PROCEDURE
Corewell Health East - Hemoglobin S Solubility Testing of Patients - Sickledex Method - Dearborn, Troy

This Procedure is Applicable to the following Corewell Health sites:
Corewell Health Beaumont Troy Hospital, Corewell Health Dearborn Hospital

Applicability Limited to: N/A

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Functional Area: Clinical Operations, Laboratory

Lab Department Area: Lab - Blood Bank

1. **Principle**
   This document will provide the policy and procedural steps that will be used to test patient samples for the presence of hemoglobin S (Hb-S).
   A. SICKLEDEX® is a qualitative solubility test to test 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.
   B. Patient samples are tested for Hb-S as described in this document for screening purposes only. It does not distinguish between sickle cell disease and sickle cell trait. Confirmatory hemoglobin evaluation testing performed in other laboratories is used to diagnose patients with sickle cell disease.

2. **Responsibility**
   Personnel who have completed the competency requirements will perform this testing.

3. **Definitions**
   A. Cloudy, turbid suspension
      1. A suspension that is sufficiently opaque to obscure the ruled lines on the test tube rack, so they are not visible when looking through the test tube.
   B. Transparent suspension
      1. A suspension that is clear enough that the ruled lines on the test tube rack are visible when looking through the test tube.
   C. Hb-S
      1. Hemoglobin S
4. **Specimen**
   A. No patient preparation is required prior to specimen collection.

B. The preferred specimen is a 4 ml K_2EDTA sample with affixed identifying label.
C. Clotted samples must never be tested.
D. Samples that have been refrigerated at 1°C to 10°C for up to 45 days may be tested.
E. Specimens must be centrifuged to obtain packed cells for testing.

5. Reagent/Equipment Needed

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 used 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.
Warning: Sodium Hydrosulfite is a flammable solid and a strong reducing agent. Keep container tightly closed and dry. Avoid contact with eyes and skin. Refer to SDS.
C. The reconstituted SICKLEDEX® solubility buffer is prepared by reconstituting the SICKLEDEX® reagent powder in the SICKLEDEX® solubility buffer, as described in the Procedure of 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.
E. 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.
F. Dispenser caps (for the working SICKLEDEX® solubility buffer)
G. Calibrated pipette; either manual or electronic
H. Pipette tips
I. 12x75mm test tubes

6. Quality Control

A. Positive and negative commercial controls are tested with each batch of testing to check for proper test procedure and reagent reactivity and to compare unknown specimen test results with control test results to ensure proper reporting.
B. As an alternative, known positive (AS) and negative (AA) patient samples may be obtained from special testing along with patient hemoglobin electrophoresis reports.
C. Inability to obtain the expected results for the positive and negative controls may indicate deterioration of the controls.
D. Moderate darkening of the supernatant of the controls is normal; however darkly colored supernatant may be indicative of product deterioration.
E. The Sickle-Chex® controls must be labeled with the date opened, the expiration date, and the technologist's initials.
F. The reconstituted buffer must be labeled with the reconstitution date, the new expiration date, and the technologist's initials.
G. Hb-S controls are documented on the Sickle Screen QC Log, which is in Transfusion Medicine policy, Corewell Health East - Quality Control of Blood Bank Reagents - All Beaumont Hospitals v.3 or on the HgB-S Downtime Worksheet attached to this SOP.

7. Procedure

A. Before you get started:
    1. Verify that reagents and controls are within their expiration date.

2. Allow the controls and patient samples 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 below in 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.
   a. Allow 1 bottle of SICKLEDEX® solubility buffer and 1 vial of SICKLEDEX® reagent powder to warm to room temperature (18°C – 30 °C).
   b. Add the contents of the SICKLEDEX® reagent powder vial to the bottle of SICKLEDEX® solubility buffer.
   c. Place a dispenser cap on the bottle. Dissolve the reagent powder completely with vigorous agitation.
   d. 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.
      1) **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 for Hemoglobin S

1. Remove the positive and negative controls from the refrigerator. Allow them to warm to room temperature (18°C – 30 °C) for 15 minutes before use.
2. Centrifuge all patient samples for 5-10 minutes at 3500 RPM to obtain proper cell separation.
3. Label a 12 x 75 mm test tube with the patient identifier for each sample to be tested. Also label a tube for the positive control and another for the negative control.
4. 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).
5. 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.**
6. Pipet 10 µL packed RBCs from the bottom of the tube and add it to the correspondingly labeled test tube.
7. Ensure the positive and negative controls have had time to warm to room temperature (18°C – 30 °C) for at least 15 minutes before use. 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.
8. 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.

