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Comparison of haemoglobinometry by WHO Haemoglobin Colour Scale and copper sulphate against haemiglobincyanide reference method

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Summary Although estimation of haemoglobin is essential for diagnosing anaemia and assessing its severity, many health centres in developing countries do not have the facilities for haemoglobinometry. The WHO Haemoglobin Colour Scale (HCS) method is a simple and inexpensive clinical device that was recently developed in order to diagnose anaemia in such centres. In Indonesia, the copper sulphate specific gravity method is used for blood donor screening and also in primary health clinics in the rural and remote areas. In this study, the HCS method is compared with the copper sulphate method and with an earlier paper scale, the Tallquist method, against the standard haemiglobincyanide spectrophotometric method. The HCS method showed an acceptable level of precision and accuracy for use as a reliable screening tool to diagnose anaemia in patients and also for blood donor screening.

Keywords Haemoglobin Colour Scale, copper sulphate, haemoglobinometry, anaemia screening, blood donors

Introduction

Haemoglobin measurement is essential for diagnosis of anaemia, for assessing its severity and for management of patients. There are several methods that can be used to measure the haemoglobin concentration in the blood. Most tests are based on the measurement of colour changes when reagent is added to blood, ranging from spectrophotometry against a reference standard (ICSH, 1995), to a simple paper scale as originally proposed by Tallqvist (1900); another method for screening at a given level of haemoglobin is the measurement of the density of blood by means of a copper sulphate solution of specified specific gravity (Philipps, 1950). Measurement such as haemiglobincyanide (HiCN) is the recommended method of the International Committee for Standardization in Haematology (ICSH), but for this a spectrophotometer or photometer and stable electricity are needed. In areas where such facilities are not accessible, such as in the rural and remote areas in Indonesia, this method is not feasible. In many of the Primary Health Care centres, the Tallquist method is still used for determining haemoglobin values, while the Indonesian Red Cross Blood Banking Unit (1998) uses the copper sulphate method ($CuSO_4$) at a specified cut-off point for selecting non-anaemic prospective blood donors (Tyler *et al.*, 1999).

The Haemoglobin Colour Scale (HCS) is a recently introduced method for estimating haemoglobin which has been developed for WHO. It is based on comparison of the colour of a drop of blood absorbed on a special test strip with a range of colour shades equivalent to haemoglobin of 4, 6, 8, 10, 12 and 14 g/dl, respectively. Its principle is the same as for the Tallquist method, but altered beyond recognition by modern technology (Stott & Lewis, 1995; Lewis, Stott & Wynn, 1998): after extensive trials a special chromatography paper 31ET Chr (Whatman International, Maidstone, UK) was selected for use as the test strip matrix for the drop of blood, selected dye mixtures representing the different haemoglobin concentrations were identified by analysis of the colour spectra of those haemoglobins, computerized print was used to print the colour shades on a card which was then bound into a specially designed mount. The end result is a simple and inexpensive device that can be easily used in small health centres in remote or rural areas (Munster *et al.*, 1997; Tatsumi *et al.*, 1999). The aim of our study was to establish its clinical utility and

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to assess the possibility of using it as an alternative test in screening donors for anaemia in blood transfusion centres and primary health care centres.

Materials and methods

Study design

Blood from the donors in the Indonesian Red Cross Blood Transfusion Centre, Jakarta, was used to obtain reference value for haemoglobin concentration and to calculate the correlation between the HiCN reference method and the HCS, the Tallquist Scale and the copper sulphate method, respectively. Blood from the Haematology unit (Clinical Pathology Department, Cipto Mangunkusumo Hospital, Jakarta) was used to calculate the sensitivity and specificity of the HCS and copper sulphate method for detecting anaemia and estimating the degree of anaemia. For blood donor screening, the discrimination point for anaemia was set at 12 g/dl, as defined by WHO (Evatt *et al.*, 1992).

