four component mixtures, with  $\alpha_1$  fraction inseparable from albumin. Nowadays, for diagnostic purposes, zone electrophoresis in a porous medium is applied which provides visualization of five sharply separated zones. This method also allows a permanent record of the result.

## 2. SPE by Agarose Gel

One of the most commonly used media for electrophoresis is agarose, a neutral fraction of agar obtained by separating agarose from agaropectin. It is applied for separation of proteins in serum, urine, and cerebrospinal fluid, hemoglobin types, lipoproteins, etc. Agarose gel has low affinity to proteins, it is practically free of ionizable groups thus performing little endosmosis, although this could be modified according laboratory's specific needs. The pores size allows separation based principally on the charge-to-mass ratio of the protein. The electrophoretic time for most of the routine techniques is 20 to 30 minutes depending to the manufacturer's instructions and the whole test including fixation, staining, and drying extends the process to 1 hour. Prepacked gels may differ in size according to the manufacturer and the number of sample application points. Usually 7 to 10 samples are applied at the same time reducing the cost of a single test and allowing comparison between electrophoregrams along with processing each single plot. The simultaneous fractionating of different samples on the same gel surface improves the assessment of the results, especially in cases of discrete deviations from the control sample revealing some not that obvious signs of dysproteinemia.

Agarose gel provides an excellent densitometric quantification of stained with protein-specific dyes zones

because after decoloration of the background it becomes absolutely transparent. The photometric optical system measures the absorbance of each band, and then the microprocessor integrates the area under the displayed peaks and reports each band as percent from the total protein in the sample. Slight differences in buffer's properties, electrophoretic time, and parameters of the electric field applied may lead to variations in plot's length. In most of the cases it compromises neither the densitometric readings, nor the visual assessment of the electrophoregram. One of the advantages of densitometers includes the ability of scanning plots varying from 25 to 110 mm in length.

Serum protein electrophoresis is used mainly as a routine screening test for dysproteinemia including the presence of paraproteins. The relative quantity of major serum protein components is also a primary focus of the interpretation (Jacoby et al., 2000). The clinical presentation of diseases related to monoclonal protein is often obscure at the onset and usually plasma cell dyscrasia is suspected when complications have already come forward. These include unexplained bone pain, bleeding, frequent infections, anemia, and amyloidosis (Fernández de Larrea et al., 2015). Other indications may be hypercalcaemia, pathologic fractures, hypergammaglobulinemia, or immunoglobulin deficiency, peripheral neuropathy, and unexplained proteinuria. M-gradient is always present in plasmocytoma, Waldenström's macroglobulinaemia and associated neoplasias of the lympho-plasma-cellular tissue, however, it can also be observed in carcinomas, chronic liver diseases and chronic inflammatory processes as well as in clinically healthy persons in individual cases (Zimmerman, 1976). In some cases, unusual bands may be artifacts which appear due to preanalytical errors concerning sampling or imprecise technical implementation, but they are easily recognized by experienced specialists in charge of the results interpretation. Application of plasma would yield a sharp band in beta-gamma region where fibrinogen migrates. Sometimes extremely elevated CRP may be detectable as an "M-spike" on serum protein electrophoresis (Rader et al., 1983).

### 3. Evaluation of Abnormal Patterns

#### Albumin

Albumin is the most abundant of serum proteins comprising 52-69% of normal serum proteins. Albumin contributes to sustaining sufficient colloid osmotic pressure to counterbalance hydrostatic pressure and also transports various substances, including exogenous drugs.

Bisalbuminaemia is characterized by two sharp bands of albumin called alloalbumins showing different electrophoretic mobility and described as fast or slow type variants (Kobayashi et al., 1995). This finding can be inherited or acquired, and is rarely encountered serum protein anomaly (Chhabra et al., 2013) and in most of the cases incidentally detected. Inherited bisalbuminaemia is a rare genetic disorder, also found by chance (Šimundić et al., 2009), and the acquired type is usually related with patients receiving high doses of  $\beta$ -lactams, patients with diabetes mellitus, chronic kidney disease, Alzheimer's disease, and pancreatic disease, commonly complicated with ruptured pseudocyst.

