A simple and reliable method for estimating haemoglobin

G.J. Stott[1] & S.M. Lewis[2]

A new colour scale has been devised for estimating haemoglobin levels by matching the blood sample with ten levels of haemoglobin (3, 4, 5, 6, 7, 8, 9, 10, 12 and 14 g/dl) on the scale. Preliminary results show good correlations with spectrophotometric readings. The new device is being field tested and if the initial promise is confirmed, will provide a simple and reliable method for estimating haemoglobin where laboratory facilities are not available.

Methods for assessing haemoglobin levels by matching a drop of blood on a piece of blotting paper against a colour scale have been widely used in health centres in developing countries for the detection of anaemia. In theory, they are attractive because of their simplicity, portability and low cost. In practice, they are so grossly inaccurate, especially at lower haemoglobin levels, that they have little value.

Nevertheless, it is realized that if a suitable version of a device based on the principle of direct comparison of fresh blood with a reliable colour scale could be developed it would serve a very useful purpose, not only for field use but also in hospitals, health centres, clinics and general practice, especially in situations where a reasonably reliable assessment, rather than great accuracy and precision, is what is needed to permit a clinical decision and action.

The first problem was therefore to identify the factors responsible for the wide margin of error found in the available colour scales and to overcome them so as to develop a reliable method suitable for field use.

Causes of inaccuracy and imprecision

The main sources of error in the use of the haemoglobin colour scales currently available are:

(1) The colours of the printed scales vary between manufacturers and may not even look like blood at all, particularly at lower haemoglobin levels, perhaps because they were not prepared by matching with fresh blood.

(2) The absorbent papers supplied with these colour scales also show much variation and are unsuitable because:

— the blood spreads unevenly and too widely, causing dilution of the colour;

— the blood takes too long to be absorbed, especially at higher haemoglobin levels (from several seconds to many minutes), which is inconvenient for practical use; and

— the paper is too thin and therefore very translucent when damp.

(3) The design of the haemoglobin scale booklets, with their absorbent papers in front and the colour scale at the back, makes it impossible to use them without light entering from behind the scale; the blood stains on the test paper, being damp and translucent, then become unmatchable.

(4) Even if the colour scale and the test and background papers are held close together, light is reflected from the white background through the damp blood stain, giving too bright a colour which cannot be matched.

(5) The circular apertures of 5–6 mm diameter in the colour scales, through which the blood stains are viewed, are too small to permit proper matching because the margin casts a shadow.

Development of a new method

By eliminating, as far as possible, the identified sources of error it proved possible to develop a device and colour scale with absorbent papers that gave reproducible results and an acceptable degree of accuracy.

Blood standards

The study was based on the colours observed with a set of blood samples of known haemoglobin content,

[1] Programme on Health Technology, World Health Organization, 1211 Geneva 27, Switzerland. Requests for reprints should be sent to this author.

[2] Senior Research Fellow in Haematology, Royal Postgraduate Medical School, London, England.

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as measured by spectrophotometry in accordance with the ICSH reference method.[a] Their haemoglobin levels were adjusted by adding autologous plasma to obtain samples in the range 3–14 g/dl, in 1 g/dl steps.

Absorbent test papers

Desirable qualities in an absorbent paper for use with a haemoglobin colour scale are:

— the paper should absorb the blood drop quickly and lose its sheen almost immediately;

— the paper should be thick enough not to allow too much reflection of light through it when held against a pale background while still damp. (Note: even if the sheen disappears almost at once, the blood stain remains damp for 15–20 minutes depending on the ambient temperature and humidity); and

— the paper when stained with blood at low haemoglobin levels should have as uniform a colour as possible without too much interference from white fibres which prevents satisfactory matching.

Absorbent papers have very different qualities depending on their texture, composition, pore size, flow characteristics, fluid spread and pH. A number of Whatman filter and chromatographic papers were examined, namely: 3 MM Chr, 4 Chr, 17 Chr, 31 ET Chr, BP 87, D 28, F 178–10, F 427-01, and F 427-02; and also papers 557, 598 L, 2316, 2292 and 3469, produced by Schleicher and Schuell. Some were too thin, others too thick; and some absorbed the blood drops easily, but others poorly.

