TRAINING UPDATE

Lab Location:	SGAH and WAH	**Date Implemented:**	11.9.2012
Department:	Blood Bank	**Due Date:**	11.30.2012

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:
Sickle Cell Screen
Description of change(s):
• Use a MLA PIPETTE to measure the volume of the buffer. Do not "wing it." • Be sure you mix the thoroughly after you add red cells to the buffer. This may require parafilming the tube and inverting several times. • Units that are sickle positive cannot be used for transfusion to neonates or patients with sickle markers, but they CAN be transfused to the general population.

EMPLOYEE SIGNATURES

I have read and understand the procedure described above:

Name	**Signature**	**Date**

Technical SOP

Title	**Sickle Cell Screen**		
Prepared by	Stephanie Codina	Date:	04/28/2011
Owner	Stephanie Codina	Date:	04/28/2011

Laboratory Approval	**Local Effective Date:**		
Print Name and Title	**Signature**		**Date**
Refer to the electronic signature page for approval and approval dates.			

Annual Review			
Print Name	**Signature**		**Date**

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1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Sickle Cell Screen	Manual	BSCKL

Synonyms/Abbreviations
Sickledex, Sickle Cell Anemia Screen, Sickle Cell Hemoglobin Screen, Dithionite Test, Sickle Cell Anemia, Sickle Test, Hemoglobin S Test, Hemoglobin S Screen

Department
Blood Bank

2. ANALYTICAL PRINCIPLE

Sickle cell disease is an inherited condition characterized by the presence of hemoglobin S (Hb-S). Hb-S exists in a homozygous state (S/S) known as sickle cell anemia or in a heterozygous state (A/S) known as sickle cell trait.

Deoxygenated Hb-S is insoluble in the presence of a concentrated phosphate buffer solution and forms a turbid suspension that can be easily visualized. Normal hemoglobin A and other hemoglobins remain in solution under these conditions. These different qualitative outcomes allow for the detection of sickle cell disease and its traits.

This test uses Saponin to lyse the red blood cells. Sodium hydrosulfite then reduces the released hemoglobin. Reduced Hb-S is insoluble in the concentrated phosphate buffer and forms a cloudy, turbid suspension. Other sickling hemoglobin subtypes may also give a positive result.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	None
Specimen Collection and/or Timing	None
Special Collection Procedures	Clotted blood cannot be used.

3.2 Specimen Type & Handling

Criteria		
Type -Preferred	Whole blood (EDTA) Blood blood (donor segments) mixed with ACD, CPD, CPDA-1 anticoagulants. Red blood cell units with additive solutions can be used.	
-Other Acceptable	Whole blood (Heparin, Citrate) Hemolyzed blood	
Collection Container	Lavender top tube (EDTA) Donor segments from red blood cell units	
Volume - Optimum **- Minimum**	3mL 20µL	
Transport Container and Temperature	Same as above, at room temperature	
Stability & Storage Requirements	Room Temperature:	24 hours
	Refrigerated:	<45 days
	Frozen:	Unacceptable

Criteria	
Timing Considerations	Test patient specimens as soon as possible following collection. Donor segments may be tested up to the expiration date of the unit.
Unacceptable Specimens & Actions to Take	Frozen, Incomplete or incorrect labeling – refer to procedure "Sample Specifications for Blood Bank Testing" for details.
Compromising Physical Characteristics	Refer to section 14.
Other Considerations	None

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
SICKLEDEX test	Streck, Cat.#217657

4.2 Reagent Preparation and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.

Reagent A	SICKLEDEX Reagent Powder, 1 vial
Reagent B	SICKLEDEX Solubility Buffer, 1 bottle
Preparation	Bring buffer and reagent powder to room temperature before mixing. Add contest of one vial of SICKLEDEX reagent powder (reagent A) to one bottle of SICKLEDEX solubility buffer (reagent B). Cap the bottle. Shake vigorously to dissolve the reagent powder completely.
Storage	2-10°C until expiration date on label of reconstituted reagent.
Stability	45 days when refrigerated.
Special Handling	Working solution must be at room temperature (18-30°C) prior to testing.

