Al-Rasheed University College
Medical Analysis Department
Clinical Chemistry Lab.
Fourth Stage

كليـــــة الرشيد الجامعـــــة
قســـــم التحليلات المرضيــة
مختبر الكيميـــاء السريريـــة
المرحلـــــة الرابعـــــة

Serum Protein Electrophoresis (SPE)

Lecture (2)

Submitted by:
Ahmed Abdul Sattar
Assistant Lecturer

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Proteins are large biological molecules, or macromolecules, consisting of one or more long chains of amino acid residues.

Proteins perform a vast array of functions within living organisms, including:

- Catalyzing metabolic reactions.
- Replicating DNA.
- Responding to stimuli.
- Transporting molecules from one location to another.

Proteins differ from one another primarily in their ***sequence of amino acids***, which is dictated by the nucleotide sequence of their genes, and which usually results in folding of the protein into a specific three-dimensional structure that determines its activity.

Serum protein electrophoresis (SPEP) is a laboratory technique that is used to determine the levels of some types of proteins in a blood sample. Serum is the liquid part of your blood. Protein electrophoresis separates proteins based on their ***size*** and ***electrical charge***. There are a number of reasons why a doctor may order this test. One is to test for a cancer called multiple myeloma; another is to screen for multiple sclerosis, a chronic disease of the nervous system. SPEP is used to help diagnose or monitor a variety of different diseases or disorders that have abnormal proteins or protein levels. Electrophoresis is not usually used by itself to diagnose a disease. Instead, it is used along with other laboratory tests to provide more information to help with diagnosis.

Too much or too little protein can cause problems. The five groups of proteins usually considered during an SPEP test are:

➲ Components of Serum Protein Electrophoresis:-

The pattern of serum protein electrophoresis results depends on the fractions of two major types of protein: albumin and globulins. Albumin, the major protein component of serum, is produced by the liver under normal physiologic conditions. Globulins comprise a much smaller fraction of the total serum protein content. The subsets of these proteins and their relative quantity are the primary focus of the interpretation of serum protein electrophoresis.

Albumin, the largest peak, lies closest to the positive electrode. The next five components (globulins) are labeled alpha1, alpha2, beta1, beta2, and gamma. The peaks for these components lie toward the negative electrode, with the gamma peak being closest to that electrode. Figure (1) shows a typical normal pattern for the distribution of proteins as determined by serum protein electrophoresis.

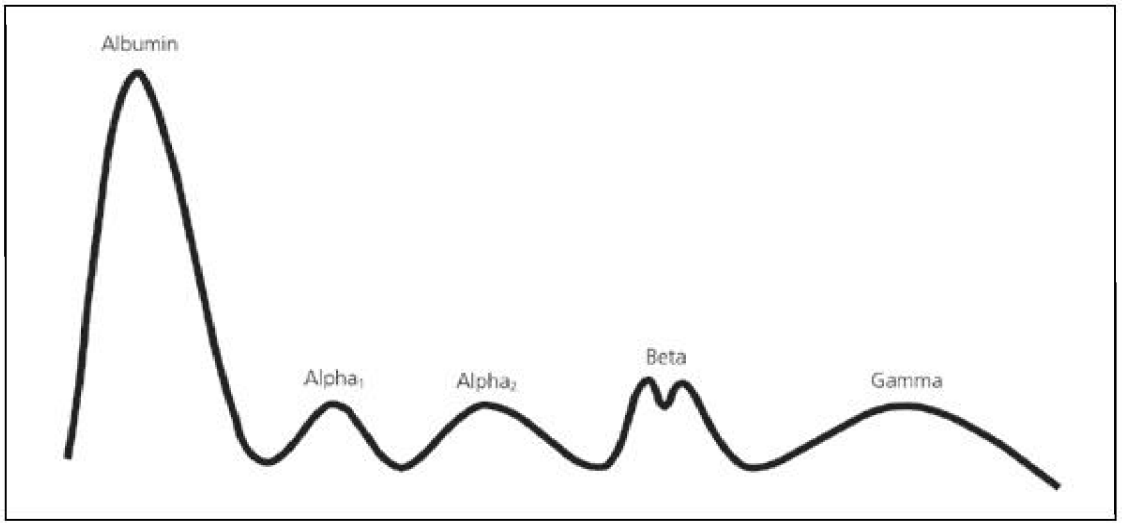

Figure 1

Typical normal pattern for serum protein electrophoresis.

