Jordan univ.2018

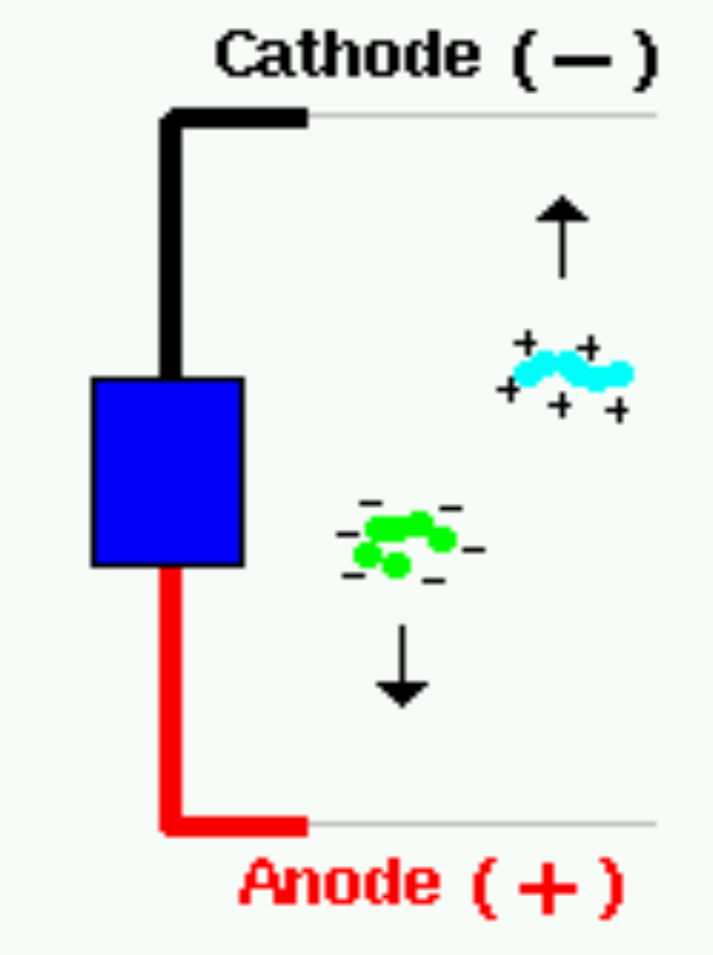
LABORATORY INSTRUMENTATION AND MEASURMENTS METHOD

lect5

Principle of Separation

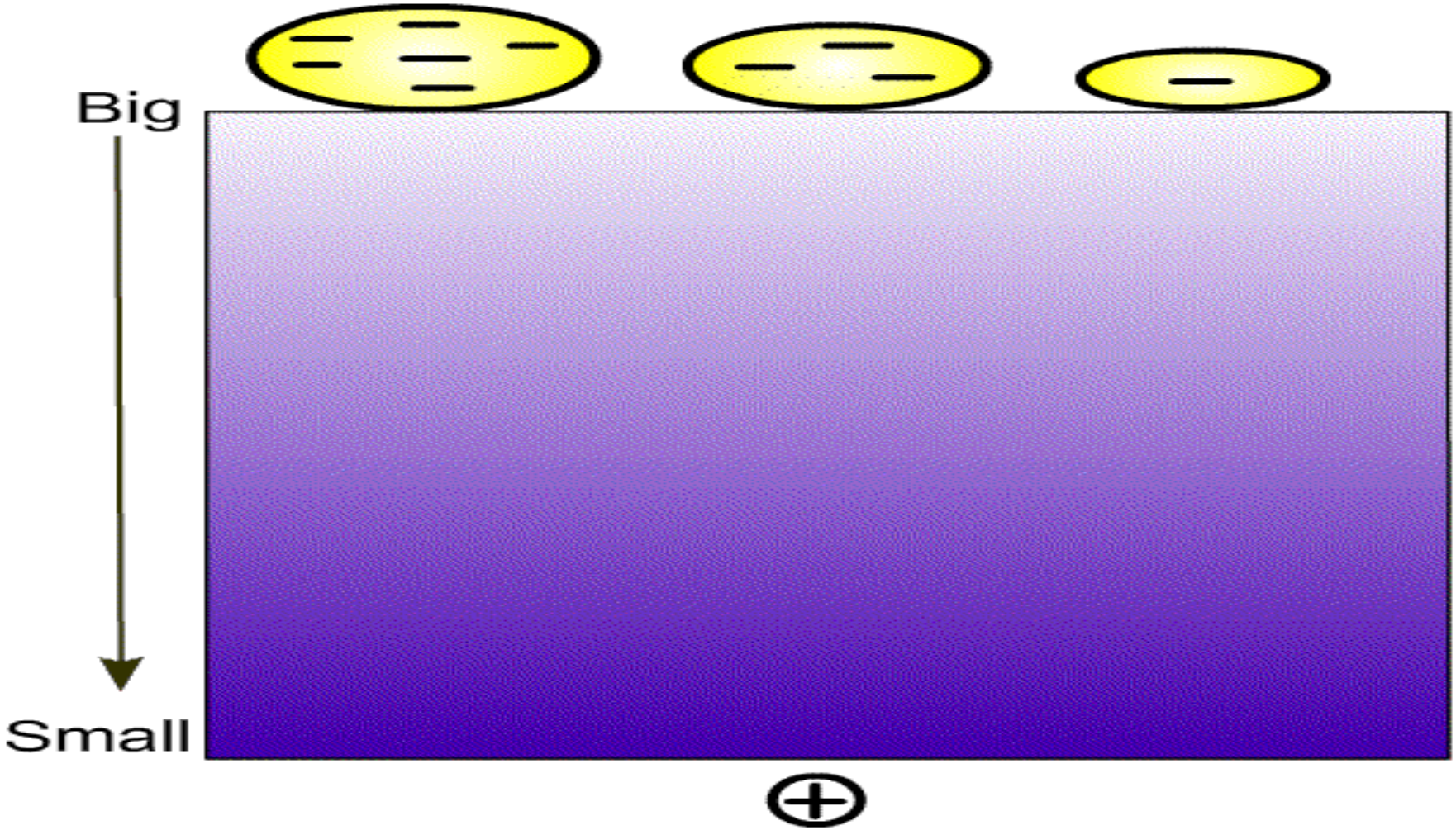
- According to charge
- According to size

