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A COMPARATIVE STUDY OF TOTAL HEMOGLOBIN MEASUREMENT TECHNOLOGY: NONINVASIVE PULSE CO-OXIMETRY AND CONVENTIONAL METHODS

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A COMPARATIVE STUDY OF TOTAL HEMOGLOBIN MEASUREMENT TECHNOLOGY: NONINVASIVE PULSE CO-OXIMETRY AND CONVENTIONAL METHODS

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DISCLOSURE

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ABSTRACT

Hemoglobin can be measured on a variety of devices using different principles of operation. Noninvasive pulse CO-oximetry represents the latest development in hemoglobin measuring technology. The technology uses principles similar to pulse oximetry to measure total hemoglobin, oxyhemoglobin, reduced oxyhemoglobin, carboxyhemoglobin and methemoglobin. Similar to the introduction of pulse oximetry to the medical field, pulse CO-oximetry has been met with skepticism. Since the technology is noninvasive and provides continuous monitoring in comparison to invasive and discrete techniques used in other methods, CO-oximetry purportedly provides an advantage in patient care. The purpose of this research is threefold: (a) to review the various underlying principles of measuring hemoglobin, (b) to compare the results of clinical studies determining the efficacy of the new pulse CO-oximeter technology, and (c) to provide a technical and financial basis for implementation of pulse CO-oximetry into a hospital or institution. These combined outcomes will determine the practicality of non-invasive pulse CO-oximetry in improving patient care, given the advantage of noninvasive, continuous monitoring.

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1. INTRODUCTION

Since hemoglobin concentration and oxygen saturation are indicative of a patient's ability to transport oxygen, it is routinely monitored or tested in any instance where oxygen transport is thought to be compromised. Additionally, hematocrit and total hemoglobin concentration are used perioperatively to monitor patients during procedures with a high risk of blood loss or hemorrhage. The measurements are used in guiding clinical decisions to treat low blood volume, or anemia, through medications or blood transfusion.

Due to the risks associated with allogeneic blood transfusions, blood management strategies should attempt to stabilize a patient's condition by alternative means before resorting to transfusions. Studies have shown that a restrictive strategy of red blood cell transfusion is at least as effective as and possibly superior to a liberal transfusion strategy in critically ill patients [1]. A new noninvasive technology, pulse CO-oximetry monitoring, purportedly provides instantaneous hemoglobin monitoring, similar to how a pulse oximeter monitors oxygen saturation. Much like the implementation of pulse oximetry in monitoring or diagnosing hypoxemia, noninvasive pulse CO-oximetry requires an understanding of the methods of operation compared to the typical point of care or laboratory devices used to measure hemoglobin. A comparison of the applications, accuracy, and costs associated with the various methods of hemoglobin measurement will assist in defining the role of noninvasive pulse CO-oximetry in guiding clinical decisions.

2. BACKGROUND

A CO-oximeter is a device that is used to detect hypoxia, a condition in which tissue is deprived of oxygen. CO-oximeters measure relative blood concentrations of

oxygenated and reduced hemoglobin. Additionally, CO-oximeters measure the dysfunctional hemoglobins or dyshemoglobins; these include relative blood concentrations of carboxyhemoglobin and methemoglobin [2]. Total oxygen content in blood is dependent on two key variables: the concentration of hemoglobin, typically measured in grams per deciliter (g/dL), and the percentage of available hemoglobin that is bound with oxygen. Several devices are used to measure the concentration of hemoglobin, or total hemoglobin (tHb); these devices use spectrophotometry- and conductivity-based methods, in addition to the multi-wavelength pulse technology used in the recently introduced noninvasive pulse CO-oximeter.

Some devices, such as those based on the Coulter Principle, are lab-based, provide discrete measurements and require the collection of arterial or venous blood samples for analysis. Point of Care Testing (POCT) devices are also available to measure total hemoglobin at the bedside using conductivity and spectrophotometric technologies. These devices provide only a discrete measurement and require either a capillary, venous or arterial blood sample. Pulse oximetry allows non-invasive continuous measurement of the percentage of hemoglobin in the arteries with bound oxygen.

2.1 HEMATOCRIT

Hemoglobin molecules in arterial blood, in normal physiologic state, are fully saturated with oxygen, expressed as oxygen saturation in percentage. A typical healthy adult has an arterial sampled oxygen saturation of 97-100%. Oxygen saturation can be measured noninvasively with a pulse oximeter. The oxygen carrying capacity of hemoglobin can be compromised by dyshemoglobins, molecules that bind with the iron of the hemoglobin, forming a stronger bond than oxygen. Traditional pulse oximetry is not capable of measuring the common dyshemoglobins: carboxyhemoglobin (COHb) and methemoglobin (MetHb) and Sulfhemoglobin (SulfHb), sometimes resulting in false

measurements. Invasive determination of hemoglobin levels remains the most commonly used method to evaluate the oxygen carrying capacity of blood.

