

# Clinical Significance of Electrophoresis in the Management of Patient

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The history of electrophoresis is even newer and shorter than that of paper chromatography. It was probably discovered in 1873 by Koning, it was not till 1946, however, that paper electrophoresis had its real beginnings. Again, it was the team of Consden, Gordon and Martin who started the ball rolling[1]. They reported the successful separation of charged particles by electrophoresis in silica gels. This led others to try paper as the supporting medium and almost simultaneously there appeared a spate of papers from various laboratories[2]. To name but a few of the early leaders in the field were Wieland and Grassmann in Germany, Durrum in America and Flynn in England (1948-1950). The early workers were mainly intrigued by the possibility of diagnosing disease by studying the changes in serum proteins as revealed by paper electrophoresis[3]. Even today this is still one of the major fields of investigation in paper electrophoresis, but the scope of the techniques is steadily being extended into almost every biological field, particularly when it is used in conjunction with paper chromatography as a two way procedure i.e., coupling the two techniques of chromatography and electrophoresis, to provide two way separations. Either techniques may be used for the first one-way run and is then followed by the other. All that is necessary is to ensure that after the first run, the solvent which is a buffer can be completely removed from the paper in readiness for the second run. In fact, it can be truly said that the history of paper electrophoresis still lies before it.

Electrophoretic patterns are utilized all over the world to acquire vital information of diagnostic importance which may not be apparent from the history, routine biochemistry, haematology or urinalysis. Certain patterns are very valuable in identifying diseases which may not have detected by routine diagnostic work up. The abnormalities in electrophoretic patterns are better correlated with the patients clinical data. Elevated and decreased quantitative values can be identified.

Electrophoretic patterns can be obtained for haemoglobin, serum proteins, cerebrospinal fluid and serum isoenzymes of lactate dehydrogenase, creatine kinase, alkaline phosphatase and gamma glutamyl transferase.

Electrophoresis has gained a secure position in terms of a valuable diagnostic aid for multiple myeloma, macroglobulinemia, nephrosis, exudative enteropathy, and hepatic cirrhosis, as well as various other diseases. However, many diseases that includes metabolic infections and neoplastic disorders are associated with non specific alterations of serum proteins[4].

Electrophoresis yields adequate separation and estimation of various serum proteins, most frequently in patterns or configurations consistent with several diseases. These patterns usually involve alterations in concentrations of one or more of the fractions, that is, albumin, alpha and beta globulins, and gamma globulin. It is the pattern of serum protein changes rather than alterations in concentrations of the individual serum protein fractions that has provided the maximum diagnostic usefulness. It must be kept in mind that the serum protein patterns or configurations are usually more informative and consistent with a pathologic lesion than isolated absolute values of individual fractions.

Electrophoretic techniques have been refined to a level where their practical utilization in the clinical laboratory has become an accessible, rapid, convenient and inexpensive procedure[5]. A system for haemoglobin and serum proteins electrophoresis has been set up at Shaikh Zayed Hospital.

The definition of electrophoresis is very simple 'Electro' means current and 'phoresis' mean fractions. Thus by applying a constant current, for a fixed time, at a known pH of the desired buffer, fractionation of the various bands of proteins, iso-enzymes etc. are obtained.

Different proteins have different molecular weights (sizes) and at a desired pH (which plays a critical role) have different electrical charges. Under the influence of an electric field, different proteins move at

different rates depending on their charges and molecular weights. Thus the separation of serum proteins as bands is obtained shown in Figs. 1 & 2).

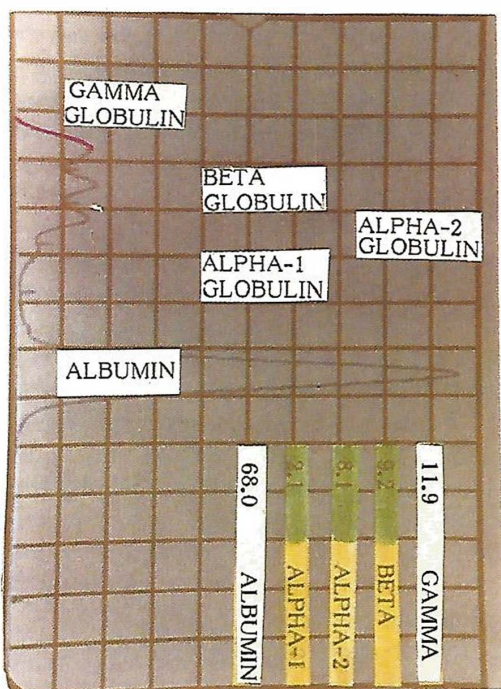


Fig. 1: Normal densitometric patterns of serum protein electrophoresis in terms of percentages.

