Studies in methods of haemoglobin estimation suitable for use in public health programmes

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In a search for a simple method of haemoglobin estimation which could be used by paramedical personnel in public health programmes, Talkin's procedure, colour matching against a single colour standard and specific gravity determination of whole blood were investigated. In the hands of eleven observers all the three methods gave a similar degree of error, which was unacceptably large. It is concluded that none of these three methods is suitable for use in screening population groups for anaemia.

Studies in different parts of India have shown a high prevalence of nutritional anaemia, especially in certain vulnerable population groups such as pre-school children and pregnant women. Public health action to deal with this situation may be attempted either by giving appropriate therapy to everyone in the vulnerable groups (e.g. iron to all pregnant women) or by confining treatment to the more anaemic in the population. If the latter approach is to be used, a suitable method must be available whereby paramedical field workers can detect the more anaemic individuals who are to receive therapy and can subsequently monitor their response to treatment.

The internationally recommended method for the measurement of haemoglobin concentration involves the accurate measurement of a small volume of blood which is mixed with a measured volume of potassium cyanate solution. The optical density of the resultant solution is then determined in a photometric colorimeter. This technique can, therefore, only be carried out by relatively skilled personnel working under reasonable conditions and is not suitable for use in rural public health programmes. Some simpler method of haemoglobin estimation which can be applied by rural health workers to screen people for the presence of anaemia is obviously desirable.

This study was undertaken to evaluate the reliability of three possible methods of haemoglobin estimation—two by visual colour matching and one by specific gravity determination.

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Material and Methods

The three different methods of haemoglobin estimation evaluated were:

(i) The classical colour matching procedure of Tallquist\textsuperscript{a} using a commercially obtained book with colour standards ranging from 30-100 per cent. (100 per cent $=15.6$ g. per cent of haemoglobin).

(ii) A colour matching procedure using a single colour standard, made in the laboratory from oil paints, to match the colour of a drop of blood with a haemoglobin concentration of 8 g. per cent.

(iii) Specific gravity determination of whole blood\textsuperscript{b} using copper sulphate solution of specific gravity 1.042.

Four hundred cc. of blood was collected in anticoagulant (versine or sodium citrate) on different occasions from three individuals. The cells and plasma were separated and 50 samples, with different haemoglobin concentration, were reconstituted with mixture of differing proportions of the two. The specimens were numbered randomly.

Eleven observers, two doctors, four trained laboratory technicians, three auxiliary nurse midwives and two attenders took part in the study. Each observer examined the 50 specimens first by one method, then by another and then the third, in random order, on each of three occasions. Thus, each observer made a total of 150 observations with each of the three methods, making a total of 1650 observations with each method. All results were recorded independently by each observer for each method. Care was taken to see that all specimens were kept adequately mixed at all times.

For the Tallquist and single colour comparison, the observers were instructed that a drop of blood should be put on standard filter paper and the colour matching should be done in day light as soon as the sheet had gone from the drop, indicating partial drying. For the copper sulphate method they were instructed to record whether the drop of blood rose to the surface ($Hb<7.5$ g.), was suspended in the solution ($Hb=7.5$ g.) or sank to the bottom ($Hb>7.5$ g.).

The true haemoglobin concentration of each sample was determined by a trained technician using the cyanmethaemoglobin method, standardised against a reference standard of the International Committee for Standardisation in Haematology.

Results and Discussion

The specimens were arranged according to their true haemoglobin concentration in groups of one gram of haemoglobin, 1.5 g. per cent to 2.4 g. per cent etc. and the percentage of observations on the specimens in each group, which were adjudged to be equal to, or greater than the standard was calculated, and the results plotted at each haemoglobin level. The results are shown graphically (Graphs 1 to 4).
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The utility of any screening test for anaemia depends on the reliability with which the test can be applied, by even relatively unskilled people, to distinguish those individuals with a haemoglobin concentration below a given standard, from those with a haemoglobin concentration equal to or above the standard. In Graphs 1 to 3 the slope of the graphs is an index of this reliability—the steeper the graph, the more sharply can those samples with a haemoglobin concentration below a certain point be defined.

The sigmoid shape of the curves suggests that there is a fairly uniform distribution of observations about a mean and it is probable that there are as many false low observations as there are false high ones. If this be so, the point at which the curves cut the 50 per cent mark may be taken to represent the actual value of the standard concerned. On this basis it will be seen that the stated values for the standard Talquist are quite different from the actual values (Table I). It was not possible to determine with certainty whether this was due to the colour standard being wrongly constructed or whether the standard colours had faded. However, in that the 100 per cent standard was, if anything, darker than it should have been, while the others were lighter, it suggests that the error was in the original colour reproduction.

