STOP THE PRESSES

Point of Care Haemoglobin Estimation

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Citation

Introduction

The prevalence of anemia in India is still unacceptably high at 53.1 % in non-pregnant women, 50.3% in pregnant women, 58.4% in children 6 months and 22.7% in adult men(1) and remains a major public health problem. For screening, tracking as well as monitoring the progress of anemia in both in primary and community health care for public health programs, hemoglobin needs to be estimated accurately.

In recent years, point of care (POC)testing is increasingly being used and is defined as the any test performed in closest proximity to the patient outside the controlled conventional laboratory site and which provides rapid results(2)

Point of care (POC) hemoglobin estimation needs to be accurate, reliable and valid to get good estimates of prevalence of anemia particularly in resource poor settings including primary health care centers and community centres and field settings(3). Both quantitative and qualitative methods have been used for POC testing (3).

Blood collected from individuals are used to estimate hemoglobin and thereby to determine the extent of anemia prevalence in a population. Hemoglobin is usually measured on venous blood or capillary blood and sometimes in clinical situations in arterial blood (4). In controlled settings as in a hospital laboratory, presently automated analyzers are used. However, in field and emergency situations portable analyzers are routinely used. In general, measurement of venous blood in a clinical laboratory using the cyanmethemoglobin method or using automated analyzers are considered the gold standard for estimating the prevalence of anemia.

Several factors in measurement could contribute to variability in hemoglobin estimates. The variability between instruments, the type of blood sample collected and measurement issues while collecting blood could contribute to the variability in estimates of hemoglobin.

This background paper provides information on the different methods used for estimation of hemoglobin, the factors that affect the results of POC testing of hemoglobin estimates in clinical and field settings and the resultant public health implications.

Methods of hemoglobin estimation

The various instruments presently commonly used, the principles of analysis and the pros and cons of using the instrument are presented in Table 1. Apart from the gold standard of cyanmethemoglobin method and the automatic analyzers, the POC testing for hemoglobin estimation uses 3 types of technologies: (1) smaller bench top analyzers which are smaller versions of laboratory analyzers providing a full blood count, red blood cell indices and either a 5- 3-part white cell differential (2) handheld devices using invasive methods (venous, capillary or arterial blood) (3) handheld devices using non-invasive technology (2,5).

Factors affecting POCT of hemoglobin:
(**Sub Headings:** Study Type, Study Population, Study Area, Study Duration, Sample Size

calculation, Inclusion Criteria, Exclusion Criteria, Strategy for collection, Working Definition, Ethical Approval, Consent, Data Analysis - Software, Flow Diagram Required)

Instrument used to estimate hemoglobin:

As early as 1948, it was noted that no method measures hemoglobin directly, as all methods involve computations which are not equally accurate(11). Hence inherent instrument differences in hemoglobin values persist due to precisely the same reason coupled with different technologies used.

For accuracy and reliability of measurement, both sample collection and analysis technique are critical (12). A method could be precise providing close values across repeated measurements but need not be accurate, as for example an instrument could repeatedly give a value close to 10.5 g/dL of hemoglobin while the actual value may be 11.5 g/dL. Previous evaluations of the HemoCue have revealed conflicting results, with some studies presenting a good precision and accuracy of this equipment, and others emphasizing the need of a better methodological control. In the study by Paivaa et al(8), the HemoCue provided higher values of hemoglobin compared to the automated analyzer. There was high agreement between the diagnosis of anemia by both equipment (k=0.81; p<0.01).

Hemoglobin values from venous blood samples of 33 adult subjects were compared using the HemoCue 201 and the automated analyzer (Beckman Coulter) at the laboratory at St John's Medical College, Bangalore (unpublished, Kurpad AV). The mean values using the automated analyzer was 11.5 ± 0.88 g/dL while that measured using the HemoCue was 12.0 ± 1.1 g/dL with the mean difference being 0.45 ± 1.36 g/dL with values in the HemoCue being higher. The lower limit of agreement was -2.24 g/dL and the upper was 3.15 g/dL.