Materials

One drop of capillary blood (about 20 µl) and 2 ml of venous blood collected in disodium-EDTA were obtained from 240 healthy blood donors (120 males and 120 females) at the Transfusion Blood Center Indonesian Red Cross (Jakarta). The capillary blood was used to estimate haemoglobin concentration by the HCS; the EDTA samples were used for repeating the haemoglobin determination by the HCS, and also with the old paper scale, copper sulphate and the HiCN method.

From the Hematology unit (Clinical Pathology Department, Cipto Mangunkusumo National Referral Hospital, Jakarta) 150 blood samples were collected into disodium-EDTA and analysed with the four method for haemoglobin determination. The blood samples were either obtained from specimens that had been collected for routine clinical laboratory tests or specifically for this study from volunteers in accordance with ethically approved procedure.

Methods

Haemoglobin Colour Scale

A drop of blood (20 µl) was applied to a test strip of paper to form a blood spot of approximately 1 cm. After 30 s and not later than 2 min after application of blood, the colour was compared with the set of colour shades by holding the test strip behind the scale apertures avoiding direct sunlight or marked shadows. The haemoglobin value recorded corresponded to the closest colour standard match or intermediate between two colours at 1 g/dl intervals. This method were evaluated using non-anticoagulated capillary blood and the disodium-EDTA blood, performed twice by one technician and confirmed by a medical doctor (Lewis *et al.*, 1998; Ingram and Lewis, 2000).

Tallquist Paper Scale method

This differs from the HCS in that the colours range from yellow to brown in a scale of 10–100%, which is claimed to represent a haemoglobin range from 1.58 to 15.8 g/dl.

Copper sulphate method

Working solutions of $CuSO_4$ with specific gravity range of 1.035–1.062 (representing haemoglobin value of 3–17 g/dl) were prepared fresh and calibrated each day. A drop (about 20 µl) of blood was dropped into each of a series of tubes containing copper sulphate solutions and observed. The tube where the drop of blood neither rises nor falls indicates the specific gravity of the blood. The haemoglobin value was calculated as:

$$\text{Hb (g/dl)} = 480 \times (\text{SG of } CuSO_4 - 1.026).$$

Haemiglobincyanide method

Twenty millilitre of blood were added to 5 ml of cyanide-ferricyanide solution; the absorbance was read using a spectrophotometer (Perkin Elmer Corporation, Wellesley MA, USA), and the haemoglobin values were calculated using the ICSH/WHO International standard. A fresh working solution was prepared each day. The measurement obtained by this method were used as gold standards for the other methods (ICSH, 1996).

Preliminary study

For each of the methods, within-run precision was determined by 10 consecutive measurements and between-run precision over 10 days, using commercial blood control material Equinox 16T (Hematronix, Plassay, Limerick, Ireland).

Statistical analysis

Statistical analysis (Santoso, 1999) was performed from the data collected using Statistical product and Service Solution (SPSS) 10.0 for Windows (Microsoft, Seattle, WA, USA).

Table 1. Precision of the methods for haemoglobin determination

	HCS (1 g/dl intervals)	$CuSO_4$ (0.5 g/dl intervals)	HiCN (0.1 g/dl intervals)
Within-run consecutively			
1.	14	13.5	13.7
2.	14	14	13.8
3.	14	14	13.8
4.	14	13	13.8
5.	14	14	3.8
6.	14	13.5	13.8
7.	14	13	13.8
8.	14	13.5	13.8
9.	14	13.5	13.7
10.	14	14	13.7
Mean (g/dl)	14	13.6	13.77*
SD (g/dl)	0	0.39	0.05
CV (%)	0	2.86	0.36
	WHO Colour Scale (1 g/dl intervals)	Copper sulphate (0.5 g/dl intervals)	HiCN (0.1 g/dl intervals)
Between-runs (daily)			
1.	14	14	13.7
2.	14	14	13.8
3.	14	14	13.8
4.	14	13.5	13.8
5.	14	13	13.7
6.	14	13	13.8
7.	14	14	13.8
8.	14	14	13.8
9.	14	13.5	13.7
10.	14	13.5	13.7
Mean	14	13.65	13.76*
SD	0	0.41	0.05
CV (%)	0	3	0.36

*If rounded to nearest integral number the mean is 14 g/dl and CV zero.

