

Original Article

Comparative Evaluation of Pre-Donation Haemoglobin Screening Methods

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Abstract

Background: The pre-donation haemoglobin (Hb) estimation is the only laboratory test done on blood donors to determine an individual's eligibility to donate blood with an intention to prevent bleeding an anaemic donor. With availability of wide range of screening methods, no single technique has emerged as the most appropriate and ideal for screening blood donors. The primary objective of the study was to compare results of copper sulphate method and haemoglobin meter with the gold standard Cyanmeth hemoglobin (CyanHb) method.

Materials and methods : Prospective observational study done in 238 blood donors. Sample analyses were done using Copper sulphate solution, haemoglobin meter and Cyanmeth haemoglobin method.

Results : Mean values of three methods Cyanmeth haemoglobin, and Haemoglobin meter methods are 12.5 ± 2.2 , and 13.08 ± 1.61 respectively. Haemoglobin values obtained by haemoglobin meter and copper sulphate methods were comparable, with sensitivity of 94.25% and 94.47% respectively.

Conclusion : CuSo4 method and haemoglobin meter can be used alternatively based on resources available. The higher values obtained by CyanHb method could be due to turbidity factor of the lipids in the blood sample.

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Introduction

Pre donation haemoglobin screening is among the first and foremost tests done for blood donor to prevent blood collection from an anaemic donor. Therefore, an accurate and reliable method for haemoglobin estimation is essential.^[1] Estimates of prevalence of anaemia depend on the methods used for assessing haemoglobin concentration and on the cut-off point applied. The choice of method for measuring haemoglobin concentration depends upon performance of the method, conditions under which the blood will be collected such as remoteness of the location, whether laboratory support is available, whether staffs are experienced, and whether the study population is cooperative.^[2]

There are various methods of haemoglobin estimation with its own advantage and limitations. Cyanmet haemoglobin (CyanHb) method is the method

recommended by International Council for Standardization in Haematology (ICSH) and method recommended by Indian Drugs and Cosmetics Act, 1940 for haemoglobin estimation. Haemoglobin estimation by this method is cheaper, but has the disadvantage of taking long time, requires venipuncture before blood donation and has limited usage in remote areas as it requires accurate dilution of blood and electric power.^[1,3,4]

The Hemometer/ haemoglobin photometer/ haemoglobin meter is a simple, portable device, requires only a small sample of capillary blood, does not require refrigeration or even electricity and gives immediate, digitally displayed results. This method is very useful in resource limited areas.

The copper sulphate (CuSO₄) method is the traditional method being used at many blood centres which is a cheap and an easy method but does not provide an acceptable

degree of accuracy.^[5, 6] But CuSO₄ method gives accurate results, if strict quality control is applied.

Despite the availability of various methods for measuring donor haemoglobin, no single technique has emerged as the most suitable for haemoglobin estimation in blood donation setting.^[7]

Any new method for haemoglobin screening should save time and money. So there is a need for a quick and reliable method of haemoglobin estimation that is feasible and time saving in order to screen the blood donors and to decide whether or not to defer them for blood donation, especially in remote areas and field camps.

The primary objective of the study was to compare results of copper sulphate method and Haemoglobin meter with gold standard Cyanmeth haemoglobin method.

Materials and methods

The prospective study was conducted on 238 voluntary blood donors attending routine donor sessions at tertiary care hospital based blood centre in South India over a period of 3 months. Two ml of venous blood sample in EDTA were drawn from apparently healthy donors after obtaining their consent. The samples were analysed using three different methods of haemoglobin estimation. Haemoglobin estimation by Cyanmeth haemoglobin method (Photoelectric colorimeter – 113, Systronics), CuSO₄ specific gravity method and Haemoglobin meter (Mission, Haemoglobin testing system, Acon laboratories, Inc. USA) was done without delay by single trained technician. The working CuSO₄ solution was prepared (specific gravity 1.053) and standardised everyday using standard operating procedure. The functioning of the Mission, Haemoglobin testing system was checked everyday using known controls / specimens as per manufacturer's instructions. Quality control (QC) and calibration of photoelectric colorimeter was done as per standard operating procedure.

The Mission Hb Haemoglobin testing system is intended for the quantitative determination of Haemoglobin that analyses the intensity and colour of light reflected from the

reagent area of test strip.

