Investigation of sickle cell disease
Sickle cell disease

- Sickle cell disease is an inherited disorder which affects people from Africa, India, Middle East and the Mediterranean region.
- The main forms of sickle cell disease found in tropical countries are:
  - *Homozygous sickle cell anemia* (HbSS)
  - *Sickle cell hemoglobin C* (HbSC)
  - *Sickle cell thalassaemia*
  - *Sickle cell trait* (HbAS)
Hematological investigation of sickle cell disease

- Measurement of hemoglobin
- Sickle cell slide test to detect HbS in red cells.
- HbS solubility filtration test to differentiate sickle cell anemia from other sickle cell disorders.
- Examination of a Romanowsky stained thin blood film for features associated with sickle cell disease.
- Hemoglobin electrophoresis
Sickle cell slide test

- This test is simple to perform and requires only a single reagent. It does not however, differentiate between sickle cell disease and sickle cell trait.
Principle of test

• Blood is mixed on a slide with a chemical reducing agent (Freshly made 20 g/l (2% w/v) sodium metabisulphite or sodium dithionite solution), covered with a cover glass, and incubated at room temperature for up to 1 hour or more. The reducing agent deoxygenates the hemoglobin in the red cells providing the conditions for cells containing HbS to sickle.
**Test method**

1- Deliver one drop of patient’s capillary blood or well mixed venous blood on a slide (marked P). Add an *equal* volume of fresh reducing reagent, mix, and cover with a cover glass. Exclude any air bubbles.

- *Note*: When the patient is severely anemic, use 2 drops of blood or preferably use a plasma reduced blood sample.
2- Set up a *Negative Control*: Deliver one drop of blood from a person that does not have a sickle cell disorder on a slide marked ‘Neg Control’. Add an *equal* volume of fresh reducing reagent and mix. Cover with a cover glass. Exclude any air bubbles.

- If a blood from a known sickle cell trait person is available, set up also a *Positive Control*. 
3- Place the slides in a container (plastic box or petri dishes) with a damp piece of blotting paper or tissue in the bottom to prevent drying of the preparations. Close the container and leave at room temperature.

4- After 10–20 minutes, examine the patient’s preparation microscopically for sickle cells. Focus the cells first with the 10 objective and examine for sickling using the 40 objective.

• When the preparation is negative, examine it again after 1–2 hours
HbS solubility filtration test

• When reagents are available, this test should be performed in preference to the sickle cell slide test because it provides information about the different sickle cell disorders.
Principle of the test

• Blood is mixed in a phosphate buffer-saponin solution containing sodium dithionite and filtered. In its deoxygenated form, HbS is insoluble. HbSS is indicated by a red precipitate on the filter paper with a pale yellow filtrate. Other forms of hemoglobin are soluble when in a reduced state.
Reagents

• Phosphate buffer-saponin pH 7.1 Store at 2–8 C. Renew every 3 months or if it becomes turbid.
• Sodium dithionite powder.
• To make the working reagent:
  – Measure 20 ml of buffer-saponin solution.
  – Add 0.2 g sodium dithionite and mix gently until the chemical is dissolved.
• Note: The working reagent is not stable. It can be used only on the day it is prepared.
Test method

1- Pipette 2 ml of working reagent into a test tube approximately 13 77 mm.

2- Wash in 100 l (0.1 ml) of capillary blood or well mixed venous blood.

   *Note:* When the hemoglobin is below 70 g/l (7g/dl), use twice the volume of blood or if a venous blood sample, use plasma reduced blood (remove about half the plasma).

3- Mix well and filter through a small (5.5 cm diameter) No. 1 filter paper.

4- Note the color of the solution (pale yellow, pink red, or dark red) and whether there is any red precipitate (insoluble reduced HbS) on the filter paper.
Results after filtration

- HbSS . . . . . . Clear pale yellow filtrate. Abundant red precipitate on filter paper
- HbAS. . . . . . Clear pink filtrate. Small amount of red precipitate on filter paper
- HbAA (normal) . . . . . . . Dark red fluid (soluble reduced Hb) with no precipitate on filter paper
• Report the HbS solubility filtration test as:
  – ‘Positive for sickle cell anaemia’ when result shows HbSS appearance.
  – ‘Positive for sickle cell haemoglobin’ when result shows HbAS appearance.
  – ‘Negative for HbS’ when result shows HbAA appearance.