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| **SOP Number:** | CH-070 | **Creation Date:** |  |
| **Department:** | Hematology | **Effective Date:** |  |
| **Author:** | R.J. Bernshausen MT(ASCP) | **Version:** | 1.0 |

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| Applicable Standards | | | |  | Version History | | | |
| Standard | | Organization | |  | Version | Effective Date | | Deactivation Date |
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| Review History (Up to the Last 15 Occurrences) | | | | | | | | |
| Date | Version | | Revision Type | | | | Review By | |
|  |  | | Director Review | | | | System Laboratory Medical Director, Joe A. Lewis, M.D., F.C.A.P. | |
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| Distribution |
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**CLINICAL SIGNIFICANCE**

Sickle cell anemia is a chronic hemolytic anemia that occurs in individuals who are homozygous for the hemoglobin S gene, i.e. S/S. Hemoglobin S comprises from 70­90% of the hemoglobin present in individuals that are homozygous for the hemoglobin S gene. Heterozygous individuals (A/S) are carriers of the sickle cell trait and their total hemoglobin content may contain up to 50% of the S hemoglobin.

When an individual with sickle cell anemia is exposed to periods of decreased oxygen availability, the hemoglobin S forms elongated tactoids (liquid crystals) within the erythrocyte. This may cause vascular occlusions that leads to accumulated damage to the kidney, heart, lung, eyes and other organs. The disease is usually life shortening and its most common symptom is expressed as chronic hemolytic anemia. Heterozygous individuals are usually asymptomatic but under certain conditions, such as hypoxia during surgery or severe pneumonia, sickle cell syndrome may occur.

**PRINCIPLE**

The SickleScreen Kit is a modified Nalbandian procedure based upon differential solubility. Red blood cells are lysed by a surfactant. The released hemobglobin is reduced by sodium hydrosulfite. Reduced Hemoglobin S is insoluble in concentrated phosphate solutions and forms a turbid suspension. Normal Hemoglobin A and most other hemoglobins remain in solution under these conditions. Both sickle cell disease and sickle cell trait can be detected with this procedure.

**SPECIMEN:**

**Patient Preparation:**

No special patient preparation is required.

**Type:**

Specimens may be collected in evacuated tubes containing either EDTA, sodium or lithium heparin or sodium citrate. For specimens collected in EDTA, or sodium or lithium heparin, an absolute minimum volume of 1.0 ml is necessary to maintain a proper blood to anticoagulant ratio. Specimens may also be collected in a 2.7 or 1.8 ml evacuated tube containing 3.2% Sodium Citrate (ratio of 9 parts blood to 1 part anticoagulant). Full blood volume is required for this to be an acceptable specimen

The specimen must meet the criteria outlined in the **Specimen Rejection Policy**.

**Handling Conditions:**

All specimens for this test must be well-mixed prior to processing. Specimens may be stored at 2-8 °C for up to 2 weeks prior to testing.

**EQUIPMENT AND MATERIALS:**

**Equipment:**

1. Tube reading rack.
2. Pacific Hemostasis Phosphate Buffer
3. Pacific Hemostasis Reaction Vials (contains sodium hydrosulfite powder)
4. Streck Sickle-Chex Positive and Negative controls.
5. 50 µL pipette
6. Timing device

**Storage Requirements:**

1. **Sickle-Chex Positive and Negative controls:** Unopened vials are stable through the expiration date on the container. Once opened the controls are stable for 100 days when stored at 2-10 °C.

Sickle-Chex controls are made from human source material which tested non-reactive for antigens to Hepatitis B (HbsAg), negative by tests for antibodies to HIV (HIV-1/HIV-2) and Hepatitis C (HCV), non-reactive to HIV-1 RNA and HCV RNA by licensed NAT and non-reactive to the Serological Test for Syphilis (STS) using techniques specified by the U.S. Food and Drug Administration. Since no known test method can assure complete absence of human pathogens, this product should be handled with appropriate precautions and disposed of as if it were infectious medical waste.

Inability to obtain expected values may indicate product deterioration.