It was concluded that the paper Whatman 31 ET Chr gave the best results, with the Schleicher and Schuell paper 2992 as second best. A drop of blood is absorbed and loses its sheen on these papers in a few seconds at high haemoglobin levels, and in one or two seconds at lower levels, and gives a regular round stain with limited spread.

Colour standards

To develop a colour scale corresponding to the appearance of the blood sample at the different haemoglobin levels (3–14 g/dl), drops of blood were placed on test strips of Whatman 3 ET Chr paper and, as soon as the blood stains had lost their sheen, the spectral characteristics of their colours were measured by a computerized analytic spectrophotometer.

To meet these specifications, printing inks (resistant to fading due to ultraviolet rays) were prepared from pigments of the three primary colours and a neutral diluent. The different shades were then printed on strips of acid-free paper at a defined ink thickness, and dried and varnished.

Lighting

Colour matching of a blood stain on absorbent paper with the shades on a colour scale is influenced by the type of light (daylight, tungsten, or fluorescent tube), the angle at which the light is reflected, and the visual interference from a white or coloured background.

It was found that the best results were obtained when the scale is held at an angle of 45° in daylight (but not bright sunlight), with the light coming from behind or over the shoulder of the observer, and with a neutral pale-grey matt background to the colour scale.

Effect of time on the colour of blood stains

Blood stains on absorbent paper change in colour with time, as the haemoglobin converts to the reduced form and to methaemoglobin. This change begins after a couple of minutes and comparisons with the colour scale must therefore be made as soon as the sheen disappears.

Preparation of a reliable colour scale

With the experience from many studies of the various sources of error and how to overcome them, an improved device with a new colour scale was prepared with the following main features:

— there are ten colour standards corresponding to the colour of blood stains on Whatman 31 ET Chr paper at haemoglobin levels of 3, 4, 5, 6, 7, 8, 9, 10, 12 and 14 g/dl;

— the colour standards (20 × 40 to 60 mm) are printed in a continuous row without any separation so as to allow matching of test strips also against one side of the colour scale;

— circular apertures of 8–9 mm diameter are placed in the centre of each colour standard (to facilitate comparisons when test strips are placed behind the colour scale);

— the colour scale is mounted on a rigid white polyvinyl chloride or polypropylene sheet or thick card with a neutral pale-grey matt background of laminated board for easy cleaning; and

— the test papers (12–15 × 60 mm) are supplied in small packets separately from the colour scale.

[a] **International Council on Standardization in Haematology.** *Journal of clinical pathology*, 1978, **31**: 139–143.

Practical features

It is essential that a device of this type should:

— be inexpensive and not require batteries, cuvettes, chemicals or maintenance;

— be reliable, durable and replaceable at low cost;

— be suitable for use also by relatively junior health staff (provided they are instructed in the method);

— allow the detection of mild, moderate or severe degrees of anaemia (whatever the age, sex, state of pregnancy, or altitude);

— permit health staff to detect improvement or deterioration in individual patients following therapy;

— provide the basis for a set of guidelines for the recognition and management of anaemia (especially where laboratory facilities are not readily available) in children, pregnant women, adults and those with malaria or other parasitic conditions (e.g., ancylostomiasis); and

— allow the identification of potential blood donors (i.e., those with haemoglobin levels at or above 12 or 14 g/dl, depending on national norms).

The ten colour standards in the scale make it possible to achieve a fair degree of precision in estimating haemoglobin levels (0.5 g/dl) but the number could be reduced if desired, although this would increase the margin of error at some haemoglobin levels. Matching of test strips may be done from behind or at the side of the colour scale, depending on local lighting conditions. The design of the card ensures that it remains closed when not in use so as to reduce the risk of colours fading.

Besides the instructions for use, the device will include brief guidelines for the management of anaemia under field conditions, which may be modified to suit the local health situation.