Figure 1: Comparison of hemoglobin values between the Automated analyzer and the HemoCue 201

Measurement of hemoglobin concentration by HemoCue in pregnant women provides lower precision than that of automated analyzers, and its precision also varies by sample type (i.e. whether the sample is taken from a capillary or vein) (13).

A review by Sobhy et al (13) on diagnostic accuracy of POCT of hemoglobin versus reference laboratory tests during pregnancy reported that Copper sulfate provided 97% sensitivity (95% confidence interval [CI] 88%–100%) and 71% specificity (95% CI55%–85%); the Sahli method provided 86% sensitivity (95% CI 75%–94%) and 83% specificity (95% CI 68%–93%); and HemoCue provided 85% sensitivity (95% CI 79%–90%) and 80% specificity (95% CI 76%–83%). Sari et al (14), compared values obtained through direct cyanmethemoglobin, indirect cyanmethemoglobin and HemoCue using both venous and capillary blood. Using the indirect cyanmethemoglobin method prevalence of anemia was reported to be between 31 to 38%. The direct cyanmethemoglobin method and the HemoCue indicated a prevalence of 14 to 18%. (Sari et al, 2001). In an Indian study of 888 adolescent girls (Bansal et al 2016) aged 11-18 years residing in an urban slum in Delhi, hemoglobin was measured by both direct and indirect cyanmethemoglobin methods on venous blood. For the indirect method, a spot of blood was collected on Whatman's filter paper and then eluted into the Drabkin's solution and the hemoglobin measured. The estimated mean hemoglobin levels recorded were 116.1 ± 12.7 using the direct method and 110.5 ± 12.5 g/l using the indirect method with a mean difference of 5.67 g/l (95% confidence interval: 5.45 to 5.90, P<0.001) which translates to a difference of about 0.567 g%. The prevalence of anaemia was reported as 59.6 by the direct method and 78.2 per cent by the indirect method. The sensitivity and specificity of indirect cyanmethemoglobin method were 99.2 (95% CI: 98.0 to 99.7%) and 56.4 (95% CI: 51.3 t0 61.4) per cent, respectively. Thus a higher prevalence of anemia could therefore be recorded using the indirect method of collection. The authors however, suggested the use of a correction factor of 0.567 g% hemoglobin.

An Indian study (15) on children 1 to 6 years of age reported mean values of 9.33 ± 2.72 by Hemocue and 8.14 ± 2.45 by cyanmethemoglobin method with prevalence of anemia at 66% with the HemoCue and 88% with the cyanmethemoglobin method. Compared to the cyanmethemoglobin method, sensitivity of Hemocue method was 0.75 and specificity 1.0. There was an overestimation by 10 to 15% in hemoglobin values when the HemoCue was used.

The diagnostic accuracy of using HCS against clinical accuracy solely by clinical signs indicated that sensitivities ranged from 33% to 96% and specificities from 14% to 100%. However, in resource poor settings it was noted that accuracy of diagnosis

was significantly increased as 48% of diagnosis of mild to moderate anemia could be missed by resorting to clinical examination alone and HCS could reduce the proportion to 20% (16).

Type of blood sample:

Studies have used capillary, venous and/or arterial blood to evaluate the effect of the type of blood sample on hemoglobin measurements.

Yang et al (17) examined the differences between capillary, venous and arterial blood measured in an automated analyzer in adults. Coefficient of variation (CV) between finger prick and venous blood for hemoglobin measured was 2.45 ± 1.32. The CV was significantly higher as measured with the capillary or arterial blood than with the venous blood.

Chen et al (18) , studied forty-two intensive care unit patients and compared the hemoglobin measurements obtained in capillary, arterial, and venous blood samples, analyzed by the HemoCue and an automated counter. The HemoCue gave repeatable results when hemoglobin estimates were made using either venous or arterial samples, but capillary estimates were found to be significantly less repeatable.