(1) According to charge



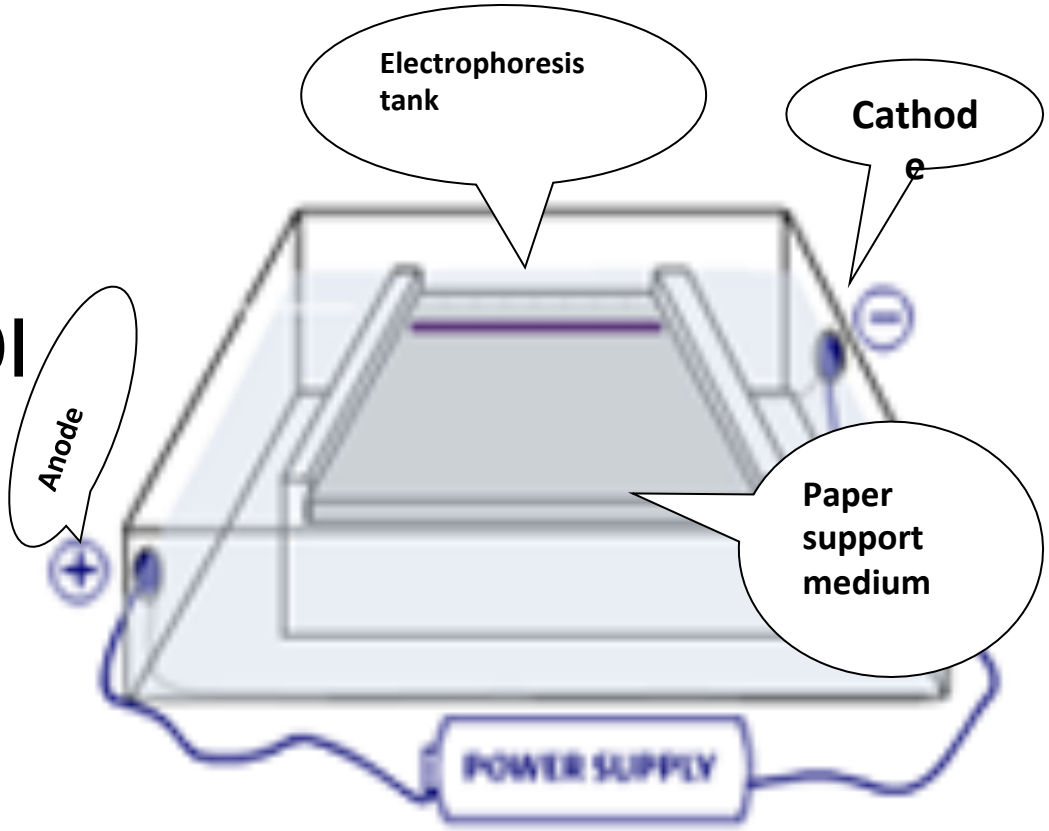
- **When charged molecules are placed in an electric field, they migrate toward either the positive (anode) or negative (cathode) pole according to their charge.**

(2) According to size

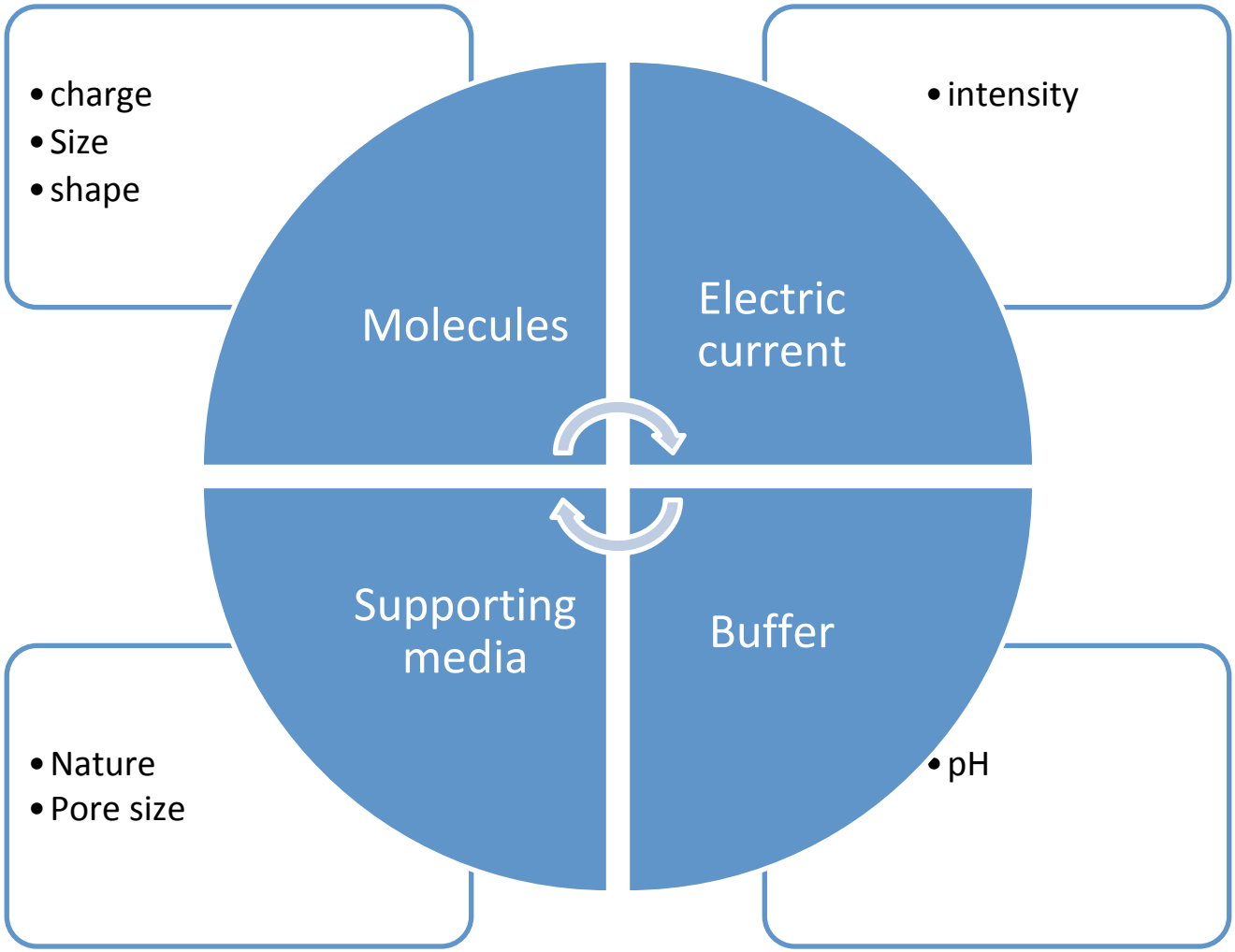


Instruments and Reagents

- Power supply
- Tank
  (Buffer ,Suppoı
  ting media)
- Detection and
  Quantification



