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An evaluation of methods of screening for anaemia*

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Screening methods for anaemia were selected for testing on the grounds of cheapness, simplicity, sturdiness, accuracy and independence of mains electricity or batteries. The methods evaluated were the copper sulfate method, the Dare haemoglobinometer, the Lovibond comparator, the A. O. Spencer haemoglobinometer, and the Tallqvist method. A new device, the Carib haemoglobin comparator, was developed. The Dare and Lovibond instruments were found to be inaccurate in the laboratory. The other instruments were tested by primary health care workers in clinics in Jamaica. The Carib haemoglobin comparator and the copper sulfate method were found to be accurate, easy to use, and cheap. Both methods are considered to be useful for screening for anaemia at primary health care level.

Anaemia is found throughout the world, and can be particularly serious when it occurs in pregnant women (*1*, *2*). In general, diagnosis of anaemia depends on measurement of the concentration of haemoglobin in the blood. This can be done by assessing: (1) the colour of the blood (since haemoglobin is the main pigment); (2) the erythrocyte volume fraction; or (3) the specific gravity of the blood.

There is a need for a simple screening method for anaemia (*2*) for use by public health workers in the field. The present study was carried out to determine the most appropriate method of screening for anaemia in clinics or during home visits by primary health care workers in Jamaica. The available screening methods were reviewed and tested for reliability and ease of use, both in the laboratory and in the field. In addition, we developed and tested a new screening method, based on colorimetry.

REVIEW OF AVAILABLE METHODS

Any method of screening or monitoring individuals for anaemia at primary care level should be cheap, simple to operate, sturdy enough for field use, dependent neither on mains electricity nor batteries, and reasonably accurate. It should also use a minimum of materials that require regular replacement and should give immediate results.

After a careful review of the available technology, we concluded that several methods were worthy of further study. These were: the copper sulfate method (*3*, *4*), the Dare haemoglobinometer (*5*, *6*), the Lovibond comparator (*7*), the A. O. Spencer haemoglobinometer (*8*, *9*), and the Tallqvist method (*10*, *11*). These methods are described below.

Copper sulfate method

The copper sulfate method is based upon the observation that the specific gravity of blood is greatly influenced by its erythrocyte volume fraction. Serum protein does not have a large effect on specific gravity, and can thus be neglected.

A blood droplet is allowed to fall into copper sulfate solution, with a specific gravity equivalent to that of blood with a haemoglobin content of 100 g/l, from about 1 cm above the surface. The momentum of the droplet takes it to 1-2 cm below the surface within about 5 seconds. The movement of the drop over the next 5-10 seconds is observed. If it continues to fall, the haemoglobin level is judged to be more than 100 g/l. If it rises, the haemoglobin is taken as less than 100 g/l, and the test is repeated in copper sulfate solution with a specific gravity equivalent to a haemoglobin level of 80 g/l. Consequently, the haemoglobin level of every sample is categorized as below 80 g/l, between 80 and 100 g/l, or over 100 g/l. Detailed instructions for preparation of the solutions and use of the method in clinics are given elsewhere (*12*).

Dare haemoglobinometer

A small glass reusable chamber is filled with whole blood by capillary action. (Alternatively, a disposable chamber of blotting-paper may be used.) The

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chamber is illuminated with a battery-lit bulb and viewed through a red filter; the intensity of emergent light is compared with a graded standard.

Lovibond comparator

A measured quantity of whole blood is diluted with a measured quantity of dilute ammonia solution made up with distilled water and placed in a comparator tube. Haemolysis occurs resulting in a clear red solution. Ammonia solution is placed in an identical comparator tube. Coloured glass filters are rotated in front of the clear tube and a comparison of colour intensity made by viewing transmitted daylight or electric light through both tubes.

A. O. Spencer haemoglobinometer

A drop of whole blood is placed in a glass chamber and haemolysed by agitating gently with a stick impregnated with saponin. The chamber is covered with a glass plate and placed in front of a battery-lit bulb emitting green light; the intensity of colour of the sample is compared with a graded standard on a split screen.

Tallqvist method

A drop of whole blood is placed on a strip of standard blotting-paper and allowed to dry. The intensity of colour is compared with a range of standard colours printed on paper.