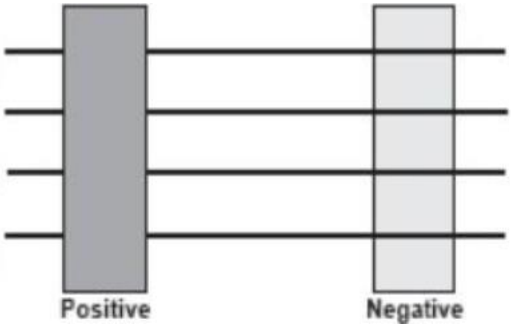
9. Mix the contents of all tubes thoroughly by swirling each tube several times. Return each test tube to the test tube holder.
10. Return all reagents and controls to the refrigerator. For the positive and negative controls, wipe the threads of the vials and caps before returning to refrigeration.
11. Allow the tubes to stand at room temperature (18°C – 30 °C) for at least 6 minutes.
    a. Positive results will occur 6 minutes after the addition of the RBCs to the working buffer solution.
    b. Results may be observed for up to 60 minutes.
    c. Negative donor results may not clear as quickly or completely as the negative control.
12. 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.
13. If patient result is positive:
    a. Testing should be repeated using washed cells before reporting.
        i. Wash patient specimen once with saline and centrifuge.
        ii. Decant the supernatant.
        iii. Repeat test with the washed packed cells.
    b. Show both original and repeat tests to a second technologist for confirmation before reporting positive results.
    c. Hemoglobin Evaluation is automatically ordered by LIS on all positive screening results. Send specimen to Special Chemistry for confirmatory Hemoglobin Electrophoresis testing.
14. Document the results of the quality control on the Sickle Screen QC log or on the HgB-S Downtime Worksheet as mentioned above.
15. Document Hb-S results for the patients in Beaker LIS as described in the attachment ***Resulting Patient Sickle Cell Screen Tests.***

8. Results/Interpretation

A. Interpret the Hb-S test as positive or negative, as described below. Hb-S results for the patients are documented in Beaker as described in the attachment ***Resulting Patient Sickle Cell Screen Tests.***

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.



B. **Expected Results** Normal = Negative
The estimated frequency of Hgb S/S in the U.S. population is one in four thousand (0.025%) and one in one hundred (1%) for the heterozygous genotype. The homozygous form of Sickle Cell Disease affects 0.3% of the black population. The heterozygous form of the disease (Sickle Cell Trait) affects more than 8% of the black population.

9. Limitations

Entities will reference associated Documentation contained within this document as applicable
Printouts of this document may be out of date and should be considered uncontrolled.

A. False negative results may occur in patients if:
   1. The working buffer is not allowed to come to room temperature before testing
   2. Hb-S concentration is below the sensitivity of Sickledex
      a. Testing patients younger than 6 months of age (due to the presence of Hgb F)
      b. Patient had transfusion which decreases Hb-S below 30%
   3. 10 x 75 test tubes are used in error
B. False positive results may occur in patient if:
   1. Samples have abnormal proteins, or elevated levels of protein
   2. Samples have hyperlipidemia
   3. Patient had transfusion with Hb-S positive blood
   4. Patient suffers from extremely high hemoglobin, erythrocytosis, or leukocytosis
   5. Patient has rare sickling hemoglobin subtype such as Hb-C Harlem, Hb-C Georgetown, Hb-C Ziguinchor and Hb-S Travis.

10. Revisions

Corewell Health reserves the right to alter, amend, modify or eliminate this document at any time without prior written notice.

11. References

A. SICKLE-Chex® Instructions for Use, Streck, 350413-16, 2023-07.
B. SICKLEDEX® Instructions for Use, Streck, 350430-27, 2024-05.

12. Procedure Development and Approval

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13. Keywords

Not Set