

## UPDATE

# Use of Point of Care Testing (POCT) in measurement of hemoglobin

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<a href="#">Abstract</a>	<a href="#">Introduction</a>	<a href="#">Methodology</a>	<a href="#">Results</a>	<a href="#">Conclusion</a>	<a href="#">References</a>	<a href="#">Citation</a>	<a href="#">Tables / Figures</a>
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## Abstract

Point of care tests (POCT) are critical to success of public health programs like anemia control program which involve measurement of biomarkers; as they help in prompt decision making during first (and in many scenario the only) contact with the targeted beneficiary. There are many methods currently in use for point of care testing for hemoglobin estimation and include manual methods like Sahli's method, Indirect cyanmethemoglobin method, WHO Hemoglobin Color Scale method; digital hemoglobinometers including the newer non-invasive devices. The current background paper reviewed available published literature regarding performance of different POCT methods for hemoglobin estimation. Available literature indicates that invasive digital hemoglobinometers have shown a reasonable performance for use as POCT in facility and community settings both for clinical diagnosis as well as surveys. Adequate training of front line workers for use of digital hemoglobinometers and adherence to standard operating procedures (SOPs) is essential to reduce errors/biases. Non-invasive digital hemoglobinometers seem to be promising new option for POCT which obviates the need for drawing blood sample (either by venous or capillary method) but further research and development is required before their use in programs.

## Background

Hemoglobin (Hb) is the vehicle molecule responsible for carrying oxygen from lungs to tissues. Hemoglobin was discovered by Hünefeld in 1840. In 1959, Max Perutz determined the molecular structure of hemoglobin by X-ray crystallography (1). Over the years estimation of blood hemoglobin became an important domain in public health. In similar way, methods of hemoglobin estimation evolved over the years. The first clinical test of Hb measurement devised more than 100 years ago involved adding drops of distilled water to a measured volume of blood until its color matched that of an artificial colored standard (2,3).

Later modifications (4) involved first saturating blood with coal gas (carbon monoxide) to convert hemoglobin to the more stable carboxyhemoglobin. Modern hemoglobinometry dates from 1950s,

following development of spectrophotometry and the hemoglobin cyanide or cyanmethemoglobin method (5). Adaptation of this method and others for use in automated hematology analyzers followed. Over the past two decades advances have focused on development of methods which allow point-of-care testing (POCT) of hemoglobin.

## Point of care testing:

The term POCT is coined for the laboratory tests that are performed close to the patient i.e. at or near the site of provision of clinical care using easy operable analyzers. In several situations, these POCT techniques might suitably replace traditional laboratory set-up-based testing (6). The World Health Organization (WHO) in the 1970's had emphasized for a haemoglobin estimation method that is simple, cheap with robust technology, made useful to primary health care physician, health

workers in the field settings. POCT is essential in situations like conducting laboratory tests in rural settings, large scale screening camps/studies, and blood donation camps as it gives rapid point of care results, which help in further decision making.

#### **Available methods for measuring hemoglobin at point-of-care:**

##### **A. Invasive methods:**

- a. Direct cyanmethemoglobin method (gold standard)
- b. HemoCue method
- c. Sahli's method
- d. Hb color scale method
- e. Copper sulphate method

##### **B. Invasive methods with reagent free cuvettes:**

- a. HemoCue 301
- b. DiaSpect

##### **C. Non-invasive methods:**

- a. Occlusion spectroscopy (NBM 200)- ring shaped sensor and cuff
- b. Pulse co-oximetry
- c. Transcutaneous Reflection Spectroscopy (HemoSpect)

#### **Methodological details of various POCTs:**

Cyanmethemoglobin method is a spectrophotometric method and considered as gold standard for hemoglobin estimation. Nearly 40 years after it was first adopted as the reference method for measuring hemoglobin by the International Committee for Standardization in Hematology (ICSH), the hemiglobincyanide (HiCN) test still remains the recommended method of the ICSH (7) against which all new Hb methods are judged and standardized. In this method, blood is diluted in a solution containing potassium ferricyanide and potassium cyanide. Potassium ferricyanide oxidizes the iron in heme to the ferric state to form methemoglobin, which is converted to hemiglobincyanide (HiCN) by potassium cyanide. Most hemoglobin derivatives (oxyhemoglobin, methemoglobin and carboxyhemoglobin, but not sulfhemoglobin) are converted to HiCN and therefore taken into measurement by this method. The major advantages of this method are that it is inexpensive and there is a standard HiCN solution manufactured and assigned a concentration value according to very precise criteria laid down and reviewed periodically by the International Council for Standardization in Hematology (ICSH).

