CLS 291 Clinical Hematology 1

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Lecture 10

Screening Tests for Sickle Cell Anemia: Sickling and Solubility Test

Outlines

I. Background

i. Normal Hemoglobin Production

ii. Hb S Mutation and Polymerization

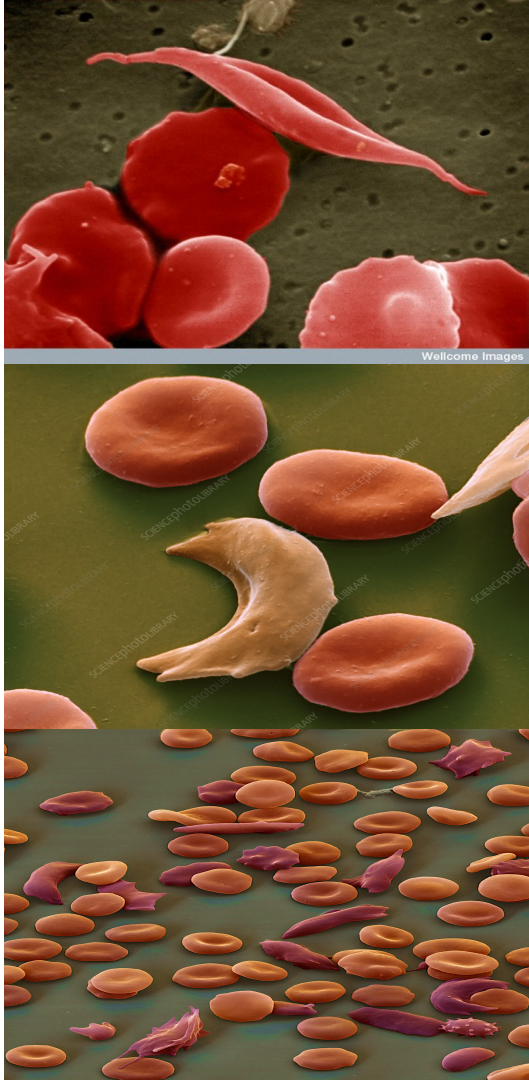
iii. Hereditary of Sickle Cell Anemia

iv. Abnormal Hemoglobin

II. Lab Tests for the Diagnosis of Sickle Cell Anemia

i. Sickling Test

ii. Sickle Solubility test



Normal Hemoglobin Production

Hb A

- Red blood cells with normal hemoglobin are smooth, disk-shaped, and **flexible**. Thus, they can move through the blood vessels easily.
- **Hb A** can tolerate low oxygen tension.
- Pure **deoxy-Hb A** is totally **soluble** in concentrate phosphate buffer.
- If a person inherits the normal Hemoglobin A gene from each parent, the Hb genotype is HbAA.

