

LINE SCALE



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SAS #70-PISCT
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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

For Technical Assistance Call 800-275-2326
Outside the USA Call 210-699-8800



SA Scientific, Inc.
4919 Golden Quail, San Antonio
Texas 78240 USA

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.³ Hb-S when deoxygenated, is insoluble in concentrated phosphate buffer and produces visible turbidity. Since almost all other hemoglobins (i.e. Hb-A, -F, -C, -E, -D) are soluble, blood specimens containing Hb-S are easily identified.

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.^{4,5}

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.

STORAGE

Before use, store the kit at 15° to 30°C. After adding lysing reagent, store the Sickie Cell Buffer at 2° to 8°C. Store Sickie Cell Lysing Reagent at 15° to 30°C. Store Sickie 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 or from finger stick into tubes containing anticoagulant (heparin, EDTA, ACD). Specimens can be kept for 1 to 2 weeks at 2° to 8°C. Clotted 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 SICKIE CELL BUFFER

Add the entire contents of one vial of Sickie Cell Lysing Reagent to 50ml of Sickie 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 Sickie Cell Buffer to 12 x 75mm tube.
2. Add 20µl of well mixed whole blood or control specimen and mix thoroughly and gently. If testing a specimen with a hemoglobin below 7gm/dl, add 40µl of whole blood.
3. Allow to stand for 5 minutes but no longer than 30 minutes at room temperature (20° to 30°C).
4. Read the results by looking through the test tube at the Line Scale (provided in this insert) Hold the test tube upright and perpendicular approximately 3cm from the Line Scale. Adequate illumination is necessary.

INTERPRETATION OF RESULTS

Positive Results - If Hb-S or any sickling hemoglobin is present, the solution will be sufficiently turbid to prevent reading of the Line Scale through the test tube. All doubtful tests, as well as all positive tests, should be confirmed by electrophoresis. Positive results can be further checked by performing the following steps:

1. Transfer 2.0ml of Working Sickie Cell Buffer to 12 x 75mm test tube.
2. Add 5 drops of Sickie Cell Urea Reagent and mix.
3. Add 20µl of positive whole blood and gently swirl to mix.
4. Let stand for 5 minutes but no longer than 30 minutes. Read against the Line Scale.

The presence of Hb-S will be indicated if the solution becomes clear in the presence of the Sickie Cell Urea Reagent. The only known variant that will also clarify with the Urea Reagent is HbS/C (harlem).⁴ This step does not replace confirmatory testing for positive results.

Negative Results - If a sickling hemoglobin is not present, the solution will be clear enough to allow the Line Scale to be seen through the test tube.

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 40 µl of the sample. Doubling the volume of anemic blood in an effort to have adequate sample of hemoglobin is well documented.^{10,11,12} 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.^{4,5} 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.⁷
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 20µl of the packed erythrocytes.
7. Abnormal elevations of total serum protein levels reportedly cause coarse flocculation, which may be incorrectly interpreted as positive for Hb-S.¹² 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.

QUALITY CONTROL

Quality control samples should be used routinely to monitor test performance.⁸ 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.¹⁰ Specimens containing normal hemoglobin and HbA/C, HbC/C, HbA/F, and

thalassemia reportedly produce negative results.¹³ However, low solubility variants such as HbH, King's County and Stanleyville II may show false positive results.^{4,5}

The homozygous form of Sickie 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.

PERFORMANCE CHARACTERISTICS

A total of 166 consecutive clinical samples were tested on specimens submitted for sickle cell testing. Samples were assayed with SAS™ Sickie 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 SAS™ Sickie Cell Test correctly identified all specimens. A blind study was performed to determine the reproducibility and repeatability of test results with the SAS™ Sickie 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.