1. **SickleScreen Reaction Vials:** The glass vials contain sodium hydrosulfite. Upon receipt, reagent should be dated and stored at room temperature (15-30 °C). Protect from light and moisture. Reaction vials are stable until expiration date marked on the container. If a vial is opened, it must be used within 12 hours after opening. Once phosphate buffer is added to the reaction vial, the resulting solution must be used within 4 hours.
2. **SickleScreen Phosphate Buffer**: Upon receipt, reagent should be dated and stored at room temperature (15-30 °C). Reagent is stable until expiration date printed on container. When placed in use, write open date on container.

**Performance Parameters:**

Of twenty samples analyzed by hemoglobin electrophoresis, ten were confirmed NA (>1= 95% Hemoglobin A). the remaining 10 were confirmed A/S (37-46% Hemoglobin S). When tested using the SickleScreen Kit, all A/A samples were correctly reported as negative. All A/S samples were correctly reported as positive. Multiple kit lots were used.

***CALIBRATION:***

No calibration is required for this procedure

***QUALITY CONTROL:***

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Sickle-Chex Positive (A/S) and Sickle-Chex Negative controls should be run each time a patient is tested to ensure the proper functioning of the test system.

Results of these controls should be entered into the LIS for documentation purposes.

***PROCEDURE — STEPWISE:***

1. Remove one reaction vial from the storage tray for each patient and control to be tested. Properly label each reaction vial to be used.
2. Gently tap the reaction vial to break up any clumps and to bring all of the sodium hydrosulfite powder to the bottom of the tube.
3. Uncap all reaction vials to be used and place the vials in the reading rack. Open reaction vials are stable for only 12 hours.
4. Using the dispenser cap, squeeze phosphate buffer into each reaction vial to the marked "Fill to this level" on the rack (approximately 4 mLs).
5. Add 50 microliters of well mixed, anti-coagulated whole blood or 3 drops of control into the reaction vial.
6. Cap each reaction vial and invert 10-12 times to thoroughly mix the sample with the reaction vial contents.
7. Let reaction vials incubate at room temperature in the tube reading rack for at least 10 minutes but no more than 20 minutes.
8. At the end of the incubation period observe the reaction vials:
9. **Positive:** If hemoglobin S or any other sickling hemoglobin is present, the solution will be turbid and the lines embossed on the tube reading rack behind the reaction vial will not be visible through the tube's contents.
10. **Negative:** if no sickling hemoglobin is present, the lines will be visible through the reaction tube's contents.

***CALCULATIONS:***

There are no calculations for this procedure.

***REPORTING RESULTS.***

**Reference range:**

Normal for this test is a negative result.

**Procedures For Abnormal Results:**

There are no special procedures for abnormal results; however, this is a screening test only and all positive or questionable results should be further evaluated. Whenever a positive result is reported out in the LIS is it accompanied with the following message:

*“Recommend further testing (i.e. Hemoglobin Electrophoresis).”*

**Reporting Format:**

Enter results into the **LIS** using the following table:

**Table CH70-01**

|  |  |
| --- | --- |
| **RESULT** | **RESULT  MNEMONIC** |
|  |  |
| NEGATIVE | N |
| POSITIVE | P |

***PROCEDURE NOTES:***

1. When the hemoglobin concentration of the sample is 8 g/dl or less, the sample volume should be increased to 100 microliters as severe anemia can cause false negative results.

***LIMITATIONS OF THE PROCEDURE:***

1. Samples from infants less than 6 months old cannot be used with this procedure as high concentrations of hemoglobin F can cause false negative results.
2. Rare hemoglobin variants such as Hemoglobin C Harlem or Hemoglobin C Georgetown can cause a false positive sickling reaction.
3. False positive reactions can also be caused by patients with multiple myeloma , cryoglobulinemia and other dysglobulinemias. This can be remedied by washing the patient's red blood cells in physiologic saline and using this as the test sample.

***REFERENCES****:*

* Pacific Hemostasis Division, SickleScreen Sickling Hemoglobin Screening Kit, Product number 251-050, Fisher Diagnostics., March 1998.

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