CARIB HAEMOGLOBIN COMPARATOR

From the review of available methods for estimating haemoglobin level, it was concluded that none of the techniques entirely satisfied the criteria of ease of use, cheapness, and freedom from reliance on regular supplies. A new instrument, the Carib haemoglobin comparator, was therefore designed and built in the Physics Department of the University of the West Indies.

Specifications

In order to keep down the construction costs, it was decided not to attempt to measure the haemoglobin level itself but to categorize each patient as severely anaemic, moderately anaemic, or not anaemic. This corresponds to the three categories given by the copper sulfate method, which had proved satisfactory when used in antenatal clinics in Jamaica (*13*, *14*). In addition, it was decided that the apparatus should incorporate a filter to exclude all but the principal wavelength of light absorbed by haemoglobin; in this way, errors caused by confusion between colour differences and differences in transmission or reflection of light would be minimized.

The instrument was thus designed to provide easily interpretable results, be independent of the electricity supply, and to have a low cost. A detailed report on the instrument has been published elsewhere (*15*).

We should have liked to use untreated whole blood in the instrument, but it was found necessary to haemolyse the samples. Commercially-produced saponin sticks for this purpose are expensive, but it was possible to produce saponin sticks very cheaply using toothpicks dipped in a saponin solution and dried.

Method of use

A drop of blood is placed in a transparent chamber, haemolysed with a saponin-coated stick, and covered with a glass plate. This is placed in the instrument alongside two filters (corresponding to haemoglobin levels of 80 g/l and 100 g/l) of a neutral density type. Commercial photographic filters of the appropriate densities were used. Daylight or interior lighting is viewed through the sample and comparison filters and through a green filter which removes colour differences between them. The intensity of emergent light is compared and the haemoglobin level is thus classified as > 100 g/l, between 80 and 100 g/l, or < 80 g/l.

LABORATORY TESTING

All the techniques were tested and compared in the laboratory by two experienced technicians, who had spent at least an hour familiarizing themselves with each instrument.

Non-anaemic blood was diluted with isotonic saline to give 6 samples with haemoglobin concentrations of 59 g/l, 82 g/l, 94 g/l, 97 g/l, 116 g/l, and 126 g/l, respectively. Each sample was tested 4 times on each instrument by each technician, in a random fashion. The technicians were unaware of the true haemoglobin levels of the samples.

The cyanmethaemoglobin method (*16*) was used as the control against which the other methods were assessed. The values were read on a Beckman 26 spectrophotometer. All the samples used in the laboratory study were also assessed on a Coulter counter.

Analysis

Two methods were used for the statistical analysis of the results. The Dare and Spencer haemoglobinometers, which measure actual haemoglobin levels, were subjected to an analysis of variance. The Carib haemoglobin comparator and the copper sulfate method, which group samples as being above or

below a given cut-off point, were assessed for sensitivity and specificity. The Tallqvist method was assessed by both techniques, and the Lovibond comparator was considered as a screening instrument and treated accordingly.

Results

The standard deviations from the actual values of the results given by the Dare, Spencer, and Tallqvist instruments are given in Table 1. The Spencer instrument gave the most accurate results; the standard deviation for the Dare instrument was unacceptably high, with both disposable and reusable chambers.

Table 2 sets out the sensitivity and specificity at 80 g/l and 100 g/l of the screening methods tested.

Table 1. Standard deviations of the haemoglobin levels given by the different techniques

Instrument	Standard deviation (g/l)
Dare with disposable chamber	19.1
Dare with reusable chamber	20.3
Spencer haemoglobinometer	3.8
Tallqvist method	9.6

Table 2. Sensitivities and specificities of the instruments tested in the laboratory

Method	Level (g/l)	Sensitivity[a] (%)	Specificity[b] (%)
Carib haemoglobin comparator	80	100	100
	100	100	100
Copper sulfate	80	100	80
	100	100	100
Lovibond	80	100	58
	100	98	35
Tallqvist	80	100	90
	100	88	75

[a] Sensitivity = $\frac{\text{No. of samples correctly indicated as being below the cut-off point}}{\text{True no. of samples with value below the cut-off point}} \times 100$

[b] Specificity = $\frac{\text{No. of samples correctly indicated as being above the cut-off point}}{\text{True no. of samples with value above the cut-off point}} \times 100$

The Lovibond instrument gave an unacceptable specificity at both levels. The Carib haemoglobin comparator, the copper sulfate method, and the Tallqvist method gave satisfactory results.

