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-Vivo Diagnostic Use

READ ALL INSTRUCTIONS BEFORE BEGINNING THE ASSAY

INTENDED USE
The SAS™ Sickle Cell Test is intended to be used as an aid in the qualitative detection of hemoglobin S (Hb-S) in anticoagulated whole blood. The test does not distinguish between sickle cell disease (HbSS) and sickle cell trait (HbSA). This test is not recommended for use on newborns under 3 months of age. This test is for professional use.

INTRODUCTION
Sickle cell disease is an inherited disease which is characterized by the presence of an abnormal hemoglobin (Hb-S). In normal adults, 95% or more of the hemoglobin is present as hemoglobin A (Hb-A). Infants younger than 3 months of age may have low levels of Hb-S which may not be detectable by this methodology. Hemoglobin S can be inherited in the homozygous state (S/S), which results in sickle cell anemia, or in heterozygous state (A/S), which is usually the benign, asymptomatic sickle cell trait. Hemoglobin S can also occur in the presence of other abnormal hemoglobin, i.e. Hb-C (S/C), thalassemia (S-thal), or Hb-D (S/D). These are referred to as the sickle cell variants and can produce symptoms of varying severity.

PRINCIPLE OF THE TEST
Erythrocytes are lysed by saponin, and the released hemoglobin is deoxygenated by dithionite in a concentrated phosphate buffer. When a positive specimen is identified, the addition of urea to the reaction mixture will cause the solution to become clear if Hb-S is present. If the solution remains turbid after adding urea, a non Hb-S is indicated. Electrophoresis assay is required for conclusive identification.

The method presented here is based upon a modified Nalbandian procedure.1,3

REAGENTS & MATERIALS PROVIDED
1. Sickle Cell Buffer - contains Potassium Phosphate, Saponin and Sodium Azide. It is stable until the expiration date noted on the label when stored at 15° to 30°C. A Working Sickle Cell Buffer must be prepared prior to testing. Add the entire contents of one vial of Sickle Cell Lysing Reagent to 50ml of Sickle Cell Buffer and mix completely by swirling. The Working Sickle Cell Buffer is stable for 30 days from date of preparation when stored at 2° to 8°C.
2. Sickle Cell Lysing Reagent - contains Sodium Hydrosulfite powder. It is stable until the expiration date noted on the label when stored at 15° to 30°C.
3. Sickle Cell Urea Reagent - contains Urea and Sodium Azide. It is stable until the expiration date noted on the label when stored at 15° to 30°C.

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

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STORAGE
Before use, store the kit at 15° to 30°C. After adding lysing reagent, store the Sickle Cell Buffer at 2° to 8°C. Store Sickler Cell Lysing Reagent at 15° to 30°C. Store Sickler Cell Urea Reagent at 15° to 30°C. Bring all reagents to room temperature before use.

SPECIMEN COLLECTION
Whole blood can be collected by venipuncture from finger stick into tubes containing anticoagulant (heparin, EDTA, ACD). Specimens can be kept for 1 to 2 weeks at 2° to 8°C. Cletted blood and blood collected on filter paper is not appropriate for use.

MATERIAL REQUIRED BUT NOT PROVIDED
Pipettes
Test tubes 12 x 75mm
Timer

PREPARATION OF WORKING SICKLE CELL BUFFER
Add the entire contents of one vial of Sickler Cell Lysing Reagent to 50ml of Sickler Cell Buffer and mix completely by swirling. The working buffer may be used for 30 days from date of preparation if stored at 2° to 8°C.

PROCEDURE
1. Transfer 2.0ml of the Working Sickle Cell Buffer to 12 x 75mm tube.
2. Add 20µl of whole blood to the Working Sickle Cell Buffer and mix completely by swirling. The working buffer may be used for 30 days from date of preparation if stored at 2° to 8°C.
3. Allow to stand for 5 minutes but no longer than 30 minutes. Read against the Line Scale.

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 7gm/dl, the test should be performed using 40µl of the sample. Doubling the volume of anemic blood in an effort to have adequate sample of hemoglobin is well documented.11 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.
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.33 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.

EXPECTED VALUES
Specimens containing Hbs/S, Hbs/A, Hbs/C, Hbs/D, Hbs/C-thalassemia and Hbs/N (Baltimore) reportedly produce positive results.11 Specimens containing normal hemoglobin and Hbs/A/C, Hbs/C, Hbs/A/F, and thalassemia reportedly produce negative results.13 However, low solubility variants such as HbH, King's County and Stanley 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 Arab 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.

PERFORMANCE CHARACTERISTICS
A total of 166 consecutive clinical samples were tested on specimens submitted for sickle cell disease testing. Samples were assayed with SAST™ Sickle Cell Test and another commercially available test according to the respective assay procedure. Both methods identified 42 positive and 124 negative specimens. The results demonstrated a 100% agreement between both tests for all specimens tested. Hemoglobin electrophoresis was performed on 22 specimens which identified 11 positive and 11 negative specimens. The SAST™ Sickle Cell Test correctly identified all specimens. A blind study was performed to determine the reproducibility and repeatability of test results with the SAST™ Sickle Cell Test. Testing was performed with a panel of specimens which included 8 known negative specimens and 10 known positive specimens containing Hbs ranging from 20 to 68%. The testing was performed using 3 different lots, on 3 different days, by 3 different investigators at one site. The results showed a 100% correlation within runs, from run to run, and between investigators for all specimens. A limits of detection study was done which found that this test can safely detect 20% levels of Hbs.

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.

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