Rapid Solubility Test for Detection of Hemoglobin S

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We have devised a rapid screening test for use in detecting sickling hemoglobins. This test depends on the insolubility of sickling hemoglobins in 2.24 molar phosphate buffer. Sickling hemoglobins form a turbid suspension in this test, in which positive and negative results, respectively, appear very much like those for the "Sickledex" test.

**Additional Keyphrases** solubility of Hgb S • electrophoresis • techniques for detecting hemoglobin S compared • "Sickledex" • sickling hemoglobins • tests for sickle cell disease • turbidimetric tests

Several screening methods for detecting sickling hemoglobins are presently used in the clinical laboratory (1–6). The present procedure depends on the insolubility of reduced hemoglobin S in a 2.24 mol/liter phosphate buffer. The commercially available "Sickledex" (Ortho Diagnostics, Raritan, N.J. 08869) is also thought to be based on this principle (6).

Our method was compared with the standard sodium metabisulfite procedure and with the Sickledex test. In addition, results of the screening methods were verified by electrophoresis.

This report presents a reliable, sensitive, and inexpensive means of detecting hemoglobin S and other sickling hemoglobins. A turbidimetric test, it is performed similarly to the Sickledex test.

**Materials and Methods**

**Apparatus**

_Sample_ (Oxford Laboratories, San Mateo, Calif. 94401) were used to perform the Sickledex test and the solubility test.

_The "Agarose Film Cassette System"_ (Analytical Chemists, Inc., Palo Alto, Calif. 94301) was used as directed for hemoglobin electrophoresis. The electrophoresed hemoglobins were scanned with a densitometer (Joyce Loebl & Co., Ltd., Gateshead-on-Tyne, England).

**Reagents**

All chemicals were reagent grade, and distilled, de-ionized water was used for all solutions. Samples of whole blood were collected in EDTA in a glass test tube ("Vacutainer"; Becton, Dickinson & Co., Rutherford, N.J. 07070).

_Sodium metabisulfite_ (Na2S2O4) (Fisher Scientific Co., Fairlawn, N.J. 07410). An aqueous 0.106 mol/liter solution.

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

_The solubility test reagent_ consists of, per liter: 1.31 mol of K2HPO4, and 0.95 mol of KH2PO4 (J. T. Baker Chemical Co., Phillipsburg, N.J. 08865), 57.4 mmol of Na2S2O4 (Fisher), and 2.5 g of saponin (Matheson, Coleman & Bell, Inc., Norwood, Ohio 45212). Final concentration of phosphate: 2.26 mol/liter (pH 6.96).

**Procedure**

One hundred and ten whole blood samples were all examined by a series of four procedures. Before the tests were made, seven of the specimens had been found to be hemoglobin SS by electrophoresis. During testing, a total of 10 specimens was found to be abnormal.

The sodium metabisulfite sickle cell test was performed on microscope slides, with use of whole blood samples. A 0.105 mol/liter solution of Na2S2O4 was used as a reducing agent and Petri dishes were used to contain the slides. Sickling was assessed at 30 min, 1 h, and 3 h (3).

The Sickledex test was performed according to directions. The solubility test was performed

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1 Manufacturers' names and products are given herein as scientific information only and do not constitute an endorsement by the United States Government.

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by adding 2 ml of the phosphate buffered reagent to 12 × 75 mm test tubes. This was followed by the 20-μl sample of mixed whole blood. The solutions were mixed and examined for turbidity after 10 min.

For electrophoresis, the hemolysate was prepared according to the method of Goldberg (4), in which the cells are washed three times in physiological saline, centrifuged, and lysed with distilled water. The Agarose Film Cassette System includes an agarose gel (12 g/liter) on a galactose-polymer film backing and barbital buffer (0.05 mol/liter) pH 8.6. Electrophoresis was for 35 min.

Results

Of the 110 samples run, three contained hemoglobin SA, seven contained hemoglobin SS, and the rest contained normal adult hemoglobin AA. Results by the four methods agreed completely. The solubility test proposed here seems to be as sensitive after standing approximately 10 min as the Sickledex test (Figure 1).

Discussion

In 1953, Itano (1) described poor solubility in phosphate buffers of reduced hemoglobin mixtures containing hemoglobin S. Since then a number of laboratory tests have been developed in which this principle is used to detect hemoglobin S (2, 5). Diggs et al. developed the Sickledex test (Diggs, L. W. et al., Scientific Exhibit, 1968). We and others (6) think that the Sickledex test is a modification of the same principle. The solubility test reported here is performed in a manner similar to the Sickledex test. It requires a similar amount of test reagent, and the final mixture should stand at room temperature for approximately 10 min after mixing. The cost of the Sickledex test is approximately 48 cents per sample; the cost of the solubility test is less than 4 cents per test.

It is important that the final buffer concentration be no less than 2.24 mol of phosphate per liter. When a 20-μl aliquot of whole blood sample is added to the 2 ml of buffer (2.26 mol of phosphate per liter) in the test tube, the final concentration of the phosphate becomes 2.24 mol/liter. The difference in solubility between hemoglobin S and hemoglobin A is critical at this buffer concentration. If the hemoglobin concentration is so low that it is necessary to add 40-μl of sample hemoglobin, then the corresponding buffer concentration of the reagent should be 2.28 mol/liter phosphate to give a final phosphate buffer concentration of 2.24 mol/liter.

An alternative method for preparing the solubility test reagents is to use a solution of ascorbic acid (11 mmol/liter) and sodium metabisulfite (105 mmol/liter) as combined reducing agents instead of sodium dithionite (7).

Working reagents for both the Sickledex test and the solubility test are stable for 3-4 weeks if kept at 4°C when not in use. The reducing reagent, sodium dithionite, is the limiting factor in the stability of the solubility test reagent.

The solubility test is currently being automated in this laboratory for large-scale screening studies.

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References