Factors affecting the rate of migration of charged molecules:

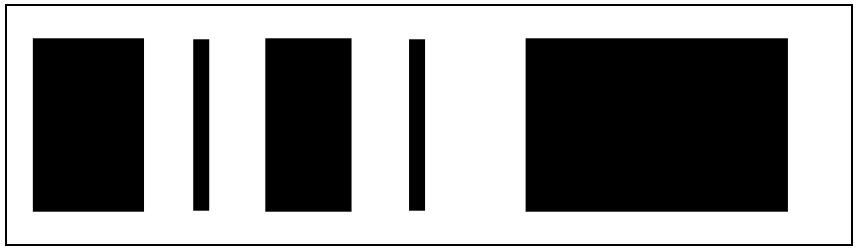



1
2
3
4
5
6

Electrophoresis pattern of normal serum proteins

1 2 3 4 5

Alb α1 α2 β γ



Densitometer scanning from cellulose acetate strip.

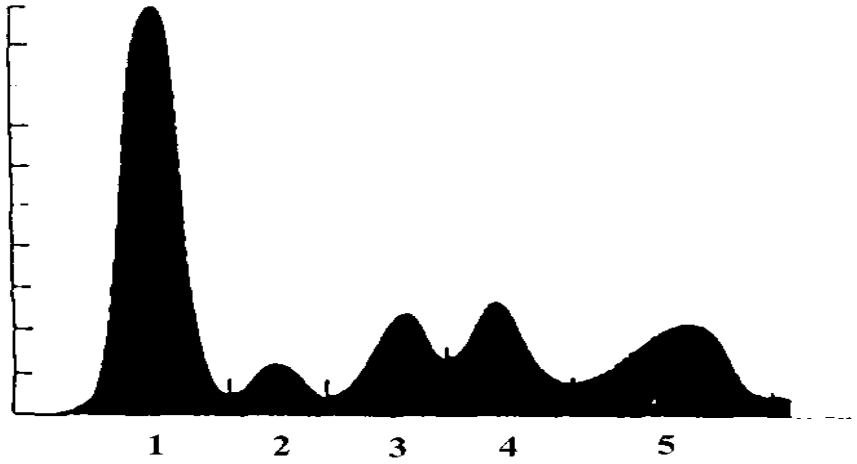
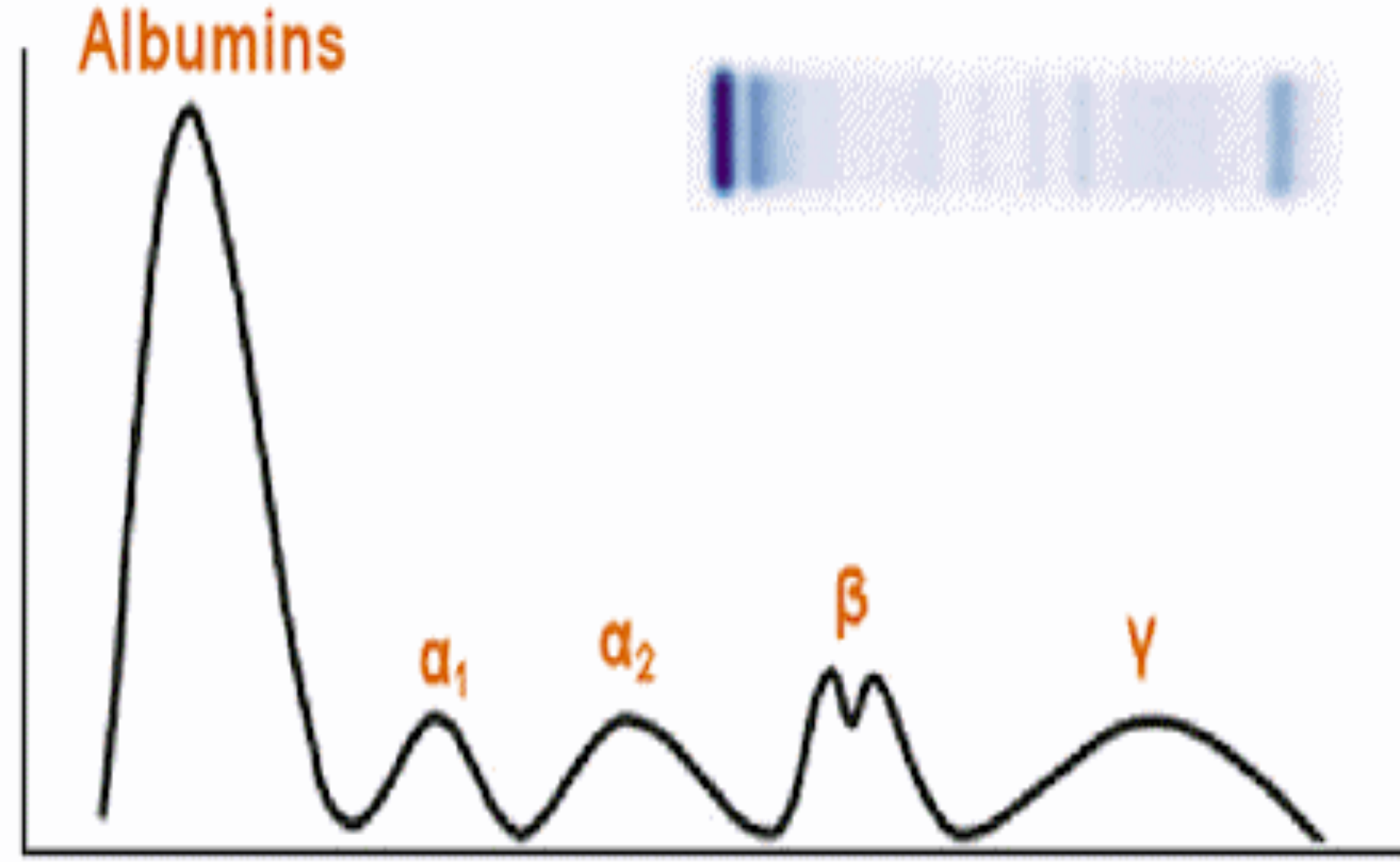