1. **albumin:** transports substances; plays a role in tissue growth and repair. The albumin band represents the largest protein component of human serum. The albumin level is decreased under circumstances in which there is less production of the protein by the liver or in which there is increased loss or degradation of this protein. Malnutrition, significant liver disease, renal loss (e.g., in nephrotic syndrome), hormone therapy, and pregnancy may account for a low albumin level. Burns also may result in a low albumin level. Levels of albumin are increased in patients with a relative reduction in serum water (e.g., dehydration).
2. **alpha-1 globulins:** the major alpha-1 globulin is called alpha-1-antitrypsin, which is produced by the lungs and liver, and increase with inflammatory diseases. The alpha1-protein fraction is comprised of alpha1-antitrypsin, thyroid-binding globulin, and transcortin. Malignancy and acute inflammation (resulting from acute-phase reactants) can increase the alpha1-protein band. A decreased alpha1-protein band may occur because of alpha1-antitrypsin deficiency or decreased production of the globulin as a result of liver disease.
3. **alpha-2 globulins:** a class of proteins that have many functions in the body, but are involved in inflammation
4. **beta globulins: move substances;** support immunity, and in conditions like high cholesterol and atherosclerosis. The beta fraction has two peaks labeled beta1 and beta2. Beta1 is composed mostly of transferrin, and beta2 contains beta-lipoprotein. IgA, IgM, and sometimes IgG, along with complement proteins, also can be identified in the beta fraction.
5. **gamma globulins:** support the immune system and are increased in multiple myeloma, as well as some autoimmune conditions like rheumatoid arthritis and systemic lupus erythematosis. Much of the clinical interest is focused on the gamma region of the serum protein spectrum because immunoglobulins migrate to this region. It should be

noted that immunoglobulins often can be found throughout the electrophoretic spectrum. C-reactive protein (CRP) is located in the area between the beta and gamma components.

➲ **Normal results for STP & SPEP testing:**

	Test	%	g/dl	g/L
	Serum Total Protein	*Newborn*	5.2– 9.1	52– 91
		Adult	6.6 – 8.3	66 – 83
1	Albumin	60% - 75%	3.6 - 5.2	36-52
2	Alpha-1 (α-1)	1.7% - 5%	0.1 - 0.4	1 - 4
3	Alpha-2 (α-2)	6.7% - 12.5%	0.4 - 1	4 - 10
4	Beta (β)	8.3% - 16.3%	0.5 - 1.2	5 - 12
5	Gamma (γ)	10.7% - 20%	0.6 - 1.6	6 - 16

➲ **Abnormal Results for SPEP testing May Mean:**

	Test Result	Possible Condition(s)
Albumin	**Levels higher than normal**	**Dehydration**
	Levels lower than normal	**Kidney or liver disease, a condition involving inflammation, poor nutrition**
	Test Result	**Possible Condition(s)**
Alpha-1 Globulin	**Levels higher than normal**	**Disease leading to acute inflammation, condition may be**
	Levels lower than normal	**Liver disease, congenital emphysema, (rare)**
	Test Result	**Possible Condition(s)**
Alpha-2 Globulin	**Levels higher than normal**	**Kidney disease, nephronic disease leading to inflammation, condition may be chronic or acute**
	Levels lower than normal	**Liver disease, poor nutrition, breakdown of red blood cells**
	Test Result	**Possible Condition(s)**
Beta Globulin	**Levels higher than normal**	**Anemia, high cholesterol**
	Levels lower than normal	**Poor nutrition, liver cirrhosis**
	Test Result	**Possible Condition(s)**
Gama Globulin	**Levels higher than normal**	**Rheumatoid arthritis, infection, liver cirrhosis, inflammatory disease, multiple myeloma, lymphoma**
	Levels lower than normal	**Immune disorders and deficiencies**

➲ Procedure:

STEP-BY-STEP METHOD

A. Titan III Plate Preparation:

1. Properly code the required number of Titan III Plates by marking on the glossy, hard side with a Helena marker. It is suggested that the

identification mark be placed in one corner so that it is always aligned with sample No. 1.

