Spot Test for Detection of Sickling Hemoglobin

Sally Kelly and Lucille Desjardins

We designed a simple spot-plate method for detecting sickling hemoglobins and distinguishing hemoglobin SS from AS in samples of dried blood on filter paper. The test depends on the differential solubility of sickling hemoglobins in phosphate solutions with and without 3 molar urea. Paired phosphate and phosphate-urea hemolysates form color combinations that match those from control hemoglobins.

We describe a simple, noninstrumental procedure for detecting hemoglobin S in samples of blood on filter paper, with use of small volumes of reagents. The method depends on the differential solubilities of reduced sickling and nonsickling hemoglobin in phosphate solutions (1, 2) or Sickledex reagent (3) and the apparent solubilizing effect of urea on reduced hemoglobin S (3). The color of paired phosphate and phosphate-urea hemolysates distinguishes hemoglobin SS from AS and other hemoglobin types.

Materials and Methods

Apparatus

"Dispsoo Trays" (Linbro Chemical Co., New Haven, Conn. 06511) were the reaction vessel.

"PKU cards" (printed by Moore Business Forms Inc., Albany, N.Y. 12203) of Schleicher and Schuell Filter Paper No. 903 (Schleicher and Schuell, Keene, N.H. 03431) were saturated with samples of capillary or venous blood at least 1 h before testing.

Reagents

Sickledex reagent (Ortho Diagnostics, Raritan, N.J. 08869). Made according to the supplier's directions.

Sickledex reagent with 3 molar urea. 1.80 g of urea (NH₂CONH₂), (Fisher Scientific Co., Fairlawn, N.J. 07410) in 10 ml of Sickledex reagent.

Solubility test reagent was made as described (2), consisting of, per liter: 1.31 mol of K₂HPO₄ (Fisher), and 0.95 mol of KH₂PO₄ (J. T. Baker Chemical Co., Phillipsburg, N.J. 08865), 57.4 mmol of Na₂S₃O₃ (Fisher), and 2.5 g of saponin (Fisher).

Solubility test reagent with 5 molar urea. 1.80 g of urea in 10 ml of solubility test reagent.

Control hemoglobins in the spot test were made from blood samples of the hemoglobin types AA, AS, SS, SC, AC, and AJ, identified by electrophoresis. Control hemoglobins for electrophoresis were commercial preparations of hemoglobins AS, AC, SC, and AA (Gelman Instrument Co., Ann Arbor, Mich. 48106).

Procedure

Samples of whole blood from 73 Negro children and adults, dried on PKU cards, were examined by the spot test and by cellulose acetate electrophoresis. These spot-test observations were made before electrophoresis.

Spot tests were carried out in two adjacent cups of a clear plastic tray, in which the samples, 5-mm discs of blood-saturated filter paper, were overlaid with 0.2 ml of reagent. Reagent without urea was
placed in one cup and reagent with urea in the other. The solutions were stirred gently 30 min later and were observed at 60 min against a white background. The tests were carried out with both Sickledex and “solubility test reagents.”

Several discs per sample were eluted with 0.2 ml of “Hemolysate Reagent” (Helena Laboratories, Beaumont, Tex. 77704) in 75 × 12 mm tubes or with distilled water overnight at 1°–5°C for electrophoresis on cellulose acetate membranes with 10 mm buffer (13 mmol/liter, pH 8.9–9.3) for 40 min at 400 V (4).

Results

The hemolysates of the various control hemoglobin samples were of different colors. Paired as phosphate and phosphate–urea hemolysates, they formed patterns consistent with the electrophoretic hemoglobin type (Table 1). The two hemolysates of hemoglobin A, for example, were more highly colored than those of hemoglobin AS, SS, or SC. The paired hemolysates of hemoglobin SS were only faintly colored and were distinguishable from those of hemoglobin AS or SC (Figure 1).

Paired hemolysates from the test samples matched the color combinations of one or more control hemoglobins, and provisional hemoglobin types were assigned according to the best match. The color match agreed, in all instances, with the electrophoretic characterization: 54 were classified in both tests as hemoglobin A, 16 as hemoglobin AS, and 3 as hemoglobin SS.

That the color differences were not the result of lower hematocrit values was verified by comparing the reactions of normal hemoglobin SS and hemoglobin AS blood samples diluted with serum to give hematocrit values as low as 10%. The paired hemolysates from samples of hematocrit values of 15% and above formed the same color combinations as the corresponding samples of normal hematocrit value; “anemic blood,” therefore, should not be considered a likely cause of “false positive” results. A sample of hemoglobin SS blood, furthermore, of which the hemoglobin F component was 30% and the hemoglobin was 22%, gave a color test reaction of hemoglobin AS, i.e., 1 “false negative” result for hemoglobin SS, and illustrates a limitation of the test.

Paired hemolysates from samples with other types of hemoglobin were prepared and their color combinations observed. All but one of the following were those of hemoglobin A or AC: newborns with hemoglobin F, a healthy adult with hemoglobin AJ, a patient with a hemoglobin A, fraction of 5.9% (β-thalassemia minor), and anemic patients of Mediterranean ancestry whose electrophoretic patterns were normal. One from the latter group formed the color combination of hemoglobin AS or SC, the cause of which is under study.

Hemolysate colors formed were more vivid and the test results easier to interpret in “solubility test” reagents (B) with (u) and without urea

From top to bottom: Hemoglobin AA, AJ, AC, AS, SS, SC, F. In color, differences are more striking

Discussion

The spot test is an inexpensive, microscale, screening method that will distinguish hemoglobin SS from AS without instrumentation. It combines the principles of the modified Sickledex tube test (5) with the convenience of a spot-plate method (6). Using “solubility test reagents” (2), the costs of spot test reagent are about $3 per test, including
chemicals, PKU cards, and plastic trays.

We attribute the differential hemolysate colors to the proportions of hemoglobin S in the samples. Insoluble in phosphate solutions of the molarity used in the test (1), hemoglobins SS or AS form dilute hemolysates that are clear or yellow. Hemolysates of these hemoglobins in phosphate solutions containing 3 molar urea are apparently more concentrated, presumably because urea increases the solubility of hemoglobin S, secondary to breakage of hydrophobic bonds and dispersal of the nematic liquid-crystal system (3). Conversely, non-S hemoglobins with the phosphate solubility of hemoglobin A form concentrated hemolysates with or without urea. We consider the spot test analogous to the dithionite and urea-dithionite tube tests (6), except for the indicator reaction, which, in the latter, makes use of the light dispersal, rather than solubility, property of hemoglobin S's nematic liquid-crystal system. We anticipate, however, that the delicate color changes in our test may be seen only in a reaction mixture of the geometry we describe.

The dried-blood-spot samples are convenient for mailing, identification, and storage, and lend themselves readily to confirmation of hemoglobin type by qualitative hemoglobin electrophoresis.

The same cautions and limitations outlined for the "solubility test reagents" (2) pertain to their use in our test.

References