



National Standard Operating Procedure for Sickle Solubility Test

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Sultanate of Oman
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Acronyms

SCD	Sickle cell disease
HbS	Hemoglobin S
RBC	Red blood cells
HPLC	High performance liquid chromatography
EDTA	Ethylenediaminetetraacetic acid
ID	Identification
QC	Quality Control
CBB	Central blood bank
CMLSO	Chief Medical Laboratory Scientist Officer
DLS	Diagnostic laboratory services

1. Purpose

To provide a standardized step-by-step procedure for performing the sickle solubility test to detect the presence of sickle hemoglobin (HbS) in blood samples.

2. Scope

This SOP applies to all medical laboratory technicians performing hematology tests. It is intended for use as a screening test only. Confirmation of HbS should be performed by hemoglobin electrophoresis, HPLC, or molecular methods.

3. Definitions

3.1. Sick cell anemia: Hemoglobin S is inherited in the homozygous state (SS) i.e. two sickle cell genes, one from each parent, which results in sickle cell anemia, a severe disease.

3.2. Sick cell trait: Hemoglobin S inherited in the heterozygous state (AS) i.e. only one sickle cell gene from either parent and one normal gene from the other, which result in sickle cell trait, asymptomatic except under circumstances of reduced oxygen tension (e.g. extreme physical exertion).

3.3. Combined heterozygous sickle cell disease: Hemoglobin S is inherited along with other abnormal hemoglobins, a state called combined heterozygous including HbC (SC), β -thalassemia (S- β -thal), HbD (SD) and HbE (SE)..etc. These are sickle cell variants and can produce symptoms of varying severity.

3.4. HPLC: is an analytical technique used to separate, identify, or quantify each component in a chemical mixture. It is used to identify and quantify different Hb variants in blood.

4. Procedure

4.1. Clinical background:

Sickle cell disease (SCD) is one of the most common inherited hemoglobin disorders worldwide. Haemoglobin S can be inherited in a homozygous state (SS), heterozygous state (AS) or combined heterozygous. It forms long, rope-like structures called polymers when deoxygenated (oxygen deprived) which then aggregates within the red cells causing distortion of the shape of red blood cells (RBCs) to form sickle-shaped and angulated red cells. Early detection of sickle

hemoglobin (HbS) is essential for proper diagnosis, patient management, genetic counseling, and prevention strategies.

The sickle solubility test is a simple, rapid, and cost-effective screening method used to identify the presence of HbS in blood samples. While this test does not differentiate between sickle cell trait (heterozygous HbAS) and sickle cell disease (homozygous HbSS), it serves as an important first-line tool for mass screening and for use in laboratories with limited resources. Confirmatory testing using methods such as hemoglobin electrophoresis, high-performance liquid chromatography (HPLC), or molecular techniques is required to establish a definitive diagnosis.

4.2. Principle:

Hemoglobin S (HbS) is relatively insoluble when exposed to a reducing agent and a buffer. The test reagent contains a lysate to lyse the red cells and release the hemoglobin and a reducing agent to reduce the released hemoglobin. The test reagent, buffer and patient sample are mixed. A turbid suspension results in the presence of HbS due to precipitation, while normal hemoglobin (HbA) and other hemoglobins remain soluble and the solution stays clear. The degree of turbidity is visually compared against a control.

4.3. Pre-analytical phase:

4.3.1. Sample Requirements

- About 2-4 ml of venous blood collected into preferably EDTA tubes (other anticoagulants maybe acceptable as per the manufacturer recommendation).
- Specimens should be transported at room temperature 18 – 30 °C and can be store in the refrigerator of 2 – 8 °C.
- Samples should be returned to room temperature, for approximately 30 minutes, before analysis.
- Sample can be processed within 7 days of collection if stored in the refrigerator at 2–8 °C.
- Criteria for specimen rejection:
 - Inappropriate (missing/inadequate patient information) labelling of the sample.