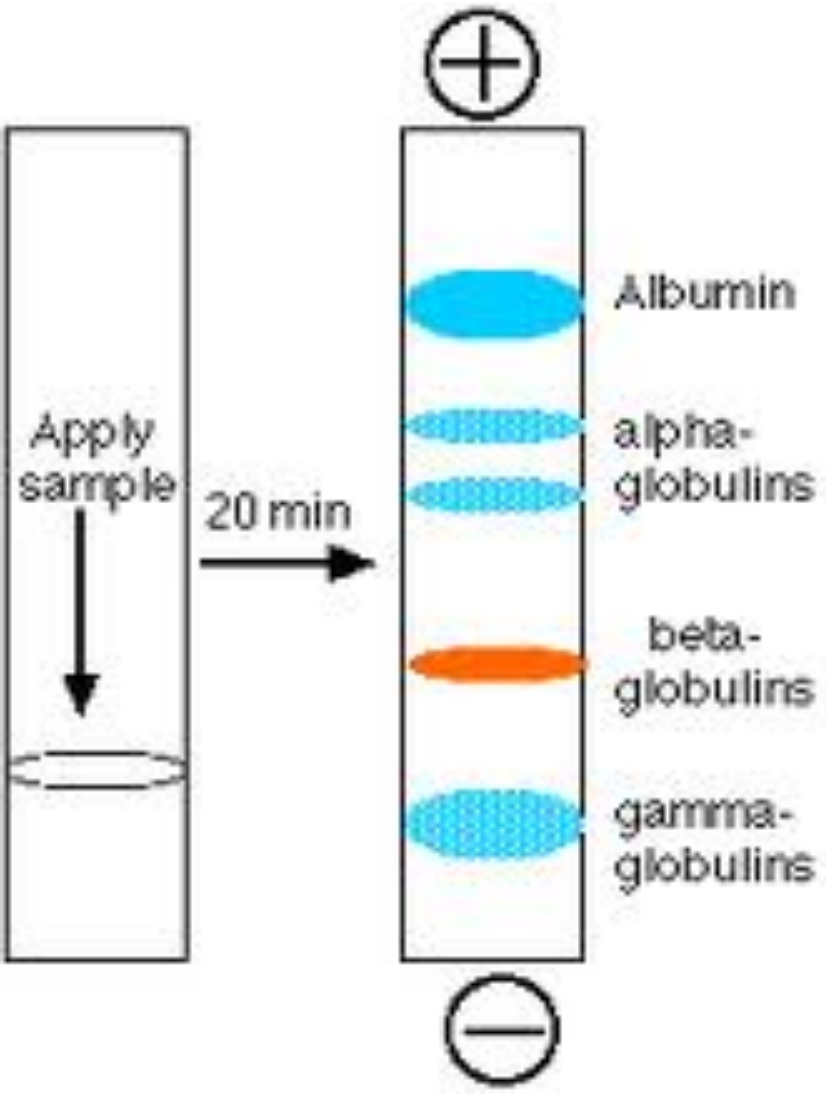
Figure 1: Normal electrophoretic graph and Blood Proteins



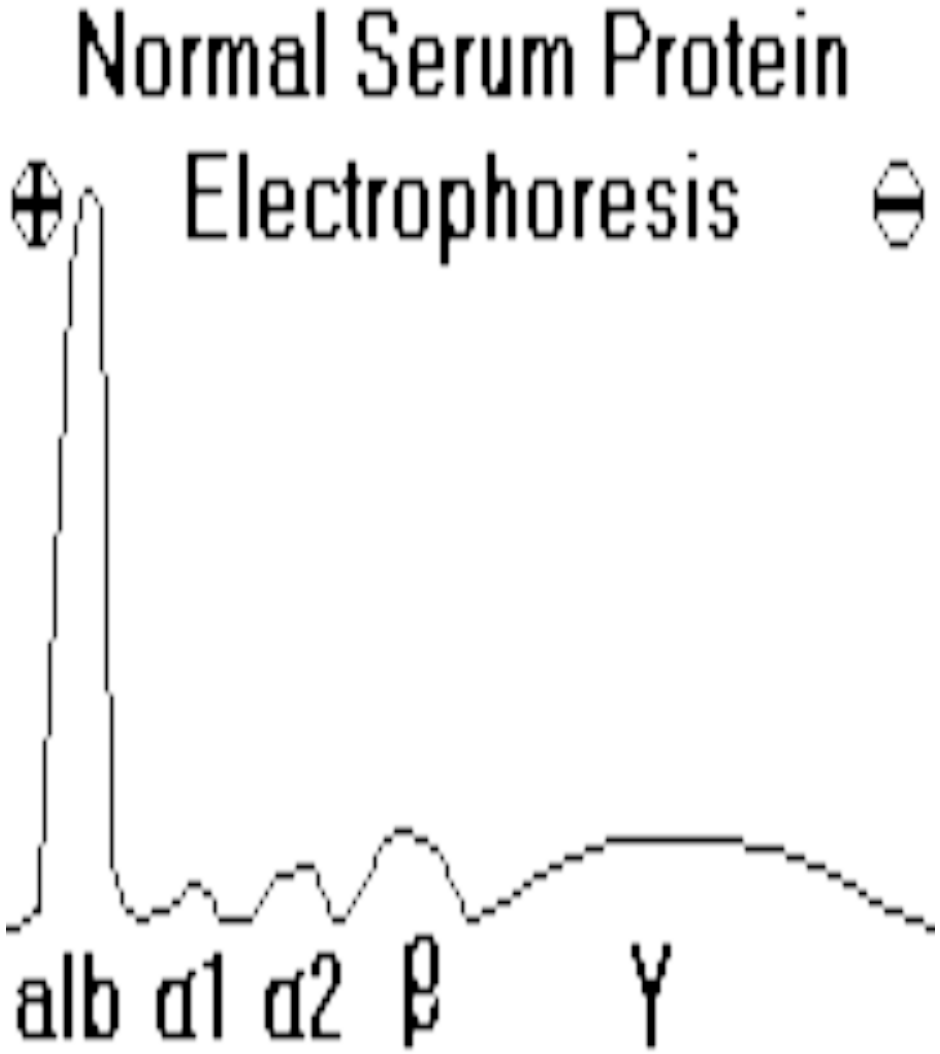
Interpretation of the results:

- **Albumin** is the fastest protein , forming the largest band , and is closest to the positive electrode.