Absolute decrease in albumin concentration in the context of inflammation includes pathological mechanisms such as increased vassal permeability and losses into extravascular space, increased local cellular consumption, direct inhibition of cytokines resulting in decreased synthesis, and increased concentration of acute phase reactants. In patients with hepatic diseases the synthesis is unaffected even in cases of severe parenchymal injury and the mechanisms leading to hypoalbuminemia include increased synthesis of immunoglobulins, loss in extravascular space, or direct inhibition of toxins, including alcohol. It's important to note that albumin levels do not correlate well with severity, prognosis, and total hepatic function in patients with acute hepatitis and cirrhosis of the liver. Impaired renal function presented with proteinuria is another possible reason for occurring hypoalbuminemia. The pathological mechanisms reveal increased filtration due to blocking of acidic groups such as glycosylation in diabetes mellitus, increased glomerular filtration rate in nephrotic syndrome, proximal tubular injury, and some physiological conditions such as fever and extreme physical activity. Albuminuria in individuals with diabetes and hypertension is proved to be predictive for developing of kidney diseases. In all these cases albumin band on serum protein electrophoresis is less intensively stained and a comparative visual evaluation is acceptable only versus a normal control pattern. Densitometric scan will reveal a smaller peak of albumin on account of some of the other protein fractions.

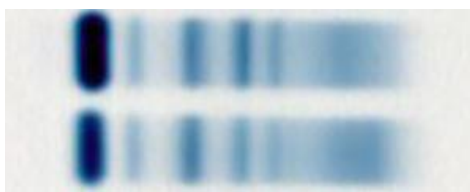


Figure 1: Normal and hypoalbuminemia SPE patterns

#### Alpha-1 Fraction

Alpha1 Fraction is comprised of alpha1-antitrypsin (AAT), thyroid-binding globulin (TBG) and transcortin. Usually it is increased in the context of acute infection and inflammatory disorders. The increased intensity may combine with decreased albumin and increased alpha2-globulins, thus forming the common pattern of acute inflammation. At the other hand, chronic inflammation, frequently associated with autoimmune diseases, chronic liver disease, chronic infection, and malignancy is characterized by increased alpha1-globulins with or without increased alpha2-globulins and polyclonal elevation of gamma region. Increased alpha1-fraction may be observed in pregnancy.

Decreased intensity of alpha1-globulin fraction is observed in nephrotic syndrome, exudative skin disorders and gastroenteropathies. It usually combines with decreased albumin, increased alpha2- and beta-globulins forming the pattern of protein losing disorders. Absent staining of alpha1 fraction is observed in AAT deficiency, an inherited condition related to defective synthesis of AAT where the synthesized AAT has irregular shape which doesn't allow the protein to leave the hepatocytes and reach the lungs where it normally inhibits the neutrophil elastase. AAT deficiency causes degradation especially to lung tissue and eventually leads to characteristic manifestations of pulmonary emphysema (DeMeo et al., 2004).

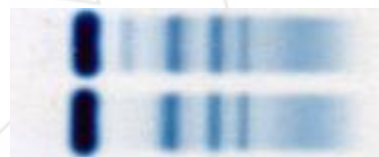


Figure 2: Normal and isolated AAT deficiency patterns

#### Alpha-2 Fraction

The proteins contributing to alpha2-globulin fraction are ceruloplasmin (CPL), alpha2-macroglobulin (A2M), and haptoglobin (HPT). Increased intensity in alpha2-band is usually observed in inflammatory states, nephrotic syndrome, oral contraceptive use, steroid use, and hyperthyroidism- in acute and chronic inflammation patterns and protein losing disorders. CPL carries more than 95% of the total copper in healthy human plasma (Hellman et al., 2002). It is also a copper-dependent oxidase, associated with possible oxidation of ferrous into ferric iron, therefore assisting in its plasma transport in association with transferrin (Song et al., 2013). CPL is found elevated as an acute phase reactant and in some genetic defects. Decreased CPL levels may be related to nutritive copper deficiency, Menkes disease and Wilson disease. A2M has normally higher concentration in children, probably as a defensive mechanism against a broad spectrum of proteases (Rehman et al., 2013). In the context of nephrotic syndrome the elevated A2M levels compensate the renal loss of low molecular weight proteinase inhibitors. HPT binds free plasma hemoglobin, which allows degradative enzymes to gain access to hemoglobin while at the same time preventing renal loss of iron and protecting the kidneys from damage by hemoglobin. HPT is a natural bacteriostatic against iron requiring bacteria such as E. coli. HPT rapidly decreases in hemolysis, ineffective erythropoiesis and liver

