TRAINING UPDATE

Lab Location:	SGAH and WAH	**Date Implemented:**	10.20.2014
Department:	Blood Bank	**Due Date:**	10.31.2014

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Sickle Cell Screen and Worksheet

Description of change(s):

1. Due to a recent FDA-reportable event, the sickle cell worksheet has been redesigned. The new sheet is similar to the antigen typing form. Reagents, controls, and testing of both patients and units will be documented on the form. Once complete the form will go into the bin for second tech review.

2. The following were also added to the form:

    a. Check box to indicate the blood product label was updated to reflect the HbS-negative status
    b. Billing of units
    c. Review of controls
    d. Review that reagents were used within expiration

3. The procedure has been updated to reflect the new form.

4. The LIS instructions were updated for clarity (content did not change)

Electronic Document Control System

Quest Diagnostics

Document No.: AG.F102[2]
Title: SICKLE CELL SCREEN TESTING WORKSHEET
Owner: LESLIE.X.BARRETT LESLIE BARRETT
Status INWORKS
Effective Date: 15-Nov-2014 **Next Review Date:**

Quest Diagnostics

Shady Grove Adventist Hospital
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: _____

Electronic Document Control System

Quest Diagnostics

Document No.: SGAH.BB105[3]
Title: SICKLE CELL SCREEN
Owner: LESLIE.X.BARRETT LESLIE BARRETT
Status INWORKS
Effective Date: 15-Nov-2014 **Next Review 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.		

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

TABLE OF CONTENTS

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

Form revised 10/31/02

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 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.
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

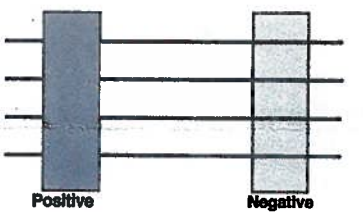
8. PROCEDURE

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. 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
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 worksheet.

Step	Action
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. 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. 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 variant, 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, 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.
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

The employee has 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.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

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 (AG.F102)
Current package insert for SICKLEDEX

17. REFERENCES

1. Fung, Mark K., Grossman, B.J., Hillyer, C.D., and Westhoff, C.M. 2014. Technical Manual of the AABB, 18th ed. AABB Publishing, Bethesda, Maryland.
2. Standards for Blood Banks and Transfusion Services, 2014. AABB, 29th 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.

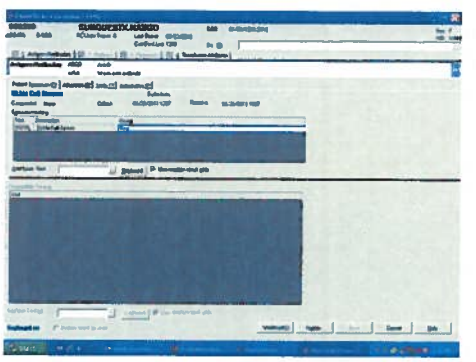
18. REVISION HISTORY

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
001	6.24.2014	19	Updated/added appendices to reflect new Sunquest process for resulting units and printing the HbS neg comment on blood product labels.	SCodina	NCacciabeve
		Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	LBarrett	
2	10.14.14	8	Updated worksheet; updated SOP to reflect addition of second tech review and changes to worksheet.	SCodina	NCacciabeve
		App D	Added step to add the HBSN special testing of units to the LIS.		

19. ADDENDA

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.

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.