Since hemoglobin transports oxygen from the lungs to the peripheral tissues, it is frequently used to indicate a patient's ability to transport oxygen. Hematocrit (HCT), the ratio of red blood cells (RBCs) to total blood volume is also useful in determining oxygen transport function. Hemoglobin accounts for approximately one-third of the red blood cell volume or HCT. To understand the relationship between the two, it is helpful to understand the composition of blood.

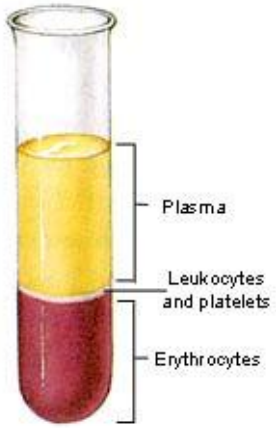

Figure 1: Diagram of packed cell column showing separation of cells and plasma after centrifugation.

Blood contains red blood cells (RBCs), white blood cells (WBCs) and platelets. These components are termed the 'formed elements'. HCT, sometimes referred to as packed cell volume (PCV), is a measurement of RBC volume, typically about 45% [3]. HCT can be measured using a manual method, called microhematocrit, in which a slender capillary tube of whole blood is spun down in a centrifuge [4]. The blood components separate as seen in Figure 1. The dense RBCs move to the bottom of the tube; the WBCs and platelets form a thin layer on top known as the buffy coat. This stacked column is known as a packed cell column. From the packed cell column, the volume of RBCs is compared to the total volume of whole blood to determine HCT; this value is typically reported as a percentage. Normal values, ranging from 32% to 61%, are listed in Table 1 in percentage as well as liters of packed cells per liter of blood (L/L) [4]. The remaining volume of blood, in addition to the formed elements, is clear yellow-tinted plasma.

Table 1
Hematocrit Reference Ranges

		AVERAGE		RANGE	
		Percent (%)	SI Units (L/L)	Percent (%)	SI Units (L/L)
Adults	Males	47	0.47	42–52	0.42–0.52
	Females	42	0.42	36–48	0.36–0.48
Children	Newborn	56	0.56	51–61	0.51–0.61
	1 year	35	0.35	32–38	0.32–0.38
	6 years	38	0.38	34–42	0.34–0.42

Data from Estridge, Barbara [4].

2.2 THE COULTER PRINCIPLE

In the mid-1950s, Wallace H. Coulter and his brother Joseph R. Coulter, Jr. introduced what today is known as the Coulter Principle [5]. Their principle counts and sizes cells by detecting and measuring changes in electrical resistance as the cells pass through a small aperture. The apparatus depicted in Figure 2 has a sample tube suspended in a larger sample beaker. Cells are suspended in a conductive liquid which acts as an insulator. As each cell passes through an aperture in the smaller sample tube, it momentarily increases the electrical resistance of the path between the two submerged electrodes. Resulting signal pulses between the electrodes are amplified and counted. The number of pulses indicates particle count, and the size of the electrical pulse is proportional to the cell volume [6].

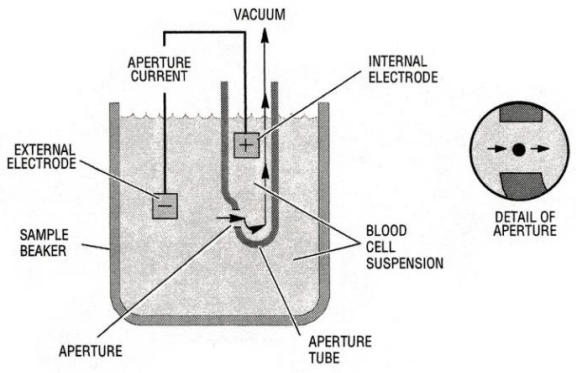

Figure 2: Functional schematic depicting the Coulter Principle for counting and sizing particles

Figure from Coulter International Corp., Coulter Corporation [6].