diseases. It is significantly decreased in newborns in the context of functional liver immaturity. The SPE pattern of hemolysis reveals decreased alpha2-band intensity and appearance of additional hemoglobin band between alpha2 and beta1 regions.

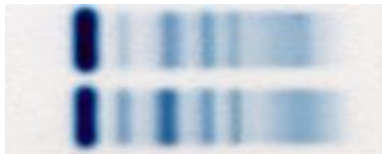


Figure 3: Normal and elevated alpha2-globulins patterns

### Beta Fraction

Beta fraction has two peaks- beta1 and beta2. Beta1 is comprised mostly of transferrin (TFR) and beta2 contains beta-lipoprotein. IgA, IgM, and IgG, along with complement proteins, can also be identified in beta fraction. Evaluation of the two separate peaks is usually troubled due to the variable electrophoretic mobility of immunoglobulins. SPE reveals increased beta region intensity in hyperlipidemia and iron-deficiency anemia. The differential diagnosis of anemia may be supported by SPE revealing normal beta band intensity in anemia in chronic disease and increased in iron deficiency. Beta region is decreased in acute inflammation as TFR is an acute phase reactant. It is also decreased in nephrotic syndrome, malnutrition and protein losing enteropathy. Congenital atransferrinemia yields extremely low intensity of beta region, iron excess, but severe hypochromic anemia not responding to iron supplementing therapy. Significant increase of beta region intensity may be caused by beta2-microglobulin (B2M) elevation as a result of total tubular dysfunction or B-lymphocyte related malignancies. Extremely elevated B2M is a marker predicting poor outcome after kidney transplantation and in multiple myeloma. C3 is liable and decreases with storage resulting in much variation in beta2 region. Extreme elevation of LDL results in both quantitative and qualitative changes in beta2 fraction as the band has a typical artifact-like appearance, easily distinguished by experienced specialists. This particular evaluation reveals the role of visual assessment of SPE. Sometimes elevated beta band intensity may be caused by underlying monoclonal bands in this region in the context of light chain disease, amyloidosis, heavy chain disease, IgD and IgE monoclonal gammopathies.

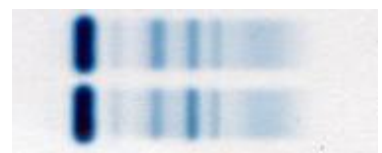


Figure 4: Normal and isolated beta1 elevation patterns

### Beta-Gamma Region

Immunoglobulins are usually distributed in gamma region. IgA has the most anodal mobility stepping into the beta-gamma interzone. Polyclonal elevation of IgA causes a beta-gamma fusion (s. beta-gamma bridge) in patients with cirrhosis, respiratory infection, skin diseases, rheumatoid arthritis. A critical preanalytical error may result in the appearance of a sharp fibrinogen band in beta-gamma region due to plasma application instead of serum. Occasionally,

blood drawn from heparinized patients does not fully clot, also resulting in a visible fibrinogen band which can be mistakenly interpreted as a monoclonal immunoglobulin (most often IgA).

### Gamma Region

SPE is most often appointed as a screening test for monoclonal gammopathies. Immunoglobulins are generally the only proteins present in normal gamma region. A normal gamma zone appears as a smooth diffuse blush with no asymmetry or sharp peaks. The extended gamma region is a result of both electrophoresis and the reverse process-electroendosmosis (EEO). EEO determines a partial gamma globulins migration towards the cathode, providing a stretched gamma image where discrete qualitative changes may be detected and accurately interpreted.

