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| Department: | Laboratory and Blood Bank (Haematology) | | |
| Document: | Internal Policy and Procedure | | |
| Title: | Sickling Test | | |
| Applies To: | All Laboratory Staff | | |
| Preparation Date: | January 06, 2025 | Index No: | LB-IPP-056 |
| Approval Date: | January 20, 2025 | Version : | 2 |
| Effective Date: | February 20, 2025 | Replacement No.: | LB-IPP-056(1) |
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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° c
- 4.1.6 Stability : 1 – 2 weeks at 2°-8° 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^o-8^o C

6. RESPONSIBILITIES:

- 6.1 The assigned technician

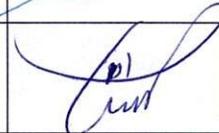
7. APPENDICES:

- 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:

| | Name | Title | Signature | Date |
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