Status **Scheduled** PolicyStat ID **11703647**

Beaumont

Origination	6/26/2022
Last Approved	5/27/2022
Effective	6/26/2022
Last Revised	5/27/2022
Next Review	5/26/2024

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Area	Laboratory-Blood Bank
Applicability	All Beaumont Hospitals

Hemoglobin S Testing of Donor Units

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document will provide policies and procedural steps that will be used to test segments from donor red blood cell (RBC) units for hemoglobin S (Hb-S).

II. PRINCIPLE:

SICKLEDEX® is a qualitative solubility test for testing for the presence of sickling hemoglobins in human blood. SICKLEDEX® uses Saponin to lyse the test RBCs and release the hemoglobin. Sodium hydrosulfite then reduces the released hemoglobin. Reduced Hb-S is insoluble in the 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 visual detection of sickle cell disease and its traits.

III. SCOPE:

A. Patient samples are not eligible for Hb-S testing as described in this document.

B. Hb-S testing of donor units at Grosse Pointe are performed in Grosse Pointe Hematology. The appropriate results are appended in the Blood Bank computer as described below.

C. Hb-S testing of donor units for Taylor, Trenton and Wayne are referred to Dearborn or Royal Oak Blood Banks for testing as described in this document.

IV. GENERAL INFORMATION:

RBCs from donor samples may be tested for Hb-S in the following situations:

A. All neonatal patients that require transfusion shall receive Hb-S negative RBCs.

B. Any known sickle cell disease or thalassemia patient shall receive Hb-S negative RBCs as described in Transfusion Medicine policy, Policies Specific to Patients with Sickle Cell Disease and Thalassemia. For example:

    1. Sickle cell anemia, the homozygous state of HbSS.
    2. Sickle cell β -thalassemia, the compound heterozygous state of HbS and β -thalassemia.
    3. Sickle cell hemoglobin C (HbSC), the compound heterozygous state of HbS and hemoglobin C.
    4. α -thalassemia or β -thalassemia, major or minor.

C. Any donor unit that is to be stored frozen must be tested and confirmed Hb-S negative before freezing. Refer to Transfusion Medicine policy, *Freezing Red Blood Cells*.

V. POLICIES:

A. All reagents, controls, and donor units must be used within their expiration date when testing for Hb-S.

B. Hb-S results for the donor units are documented as described in the Blood Bank CDM - *Resulting HgbS Units*.

C. All Hb-S negative units that are tested at Beaumont Health must have the **HGBSN** attribute added to the unit as described in Blood Bank CDM - *Add/Delete/Edit/Display Unit Attributes*.

VI. SPECIMEN COLLECTION:

The specimen is packed RBCs from a donor unit segment.

VII. REAGENTS:

A. SICKLEDEX® solubility buffer (a 2.3 M potassium phosphate buffer solution with 0.1% 2-chloroacetamide preservative). Store tightly capped at 2°C - 30°C in the refrigerator. Do not use beyond the expiration date.

B. SICKLEDEX® reagent powder vials (contain Saponin and sodium hydrosulfite). Store tightly capped at 2°C - 30°C in the refrigerator. Do not use beyond the expiration date.

C. The reconstituted SICKLEDEX®solubility buffer is prepared by reconstituting the SICKLEDEX® reagent powder in the SICKLEDEX® solubility buffer, as described in procedure XI.B *Preparation of the Reconstituted Sickledex Solubility Buffer* in this document.

    1. Expires 45 days after the date of preparation, not to exceed the original expiration of the buffer or reagent powder.
    2. Stored tightly capped at 2°C - 10°C in the refrigerator. DO NOT FREEZE.
    3. Allowing the entire bottle of the reconstituted buffer to warm to room temperature may reduce the open-bottle stability.
    4. A slight sediment may form during storage.

D. Sickle-Chex® controls (Hb-S positive and negative controls, stabilized human RBCs in a preservative medium, manufactured by Streck). Expires 100 days after opening, not to exceed the original expiration date. Stored tightly capped at 2°C - 10°C in the refrigerator.

VIII. EQUIPMENT:

A. Plastic test tube holder, with 2 mL line marking and additional black lines which are used to assess the test solution in the test tubes for turbidity.