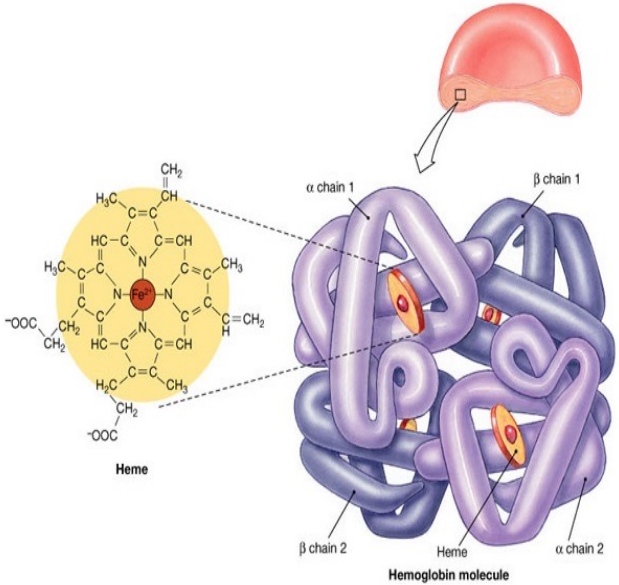
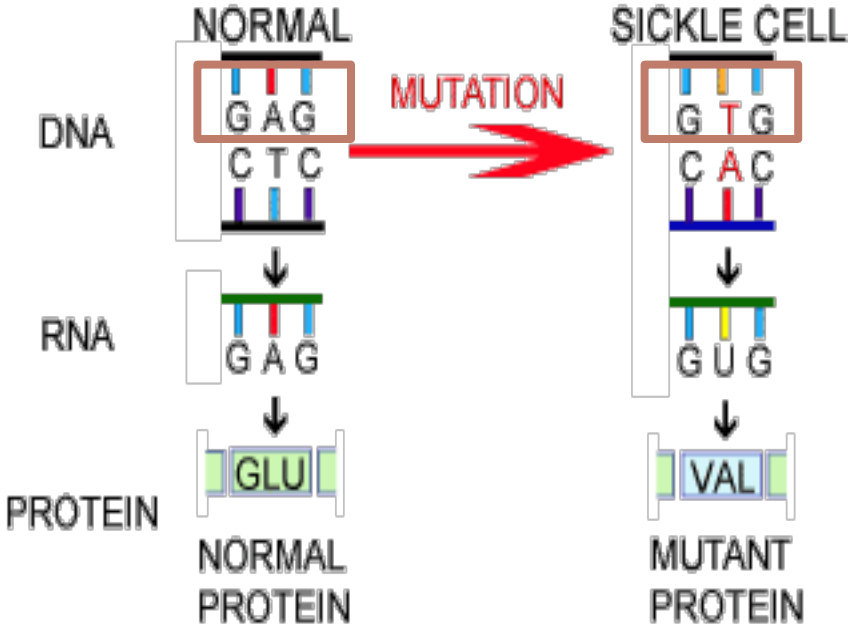


Table 2.3 Normal haemoglobins in adult blood.