- The next 4 bands are globulins; **alpha 1**, **apha 2**, **beta** and **gamma** globulins, respectively.



Separating serum proteins by electrophoresis



Serum Protein Electrophoresis is useful in detecting: clinical disorders

Albumin

Hypoalbuminemia

- **Liver disease**
- **Kidney disease**

∝ 1 – antitrypsin deficiency

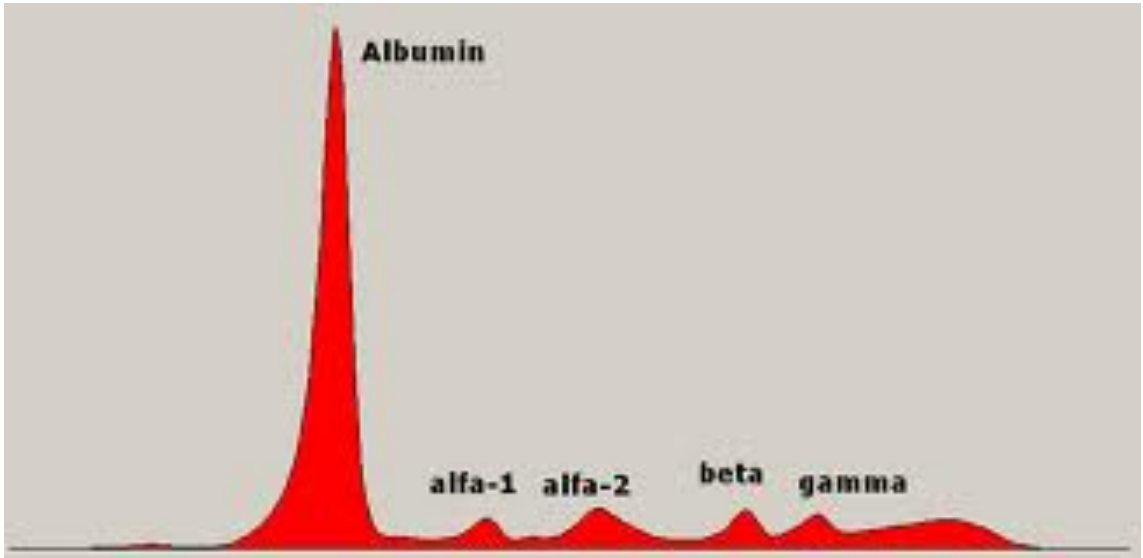
Immunoglobins

Hypogammaglobinemia

Hypergammaglobinemia

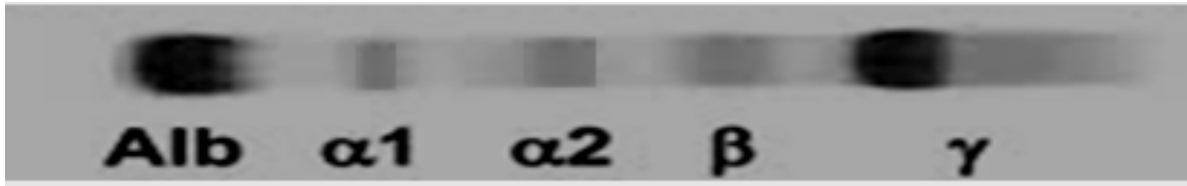
Interpretation of results require knowledge of common patterns. You should always interpret according to the normal pattern

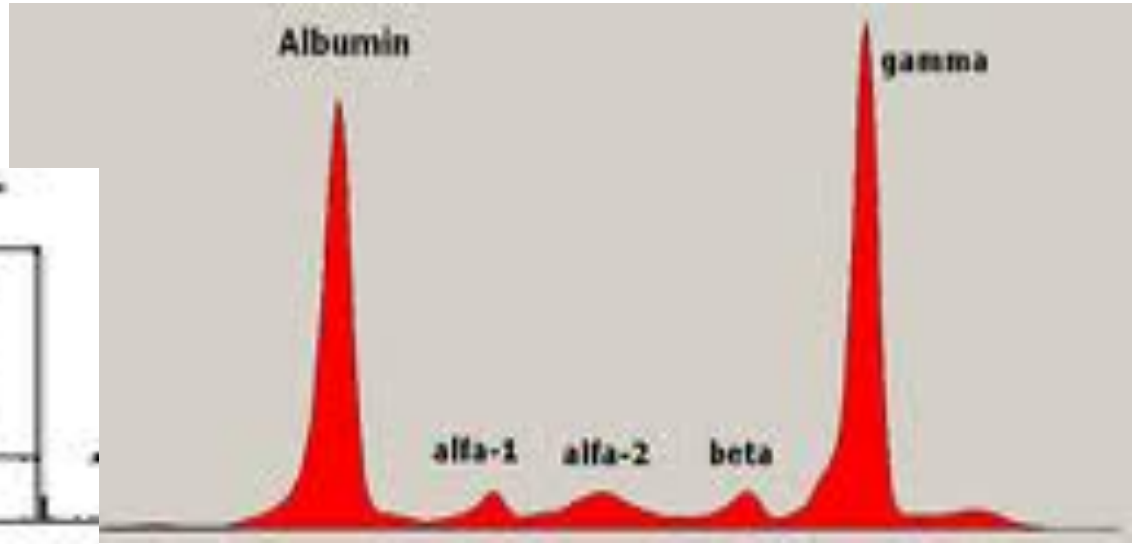
Normal Pattern

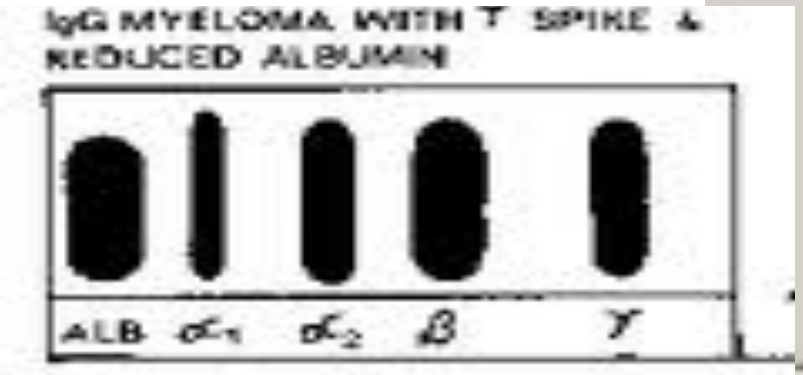


Monoclonal Gammopathy Pattern

The large spike in the gamma region is indicative of an increase in one immunoglobulin class typically seen in monoclonal gammopathies such as multiple myeloma.

