Journal of Applied Health Sciences and Medicine, 4(11):1–5, 2024. DOI: 10.58614/jahsm4111

Journal of Applied Health Sciences and Medicine

Journal homepage: https://www.jpub.org/journal-details.php?journal-id=39

Research Article

Development of Serum Protein Electrophoresis Examination using Agarose Gel membrane

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Article Info

Keywords: *Agarose gel membrane, Serum protein, Patients with kidney disorders, Patients with liver disorders, Rheumatoid Arthritis Patients.*

Received: *24 July 2024*
Accepted: *25 October 2024*
Published: *3 December 2024*

Abstract

Serum protein electrophoresis on agarose gel membrane is a versatile protein fraction separation technique that can be applied to many analyses, especially in clinical laboratories. The development of serum protein electrophoresis examination was conducted to complement the clinical chemistry course. *Objective:* to develop a protein identification test that is still not applied, because the full automatic method has dominated the market, so that clinical laboratories in remote areas cannot afford to buy protein electrophoresis equipment, even though this test is very important to accelerate the diagnosis of liver, kidney, and autoimmune disorders. *Methods:* sampling serum of normal individuals and hepatitis patients, respondents each to determine the composition of the five protein fractions by electrophoresis, whether there is a difference. *Results:* in the serum of normal individuals, the albumin fraction dominated among other protein fractions, while in the serum of hepatitis patients, the gamma globulin fraction was more dominant than the albumin fraction. *Conclusion:* agarose gel membrane can be used as a routine examination to distinguish patients with liver disorders, ginkal and rheumatoid arthritis.

1. Introduction

Protein electrophoresis is a test to qualitatively and quantitatively measure specific protein fractions in the blood (when bands are read on a densitometer). This test separates protein fractions in the blood based on their molecular weight and electrical charge. Protein electrophoresis tests are often used to find abnormal substances called M proteins [1]. The presence of M proteins can be a sign of a type of cancer called myeloma, or multiple myeloma. Myeloma affects white blood cells called plasma cells in the bone marrow. Protein electrophoresis tests are also used to diagnose other conditions that affect plasma cells [2]. These include Waldenström's macroglobulinemia, Monoclonal Gammopathy of Undetermined Significance (MGUS), and primary amyloidosis. The amount of protein in the blood when too much or too little can cause health problems. There are 5 groups of proteins that are usually considered in protein electrophoresis tests, namely: albumin is a protein that transports substances and plays a role in tissue growth and repair. Second, alpha-1 globulin: primarily called alpha-1-antitrypsin, which is produced by the lungs and liver and increases with inflammatory diseases. Third, alpha-2 globulins this class of proteins has many functions in the body and is involved in inflammation. Fourth, Beta globulins these proteins move substances, support immunity, and increase in number in multiple myeloma disease and conditions such as high cholesterol and atherosclerosis. Fifth gamma globulin this type of protein supports the immune system and is highly increased in multiple myeloma disease, as well as some autoimmune conditions such as rheumatoid arthritis and systemic lupus erythematosus [3]. In protein electrophoresis, proteins are separated through electrical forces and electroendoosmotic forces. The net charge of a protein is based on the amount of its amino acid charge, and the pH of the buffer. Proteins are applied to a solid matrix such as an agarose gel, or cellulose acetate membrane in a liquid buffer, and an electric current is applied. Negatively charged proteins will migrate towards the positively charged anode. Albumin has the most negative charge, and

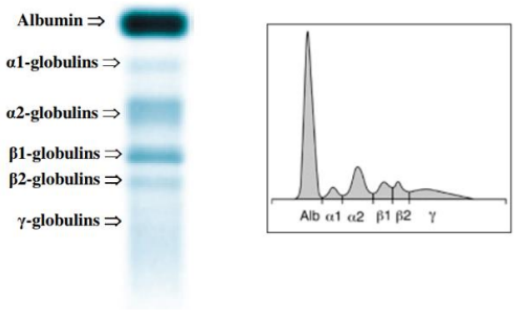

Figure 1: Electrophoresis results of serum proteins on agarose gel.

will migrate fastest towards the anode. Endoosmotic flow is the movement of liquid towards the cathode, which causes proteins with weaker charges to move backwards from their site of application. Gamma globulin proteins are the largest in size and have the least electronegativity, so they migrate last. Electrophoretic band images are useful for establishing the diagnosis of liver, kidney and autoimmune disorders [4]. liver, kidney and autoimmune disorders [4].

Until now, electrophoresis examination of serum protein with agarose gel membrane has not existed in the clinical laboratory, so the development of protein electrophoresis examination is needed. [5] Serum protein electrophoresis in 0.5% agarose Serum protein electrophoresis in agarose gel is still one of the basic examinations in clinical chemistry, and in practicum which serves to separate protein fractions by horizontal electrophoresis [6].

