SICKLE CELL TEST

☒ St. Joseph Medical Center Tacoma, WA ☐ St. Clare Hospital Lakewood, WA ☐ St. Elizabeth Hospital Enumclaw, WA
☐ St. Francis Hospital Federal Way, WA ☐ St. Anthony Hospital Gig Harbor, WA ☐ Highline Medical Center Burien, WA ☐ PSC

PURPOSE

To provide instructions for performing the sickle cell test.

BACKGROUND

Hemoglobin S (Hb-S) is an inherited condition characterized by the presence of Hemoglobin S (Hb-S). Hb-S exists either in the homozygous (S/S) state known as Sickle Cell Anemia or in the heterozygous (A/S) state known as Sickle Cell Trait. Homozygous individuals (S/S) commonly exhibit symptoms of severe hemolytic anemia and/or vascular occlusions. Heterozygous individuals (A/S) are usually asymptomatic. Hb-S may also be found with other abnormal hemoglobins or with thalassemia.

Hb-S tends to form sickled-shaped tactoids within erythrocytes under conditions of low oxygen tension. The formation of these irreversibly sickled red cells causes the onset of the acute symptoms. Detection of both the homozygous and heterozygous condition is important so high-risk individuals can be identified and their symptoms reduced.

Donor red blood cells to be given to patients with Sickle Cell Disease or to be used for exchange transfusion in infants must also be tested for Hemoglobin S and must be negative for this hemoglobin variant. An exception to this can be made in urgent situations where there is no time to complete the testing prior to transfusion.

SPECIMEN REQUIREMENTS

- Any anti-coagulated specimen or whole blood from heel or finger (not clotted).
- Samples may be stored at 1-10C for up to 45 days prior to testing
- Never use clotted blood

Please note that the new reagent package insert does NOT state that the use of hemolyzed blood is acceptable. This has been removed from the WI.

REAGENTS/EQUIPMENT

- Sickledex Solubility Buffer
- Sickledex Solubility Reagent Powder
- Dispenser caps for Solubility Buffer
- Sickle-Chex Pos and Neg controls
- 12 X 75 test tubes
- Test tube rack
- 20 µL MLA pipet with disposable tips

REAGENT PREPARATION

The working solubility buffer must be prepared before screening can be performed.

1. Bring buffer and reagent powder to room temperature before mixing.

2. Add the entire 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. Shake vigorously to dissolve the reagent powder completely.

5. Write the date prepared in the space provided on the solubility buffer bottle.

6. Store the working solubility buffer tightly capped at 2-10C when not in use. DO NOT FREEZE.

7. A slight sediment may form during storage. This will not interfere with results.

8. Reconstituted buffer must be used within 45 days.

CONTROL PREPARATION

1. Allow the vials to warm to room temperature for 15 minutes before use.

2. To mix:
   - Hold vial horizontally between the palms of the hands and roll the vial back and forth for 20-30 seconds
   - Mix by rapid inversion to ensure the cells are suspended
   - Vials stored for an extended period of time may require extra mixing
   - Gently invert the vials 8-10 times immediately before sampling.

3. Open vial is stable for 100 days when stored at 2-10 C (not to exceed the expiration date stated on the product vial). Write open date on vial. Indicate 100 day outdate on box of controls.

TESTING INSTRUCTIONS

It is NOT necessary to wash the cells of all SIC positive patients. This should be done only when coarse flocculation is observed when trying to read the lines through the test tubes**.** Such coarse flocculation is caused by elevated protein levels and is uncommon. You may check chemistry results for total protein if you have a question as to whether or not washing is indicated. See Limitations section.

1. Label tubes for controls, patients, and donor units.

2. Dispense 2.0 ml of cold, working, reconstituted Sickledex Solubility Buffer into each tube. **Return the bottle of working Solubility Buffer to the refrigerator immediately. Allow the solution in the test tubes to warm to room temperature (18-30C) for a minimum of 10 minutes before use. The use of reagents below room temperature may give false results.**

3. Deliver 20 µl of whole blood sample or 10 µl of packed red cells to the appropriate tube containing working Sickledex Solubility Buffer, rinsing the pipet in the test solution.

   Note: When testing specimens from severely anemic patients (≤ 15% Hct), centrifuge the sample 5-10 minutes at 1200 rpm. Pipet 10 ul packed cells from the bottom of the tube and add to the working Sickledex Solubility Buffer.

4. Sickle-Chex Controls may be added to the appropriate tube by inverting the Control vial and holding it vertically over the tube while dispensing one drop of control (20 µl) into working Sickledex Solubility Buffer.