The HCT is calculated from the RBC count and the mean corpuscular volume (MCV), using the following equation:

$$\text{Hematocrit (\%)} = (\text{RBC} \times \text{MCV})/10$$

2.3 HEMOGLOBIN AND COMMON DYSHEMOGLOBINS

Hemoglobin is composed of a heme molecule and globin protein. The smallest unit of the heme is the pyrrole which is formed when succinyl-CoA binds with glycine. Four of the pyrrole molecules combine to form porphyrin or protoporphyrin IX [7]. These molecules combine with ferrous iron (Fe^{++}) to form the heme molecule. Each heme molecule combines with a long polypeptide chain known as a globin, forming a hemoglobin chain. The globin is a protein of 574 amino acids in four polypeptide chains [3]. Each hemoglobin chain contains an atom of iron capable of binding loosely with one molecule of oxygen; therefore, each hemoglobin molecule containing four hemoglobin chains can transport four oxygen molecules. There are approximately 270,000,000 hemoglobin molecules in each red blood cell [3]. Hemoglobin concentration is typically measured in grams per liter (g/L) or grams per deciliter (g/dL). Table 2 lists the hemoglobin reference ranges for newborns, children, adult males and adult females [4].

Table 2
Hemoglobin Reference Ranges

Age/Gender	Grams/Deciliter (g/dL)	Grams/Liter (g/L)
Newborn	16-23	160-230
Children	10-14	100-140
Adult males	13-17	130-170
Adult females	12-16	120-160

Data from Estridge, Barbara [4].

Carbon monoxide (CO) is a gas produced by the combustion of carbon-containing fuels from sources such as portable generators, oil heaters, kerosene burners, propane heaters, faulty furnaces, motor vehicles, stoves, gas ranges and gas heaters [8]. Once

inhaled, carbon monoxide can bind to hemoglobin to form carboxyhemoglobin (COHb), reducing the oxygen carrying capacity of the RBCs due to direct competition for the binding sites. The delivery of oxygen is further impaired due to allosteric effects that increase hemoglobin affinity for oxygen at remaining binding sites [9]. Research shows that COHb is normally found in less than 2.9% of the available oxygen-binding sites of hemoglobin. Additionally, elevated levels of carboxyhemoglobin are found in smokers ranging from 5 to 10% [9,10].

Carboxyhemoglobinemia, anemia induced by carbon monoxide poisoning (COP), presents clinically with varying symptoms that typically correlate with the level and duration of the exposure and the patient's prior clinical condition. While symptoms can include headache, dizziness, nausea fatigue and weakness, some patients may present asymptomatic, making COP difficult to diagnose. COP can cause decreased myocardial function, respiratory alkalosis, metabolic acidosis, pulmonary edema, multiple organ failure and ultimately death under extreme exposure without immediate treatment [8].

Treatment should begin with removal of the noxious agent. The half-life of COHb while breathing room air is 5.3 hours; while breathing 100% oxygen at sea level the half-life is reduced to approximately 1 hour. With 100% oxygen at 3 atmospheres, the half-life is further reduced to 23 minutes. Due to this reduction in half-life, hyperbaric oxygen therapy is the 'gold standard' treatment in severe cases of Carboxyhemoglobinemia [11]. There is little correlation between clinical symptoms and COHb concentration; typically, a COHb concentration above 25% is considered sufficient to indicate hyperbaric oxygen therapy [11].

Methemoglobin (MetHb) is another dysfunctional form of hemoglobin incapable of transporting oxygen. Methemoglobinemia occurs with elevated levels of MetHb.

Hemoglobin contains iron in the ferrous state (Fe^{++}) whereas MetHb contains iron in the ferric state (Fe^{+++}). Similar to COHb, heme molecules in MetHb are not only incapable of transporting oxygen molecules, but they also have an allosteric effect, increasing the affinity of the remaining heme groups for oxygen. Exogenous causes of Methemoglobinemia include commonly prescribed drugs, herbicides, pesticides, chemical fumes, petrol octane boosters, and inhaled nitric oxide. Table 3 lists medications that have been documented to contribute to Methemoglobinemia.

Table 3
Medications Documented To Contribute to Methemoglobinemia

Benzocaine	Flutamide	Nitroprusside	Pyridium
Celecoxib	Lidocaine	Nitrous oxide	Riluzole
Cetacaine	Methylene blue	Phenazopyridine	Silver nitrate
Chlorates	Metoclopramide	Phenol	Sodium nitrate
Dapsone	Nitrates	Prilocaine	Sulfonamides
Dimethylsulfoxide	Nitric oxide	Primaquine	
EMLA topical anesthetics	Nitroglycerin	Procaine	

Data from Barker, SJ [8].

