CLS 291 Clinical Hematology 1

جامعة الملك سعود
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Lecture 11

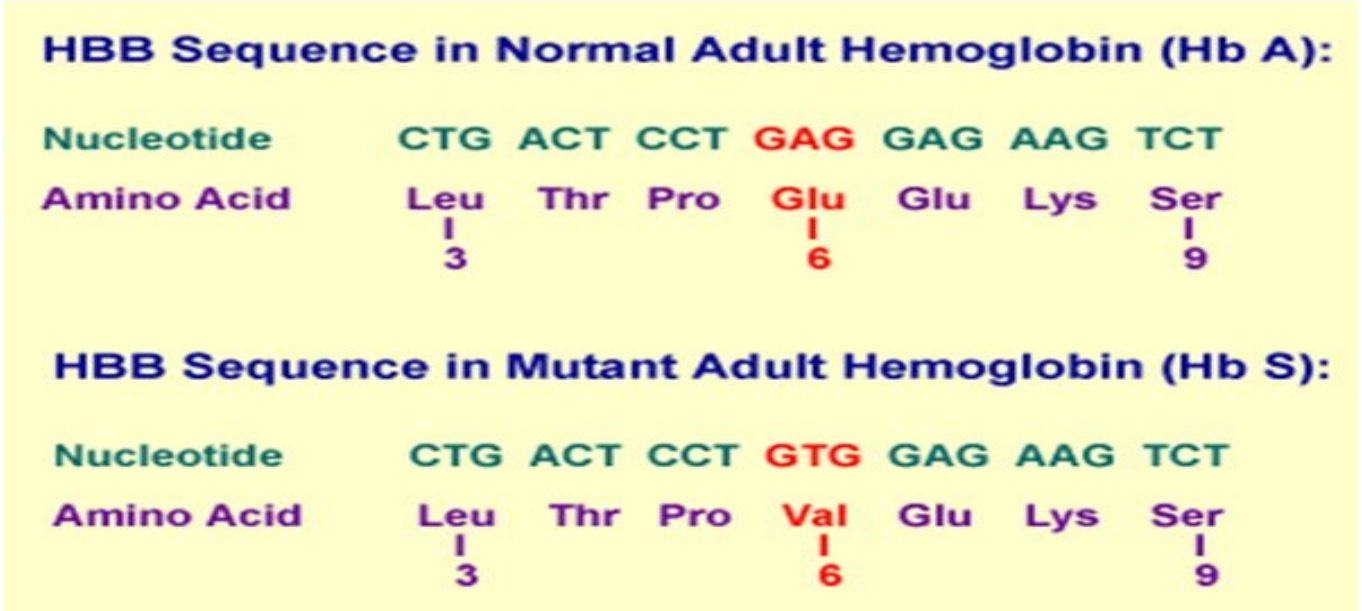
Hemoglobin Electrophoresis

Hb variants

- There are a number of abnormal Hb, such as:
  - Hb S
  - Hb C
  - Hb O
  - Hb D
  - Hb H

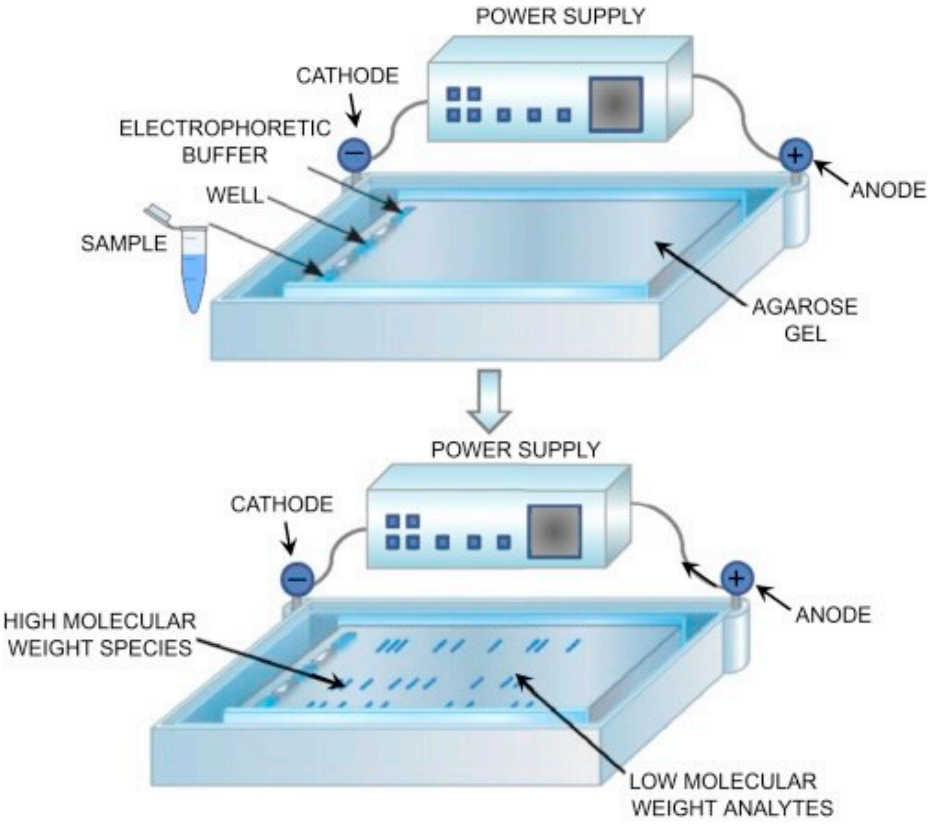
Hemoglobin S Mutation

- There is a substitution of an amino acid in the β chain.
- Glutamic acid is substituted by Valine at position 6 of the β chain.