There have been conflicting results from studies that have compared specifically the use of capillary or finger stick method with the venous blood for measurement of hemoglobin with some studies showing no significant difference, some showing higher values with use of the capillary blood, and some lower values (5) (Chaudhary et al). In a study by Paiva et al (8), the HemoCue showed low repeatability of Hb measurements in duplicate in capillary (CR=0.53 g/dL, CV=13.6%) and venous blood (CR=0.53 g/dL, CV=13.6%). There was high agreement between Hb in capillary blood by the HemoCue and in venous blood by the counter (ICC=0.86; p<0.01). Hemoglobin levels in capillary blood were higher than those in venous blood (12.4 and 11.7 g/dL, respectively; p<0.05). They concluded that HemoCue seems to be more appropriate for capillary blood but requires adequate training of the measurers (8). The estimate of hemoglobin measured on capillary blood was higher than venous blood in both children and adults. A difference of +0.17 g/dl (p= 0.15) was noted in children while a difference of +0.59 g/dl (p< 0.0001) for samples from adults was noted when using the Hemocue (19).

In a study of 36,000 paired venous and capillary samples of blood donors, measured capillary hemoglobin was 12.4 g/dL in females (n =25 762),

and 13.4 g/dL in males (n= 10 496). In general, the mean measured capillary values were lower than the venous values, by 1.07 g/dL in males and 0.67 g/dL in females. It was noted that as the hemoglobin level increased the difference between capillary and venous blood decreased (20,21). The mean difference between capillary and venous hemoglobin increased in females from 45-50 years, while in males there was a gradual decline with age. The gap between capillary and venous blood is attributed to the Fähreus effect where there is a relative dilution effect in capillaries due to the difference in flow speeds between the centrally concentrated cells which are faster and the peripheral displaced plasma which flows much slower. This difference is based on age, sex and red cell count, with higher viscosity with higher red cell count. Further, there up-regulation by estrogen in pre-menopausal women by increasing the nitric oxide production leading to vaso-dilution could modulate the Fahreus effect (21).

In children and in adults a higher hemoglobin concentration was reported in venous blood compared to capillary blood, with mean difference being higher in children at about 0.5 g/dL. Thus prevalence of anemia is likely to be much lower when hemoglobin is estimated using venous blood (22). It was observed that only when a deep puncture was made and when at least 0.5 ml of blood was collected results obtained through venous and capillary blood matched reasonably well.

Other factors:

There could be both anatomical and technical reasons that affect the hemoglobin result. For methods using finger prick, with the source of blood being the loop capillaries fluctuation in values is possible with the temperature of the skin, the extent of skin penetration and the dilution by fluids from the extracellular tissue due to the variation in skin pressure. The size of the lancet used, the way in which it is applied and inherent individual level difference in blood droplets from capillaries could affect the result (5). Postural factors also affect the hemoglobin levels with studies among blood donors indicating differences in hemoglobin levels with standing and recumbent postures. Standing increases hemoconcentration in the lower extremities with intravascular fluids moving into the interstitial spaces compared to the recumbent position when hemodilution occurs (5,23).

Physiological factors such as puberty, menopause, climate acclimatization, fitness level, lean body mass and some acute factors such as posture and level of hydration also affect hemoglobin levels (21).

Synthesis and way forward:
Hemoglobin measured through different sources do show variability in values obtained due to several reasons such as instrument variability, type of blood samples and due to other factors. It should be noted that when testing accuracy and reliability of POC hemoglobin estimates, typically when venous blood levels are compared, the whole blood sample collected can divided into 2 for measurement on the gold standard and the POC testing device, which is a problem as capillary blood is not accessed. The same, however, cannot be done when using the POC testing device where in most POC situations capillary blood is used. Capillary blood cannot be divided into 2 and measured on each device. Most investigators have compared venous hemoglobin by gold standard versus fingerstick hemoglobin by POC testing or venous blood on both devices. The problem in such cases is that 2 differences arise due to firstly the instrument and secondly to sampling method. Some investigators have tested venous blood on the gold standard and the fingerstick capillary blood on the POC testing device which means 2 pricks on the individual being tested.

