Creative Imagination at Work

Demonstration of Hb C Crystals

by John Martone

When the reading of a blood smear reveals a non-artifactual leucocytosis ("target" cells), the presence of Hb C must be considered. If the red blood cells are normocytic or microcytic, the chances for the presence of Hb C are increased. If these features are found in the blood smears of blacks, the chances are further increased because most cases in which Hb C is present are found among blacks.

Recently we performed a complete blood count on a black patient. The smear revealed 70% target cells with an occasional drepanocyte. It also revealed "boomerang-shaped" red cells filled with hemoglobin. The evidence indicating the presence of Hb SC was strong and eventual hemoglobin electrophoresis confirmed the suspicion.

Since hemoglobin electrophoresis was not scheduled immediately, two tests were performed to determine quickly the diagnosis of Hb SC. The first test, a sickle cell preparation using the standard sodium metabisulfate method, proved positive. In the second, an Hb C crystal preparation was used.

This procedure involved the preparation of a 3% saline solution. EDTA-anticoagulated blood was used. The blood was centrifuged, and the plasma removed. Then a two-part saline, one-part packed cell dilution was made. The dilution was gently mixed and allowed to stand for at least one-half hour. Although 12 hours is necessary for good C crystal manifestation, crystals can be seen after one-half hour.

The preparation was made by applying a large drop of the mixed cell dilution to a glass microscope slide. A coverslip was placed over the drop and the slide was then inverted upon a 4 x 3 sponge gauze and gently pressed to remove the excess blood dilution. The edges were sealed with petrolatum to prevent evaporation and to keep the cells stationary while they were being viewed.

The slide was viewed under oil using the oil immersion lens. Red cells containing Hb C first appear as pherocytic cells. The Hb C, not being soluble, becomes concentrated within the red cell as time passes. The hemoglobin crystalizes and appears as soft-edged squares or rectangles, or as groups of segments along the inner red cell wall. The remainder of the red cell appears clear.

For stained smears, the 3% saline-packed cell dilution is used, and the specimen is smeared at a higher wedge angle to concentrate the cells. The smears are allowed to dry and then are fixed in methyl alcohol for 10 seconds. Staining is done in the usual manner.

Hb C crystals can be demonstrated in the blood of patients with Hb AC, SC, or CC. In patients with Hb AC, crystals are seen usually within one hour and only within that hour. The crystals are seen as "doughnuts" in the red cell.

This method is a quick and interesting way to establish the presence of Hb C, although hemoglobin electrophoresis is necessary to document results.

Transfer Packs
Make Ice Packs

by Ronald Goldstein, MT, and Anthony Go, Jr., MT(ASCP)

Blood Bankers, instead of discarding all those transfer packs from double-pack units transferred as whole blood, remove them, open the inline needle, and fill with water through a wash bottle. Knot the end close to the bag, label as ice, and freeze. They make terrific ice packs for packaging blood for shipment.
Preparation of Calibration Curve

1. Into 19-mm cuvets, pipette the solutions indicated below:

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Standard Solution</th>
<th>Substrate (ml)</th>
<th>Phosphate Buffer</th>
<th>Activity mIU</th>
</tr>
</thead>
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<td>1</td>
<td>0.00</td>
<td>1.00</td>
<td>0.20</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>0.95</td>
<td>0.20</td>
<td>8</td>
</tr>
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<td>3</td>
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<td>0.90</td>
<td>0.20</td>
<td>17</td>
</tr>
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<td>5</td>
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</tr>
<tr>
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<td>84</td>
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<tr>
<td>8</td>
<td>0.60</td>
<td>0.40</td>
<td>0.20</td>
<td>100</td>
</tr>
</tbody>
</table>

2. Incubate for 60 minutes at 37 °C.
3. Add 1.0 ml of color reagent and incubate at 37 °C for 15 minutes.
4. Add 10.0 ml of 0.4 N sodium hydroxide to each cuvet, mix, and allow to stand at room temperature for five minutes.
5. Read the %T of each cuvet against tube no. 1 at 505 nm within 30 minutes.
6. Plot a calibration curve of the %T versus the corresponding units on semilog paper. The resulting plot is not linear.

Reagents

1. Phosphate buffer, 0.1 M, pH 7.4
   Add 2.178 g of potassium dihydrogen phosphate (KH₂PO₄) and 11.926 g of disodium hydrogen phosphate (Na₂HPO₄) to a 1 000-ml volumetric flask. Dissolve in 800 ml of water and check pH. If necessary, add 5 N hydrochloric acid or 5 N sodium hydroxide to adjust pH to 7.4. Dilute to volume with water and recheck the pH. Add a few drops of chloroform as a preservative. Stable for one year at room temperature.

2. Substrate solution
   Dissolve 1.78 g of D, L-alanine and 30 mg α-ketoglutaric acid in 0.1 M phosphate buffer (pH 7.4). Add 0.5 ml of 1 N sodium hydroxide and dilute to 100 ml in a volumetric flask with 0.1 M phosphate buffer (pH 7.4). Measure the pH; if necessary adjust to 7.4 with 5 N hydrochloric acid or 5 N sodium hydroxide. Add a drop of chloroform as a preservative. Stable for six months when stored at 4–7 °C.

