Solubility Test for Hemoglobin S

Principle

This hemoglobin S screening test is based on the relative insolubility of hemoglobin S when combined with sodium dithionite, a reducing agent.\(^1,2,3,4\) When whole blood is mixed with the reducing agent, saponin lyses the erythrocytes and hemoglobin is released. If hemoglobin S is present, it will form liquid crystals and give a turbid appearance to the solution. A transparent solution is seen with other hemoglobins that are more soluble in the reducing agent.

Reagents and Equipment

1. Stock solution:

   \[
   \begin{align*}
   & \text{K}_2\text{HPO}_4, \text{anhydrous} \quad 216 \text{ g} \\
   & \text{KH}_2\text{PO}_4, \text{crystals} \quad 169 \text{ g} \\
   & \text{Saponin} \quad 10 \text{ g} \\
   & \text{QS with deionized water to 1 liter}
   \end{align*}
   \]

   (Store reagent at 4°C for one month)

2. Working solution:

   Prepare sufficient quantity for the day by adding 5 mg sodium dithionite (\(\text{Na}_2\text{S}_2\text{O}_4\)) to 1 mL of stock solution.

3. Test tubes, 12x75 mm

4. Micropipet, 0.02 mL

5. Micropipet tips

6. Pipets, 2.0 mL

7. Paper-board test tube holder — reading card should have 14-point or 18-point black type in straight lines on a white background, approximately 0.5 cm apart. Tubes should be held 2.5 cm from the reading card.
Quality Control

A positive control (A/S) containing 30-45% Hb S and a negative control (A/A) should be analyzed with each patient specimen.

Specimen

Whole blood anticoagulated with EDTA, heparin, or sodium citrate is acceptable. Specimens may be stored at 4°C for up to three weeks before testing.

Procedure

1. Allow reagents and specimens to warm to room temperature prior to performing this test.
2. Pipet 2.0 mL of working solution into a labeled 12 x 75 mm test tube.
3. Add 0.02 mL of whole blood to the appropriately labeled test tube.
4. Mix the contents thoroughly.
5. Incubate the tubes for five minutes in the test tube holder at room temperature.
6. Read for turbidity.

Results

A positive result is indicated by a turbid suspension through which the ruled lines are not visible. A negative result is indicated by a transparent suspension through which the ruled lines are visible (Figure 7-13).

Comments

1. This is a qualitative test and does not distinguish between hemoglobin S disease (S/S) and hemoglobin S trait (A/S). To confirm the presence of Hb S and differentiate between the two states, a hemoglobin electrophoresis at an alkaline pH should be performed.
2. Other abnormal hemoglobin variants are known to cause sickling and will give a positive solubility test. These variants include Hb C Harlem, Hb S Travis, and Hb C Ziguinchor. To differentiate these variants from HbS, a hemoglobin electrophoresis at an alkaline pH should be performed. On occasion, a hemoglobin electrophoresis at an acid pH may be needed to complete the differentiation.
3. Technical sources of error include:
   a. Inactive or outdated reagents
   b. Reagent below room temperature
   c. Improper mixing of specimen with reagent
   d. Improper interpretation of results

4. Physiologic sources of error include:
   a. Erythrocytosis, hyperglobulinemia, extreme leukocytosis, or hyperlipidemia may cause false positive results. Use of packed erythrocytes (0.01 mL) will correct this problem.
   b. An anemic individual (< 7.0 g/dL) may have a false negative result. Use of packed erythrocytes (0.01 mL) will correct for this.
   c. Recent transfusion with normal erythrocytes may cause a false negative result.
   d. If an infant is younger than 6 months, false negatives may occur due to low concentration of Hb S.

References


