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An improved solubility test for haemoglobin S

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Because conventional methods of testing for sickling are time consuming and liable to error other tests have been suggested for the detection of haemoglobin S (Hb S) (Ballard, Radcliff, Sakhadeo, and Schorr, 1970; Canning and Huntsman, 1970; Huntsman, Barclay, Canning, and Yawson, 1971; Cook and Raper, 1971). Most of these solubility tests for Hb S are adaptations of the quantitative precipitation methods introduced by Itano (1953) and Goldberg (1958). Published accounts leave doubt how well such tests behave in normal routine use. They are not suitable for testing samples from neonates since these may contain only a small proportion of Hb S. Occasionally they give false positive results due to heavy precipitates of plasma proteins. The volume of blood used is usually increased if the packed cell volume of the sample is low, in order to reduce the chance of a false negative result. This practice increases turbidity caused by precipitation of plasma proteins. These drawbacks are minimized in the method which follows.

Materials and Methods

The solubility test was developed from the procedure described by Goldberg (1958). A stock buffer solution was prepared by dissolving 33.8 g KH_2PO_4 (anhydrous), 43.4 g K_2HPO_4 (anhydrous), and 1.0 ml of 10 g/100 ml aqueous saponin solution in water to a final volume of exactly 200 ml.

For individual tests, 30 to 40 mg of sodium dithionite was measured with a small scoop and dissolved in 4 ml of stock buffer. The blood samples were allowed to sediment or were lightly centrifuged. Approximately 0.1 ml of partially packed red cells was mixed with the solution of dithionite in buffer, and the mixture left to stand for five minutes. Samples containing Hb S gave a persistent turbidity. Results were confirmed by inspecting a few drops of the mixture with the low power of a microscope. Red cells containing Hb S retained their form, but if Hb S was not present the cells were lysed and their con-

tents dispersed. Plasma protein precipitates appeared amorphous.

The majority of the samples tested were collected from negroid patients attending the dental, obstetric, or surgical departments. The haemoglobins present were not known to the technicians carrying out the tests, but were determined independently using cellulose acetate electrophoresis. Agar gel electrophoresis was used to distinguish Hb S from Hb D.

A preliminary comparison was made of the sickle cell test (using 2% buffered sodium metabisulphite) and the solubility test read by naked-eye inspection for turbidity. When sufficient blood was available, the tests were set up in parallel and the remains of the sample were analysed by haemoglobin electrophoresis. When it became clear that the solubility test was at least as sensitive as the sickle cell test, the latter was abandoned. Subsequently, one technician inspected the solubility tests for turbidity, a second read them with a microscope. Both observations were recorded for comparison with the haemoglobin phenotype.

Results

The sickle cell test gave false negative results with 5.3% of blood samples containing Hb S (standard deviation of proportion, 2.5%). Two errors were potentially serious, but were detected by the solubility test (Table I)

Electrophoresis Result	No. of Sickle Cell Tests	No. of Solubility Tests
Sickle cell trait	63 (2) ¹	66 (0)
Sickle cell anaemia	7 (1)	7 (0)
Hb SC disease	2 (1)	2 (0)
Not electrophoresed, both tests positive	4	4
Total samples Hb S positive	76 (4 = 5.3%)	79 (0)
Normal phenotype	380 (0)	399 (4)
Hb C trait	17 (0)	17 (0)
Hb A, A ₂ and A ₁	1 (0)	1 (0)
β -thalassaemia trait	3 (0)	3 (0)
Not electrophoresed, both tests negative	29	29
Total samples Hb S negative	430 (0)	339 (4 = 0.9%)

Table I Preliminary comparison of the sickle cell and solubility tests

¹Numbers of errors in parentheses.

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In the second part of the study, the proportion of sicklers not detected by the solubility test was 1.1% (two samples). The proportion of false positive results observed for samples not containing Hb S was acceptably small (0.7%). Microscopy detected the presence of Hb S in four samples judged negative by naked-eye inspection. One of these samples was from an adult with the sickle cell trait, the others were from neonates (Table II).

Haemoglobin S Present		Haemoglobin S Absent	
Diagnosis	No. of Samples	Diagnosis	No. of Samples
Sickle cell trait	167 (2) ¹	Normal	956 (6)
Sickle cell anaemia	6 (0)	Hb C trait	41 (1)
Hb SC disease	6 (0)	Hb D trait	1 (0)
Sickle cell/ β thalassaemia	1 (0)	β thalassaemia trait	7 (0)
Hb F, S, and Barts (neonate)	1 (0)	Hb A, A ₂ and A' ₂	9 (0)
Hb F, S, and A	2 (0)	Persistent Hb F	1 (0)
		Normal cord blood (Hb F, some Hb A)	7 (0)
Total	183 (2 = 1.1%)	Total	1022 (7 = 0.7%)

Table II Solubility test results by microscopy on 1205 samples submitted for routine testing

¹Errors in parentheses.

Hb S was present in 15.1% of all samples.

Comment

A test for haemoglobin S (Hb S) must reliably detect patients with clinically important sickling disorders. For routine use in the hospital laboratory such a test should also be rapid, simple, and inexpensive. The test described fulfils these requirements. It is easier to perform than the sickle test and no less reliable. In this laboratory, adoption of the solubility test has resulted in a considerable saving of time.

The use of partially packed cells corrects for anaemia and reduces turbidity due to plasma proteins. Although an accurate result may usually be obtained by naked-eye inspection for turbidity, microscopy increases the sensitivity of the test and

decreases the chance of a plasma protein precipitate being mistaken for Hb S. If macroscopic reading is neglected altogether, the test may be performed upon as little as 1 μ l of red cells. This method is specially suited to paediatric use.

The stock buffer keeps well at room temperature for up to four weeks, but false positive findings may result from the preparation of a solution which is too concentrated, or from the use of defective reagents. Not all batches of saponin are equally suitable; a sample should be used which will clear turbidity from a normal control within three minutes. Concentrated saponin solution may be stored at -20°C for several months. Positive and negative controls should be included with each batch of tests to ensure that the reagents are satisfactory and to reduce the chances of a technical error escaping detection.

The presence of African as well as West Indian communities in the area served by this hospital probably accounts for the comparatively high proportion of samples containing Hb S. The great majority of patients tested were attending hospital for reasons bearing no recognized relationship to any haemoglobinopathy. It is unlikely that selection of patients or samples influenced the results of either the sickle cell or the solubility tests.

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