Methemoglobinemia can often go unnoticed or undiagnosed due to the ambiguous nature of the flu-like symptoms at low levels of MetHb concentrations. Patients can present asymptomatic at concentrations less than 15%. Concentrations greater than 70% can lead to mortality. As with any poisoning, removal or elimination of the toxic or noxious agent is appropriate. Intrinsic enzyme systems will convert MetHb to hemoglobin at a rate of approximately 15% per hour in healthy individuals [8]. Treatment for elevated levels begins with maximizing the oxygen carrying capacity of normal hemoglobin and then facilitating the reduction of MetHb. Supplemental oxygen should be given followed by the administration of intravenous methylene blue for moderate to severe cases. Methylene blue has been shown to induce Methemoglobinemia in some patients if injected too rapidly. Doses greater than 15 mg/kg may induce hemolysis and should be avoided [9]. Some patients may present with rebound methemoglobinemia

after a positive response to therapy. In cases of severe methemoglobinemia, a blood transfusion may be necessary.

2.4 TYPES OF ANEMIA

Since hemoglobin concentration and oxygen saturation are indicative of a patient's ability to transport oxygen, it is routinely monitored or tested in any instance where oxygen transport is thought to be compromised, such as anemia. Additionally, hematocrit and total hemoglobin concentration are used to perioperatively monitor patients during procedures with a high risk of blood loss or hemorrhage. The measurements are used to guide clinical decisions in treating low blood volume, or anemia, through medications or blood transfusion. Anemia is defined by a significant reduction in either the number of RBCs per unit volume of whole blood, or more specifically too little hemoglobin in the cells [12]. Causes of anemia can be hereditary or extrinsic.

Blood loss anemia occurs due to rapid blood loss or hemorrhage, resulting in reduced red blood cells and hemoglobin in the body. The body can replace the plasma of the blood in 1 to 3 days, leaving a low concentration of red blood cells. The red blood cell concentration can return to normal within 3 to 6 weeks [7].

Exposure to gamma ray radiation, excessive x-ray radiation, industrial chemicals or drugs can inhibit production of erythroblasts within the bone marrow, a condition termed bone marrow aplasia. Extreme exposure results in complete destruction of bone marrow and is followed in a few weeks by lethal anemia, known as aplastic anemia [7].

Since erythroblast production in the bone marrow requires vitamin B_{12}, folic acid, and intrinsic factor from the stomach mucosa, reduction of any one of these can lead to slow reproduction in the bone marrow. This allows the red blood cells to grow too large

forming megaloblasts. Megaloblastic anemia occurs because the megaloblasts are oddly shaped, oversized and have fragile membranes. These cells rupture easily, leaving the person with an inadequate number of red blood cells [7].

Hemolytic Anemia is caused by different abnormalities in the red blood cells, most hereditarily acquired. These abnormalities can cause the cells to be more fragile and rupture easily. The number of red blood cells formed may be normal, but the lifespan of the cells is often short. The cells are destroyed faster than they can be formed. In hereditary spherocytosis the cells are small and spherical rather than biconcave discs. Since they don't have the typical bag-like structure, the cells cannot withstand compression forces and rupture easily in tight vascular beds. Sickle cell anemia is present in 0.3-1.0 percent of West African and American blacks. The cells have an abnormal type of hemoglobin. This hemoglobin, when exposed to low concentrations of O_2, precipitates into long crystals inside of the red blood cell and damages the cell membrane [7].

2.5 MANAGING BLOOD LOSS ANEMIA

Gastrointestinal, obstetric, gynecological, and various surgical procedures can all occur with high risk of hemorrhage, potentially leading to anemia. Anemia is also a common occurrence in critical care or intensive care units (ICU). There are many risks associated with obstetrics and gynecology. During pregnancy, clinicians should be prepared to use appropriate interventions to prevent or manage hemorrhage from conditions such as antepartum hemorrhage, an ectopic (extrauterine) pregnancy, miscarriage, placenta previa, placental abruption, and postpartum hemorrhage (PPH) [13]. During gastrointestinal procedures, blood loss can occur due to peptic ulcer hemorrhages, bleeding gastroesophageal varices, Mallory-Weiss tears, Dieulafoy's lesions, gastrointestinal angiomata and other conditions. Additionally, lower GI bleeding

can occur due to diverticular hemorrhage, angiodysplasias, anorectal hemorrhage, postpolypectomy site bleeding, inflammatory bowel disease and Meckel's diverticulum [14].