	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

Hb S Mutation and Polymerization

- Hb S: is abnormal Hb resulting from a mutation in the **beta-globin gene.**

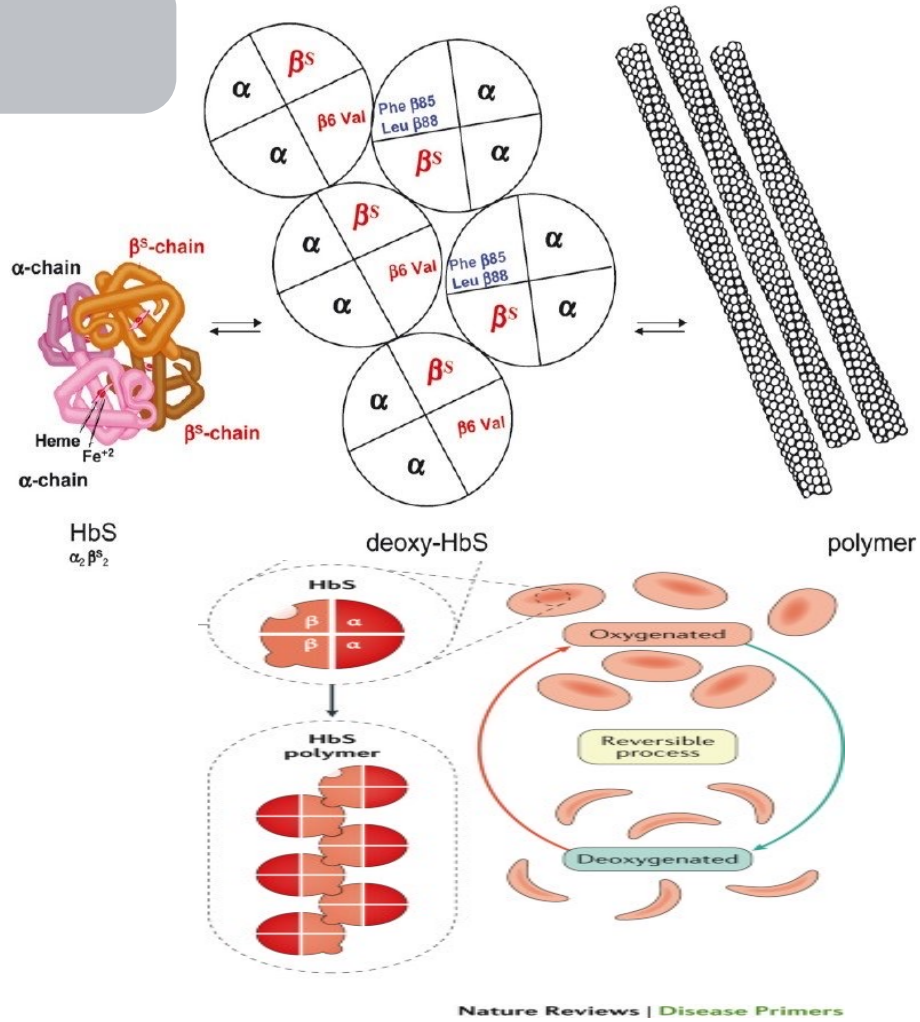


Primary Structure	Secondary and Tertiary Structures	Quaternary Structure	Function	Red Blood Cell Shape
Normal hemoglobin: 1 Val, 2 His, 3 Leu, 4 Thr, 5 Pro, 6 Glu, 7 Glu	β subunit	Normal hemoglobin (α, β, α, β)	Molecules do not associate with one another; each carries oxygen.	10 μm
Sickle-cell hemoglobin: 1 Val, 2 His, 3 Leu, 4 Thr, 5 Pro, 6 Val, 7 Glu	Exposed hydrophobic region; β subunit	Sickle-cell hemoglobin (α, β, α, β)	Molecules crystallize into a fiber; capacity to carry oxygen is reduced.	10 μm

Hb S Polymerization

- Under low-oxygen tension, HbS chains **polymerize** and form **rigid**, **rod-like fibers** that **precipitate** inside RBCs, resulting in the characteristic crescent (sickle) shape.

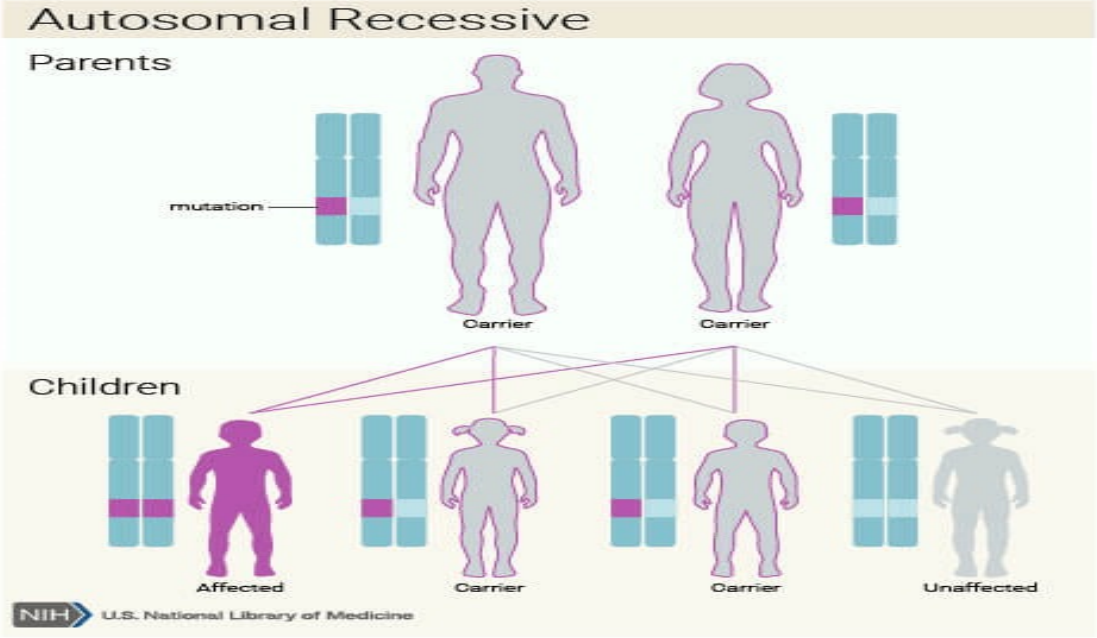
- **Low oxygen tension can be caused by:**
  - Change temperature
  - High altitude
  - Dehydration
  - Stress



