HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER
MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Haematology)		
Document:	Internal Policy and Procedure		
Title:	Sickling Test		
Applies To:	All Laboratory Staff		
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1. PURPOSE:

1.1 Elucidating the procedure of qualitative detection of hemoglobin (Hb) S in blood.

2. DEFINITONS:

N/A

3. POLICY:

3.1 Qualitative determination of haemoglobin S (HbS) in blood using a phosphate solubility method.
3.2 Principle:
- 3.2.1 Erythrocytes are lysed by saponin
- 3.2.2 Released hemoglobin is reduced by dithionite in a Phosphate Buffer
- 3.2.3 Reduced Hb S is characterized by its very low solubility

4. PROCEDURE:

4.1 Specimen Requirement

- 4.1.1 Specimen Type : Whole blood
- 4.1.2 Tube Type : K-EDTA (Lavender) Tube
- 4.1.3 Amount Required : 2 ml
- 4.1.4 Delivery Arrangement: As soon as possible and should not exceed 30 minutes from collection time
- 4.1.5 Temperature Restriction: 37^{0} c
- 4.1.6 Stability : 1 – 2 weeks at 2^{0}-8^{0} c
- 4.1.7 Unacceptable SP:
  - 4.1.7.1 Clerical Error
  - 4.1.7.2 Clotted
  - 4.1.7.3 QNS
- 4.1.8 Time of the test: Daily morning

4.2 Steps

- 4.2.1 Mix by inversion and allow to stand at RT for 15 minutes
- 4.2.2 Read the test by holding the test vial approximately 3 cm from the lined scale on the card provided with the kit
- 4.2.3 Severe anemia (< 7 g/dL) = use 40 uL sample instead of 20
- 4.2.4 For Positive Test add 2 ml of Sickling Solution to new tube
- 4.2.5 Add 300 uL of sickle cell urea reagent and mix.
- 4.2.6 Add 0.02 ml of Positive specimen
- 4.2.7 Mix the tube by inversion

4.2.8 Allow to stand at RT for 5 minutes
4.2.9 Read the test as.
4.2.9.1 Solution clear: The test must be confirmed by Hb electrophoreses
4.2.9.2 Solution still turbid: A non S-Sickling Hb is indicated

4.3 **Results Interpretation**: In normal adults, 95% or more of Hb is present as Hb A
4.3.1 Hb s can be inherited in the homozygous (S/S) pattern (Sickle Cell Anemia)
4.3.1.1 Hb s: 80-100 %
4.3.1.2 Hb A: absent
4.3.1.3 Hb F: small amount
4.3.2 Hb S can be inherited in heterozygous (A/S) pattern (Sickle cell Trait)
4.3.2.1 Hb s: 20 – 40%
4.3.2.2 Hb A 60-80%
4.3.2.3 Hb F: Small amount
4.3.3 RBC in person with Sickle Cell Anemia make an abnormal Hb (Hb S) which are rigid and deformed and take on a "Sickle ' or crescent shape. This causes the RBC to not be inflexible enough to squeeze through small blood vessels and may result in blood vessels being blocked.
4.3.4 Positive Results:
4.3.4.1 The lines behind the test tube are not visible (Hb S or other Sickling hemoglobin is present
4.3.5 Negative Results
4.3.5.1 The lines are seen through the test tube(No Sickling Hemoglobin is present

4.4 **Test Limitation**
4.4.1 False Positive Results occur in:
4.4.1.1 Severe anemia (< 7 g/dL) = the test must be rerun with 40 uL sample
4.4.1.2 Polycythemia
4.4.1.3 Multiple Myeloma
4.4.1.4 Cryoglobulinemia and other disglobulinemias
4.4.1.5 Up to 4 months after transfusion with RBC having Sickle Cell Trait
4.4.2 False Negative Results occur in:
4.4.2.1 Infant younger than 3 months
4.4.2.2 Coexisting Thalassemia or Iron Deficiency
4.4.2.3 Hb S concentration < 15 % of total Hb

5. MATERIALS AND EQUIPMENT:

5.1 Reagents & Supplies
5.1.1 Sickle Cell Buffer
5.1.2 Dithionite Reagent
5.1.3 Sickle Cell Urea Reagent
5.2 Preparation of Sickling Solution
5.2.1 Add the entire contents of one vial dithionite reagent to bottle of sickle cell buffer.
5.2.2 Mix by swirling
5.2.3 Stability after preparation : 1 month at 2^{0}-8^{0} C

6. RESPONSIBILITIES:

6.1 The assigned technician

7. APPENDICES:

7.1 N/A

8. REFERENCES:

8.1 Hematology, Williams et al, Ed. 4, 1990 (1722).
8.2 Clinical Interpretation Of Laboratory Tests, Widman, Ed. 91983(43)

9. APPROVALS:

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