Over 100 proteins have been identified in the serum[6,7]. Those in high concentrations were easy to measure, while those in low concentrations are difficult to separate; elaborate immunological techniques are applied. Electrophoresis of serum proteins is rather easy while haemoglobin electrophoresis is done in a more elaborate method.

Presently (at Shaikh Zayed Hospital) we are using cellulose acetate strips to scan the bands by means of a densitometer. We are in the process of developing and acquiring advanced supporting media which would have the ability to separate proteins in a very distinctive manner. The medium listed below have different types of modalities in terms of resolution of the bands:-

1. Paper Strips
2. Cellulose Acetate Strips
3. Agar gels-thin layers
4. Agarose (a purified agar derivative)
5. Starch
6. Acrylamide or Polyacrylamide-with this media

serum proteins can be separated upto 15 fractions.

The time required for separation and the degree of resolution, are the two major criteria for selection of any type of media. Cellulose acetate strips have the following advantages:-

- i) maximum economy
- ii) fast operation and
- iii) effective separation

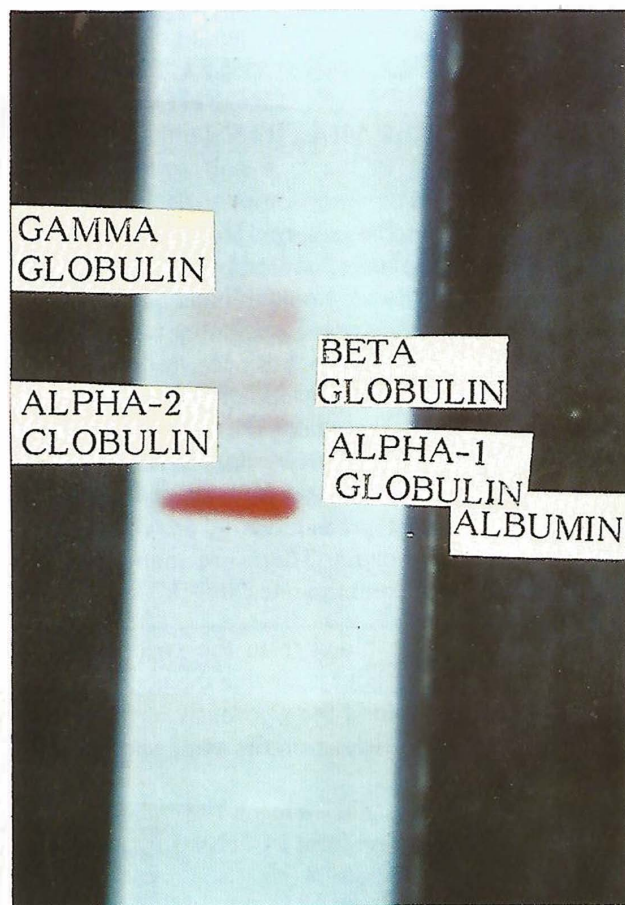


Fig. 2: Serum protein electrophoretic pattern on cellulose acetate strips developed at Shaikh Zayed Hospital.

These ensure reliability, reproducibility and greater sensitivity. Cellulose Acetate strips give a very clean and sharp separation of individual zones; this being the key to a swift and accurate interpretation and quantitation of the protein bands of a variety of substances e.g., serum proteins, haemoglobin, glycoproteins, lipoproteins and many enzymes.

Serum protein electrophoresis[8,9] is probably the most widely used electrophoretic applications which can

be used in many different clinical situations. An elaborate attempt has been made in this paper to scan all the relevant literature, present only the universally accepted findings[10,11], and to high light them. Serum is a liquid part of blood which is separated after coagulation of blood, it constitutes about 40-50% of blood. Plasma is about 50-60% of whole blood. The only difference between plasma and serum is that serum lacks all the clotting factors. Whenever possible, use serum as many anti-coagulants interfere with the chemical analysis, particularly the enzymes.