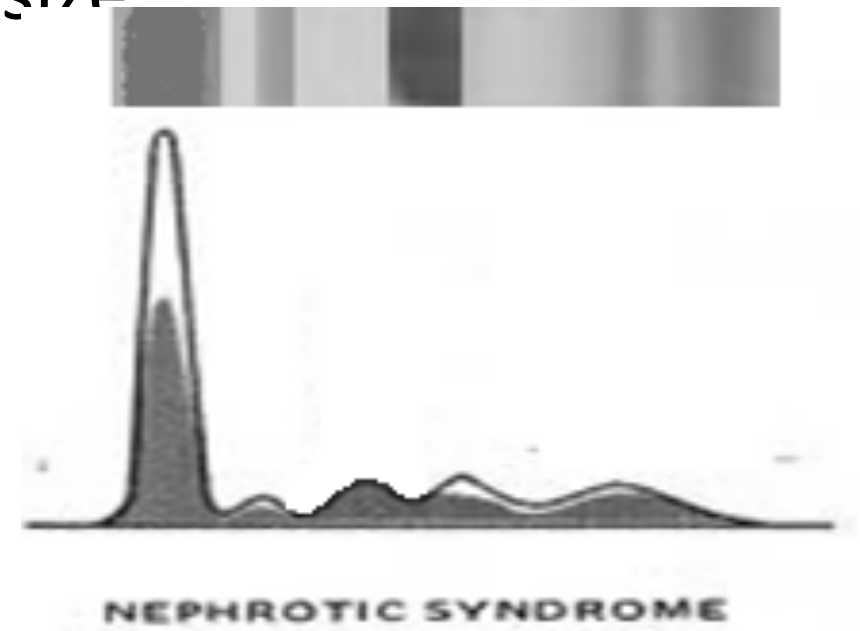






Nephrotic Syndrome

The nephrotic syndroma pattern is characterized by decreases in all protein fractions except ∝-2. ∝-2-Macroglobulin is retained by the kidney owing to its large molecular size



Hemoglobin electrophoresis

- is a test that measures the different types of the oxygen-carrying substance (hemoglobin) in the blood.
- Hempoglobin electrophoresis is performed to find out abnormal forms of hemoglobin (hemoglobinopathy).

Hemoglobin electrophoresis

- Many different types of hemoglobin (Hb) exist. The most common ones are HbA, HbA2, HbF, HbS, HbC, Hgb H, and Hgb M.
- Healthy adults only have significant levels of HbA and HbA2.
- Some people may also have small amounts of HbF (which is the main type of hemoglobin in an unborn baby's body). Certain diseases are associated with high HbF levels (when HbF is more than 2% of the total hemoglobin).

Hemoglobin electrophoresis

- HbS is an abnormal form of hemoglobin associated with sickle cell anemia. In people with this condition, the red blood cells have a crescent or sickle shape. These misformed cells then break down, or can block small blood vessels.
- HbC is an abnormal form of hemoglobin associated with hemolytic anemia. The symptoms are much milder than they are in sickle cell anemia.

Normal Values

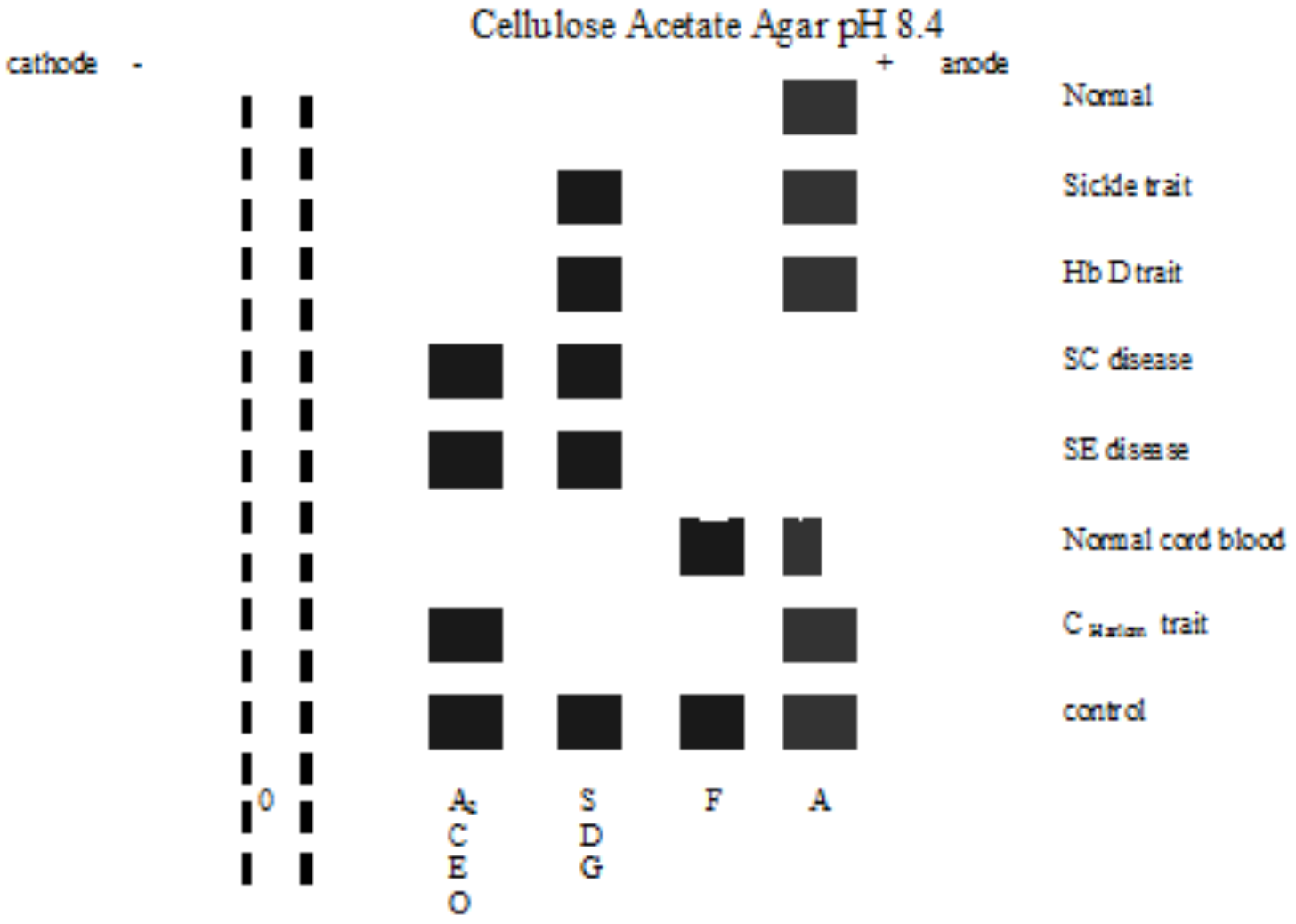
- **In adults**:
- Hgb A1: 95% to 98%
- Hgb A2: 2% to 3%
- Hgb F: 0.8% to 2%
- Hgb S: 0%
- Hgb C: 0%
- **In infants and children**:
- Hgb F (newborn): 50% to 80%
- Hgb F (6 months): 8%
- Hgb F (over 6 months): 1% to 2%