Electrophoresis

- Electrophoresis uses an electrical current to separate normal and abnormal types of hemoglobin in the blood.
- Hemoglobin types have different electrical charges and move at different speeds.
- An abnormal amount of normal hemoglobin or an abnormal type of hemoglobin in the blood may indicate a disease state.



Types of Electrophoresis

Types of Electrophoresis:

1. Cellulose Acetate Electrophoresis at Alkaline pH 8.0
2. Citrate Agar Electrophoresis at pH 6.0
3. Agarose Gel Electrophoresis.

- The automated method to detect the Hb variant is Automated Hb electrophoresis or Automated High-Performance Liquid Chromatography (HPLC)

- Technical factors affect Hb mobility:

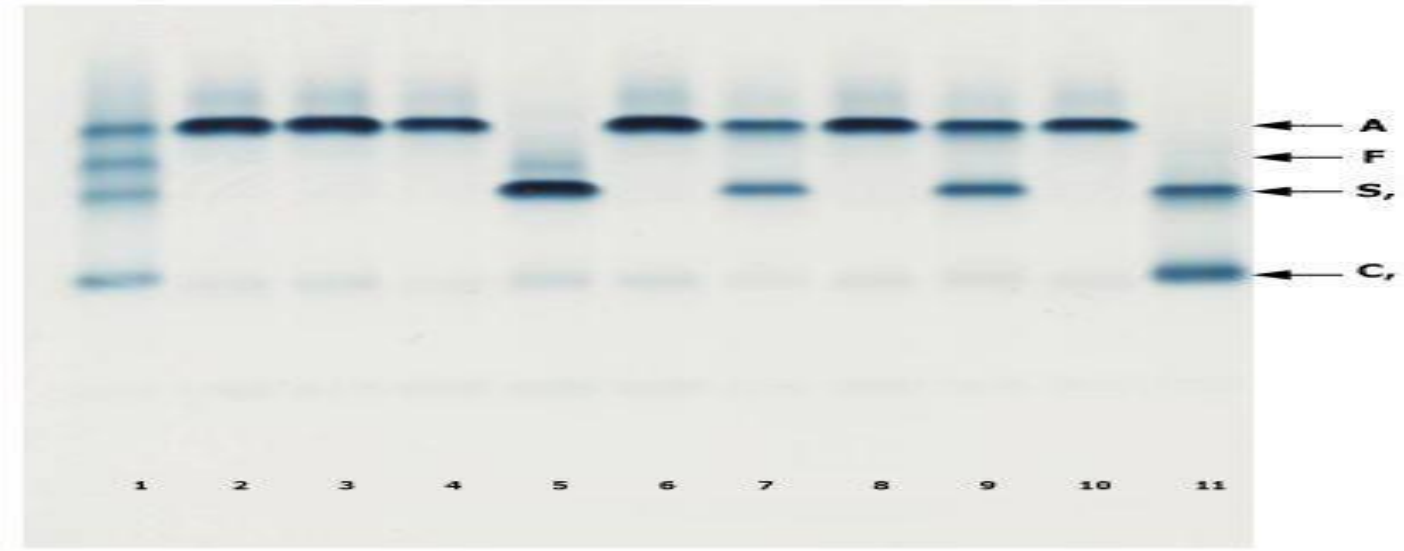
    1. The intensity of the electrical field.
    2. Nature of charged particles on specific pH.
    3. Medium in which the movement may occur.

Cellulose Acetate Electrophoresis at Alkaline pH

Principle

- At alkaline pH, haemoglobin is a negatively charged protein and when subjected to electrophoresis will migrate toward the anode (+).
- Structural variants that have a change in the charge on the surface of the molecule at alkaline pH will separate from Hb A.
- Haemoglobin variants that have an amino acid substitution that has no effect on overall charge will not separate by electrophoresis.