Despite this, POC testing of hemoglobin is still useful as it is a rapid method that facilitates rapid diagnosis (24). Although POC determination of hemoglobin is rapid and cost-effective, these values should be further confirmed by standard methods used in laboratories. A test should have high sensitivity if screening for anemia is used to decide which patients should be referred for further investigation and treatment (13). Using a test with high sensitivity but low specificity leads to unnecessary referrals for further investigation and so adds to costs for the health service. Hence decisions on the methods to detect the level of hemoglobin and consequently the prevalence of anemia should be carefully considered especially in resource poor settings.
Standardized training and supervision are important to get accurate and reliable estimates through POC hemoglobin. Even in clinical practice, POC testing of hemoglobin is found to be a simple and useful screening test. It is only in a smaller proportion of cases that the full blood count is required and further

investigation is warranted only if clinical signs or a patient's history indicates that further tests need to be done (25).

In resource poor settings, the Hemocue is largely used for POC estimation of hemoglobin both in the clinic/hospital setting or in the community setting. The HemoCue is a portable device, requiring only a small sample of capillary or venous blood which is simple to use and relatively inexpensive with access to refrigeration or even electricity not being required, and gives a digitally displayed hemoglobin value immediately (26). To reduce inter- and intra-individual variability it is essential to have good practice and training of all technicians to get accurate and reliable measurements particularly when capillary blood is estimated (12). To ensure good results the technique of sticking fingers and getting a large enough blood drop should be done through a standardized protocol as these are 2 error prone steps but the key to getting good results. By standardizing techniques of blood collection and by maintaining standardized protocols for analysis, some of the errors in measurement could be overcome.

There have been contrary views on the differences in hemoglobin concentrations in males and females. Some investigators have questioned the present normal reference ranges of hemoglobin values as the values were derived from apparently normal populations without defining the population base as it is not clear what sampling techniques was used and what criteria were employed to define these ranges (27). Rushton and Barth (27) contend that these values were possibly derived from iron deficient women and hence the lower range in women while no gender difference exists. There are no significant gender differences in pre-pubertal hemoglobin levels until the onset of menstruation in women until many years after the menopause, when levels become similar again, although population data from some countries indicate that no gender differences are likely to exist. If this is the case this could imply that the present ranges are low for women and that the actual percentage of women with high anemia levels could be much higher. However, the authors conclude that to demonstrate that lower limits of hemoglobin and ferritin are the same in both genders after matching for age and weight, studies with iron replete individuals (serum

ferritin above 100 ug/dL) are required. Murphy (21) argues that the difference between males and females in venous hemoglobin and red cell mass is likely due to sex hormonal levels with androgen levels in males having a higher stimulatory effect.

The RISE study showed that capillary hemoglobin measurement systematically overestimated hemoglobin value compared to venous values in severely iron-depleted among blood donors (23). Values were higher than venous blood when values were closer to the higher clinical range, but lower in the lower side of the clinical range. Although capillary blood was a good predictor of venous hemoglobin, females with low normal hemoglobin and anemic donors were found to be incorrectly classified (5,23). Thus, with India having a high anemic population with maybe iron depletion, values from POC testing of hemoglobin need to be viewed with caution.

Further, the Fåhreus effect also plays a role when capillary blood is used as diameters of the vessel determine the mean content of the red blood cells with a decrease in red blood cells due to decrease in flow rate in the narrower blood vessels whether capillaries, venules or arterioles particularly in vessels below 300 µm in diameter. The mean hematocrit (21,28). The mean hematocrit levels in vessels with diameters below 300 µm varies in proportion to the mean diameter of the blood vessel. Added to this the microvasculature in females contains higher red cells per volume blood than males although the average red blood cell mass per unit body weight is lower in females as microvascular vasodilation increases the red cell count through the Fåhreus effect (20,21). As a consequence, several factors are likely to play a role in the measured value of hemoglobin obtained.

POC hemoglobin estimation is still a useful method to detect anemia. But efforts to reduce errors by using standardized protocols is necessary with both internal and external quality control measures taken.

