University of Washington Medical Center 1959 NE Pacific Street. Seattle, WA 98195 Transfusion Services Laboratory Policies and Procedures Manual	Original Effective Date: 03-11-2016 Revision Effective Date: 06-07-2022~~07-26-2021~~	Number: PC-0036.043

TITLE: Testing and Provision of Hemoglobin S Negative Blood

PURPOSE:
To provide instructions for performing sickle cell testing on red blood cell components using the SickleDex kit and controls and provide hemoglobin S negative components

PRINCIPLE & CLINICAL SIGNIFICANCE:
Principle
SickleDex is a qualitative solubility test kit used to detect the presence of sickling hemoglobins (HbS) in blood or control material. *SickleDex* uses saponin to lyse the red blood cells. Sodium Hydrosulfite then reduces the released hemoglobin. Reduced HbS is insoluble in the concentrated phosphate buffer and forms a cloudy, turbid suspension.

Clinical Significance
Transfusion of units negative for hemoglobin S reduces tissue hypoxia in patients with Sickle Cells Disease (SCD) and reduces the risk of cellular breakdown from the hemoglobin S positive RBCs in neonates

POLICIES:
- HbS negative red blood cells components shall be provided to patients for the following:
  - Sickle cell disease, sickle cell trait and thalassemia, Diamond Black-fan anemia
  - Red blood cell exchanges (adult and neonatal)
  - Children < 4 months old and intrauterine transfusions
- ~~SCD patients~~Patients needing HbS negative red cell components must be flagged in Sunquest with the NHBS attribute to prevent release of untested units. ~~Refer to SOP: *Blood Administrative Data Entry Updates*~~

SPECIMEN REQUIREMENTS:
Blood from donor segments stored at 1-6°C for up to 45 days

REAGENTS/SUPPLIES/EQUIPMENT:

Reagents:	Supplies:	Equipment:
• SickleDex Kit • Sickle-Chex Controls	• 12 x 75 mm test tubes • Adjustable pipette • Disposable pipette tips • Blood bank transfer pipettes	Test tube rack with lines

QUALITY CONTROL:
Quality control is performed with each batch of testing

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INSTRUCTIONS:

TABLE OF CONTENTS:

Working Solubility Buffer Preparation
SickleDex Testing

Working Solubility Buffer Preparation

STEP	ACTION
1	Bring buffer and reagent powder from the SickleDex Kit to room temperature before mixing
2	Add contents of one vial of SickleDex Reagent Powder to one bottle of SickleDex Solubility Buffer
3	Place a white dispenser cap on the bottle of working solubility buffer
4	Agitate the buffer vigorously until the reagent powder is dissolved
5	Label the buffer container with the following after reconstitution: • Reconstitution date • 45-day expiration date • Tech ID

SickleDex Testing

STEP	ACTION
1	Label 12 x 75 mm tubes for each donor unit to be tested, 1 positive control and 1 negative control
2	Allow controls ~~and buffer~~ to warm to room temperature (18°C to 30°C) for approximately 15 minutes before use
3	Place tubes in testing rack and fill each tube with working SickleDex Solubility Buffer to the red line on rack (approximately 2 mL) **NOTE:** Allow tubes with buffer to sit at room temp for atleast 10minutes. Return buffer to refrigerator immediately after use
4	Mix controls by holding vertically between hands and rolling the vials back and forth for 20-30 seconds followed by inverting end over-end 20 times. **NOTE:** Visually inspect the bottom of the vial to ensure all cells are suspended
5	Add 1 drop of each control into the appropriate labeled control tube **NOTE:** The control MUST be inverted and held vertically directly over the test tube to ensure accurate delivery. Wipe threads on each control before returning cap, if necessary
6	Add 10~~20~~uL of ~~whole blood~~packed cells and swirl the contents to mix **NOTE:** If donor segment ~~does not appear~~appears to contain whole blood, ~~(due to settling prior to sealing),~~ use 2~~1~~0uL of ~~packed red blood cells for testing~~whole blood for testing

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STEP	ACTION
7	Allow tubes to stand in the testing rack at room temperature for at least 6 minutes and no longer than 60 minutes
8	Read reaction macroscopically by looking through the test tubes at black lines on the back of the testing rack If result read betweenat 6-60mins is: Negative → Acceptable to report if result is clearly negative **Note:** If reading at 6 minutes is indeterminate, incubation can be extended up to 60 minutes ~~Incubate sample for a total of 60 mins to confirm negative result~~ Positive → Further incubation is not required
9	Go to section Result Reporting

