SAS™ SICKLE CELL TEST

(Modified Nalbandian)

For the Qualitative Determination of Hemoglobin S (Hb-S) in Blood

Store at Room Temperature 15° - 30°C

For In-Vitro Diagnostic Use

Precautions

1. Do not pipette reagents by mouth.
2. In case of contact with reagents, flush affected area with large amounts of water. If irritation persists, seek medical attention.
3. Any cloudiness observed in the Sickle Cell Buffer which will not readily dissolve upon mixing may indicate reagent deterioration.
4. If the Sickle Cell Lysing Reagent powder becomes damp and lumpy prior to use, it should be discarded.
5. Reagents in this kit contain Sodium Azide as a preservative which may react with lead or copper in plumbing to form potentially explosive metal azides. Upon disposal, always flush with large volumes of water to prevent azide buildup.
6. All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
7. The Sickle Cell Lysing Reagent contains Sodium Hydrosulfite, which is a flammable solid, and a strong reducing agent. In case of contact with eyes or skin, promptly flush exposed areas with plenty of water for at least 15 minutes. Seek medical attention if irritation persists.

BIBLIOGRAPHY

11. Modified Nalbandian procedure.

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3. This test is not appropriate for use in newborn screening or testing for hemoglobinopathies using dried blood specimens.

4. Rare sickling hemoglobins reportedly also give positive test results with this procedure. Some of these include Hemoglobin C (Harlem), Hemoglobin C (Georgetown), Hemoglobin H (a Heinz body forming hemoglobin), and other low solubility hemoglobins such as King’s County and Stanley II. In patients who have had a splenectomy and have unstable hemoglobins, the test may appear positive due to the presence of numerous insoluble erythrocyte inclusions.

5. In all cases where abnormalities are suspected or indicated, electrophoretic confirmation is recommended and necessary to identify specific genotypes.

6. Samples that are highly lipemic or are borderline positive should have the following procedure performed:
   - Centrifuge the whole blood specimen at 1500xg for 10 minutes and carefully remove the supernatant plasma and discard. Repeat the test using 20 µl of the packed erythrocytes.
   - Abnormal elevations of total serum protein levels reportedly cause coarse flocculation, which may be incorrectly interpreted as positive for sickle cell. When this occurs, wash the specimen once with normal physiological saline and centrifuge. Decant the supernatant and re-suspend the cells to the original volume using normal group compatible serum. Retest with the washed specimen.

LIMITATIONS

1. Severe anemia can cause false negatives. If the physician suspects this condition, a hemoglobin determination is necessary prior to testing. If the patient's hemoglobin is below 7 gm/dl, the test should be performed using 40 µl of the sample. Doubling the volume of anemic blood in an effort to have adequate sample of hemoglobin is well documented. The necessity of determining hemoglobin levels prior to testing must be established by individual laboratory guidelines.

2. False negative results may occur when:
   - a) the Hb-S concentration is less than 20% of total hemoglobin. This can occur when a patient is transfused with blood from a donor with HB-S trait.
   - b) when testing infants younger than 3 months of age. It is recommended not to use this test before 3 months of age.

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QUALITY CONTROL

Quality control samples should be used routinely to monitor test performance. It is recommended to use positive and negative sickle cell controls during each day of testing and/or according to individual laboratory guidelines.

EXPECTED VALUES

Specimens containing HbS/S, HbS/A, HbS/C, HbS/D, HbS-thalassemia and HbS/N (Baltimore) reportedly produce positive results. Specimens containing normal hemoglobin and HbA/C, HbC/C, HbA/F, and thalassemia reportedly produce negative results. However, low solubility variants such as HbH, King’s County and Stanleyville II may show false positive results.

The homozygous form of Sickle Cell Disease affects 0.3% of the black population. In America and Africa, Hb-A/S is the most common hemoglobin variant, approximately 8% in African Americans (heterozygous form) and 30% in African Blacks. The mutation probably originated in Central Africa and spread to countries bordering the Mediterranean Sea, including the non-black people of these areas, e.g., Italy, Greece, Turkey and some of the Arabic nations. The heterozygous state does not cause anemia or shortened red cell life span, but one in 6000 African Americans is homozygous for Hb-C.