5. CALIBRATORS/STANDARDS

N/A

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Sickle-Trol Sickle-Cell Hemoglobin Controls A/S and A/A erythrocytes	Dade Behring, Inc. B4585-10

6.2 Control Preparation and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation.

Control Name	Sickle-Trol Sickle-Cell Hemoglobin Control A/S erythrocytes (positive)	Sickle-Trol Sickle-Cell Hemoglobin Control A/A erythrocytes (negative)
Contents	Packed red blood cells	Packed red blood cells
Preparation	Remove controls from refrigerator and allow to warm at room temperature for 15 minutes. Immediately prior to use, resuspend the cells by rolling the vial horizontally between the palms of the hands for 20-30 seconds. Ensure all cells are suspended. Do not shake.	
Storage & Stability	Store at 2-8°C when not in use. When stored properly, the product is stable for 100 days from the open date or the expiration date, whichever is sooner.	
Frequency	Assay once with each run.	Assay once with each run.
Current Ranges	Positive	Negative
Corrective Action	If any control value is unacceptable, DO NOT REPORT PATIENT RESULTS. Consult a supervisor.	

6.5 Review Patient Data

N/A

6.6 Documentation

Record quality control results each day the assay is performed.

6.7 Quality Assurance Program

The laboratory participates in CAP proficiency testing.
Training and competency testing must be established for all technical employees performing this assay.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

N/A

7.2 Equipment

N/A

7.3 Supplies

- 12 x 75 mm test tubes
- Test tube rack with lines
- 2.0 mL calibrated MLA pipette
- 20 µL calibrated MLA pipette
- 10 µL calibrated MLA pipette
- Centrifuge
- Timer

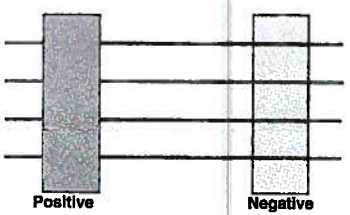
8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

Step	Action
1	Confirm specimen acceptability and specimen labeling per procedure, "Sample Specifications for Blood Bank Testing."
2	Perform a history check on the patient to determine if recent transfusion has occurred. False positive and false negative results may occur in patients who have had recent blood transfusion.

Step	Action
3	If units are being tested, select a segment from each unit that is otherwise suitable for crossmatch to the patient (antigen negative, meets transfusion specifications, etc). Label the test tube holding the segment with the full unit number. Refer to procedure, "Red Blood Cell Transfusion in Sickle Cell Disease."
4	Remove the buffer and controls from the refrigerator.
5	Obtain a "Sickle Cell Screen Testing Worksheet" and document the following on the worksheet: A. Date of testing B. Tech identification C. Patient name D. Patient medical record number E. Unit number (write "NA" if no units are tested) F. Positive control lot number and expiration date G. Negative control lot number and expiration date H. Buffer lot number and expiration date
6	Label a test tube for each specimen to be tested. A. Labeling standards are detailed in the policy "Sample Specifications for Blood Bank Testing." B. Label one test tube for the positive control. C. Label one test tube for the negative control. D. Label one test tube for each patient specimen and/or donor unit to be tested. a. Patient identifiers include the patient's first and last initials or the first 3 letters of the patient's last name. b. Unit identifiers include the last 3 digits of the unit number. c. Additional identifiers must be used if needed to differentiate between patients or units.
7	Dispense 2.0 mL of cold working SICKLEDEX Solubility Buffer into a clean, labeled 12 x 75 mm test tube. A. Return the working solubility buffer to the refrigerator immediately after use. B. Allow the test tubes containing the working solution to warm to room temperature. **The use of reagents below room temperature can yield false results.**