Turbidity due to proteins, cellular matter and lipids is a potential problem with spectrophotometric estimation of any blood constituent, including hemoglobin. The large dilution (1:251) of sample largely eliminates the problem, but falsely raised Hb results can occur in patients whose plasma protein concentration is particularly high (8,9). Heavily lipemic samples and those containing very high numbers of white cells (leucocytes) can also artifactually raise Hb (10). Other disadvantages are that it requires trained or skilled personnel for measurement, quality assured laboratory set-up, and cyanide is also considered a potential hazardous waste.

Cyanide free techniques were then developed which gave rise to Sodium Lauryl Sulphate method and Azide- Methemoglobin (Vanzetti's method).

#### **Sodium Lauryl Sulphate method:**

Sodium Lauryl Sulphate (SLS) is a surfactant which breaks down erythrocytes and rapidly forms a complex with the released hemoglobin. The product SLS-MetHb is stable for a few hours and has a characteristic spectrum with maximum absorbance at 539 nm. The method has been adapted for automated hematology analyzers. The reagent is non-toxic, and It is also less prone to interference by lipemia and increased concentration of leukocytes (11).

#### **Azide methemoglobin method (Vanzetti's method):**

This method is based on conversion of hemoglobin to a stable colored product azide-methemoglobin which has an almost identical absorbance spectrum to that of HiCN (12).

All these methods require specific auto-analyzer in laboratory setting. Development of WHO colour scale method and portable hemoglobinometers took place for measurement of Hb in settings where there is no laboratory.

#### **WHO hemoglobin color scale (HCS):**

HCS was developed for World Health Organization (WHO). This low-technology test has huge significance for the economically deprived countries. In areas where there are no laboratory facilities and insufficient resources to fund more sophisticated POCT hemoglobinometers, it is virtually the only means of determining Hb (13). The HCS test is based on the simple principle that the color of blood is a function of Hb. A drop of blood is absorbed onto

paper and its color compared with a chart of six shades of red, each shade representing an equivalent Hb level.

Pooled sensitivity of the HCS to diagnose anaemia was 80% (95% CI: 68–88) and specificity 80% (95% CI: 59–91) (14) when compared with clinical screening of anemia with respect to Gold Standard. In this review, nine studies used HemoCue as gold standard.

### **Portable hemoglobinometers:**

Hemoglobinometers are of great use in point-of-care setting especially in field conditions.

**HemoCue 201+:** This is the most common hemoglobinometer used in the field for providing point-of-care screening for anemia in all age groups. Within the microcuvette, sodium deoxycholate haemolyses the red blood cells releasing haemoglobin. Sodium nitrite then converts the haemoglobin to methemoglobin which, together with sodium azide, gives azide methemoglobin. The absorbance of the sample is measured at two wavelengths (570 and 880 nm) in order to compensate for turbidity in the sample. The haemoglobin level is then calculated by the meter and displayed on the screen.

### **Challenges with hemoglobin estimation in field setting:**

Biological variation poses a major challenge in measurement of Hemoglobin. Most methods are affected by certain physiological factors thus making the readings unreliable. The factors which can affect the Hb readings are sex of the individual [Expected venous Hb value is 0.5 to 0.8 g/dL lower for women compared to men]; blood specimen site (capillary versus venous); age; hydration status; diurnal variation; smoking status; pregnancy status and others (15). However, the method for Hb estimation should minimize the errors to as minimum as possible in a cost-effective way.

At a national level, we need to find easier methods due to resource-constraint, lack of laboratory settings, lack of manpower or equipment for blood storage and transport in all parts of the country. For field conditions, we may need to identify the most feasible, cost-effective and convenient method for

Hb estimation which may be hassle-free in all parts of the country.

### **Hemocue as a POCT?**

Under field conditions with no or fewer resources, HemoCue is a promising tool for estimation of haemoglobin (16). Even a non-technical person can operate the instrument and interpret the result easily. HemoCue requires only fewer amounts of blood (10 µL) and also requires less turnover time when compared to the automated analyzer (17).

