TRAINING UPDATE

Lab Location:	SGMC and WAH	**Date Implemented:**	11.18.2016
Department:	Blood Bank	**Due Date:**	12.15.2016

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Sickle Cell Screen

Description of change(s):

Manufacturer's instructions were updated; changes are reflected in the SOP:

1. Sickle controls must be mixed thoroughly prior to use. Instructions for mixing were added to the procedure.

2. The manufacturer's instructions state sickle screens can be read after 6 minutes. HOWEVER, Streck states "not all positives will show after 6 minutes." The procedure was updated to indicate we can call positives after 6 minutes, but negatives must be incubated for 15 minutes.

Electronic Document Control System

Document No.: SGAH.BB944[0]
Title: Sickle Cell Screen
Owner: LESLIE BARRETT
Status INWORKS
Effective Date: 17-Dec-2016

Next Review Date:

Non-Technical SOP

Title	**Sickle Cell Screen**
Prepared by	Stephanie Codina Date: 11.16.2016
Owner	Stephanie Codina Date: 11.16.2016

Laboratory Approval

Print Name and Title	Signature	Date
Refer to the electronic signature page for approval and approval dates.		

Local Issue Date: Local Effective Date:

Review:

Print Name	Signature	Date

Form revised 3.1.00

TABLE OF CONTENTS

1. PURPOSE

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.

2. SCOPE

This procedure applies to any patient sample or donor unit for which sickle screening is ordered or needed per protocol.

3. RESPONSIBILITY

All blood bank staff members must understand and adhere to this procedure for performing sickle screens.

4. DEFINITIONS

N/A

5. SPECIMEN

Criteria	
Type -Preferred	Whole blood (EDTA) Red cells (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	3mL
- Minimum	20µL
Transport Container and Temperature	Same as above, at room temperature
Stability & Storage Requirements	Room Temperature: 24 hours
	Refrigerated: <45 days
	Frozen: Unacceptable
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.

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

6. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used.

6.1 Reagent Summary

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

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6.2 Reagent Preparation and Storage

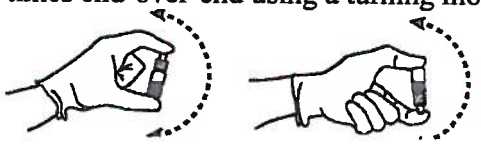
Assay Kit	
Reagent a	SICKLEDEX Reagent Powder, 1 vial
Reagent b	SICKLEDEX Solubility Buffer, 1 bottle
Container	SICKLEDEX solubility buffer bottle provided in kit.
Storage	2-10°C until expiration date on label of reconstituted reagent.
Stability	45 days after reconstitution when stored refrigerated and tightly capped.
Preparation	Bring buffer and reagent powder to room temperature before mixing. Add contents 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. Working solution must be at room temperature (18-30°C) prior to testing.

7. QUALITY CONTROL

7.1 Controls Used

Controls	Supplier and Catalog Number
Sickle-Chex	Streck, Cat. #217654

7.2 Control Preparation and Storage

Control	Sickle-Chex positive and negative controls
Preparation	Remove control vials from refrigerator and allow to warm at room temperature (18-30°C) for 15 minutes. To mix (do not mix mechanically or vortex): A. Hold the vial vertically and roll each vial between the palms of the hands for 20-30 seconds.  B. Continue to mix by holding the vial by the ends between the thumb and finger and rapidly inverting the vial 20 times end-over-end using a turning motion of the wrist.  C. Visually inspect the bottom of the vial to ensure all cells are resuspended and sample immediately after mixing. D. Visually inspect the vial for signs of deterioration or darkly colored supernatant. Do not use product that appears abnormal.

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.

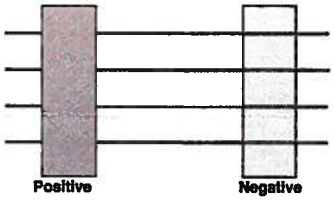
8. PROCEDURE

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.

All parts of this kit should be used together. Reagents from different boxes should not be mixed.

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. Verify the hematocrit of the sample. False results can occur when the hematocrit is less than 15%.
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. Accession Number F. Unit numbers to be tested G. Positive control lot number and expiration date H. Negative control lot number and expiration date I. Buffer lot number and expiration date

Step	Action
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	Pipette 2.0 mL of cold working SICKLEDEX Solubility Buffer into each 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.**
8	Once the working solution and controls have warmed to room temperature, add red blood cells to each appropriately labeled tube. Be sure to mix the controls per section 7.2 of this procedure. 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 or negative control to the corresponding tube. For accurate delivery volume, the control vial **must** be inverted and held vertically directly over the test 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.

