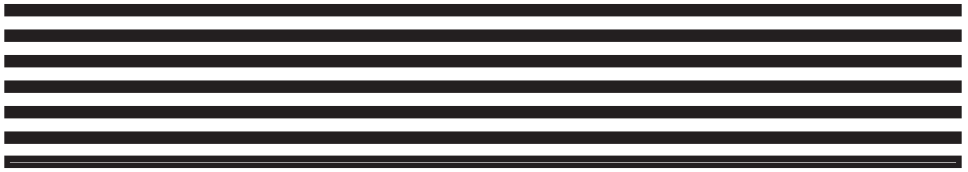
PERFORMANCE CHARACTERISTICS

A total of 166 consecutive clinical samples were tested on specimens submitted for sickle cell testing. Samples were assayed with **ASI 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 **ASI Sickle Cell Test** correctly identified all specimens. A blind study was performed to determine the reproducibility and repeatability of test results with the **ASI Sickle Cell Test**. Testing was performed with a panel of specimens, which included 8 known negative specimens and 10 known positive specimens containing Hb-S 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 Hb-S.

WARRANTY

This product is warranted to perform as described in its labeling and ARLINGTON SCIENTIFIC, INC. literature. ARLINGTON SCIENTIFIC, INC. disclaims any implied warranty of merchantability or fitness for a particular purpose and in no event shall ARLINGTON SCIENTIFIC, INC. be liable for consequential damages.

Line Scale



REFERENCES

1. Widmann, F.K.; Clinical Interpretation of Laboratory Tests, F.A. Davis Co., Philadelphia, pp. 57-60, 1983.
2. Neel, J.V.; *Blood The Journal of Hematology*, 6:389, 1951.
3. Itano, H.A.; *Arch. Biochem. Biophys*., 47:148, 1953.
4. Nalbandian, R.M., et al.; Dithionite Tube Test—A Rapid, Inexpensive Technique for the Detection of Hemoglobin S and Non-S Sickling Hemoglobin. *Clin Chem*., 17:1028-1032, 1971.
5. Nalbandian, R.M., et al.; Automated Dithionite Test for Rapid, Inexpensive Detection of Hemoglobin and Non-S Sickling Hemoglobin. *Clin Chem*. 17:1033-1037, 1971.
6. Calam, R.R.; *Selected Methods of Clinical Chemistry*, Vol.9, Eds. By W.R. Faulkner and S. Meites, AACC Pub., Washington, pp.3-10, 1982.
7. Fairbanks, V.F. and Klee, G.G.; Textbook of Clinical Chemistry, Ed. N.W. Tietz, W.B. Saunders Co., Philadelphia, pp1540-1541, 1986.
8. Whitehead, T.P.; Quality Control in Clinical Chemistry, John Wiley & Sons, New York, 1977.
9. Kaplan, L.A., Pesce, A.T.; *Clinical Chemistry*, 2nd ed. The C.V. Mosby Co. Missouri, 1989.
10. Ballard, M.S., et al.; A new Diagnostic Test for Hemoglobin S. *Jour. of Pediatrics*, Vol 76, pp.117-119, 1970.
11. Diggs, L.W., Walker, R.; Technical Points in the Detection of Sickle Cell Hemoglobin by the Tube Test. *Amer. Jour. of Med. Tech*, Vol 37, pp.33-35, 1971.
12. Loh, W.P.; Evaluation of a Rapid Test Tube Turbidity Test for the Detection of Sickle Cell Hemoglobin. *Amer. Jour. of Clin. Path.*, Vol 55, pp.55-57, 1971
13. Canning, D.M., Huntsman, R.G.; An Assessment of Sickledex as an alternative to the Sickling Method. *Amer. Jour. of Clin. Path*., 23(8): pp.736-737, 1970.
14. National Committee for Clinical Laboratory Standards. Solubility Test for Confirming the Presence of Sickling Hemoglobins: Approved Standard. NCCLS, Publication H10-A. Villanova, PA: NCCLS, 1996.

ASI TECHNICAL SUPPORT: (800) 654-0146

Distributed By:

ARLINGTON SCIENTIFIC

(800) 654-0146 (801) 489-8911 Fax (801)489-5552
1840 N. Technology Dr., Springville, UT 84663
www.arlingtonscientific.com

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ASI

SICKLE CELL TEST

For in vitro diagnostic use

Catalog Number	Kit Size
200025	25 Tests
200100	100 Tests

CPT Code: 85660

INTENDED USE

The **ASI 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 (HbS/S) and sickle cell trait (HbS/A). This test is not recommended for use on newborns under 3 months of age. These materials are intended to be acquired, possessed and used only by healthcare professionals.

SUMMARY AND EXPLANATION

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 the 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 PROCEDURE

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

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 50 ml 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.

Sickle Cell Lysing Reagent – contains Sodium Hydrosulfate powder. It is stable until the expiration date noted on the label when stored at 15° to 30° C.

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.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

1. Do not pipet by mouth.
2. **ASI Sickle Cell Reagents** contain sodium azide. Azides in contact with lead and copper plumbing may react to form highly explosive metal azides. When disposing of reagents containing azide, flush down the drain with large quantities of water to prevent azide build-up.
3. In case of contact with reagents, flush affected area with large amounts of water. If irritation persists, seek medical attention.
4. Any cloudiness observed in the Sickle Cell Buffer, which will not readily dissolve upon mixing, may indicate reagent deterioration.

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 attention if irritation persists.
7. Do not smoke, 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° to 30° C. After adding lysing reagent, store the Sickle Cell Buffer at 2° to 8° C. Store Sickle Cell Lysing Reagent at 15° to 30° C. Store Sickle Cell Urea Reagent at 15° 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° to 8° C. Clotted blood and blood collected on filter paper is not appropriate 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:

	25 Tests	100 Tests
SICKLE CELL LYSING REAGENT	1 gm	4 gm
SICKLE CELL UREA REAGENT	4.0 ml	17.0 ml
SICKLE CELL BUFFER	50 ml	200 ml

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° 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° 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).[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.

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.[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.

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 Hb-H, King's County and Stanley II may show false positive results.[4,5]

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 (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 1 in 6000 African Americans are homozygous for Hb-C.