The time taken in the laboratory for each test and the assessments of the laboratory assistants regarding the ease of operation of each instrument are set out in Table 3. They found the copper sulfate method was easy to use; it was also the fastest. However, they preferred the Spencer haemoglobinometer. They found both Dare instruments were hard to use and had difficulties with colour matching in the Dare, Lovibond, and Tallqvist methods.

Stability of copper sulfate solution

The specific gravity of copper sulfate solution kept in closed glass or plastic containers was checked regularly over a period of a year; no change was detected. Solution kept in open vessels in various environments showed a negligible change in specific gravity over an 8-hour period.

We conclude that no errors would result from deterioration of the solution, either during storage or during a clinic session.

Conclusions

The Dare haemoglobinometers and Lovibond haemoglobin field kit performed unsatisfactorily. The Tallqvist method gave good results, but the laboratory technicians did not like it.

Table 3. Time taken for each estimation and ease of operation of the methods tested

Method	Mean time for one estimation (s)	Ease of operation[a]	Observations of technicians
Carib haemoglobin comparator	84	4	Sample holders need improvement
Copper sulfate	60	1	Easy to use
Dare (disposable chamber)	152	6	Matching colours very difficult
Dare (reusable chamber)	210	7	Matching colours very difficult
Lovibond	73	5	Matching colours difficult
Spencer	92	2	Preferred instrument
Tallqvist	120	3	Matching colours difficult

[a] Ranked by the technicians from 1 to 7, in order of increasing difficulty.

The Carib haemoglobin comparator, copper sulfate method, and Spencer haemoglobinometer functioned well and the copper sulfate solutions showed no deterioration with storage over a year or after exposure to the atmosphere for 8 hours.

FIELD TESTING

Four methods were chosen for field assessment in clinics. These were the Carib haemoglobin comparator, the copper sulfate method, the Spencer haemoglobinometer, and the Tallqvist method. The purpose was to assess the ease of operation and accuracy of each instrument as a screening device.

Each method was tested in a separate urban government public health clinic in Jamaica. The technique was first demonstrated to the clinic nurses and community health aides in the respective clinics. Then the health aides were trained until they could use the method correctly and were confident and at ease with it. Two aides in each clinic were then chosen to test the blood of at least 50 patients using the method in which they had been trained. All those chosen had had at least 5 years of secondary education, and all but one had worked for at least 4 years as a health aide. The blood for the test was obtained either from a finger prick or intravenously with the free and informed consent of the patients. At the same time, 20 μl of venous blood were taken from each patient, added to 5 μl of Drabkin solution, and stored in a refrigerator in the laboratory. The haemoglobin level of this sample was estimated by the cyanmethaemoglobin method within, at most, 72 hours (usually within 24 hours) of being taken.

Ease of operation

The Carib haemoglobin comparator was well liked by the health aides who found no difficulty in its use. The necessity for washing and drying the sample holder between each test did not make the instrument unpopular. If the area where the tests were being carried out was dark, the examiner had to move to a sunlit area to read the result.

The copper sulfate method was found to be very easy to use. However, some months after the completion of the study, when the copper sulfate method was in use in many clinics, some lack of confidence in its use was expressed by nurses. There are thought to be three main reasons for this: (1) the test was felt to be less "scientific" and thus less reliable than other tests; (2) there was a lack of appreciation that the behaviour of the drop over the first 10–15 seconds is the important factor; and (3) there was incomplete understanding of the concept of screening (so that, for example, "over 100 g/l" may seem not so useful a statement as, say, "112 g/l"). The first two reasons can be related to the need for careful training and demonstration rather than to a fault in the method itself. The third emphasizes the need to teach the operational consequences of these investigations.

The Spencer haemoglobinometer was very well liked. The nurses and health aides found it easy to use and had no problems in colour matching. No problems developed in the instrument during the two months of testing.

The health aides had great difficulty in matching the colours with the Tallqvist method, which was not liked.

Accuracy

Few of the patients at the clinics had haemoglobin levels below 80 g/l, so that it was not possible to make meaningful comparisons at this level.

The statistical indices of sensitivity and specificity (*17*) at 100 g/l were calculated for each instrument, as had been done in the laboratory testing. Positive and negative predictive values were also calculated (*18*). The positive predictive value indicates the proportion of positive results that are correct and the negative predictive value indicates the proportion of negative results that are correct.