Serum protein electrophoresis using densitometry. The electrophoresis bands are useful for distinguishing the five main bands of normal serum electrophoresis forms.

Serum protein electrophoresis in 0.5% agarose Serum protein electrophoresis in agarose gel is still one of the basic examinations in clinical chemistry, and in practicum which serves to separate protein fractions by horizontal electrophoresis. In this electrophoresis setting, the protein is native, because it is not denatured. In alkaline buffer (pH 8.5-9.0) the protein fraction will be negatively charged and will migrate from the negative electrode (cathode) to the positive electrode (anode). Agarose gels, unlike acrylamide, have large pores for protein movement, consequently proteins are separated according to their surface charge density. This separation results in the albumin fraction (moving the farthest), followed by alpha-1, alpha-2, beta, and gamma globulins [7].

There are 5 groups of proteins that are usually considered during the Serum Protein Electrophoresis (SPEP) test [8] are Albumin This protein transports substances and plays a role in tissue growth and repair. Alpha-1 globulin primarily called alpha-1-antitrypsin, which is produced by the lungs and liver and increases with inflammatory diseases. Alpha-2 globulin this protein has many functions in the body and is involved in inflammation. Beta globulins these proteins move substances, support immunity, and increase in number in multiple myeloma and conditions such as high cholesterol and atherosclerosis. Gamma Globulin These proteins support the immune system and are increased in multiple myeloma, as well as some autoimmune conditions such as rheumatoid arthritis and systemic lupus erythematosus [9].

SPEP testing is necessary if symptoms and conditions affecting protein in the blood serum are experienced, such as unexplained weight loss, bone pain or frequent fractures, fatigue, weakness, nausea, constipation, excessive thirst, and back pain [10].

Some of the conditions that can cause the above symptoms are: cancer, thyroid, diabetes, anemia, liver disease, malnutrition, autoimmune diseases, and multiple sclerosis. [11] The various fractions of these proteins in the body have different functions. This means that high or low levels of the five types of proteins examined during the test can indicate different diseases. For example: elevated albumin levels mean dehydration, while lower than normal levels mean kidney, liver or a condition involving inflammation or poor nutrition. Alpha-1 globulin with a high level means dehydration and when the level is lower than normal, it means kidney or liver disease, and a condition involving inflammation or poor nutrition. [12] Alpha-2 globulin with a higher than normal level indicates kidney disease or a disease that causes inflammation (chronic or acute conditions), when the level is lower than normal, it means liver disease, poor nutrition, or red blood cell damage. Beta globulins with higher than normal levels indicate anemia, multiple myeloma, and high cholesterol, while lower than normal levels indicate poor nutrition or liver cirrhosis. [13] Gamma globulins with higher than normal levels indicate rheumatoid arthritis, infection, liver cirrhosis, inflammatory diseases, multiple myeloma, and lymphoma, while lower than normal levels indicate immune deficiency [14].

Currently in private clinical laboratories, protein electrophoresis using the capillary electrophoresis method is an analytical liquid-base separation technique. Capillary electrophoresis separates biomolecules or ions in capillary channels based on their electrophoretic mobility under the influence of an electric field. The movement of molecules during the application of high voltage is based on the electrophoretic mobility and the mobility of the electroosmotic flow buffer (EOF) inside the capillary. [15] Electrophoretic mobility of analytes or solutes depends on the size and shape of the molecules and their electric charge. Capillary electrophoresis systems are widely used in analytical biochemical processes, such as nucleic acid analysis, coenzyme separation, protein identification, genome sequencing, and DNA fingerprinting. This method has good efficiency and selectivity, but it is wasteful of electricity because it uses high-voltage electric current and the equipment is expensive, so the inspection rate is also expensive.

In order to minimize the cost of testing for liver disorders and autoimmune diseases, protein electrophoresis was developed using cellulose acetate membrane with a horizontal electrophoresis device that is cheap, and does not waste electricity, so that the cost of the examination can be reduced and the examination can be carried out in a simple laboratory.

