A rapid whole blood solubility test to differentiate the sickle-cell trait from sickle-cell anaemia

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SYNOPSIS A simple and rapid screening test which differentiates sickle-cell trait and sickle-cell anaemia is described. The test utilizes 0.1 ml of whole blood and is based on the low solubility of reduced sickle haemoglobin. Results intermediate between the sickle-cell trait and sickle-cell anaemia are obtained in unusual cases of sickle-cell anaemia with high foetal haemoglobin.

The need to supplement the results with haematological and electrophoretic techniques is discussed.

There is an increasing demand by surgeons and anaesthetists that Negro patients should be screened for sickle haemoglobin before any operative procedure.

Two methods are currently used: (1) the sickling test (Daland and Castle, 1948) is simple and cheap to perform but false positive and false negative results are not uncommon (Schneider, Alperin, and Lehmann, 1967); (2) Sickledex (Ortho) is a proprietary preparation, which detects sickle haemoglobin by precipitation. This test is simple and more rapid than the described test with the added advantage that no reagents need be weighed. It appears to be reliable (Diggs, Schorr, Ascari, and Reiss, 1968; Loh, 1968: Canning and Huntsman, 1970) but it is expensive and may be prohibitively so if large numbers of examinations are required.

Both methods merely detect the presence of sickle haemoglobin and neither of these techniques will distinguish the sickle-cell trait from sickle-cell anaemia. The technique described below is cheap, reliable, and rapid and will in addition distinguish between the sickle-cell trait and sickle-cell anaemia. It is based on the Itano solubility test (Itano, 1953) adapted by Goldberg (1958) for capillary samples. In the described test a whole blood sample is used instead of a prepared haemolysate, the cells being haemolysed by saponin. Some guidance as to the probable findings in sickle-cell haemoglobin C disease, sickle-cell thalassaemia and sickle-cell anaemia with unusually high foetal haemoglobin is also given.

Materials and Methods

The following blood samples were examined:

<table>
<thead>
<tr>
<th>Sample</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>103</td>
</tr>
<tr>
<td>Sickle-cell trait</td>
<td>56</td>
</tr>
<tr>
<td>Sickle-cell anaemia</td>
<td>12</td>
</tr>
<tr>
<td>Sickle-cell thalassaemia</td>
<td>6</td>
</tr>
<tr>
<td>Sickle-cell trait with hereditary persistence of high foetal haemoglobin</td>
<td>1</td>
</tr>
<tr>
<td>Sickle-cell haemoglobin C disease</td>
<td>6</td>
</tr>
<tr>
<td>Sickle-cell haemoglobin D disease</td>
<td>1</td>
</tr>
<tr>
<td>Haemoglobin C trait</td>
<td>10</td>
</tr>
<tr>
<td>Haemoglobin D trait</td>
<td>1</td>
</tr>
<tr>
<td>Haemoglobin E trait</td>
<td>1</td>
</tr>
</tbody>
</table>

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Potassium dihydrogen phosphate (anhydrous) . . . . . . 33·78 g
Di-potassium hydrogen phosphate (anhydrous) . . . . . . 59·33 g
White saponin . . . . . . . . . . . . 2·50 g

Dissolve in distilled water up to a final volume of 250 ml.
Store at 4°C.

**Working Solution**

Sodium dithionite, 0·1 g, is dissolved in 10 ml of buffer before testing. This is sufficient for five tests.

Then 1·9 ml of working solution is added to a round-bottomed Kahn tube (77 × 13 mm) and 0·1 ml of whole blood is washed into the solution and thoroughly mixed. If the haemoglobin level is below 7 g/100 ml 0·2 ml whole blood is used. The tube is centrifuged at 2,500 to 3,000 rpm (centrifuge radius 20-15 cm respectively) for five minutes. The centrifuge must not be braked. Alternatively, the test solution may be filtered through 5·5 cm diameter (Whatman no. 1) filter paper. This process will take about 10 minutes. When haemolysates are analysed the test solution must be filtered.