5. Mix tubes thoroughly by swirling and place in test tube rack.

6. After sampling Sickle Chex controls, wipe threads of both vial and cap before replacing cap and returning to the refrigerator

7. Allow to stand at room temperature (18-30C) for a minimum of 6 minutes. Observe for turbidity. Read results between 6 and 60 minutes.

8. Read tubes by placing in lined Test Tube holder and observing macroscopically for turbidity by looking through the test tubes at the black lines of the test tube rack.

INTERPRETATION OF RESULTS

- A **positive** test for Hb-S in indicated by a cloudy, turbid suspension through which the ruled lines are not visible.
- A **negative** test in indicated by a transparent suspension through which the ruled lines are clearly visible.

Note: Sickle cell controls are designed to verify the activity of the reagent. Negative patient results may not clear as quickly or as completely as the control.

LIMITATIONS

- False positives may occur in patients with erthrocytosis, hyperglobulinemia, extreme leukocytosis, or hyperlipidemia. Coarse flocculation may occur in these samples due to elevated levels of total serum protein.

  Note: These patient samples may be washed in normal physiologic saline, centrifuged, and 10µl of the packed cells used for testing.

- False positives or false negatives may occur in patients with severe anemia (≤ 15% hematocrit)
- False negatives may occur in infants under six months of age due to elevated levels of Hemoglobin F
- False positives or false negatives may occur in patients with a recent blood transfusion
- Positive results may occur in patients with some rare sickling hemoglobin subtypes such as Hemoglobin C Harlem or Hemoglobin C Georgetown.

CERNER ORDERING

The order code for the Sickle Cell Test is **SIC.**

RESULTING

1. At the select prompt enter TSB.
   - Work Center: 500 Test Site: 500
   - Enter "SIC" in Test Name field
   - You will see "Retrieve already performed QC results? Y". Override by typing in "N" and press Enter (unless control results were previously entered separately for this run)

2. The positive and negative controls and any outstanding patient tests will load.

3. Result controls as POS and NEG.

4. **If patient result is NEGATIVE:**
   - Type in NEG in the result field. Press Enter
   - You will see "**Results correct (Y/N/R/D) Y**" at the bottom of the screen. Enter through the Y.
   - You will see "**Ver / Perf info correct? (Y)es, (N)o, (B)ackdate: Y**" Enter through again.
   - You will see "**procedures SIC thru SIC performed at (date) (time)**". Press Enter several times until the phrase clears out.

5. **If patient result is POSITIVE**:
   - Press the Footnote key (F11) then press Enter
   - Press the Footnote key (F11) again
   - Type POS into the patient result field, then press Enter
   - The footnote opens
   - In the chartable comment area (above the line) press PF1 (Num Lock) key and then press G
   - Enter through the ID field
   - At the Template field, type in **SICKLE**
   - The following comment is printed automatically:

     "The method used to obtain this result is for screening purposes only. Further studies, such as Hgb Electrophoresis, are recommended"

   - Press the Home key (F8)
   - You will see "**Results correct (Y/N/R/D) Y**" at the bottom of the screen". Enter through the Y.
   - You will see "**Ver / Perf info correct? (Y)es, (N)o, (B)ackdate: Y**" Enter through again.
   - You will see "**procedures SIC thru SIC performed at 1/26/14 1315**". Press Enter several times until the phrase clears out.
   - Double check in OID to be sure test resulted and was footnoted.

WHEN TESTING DONOR UNITS

1. Ordering: At select prompt enter LTU. Enter Unit Number where indicated. At unit test center, SIC

2. Resulting: Enter unit SIC result using TSB as above.

3. Order and result only one unit at a time, ie: order in LTU, Result in TSB, then return to LTU and TSB separately for each unit tested.

4. Write charges for unit testing in the charge book.

.

REFERENCES

Streck Sickledex Kit Package Insert

Streck Sickle-Chex Control Package Insert

DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
1. New reagent package insert changes 2. Put into updated document control format 3. Added statement that RBC's given to Sickle Cell Anemia patients and used for exchange transfusions must be negative for Hb-S unless there is an urgent situation where there is not enough time to test. 4. Clarified resulting steps as they could not be followed as written 5. Clarified that only rare POS patients need to have their cells washed and retested			
☐ ***No significant change to process in above revision. Per CAP, this revision does not require further Medical Director approval.***			
Committee Approval Date	☐ **Date:** ☒ **N/A – revision of department-specific document which is used at only one facility**	**Medical Director Approval *(Electronic Signature)***	