

Total Hemoglobin Measurements: Accuracy of Laboratory Devices and Impact of Physiologic Variation

Summary

The clinical measurement of total hemoglobin has inherent variability. CO-Oximeter and point-of-care (POC) devices that are commonly used to measure hemoglobin have been shown to vary up to ± 1.2 and ± 1.3 g/dL, respectively. Additionally, there are a variety of physiologic and methodologic factors that can significantly influence hemoglobin levels in the body. Physiologic factors such as the blood source (venous or arterial), site and time of blood draws, and patient body position are recognized in the clinical literature to add variability to hemoglobin levels. Blood draw techniques such as "pushing out" blood during a fingertip capillary draw and blood-mixing errors can have an additional variability impact on hemoglobin measurement. This paper reviews these factors and expands on the inherent variability and limitations of current hemoglobin measurements based on the device used and patient assessed.

INTRODUCTION

Total hemoglobin (Hb) is one of the most frequently ordered laboratory tests, both in the hospital and physician's office. Baseline Hb levels guide many clinical diagnoses and therapeutic interventions. While anemia detection is the primary reason for ordering the test, serial assessments are often made to track disease progression, blood loss, and the efficacy of therapies designed to restore Hb values to normal levels.

Hemoglobin measurements have traditionally required an invasive blood draw. The blood is then subjected to analysis by a laboratory device, such as a CO-Oximeter. More recently, invasive Hb measurements have also been performed with point-of-care devices. Although a laboratory or POC device may specify a narrow measurement accuracy specification, the reported accuracy range in the clinical setting is often wider than the specification. In addition, the subject's own physiology can affect Hb measurements.

This paper reviews the available literature on the accuracy and variability of laboratory and POC devices, as well as the physiological factors that affect Hb measurement.

INVASIVE LABORATORY DEVICES

CO-Oximeters are the gold standard for Hb measurement and analyze hemolyzed blood using spectrophotometric detection. The accuracy of CO-Oximetry is a function of multiple variables, including the device method (number of wavelengths of light used), device model type, and the proper handling of the blood analyte.

Intradevice comparison is Hb variability from the same blood sample on the same device. Interdevice comparison is Hb variability from the same sample on different devices. There is no standard procedure for checking the measurement error of the laboratory CO-Oximeter.¹ Bland and Altman pointed out that both reference devices and test devices produce and/or contain inherent errors.²

Gehring, et. al. performed a 36-patient study using the same blood sample analyzed on two identical devices from 5 different CO-Oximeter manufacturers. As Table 1 demonstrates, there was significant intradevice variation in the Hb measurements.

Table 1. Intradvice comparison of Hb measurement of the same blood sample on two identical devices

Intradvice Comparison	Brand A	Brand B	Brand C	Brand D	Brand E
Mean (g/dL)	- 0.8	- 0.3	- 0.4	0.0	0.4
Standard deviation (g/dL)	0.3	0.2	0.9	0.1	1.2

In another study using a Hb calibration control device as the reference standard, 31 CO-Oximeters were evaluated from five different manufacturers using four levels of control solutions.³ As table 2 shows, CO-Oximeter measurements of Hb may vary as much as 0.9 g/dL between devices in a normal Hb range.

Table 2. Variation in Hb measurement across 31 CO-Oximeter devices

Comparison	Level 1	Level 2	Level 3	Level 4
Mean Hb Value of Control (g/dL)	5.4	8.4	13.8	17.4
Standard deviation: all devices (g/dL)	0.3	0.3	0.4	0.5
Range/Level (g/dL)	5.0-5.4	8.0-8.6	13.3-14.2	17.1-17.7

INVASIVE POINT-OF-CARE DEVICES

Use of point-of-care hematology analyzers has become more frequent in the last decade because of their ability to provide quicker test results with portable devices and smaller samples, typically from a capillary source at the fingertip. However, it is well understood that POC devices for Hb offer reduced accuracy compared to laboratory devices. Factors that affect POC device accuracy include device method, the size of the blood sample, and strong potential for confounding elements with capillary blood.

For the assessment of Hb and hematocrit (Hct) in POC devices, two principles of operation exist:

1) Spectrophotometric—typically employed to measure Hb and calculate Hct; and 2) Conductometric—typically employed to measure Hct and calculate Hb.

Spectrophotometric Analysis

The photometric determination of Hb in POC devices typically involves a small but invasive blood sample, usually obtained from a finger stick. The finger stick draws capillary blood, although POC devices can also sample venous blood. Capillary blood is typically the substrate used for analysis, but validation literature clearly demonstrates a significant variability in capillary blood measurements compared to calibrated laboratory references. This variability is a function of both the device method and the result of using a small sample from the capillary bed where pressure can create dynamic fluid shifts. For example, if a clinician needs to push the finger to extract enough capillary blood, this forces a greater amount of plasma concentration into the blood sample and compromises the measurement. The most commonly used spectrophotometric POC device for Hb assessments is the HemoCue (Qwest Diagnostics, Lake Forest, CA).