In comparing the results, which are shown in Table 4, it must be remembered that each method was tested

Table 4. Sensitivity, specificity, and positive and negative predictive values of four screening methods, at a haemoglobin level of 100 g/l

Method	Sensitivity	Specificity	Positive predictive value[a]	Negative predictive value[b]
Carib haemoglobin comparator	72.6	96.5	84.2	94.3
Copper sulfate method	87.5	98.9	87.5	98.9
Spencer haemo-globinometer	77.5	95.9	93.9	88.6
Tallqvist method	60.5	59.1	46.0	72.2

[a] Positive predictive value $= \frac{\text{No. of anaemic people so recognized}}{\text{Total no. diagnosed as anaemic}} \times 100$

[b] Negative predictive value $= \frac{\text{No. of non-anaemic people so recognized}}{\text{Total no. diagnosed as non-anaemic}} \times 100$

in a different clinic. Thus the operators and the patients were different.

The clinic test showed the Tallqvist method to be not sufficiently reliable, while the Carib haemoglobin comparator, the copper sulfate method, and the Spencer haemoglobinometer all performed with satisfactory accuracy under clinic conditions in Jamaica.

CONCLUSIONS

Our purpose in this study was to find an anaemia screening device for use at the primary health care level, that would be accurate, acceptable, cheap, simple to operate, sturdy, and easy to use, and that would give immediate results.

We conclude that the Spencer instrument is accurate, acceptable, and sturdy in clinic use. It is, however, too expensive for use at the primary health care level in most countries.

The copper sulfate method is an accurate screening technique, is very cheap and easy to use in primary health care, and is an excellent method for use in clinics. The copper sulfate solutions need to be made up at a central point and distributed every few months.

The Carib haemoglobin comparator is accurate, simple to operate and portable and would be fairly cheap if manufactured on a large scale. We consider that it has great possibilities.

None of the remaining methods — the Dare haemoglobinometer, the Lovibond comparator, and the Tallqvist method — performed satisfactorily.

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RÉSUMÉ

EVALUATION DES MÉTHODES DE DÉPISTAGE DE L'ANÉMIE

Les instruments utilisables au niveau des soins de santé primaires pour dépister les sujets exposés à l'anémie doivent être bon marché, simples à utiliser, robustes, fonctionnant sans source d'électricité et raisonnablement précis entre les mains d'auxiliaires de santé; enfin, ils ne doivent nécessiter au plus qu'un petit nombre d'éléments à remplacer régulièrement et ils doivent fournir des résultats immédiats.

Après avoir passé en revue les méthodes disponibles, on a retenu, en vue d'essais de laboratoire, la méthode au sulfate de cuivre et celles de Dare, Lovibond, A. O. Spencer et Tallqvist. De plus, on a mis au point et expérimenté un nouvel instrument, l'hémoglobinomètre Carib.

Avec ce dernier appareil, un comparateur colorimétrique, on hémolyse par la saponine une goutte de sang placée dans une chambre transparente. On observe la lumière du jour ou la lumière d'une source à incandescence en interposant sur le trajet lumineux d'une part l'échantillon d'autre part deux filtres neutres permettant des comparaisons d'intensité lumineuse (et correspondant, dans notre essai, à des taux d'hémoglobine de 80 et de 100 g/l) ainsi qu'un filtre vert qui élimine les différences de couleur. L'observateur note si le sang lui apparaît plus foncé que le filtre foncé, plus clair que le filtre clair ou d'intensité intermédiaire et il en déduit l'ordre de grandeur du taux d'hémoglobine.

Chaque instrument a été essayé en laboratoire par deux techniciens qualifiés et l'on a comparé les résultats obtenus aux valeurs «exactes» fournies par la méthode à la cyanméthémoglobine.

Avec l'instrument de Dare, l'écart-type a été trop élevé pour être admissible, tandis que l'instrument de Lovibond ne présentait pas une spécificité acceptable aux points limites (80 et 100 g/l). En revanche, l'hémoglobinomètre Carib, la méthode au sulfate de cuivre et la méthode de Tallqvist ont donné des résultats satisfaisants.