## **PRACTICAL FACTORS AFFECTING THE QUALITY OF ELECTROPHORETIC SEPARATIONS**

### **Sample's Collection**

The specimen to be collected should always be in a clean, dry glass test tube, preferably supplied by the laboratory, or the blood should remain in the syringe after drawing it from the patient and that sample should be sent to the lab. (right away) in order to process it, so that the sample can be run without any further delay. Whatever type of container is used, it must be scrupulously clean and dry. For serum proteins electrophoresis, only 0.5 ml clotted blood is sufficient. One of the important precautions in drawing blood is always to avoid hemolysis. There are many causes of hemolysis and some common ones are<sup>12</sup>.

- i) Forcing the blood from the syringe into the test tube without removing the needle.
- ii) Mixing of blood too vigorously
- iii Repeated pricking on the same veni-puncture site.
- iv) Moisture in the syringes (i.e., glass syringes when removed from autoclave).

### **Time and place of Collection**

The ideal way is to collect the sample or specimen at the place of examination. The reason being the crucial time interval between the collection of the sample and its examination. Secondly, an effort should be made to keep the sample at ambient temperature. Though seemingly trivial these factors are important in preventing erroneous results.

### **Avoidance of Contamination**

All efforts should be made to avoid any type of contaminants while taking out the serum from the test tube. Always rinse the pasteur tube or tips 3-4 times

with distilled water. Then draw a small amount and with that serum, rinse the tips or pasteur tube, discard that serum. Now draw the serum and process it for examination.

### **Transportation of the sample**

If the sample of serum has to be transported from one place to another during the hot season, make sure that the sample is kept frozen. Otherwise the results may deviate. There are several basic clinical situations in which a clear picture can be obtained from serum protein electrophoresis[12-15].

### **Buffer pH, Ionic strength and Composition**

High ionic strength yields distinct separations, but the mobilities of the fractions are low, giving a crowded pattern; low ionic strength gives faster rates of migration but more diffuse patterns. The ideal ionic strength of the buffer yields optimum results. The composition of the buffer affects the quality of separation to only a small extent. Barbitol based buffers are almost universal for proteins; TRIS buffers are used for hemoglobins.

### **Evaporation and heating**

In order to minimise the evaporation and heating, the enclosed volume of the electrophoretic tank is kept small and the tank is carefully sealed, as a result the inside atmosphere rapidly becomes saturated. Since heating is proportional to the square of the current, the voltage used should be just adequate to give a good separation in a reasonable working time.

### **Sample Application**

The sample is applied as a sharp, narrow band in order to minimise diffusion. The applicator must be scrupulously clean and undistorted. In agarose gel and starch gel a narrow slot is created in the gel. The sample is transferred with either a fine pipette or a serrated plastic edge.

### **Staining and Cleaning**

For the best results, the combined stain-fixative should not be used more than 8-10 times, and dehydration and cleaning should be done after every 3rd use. One of the chief advantages of cellulose acetate as a support medium is the ease with which the separated fractions can be stained plus the ability to make the membrane crystal clear for subsequent scanning of the pattern of separated fractions.

### Scanning and Quantifications

A densitometer is a sophisticated scanner. It is a device that moves the stained, cleared strip past a light source and filter-slit-photometer system. No matter how elaborate the system, the quality of the results are determined by the stained and cleaned strip of cellulose acetate.

The following groups of disease can be diagnosed by electrophoresis.

#### Liver Diseases

The globulin bands used are gamma globulin beta, alpha-2 and gamma globulin bands. Three distinct stages of liver diseases can be seen.

- a) Acute inflammatory processes
- b) Chronic hepatitis
- c) Cirrhosis

#### Kidney Diseases[16,17]

The bands assessed are albumin and alpha-2 globulins. The variation in the bands gives a clue to the degree of nephritis/nephrotic syndrome.

#### Inflammatory diseases [18,19]

In such cases, the bands assessed are alpha-2, alpha-1, beta and gammaglobulin bands. Three situations can be delineated:

- i) acute inflammations e.g., sepsis
- ii) sub-acute or chronic inflammations
- iii) tumors

#### Immunological System Defects[20,21]

Cases of hypo gammaglobulinemia associated with immunological defects (e.g., an antibody deficiency). The bands assessed are gammaglobulins.

#### Paraproteinaemias[22]

All globulin bands are assessed which assume, steep-pointed spikes when scanned on a densitometer. Monoclonal gammaglobulinemias of beta or gamma type are the most common. They may be associated with Bence Jones proteins in the urine.