2. Materials and Methods

Tools and Reagents

The tool used is a horizontal electrophoresis equipped with a power supply to create an electric current. The comb of the agarose gel electrophoresis device is used as an applicator, painting/washing container, and seeding [14]. Reagents, Agarose gel from Vivantis (PC0701-

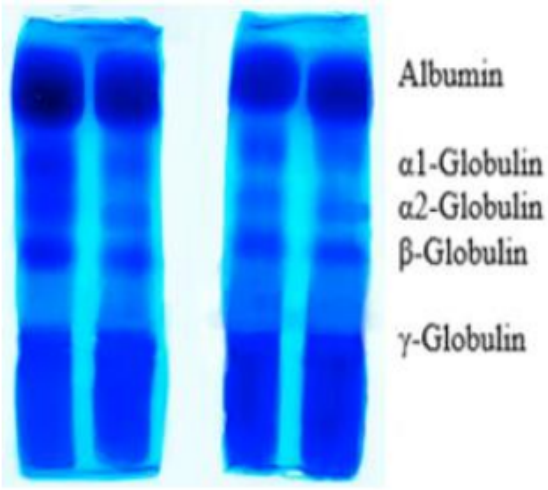

Figure 2: Serum of Normal Individuals

1900) is Agarose Gel Electrophoresis, for Molecular Biology, 100 Gram, TEB Buffer pH 8.6 (TBE Buffer 10x Stock Recipe: 108 g tris base, 55 g boric acid, 900 ml double-distilled H2O, 40 ml 0.5 M EDTA solution (pH 8. 0) Adjust volume to 1 L, NaCl, Aquadest, Glacial acetic acid, Coomassie brilliant blue G-250 dye: 10% (v/v) acetic acid/0.006% (w/v) Coomassie brilliant blue G-250 (Bio-Rad; store several months at room temperature), Isopropanol fixation solution: 25% isopropanol/10% acetic acid (v/v; store for several months at room temperature), 10% (v/v) acetic acid destaining solution, Methanol, Bromophenol Blue.

Data collection procedures

Procedure for Preparation of 0.5% Agarose Gel, 0.5% agarose gel by mixing buffer and solid agarose in 50 mL of TEB buffer pH 8.6 to 0.25 g of agarose in an Erlenmeyer, insert a stirring rod, then cover with aluminum foil, and heat to boiling while stirring continuously. This amount of agarose is for two gels, Once the solution starts to boil, the heater is turned off and allow the agarose to cool to approximately 60^{o}C, Then pour 25 mL of agarose and attach one comb to one side of the tray. Allow the gel to set for at least one hour. Pour 250 mL of cold electrophoresis buffer back into the gel, carefully remove the comb (wells should not break) and allow the gel to reach equilibrium for 5 minutes.

Serum Protein Electrophoresis Procedure, Assemble the electrophoresis equipment by inserting silicone seals into the grooves on both sides of the gel tray, and positioning the tray into the chamber, so that the gel faces the side wall of the chamber, Application of serum samples into the wells: below the surface of the buffer, 10µL of serum mixed with 1 µL Bromophenol Blue reagent so that the protein fraction rate is visible when electrophoresis is performed at 25 volts for 45 minutes or 100 volts for 30 minutes, close the chamber and connect it to the power source. Set the voltage at 100 V and let the electrophoresis run until the Bromphenol Blue reaches about 0.5 cm from the edge of the gel or the electrophoresis lasts about 30 minutes, the power supply is turned off, and the cables and tray are removed. In a plastic container with a lid, add ∼10 gel volume of isopropanol fixation solution.

Remove the gel from the electrophoresis unit and place it in the fixation solution. Place the container on a shaker for 10 to 15 minutes for 1 mm thick gels or 30 to 60 minutes for 1.5 mm thick gels in fresh fixation solution. The solution is removed from the petridish by suction (because when poured, the gel will break because it is soft), 5 to 10 gel volumes of Coomassie brilliant blue G-250 rapid staining solution are added. Shake for 30 minutes to 1 hour for 1 mm thick gel or 2 to 3 hours for 1.5 mm thick gel. Staining of protein bands becomes apparent within 5 to 15 minutes. Remove the dye solution by adding 5 to 10 volumes of 10% acetic acid and stirring gently. Removing the dye takes one to two hours, until a clear background with blue protein bands appears.

For storage, place the gel in a resealable plastic bag at 4°C in 10% acetic acid, then evaluate the gel by electroforeogram. [16] Observe the pattern/image and try to identify the visible protein bands (qualitative) or by Densitometer (quantitative). For a permanent record, scan or photograph the gel, or dry the gel as follows; place a wet piece of Whatman 3MM filter paper on the gel dryer and position the gel (which has been rinsed with water) on the filter paper. Cover the gel with a piece of plastic wrap and then add a second piece of wet filter paper. Dry for 1 to 2 hours at ∼80°C or according to the gel dryer instructions. As an alternative gel drying method, a porous membrane is used to clamp the gel in a buffer frame. In this case, dry the gel on a table at room temperature overnight [17].

