**PRINCIPLE OF THE PROCEDURE**

Erythrocytes are lysed by saponin, and the released hemoglobin is deoxygenated by dithionite in a concentrated phosphate buffer\(^1\). 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 can produce symptoms of varying severity. When a positive specimen is identified, the addition of urea to the reaction mixture will cause the solution to become cloudy. A rapid, inexpensive technique for the detection of Hemoglobin S and Non-S Sickling Hemoglobin. **Performance of the test:**

1. Prepare a panel of specimens, which included 8 known negative specimens and 10 known positive specimens containing Hb-S ranging from 20 to 68%.
2. 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 Hb-S.

**REFERENCES**

5. If the Sickle Cell Lysing Reagent powder becomes damp and lumpy prior to use, it should be discarded.
6. The Sickle Cell Lysing Reagent contains Sodium Hydrosulfate, 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 advice if irritation persists.
7. Do not store, eat, drink or apply cosmetics in areas where plasma/serum samples are handled.
8. Any cuts, abrasions or other skin lesions should be suitably protected.
9. All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.

HANDLING AND PROCEDURAL NOTES
1. In order to obtain reliable and consistent results, the instructions in the package insert must be strictly followed. Do not modify the handling and storage conditions for reagents or samples.
2. Do not use past the expiration date indicated on the kit.
3. Do not interchange components of one kit with those of another kit.

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

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

NOTE: Packed red blood cells can be used instead of whole blood to minimize false positives and false negative reactions.14

PERFORMANCE OF THE TEST
Materials Provided:

<table>
<thead>
<tr>
<th></th>
<th>25 Tests</th>
<th>100 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>SICKLE CELL LYSING REAGENT</td>
<td>1 gm</td>
<td>4 gm</td>
</tr>
<tr>
<td>SICKLE CELL UREA REAGENT</td>
<td>4.0 ml</td>
<td>17.0 ml</td>
</tr>
<tr>
<td>SICKLE CELL BUFFER</td>
<td>50 ml</td>
<td>200 ml</td>
</tr>
</tbody>
</table>

Additional Material Required:
1. Pipets
2. Test tubes 12 x 75 mm
3. Timer

PREPARATION OF WORKING SICKLE CELL BUFFER
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 buffer may be used for 30 days from date of preparation if stored at 2°C to 8°C.

ASSAY PROTOCOL
1. Transfer 2.0ml of the Working Sickle Cell Buffer to a 12 x 75 mm 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 7 g/dl, add 40 µl of whole blood.
3. Allow to stand for 5 minutes but no longer than 30 minutes at room temperature (15°C to 30°C).
4. Read results by looking through the test tube at the Line Scale (provided in this insert). Hold the test tube upright and perpendicular approximately 3 cm from the Line Scale. Adequate illumination is necessary.

QUALITY CONTROL
Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory’s standard Quality Control Procedures. Controls with graded reactivity should be included. If control samples do not yield the expected response, the assay should be considered invalid and the assay repeated. If the repeat assay does not elicit the expected results for the control samples, discontinue use of the kit and contact ASI Technical Support at 800.654.0146.

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 2.0 ml of Working Sickle Cell Buffer to 12 x 75 mm test tube.
2. Add 5 drops of Sickle Cell Urea Reagent and mix.
3. Add 20 µl of positive whole blood and gently swirl to mix.
4. Let stand for 5 minutes but no longer than 30 minutes. A positive interpretation should not be determined before 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 Sickle Cell Urea Reagent. The only known variant that will also clarify with the Urea Reagent is HbS/C (Harlem). 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.
1. Negative results may be interpreted as early as 5 minutes.

LIMITATIONS OF THE PROCEDURE
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 g/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 the 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. b) when testing infants younger than 3 months of age. It is recommended not to use this test on infants younger than 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 1500 x g for 10 minutes and carefully remove the supernatant plasma and discard. Repeat the test using 20 µl 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.13 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. Re-test with the washed specimen.

EXPECTED VALUES
Specimens containing Hbs/S, Hbs/A, Hbs/C, Hbs/D, Hbs-thalassemia and Hbs/S-N (Baltimore) reportedly produce positive results.16 Specimens containing normal hemoglobin and HbaA/C, HbcC/C, HbA/F, and thalassemia reportedly produce negative results.13 However, low solubility variants such as Hb-H, King’s County and Stanley II may show false positive results.1,3

The homozygous form of Sickle Cell Disease affects 0.3% of the black population. In America and Africa, Hbs/A is the most common hemoglobin variant, approximately 8% in African Americans (homozygous 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 homozygous state does not cause anemia or shortened red cell life span, but 1 in 6000 African Americans are homozygous for Hb-C.