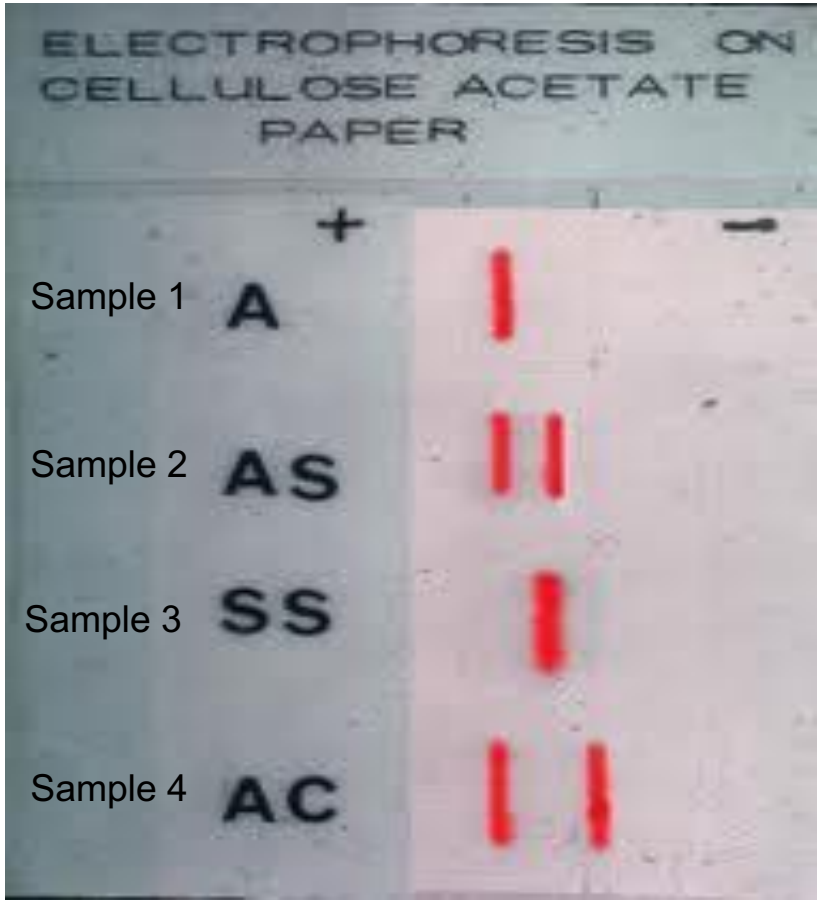
Electrophoresis Result Reading

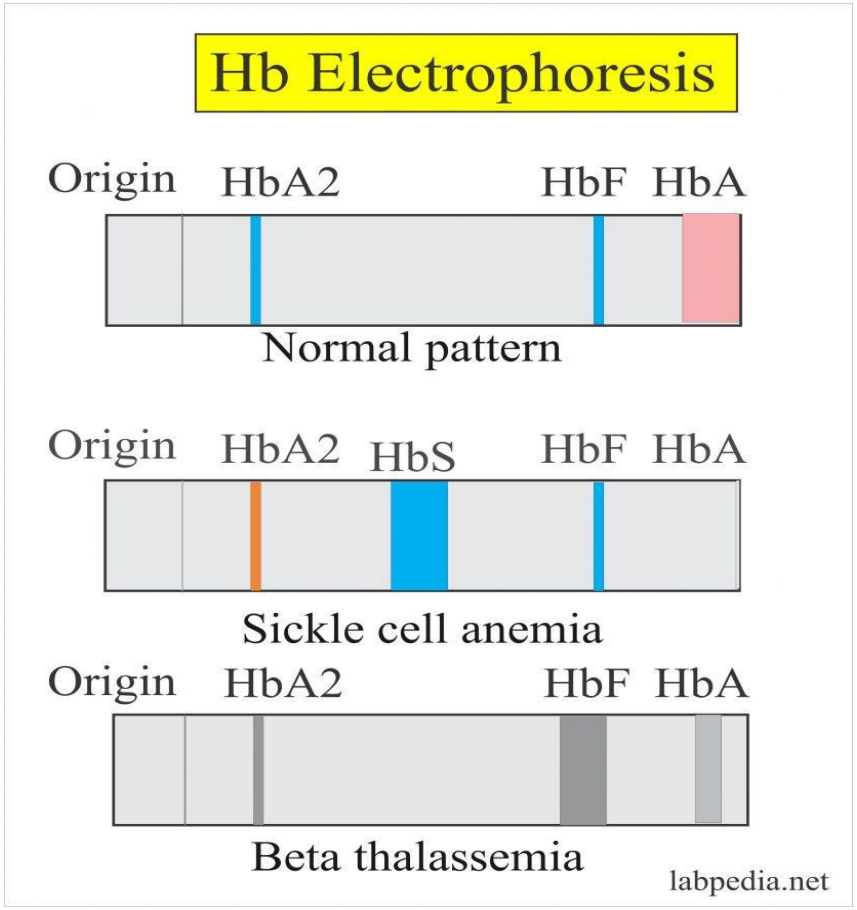


- This is an example of hemoglobin electrophoresis run at alkaline pH on cellulose acetate.
- The electrophoretic positions of the more common hemoglobins are shown on the right.

- Lane 1 is a commercial standard containing approximately equal amounts of hemoglobins A, F, S, and C.

