

Electrophoresis

Definition:-

Electrophoresis is a technique used to separate different elements (fractions) of a blood sample into individual components. Serum protein electrophoresis (SPEP) is a screening test that measures the major blood proteins by separating them into five distinct fractions: albumin, alpha1, alpha2, beta, and gamma proteins. Protein electrophoresis can also be performed on urine.

Purpose:-

Protein electrophoresis is used to evaluate, diagnose, and monitor a variety of diseases and conditions. It can be used for these purposes because the levels of different blood proteins rise or fall in response to such disorders as cancer, intestinal or kidney protein-wasting syndromes, disorders of the immune system, liver dysfunction, impaired nutrition, and chronic fluid-retaining conditions.

The movement speed of DNA:-

Depending on : -

- 1 - the power of the electric field.
- 2 - buffer solution.
- 3 - Intensity agarose Gel .
- 4 - The size of the DNA .

• small-sized molecules of DNA will move more quickly than larger particles.

Note: - Careful and attentive when the boiling process in agarose solution will become very hot, and the increase in heating possible that vaporizes a solution agarose.

Keeping the gel

- Methyl Alaithidiom works on correlation with DNA to help the process of radiation under ultraviolet.

Note: -

Methyl Alaithidiom carcinogen should be wearing gloves and caution in dealing with them.

Parts of device:-

1. Power supply
2. Cover
3. Gel tank
4. Electrical leads
5. Casting tray
6. Gel combs

Prepare Electrophoresis dish

- Run past the edges of the dish and then put the combs.
- put the dish on a flat surface, with a note not to touch the surface of the dish harrows Electrophoresis.

Adding the gel

- agarose solution should drown harrows but not entirely.
- care to remove air bubbles resulting from the heating process.

- leave agarose solution to cool down, to freeze for the duration (30-45 minutes).
- When it cools agarose gel will be flexible, semi-transparent, compared with its shape before cooling
- Remove the combs and pasting.
- Put the electrophoresis gel dish within the basin.
- Set up buffer solution in the basin electrophoresis until cover the surface of the gel, make sure that the solution is included in all the holes.

Add the sample

- DNA sample mostly be transparent, so mix with dye (3 Maekerolatr +5 Maekerolatr of the sample).
- Very carefully down the sample Pipette electronic slots inside the gel.

Moving of gel

- Cover the basin Gel by its cover of the device taking into account that each pole in its place.
- Open the power source.
- DNA will leave to the anode (the elevator).

Article Alaithidiom require ultraviolet until sacrificed DNA during the process of filming.

Normal results

The following values are representative, although there is some variation among laboratories and specific methods. These values are based on the agarose system.

- total protein: 5.9-8.0 g/dL

- albumin: 4.0-5.5 g/dL
- alpha1 globulin: 0.15-0.25 g/dL
- alpha2 globulin: 0.43-0.75 g/dL
- beta-globulin: 0.5-1.0 g/dL
- gamma globulin: 0.6-1.3 g/dL

Abnormal results

Albumin levels are increased in dehydration. They are decreased in malnutrition, pregnancy, liver disease, inflammatory diseases, and such protein-losing syndromes as mal absorption syndrome and certain kidney disorders.

Alpha1 globulins are increased in inflammatory diseases. They are decreased or absent in juvenile pulmonary emphysema, which is a genetic disease.

Alpha2 globulins are increased in a kidney disorder called nephrotic syndrome. They are decreased in patients with an overactive thyroid gland (hyperthyroidism) or severe liver dysfunction.

