

# Hemoglobin Electrophoresis

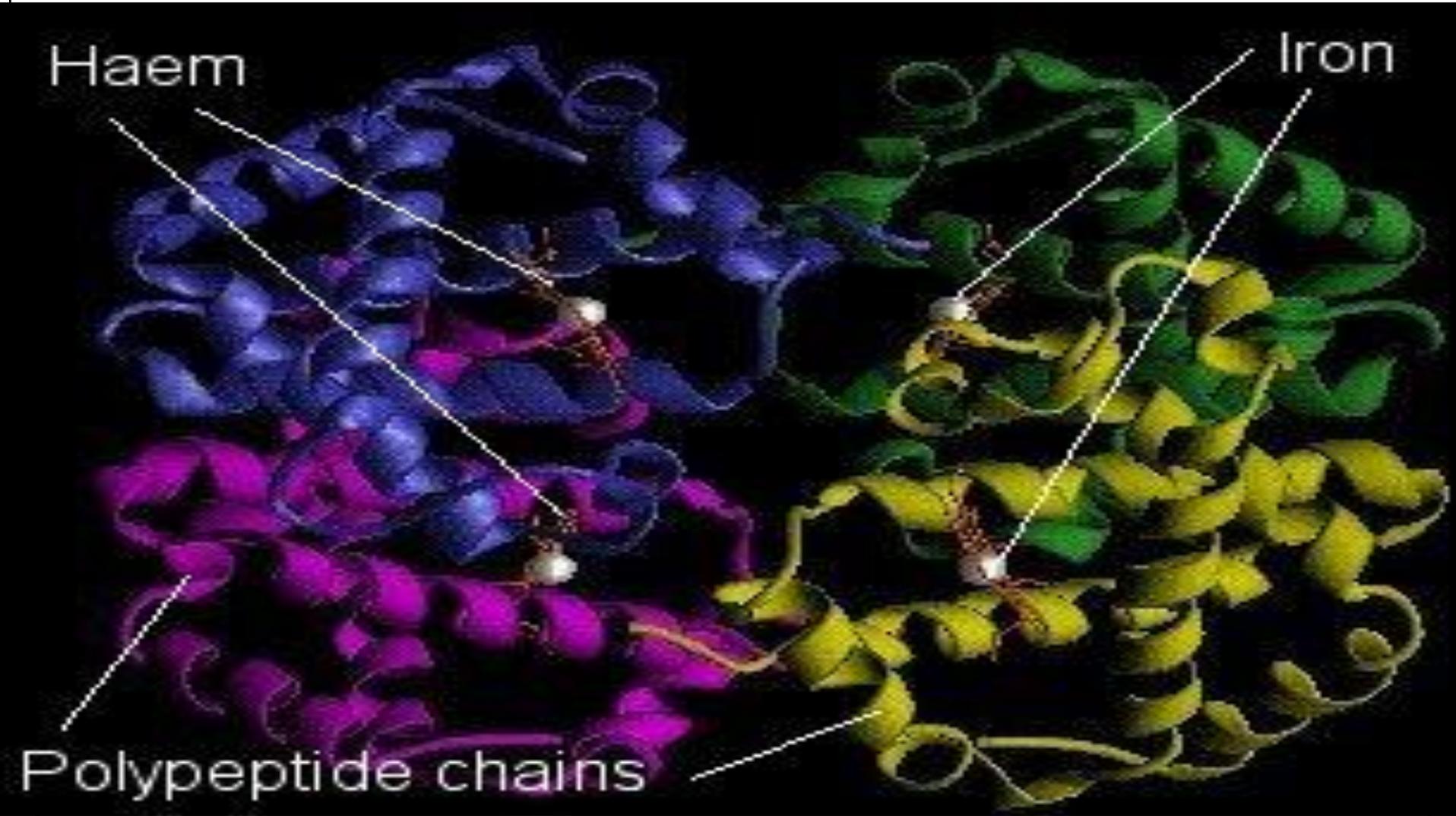
Prepared by  
Hadeel AL Sadoun

# Hemoglobin

Haem

Iron

Polypeptide chains



# Hemoglobin

Hemoglobin (Hgb) is composed of many different types, the most common being A<sub>1</sub>, A<sub>2</sub>, F, S, and C.

## Hemoglobin found in normal RBC's:

*Major component of hemoglobin*

- **Hg A** > 95 %

*Minor components*

- **Hg A<sub>2</sub>** approx: 2-3% of total Hgb
- **Hg F**: exist in minimal quantities in adult <2 % but major quantity in fetus 99% - 100%.
- Increased level of Hg F in adult considered abnormal
- Mostly Found in thalassemia, and hereditary persistence of fetal hemoglobin

# Hemoglobin

## Hemoglobin found in abnormal RBC's:

- **Hgb S:**

- associated with the disease of sickle cell anemia
- occurs predominantly in African-Americans.
- Distinguished in film by crescent-shaped (Sickle cells) red blood cell.
- Sickle cells have short life span thus this patient will have anemia

- **Hgb C:**

- hemoglobin variant found in African Americans.  
Red blood cells containing Hgb C limited survival and easily destroyed red blood resulting in mild to severe haemolytic anaemia.