Electrophoresis Result Reading





Electrophoresis Result Reading

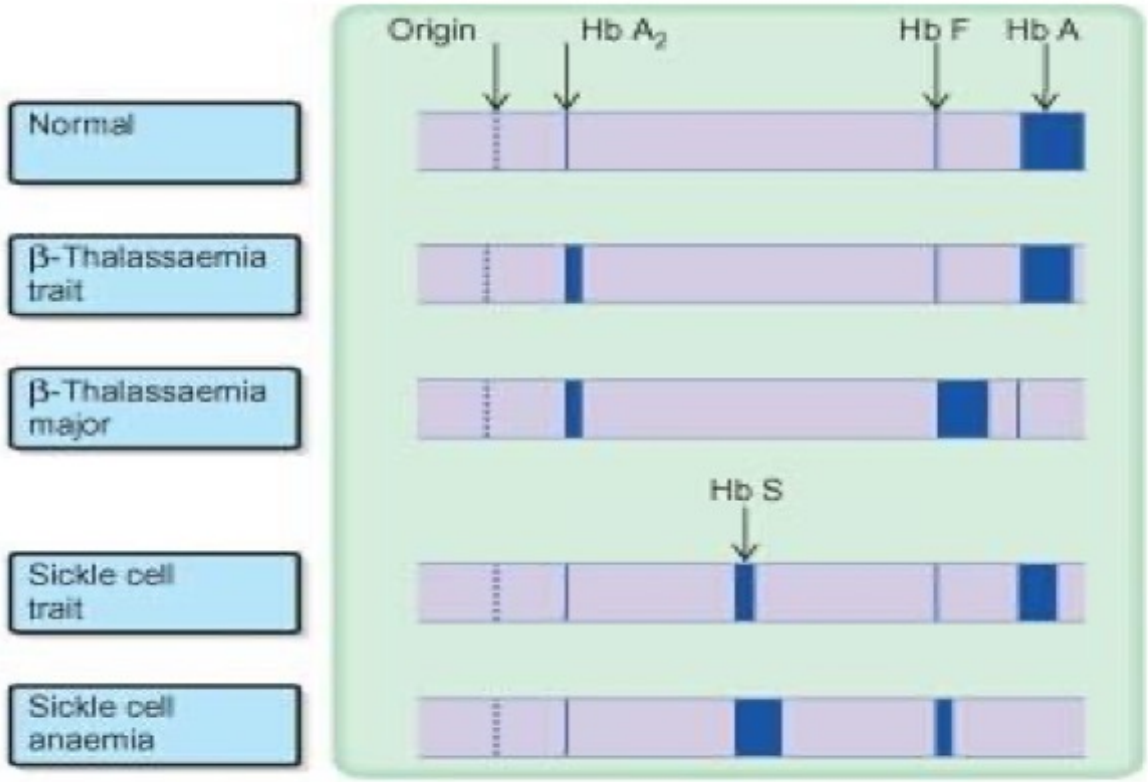
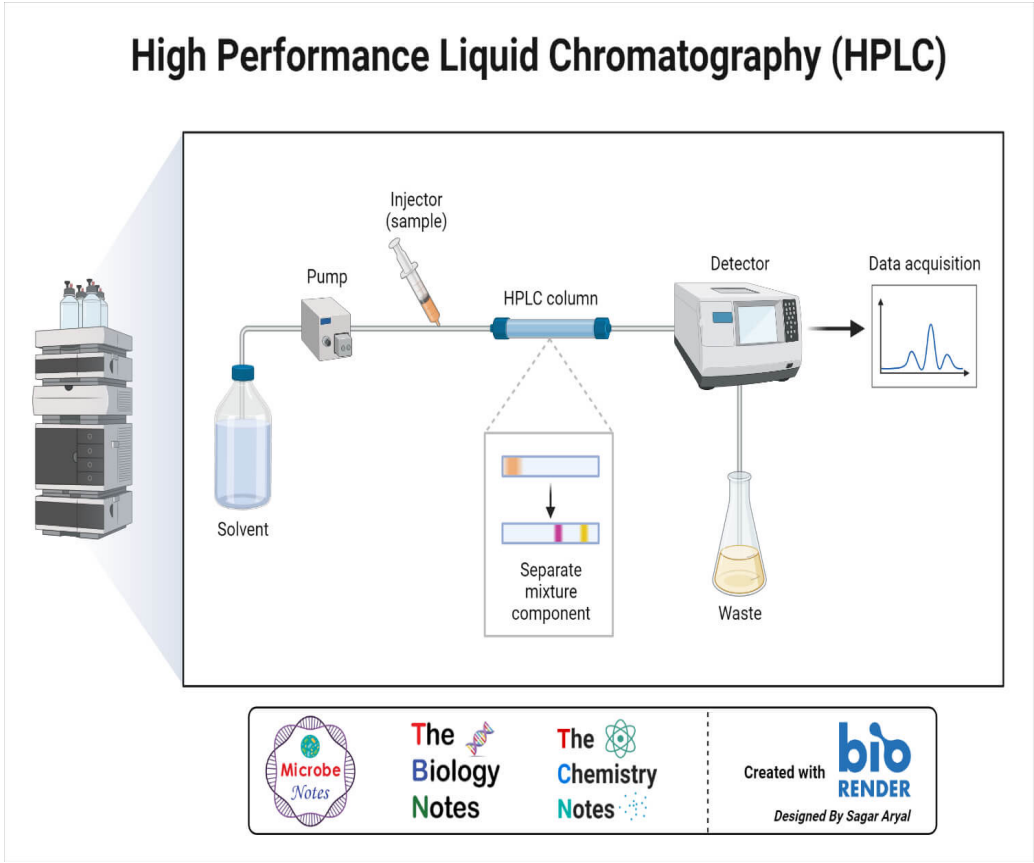


Table 2.3 Normal haemoglobins in adult blood.

	Hb A	Hb F	Hb A_2
Structure	$\alpha_2\beta_2$	$\alpha_2\gamma_2$	$\alpha_2\delta_2$
Normal (%)	96–98	0.5–0.8	1.5–3.2

- Results are qualitative and quantitative.
- The different types of Hb can be identified (qualitative).
- The amount of each separated hemoglobin is measured (quantitative).