Step	Action
10	Allow the samples to rest at room temperature (18-30°C) for at least 6 minutes. Set a timer. The maximum time in which tests can incubate is 60 minutes. Note: Not all positive reactions will be visible after 6 minutes. Negatives should be incubated for a minimum of 15 minutes prior to interpreting.
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 worksheet.
13	Enter the sickle screen results in the LIS for the patient or unit tested per appendices A and B.
14	Manually bill for sickle testing performed on units per appendix C. Document the accession number to which the sickle testing was added on the worksheet.
15	Add the sickle results to the ISBT labels for all negative units per appendix D.
16	Place the worksheet in the designated bin for second tech review. A different tech must perform the following within 2 shifts of testing: A. Verify that all reagents are within the designated expiration date. B. Verify that control results are acceptable. a. The positive control must yield a positive result. b. The negative control must yield a negative result. C. Verify that billing for donor units is performed accurately. D. Verify that sickle results were entered correctly for each patient and donor unit. Second tech review is documented on the worksheet.

9. REPORTING RESULTS

Results are reported as positive, negative, or inconclusive based on whether or not the lines are visible through the solution as outlined in the procedure.

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.

10. LIMITATIONS OF METHOD

A. False positive results may occur in patients with erythrocytosis, hyperglobulinemia, extreme 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.
B. False positives and false negatives may occur in patients with severe anemia (≤15% hematocrit).
C. False negatives may occur in infants <6 months in age due to elevated levels of hemoglobin F.
D. False positives or false negatives may occur in patients with a recent blood transfusion.
E. Positive results may occur in patients with some rare sickling hemoglobin subtypes such as Hemoglobin C Harlem or Hemoglobin C Georgetown.
F. 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.

11. RELATED DOCUMENTS

SOP: Red Blood Cell Transfusion in Sickle Cell Disease
SOP: Sample Specifications for Blood Bank Testing
Form: Sickle Cell Screen Testing Worksheet (AG.F102)

12. REFERENCES

A. Fung, MK, Grossman, BJ, Hillyer, CD, and Westoff, CM. 2014. Technical Manual of the AABB, 18th ed. AABB Publishing, Bethesda, Maryland.
B. Standards for Blood Banks and Transfusion Services, 30th ed. 2016. AABB Publishing, Bethesda, Maryland
C. Sickle-Chex Instructions for Use, Streck, La Vista, NE. Insert Code 350413-12, 06/2016.
D. Sickledex Instructions for Use, Streck, Omaha, NE. Insert Code 350430-19, 06/2015.

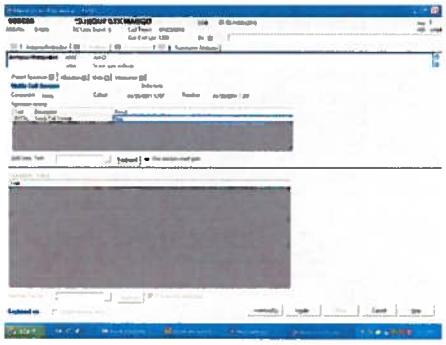
13. REVISION HISTORY

Version	Date	Reason for Revision	Revised By	Approved By
		Supersedes SGAH.BB105.3, WAH.BB98.3		

14. ADDENDA AND APPENDICES

Appendix A: LIS Entry of Patient Sickle Testing
Appendix B: LIS Entry of Unit Sickle Testing
Appendix C: Billing of Unit Sickle Testing
Appendix D: Adding the "Hb S Negative" Comment to the Blood Product Label

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.

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Appendix B
LIS Entry of Unit Sickle Testing

Step	Action
1	Access Sunquest function, "Blood Product Testing."
2	At the "Unit #" prompt, scan the unit number of the unit to be resulted.
3	At the "Component" prompt, scan the E code for the product testing. This will autofill both the "component" and "division" fields.
4	Click the "Add" button.
5	Repeat steps 2-4 for each additional unit to be resulted.
6	When all units have been selected, click the "Continue" button.
7	The first unit will move to the right-hand side of the screen. Verify the unit number at the top of the screen for accuracy before proceeding.
8	In the "Test" column, type ";AO" then press the tab key.
9	The pop-up message, "Confirm adding test: AO" will appear. Click the "Yes" button.
10	The description "Ag/Aby Info (on units)" will appear.
11	In the "Result" column, type the interpretation of the unit sickle test then press the "tab" key. A. Type ";HBSP" for Hemoglobin S positive. B. Type ";HBSN" for Hemoglobin S negative.
12	Click the "Save" button.
13	If additional units need to be resulted, click the "Continue" button and perform steps 7-12.