2. Soak the plates for 20 minutes in diluted Electra HR buffer. The plates should be soaked in the Bufferizer according to the instructions for use included with the Bufferizer. Alternately, the plates may be wetted by slowly and uniformly lowering a rack of plates into the HR Buffer such that air is not trapped in the plates. The same soaking buffer may be used for soaking up to 12 plates or for approximately one week if stored tightly closed. If used for a more prolonged period residual solvents from the plate may build up in the buffer or evaporation may alter buffer concentration.

B. Electrophoresis Chamber Preparation

1. Pour approximately (100 ml) of diluted HR Buffer into each of the outer sections of the electrophoresis chamber. Do not use the same buffer in which the plates were soaked for electrophoresis.
2. Wet two disposable wicks in the buffer. Stand them lengthwise (on edge) in the buffer compartments. Fold the top edge of each wick over each support bridge, making sure the bottom edge is in the buffer and touching the bottom of the chamber. Press the top edge down over the bridge until the wick makes contact with the buffer, and there are no air bubbles under the wicks.
3. Cover the chamber to saturate the air with buffer. Discard electrophoresis buffer after use.

C. Sample Application

1. Fill each well in the sample plate with 3 µL of sample using the microdispenser. Expel the samples as a bead on the tip of the glass tube; then touch this bead to the well. Cover the samples with a glass slide if they are not used within 2 minutes.

2. Prime the applicator by depressing the tips into the sample wells 3 or 4 times. Apply this loading to a piece of blotter paper. Priming the applicator makes the second loading much more uniform. Do not load applicator again at this point, but proceed quickly to the next step.
3. Remove the wetted Titan III Plate from the buffer with the fingertips and blot once firmly with a blotter. Before placing the plate in the aligning base, place a drop of water or buffer on the center of the aligning base. This prevents the plate from shifting during the sample application. Place the plate in the aligning base, cellulose acetate side up, aligning the bottom edge of the plate with the black scribe line marked "CENTER APPLICATION". The identification mark should be aligned with sample No. 1.
4. Apply the sample to the plate by gently depressing the applicator tips into the sample well 3 or 4 times and promptly transferring the applicator to the aligning base. Press the button down and hold it 5 seconds

D. Electrophoresis

1. Quickly place the plate(s) cellulose acetate side down, in the electrophoresis chamber. Place a weight (glass slide, coin, etc.) on the plate(s) to insure contact with the wicks. Cover the chamber securely and wait 30 seconds for the plate(s) to equilibrate.
2. Electrophorese the plate(s) for 15 minutes at 180 volts. Power must be applied within 5 minutes after the plate(s) has been placed in the chamber.

E. Visualization of the Protein Bands

1. At the end of the electrophoresis time, remove the plate(s) from the chamber. Place them in 40-50 mL of Ponceau S stain (sufficient volume to cover the plate(s) for 6 minutes. When staining 2 or more plates, carry out the protocol vertically in a rack. The stain may be reused until the plate background contains stain precipitate.

2. Destain in 3 successive 2 minute washes of 5% acetic acid or until the plate background is white. The plates may be dried and stored as a permanent record at this point if stored in a plastic envelope to protect the surface. If a transparent background is desired (i.e. for densitometry), proceed to the next step.

If using Clear Aid Solution:

3. Dehydrate by rinsing the plate in two absolute methanol washes for two minutes each wash. Allow the plate to drain for 5-10 seconds before placing in the next solution.
4. Place the plate into the clearing solution for 5-10 minutes.
5. Drain off excess solution. Then place the plate, acetate side up, onto a blotter, and into a drying oven at 50-60°C for 15 minutes or until dry.

If using PermaClear Solution:

3. Place the plate(s) into the diluted PermaClear clearing solution for 2 minutes.
4. Drain off excess solution by holding plate(s) vertically for 1 minute. Then place the plate, acetate side up, onto a blotter, and into a drying oven at 50-60°C for 15 minutes or until dry.

RESULTS:

The fastest moving band, and normally the most prominent, is the albumin band found closest to the anodic edge of the plate. The faint band next to this is alpha1 globulin, followed by alpha 2 globulin, beta, and gamma globulins.