Example of HPLC result



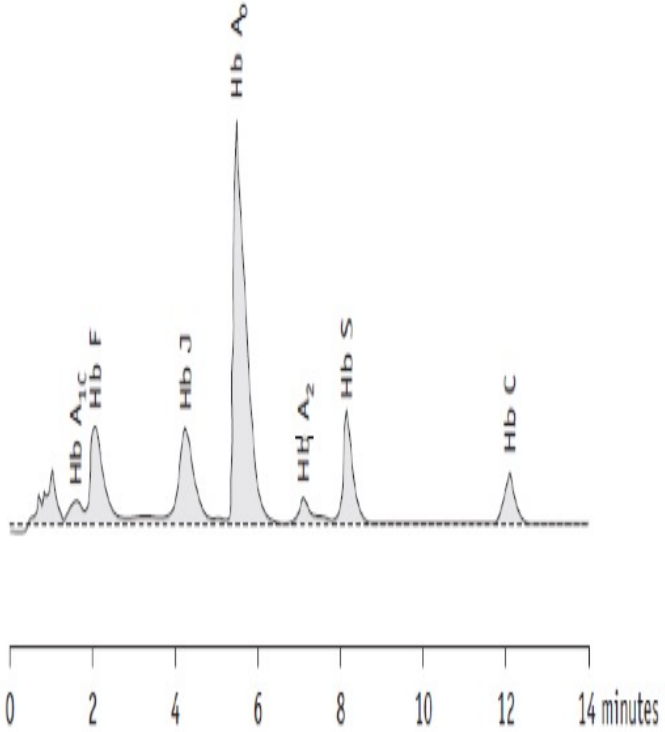


Figure 14.7 A mixture of haemoglobins separated by high-performance liquid chromatography (HPLC).

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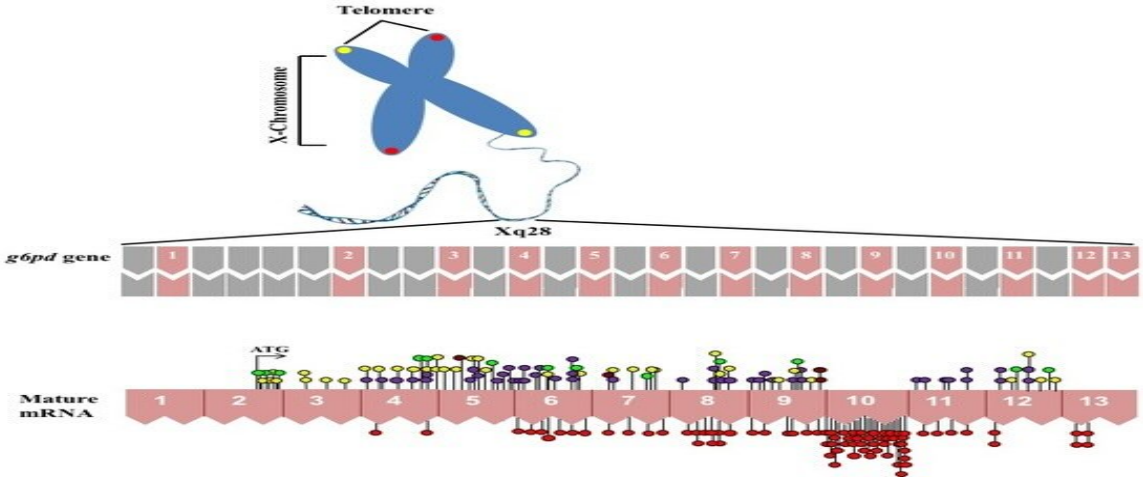
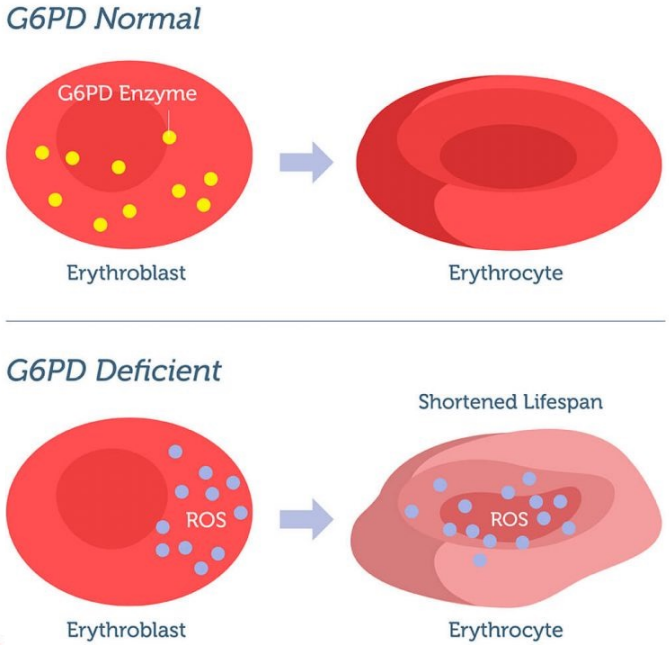
Lecture 12

Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency

G6PD Deficiency

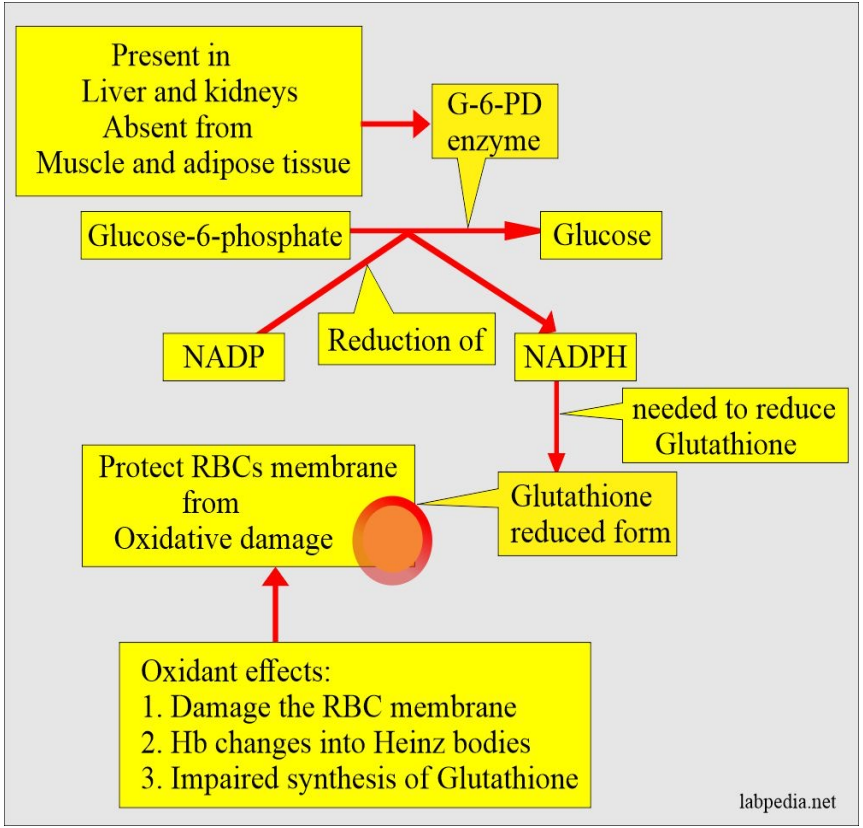
- G6PD deficiency is the most common enzymatic disorder of RBCs.
- Many gene variants were detected (around 180 variants).
- G6PD deficiency is an X-linked disease.