B. Pipette capable of dispensing 10 µL packed RBCs.

IX. SUPPLIES:

A. Dispenser caps (for the working SICKLEDEX®solubility buffer)

B. Disposable 12 x 75 mm test tubes, glass or plastic

C. Disposable pipette tips

X. QUALITY CONTROL:

A. Positive and negative controls are tested with each batch of donor RBC tests and must perform as expected.

1. These results shall be documented in the Blood Bank computer system.
2. Moderate darkening of the supernatant of the controls is normal; however darkly colored supernatant may indicate product deterioration.
3. Inability to obtain the expected results for the positive and negative controls may indicate deterioration of the controls.
4. The Sickle-Chex® controls must be labeled with the date opened, the expiration date, and the technologist's initials.
5. The reconstituted buffer must be labeled with the reconstitution date, the new expiration date, and the technologist's initials.

B. Hb-S results for the donor units are documented as described in the Blood Bank CDM - *Resulting Unit HgbS Tests*.

XI. PROCEDURE:

A. Before You Get Started

1. Verify that all reagents, controls, and donor units are within their expiration date.
2. Allow the controls and segments from donor units to warm to room temperature (18°C – 30°C), approximately 15 minutes.
3. Determine whether a sufficient volume of in-date, reconstituted buffer is available. If not, prepare as described in XI.B. *Preparation of the Reconstituted SICKLEDEX® Solubility Buffer.* DO NOT remove the bottle of reconstituted buffer from refrigeration until immediately before testing. Allowing the entire bottle to warm to room temperature may reduce the open-bottle

stability.

B. Preparation of the Reconstituted SICKLEDEX® Solubility Buffer

1. Determine whether a sufficient volume of in-date, reconstituted buffer is available. If not, prepare as described in the following steps.
2. Allow 1 bottle of SICKLEDEX® solubility buffer and 1 vial of SICKLEDEX® reagent powder to warm to room temperature (18°C – 30 °C).
3. Add the contents of the SICKLEDEX®reagent powder vial to the bottle of SICKLEDEX®solubility buffer.
4. Place a dispenser cap on the bottle. Dissolve the reagent powder completely with vigorous agitation.
5. Record the reconstitution date in the space provided on the bottle of reconstituted buffer. Also record the new expiration date and technologist's initials. The reconstituted buffer may now be used for testing.
    a. Expires 45 days after the date of preparation, not to exceed the original expiration of the buffer or reagent powder. When not in use, store tightly capped at 2 °C - 10 °C. A slight sediment may form during storage; this will not interfere with test results.

C. Testing of Donor Units and Controls

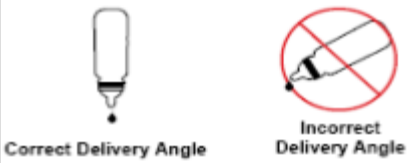
Follow the steps below to test donor units and controls for Hemoglobin S.

1. Remove the positive and negative controls from the refrigerator. Set a timer for 15 minutes and allow the controls to warm to room temperature (18°C – 30°C) before use.
2. Label a 12 x 75 mm test tube with the donor number for each unit to be tested. Also label a tube for the positive control and another for the negative control. Stickers from the donor bag may be used to label the test tube.
3. Place each test tube in the plastic test tube holder (has a 2 mL line marking and additional lines which are used to assess the test solution in the test tubes for turbidity).
4. Dispense 2 mL of **cold** reconstituted buffer to each labeled tube (to the 2 mL line) and allow the contents of the tubes to warm to room temperature (18°C – 30°C) for at least 10 minutes.
    a. The use of reconstituted buffer solution below room temperature may give false results.
    b. DO NOT remove the bottle of reconstituted buffer from refrigeration until immediately before testing. Allowing the entire bottle to warm to room temperature may reduce the open-bottle stability.
5. Obtain a labeled segment of each donor unit to be tested. Transfer 10 µL packed RBCs to the correspondingly labeled test tube. Examples of methods to obtain packed RBCs from a segment:
    a. Use scissors to cut the segment at the end containing packed cells and place the

pipette tip into the segment opening.

b. Empty the contents of the segment into a labeled 12 x 75 mm test tube, centrifuge to obtain packed RBCs, and place the pipette tip into the bottom of the tube to obtain packed RBCs.