#### Defective Proteinemias[23]

These are rare conditions and individual anaemia cases will show an absence of specific globulin bands.

#### Other Significant Proteinemias

**Bis-Albuminemia-** a duplicated albumin band indicates bis-albuminaemia which may mark the onset of plasmacytoma. Bis-albumin is a serum albumin which is separated into two distinct bands[24,25]. This abnormal pattern is inherited and there is no associated disease. The individuals are asymptomatic and the total levels of albumin and other serum proteins are normal. Approximately 10 electrophoretic forms of albumin have been reported[26]. Five have travelled faster and five slower than normal albumin. The inheritance is autosomal dominant. Normal albumin migration is increased with drugs such as aspirin and penicillin and with hyper amylasemia and hyperbilirubinemia[27,28].

Pre-albumin[29,30] exists in the serum in such small quantities that it is not readily scanned by the laboratory electrophoretic procedure. Pre-albumin is a glycoprotein and is synthesized in the liver. It is especially active in the binding of T3 and T4[31,32]. It has a very short half life of 1.9 days and this makes it a sensitive indicator of any changes affecting the synthesis and catabolism of proteins[33]. Serum pre-albumin concentration is a good liver function test. Its normal concentration is 10-40 mg/dl by radio immuno diffusion[34,35]. Some of the common causes for a low serum pre-albumin are severe liver disease, congestive heart failure and burns.

**Table 1: Serum proteins**

No.	Name of Diseases	Bands Assessed
1.	Liver Disease	Alpha-2, Beta & Gamma Globulins.
2.	Kidney Disease	Albumin, Alpha-2 Globulins
3.	Inflammatory Disorders	Alpha-2, Beta 1 & Gamma Globulins
4.	Immune Disorders	Gamma Globulins
5.	Paraproteinaemias	All Globulins (Narrow based, Steep, pointed Peaks) Plasma Cyto B-J Paraproteinaemia in urine
6.	Defective proteinemia	Absence of Specific Globulin Bands
7.	Other proteinemias	Albumin Bands Duplicated Bis-Alubmin, Anaemia and onset of Plasma Cyto

Table 3: Serum proteins pattern in normal and disease states

Conditions	Total	Albumin	Alpha-Globulin	Beta-globulin	Gamma-Globulin
Normal	100 (6.5-8.0 g/dl)	53%	14%	12%	20%
Myeloma	Usually Increased	Usually Increased	Myeloma proteins may be present in any of the globulin fractions and as much as 80% of the total protein.		
Nephrosis	Decreased	Greatly Decreased	Greatly Decreased	Normal to Slightly Decreased	Slight to moderate decrease
Obstructive Jaundice (early)	Normal	Normal	Normal	Normal	Normal
Hepatitis	Usually Normal	Decreased	Slight decrease	Slight decrease	Increased
Cirrhosis	Decrease	Decrease	Frequent decrease	Slight increase	Increase
Infections & Inflammation (liver normal)	Usually Normal	Usually Normal	Increase	Bacterial Infections: marked increase viral infections: Slight increase	Increase

Table 2: Normal range by cellulose acetate electrophoresis

Fraction	Gm/100ml	Percent of Total
Albumin	3.7-5.7	54-74
<b>Globulins:</b>		
Alpha 1	0.1-0.3	1.1-4.2
Alpha 2	0.4-1.0	4.6-13.0
Beta	0.5-1.5	7.3-13.5
Gamma	0.5-1.5	8.1-19.9
<b>Total Proteins</b>	<b>6.5-8.2</b>	<b>100</b>

Table 4: Normal values of serum proteins

Pre-albumin	4%
Albumin	63%
Alpha-1-globulin	5%
Alpha-2-globulin	8%
Beta globulin	13%
Gamma globulin	7%