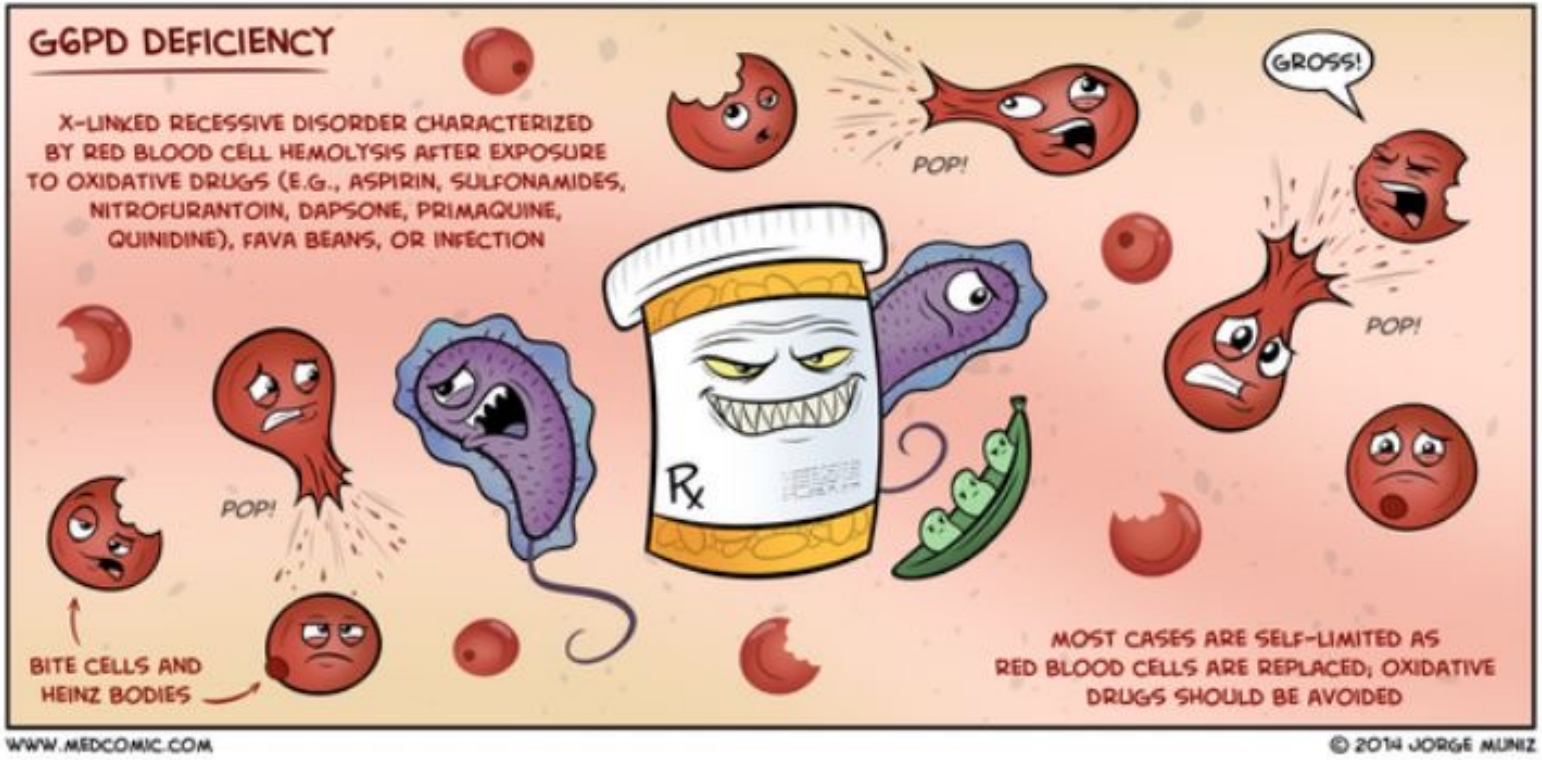


G6PD Deficiency

- G6PD enzyme functions in the protection against oxidative stress.
- The lack of G6PD leads to hemolysis during oxidative stress as a result of infection, medication, fava beans, and substance (henna).
- Oxidative stress leads to Heinz body formation and extravascular hemolysis.

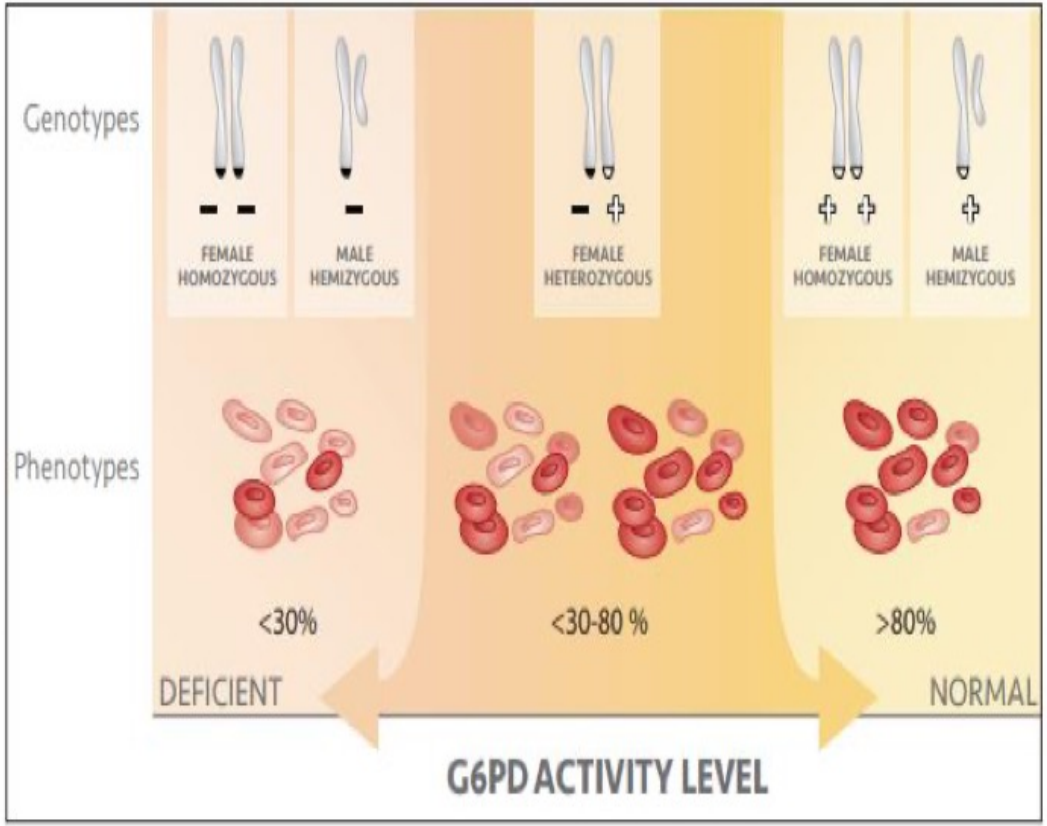


Triggers of Oxidative Stress



G6PD Deficiency

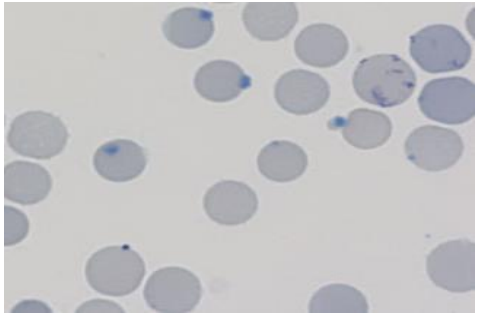
- Glucose-6-phosphate dehydrogenase deficiency ranges from mild to severe, depending on the level of enzyme activity.
  - A value >80% of normal red blood cell G6PD activity is considered G6PD normal.
  - Red cell G6PD activity less than 30% of the normal median must be regarded as G6PD deficient.