Hereditary of Sickle Cell Anemia

Hb-S genotypes:

- The **Homozygous** (S/S) genotype is found in Sickle Cell Anemia - affected patients.
  - HbSS individual has (2α2βs)
- The **Heterozygous** (A/S) genotype is found in Sickle Cell Trait (carrier patients).
  - HbSA individual has (2α1β1βs)



Abnormal Hemoglobin

- A patient can have more than one abnormal Hb:
  - HbS/HbC
  - HbS/ Thalassemia
  - HbS/HbS (sickle cell disease)
  - HbS/normal HbA **(sickle trait)**

Lab Tests for the Diagnosis of Sickle Cell Anemia

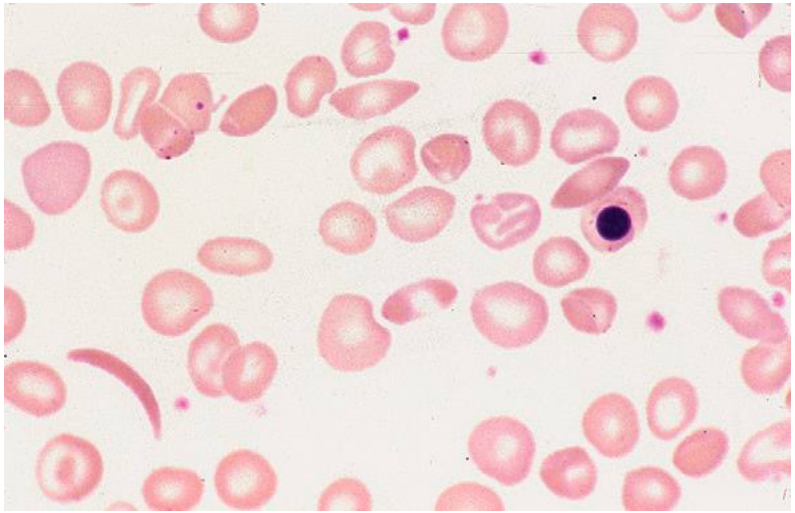
- Screening test for SCA:
    1. **Hb concentration** is low.
    2. **Blood film** shows sickle cells.
    3. **The sickling test** is positive.
    4. **The solubility test** gives a positive result.

- The screening test does not differentiate between the carrier (heterozygous) and diseased (homozygous) patients.

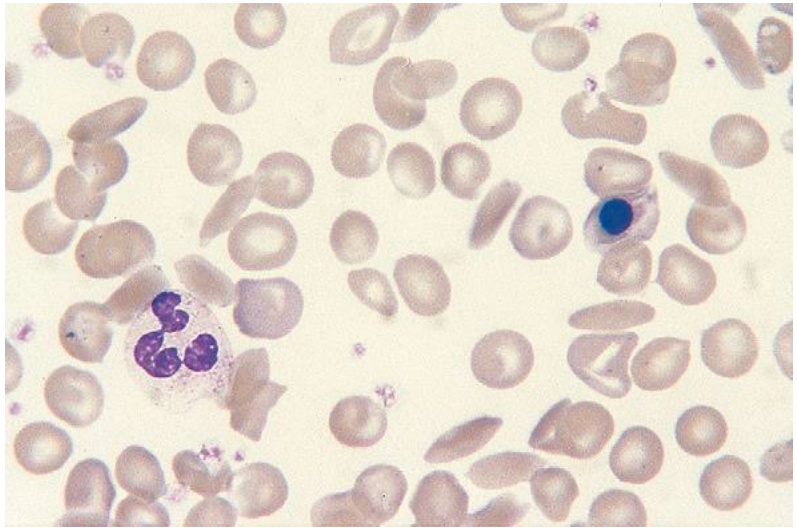
Blood Film Morphology in Sickle Cell Anemia

Blood Film Morphology of Sickle Cell Case:

- Shows Sickle cell, boat-shape, target cell, and nucleated RBC.