Step	Action
8	Once the working solution and controls have warmed to room temperature, add red blood cells to each appropriately labeled tube. A. Add 20µL of whole blood **or** 10µL of packed red blood cells from the test specimen or unit to the corresponding tube. B. Add 1 drop of positive control to the corresponding tube. C. Add 1 drop of negative control to the corresponding tube. D. If the test specimen has a hematocrit <15%, centrifuge the sample for 5-10 minutes at 1200 rpm then pipette 10µL of packed red blood cells to the corresponding test tube.
9	Mix the contents of each test tube **thoroughly** by swirling the tube several times or by capping and inverting the tube. Then, place the test tube in the test tube rack with lines.
10	Allow the samples to rest at room temperature (18-30°C) for 15 minutes. Set a timer. The maximum time in which tests can incubate is 60 minutes.
11	Read the reaction macroscopically by look through the tube at the ruled lines on the test tube rack.  A. Interpret as positive if the lines on the test tube rack are not visible through the solution. B. Interpret as negative if the lines on the test tube rack are clearly visible through the solution. C. Interpret as inconclusive if the lines on the test tube rack are vaguely visible through the solution. Note: Units that test positive using the sickle screen test should not be transfused to neonates or patients with a sickle cell marker. Positive units are acceptable for routine transfusion.
12	Document the result interpretation of each sample and control on the on the "Sickle Cell Screen Testing Worksheet."

9. CALCULATIONS

N/A

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

Positive = The lines are not visible through the solution.

Negative = The solution is clear so the lines are easily visible through the solution.

Inconclusive = The solution is cloudy and the lines are vaguely visible through the solution.

10.2 Rounding

N/A

10.3 Units of Measure

N/A

10.4 Clinical Reportable Range (CRR)

N/A

10.5 Repeat Criteria and Resulting

N/A

11. EXPECTED VALUES

11.1 Reference Ranges

Negative

Note: This test is unlikely to be positive until the patient is >6 months in age when Hg-S is present in sufficient quantities (>25%) for a positive screening test. Hemoglobinopathy evaluation is recommended for children less than 6 months old.

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

Sickling disorders are caused by the homozygous form of the sickle cell gene (sickle cell anemia), the heterozygous form of the sickle cell gene (sickle cell trait), and the combination of either with other structural hemoglobin variants or thalassemias.

Sickle hemoglobin (Hb-S) results when valine is substituted for the normally occurring glutamine residue at position 6 in the β change and intracellular crystals of deoxygenated Hb-S form, causing the red blood cell to sickle.

Hb-S is not the only hemoglobin that causes RBCs to sickle. RBCs containing $HBC_{Georgetown}$, HbI, and $Hb_{Bart's}$ also sickle. In America and Africa, Hb-S is the most common hemoglobin varian, with an incidence of the heterozygous form of approximately 8% in American blacks and 30% in African blacks.

Sickle testing of donor red blood cell units is performed to identify which units are negative for the sickle cell trait to reduce the amount of Hb-S in transfusion recipients.

13. PROCEDURE NOTES

- **FDA Status:** Approved/cleared
- **Validated Test Modifications:** None

14. LIMITATIONS OF METHOD

1	False positive results may occur in patients with erythrocytosis, hyperglobulinemia, extre leukocytosis, or hyperlipidemia. Coarse flocculation may occur in these samples due to elevated levels of total serum protein. These patient samples may be washed in normal physiologic saline, centrifuged, and 10µL of the packed cell used for testing.
2	False positives and false negatives may occur in patients with severe anemia (≤15% hematocrit).
3	False negatives may occur in infants <6 months in age due to elevated levels of hemoglobin F.
4	False positives or false negatives may occur in patients with a recent blood transfusion.
5	Positive results may occur in patients with some rare sickling hemoglobin subtypes such as Hemoglobin C Harlem or Hemoglobin C Georgetown.
6	SICKLEDEX is a qualitative screening procedure and does not differentiate between sickle cell disease (S/S) and sickle cell trait (A/S). All positive test results should be further evaluated by hemoglobin electrophoresis, when used for patient testing. This does not apply to donor screening tests.