Conductometric Analysis

The conductometric method is the principle of operation employed by I-Stat (Abbott Medical, East Windsor, NJ), a POC device used for the determination of Hb by calculation from a measured hematocrit (Hct). An invasive sample is required and special cartridges must be used. All cartridges contain multiple measurements, adding to the cost of the panel even when only the Hb value is needed. Conductivity-based Hct is considered accurate for many clinical situations but only in physiologically normal patients. The technique is prone to the same errors in measurement as spectrophotometric POC devices when measuring capillary blood. The accuracy of hematocrits from this technique is also significantly affected by changes in sodium levels, protein concentrations in the blood, and the use of plasma volume expanders, added anticoagulants, and the presence of elevated white cell counts.⁴ The conductivity method tends to underestimate the Hct, and therefore the Hb level derived from Hct. Hct derived by conductivity has been shown to be inaccurate at Hct's < 30, or Hb levels of 10 g/dL or less, limiting its ability to detect severe anemia. The manufacturer recommends against using this POC device to make transfusion decisions.⁵

While individual POC device specifications may be quoted at $\pm 7\%$ of reading, the true clinical relevancy of the data must consider all of the variables that contribute to an accumulated error in the measurement. At normal Hb levels of 13-15 g/dL, the CLIA specification variance is approximately 1.0 g/dL. In the anemic range of 10 g/dL, the target variance is 0.7g/dL. However, this summary of published studies reveals a significantly larger difference in Hb measurement between POC and laboratory devices:

- Hb measurement from capillary blood in POC devices varies 0.5 – 2.3 g/dL from reference standards ^{6, 7, 8, 9}
- Some POC devices have shown up to 10 times the variability in Hb measurement compared to a laboratory CO-Oximeter ⁷
- Hb measurements from capillary blood tend to overestimate the Hb measurement from a lab device ¹⁰

PHYSIOLOGIC VARIATION IN HEMOGLOBIN MEASUREMENTS

While laboratory and POC devices have clear variability in Hb measurement, there are also multiple sources of Hb variation within the body, including the type of blood sample, sample site, time the sample is taken, and body position.

Table 3. Factors Affecting Hemoglobin Accuracy in POC Devices

False low Hb concentration readings:	False high Hb concentration readings:
Finger squeezed, or milked, which dilutes the blood sample with interstitial fluid	Blood sample clots before cuvette filled, causing the sample to be concentrated
Site wet from alcohol solution when punctured - dilutes sample	Microcuvette not completely filled because of poor blood flow from a shallow stick
Microcuvette contains air which lowers the concentration of RBC's in the sample	

Type of Blood Sample

Laboratory devices are designed to allow sampling of either venous or arterial blood. While clinicians are often not aware of this from routine care as they do not perform simultaneous arterial and venous blood samples, it is important to understand that the Hb measurement may vary based on whether arterial or venous blood is used. Mokken, et. al.¹¹ and Yang ZW, et. al.¹² reported that arterial Hb measurements can be expected to be, on average, 0.7 – 1.0 g/dL less than the Hb measurements derived from venous blood. While the total amount of circulating red blood cells and Hb remains relatively constant whether in arterial or venous blood, the percentage of plasma concentration can vary from the arterial to venous based on a variety of physiologic factors. The amount of plasma concentration can be higher in arterial blood, potentially leading to lower concentration of Hb.

Sample Site

The sample site on the body can also affect Hb measurements. Large discrepancies were found between the values obtained from capillary blood samples from the left and right hands of the same women, with intrasubject standard deviation of 0.8 g/dL and correlation of 0.7.¹³ The wide limits of agreement indicate that two samples from different fingers of the same person could have Hb concentrations that differ by up to 2.0g/dL. Another study shows wide variation in the Hb concentration of capillary blood samples obtained from different fingers on the same individual at the same time. Inpatient variability ranged as high as 7%.¹⁴

Time

Hb measurement can vary significantly over time, even in stable patients. In a study of venous blood samples drawn from the same individuals on two different occasions, within person variances could vary as much as 2.6 g/dL in males and 2.3 g/dL in females.^{15,16} In another study, when Hb measurements were taken from the same individual on four (4) different days consecutively, intrasubject variability was 7.0% and the standard deviation was 0.8 g/dL.¹⁴

Body Position

Body position before and during the blood draw also affects Hb measurements due to the normal composition of blood, interstitial fluid shifts, and elevations of protein and white blood cells. Body position has a significant effect on venous Hb measurements due to decreases in plasma volume on assuming an upright position. Heart rate and blood pressure are higher when standing vs. sitting, which induces the movement of intravascular fluid such as plasma into interstitial compartments. This causes plasma volume to decrease and Hct and Hb levels to rise (hemoconcentration).¹⁷ Gore and colleagues showed a 6% reduction on plasma volume with standing, which changed Hb up to 2 g/dL.¹⁸ Moving from seated to standing positions for 20 minutes may result in a change in Hb concentration by >1.0 g/dL.¹⁹ The converse is also true, indicating that patients who are ambulatory may require a period of equilibration if they change body position prior to the blood draw.

CONCLUSION

A variety of factors influence Hb measurement and Hb levels in the body. The measurement of Hb can vary widely in the same patient depending on the methods used. When comparing new methods of Hb determination to existing methods, it is vital to understand the inherent variability and limitations of current Hb measurements based on the device used and the patient assessed.

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