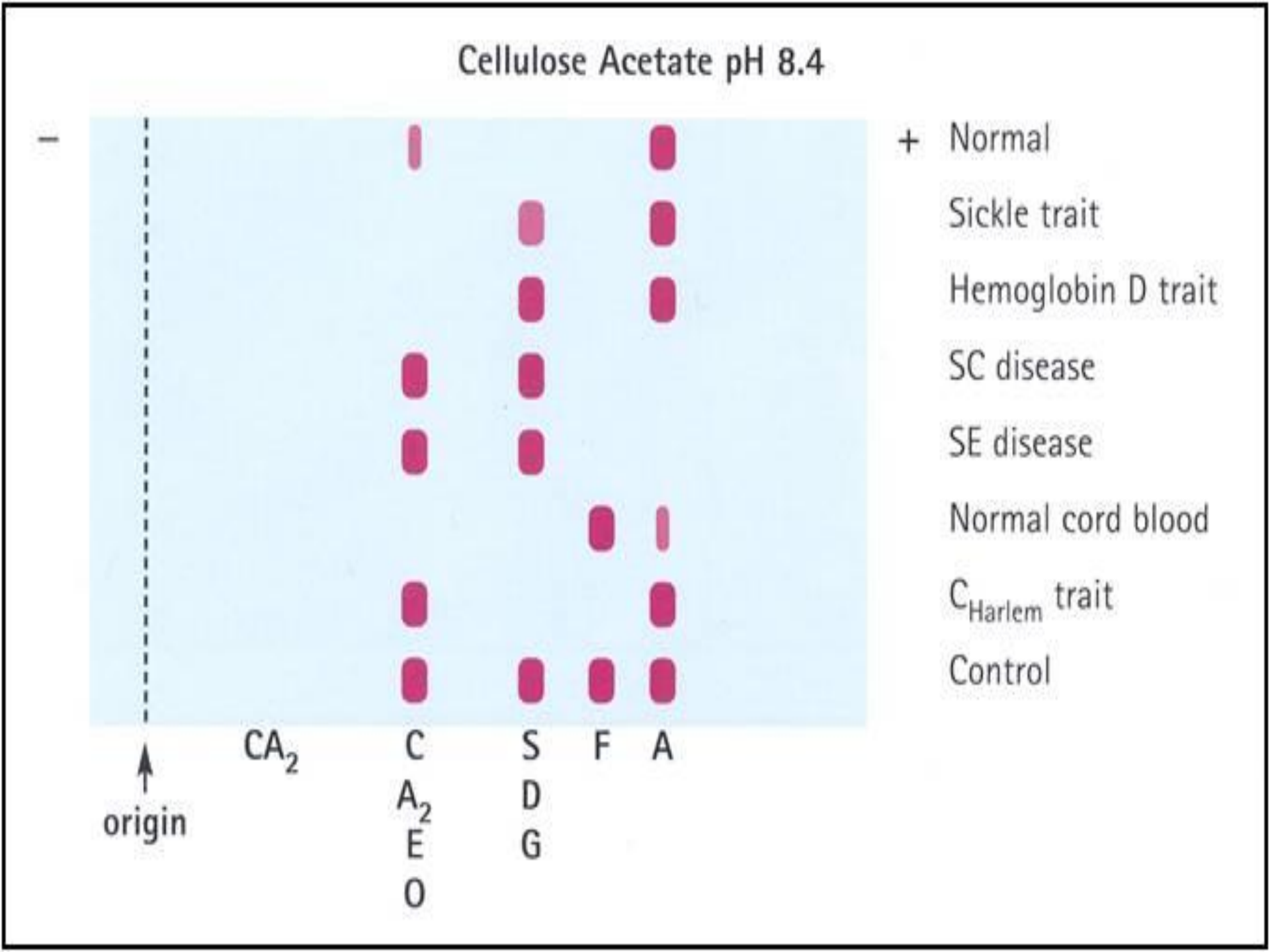
1-Cellulose Acetate At Alkaline pH

- Cellulose acetate Hb electrophoresis at alkaline pH is the primary screening procedure used to detect variant (abnormal) Hbs, of which there are several hundred.
- The major portion of normal adult Hb is A. In addition, up to 3.5% Hb A_2 is normally present, along with less than 2% Hb F. The more common mutant Hbs are S, C, E, D, G, and lepore.