Field studies

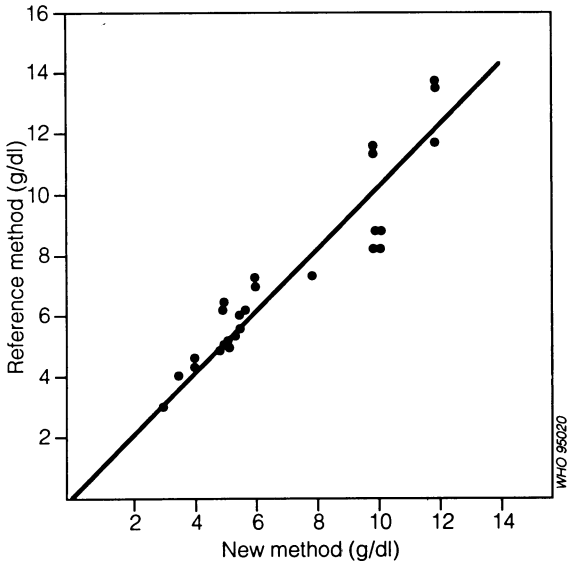
Preliminary tests

Prototype models of the device with the new colour scale were tested by independent observers. These tests showed that, with brief instruction and strict adherence to the simple technique, individual observers—with no knowledge of the actual haemoglobin levels of the blood samples or of the colour scale equivalents—could vary in their readings but there was no obvious bias (Table 1, Fig. 1). The results showed good correlations. There were some random differences in readings, especially at higher levels (mean difference, 0.85 g/dl). The intercept and slope suggest that there is a small bias (i.e., constant error). This may be overcome, if necessary, by an appropriate adjustment of the values assigned to the colour scale.

Table 1: **Comparison of colour scale device and spectrophotometric methods for haemoglobin estimation**

	Colour scale	Spectrophotometry
Mean Hb (g/dl)	6.94	7.27
No. of samples	27	27
SD	2.90	2.92
		Conclusions
Differences in means (g/dl)	0.33	No significant difference in means
SE of differences in means	0.925	
F-ratio at $v = 26$	1.011	99% probability of no significant difference in means
t-test on difference in means at $v = 26$	0.417	65-70% probability of no significant difference
Mean of differences (g/dl)	0.85	
SE of mean of differences	0.13	
t-test on differences in paired results at $v = 26$	6.405	Significant differences present between some individual results
Correlation coefficient (r)	0.9386	Perfect correlation would give $r = 1.000$; $a = 0$, $b = 1$
Intercept (a)	0.718	
Slope (b)	0.944	

Fig. 1. **Correlation of colour scale readings at actual haemoglobin levels.**



Proposed multicentre study

With these encouraging results, a larger double-blind trial involving other centres and observers, each using 10–20 blood samples at different haemoglobin levels, some of them being duplicates, is now under way. Preliminary observations indicate that a period of training is necessary to familiarize observers with the method and to enhance their ability to distinguish different shades of red.

Conclusion

We believe that this device, with its new and improved colour scale (Fig. 2), meets the required criteria for haemoglobin estimation. If the initial promise is confirmed, the device and the test strips (of suitable absorbent paper) will be made available for use in mass surveys and screening, as well as in public health and clinical services.

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Résumé

Une méthode simple et fiable de dosage de l'hémoglobine

Les centres de santé des pays en développement utilisent largement, pour la détection de l'anémie, des méthodes de dosage de l'hémoglobine qui consistent à comparer une goutte de sang déposée sur un morceau de papier buvard à une échelle colorée, mais les systèmes employés sont si imprécis, surtout pour de faibles concentrations d'hémoglobine, que les résultats ne sont guère valables.

Une étude a donc été entreprise pour rechercher les facteurs responsables de cette importante marge d'erreur et mettre au point, en modifiant la conception, les matériaux et le mode d'emploi, une nouvelle méthode et une nouvelle échelle de coloration qui donnent des résultats fiables et d'une précision acceptable.

Les teintes choisies pour la nouvelle échelle correspondent à dix concentrations d'hémoglobine dans le sang (3, 4, 5, 6, 7, 8, 9, 10, 12 et 14 g/dl). Les premiers résultats présentent une bonne corrélation avec les mesures spectrophotométriques. La nouvelle méthode fait actuellement l'objet d'essais sur le terrain, et si les premières espérances se confirment, elle constituera un moyen pratique et rapide de détection de l'anémie.

Fig. 2. **An example of a colour scale using eight colours (with apertures between the colours).**