Shows a sickled cell, boat-shaped cells, and a nucleated red cell and target cells.

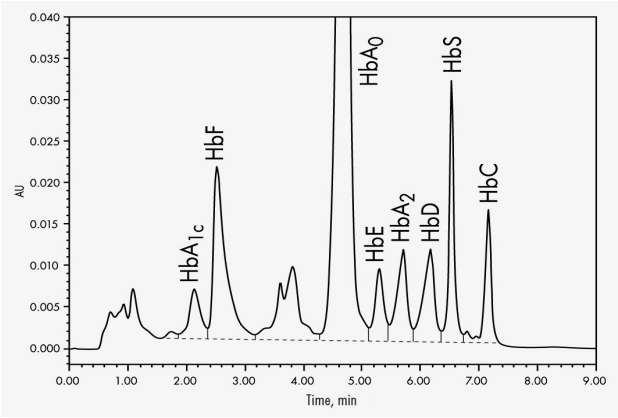


Shows a sickled cell, boat-shaped cells, and a nucleated red cell and target cells.

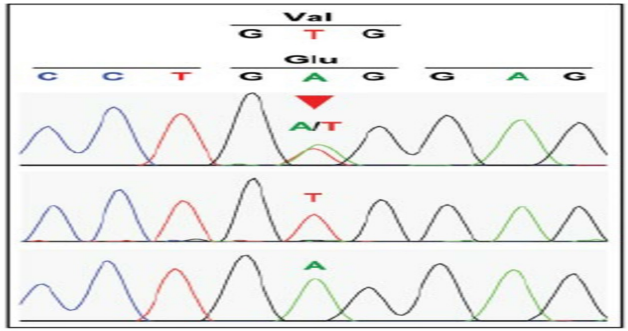
Lab Tests for the Diagnosis of Sickle Cell Anemia

- Specific test:

1. **Electrophoresis**: shows the presence of Hb S and variation in Hb A and Hb F.
2. **High-performance liquid chromatography (HPLC):** shows the presence of Hb S and variation in Hb A and Hb F.
3. **DNA analysis:** shows the mutation in the beta globulin gene.