The tests were done by trained person assigned to the job to avoid subjective bias, within one hour of sample collection. All QC's were done as per the standard operating procedure and manufacturer's recommendations.

The working CuSO₄ solution was prepared (specific gravity 1.053) and standardised every day using standard operating procedure. Results of CuSO₄ were interpreted as "pass" or "fail" at haemoglobin cut off of 12.5g/dl.

Statistical analysis

Statistical analysis was performed using SPSS version 20.0. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Haemoglobin meter and CuSO₄ method was calculated and results were compared with Cyanmeth haemoglobin method.

Results

A total 238 blood donor samples were analysed, of which 233 were males and 5 females. Donor's age ranged between 18years to 45years. A total of 26 donors were deferred due to low haemoglobin. A comparison of two different methods used in the present study against Cyanmeth method is summarised. [Table 1] Comparison of the results obtained by different methods of haemoglobin estimation against reference (Cyanmeth haemoglobin) is summarized in [Table 2] [Figure 1]. The mean haemoglobin values obtained by Cyanmeth and, Haemoglobin meter were 15.20g/dl, and 13.27g/dl respectively. The mean haemoglobin value of Haemoglobin meter was higher by 1.92. CuSO₄ method gave overall 19.3% (46/238) false results, sensitivity of 94.36%, but specificity of 50.66%. Haemoglobin meter gave overall 15.9% (38/238) false results, sensitivity 94.25%, and specificity 56.25%. Haemoglobin meter and CuSO₄ screening test inappropriately passed 4.2% (10/238) and 3.78% (9/238) donors respectively. [Table 3]

| Gender | Pass | Fail | Total number |
|------------|--------|--------|--------------|
| Male | 209 | 24 | 233 |
| Female | 3 | 2 | 5 |
| Total | 212 | 26 | 238 |
| Percentage | 89.0 % | 10.92% | 100.0% |

Table 1 : Demographic characteristics of donors (by reference method)

| Test results | Cyanmeth | | Haemoglobin meter | | CuSo4 | |
|-------------------|----------|------|-------------------|------|-------|------|
| | Pass | Fail | Pass | Fail | Pass | Fail |
| True (Hb ≥ 12.5) | 212 | 26 | 164 | 36 | 154 | 38 |
| False (Hb ≤ 12.5) | 0 | 0 | 28 | 10 | 37 | 9 |
| Total | 212 | 26 | 192 | 46 | 191 | 47 |

Table 2 : Comparison of the results obtained by different methods of haemoglobin estimation against reference (Cyanmeth haemoglobin) (n = 238)

| Test results | CuSo4 | Haemoglobin meter |
|---------------------------|-------|-------------------|
| Mean ± SD | - | 13.10 ± 2.152 |
| Sensitivity | 0.94 | 0.94 |
| Specificity | 0.50 | 0.56 |
| Positive predictive value | 0.80 | 0.85 |
| Negative predictive value | 0.80 | 0.78 |
| Likelihood ratio + | 1.88 | 1.45 |
| Likelihood ratio - | 0.12 | 0.10 |

Table 3 : Comparison between Haemoglobin meter and CuSo4 method

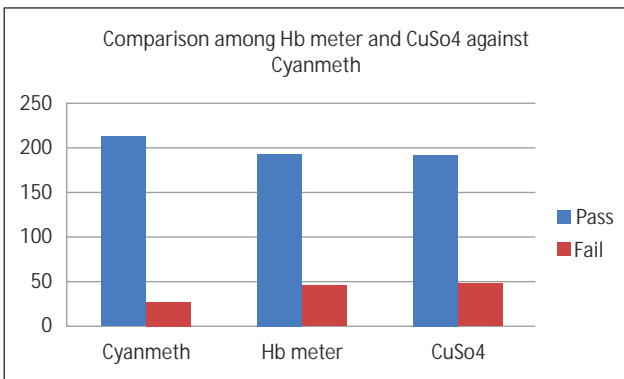


Figure 1 : Comparison among hemoglobin meter and CuSo4 method against Cyanmeth Method