15. SAFETY

You, the employee, have direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental, Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.
- Warnings of other specific hazards are noted in this procedure. Please comply with the requirements to reduce your risk of injury."

Report all accidents and injuries to your supervisor or the Environmental, Health and Safety Coordinator.

16. RELATED DOCUMENTS

SOP: Red Blood Cell Transfusion in Sickle Cell Disease
SOP: Sample Specifications for Blood Bank Testing
Form: Sickle Cell Screen Testing Worksheet
Current package insert for SICKLEDEX

17. REFERENCES

1. Roback, J.D., Combs, M.R., Grossman, B.J., Hillyer, C.D. 2008. Technical Manual of the AABB, 16th ed. AABB Publishing, Bethesda, Maryland.
2. Standards for Blood Banks and Transfusion Services, 2011. AABB, 27th ed. AABB Publishing, Bethesda, Maryland.
3. Package Insert for SICKLEDEX, Streck, Omaha, NE. Insert Code 350430-14, 02/2010.
4. Package Insert for Sickle-Chex, Streck, Omaha, NE. Insert Code 350413-10, 09/2009.

18. REVISION HISTORY

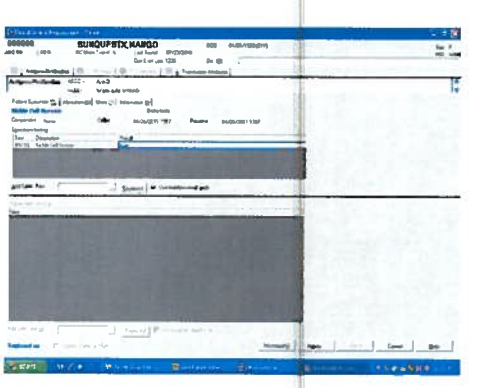
Version	Date	Section	Reason	Reviser		Approval
			Supersedes WAB007.000, SHB.007.001, SHB.011.001			
000	11.6.12	8	Updated wording in step 9 to reinforce the idea that the specimen must be mixed thoroughly. Added note that sickle units should not be used for transfusion to neonates or sickle patients but can be used for general transfusion	SCodina		NCacciabeve

19. ADDENDA

Appendix A: LIS Entry of Patient Sickle Testing
Appendix B: Billing of Unit Sickle Testing

Appendix A

LIS Entry of Patient Sickle Testing

Step	Action
1	Access SunQuest function "Blood Order Processing."
2	In the "Lookup by" prompt, click on the dropdown menu and select "Patient ID."
3	In the "Value" prompt, type the patient's medical record number and click on the "Search" button.
4	If more than one patient appears, select the correct patient by clicking on the name.
5	Click on the "Search All" button.
6	Click on the sample with the correct accession number.
7	Select the "BSCKL" test.
8	Result the test using one of the following: A. "P" for positive sickle screen B. "N" for negative sickle screen C. "I" for inconclusive sickle screen 
9	Click the "Save" button.

Appendix B

Billing of Unit Sickle Testing

Step	Action
1	Access SunQuest function "Blood Order Processing."
2	In the "Lookup by" prompt, click on the dropdown menu and select "Patient ID."
3	In the "Value" prompt, type the patient's medical record number and click on the "Search" button.
4	If more than one patient appears, select the correct patient by clicking on the name.
5	Click on the "Search All" button.
6	Click on the sample that corresponds to the crossmatch for which the units are being tested.
7	In the "Add Unit Test" field, type ";SCS" and press the "Tab" key.
8	If more than one unit is allocated to the patient, the LIS will prompt "Do you want this test added to all units in this order?" A. Select "Yes" if all units were tested. B. Select "No" if only some units were tested.
9	Click the "Save" button.
10	Note: Units are resulted per procedure "Antigen Typing." A. NSIK = negative sickle screen on a unit B. PSIK = positive sickle screen on a unit