1-Cellulose Acetate At Alkaline pH

- When an abnormal Hb is detected on cellulose acetate electrophoresis at an alkaline pH (8.2-8.6) further testing is frequently indicated: test for Hb S, quantitation of Hb A_2 and F, and citrate agar gel; acid/alkaline globin chain or neutral pH electrophoresis may also be warranted.


Cellulose Acetate Agar pH 8.4
cathode -
+ anode
Normal
Sickle trait
Hb D trait
SC disease
SE disease
Normal cord blood
C Harlem trait
control
0
A2
C
E
O
S
D
G
F
A

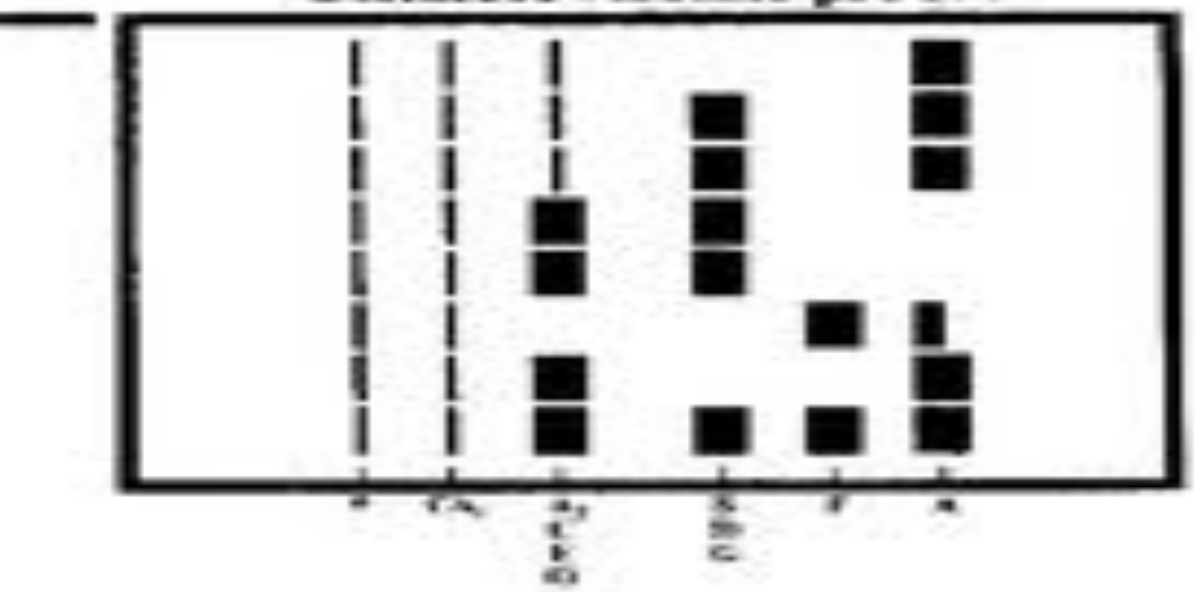

Cellulose Acetate pH 8.4
–
+
Normal
Sickle trait
Hemoglobin D trait
SC disease
SE disease
Normal cord blood
C Harlem trait
Control
origin
CA2
C
A2
E
O
S
D
G
F
A

2- Citrate Agar Electrophoresis (acid pH)

- Citrate agar electrophoresis is used to confirm variant Hbs and further differentiates Hb S from Hb D and G, and Hb C from Hb E, O_{Arab}, and C_{Harlem}. .

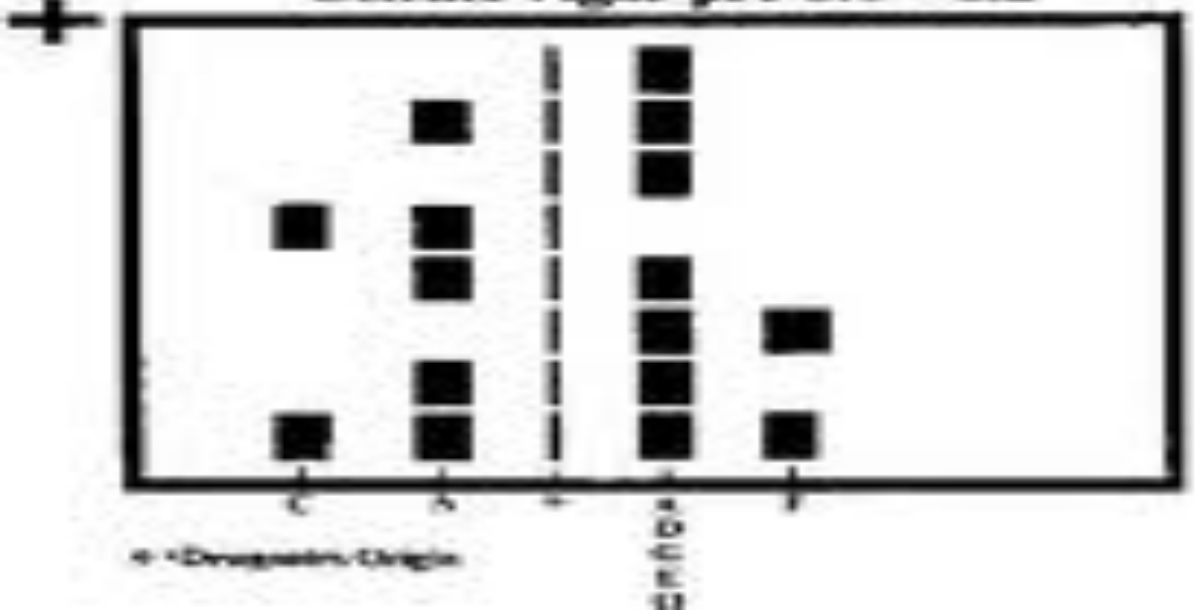
- The procedure should not be used as a screening procedure because many abnormal Hbs migrate with Hb A. However, this procedure is the method of choice when examining newborns (cord blood specimens) and infants under 3 months of age for some abnormal Hbs such as S and C because the test is able to detect quantities of Hb not easily seen by other techniques.

Cellulose Acetate pH 8.4



Normal
Sickle Trait
Hemoglobin D Trait
SC Disease
SE Disease
Normal Cord Blood
Chantem Trait
Control

Citrate Agar pH 6.0 – 6.2



Normal
Sickle Trait
Hemoglobin D Trait
SC Disease
SE Disease
Normal Cord Blood
Chantem Trait
Control