HemoCue can estimate haemoglobin levels using capillary blood, venous blood (when collected in EDTA vials) and arterial blood. However, venous blood yields better result due to higher sensitivity. Studies have reported a high sensitivity of Hemocue across the globe ranging from 82-99% (18–21) using venous blood. The lowest was seen in Sari M *et al.* (19) where HemoCue using venous blood had a sensitivity of 82.4%. However, studies by Gwetu *et al* and Tondon *et al* reported higher sensitivity of 93% and 99% when Hemocue was compared with gold standard using venous blood. They also estimated that Hemocue over estimates Hb level not more than 0.5g/dL (18,20). Though the studies by Bhaskaram *et al* and Kapoor *et al* (22,23) have indicated that HemoCue overestimates the Hb values by at least 1 gm/dL, the sample sizes were much lesser than the study by Tandon *et al.* Moreover, Morris *et al* found that replicate sampling using capillary blood may improve the reliability of the HemoCue system and reduce chance of over estimation of hemoglobin level (24). Few studies have reported that adding corrective factors to estimated value would suffice in accurate measurement of hemoglobin (23,25). Except study by Kapoor *et al* the margin of corrective factor was very small and that could be ignored (23). Though it has been observed that sensitivity of HemoCue decreases when capillary blood is used this finding is not consistent over the globe. Using capillary blood seeks adherence to prescribed standard methodology and adequate training of the person using HemoCue. Studies by Bellamy *et al* and Mendrone *et al* reported that capillary values were 3.5% and 5.9% higher respectively than the venous blood (26,27). In a study by Munoz *et al* which used standard techniques while using HemoCue when compared against auto-analyzer did not find any significant differences in between capillary and venous blood. However, they claimed that not including children in the study could have lead to

that result (28). Some studies also have quoted lesser Hb values when capillary blood was used (29,30). Similarly, in a study by Neufeld *et al*, they reported that on an average, the Hb in capillary blood was higher than in venous blood. The difference ranged from +0.17 g/dl ( $p = 0.15$ ) for samples from children measured by Hemocue, to +0.59 g/dl ( $p < 0.0001$ ) for samples from adults using the Hemocue. They also discussed that the difference could be physiology as sample inadequacy or incorrect methodology may result in hemodilution rather than hemoconcentration (31).

Most global studies reported positive correlation coefficient and good agreement (Bland-Altman) between HemoCue and cyanmethaemoglobin readings (16, 32–34). The highest being 0.99 reported by Nkrumah B *et al* (16). Indian studies have also reported positive correlation between HemoCue and Cyanmethemoglobin readings; however, the correlation was weaker when compared to global studies (35). This could be due to poor methodological techniques and/or poor-quality control. This warrants the need for training regarding operation of HemoCue machine among health care providers.

However, challenges are associated with use of Hemocue 201+. Humid conditions have seen to be hampering the accurate estimation of hemoglobin as the reagent within micro-cuvette changes with humidity (36,37). Studies have demonstrated a fall of sensitivity from 82% to 60 % of Hemocue by usage of old micro-cuvettes of 2-25 days (37). This may be tackled by individualized packaging of micro-cuvettes similar to the glucose test strips or by limited the use of cuvettes for few hours or days instead of 20-30 days even if it is stable. Another disadvantage with Hemocue was that it was relatively expensive and requires continuous replenishment of micro-cuvettes. However, direct cyanmethemoglobin method also would require replenishment of reagents, vacutainers, maintenance costs and quality assured laboratory facility for implementing these tests.

In view of lower sensitivity of HemoCue using capillary blood and possibility of marginal over estimation of Hb level, few researchers advocate use of HCS. The study by Tandon *et al* reported that sensitivity of HCS is much lower than HemoCue. Moreover, HCS was highly prone to subjective error

resulting in 25.2% false results (20). Similarly, Paddle *et al* compared HCS and HemoCue hemoglobin assay and concluded that “while considerations such as cost, and simplicity of use make the Hemoglobin Color Scale an attractive proposition, its poor accuracy renders its use questionable” (38).

**Hemoglobinometers with reagent free cuvettes:** To overcome the issues of humidity and other physical environment, a newer development has come up with respect to hemoglobinometers which utilized reagent free cuvettes unlike HemoCue 201 method. Newer hemoglobinometers namely HemoCue 301, DiaSpect requires reagent-free cuvettes which attempts to overcome few of the challenges faced by the older HemoCue versions. As this method use reagent free microcuvettes, they do not get affected by humidity and do not require special storage conditions (39). HemoCue 301 was found adequately apt for routine use in blood donation camps. The device was designed to operate at varied temperatures ranging from 0-40°C (40). The main advantage is that the cuvettes are significantly cheaper than the previous models and will not deteriorate in adverse climatic conditions. The cuvettes will be at least 30–40% cheaper than that for the previous models (41) In a study which compared DiaSpect against reference method (auto-analyzer), it was seen that DiaSpect had a sensitivity of 98% and qualified as a good screening method (42).