Detection of HbS by HPLC

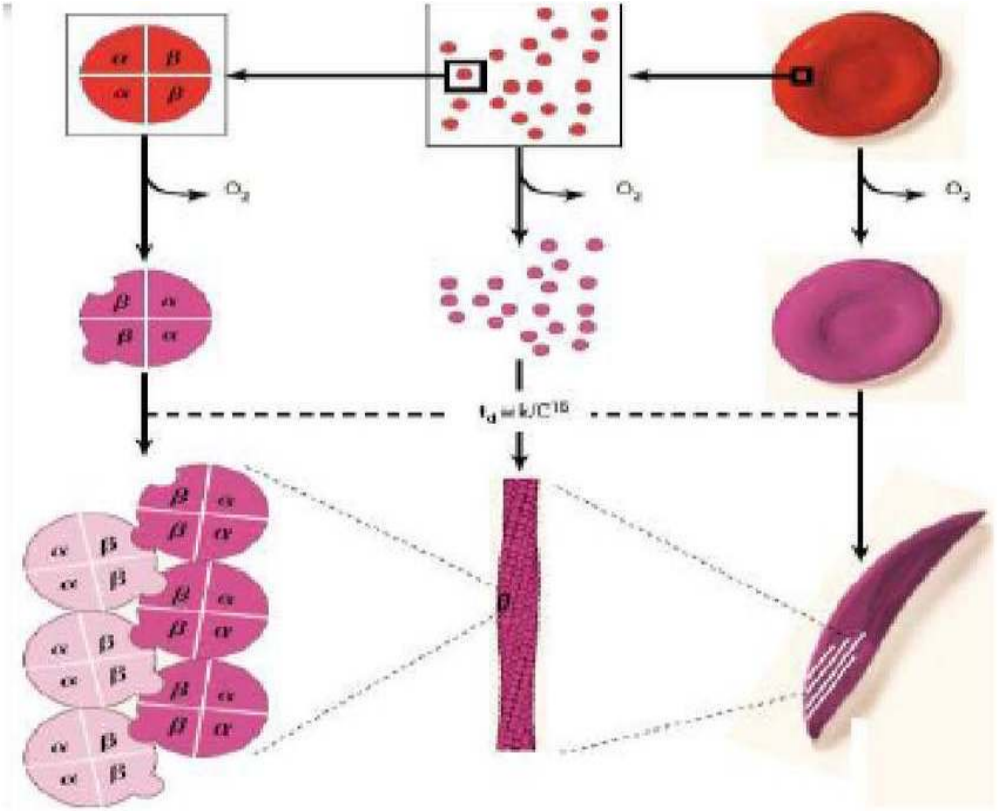


Detection of mutation by sequencing

i. Sickling Test

Principle

- Red cells which carry HbS will change their shapes from round shape to sickle shape under low oxygen tension.
- This situation can be induced in vitro by adding a **deoxygenating agent** such as sodium metabisulphite.
- Red cells with Hb A will not be affected and will maintain their rounded shape.

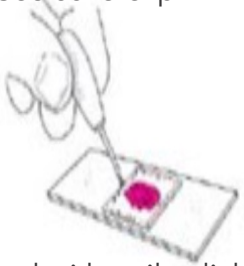


i. Sickling Test

- **Reagent**: 2% sodium metabisulphite.
- **Material**: rack, tube, slides, coverslip, pasture pipette, nail polish, EDTA Whole blood sample, filter paper.
- **Procedure:**
  1. On a tube, add 1 small drop of EDTA blood sample + 4 drops of sodium metabisulphite.
  2. Mix.
  3. Take a small drop-by pasture pipette and put on a slide and cover it with a coverslip.
  4. Seal the coverslip with nail polish. (to prevent oxygen from entering)
  5. Put it inside a wet Petri dish (wet filter paper or cotton ball).
  6. Incubate it for an hour at 37˚C.
  7. Examine it under the microscope at (40X).


Add a drop

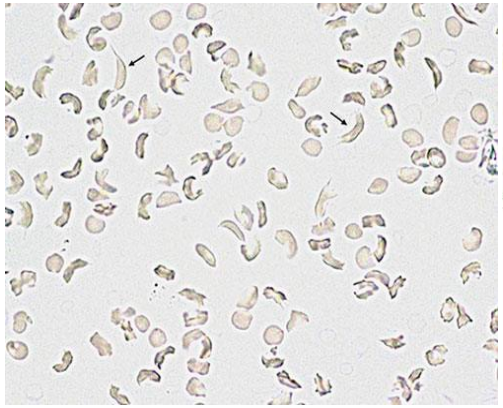

Use coverslip


Seal with nail polish

i. Sickling Test

Result interpretation:

- A positive test shows sickle cells under the microscope.
- Hb-S may be present as a homozygous trait of **Hb SS**.
- or with other hemoglobin, such as:
    - Hemoglobin A → **Hb AS**
    - Hemoglobin C → **Hb S/C**
    - Thalassemia → **Hb S /thalassemia**



- A negative test shows rounded cells under the microscope with the absence of sickle cells.
    - Indicate that the hemoglobin inside the cells is either of **Hb AA trait** or other non-sickled Hb traits.

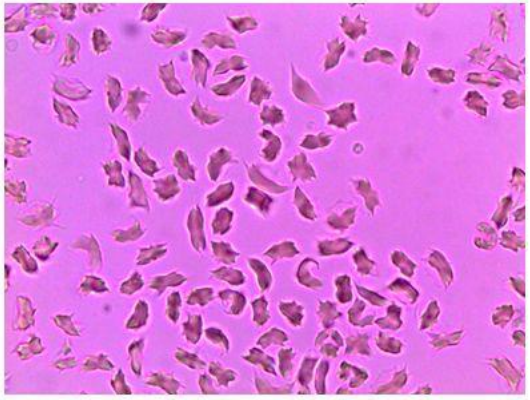
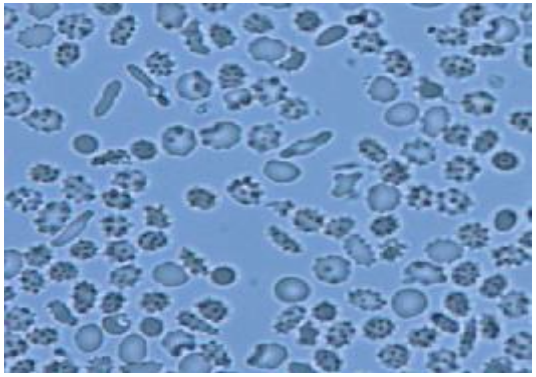