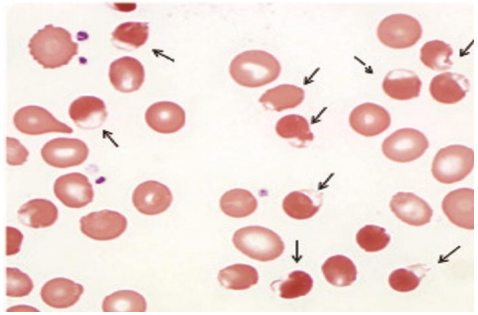


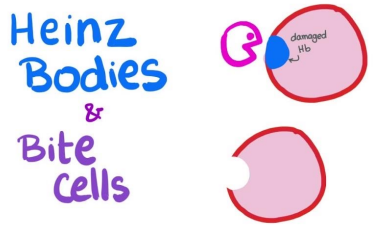
G6PD Deficiency Testing

Diagnosis of G6PD Deficiency:

1. General screening test:
   a) CBC
   b) Blood film: Bite cells, blister cells, small irregular cells, Heinz bodies, polychromasia.
2. Special Screening of G6PD deficiency:
   a) Fluorescent spot testing (FST).
3. Confirmatory test:
   - Quantitative measurement of G6PD enzymatic activity.
4. Molecular test: Detection of G6PD gene mutations.

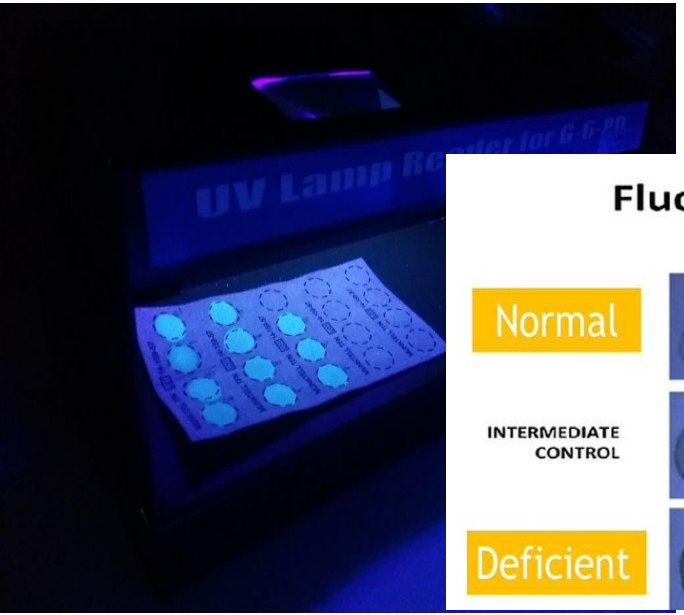


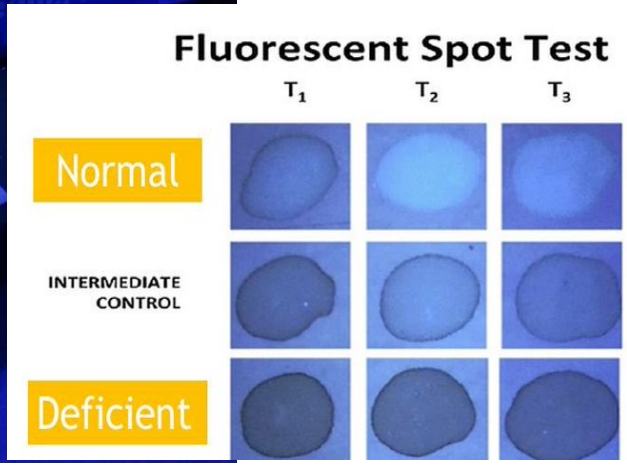


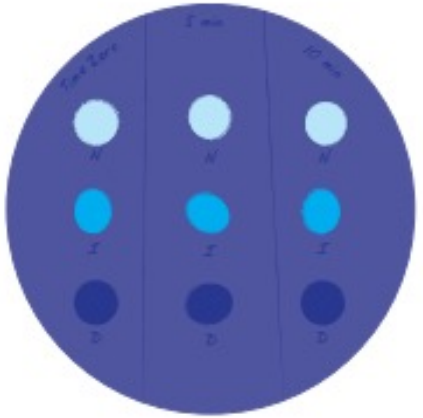


Special Screening of G6PD Deficiency

- A fluorescent spot testing (FST) for the qualitative assessment of G6PD enzymatic activity (UV-based test).



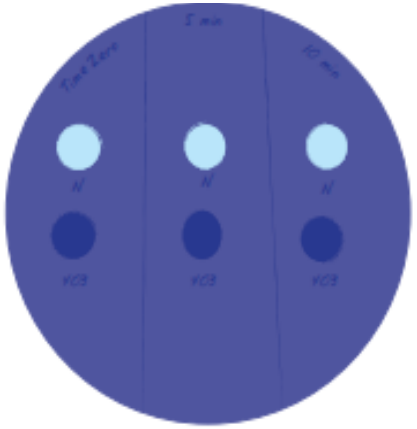




Normal G6PD enzyme activity

Intermediate G6PD enzyme activity

Deficient G6PD enzyme activity



Normal controls

Deficient blood sample results

Special Screening of G6PD Deficiency

A fluorescent spot testing (FST) principle:

- Blood is mixed with a reagent containing NADP+ and G6P. The **G6PD inside RBC** will catalase the reaction to produce NADPH which is fluorescent.
- NADPH fluorescence is directly proportional to G6PD activity, and lack of fluorescence signals G6PD deficiency