- Insufficient Quantity.
- Inappropriate Container.
- Inappropriate transport/storage
- Unknown duration of delay
- Recent history of transfusion <3 months
- Hemolyzed or clotted sample

4.3.2. Materials and Reagents

- Whole blood sample preferably EDTA tubes (other anticoagulants maybe acceptable as per the manufacturer recommendation).
- Reducing reagent solution (general: phosphate buffer, saponin/detergent, and sodium hydrosulfite or equivalent reducing agent)
- Positive control (known HbS-positive sample or commercial equivalent)
- Negative control (known HbS-negative sample or commercial equivalent)
- Test tubes
- Droppers

4.3.3. Equipment

- Test tube rack
- Adjustable Pipettes
- Refrigerator to store samples and reagents at 2 – 8 °C
- General-purpose centrifuge.

4.3.4. Safety precautions

- Handle all blood samples as potentially infectious.
- Wear appropriate PPE (gloves, lab coat).
- Disposal of waste according to biomedical waste management guidelines.
- Reagent powder: Hydrosulfite: Harmful! As it may cause fire. Harmful if swallowed. Contact with acid liberates toxic gases. Keep the container tightly closed and dry. In case of contact with eyes, rinse with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of soap. In case of fire, use dry powder and never use water.

4.3.5. Quality Control

Composed of stabilized human red blood cells in a preservative medium. The following steps are to prepare and run the QC materials:

- Remove the control vials from the fridge and warm to room temperature for 30 minutes before use.
- Mix the positive and negative controls by first rolling the vials between the palms of the hands for 20 seconds.
- Hold the vials between the thumb and finger and rapidly invert the vials 8 to 10 times using a turning motion of the wrist.
- Add the recommended amount of reagent solution to the tube with a control label.
- Dispense the recommended amount of control into the appropriately labelled control tube.
- Mix the control sample and reagent by swirling the tube or place a piece of laboratory parafilm over the top and invert the tube to ensure the sample is mixed.
- Ensure that the quality control materials do not exceed the stated expiration date or open vial stability periods.
- A positive and a negative control are run with every batch of sickle screen.
- Expected results:
 - Controls must yield the expected positive or negative outcomes before patient results can be accepted.
- Notify the senior technologist or supervisor or haematopathologist if difficulties are encountered.
- Document the QC results in the assigned QC file.

Note: If QC materials are unavailable, previously reported positive and negative patient samples from the past seven days that were tested with valid quality control samples, may be used as controls.

4.4. Analytical phase:

- Reagent preparation as per manufacturer instructions (If using previously prepared reagents pulled from the refrigerator, allow it to warm to room

temperature (18 °C to 30 °C) in the test tubes for a minimum of 10 minutes before use.

- Label the test tube with patient ID.
- Add the recommended amount of reagent solution to the tube with patient ID label.
- Add the recommended amount of whole blood to the tube.
- Mix thoroughly by inversion.
- Allow the sample to stand at room temperature (18 °C to 30 °C) for at least 6 minutes.
- Read results between 6 and 60 minutes.
- Observe the sample for turbidity against a white background with ruled lines (black colour) under good lighting.
- In case of anemia with hematocrit <15%:
 - Centrifuge the sample for 5-10 minutes at 1200 rpm. Pipet 10µl of the packed cell volume from the bottom of the tube and add it to the test tube.
- In case of flocculation due to high lipid levels, high protein levels and leukocytosis:
 - Patient samples may be washed in normal physiologic saline, centrifuged and 10µl of the packed cells used for testing.

4.5. Post analytical phase:

Interpretation of Results (see Annexes – Figure 1):

- Negative: No turbidity, background lines are visible through the tube → HbS absent.
- Positive: Turbidity present, background lines are not visible through the tube → HbS detected.

4.6. Limitations:

- Cannot distinguish between sickle cell trait (HbAS) and sickle cell disease (HbSS).
- False negatives may occur in severe anemia, recent transfusion from a normal donor to a patient carrying sickle hemoglobin, infants under six months of age due to elevated levels of HbF Hemoglobin.
- False positives may occur in polycythemia, extreme leukocytosis or hyperlipidemia and recent transfusion from a sickle cell trait donor to a normal patient.

- Positive results may occur in patients with some rare sickling hemoglobin subtypes such as Hemoglobin C Harlem or Hemoglobin C Georgetown.
- Confirmation required by advanced methods.

