Differential Solubility Test for Haemoglobin S

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SUMMARY

Two hundred and seventy-eight blood specimens were examined, both by the Sicklequik solubility test and haemoglobin electrophoresis on paper. Two hundred and sixty specimens (96.04%) were correctly identified by the Sicklequik solubility test. Forty-nine out of fifty (98%) specimens were interpreted identically when examined independently by two observers.

INTRODUCTION

The dithionite phosphate solubility test was described by Itano in 1953. It is based on the principle that Hb S is insoluble in the deoxygenated state in a high phosphate buffer solution and forms tactoids which produce turbidity. Huntsman et al (1970) modified the test to differentiate the homozygous and heterozygous states. This test also departed from the older ones in that turbidity was not used for interpretation. Sicklequik test is based on the modification of Huntsman et al. This test has been evaluated to find a quick and reliable alternative to haemoglobin electrophoresis, specially suited to the needs of peripheral clinics and hospitals where facilities for electrophoresis may not be available.

MATERIALS AND METHODS

Two hundred and seventy-eight blood samples were tested over four months. All tests were performed according to the manufacturer’s instructions. Results after centrifugation are as shown in Fig. 1. All the tests were interpreted by a single observer (V.B.). Fifty tests were interpreted as blind duplicates by a senior technician (J.M.). Blood specimens used were the samples sent to the Haematology Laboratory of the University Teaching Hospital for sickling test and Hb electrophoresis. These two tests were done independently by other technicians. All specimens were collected in ethylene diamine tetracetic acid (EDTA) and stored at 4°C, if there was any delay in performing the test. All the specimens were tested within 4 days of collection. Fifty tests were performed on whole blood and the rest on haemolysate prepared by the method of Dacie and Lewis (1975), without using commercial lysates. Haemoglobin electrophoresis was done on gel acetate paper at pH 9.1, a slight modification of the method of Dacie and Lewis (1975).

RESULTS

Correlation between Hb electrophoresis and Sicklequik solubility test is given in Table 1. 3.66% (3/82) AA specimens were falsely identified as AS. The specimens could not be further analysed to exclude hyperproteinaemia or Heinz body type of...
unstable haemoglobins. 3.75% (3/80) SS specimens were falsely identified as AS. None of these had been transfused in the past three months. However, in one of these the clinical presentation was very mild and hereditary persistence of foetal Hb cannot be ruled out.

The precision data is given in Table 2. The AS sample incorrectly identified by one observer had grey interphase as opposed to red expected in AS samples. Similarly one AS sample had only pink aqueous phase. Both these had Hb less than 5 gms% and the results became unequivocal after 0.2 ml of blood was used as per manufacturer’s instructions.

**TABLE 1**

**CORRELATION BETWEEN Hb ELECTROPHORESIS AND SICKLEQUIK SOLUBILITY TEST**

<table>
<thead>
<tr>
<th>SOLUBILITY</th>
<th>ELECTROPHORESIS</th>
<th>AA</th>
<th>AS</th>
<th>SS</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>79</td>
<td>3</td>
<td></td>
<td></td>
<td>82</td>
</tr>
<tr>
<td>AS</td>
<td></td>
<td>110</td>
<td>5</td>
<td></td>
<td>115</td>
</tr>
<tr>
<td>SS</td>
<td></td>
<td>3</td>
<td></td>
<td>77</td>
<td>80</td>
</tr>
<tr>
<td>SF</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>79</strong></td>
<td><strong>117</strong></td>
<td><strong>82</strong></td>
<td><strong>278</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table 1

**COMPARISON OF INTERPRETATION BY TWO OBSERVERS AS BLIND DUPLICATES**

<table>
<thead>
<tr>
<th>Hb</th>
<th>No.</th>
<th>Indentically Interpreted</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>AS</td>
<td>15</td>
<td>14</td>
<td>93.3</td>
</tr>
<tr>
<td>SS</td>
<td>25</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>50</strong></td>
<td><strong>49</strong></td>
<td><strong>98</strong></td>
</tr>
</tbody>
</table>

Fifty specimens were kept for over twelve hours to assess the possibility of eliminating the need to use centrifuge which may not be available at some peripheral centres. SS specimens showed a yellow aqueous phase with a large red floculate at the top, after about 4-6 hours. Differentiation between AA and SS was, however, not possible before 24 hours and even then was equivocal in many cases. The tests results remained stable for 4-5 days at room temperature.

**COMMENTS**

The test gave correct results in 267/278 of the specimens, an accuracy of 96.04%. Wilson and Schmidt (1974) reported 100% accuracy and precision in 200 tests performed by them. Warren et al (1975) reported 99.5-100% accuracy of the test. Hereditary persistence of the foetal Hb gene could not be ruled out in our false negative cases. Similarly the false positive cases could not be evaluated for dysproteinemia or Heinz body type of unstable haemoglobins.

Besides being fairly accurate, the test has many practical advantages namely:

1. Simplicity: Medical assistants or nurses can perform and interpret the test by following manufacturer’s instructions which are very comprehensive (Wilson and Schmidt, 1974).
2. Speed: It gives results almost instantaneously if a centrifuge is available.
3. Stability: The results are stable for 4-5 days.
4. Small quantity of blood required: Drawing blood in a few young children with severe anaemia may be very difficult. This test is semiquantitative and can be performed with 2-4 drops of blood obtained from a lancet puncture.

The test will be a useful tool to detect sickle cell anaemia especially at peripheral hospitals and clinics where facilities for Hb electrophoresis do not exist.

**ACKNOWLEDGEMENTS**

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**REFERENCES**