## REFERENCES

1. Browning, D R et al. Chromatography-Instrumental method series. New York. McGraw-Hill, 1969.
2. Smith, I, et al. Chromatographic and electrophoretic techniques, 4th ed. London: Heinemann, 1976.
3. Zweig, G., et al. Paper chromatography and electrophoresis. New York: Academic Press, 1976.
4. Davidsons, Israel, MS, et al. Clinical diagnosis by laboratory methods. 14th ed. W.B. Sander 1969: 527.
5. Weit-Kamp L.R., et al. Human serum albumin: twenty three genetic variants and their population distribution. Ann. Hum Genet. 1973 36: 41-9.
6. Raphael, S S., et al. Lynch's medical laboratory technology. 4th ed. 1983: 140.
7. Shandon. Bio-Scientec: a catalogue of products and systems 1, 1987: 15.
8. Frohlich, J., et al. Bisalbuminemia. a new molecular variant, albumin vancouver. Clin. Chem. 1978, 24: 912-5.
9. Harris R I, et al. The pre-albumin fraction: A useful parameter in the interpretation of routine protein electrophoresis. J Clin Pathol 1974; 27 86-9.
10. Hutchinson D R., et al. Prealbumin as an index of liver function after acute paracetamol poisoning. Lancet 1980; 120-127.
11. Schultze, H E, et al. Molecular biology human proteins. Amsterdam. Elsevier, 1986: 561.
12. Schultze, G R. How to submit a specimen for a pathological examination. 2nd ed. 1986.
13. Itano H A, et al. A rapid diagnostic test for sickle cell anaemia. J clin. Pathol 1949; 23: 781-3.
14. Josaphson A M, et al. Starch block electrophoretic studies on human Hb solutions. Blood. 1958; 13: 543-5.

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15. Kunkel H G, et al. Observations on the minor basic hemoglobin component in the blood of normal individuals and patients thalassemia. *J Clin. Invest.* 1958; 36: 1615-8.
16. Lehmann. H et al. *Man's haemoglobin* 2nd ed. Amsterdam: North-Holland Published, 1973.
17. Lingel J B, et al. A comparison of amino acid incorporation in the haemoglobin and ribosomes of narrow erythroid cells and circulating reticulocytes of severely anemic rabbits. *Biochemistry* 1963; 2: 309-11.
18. Marder V J, K. et al. Electrophoresis of haemoglobin on agar gels. Frequency of haemoglobin D in a negro population. *Bull. Johns Hopkins Hosp.* 1959; 105: 77-9.
19. Marengo-Rowe A J. Rapid electrophoresis and quantitations of haemoglobins on cellulose acetate. *J Clin Pathol* 1965; 18: 790-3.
20. Poulik M D. Starch gel electrophoresis in discontinuous system of buffers. *Nature* 1957; 180: 1477-9.
21. Shepard M K, et al. Semi-quantitative estimation of the distribution of fetal haemoglobin in red cell populations. *Bull Johns Hopkins Hosp* 1962; 110: 293-5.
22. Singer K, et al. Studies on abnormal haemoglobins. *Blood* 1957; 6: 413-5.
23. Smithies O, et al. An improved procedure for starch gel electrophoresis and further variations in the serum proteins of normal individuals. *Biochem J* 1959; 71: 585-8.
24. Weatherall D J, et al. *The thalassemia syndrome*. 2nd ed. Oxford: Blackwell Scientific Publications, 1972.
25. Weatherall D J, et al. Preliminary surveys for the prevalence of the thalassemia genes in some African populations. *Ann Trop Med Parasitol* 1971; 65: 253-6.
26. White J M, et al. The unstable hemoglobin-molecular & clinical features. *Progr Hematol* 1971; 7: 697-1.
27. White J M, et al. Globin syntheses in sideroblastic anaemia. *Br J Haematol* 1971; 20: 263-6.
28. Betke K, et al. Estimation on small percentages of foetal hemoglobin. *Nature* 1959; 184: 1877-8.
29. Boyer S H, et al. Myoglobin: inherited structural variation in man. *Science* 1963; 140: 1228-9.
30. Carrell R W, et al. A simple method for the detection of unstable haemoglobins. *Br J Haematol* 1972; 23: 615-7.
31. Sandar G, et al. *Serum proteins in health and disease*. London: Chapman and Hall 1966.
32. Farle D P. Abnormal albumins. In: Beeson P B, McDermott W, eds. *Cecil-Loeb textbook of medicine*. 12th ed. Philadelphia; W B Sanders, 1967.
33. Rosen B J, et al. The gamma globulins III the antibody deficiency syndrome (concluded). *N Engl J Med* 1966; 275: 765-7.
34. Hong R, et al. Limited heterogeneity of gamma globulin in hypogammaglobulinemia. *Science* 1967; 156: 1102.
35. Franklin E C, et al. Heavy chain disease: a new disorder of serum r-globulins. *Am J Med* 1964; 37: 332-3.

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