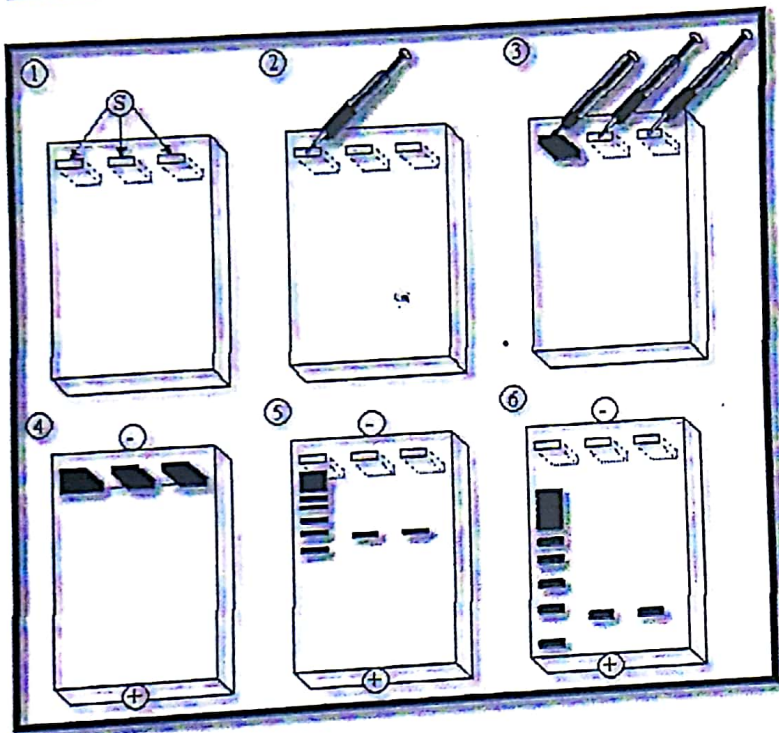
Beta globulin levels are increased in conditions of high cholesterol levels (hypercholesterolemia) and iron deficiency anemia. They are decreased in malnutrition.

Gamma globulin levels are increased in chronic inflammatory disease (for example, rheumatoid arthritis, systemic lupus erythematosus); cirrhosis; acute and chronic infection; and a cancerous disease characterized by uncontrolled multiplication of plasma cells in the bone marrow (multiple myeloma).

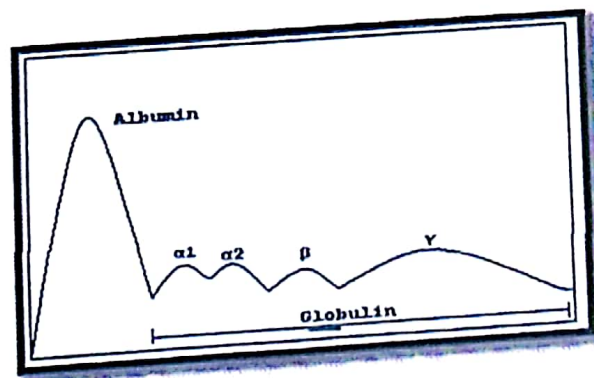
Gamma globulins are decreased in a variety of genetic immune disorders, and in secondary immune deficiency related to steroid use, leukemia, or severe infection.



Gel electrophoresis



Separation of protein



Normal separation of protein

The types of medium in electrophoresis:

1. Paper : It is Filter paper moistened with buffer, placed between electrodes, measure Amino acid, nucleotides

2. Polyacrylamide gel : used as Cast in tubes or slabs; cross linked
Measure the Proteins and nucleic acids.

3. Agarose gel : As polyacrylamide but no cross linking, measure Very large proteins, Nucleic acids and nucleoproteins, etc

Separation of protein:

Gels contain wells in which to place samples, and analytes start their migration from these points. A molecular marker can be run in one or both sides of the gel for later comparison, because it will migrate through the gel with the samples once electric current is applied. The electrophoresis buffer which covers the gel provides uniform pH and provides ions to support conductivity. When an electric current is applied across the gel, the sample molecules start moving through the gel matrix at different rates, towards the cathode if positively charged, or towards the anode if negatively charged. The voltage, as well as the time allotted for a particular run, depends on the size of the gel and the type of sample being analyzed. Running the current too long may cause samples to migrate off the gel, and turning the voltage too high may damage the samples or burn the gel itself.

The maintenance :

Cleaning the horizontal units:

- ① The Tank should be rinsed with warm water or distilled water to prevent build up salts (water at Temp. above 60°C can cause damage to the unit).
- ② The unit should be cleaned with warm water with mild detergent such as (hexan, Aliphatic, hydrocarbons)
- ③ The unit should not use following detergent: Acetone, phenol, chloroform, carbon tetrachloride, Methanol, ethanol, isopropyl alcohol.

Trouble Shooting

problem	Cause	Solution
Er 1 Error message	Over Current (400mA limitation reached)	check if the buffer concentration is appropriate. Excessive buffer concentration may cause over current issue. To clear the error message, press the START/STOP button again.
Er 2 Error message	Over Voltage (300V limitation reached)	Press START/STOP button to clear the error message contact.
Er 3	Thermal limitation reached (Output voltage < 10V)	1) check the connections 2) if Er3 error message persists, the problem may be caused by internal fan failure.
AL 1 Alarm message	Max. watts (60W) of power reached	warning message for refrance.