6. After allowing sufficient time for the positive and negative controls to warm to room temperature (18°C – 30°C), mix the contents of the positive and negative controls as follows. **Do not mix mechanically.**
    a. Hold vial vertically between the palms of the hands and roll the vial back and forth for 20 to 30 seconds.
    b. Mix by rapid inversion to ensure the cells are suspended. Vials stored for an extended period of time may require extra mixing.
    c. Gently invert the vials 8 to 10 times before sampling.
    d. The Sickle-Chex® controls must be labeled with the date opened, the expiration date, and the technologist's initials.
7. Transfer 20 µL (1 drop) of the well-mixed positive and negative controls to the correspondingly labeled test tubes. To ensure the correct delivery volume of controls, the vials must be inverted and held vertically directly over the test tube.



8. Mix the contents of all tubes thoroughly by swirling each tube several times. Return each test tube to the test tube holder.
9. Return all reagents and controls to the refrigerator. For the positive and negative controls, wipe the threads of the vials and caps before returning to refrigeration.
10. Allow the tubes to stand at room temperature (18°C – 30°C) for at least 6 minutes. Read results between 6 and 60 minutes.
    a. Positive results will typically occur 6 minutes after the addition of the RBCs to the working buffer solution.
    b. Non-patient samples (proficiency or control material) may not be evident at 6 minutes and may take up to 60 minutes to be resolved.
    c. Negative donor results may not clear as quickly or completely as the negative control.
11. Read and interpret the reactions. A reaction is read macroscopically by looking through the solution in the tube at the black lines of the test tube rack. Refer to the *Interpretation* section of this document.
12. Document the results of the quality control and the donor units in the Blood Bank computer system as described in Blood Bank CDM - *Resulting Unit HgbS Tests and* Blood Bank CDM - *Resulting the QC Rack*.

1. Add the **HGBSN** (Hemoglobin S Negative) attribute to all donor units that yield a negative result. Refer to Blood Bank CDM - *Add/Delete/Edit/Display Unit Attributes*.

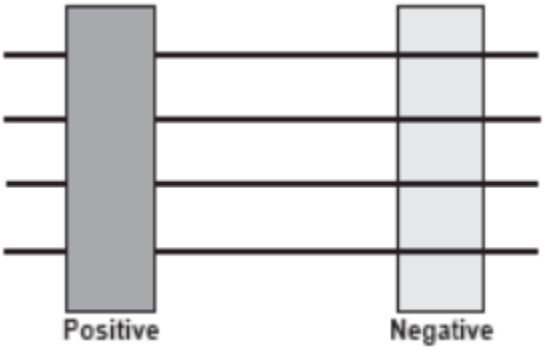
13. For all donor units, affix a unit antigen /Sickle Cell Negative label to the unit.
    1. If using the two-sided antigen label:
        a. If the result of the donor unit is Hb-S negative, document the green side of the unit antigen label.
        b. If the result of the donor unit is Hb-S positive, document the yellow side of the unit antigen label.
    2. If the result of the donor unit is Hb-S positive, document a variance and place the donor unit in quarantine.
    3. A second technologist must review and cosigns the donor unit Hb-S testing for all negative results as described in the Blood Bank CDM - *Cosigning Antigen Negative Unit Labels and Review of QC*. This includes verification that the HGBSN attribute has been added to the unit. If unable to perform cosigning at time of testing the review must occur at time of issue.

XII. INTERPRETATION:

Interpret the Hb-S test as positive or negative, as described below.

EXPECTED RESULTS

1. The reaction is read macroscopically by looking through the test tubes at black lines of the test tube rack.
2. A POSITIVE test for sickling hemoglobin is indicated by a cloudy, turbid suspension through which the black tube rack lines are NOT VISIBLE.
3. A NEGATIVE test for sickling hemoglobin is indicated by a transparent suspension through which the black tube rack lines are CLEARLY VISIBLE.



XIII. WARNINGS:

A. Saponin is a strong hemolytic agent.
B. Sodium hydrosulfite is flammable solid and is a strong reducing agent.
C. The use of reagents below room temperature can give false results.

XIV. REFERENCES:

1. SICKLE-Chex® Instructions for Use, Streck, 350413-13, 2021-08.
2. SICKLEDEX® Instructions for Use, Streck, 350430-21, 2021-08.

Approval Signatures

Step Description	Approver	Date
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