3. Color reagent
   Dissolve 200 mg of 2,4-dinitrophenylhydrazine in hot 1 N hydrochloric acid. Cool and dilute to one liter and with 1 N hydrochloric acid in a volumetric flask. Stable for one year at room temperature.
4. Sodium Hydroxide, 0.4 N
Dissolve 16 g of sodium hydroxide in about 500 ml of water in a 1-liter volumetric flask. Dilute to volume with water. Stable for one year when stored at room temperature.

5. Standard pyruvate solution, 2 \mu mol/ml
Dissolve 22 mg of sodium pyruvate in 0.1 M phosphate buffer (pH 7.4) in a 100-ml volumetric flask. Dilute to volume with 0.1 M phosphate buffer (pH 7.4). This solution must be prepared fresh just before use.

Note
Erythrocytes contain three to five times more GPT than serum. Serum with slight hemolysis is satisfactory for the assay of GPT; moderate to severe hemolysis is not acceptable.

Triglycerides
(Manual Colorimetric Method)

References
2. Frings CS, Queen C: Clin Chem 18: 709, 1972

Specimen: Serum (See note 1.)

Principle
Isopropanol extracts of serum are prepared and then treated with alumina to remove phospholipids, glycerol, and glucose. Triglycerides are then saponified to yield glycerol, which is oxidized by sodium metaperiodate to formaldehyde, which, in turn, reacts with acetylacetone to form a yellow dihydro-lutidine derivative. The absorbance of this product at 405 nm is proportional to the triglyceride concentration.

Procedure
1. To a 16 x 150-mm screw-capped culture tube containing 0.7 g of washed alumina, add 5.0 ml of isopropanol and 0.20 ml of water. Blank.
2. To another tube containing 0.7 g of washed alumina, add 5.0 ml of isopropanol and 0.20 ml of working standard. Standard.
3. To another tube containing 0.7 g of washed alumina, add 5.0 ml of isopropanol and 0.20 ml of serum. Unknown. (See notes 1 and 2.)
4. Place all tubes on a mechanical rotator for 15 minutes.
5. Centrifuge for approximately 10 minutes.
6. Transfer 2.0 ml of the clear supernatant to appropriately labeled test tubes.

7. Add 0.60 ml of saponification reagent to all tubes and mix on a vortex mixer.

8. Let all tubes stand for at least five minutes, but no longer than 15 minutes, at room temperature.

9. Add 1.5 ml of periodate reagent and mix on a vortex mixer.

10. Add 1.5 ml of acetylacetone reagent and mix on a vortex mixer. Tightly cover each tube with parafilm.

11. Place all tubes in a 65–70 °C water bath for approximately 15 minutes.

12. Remove all tubes from water bath and allow to cool at room temperature for 5–10 minutes.

13. Measure %T of standard and unknowns against blank at 405 nm using 10- or 12-mm cuvets within one hour. (See notes 2 and 3.)

Calculation

\[
\frac{A_{\text{Unknown}}}{A_{\text{Standard}}} \times 200 = \text{mg triglycerides/dl}
\]

Normal Values

30–150 mg/dl after a 10- to 12-hour fast

Reagents

1. **Washed alumina (aluminum oxide)**
   Wash alumina (ICN Pharmaceuticals, Gml H & Co., 3440 Eschwege, West Germany) with water until all of the “fines” are removed (this usually requires 8–10 washings using approximately four bed volumes of water for each wash). Dry in a 100–110 °C oven overnight. Do not allow the alumina to remain in the oven more than 18 hours. Place 0.7 g of dried alumina (a “calibrated scoop” may be used) in 16 × 150-mm screw-capped culture tubes and store at room temperature. Stable for one month.

2. **Isopropanol (aldehyde free), analytical grade**

3. **Saponification reagent**
   Dissolve 10.0 g of potassium hydroxide in 75 ml of water and add 25 ml of isopropanol. Stable for two months when stored in a brown glass bottle at room temperature.

4. **Acetylacetone reagent**
   Add 0.4 ml of 2,4-pentanedione to 100 ml of isopropanol. Stable for two months when stored in a brown glass bottle at room temperature.
5. Sodium metaperiodate reagent
Dissolve 77 g of ammonium acetate in 700 ml of water. Add 60 ml of glacial acetic acid and 650 mg of sodium metaperiodate. Dissolve the sodium metaperiodate in this solution and dilute to 1 liter with water. The order of addition of chemicals cannot be changed. Stable for two months when stored in a brown bottle at room temperature.

6. Triglyceride stock standard, (1.0 g/dl)
Dissolve 1.000 g of olive oil (Baker & Adamson No. 2043) or triolein in about 50 ml of isopropanol in a 100-ml volumetric flask. Dilute to volume with isopropanol and mix well. Stable for one month when stored in a brown glass bottle at 4–7 °C.

7. Triglyceride working standard, (200 mg/dl)
Add 2.0 ml of stock standard to a 10.0-ml volumetric flask and dilute to volume with isopropanol. Stable for one month when stored in a brown glass bottle at 4–7 °C.

Notes
1. Blood samples for the determination of triglycerides should be taken after a 10- to 12-hour fast since triglycerides are increased after an intake of most foods.

2. If the appearance of the serum is milky, a 1:5 dilution of the serum should be made prior to assay. This dilution must be taken into account in the calculation.

3. Beer’s law is followed to 500 mg of triglycerides/dl. If the absorbance of the final solution is greater than 0.8, the sample must be diluted with water and reassayed.