Results

Precision

The coefficient of variation (CV) of within-run precision for HCS at intervals of 1 g/dl, $CuSO_4$ at 0.5 g/dl and HiCN at 0.1 g/dl were 0, 2.66 and 0.36% respectively; between-run CVs were 0, 3 and 0.36% respectively (Table 1). However, when the HiCN measurements were rounded to the nearest g/dl, so as to be comparable with the HCS, the CV was negligible.

Assessment of HCS efficacy using blood donor blood and anaemic patients' blood

A comparative study of the HCS, Tallquist Scale, $CuSO_4$ and HiCN methods was carried out on the 390 blood samples described above. Good correlation was found between HiCN and HCS, between HiCN and $CuSO_4$ and between $CuSO_4$ and HCS. The correlation between HiCN and Tallquist Scale was poor (Table 2). HCS readings gave similar results with anticoagulated venous and non-anticoagulated capillary blood samples.

Normal reference values

The normal haemoglobin reference values were calculated from the 240 donors using the HCS. The data showed a non-Gaussian distribution and the reference values were

Table 2. Correlation and linear regression analysis

Reference method	Testing method	Correlation	*P*-value	Regression
HiCN	HCS	0.914	0.000	$Y = 1.044 \times -0.745$
HiCN	$CuSO_4$	0.941	0.000	$Y = 0.932 \times +0.827$
HiCN	Tallquist	0.394	0.000	$Y = 0.658 \times -3.660$
$CuSO_4$	HCS	0.893	0.000	$Y = 1.068 \times -0.347$

Table 3. Utility of HCS compared with HiCN method for diagnosing anaemia

	Haemoglobin Colour Scale		
HiCN	Anaemic	Nonanaemic	Total
Anaemic	149	1	150
Nonanaemic	0	240	240
	149	241	390
True positive	149		
False positive	0		
False negative	1		
True negative	240		
Sensitivity (%)	0.99		
Specificity (%)	1.00		
Positive predictive value	1.00		
Negative predictive value	0.99		

Table 4. Utility of $CuSO_4$ compared with HiCN method for diagnosing anaemia

	Copper sulphate		
HiCN	Anaemic	Nonanaemic	Total
Anaemic	143	7	150
Nonanaemic	0	240	240
	143	247	390
True positive	143		
False positive	0		
False negative	7		
True negative	240		
Sensitivity (%)	0.95		
Specificity (%)	1.00		
Positive predictive value	1.00		
Negative predictive value	0.97		

calculated as 13–14 g/dl for men and 12–14 g/dl for women.

Clinical efficacy of HCS for diagnosing anaemia

The results of sensitivity and specificity tests for identifying the anaemic group, and distinguishing severity of anaemia by the various methods are given in Tables 3–6.

Discussion

In the modern haematological laboratory, fully or semi-automated haematology analysers provide precise and accurate blood count (complete blood cell count) data, which are very useful in diagnosing all haematological disorders. But in developing countries such as Indonesia, only about 30% of health service providers and hospitals have such high performance blood cell analysers (Silman *et al.*, 2000). Furthermore, many primary health care centres have no stable electricity, not even equipment for manual methods and no experienced medical technologist. In such areas, many of the people suffer from nutritional anaemia and infections. Thus health workers in the Indonesian region of Puskesmas have no way to diagnose anaemia other than by clinical signs which are notoriously unreliable (Ingram and Lewis, 2000; Montresor *et al.*, 2003). Diagnosing infection with fever can be carried out without white blood cell count, bleeding can be observed without platelet count but diagnosis of anaemia remains the major problem and haemoglobin testing should be given the highest priority.