- **Hgb E:**

- found in people of Southeast Asian descent.

- **Hgb D:**

- may be present with sickle cell disease or thalassemia.

- **Hgb H**

- (heavy haemoglobin) is found in  $\alpha$  thalassemia

# Electrophoresis

- **Principle:**
  - Dissolved RBC (Hemolysate ) release the hemoglobin
  - Each type of Hgb has and electrical charge of different degree.
  - Thus, Hgb found within any blood samples can be separated and measured based on their different charges when subjected to electrical filed.
  - Hgb component can move away from each other at different speed/ rate.
  - Each can identified based on pigmented bands.
  - The bands are compared to those of normal hemoglobin (control )
- **Definition :**
  - Method used to separate Hemoglobin component in blood , Electrophoresis: process that movement of any particle in electrical filed resulted in formation of bands that's moved toward one end or another in the filed.
- **Theory:**
  - Complex interaction between Hgb , Buffer (PH) and the agar support

# Application

- To find each type of Hgb in the blood to diagnose cretin types of Hemoglobinopathies.
- To help in treatment of diseases that have abnormal Hgb in the blood.
- To know the likelihood that couple could have inherited abnormal type of Hgb that can be passed to there offspring.

# Types

- **Alkaline Electrophoresis:**

hemolysate is applied to cellulose acetate which is electrophoresed in a buffer at pH 8.4-8.6

hemoglobin proteins move from cathode to anode

- **Acid Electrophoresis:**

Necessary follow up test for confirmation of abnormal hemoglobins detected on cellulose acetate.

Hemolysate is applied to citrate agar which electrophoresed as PH 6 - 6.2

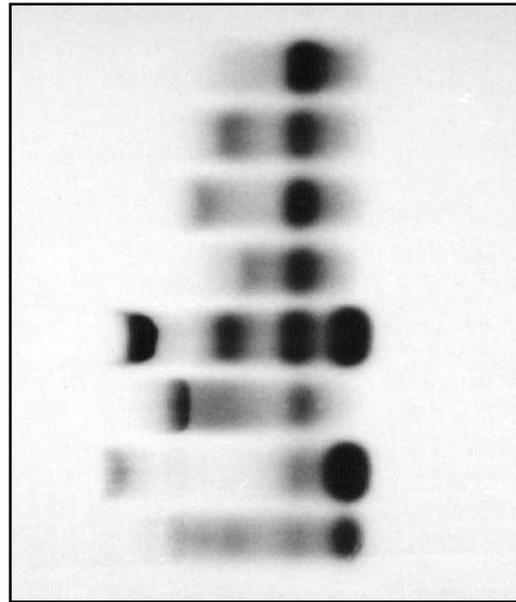
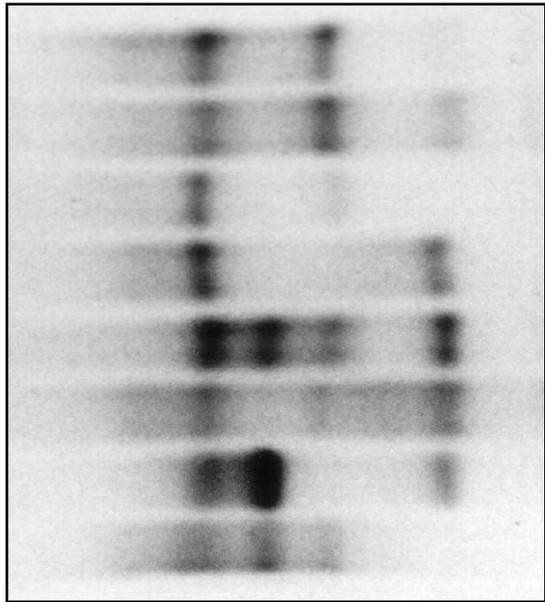
- Factor that control the movement of Hgb:

**PH**

# Hgb Electrophoresis

(+) Celloulose acetate (-)

(+) Citrate Agar (-)



1  
2  
3  
4  
5  
6  
7  
8

H  
A  
F  
D  
S  
G  
A2  
C  
E  
O

C  
S  
A  
A2  
D  
E  
G

Methemoglobin

Application point

# Application

- Control in Alkaline electrophoresis: **H A F S A<sub>2</sub>**

- **Sickle Trait**

This is a heterozygous state showing HbA and HbS and a normal amount of HbA<sub>2</sub> on cellulose acetate.

Results on citrate agar show hemoglobins in the HbA and HbS migratory positions (zones).

- **Sickle Cell Anemia**

This is a homozygous state showing almost exclusively HbS, although a small amount of HbF may also be present.

- **Sickle-C Disease**

This is a heterozygous state demonstrating HbS and HbC.

- **Thalassaemia Major**

- This condition shows HbF, HbA and HbA<sub>2</sub>.

- **Sickle Cell - Thalassaemia Disease:**

- This condition shows HbA, HbF, HbS and HbA<sub>2</sub>.

- **Hemoglobin-C Disease:**

- Disease This is a homozygous state showing almost exclusively HbC

- **Thalassaemia-C Disease:**

- This condition shows HbA, HbF, and HbC.



Take home question

What are the possible limitations of Hemoglobin electrophoresis method?