3. Results and Discussion

The results of serum protein electrophoresis in normal individuals are as follows:
Figure 3. No. 1, 2, 3, 4 Serum of patients with renal impairment with Creatinine levels of 10 mg/dL and 6 mg/dL with low α1-Globulin band thickness and almost fused with albumin (in accordance with interpretation) [18], and nos. 5, 6, 7, 8 are from the serum of patients with Rheumatoid Arthritis with RF levels of 126 IU/mL and 78 IU/mL and have high γ-Globulin band thickness, this is in accordance with the interpretation, [19] and no. 9, 10, 11, 12 are from the serum of patients with Hepatitis B with positive HBsAg, have high α2-Globulin and γ-Globulin band thickness, this is in accordance with the interpretation [3].

In the interpretation of serum electrophoresis in Hypergamma globulinemia and in patients with liver disorders, the main attention is focused on the gamma region, which consists mainly of IgG-type antibodies. The gamma-globulin zone is decreased in hypogammaglobulinemia and agammaglobulinemia. Diseases that cause increased gamma-globulin levels include Hodgkin's disease, malignant lymphoma, chronic lymphocytic leukemia, granulomatous disease, connective tissue disease, liver disease, multiple myeloma, Waldenström's

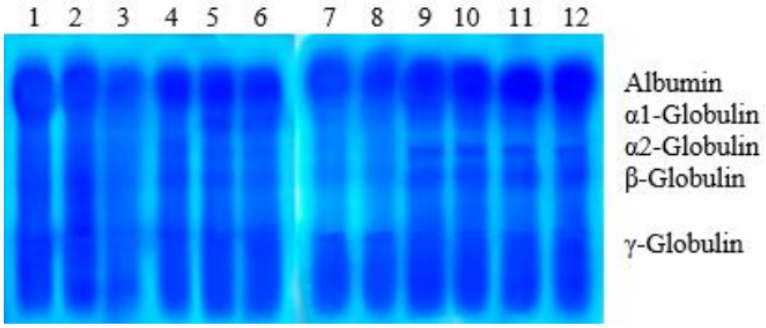

Figure 3: In Renal, Liver, and Autoimmune Impaired Individuals

macroglobulinemia, and amyloidosis [20].

Although many conditions can cause an increase in gamma regions, some disease states cause homogeneous spike-like peaks in focal regions of the gamma-globulin zone. These so-called "monoclonal gamopathies" constitute a group of disorders characterized by the proliferation of a single clone of plasma cells that produce homogeneous M proteins [21].

Features in normal individuals one to five percent of normal individuals show only oligoclonal patterns that have no clinical value. Bands without clonal types often appear in low concentrations, and are usually transient.

Features in liver disease, such as chronic liver disease can lead to increased production of globulins. These globulins are often antibodies that migrate to the β- and γ-regions, sometimes blurring the boundaries between them and this is known as the β-γ region bridge. Features in normal individuals one to five percent of normal individuals show only oligoclonal patterns that have no clinical value. Bands without clonal types often appear in low concentrations, and are usually transient.

Features in liver disease, such as chronic liver disease can lead to increased production of globulins. These globulins are often antibodies that migrate to the β- and γ-regions, sometimes blurring the boundaries between them and this is known as the β-γ region bridge. At the same time, albumin production oft [22].

Features in kidney disease. Dehydration is the main cause of hyperalbuminemia. Loss of water from the blood plasma causes a corresponding increase in the concentration of albumin and globulin. The ratio of albumin to globulin is not disturbed as both fractions are equal in concentration. The hematocrit also increases, provided that anemia does not occur at the same time [23].

Autoimmune diseases, autoantibodies are often seen in several autoimmune diseases, such as rheumatoid arthritis, Sjögren's syndrome, lupus erythematosis, progressive systemic sclerosis. Antibodies directed against viral proteins in HIV positive persons, viral hepatitis, meningitis, cytomegalovirus infection caused by C3 consumption associated with decreased beta globulin zone which can also be caused by near-defective serum samples [22].

4. Conclusion

Serum protein electrophoresis is a useful analysis for determining disease abnormalities when there is an increase or decrease in the concentration of a protein fraction. This is especially necessary when there is a suspicion of an organ abnormality. Interpretation of the results requires several similar cases, so that the pattern of protein fractions can be determined for each organ abnormality. It is important that the quality of the serum to be tested (e.g. lipemia and hemolysis) is avoided, so the development of serum protein electrophoresis techniques is tested as an important and valuable diagnostic tool in assisting the speed of diagnosis of a disease.

Article Information

Acknowledgements: Many thanks are addressed to Universitas Nahdlatul Ulama Surabaya for providing the opportunity to conduct research.
Conflict of Interest Disclosure: There are no conflicts of interest.

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