## Conclusion

Point of care testing (POCT) is critical from program point of view for anemia control program. Various invasive methods are available for point of care testing in measurement of hemoglobin and digital hemoglobinometer have shown reasonable performance for use in surveys and field settings. Non-invasive POCT seem to be promising new option which obviate need for drawing blood sample (either by venous or capillary methods) but further research and development is required to further fine tune its features and to make it suitable for Indian settings with special consideration to environmental conditions like high temperature and humidity. Moreover, adequate training of POCT handlers and adherence to universal SOPs should be mandatory to reduce inter-observer bias

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## Tables

TABLE 1 SHOWING SENSITIVITY AND SPECIFICITY OF HEMOCUE 201

S.no	Studies	Sample	Comparator	Bland-Altman	Pearson coefficient	Sensitivity (%)	Specificity (%)	Correction factor (g/dl)
1	Akhtar K et al, Aligarh, 2008 (21)		Direct cyanmethaemoglobin method			94.1	95.2	
2	Sari M et al, Indonesia, 2001 (19)	Venous	Direct cyanmethaemoglobin			82.4	94.2	
		Capillary				94.1	82.4	
3	Neufeld et al, Mexico, 2002 (31)	Capillary	Automated spectrophotometer (celldyn)			80	90	+0.3
4	Bhaskaram P et al, Hyderabad India, 2003 (22)		Cyanmethaemoglobin		$r = 0.922$	75%	100%	$0.389 + 0.831$
5	Mills F, Meadows N. London 1989 (43)		Coulter counter model S			85	94	
6	Munoz et al, Spain, 2005 (28)	Capillary	cell counter Pentra 120 Retic		$r = 0.992$ ; $P < 0.01$	96.5	94.4	
		Venous	cell counter Pentra 120 Retic			100	100	
7	Mendrone A et al 2009 (27)	Capillary	Automatic hematology analyzer (ABX Pentra 60)		$r = 0.716$	56		
8	Kapoor SK et al Haryana 2001 (23)	Capillary	Direct Cyanmethaemoglobin method					Corrective factor of minus 1.5 g/dL for Capillary minus 1.2 g/dL for venous
9	Akhtar K et al Aligarh 2008 (21)	Capillary	Direct cyanmethaemoglobin method			94.1	95.2	
10	Bahadur S et al New Delhi 2009 (35)	Capillary	Sysmex KX 21 autoanalyzer		$r = 0.40$			
11	Tondon R et al Lucknow	Venous	Automated hematology cell analyzer (ABX Micros 60, France)			99.4	84.4	

	2005 (20)							
12	Bäck SE et al Sweden 2002 (32)		ADVIA 120 (Bayer Diagnostics), GEN-S (Beckman Coulter), Sysmex XE 2100, CellDyn 4000 (Abbott), and the HemoCue B- Hemoglobin system		0.99			0.10
13	Gwetu TP, Chhagan MK. South Africa 2015 (18)	Venous	Advia 2120			93	74.5	
14	Schapkaitz et al, 2011 (44)		Coulter® LH 750 automated haematologyanalyser	Good correlation				
15	M.J. Kim et al, Korea, 2013 (45)		NBM-200; OrSense, Israel Automated hematology analyzer (LH500; Beckman Coulter, USA)	A Bland-Altman plot showed that the 2 SD difference of Hb measurements between the LH500 and the NBM-200 was >2.0 g/dL, while that between the LH500 and the HemoCue was <2.0 g/dL				
16	T.S. Levy et al, 2016, Mexico (46)	Capillary	Automated analyser	-1.51	0.183			*Also for maximo
17	Pajares et al, 2013,Spain (47)	Capillary	Coulter LH750		0.859			* Also for Diaspect
18	Nkrumah B et al,2011 Ghana (16)	Venous	Cyanmethaemoglobin	-0.39 to 0.64 g/dl	0.995 (0.994-0.996)			
			Sysmex KX21N	-0.39 to 0.69 g/dl	0.994 (0.993 to 0.995)			
19	Prakash S et al Delhi 1999 (25)	Venous	Standard blood cell coulter method					There was good correlation and hemocue showed higher results by 0.5gm/dL. corrective factor of 0.5g/dL may be applied