Sur la base des résultats de laboratoire, l'hémoglobinomètre Carib, la méthode au sulfate de cuivre, l'hémoglobinomètre, de Spencer et la méthode de Tallqvist ont été essayés dans des dispensaires de la Jamaïque en vue d'établir s'ils étaient suffisamment commodes à employer et précis pour servir de moyen de dépistage.

Chaque méthode a été expérimentée par deux agents de la santé communautaire auxiliaires ayant fait des études secondaires pendant au moins 5 ans. Ces auxiliaires ont eu beaucoup de mal à établir la bonne correspondance de couleurs

avec la méthode de Tallqvist, tandis qu'ils ont apprécié les trois autres méthodes.

On a calculé la sensibilité, la spécificité et la valeur pronostique négative ou positive (faux et vrais positifs) de toutes ces méthodes et trouvé des résultats satisfaisants dans tous les cas sauf pour la méthode de Tallqvist.

La conclusion est que l'hémoglobinomètre de Dare, le comparateur de Lovibond et la méthode Tallqvist ne donnent pas satisfaction. L'instrument de Spencer est fiable mais son coût en exclut l'emploi au niveau des soins de santé primaires; en revanche, la méthode au sulfate de cuivre et l'hémoglobinomètre Carib sont tous deux excellents en vue d'une utilisation à l'endroit même où sont mis en œuvre les soins de santé primaires.

REFERENCES

1. WHO Technical Report Series, No. 405, 1968 (*Nutritional anaemias*: report of a WHO Scientific Group).
2. WHO Technical Report Series, No. 580, 1975 (*Control of nutritional anaemia with special reference to iron deficiency*: report of an IAEA/USAID/WHO Joint Meeting).
3. PHILLIPS, P. R. ET AL. Measurement of specific gravities of whole blood and plasma by standard copper sulphate solutions. *Journal of biological chemistry*, **183**: 305-330 (1950).
4. VAN SLYKE, D. D. ET AL. Calculation of hemoglobin from blood specific gravities. *Journal of biological chemistry*, **183**: 349-360 (1950).
5. DARE, A. A new hemoglobinometer for the examination of undiluted blood. *Philadelphia medical journal*, **6**: 557 (1900).
6. FOY, H. & KONDI, A. Haemoglobin measurement in developing countries. *Lancet*, **2**: 401 (1977).
7. WORLD HEALTH ORGANIZATION. *Manual of basic techniques for a health laboratory*. Geneva, World Health Organization, 1980.
8. SEIVERD, C. E. *Hematology for medical technologists*. 3rd ed., Philadelphia, Lea and Febiger, 1970.
9. ELWOOD, P. C. & JACOBS, A. Haemoglobin estimation: a comparison of different techniques. *British medical journal*, **1**: 20-24 (1966).
10. TALLQVIST, T. W. Méthode pratique d'évaluation directe de la quantité d'hémoglobine du sang. *Archives of general medicine*, **3**: 421-425 (1900).
11. GAMMON, A. & BAKER, S. J. Studies in methods of haemoglobin estimation suitable for use in public health programmes. *Indian journal of medical research*, **65**: 150-156 (1977).
12. CARIBBEAN FOOD AND NUTRITION INSTITUTE. *The copper sulphate method for screening for anaemia*: a manual for its use. Kingston, Caribbean Food and Nutrition Institute, 1982.
13. ANDRIANASOLO, R. ET AL. An evaluation of a simplified method for screening haemoglobin in the field. *American journal of clinical nutrition*, **32**: 728-730 (1979).
14. ANDRIANASOLO, R. *An evaluation of programs to control anemias of pregnancy in Jamaica*. Ithaca, Faculty of the Graduate School, Cornell University 1980 (Ph.D. thesis).
15. BONE, R. A simple haemoglobin screening device. *Appropriate technology*, **9**: 27-29 (1983).
16. HAINLINE, A., JR. Hemoglobin. In: Seligson, D., ed., *Standard methods of clinical chemistry*. New York, Academic Press, 1958, vol. 2, pp. 49-51.
17. TALLQVIST, T. W. Ein einfaches Verfahren zur directen Schatzung der Farbestarke des Blutes. *Zeitschrift für klinische Medizin*, **40**: 137-141 (1900).
18. HABICHT, J. P. Some characteristics of indicators of nutritional status for use in screening and surveillance. *American journal of clinical nutrition*, **33**: 531-535 (1980).