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Tables

TABLE 1 COMMONLY USED METHODS OF ESTIMATION OF HEMOGLOBIN

Measurement method/Principle	Procedure	Merits	Demerits
Gold standard- Invasive			
Cyanomethemoglobin (HiCN) (5,6)	Dilution of 20 μL of whole blood in 5 mL of Drabkin's solution. Conversion of hemoglobin to cyanmethemoglobin by the addition of Potassium cyanide and ferricyanide whose absorbance is measured at 540 nm against a standard solution	1. Standard reference available 2. Internationally available reference standard calibrator	1. Toxic cyanide reagents used 2. Time consuming 3. Main cause of error is turbidity of the blood and the large dilution of the sample (20 μL of blood in 5 mL of Drabkin's solution) 4. Requires skilled trained technical personnel
Hematology Analyzers Non-cyanide method (5,6)	Compact, fully automated hematology analyzer with simultaneous analysis of 18 -22 parameters in whole blood mode and capillary blood mode	1. Accurate and reliable 2. Quick results 3. Apart from hemoglobin provides red cells, white blood cells, platelets and hematocrit	1. Requires laboratory space. 2. High and regular maintenance. 3. Not suitable for non-laboratory environments 4. Expensive
5. **Point of Care testing of Hemoglobin- Invasive**			
Sahli's method (5,6)	HCl converts hemoglobin to acid hematin, which is then diluted until the color of the solution matches that of the comparator block.	1. Used both in clinical laboratories and for field studies 2. Simple and inexpensive	1. Results are not always precise Inter-observer variability 2. Highly prone to errors due to manual pipetting
Copper sulphate method (6)	Based on specific gravity of blood. A blood droplet is put into a solution of copper sulphate with specific gravity equivalent to that of hemoglobin of 100 g/L and 80 g/L. Test results are reported as below 80 g/L, 80 to 100 g/L and above 100 g/L	1. Easy to use	1. Inaccurate as quantitative results not provided
Measurement method/Principle	Procedure	Merits	Demerits
HemoCue (201, 301) (5–8)	Quantifies absorbance of oxygenated and deoxygenated hemoglobin, while turbidity is measure and compensated for at 880 nm	1. Portable device so can be used both in clinical laboratories, hospital critical care and in large scale field studies	1. Disposable cuvettes are expensive

		2. Quick results obtained in 10 or 45 seconds 3. Simple to operate 4. Requires less blood (10 µL)	
WHO Hemoglobin Color Scale (HCS) (3,6)	The scale consists of a small card of six shades of red (lighter to darker), each representing a hemoglobin concentration of 40 g/L, 60 g/L, 80 g/L, 100 g/L, 120 g/L, and 140 g/L, respectively is used. A drop of blood is collected on a standardized chromatography filter paper and the absorption is compared with the color scale	1. Simple to use 2. Cost effective	1. Prone to individually erroneous readings
Point of Care testing of hemoglobin- Non-invasive			
Pulse Co-oximetry (SpHb sensor, Pronto-7 (4,5,9)	Emits over multi-wavelengths of light to acquire hemoglobin concentration based on light absorption through the finger. Quantitative data on hemoglobin is obtained by signal processing algorithms and adaptive filters	1. Used for continuous measurement of hemoglobin in intensive/critical care settings/operation theatres	1. Cannot be used for field studies 2. Expensive
Measurement method/Principle	**Procedure**	**Merits**	**Demerits**
Occlusion spectroscopy (NBM-200) (5,10)	A ring-shaped sensor is placed on the subject's finger. Pressure is applied using a pneumatic cuff which occludes blood flow and sends optical signal at wavelengths 600-1500 nm. The light absorption before and after the blood flow obstruction is used to estimate the hemoglobin level	Used for continuous measurement of hemoglobin in intensive/critical care settings/operation theatres	1. Cannot be used for field studies 2. Expensive
Transcutaneous reflection spectroscopy (HemoSpect) (5)	A sensor is placed on the palm side of a finger on a non-dominant hand. The sensor head emits light. The non-absorbed light is reflected to the device and this is read by the spectrophotometer	Used for continuous measurement of hemoglobin in intensive/critical care settings/operation theatres	1. Cannot be used for field studies Expensive

Figures

FIGURE 1 COMPARISON OF HEMOGLOBIN VALUES BETWEEN THE AUTOMATED ANALYZER AND THE HEMOCUE 201