### Table I. Comparison of Theoretical Haemoglobin Concentration of Talquist Standards with Observed Values.

<table>
<thead>
<tr>
<th>Theoretical Haemoglobin Concentration %</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Talquist</td>
<td>8%</td>
<td>4%</td>
<td>6%</td>
<td>8%</td>
<td>8%</td>
<td>8%</td>
<td>8%</td>
<td>8%</td>
</tr>
<tr>
<td>Observed from Figure 1</td>
<td>22</td>
<td>32</td>
<td>46</td>
<td>59</td>
<td>85</td>
<td>120</td>
<td>165</td>
<td></td>
</tr>
</tbody>
</table>

It is of interest that the slope of the graphs in Graph 1 decreases with the higher colour standards, indicating that observers had more trouble matching against the deeper red colour standards representing 80-100 per cent haemoglobin concentration than the lighter ones.

Although the single colour standard made in the laboratory was designed initially to be equal to 8 g. per cent, the results shown in Graph 2 indicate that, in fact, it was nearer to a 7 g. per cent standard. Similarly, although the copper sulphate was meant to be for a haemoglobin concentration of 7 g. per cent it also was nearer 7 g. per cent (Graph 3).

It is of interest that there was quite a wide difference between observers in their ability to apply the tests. Table II shows the total individual errors in the 150 tests for the single colour comparison and the copper sulphate method, i.e. those specimens with a true haemoglobin concentration below the standard which were said to be equal to or greater than the standard and those with at true haemoglobin concentration above the standard which were said to be less than the standard.
Graph 1. Results of Talbot's procedure. Per cent of observations on specimens in each one gram group of "true" haemoglobin concentration judged to be equal to or above the standard. Each line from left to right corresponds with increasing colour standards, 30 to 100 per cent as labelled at the top of the curve. The heavy line (80 per cent) is the one nearest to the other two standards (Graphs 2 and 3).

Graph 2. Results using single colour comparison. Per cent of observations on specimens in each one gram group of "true" haemoglobin concentration judged to be equal to or above the standard.
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**Graph 3. Results of Copper Sulphate method. Per cent of observations on specimens in each one gram group of "true" haemoglobin concentration judged to be equal to or above the standard.**

<table>
<thead>
<tr>
<th>&quot;TRUE&quot; HAEMOGLOBIN, CONCENTRATION</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of observations</td>
<td>0</td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table II. Errors of different observers.**

<table>
<thead>
<tr>
<th>Observer No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single colour comparison (out of 150)</td>
<td>19</td>
<td>27</td>
<td>19</td>
<td>8</td>
<td>17</td>
<td>22</td>
<td>31</td>
<td>14</td>
<td>18</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>Copper sulphate (out of 150)</td>
<td>19</td>
<td>21</td>
<td>11</td>
<td>8</td>
<td>20</td>
<td>22</td>
<td>31</td>
<td>13</td>
<td>22</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>Total %</td>
<td>12</td>
<td>16</td>
<td>10</td>
<td>5</td>
<td>12</td>
<td>15</td>
<td>21</td>
<td>9</td>
<td>13</td>
<td>20</td>
<td>19</td>
</tr>
</tbody>
</table>

The results of the different observers bore no obvious relationship to their general ability in the laboratory, to their level of education or to their possible motivation. One of the authors got 16 per cent of all observations wrong! Surprisingly, the accuracy of each observer was almost identical for the colour comparison and for the specific gravity test.

Graph 4 is a composite histogram showing the percentage of observer/specimens which gave a false result in each 1 g. haemoglobin group above and below the standard, for the copper sulphate method, the single colour comparison and the Tallquist standard nearest to these two—i.e. the 80 per cent standard. It will be seen that the results with all three methods were virtually identical, and that by any method
Graph 4. Histogram showing per cent error of observations on specimens whose "true" haemoglobin concentration was above or below the standard, using each of the three methods.

about 15 per cent of observations on specimens with 3 g. per cent of haemoglobin above or below the standard will be wrongly classified. Perhaps, it does not matter too much if subjects with 9-10 g. per cent haemoglobin are classified as being below 7 g. per cent, but a method which classifies 25 per cent of subjects with 5-6 g. per cent of haemoglobin, and 15 per cent of subjects with 4-5 g. per cent of haemoglobin as being above 7 g. per cent can scarcely be considered acceptable for field studies.

The studies of Van Slyke in 47 anaemic subjects showed a maximum error in the copper sulphate method of 2 g. per cent. It was therefore anticipated that the copper sulphate specific gravity method would give a better result than the colour matching ones. However, this was not so, and all three methods had a similar degree of error. The results for the copper sulphate method were similar with each of the three lots of blood and did not show any improvement with practise by the observers. It is also noteworthy that observer number 4 got all observations except 1 out of 150 within 1 g. per cent of the correct value—a result quite comparable with those reported by Van Slyke's group. It is, therefore, apparent that the inaccuracies are related to the inability of the observers to apply the method, rather than to any inaccuracy of the method itself.

It must be concluded that none of the three methods tested is suitable for field use in public health programmes and some other more reliable yet simple method for haemoglobin estimation must be sought, if populations are to be screened for anaemia by public health personnel.
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References