4.7. Documentation and Reporting:

- Record results in the laboratory information system (LIS) or logbook. (Follow local laboratory protocols).
- Report as:
 - “Sickle cell screening test in blood”: **Positive** (when the background lines are not visible).
 - “Sickle cell screening test in blood”: **Negative** (when the background lines are visible).

5. Responsibility

5.1 Laboratory Program committee:

- Ensure that document is approved by ministry of Health and distributed to all institutions.

5.2 Task force creating the document:

- Ensure that the document is in accordance with Ministry of Health guidelines and is evidence based.
- Ensure that the document is sent to the relevant individuals in different health institutions for review.
- Ensure that the document is modified after receiving relevant feedback from reviewers.
- Ensure that the document is updated where appropriate.

5.3 Laboratory Head/ supervisor:

- Review the document and provide feedback where appropriate.
- Ensure that local standard operating procedures are in line with document.
- Ensure that all staff are trained and adhere to the document

5.4 Technical staff

- Be familiar with this document and adhere to it where applicable

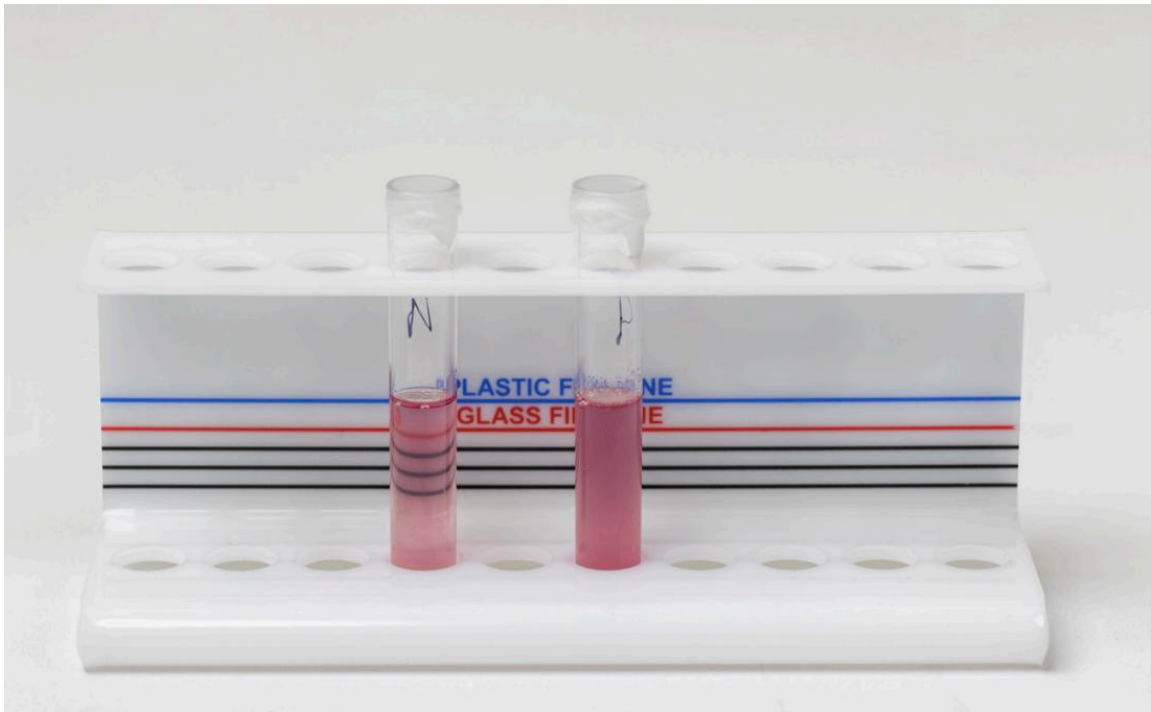
6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	August 2031

7. References

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2. Dacie JV, Lewis SM. *Practical Hematology*. 11th ed. London: Churchill Livingstone; 2011. p. 317–318.

8. Annexes



Left tube (N): Visible background lines indicate a negative Sickledex test; right tube (P): absence of background lines indicates a positive Sickledex test.