Figure 2. Sickling test of a patient with Sickle Cell Trait.



ii. Sickle Solubility test

- Deoxyhaemoglobin-S has poor solubility in concentrated phosphate buffer.

- As in the sickling test, it is used to identify the presence of Hb S and will not distinguish between the heterozygous trait (HbAS) and the homozygous disease (HbSS) trait (screening test).

Principle:

- Erythrocytes are lysed by saponin
- **released** hemoglobin is **reduced** by dithionite in a phosphate buffer.
- Reduced **Hb-S** is characterized by its very low solubility and by the formation of nematic liquid crystals (tactoids) so that in the presence of Hb-S, the solution will show turbidity.
- Hb A is more soluble in the reduced buffer and thus will show no turbidity.

ii. Sickle Solubility test

- **Reagents:** lysing buffer and sodium dithionite.
- **Materials:** rack, tubes, pasture pipette, tips, micropipette rack with lines.
- **Method:**

1. Prepare the working solution as in the leaflet.
2. Add 2 ml of working solution buffer to a glass tube.
3. Add 20 µl of EDTA blood sample.
4. **Run Positive and negative Control with every batch.**
5. Mix by inversion and allow to stand for several minutes (depending on the used kit)
6. Place the tube against a white rack on which thick bold lines have been drawn.
7. View the black lines through the solution.

- https://www.youtube.com/watch?v=fOKD_N_CKYI

ii. Sickle Solubility Test

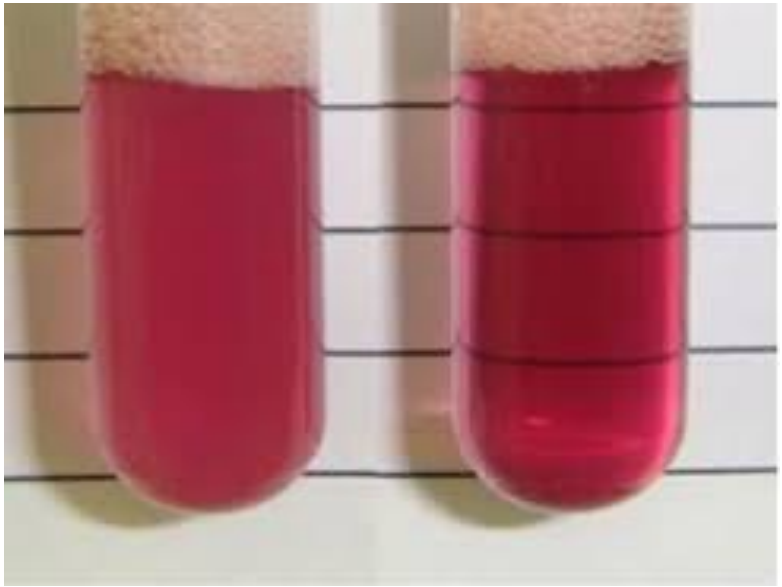
Results:

Compare with Negative and positive Control Solutions when reading the result.

- **If the tube that shows turbidity in** which the black lines are not seen is considered a **positive result**, indicating the presence of HbS.
    - All positive results should be confirmed on electrophoresis.

- A clear tube in which the black lines are seen is considered a **negative test result**, and it Indicates the absence of HbS.

Hb AS/SS Hb AA



Positive result
black lines are not seen (turbid) presence of HbS

Negative result
black lines are seen absence of HbS.