Discussion

In our study, we found that haemoglobin estimation obtained by Haemoglobin meter and CuSO4 methods were comparable. Higher haemoglobin values were obtained by CyanHb method compared to HemoCue and CuSO4 methods resulting in low specificity of Haemoglobin meter and CuSo4 method. The higher values obtained by CyanHb method may be because of the turbidity factor of the lipids in the blood sample. The cyanmet haemoglobin method is

a widely used method of estimating Hemoglobin. However, several reports indicate that results obtained using this method could be imprecise due to factors like: turbidity of the blood and the large dilution of the sample, it requires skillful technical operations in terms of accurate uptake of the blood volume into a calibrated hemoglobin pipette, careful mixing of the sample with the Drabkin's solution, measurement of absorbance in the photometer and calculation of actual value from a systematically constructed standard graph. All these manual operations are time consuming which makes this method unsuitable for large scale field studies. It is also well documented that conditions such as hyper lipidemia and Waldenstrom's macro-globulinemia can cause falsely high results for haemoglobin in the filter photometer method such as the Cyanmet haemoglobin method^[4] Cyanmet haemoglobin method is the method recommended by International Council for Standardization in Hematology(ICSH),^[8] which accepts turbidity of 0.003 absorbance units, which corresponds to 0.11g/dl haemoglobin. Higher turbidity is expected since blood donors are not fasting.^[9]

This can be overcome by using Haemoglobin meter. The advantage of this technology is that it is simple, rapid and does not require sophisticated haematological equipment. The system is designed to use capillary, venous or arterial blood. Although the Haemoglobin meter show lower measurement of haemoglobin levels, these measurements seem to fluctuate less compared to other methods

Haemoglobin meter is suitable for surveys conducted over large areas and allows screening of anaemia in remote areas without adequate laboratory facilities. It also permits decision making regarding blood donations as it provides on the spot results.^[1]

Haemoglobin screening by CuSO4 is an inexpensive and convenient method that can be used as primary screening, supplemented with Haemoglobin meter for donors rejected by CuSO4. The semi-quantitative gravimetric copper sulphate method is the traditional method being used for donor screening at many blood centres. It is easy, inexpensive and does not require venous sample. It has

been the method of choice in many countries for years for primary haemoglobin screening of potential blood donors. However, the CuSO₄ method has several disadvantages: it requires rigorous training, constant observation of staff, it does not give quantitative result of haemoglobin and always has a chance of false acceptance and deferral. It needs to undergo strict quality control and validation before it is used to screen the donors. Early reports have suggested that this method gives inappropriate failures, and a significant number of such failed donors could be recovered with alternative method of screening. On the other hand, rare cases in which there is great raise of plasma protein concentration, anaemic donors may be accepted as normal by copper sulphate method, each extra g/dL of plasma protein being equivalent to 0.7 g/dL haemoglobin. Falsely high positive results in CuSO₄ method is also due to high white cell count.

Haemoglobin screening by CuSO₄ still stands the test of time and it can be used as the primary screening method. Using Haemoglobin meter as the initial screening method could prove costly for some blood centres. The Haemoglobin level of donors rejected by CuSO₄ may be reassessed by Haemoglobin meter, to decide whether or not the donor needs to be actually deferred. This will be of utmost importance in blood donation camps where mass haemoglobin screening is done.^[7]

Where field conditions and local resources allow it, haemoglobin concentration should be assessed with the gold standard direct Cyanmet haemoglobin method. However, the Haemoglobin meter can be used for surveys

involving different laboratories or which are conducted in relatively remote areas. Haemoglobin meter costs a considerable amount of money, and this should be considered when planning and budgeting for data collection.^[2]

There are recommendations put forward by a review which should be taken into account regarding the use of Haemoglobin meter such as addressing economical, clinical and regulatory issues before its implementation, adequate training of end users, and preference for venous and arterial sampling, internal quality control and monitoring of results.^[10]

In another study, 37% of deferred donors were acceptable with HemoCue method, mentioning deferral rates would be lesser if HemoCue replaces time tested CuSO₄. Mendrone et al studied haemoglobin screening in female population and found out that HemoCue reduces the risk of accepting anaemic female blood donors without increasing the deferral of non-anaemic donors.^[11]

Conclusion

The method used for haemoglobin screening of blood donors should be simple, rapid and portable. Haemoglobin screening by CuSO₄ method can be used as the primary screening method and Haemoglobin meter can be used as confirmatory method to accept or defer a blood donor, when rejected by CuSO₄ method. Turbidity of the blood could have influenced our results of haemoglobin estimation by Cyanmet haemoglobin method which showed higher values when compared to Haemoglobin meter and CuSO₄ methods.

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