CALCULATIONS/INTERPRETATIONS/RESULTS REPORTING/NORMAL VALUES/CRITICAL VALUES

Interpretation:

If black tube rack lines are	Interpret as
CLEARLY VISIBLE thru a transparent suspension	Negative
NOT VISIBLE thru a cloudy, turbid suspension	Positive

Results Reporting in Sunquest

STEP	ACTION
1	Open 'Blood Order Processing' function
2	Assign unit to patient using 'Blood Inventory Search' or by scanning unit barcode
3	QA Warning window will open with warning message about missing NHBS attribute on unit • Check the box on the left to acknowledge QA Warning for each unit Acknowledge | Unit | Comp Type | Div # | Warning message | Have authority to override? ☐ | W141605200006 | E0382 | 00 | Patient/unit attribute incompatibility Patient's Attribute(s): NHBS Attribute(s) Missing on Unit: NHBS | Yes
4	• Order a sickle cell test on the allocated unit • Enter **;SCKL** in the 'Add unit test' box at the bottom of the screen
5	Enter the reactions according to the following tables: SKP: SCKL + SKN: SCKL - SKU: SCKL + or -

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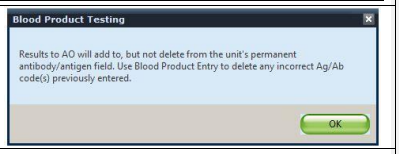
	SKP = Sickle Positive Control; SKN = Sickle Negative Control; SKU = Unit Tested
6	Enter the Interpretation in the interpretation grid: **Interpretation** / **SQ Result** / **Sunquest Hot Key** Positive / POSHBS / s Negative / NGHBS / n
7	• Click <Save> • Override QA Failure by adding a 'TDONE' comment • In the free text box, add 'Sickle Cell Testing' • Click <OK> to acknowledge unit failure on the Electronic Crossmatch Eligibility Report
8	Open 'Blood Product Testing' function
9	• Scan unit information • Click <Continue>
10	Order '**;AO**' in the 'Unit Testing' field If SCKL testing is: Then POSITIVE: Result with **;POSHBS** NEGATIVE: Result with **;NHBS**
11	Click <OK> to acknowledge the Blood Product Testing Comment 
12	Click <Save> and <Exit>
13	Go to 'Blood Order Processing' If SCKL testing on unit is: Then NEGATIVE: Go to next step POSITIVE: In the Compatibility Testing area • Uncheck 'Use reaction grid results' box • Enter '**;ND**' in the 'XM' field • Enter '**]**' in the 'TS' field for 'NOK' • Go to step 15 **NOTE:** Unit will automatically be released from patient
14	Enter comment code on unit to display the status on the Transfusion Record • Enter; CM in the 'Add unit test' box at the bottom of the screen • Enter the following code in the CM box; NHBS
15	**If performing** / **Then** Electronic crossmatch: Click <Save> Serologic crossmatch (adult): Enter results according to appropriate Crossmatch SOP then click <Save> TNRBC (Neonate crossmatch): Answer 'TS' Box Click <Save>

16	Continue to Blood Product Issue if issuing unit

CALIBRATION:
NA

PROCEDURE NOTES AND LIMITATIONS:
- Sickle –Chex controls expire 100 days after open or manufacturer's expiration, whichever date is shorter
- Donors with Hgb S concentrations less than 30.9% may not be detected
- See manufacturers insert for additional limitations on use of reagents

REFERENCES:
SickleDex Manufacturer Insert. Omaha, Nebraska: Streck; current version
Sickle-Chek Manufacturer Insert. Omaha, Nebraska: Streck; current version

RELATED DOCUMENTS:

APPENDIX:
NA

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UWMC SOP Approval:			
UWMC CLIA Medical Director	Andrew Bryan, MD	Date	
Transfusion Service Manager	Nina Sen	Date	
QA Manager	Tayler Reeves	Date	
Transfusion Service Medical Director	Monica Pagano, MD	Date	
UWMC Biennial Review:			
		Date	
		Date	

REVISION HISTORY:
04/16/18: Updated instructions for mixing controls to match revision to package insert.

06/29/2021: Added incubating negative results for 60 minutes per change in package insert and policy for providing HbS negative RBCs to Diamond-Blackfan anemia patients
05/02/2022: Updated to remove 60 min incubation requirement per manufacturer