**Results**

The appearances described below are those seen after centrifugation. After filtration, the test is assessed purely on the colour of the filtrate; the presence of a red precipitate on the paper is confirmatory evidence.

If a single test is to be performed or intermediate results are obtained it is strongly advised that a control solution is set up so that colour comparisons may be made. This is easily done by adding 0·1 ml of whole test blood to 1·9 ml working solution, previously diluted 50% with distilled water.

**Absence of Sickle Haemoglobin (Haemoglobin Types AA, AC, AD, and AE)**

A clear or opalescent red solution of reduced haemoglobin will be present, showing a variable amount of greyish protein on the surface.

**Sickle-Cell Trait**

The solution of reduced haemoglobin will be clear and pink. The sickle haemoglobin separates to the surface as a dark red band easily distinguishable from the grey protein found with a normal blood sample. Sickle-cell haemoglobin C disease and sickle-cell haemoglobin D disease give an identical result.

**Sickle-Cell Anaemia**

The solution will be clear and straw coloured, all the haemoglobin being found as a dark red band at the surface.

**Sickle-Cell Thalassaemia**

Depending on the level of normal adult and foetal haemoglobin present, the result will lie anywhere between that seen in the sickle-cell trait and sickle-cell anaemia.

**Dysproteinaemia**

Except for a variable increase in surface protein, the result will be indistinguishable from that of normal blood.

**Discussion**

The described test detects the presence of sickle haemoglobin in adult blood. A negative result permits the pathologist to reassure the clinician.

If a positive result is obtained the haemoglobin level should be determined and a blood film examined. The authors appreciate that red cell morphology may be variable, the appearances described below being 'typical'.

The great majority of positive results will be due to the sickle-cell trait. An uncomplicated sickle-cell trait carrier will have a normal haemoglobin level, a normal film, and no splenomegaly.

A patient with sickle-cell anaemia will have a haemoglobin level below 10 g/100 ml and the blood film will show hypochromia, target cells, polychromasia, and, usually, circulating sickle-cells. Splenomegaly is absent over the age of 5.

Three less common varieties of sickle-cell disease deserve special mention.

**Sickle-Cell Thalassaemia**

Even after intensive laboratory investigation, it is sometimes impossible to distinguish this disease from sickle-cell anaemia without a family study.

With the test described in this paper, if the foetal haemoglobin level is above 10%, a result intermediate between the sickle-cell trait and sickle-cell anaemia is found. The haemoglobin level will be reduced below 10 g/100 ml and the blood film shows the changes of sickle-cell anaemia, often to a lesser degree. Splenomegaly is usual and the clinical course may be relatively benign.

As expected, the unusually benign cases of sickle-cell anaemia, associated with splenomegaly and high foetal haemoglobin, which have been described in the West Indies (Serjeant, Richards, Barbor, and Milner 1968), give a result intermediate between that found in sickle-cell anaemia and the sickle-cell trait.
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A double heterozygote with sickle-cell trait and hereditary persistence of high foetal haemoglobin (Hb F = 25%) gave a result indistinguishable from that of the sickle-cell trait.

SICKLE-CELL HAEMOGLOBIN C DISEASE
Patients with this disorder are often fit, but are liable, on occasion, to sudden catastrophic sickling crises, which may result in a totally unexpected death. Anaesthesia may be a precipitating factor.

The test gives a result indistinguishable from that given by the sickle-cell trait. The haemoglobin level is usually above 10 g/100 ml but the blood film is abnormal, showing target cells with some hypochromia. Circulating sickle cells are usual. Splenomegaly is present in the majority of cases.

SICKLE-CELL HAEMOGLOBIN D DISEASE
This rare disorder gives a similar result to sickle-cell haemoglobin C disease and a clinical picture which resembles sickle-cell thalassaemia.

Whilst it is assumed that rapid electrophoretic techniques (cellulose acetate and acrylamide gel) are not available as an emergency procedure in the routine department, it cannot be over-emphasized that all specimens should subsequently be submitted to a full routine investigation.

We are indebted to Dr Graham Serjeant for forwarding to us blood samples from the West Indies.

References
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