SICKLE SOLUBILITY TEST

METHOD – SOLUBILITY TEST (VISUAL CHECK)
PRODUCT CODE – LS02

ANAMOL
THE ORIGINAL MAKERS

INSTRUCTIONS FOR USE

INTENDED USE: Test for qualitative determination of sickling hemoglobin in whole blood by solubility test method.

METHOD HISTORY

Herrick observed the sickling of erythrocytes from patients with sickle cell anaemia. Since then, over 250 structural variants of the haemoglobin molecule have been describe. Itano reported poor solubility of deoxyhaemoglobin-S in concentrate phosphate buffer. Several modifications of the original procedure have been reported.

SUMMARY

Sickle cell disease is an inherited condition characterized by the presence of Hemoglobin S (Hb-S). Hb-S exists in a homozygous state (S/S) known as Sickle Cell Anemia or in a heterozygous state (A/S) known as Sickle Cell Trait. Homozygous individuals (S/S) commonly exhibit symptoms of severe hemolytic anemia and/or vascular occlusions. Heterozygous individuals (A/S) are usually asymptomatic. Hb-S may be present with other hemoglobins, such as Hemoglobin A, C or D, or with thalassemia, a condition that interferes with the synthesis of normal hemoglobin.

PRINCIPLE

Erythrocytes are lysed by saponin and released haemoglobin 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 or non-S sickling haemoglobin, the system becomes turbid. In either case, electrophoretic confirmation is required for conclusive identification.

REAGENTS PROVIDED

SICKLE CELL BUFFER REAGENT - R1

A solution containing potassium phosphate monobasic, potassium phosphate dibasic with preservative. Keep tightly capped and protected from contamination. This can be used until expiration date printed.

SICKLE CELL POWDER REAGENT - R2

Sickle cell powder must be free flowing. Can be used until the expiration date indicated.

REAGENT STORAGE & STABILITY

Store reagents at room temperature between 4-30 °C. The reconstituted reagent is stable for 2 months when store at 2-8 °C or 3 weeks at 25 ^{0}C. Refer to preparation of working solution for additional information.

INDICATORS OF REAGENT DETERIORATION

Appearance of turbidity in the sickle cell reagent, which will not readily dissolve upon mixing, may indicate reagent deterioration. Failure to obtain accurate results in the assay of control materials may indicate reagent deterioration. Anamol cannot guarantee the stability of reagents, which have been:

1. Transferred from their original containers
2. Improperly stored
3. Contaminated during use.

PRECAUTIONS

1. Reagent contains preservatives which could be harmful. Refer to MSDS for additional information.
2. Avoid contact with skin or mucosa. Wash hands after use. Wear gloves while performing the test.
3. Patient samples should be treated as if capable of transmitting infection. Appropriate care should be taken to avoid infections, if any.
4. Discard the excess/expired reagents as per local biomedical waste management guidelines.

SPECIMEN COLLECTION

Collect whole blood in a vial containing a suitable anticoagulant (Heparin, EDTA, Oxalate) and mix thoroughly. Blood samples that have been kept for as long as 1-2 weeks at 4-8 °C are reportedly satisfactory.

PREPARATION OF WORKING SOLUTION

Working solution buffer must be prepared prior to performing the test by following the steps as mentioned below:

1. Bring buffer and reagent powder to room temperature before mixing.
2. Add the contents of one vial of Reagent Powder 2 (R2) to one bottle of Sickle Solubility Buffer 1 (R1).
3. Cover the top of the bottle of R2 by cap. Dissolve the reagent powder completely with vigorous agitation.
4. Record the reconstitution date and the reconstituted solution expiry date in the space provided on the solubility buffer bottle. You may use additional labels for recording the date of reconstitution if desired.
5. Store the working solubility buffer tightly capped at 2-8 °C when not in use.
6. Reconstituted buffer must be used within 45 days.

PROCEDURE

1. Prepare the working solution buffer as described above. If already prepared, bring the working solution buffer to room temperature before performing the test.
2. Add 2.0 ml of working solution buffer (at room temp.) reagent to the tubes and label.
3. Add 0.02 ml (20µl) of whole blood and plug it. Mix by inversion.
4. Place in the test tube rack for 5 minutes.
5. Read the test by holding the tube in Test tube stand provided. Adequate illumination is necessary

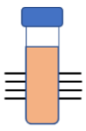
NOTE

Reagents for laboratory use only. Do not pipette by mouth. Use reagent of same lot numbers. Do not interchange the reagent of different lot numbers. All positive results should be confirmed on electrophoresis. Run Positive Control with every new run or batch.

INTERPRETATION OF RESULTS

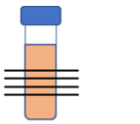
POSITIVE RESULT

If Hb-S or any other sickling haemoglobin is present, the solution is turbid and the lines behind the test vial will not be visible. Compare the turbidity of Test Solution with Negative Control Solution, if observe more turbid, say positive.



Positive Result: No lines visible

NEGATIVE RESULT



Negative Result: Lines visible

If no sickling haemoglobin is present, the clear or slightly turbid solution will permit the line to be seen through the tube. All doubtful tests, along with positive test, should be submitted for electrophoretic confirmation.

PROCEDURE LIMITATIONS

Severe anaemia will cause false negatives results, therefore, if the haemoglobin concentration is 7 g/dl or less, the samples should be re-run with 40 µl of whole blood. Blood from patients with polycythaemia, multiple myeloma, cryoglobulinemia and other dysglobulinemia cause false positives, whereas patients with over 25% Hb F present may yield false negatives. In all cases where abnormalities are indicated or suspected, electrophoretic confirmation is recommended.

QUALITY CONTROL

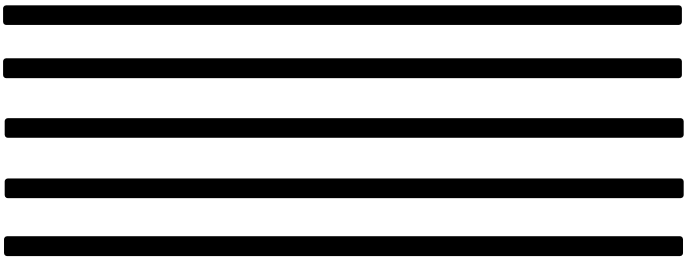
Controls should be run with each series of test. Negative controls may be collected from a normal, healthy individual. Positive controls may be purchased or obtained from samples determined to contain HbS by electrophoretic methods.

BIBLIOGRAPHY

1. Herrick, JP, Arch Intern Med. 6:517 (1910)
2. 4. Neel JV. Blood 6:389 (1951)
3. Itano, HA, Arch Biochem Biophys, 47;148 (1953)
4. Schmidt, RM and Wilson, SM J. Am Med Assoc. 225;1225 (1973)

Symbol	Explanation	Symbol	Explanation
	Manufactured By		In Vitro Diagnostic Use
	Lot Number		Read Instructions Before Use
	Catalogue Number		Storage Temperature
	Manufacturing Date		Number of Tests / Volume
	Expiry Date		Do Not Reuse
	Protect from Sunlight		Keep Dry

READING CHART



Note: To read the results, keep the tubes close to these lines. If the lines are clear with transparent solution, it indicates negative test results (refer to interpretation of negative results). If the lines are unclear (hazy or barely visible) due to turbid solution, it indicates positive result (refer to interpretation of positive results). Always run a negative control with the test for easy comparison. A positive control is recommended to be run with each test which could be commercially procured or any sample previously confirmed eletrophoretically.

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ISO 9001: 2015
ISO 13485 : 2016
GMP
CE