The HiCN method, based on the ICSH/WHO international standard is accepted as the most reliable method for

Table 5. Utility of HCS for diagnosing the degree of anaemia

	Haemoglobin Colour Scale (g/dl)					
HiCN (g/dl)	<6	6 to < 8	8 to < 10	10 to < 12	≥12	Total
<6	4	0	0	0		4
6 to < 8	2	14	7	0		23
8 to < 10	0	2	44	8		54
10 to < 12	0	0	9	59	1	69
Total	6	16	60	67	1	150
True positive	4	14	44	59	1	
False positive	2	2	16	8		
False negative	0	9	10	10		
True negative	144	125	80	73		
Sensitivity (%)	100	60.9	81.5	85.5		
Specificity (%)	98.6	98.5	83.3	90.1		
Likelihood ratio	71.4	40.6	4.9	8.6		
Positive predictive value	0.67	0.78	0.73	0.88		
Negative predictive value	1.0	0.93	0.89	0.88		

Table 6. Utility of $CuSO_4$ for diagnosing the degree of anaemia

	Copper sulphate (g/dl)					
HiCN (g/dl)	<6	6 to < 8	8 to < 10	10 to < 12	≥12	Total
<6	3	1	0	0	0	4
6 to < 8	1	16	5	1	0	23
8 to < 10	0	11	30	13	0	54
10 to < 12	0	1	8	53	7	69
Total	4	29	43	67	7	150
True positive	3	16	30	53		
False positive	1	13	13	14		
False negative	1	7	24	16		
True negative	145	114	83	67		
Sensitivity (%)	75	69.6	55.6	76.8		
Specificity (%)	99.3	89.8	86.5	82.7		
Likelihood ratio	107	6.8	4.1	4.4		
Positive predictive value	0.75	0.55	0.70	0.79		
Negative predictive value	0.99	0.94	0.77	0.81		

haemoglobinometry, but it requires a skilled technician, stable electricity and complex equipment. Other simple techniques include the Hemocue method that uses a drop of blood, giving rapid and reliable results. However the equipment and disposable cuvettes are too expensive for peripheral health centres and it requires electricity or batteries. This present study was undertaken to assess the available simple methods and to determine whether the HCS could provide an answer to the problem.

Some centres use the obsolete Tallquist Paper Scale method which has the virtue of being easy to use and cheap. But it is unreliable because the colour of the standards ranged from yellow to brown, representing haemoglobin levels that could not be compared with the real colour of blood on the test strips. The Tallquist Paper Scale is an early forerunner of the WHO HCS which has been developed as an inexpensive pocket-sized clinical device, independent on electric power, and easy to operate after a short period of familiarization and education. By contrast to the Tallquist Scale, the HCS has a range of colours that resemble the blood spots at different haemoglobin concentrations, and are thus much easier to compare. Thus, HCS is more reliable and the results showed a good correlation compared with the HiCN standard method. Calculation of the normal reference values for haemoglobin in the Indonesian population showed similar results with the HCS and the standard method.

We also assessed other methods. In Indonesia the blood bank use the copper sulphate method at a selected specific density to screen for anaemia in blood donors; this is also used in some clinical settings to determine the actual haemoglobin by means of copper sulphate solutions in a range of specific gravities. This method has many technical problems in its preparation, it requires frequent checks on the specific gravity of the preparations, it is difficult to transport and after use it is an environmentally toxic waste. Our data showed that the HCS is as reliable as the copper sulphate method for measuring haemoglobin concentration and there was also good correlation between the HCS and $CuSO_4$ in selection of blood donors. Thus, as also shown by Lewis and Emmanuel (2001) HCS could be used for routine screening of blood donors. It is certainly more practical and easier to perform than the $CuSO_4$ method.

In conclusion, our results showed that with the HCS it is possible to obtain clinically acceptable levels of precision and accuracy for haemoglobin estimation in diagnosing anaemia and for blood donor screening. It can be used anywhere and at any time without electricity, bench-equipment or technical expertise. There were no differences in results between anticoagulant venous blood and non-anticoagulated capillary blood.

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