Hypogammaglobulinemia is a diffuse symmetrical decrease in gamma region intensity normally seen in infants. Immunoglobulins are pathologically decreased in agammaglobulinemia and hypogammaglobulinemia sometimes combined with decreased albumin in the context of lymphoproliferative disorders, inflammatory bowel disease, congenital immunodeficiencies. Selective immunoglobulin deficiencies, such as IgA deficiency, are suggested by the presence of pallor in gamma zone.



Figure 5: Normal and hypogammaglobulinemia patterns

Polyclonal gammopathies are presented with diffuse increase of gamma region intensity, most often symmetrical. The most common causes are severe infection, chronic liver disease (with beta-gamma fusion), rheumatoid arthritis, systemic lupus erythematosus, and connective tissue diseases. In general, it indicates a non-neoplastic condition although is not exclusive to non-neoplastic conditions. Polyclonal immunoglobulins increase may be combined with decreased albumin. Selective polyclonal gammopathies result in asymmetrical distribution of gamma region staining without formation of a sharp peak.

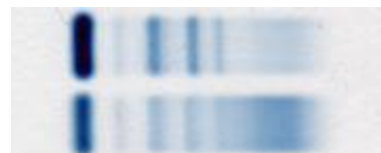


Figure 6: Normal and polyclonal gammopathy patterns

Oligoclonal gammopathies are presented by a few zones with restricted heterogeneity in gamma region. Oligoclonal bands with hypergammaglobulinemia and possibly beta-gamma fusion may be present in serum in patients responding to antigenic stimulation resulting from viral & bacterial infections, vaccines, autoimmune diseases and angioimmunoblastic lymphadenopathy. Oligoclonal bands



with decreased IgG concentration are found in chronic lymphocytic leukemia, post heart and bone marrow transplants, and common variable immunodeficiency and immunosuppressive therapy.

Monoclonal gammopathies are usually presented with a sharp band in gamma region, also known as M-spike. Clinically significant monoclonal bands should be at least as intense as the alpha1 fraction. Less intense bands are unlikely to be due to a malignant clonal expansion and are classified as monoclonal gammopathy of undetermined/uncertain significance (MGUS), which rarely progresses to multiple myeloma (Wadhera et al., 2010). Suspicious for MGUS are SPE patterns combining monoclonal band and increased acute phase fractions, transient monoclonal band evolving into an oligoclonal pattern, monoclonal band on the background of all immunoglobulin classes elevation, slightly abnormal kappa: lambda ratio. These patients should be monitored at three to six month intervals for tracking the progression of the starting condition. C-reactive protein (CRP) is an acute phase reactant which levels are normally undetectable in blood. CRP migrates in the anodal term of gamma region and its extreme elevation in acute inflammation may resemble a monoclonal band. Lysozyme may also be seen as a cathodal band in gamma region in myelomonocytic leukemia in which it is released from tumor cells. Therapeutic monoclonal antibodies (mAb) also migrate in gamma region and may be misinterpreted as a monoclonal gammopathy.



**Figure 7:** Normal and monoclonal gammopathy patterns

In most of the cases monoclonal proteins are associated with multiple myeloma and solitary plasmocytoma. Still, nearly 15% are associated with abnormal production of B-lymphocytes (lymphoma, chronic lymphocytic leukemia, macroglobulinemia of Waldenström, heavy chain disease). Broader width of monoclonal bands may be related to the amount of protein or heterogeneity of the monoclonal protein due to glycosylation.

#### 4. Conclusion

SPE is a reliable screening test for dysproteinemia used in a variety of diagnostic panels and for the diagnosis of multiple myeloma in particular. Bone pain and fatigue, pathological fractures or lytic lesions, anemia, proteinuria, renal insufficiency and hypercalcemia secondary to possible malignancy, as suggestive of an underlying plasma cell disorder, are indications for SPE appointment. SPE is generally considered in any patient with an elevated total protein, especially those with elevated globulin level relative to albumin. SPE results mark the direction of further investigations for the definitive diagnosis using immunofixation for defining the monoclonal protein and/or light chain, additional laboratory tests and other diagnostic

procedures.

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#### Author Profile

**Julieta Hristova** received the MD and PhD degrees in Medical University of Sofia in 2005 and 2015, respectively. Since 2007 she has been an assistant professor at Medical University of Sofia-Dept. of Clinical laboratory and Clinical Immunology.