Appendix C
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.

Appendix D
Adding the "Hb S Negative" Comment to the Blood Product Label

Step	Action
1	Note: This procedure is ONLY performed for Hb S negative units. Document Hb S positive units using the antigen typing tag.
2	Access Sunquest function, "Blood Product Entry."
3	Click the "Modify Unit" button in the lower, left-hand corner of the screen.
4	At the "Unit #" prompt, scan the unit number for which you want add the HbS negative comment.
5	At the "Component" prompt, scan the product code for the unit for which you want to add the HbS negative comment. Scanning the product code will autofill the component and division fields.
6	Click the "OK" button.
7	Click to open the "Ag/Ab/Attributes" tab. At the "Attribute" prompt, A. If the unit is NOT CMV-negative, type "HBSN" or scan the "HbS-negative" barcode. B. If the unit IS CMV-negative, remove the "CMVN" marker and type "CMVNHBS" or scan the "CMV seronegative HbS negative" barcode. This step will avoid the QA failure, "patient/unit ag/ab incompatibility."
8	Click to open the "ISBT Fields" tab. At the "Special Test" prompt, A. If the unit is NOT CMV-negative, type "HBSN" or scan the "HbS-negative" barcode. B. If the unit IS CMV-negative, remove the "CMVN" marker and type "CMVNHBS" or scan the "CMV seronegative HbS negative" barcode. Note: Sunquest will ONLY print the last comment entered. Example: If the unit is CMV-negative and the Hb S negative comment is added, Sunquest will only print the Hb S negative comment. The tech must ensure the correct comment code is being scanned.
9	Click the "Save" button.
10	Click the "Exit" button and access Sunquest function, "BB Label Print."
11	At the "Unit #" prompt, scan the unit number for which you want add the HbS negative comment.
12	At the "Component" prompt, scan the product code for the unit for which you want to add the HbS negative comment. Scanning the product code will autofill the component and division fields.
13	Click the "Add" button.
14	Click the "Print" button.
15	A new label will print with the "Hemoglobin S negative" comment. Adhere the label to the unit. Document the label update by putting a check mark "√" in the "Label Updated" box on the worksheet.

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Electronic Document Control System

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Title: Sickle Cell Screen Testing Worksheet
Owner: LESLIE BARRETT
Status RELEASED
Effective Date: 26-Oct-2016 **Next Review Date:** 26-Oct-2018
Implementation Date: 10/26/16 6:39 am

Review

REVIEW: **DEFAULT DOCUMENT**

Approver	Status	Sign-off Date
NICOLAS CACCIABEVE	APPROVED	10/24/16 12:56 pm
STEPHANIE L CODINA	APPROVED	10/19/16 6:20 am

Quest Diagnostics

Shady Grove Medical Center
Washington Adventist Hospital

Sickle Cell Screen Testing Worksheet

	Date	
	Tech	
	Patient Name	
	Patient MRN	
	Accession Number	
Controls	Positive Control Lot Number	
Controls	Positive Control Expiration Date	
Controls	Negative Control Lot Number	
Controls	Negative Control Expiration Date	
Buffer	Buffer Lot Number	
Buffer	Buffer Expiration Date	

Specimen Identification	Result	Label Updated?
Patient		
Positive Control		
Negative Control		

Reagents within expiration? Y or N
Controls acceptable? Y or N
Unit sickle charges (SCS):
Data entry reviewed by:

	Date	
	Tech	
	Patient Name	
	Patient MRN	
	Accession Number	
Controls	Positive Control Lot Number	
Controls	Positive Control Expiration Date	
Controls	Negative Control Lot Number	
Controls	Negative Control Expiration Date	
Buffer	Buffer Lot Number	
Buffer	Buffer Expiration Date	

Specimen Identification	Result	Label Updated?
Patient		
Positive Control		
Negative Control		

Reagents within expiration? Y or N
Controls acceptable? Y or N
Unit sickle charges (SCS):
Data entry reviewed by:

Reviewed by: _____