While blood transfusions are commonly used for the treatment of anemia, there is no universal trigger indicating blood transfusion as treatment. Current guidelines for critically ill and perioperative patients advise that, at hemoglobin values of under 7 g/dL, RBC transfusion is strongly indicated, whereas at hemoglobin values in excess of 10 g/dL blood transfusion is unjustified. For patients with hemoglobin values in the range of 7-10 g/dL, the transfusion trigger should be based on clinical indicators [15].

There are divergent views on the benefits of RBC transfusions in the critical care setting in treating anemia. One concern is that anemia may not be well tolerated by critically ill patients. A study conducted between November 1994 and November 1997 enrolled 838 critically ill patients with euvolemia, or normal blood volume, after initial treatment. 418 patients were administered a restrictive strategy of transfusion, in which red cells were transfused if the hemoglobin concentration dropped below 7.0 g/dL. The other 420 patients were assigned a more liberal strategy, in which transfusions were given when the hemoglobin concentration fell below 10.0 g/dL. Hemoglobin concentrations were maintained at 10.0 to 12.0 g/dL. The study found that the mortality rate during hospitalization was significantly lower in the restrictive-strategy group, 22.2% compared to 28.1% in the liberal-strategy group. It concluded that a restrictive strategy of red-cell transfusion is at least as effective as and possibly superior to a liberal transfusion strategy in critically ill patients. The exception to the study was those patients with acute myocardial infarction and unstable angina [16].

Complications such as infections, immunosuppression, impairment of microcirculatory blood flow, 2,3-diphosphoglycerate deficiency, and additional biochemical and physiological disturbances are associated with the use of RBC transfusions. Levels of 2,3-diphosphoglycerate have been shown to decrease with an increase in storage time [17]. 2,3-diphosphoglycerate affects the oxygen affinity of hemoglobin. Decreased levels of 2,3-diphosphoglycerate results in a decrease in the ability of hemoglobin to offload oxygen. Evidence also suggests that the transfusion of older blood, stored more than 14 days, is an independent risk factor for the development of multiple organ failure [17]. In a review of published literature on studies occurring between 1999 and 2006, Gould *et al.* reported that overall, critically ill patients who had received red blood cell transfusions had worse outcomes [17].

A recent study by Shander *et al.* found that the average total cost for transfusion ranged from $522.45 to $1183.32 per unit of RBC transfused, with a mean of 3 to 4 units transfused per patient [18]. The study took into account acquisition costs, all process steps, and all direct and indirect overhead costs of blood transfusions in surgical patients at four large hospitals.

To reduce the need for allogeneic RBC transfusions, blood cell salvage can be practiced. Devices such as the Cell Saver 5 (Haemonetics Corp., Braintree, MA), are capable of filtering blood collected in a reservoir for reinfusion [19]. A rapidly spinning Latham Bowl washes the concentrated red blood cells and returns a product equivalent to that of packed RBCs. Unprocessed salvaged blood may contain noncellular, cellular, or biochemical debris. The Cell Saver 5 system, if properly maintained and operated, eliminates 95 to 99% of unwanted contaminants [19]. Autotransfusions can also be provided to patients who donate their own blood before undergoing a procedure.

Acute normovolemic hemodilution (ANH) is an autologous blood collection technique that involves the removal of blood from a patient on the day of surgery, shortly before surgical blood loss. To maintain circulating blood volume, fluid is used to replace the blood as it is removed. The blood is collected in standard blood collection bags containing an anticoagulant agent and stored in the operating room at room temperature. The effect of whole blood withdrawal and replacement with crystalloid or colloid solution decreases arterial oxygen content, but compensatory hemodynamic mechanisms and the existence of surplus oxygen-delivery capacity make ANH a well-tolerated procedure in most patients. The major factor responsible for the increased cardiac output observed during ANH is a decrease in viscosity as the hemoglobin level declines. Low blood viscosity directly decreases systemic vascular resistance and, by improving peripheral venous flow, increases venous return to the heart. Both mechanisms result in an increase in stroke volume and cardiac output [20].

During Acute Hypervolemic Hemodilution (AHH), a fluid is infused at the beginning of surgery to achieve a reduction in the hematocrit [21]. Compared to ANH, AHH has a higher oxygen transport capacity and peripheral oxygen delivery. It may also provide a greater margin of safety in older patients.

Several physiological compensatory mechanisms also help manage blood loss; there is an inverse relationship between cardiac output and hemoglobin levels [22]. During acute hypovolemia, a condition characterized by reduced blood volume, the sympathetic stimulation preserves oxygen delivery to vital organs by increasing myocardial contractility and heart rate as well as arterial and venous vascular tone. The increased sympathetic tone diverts a decreasing cardiac output toward the coronary and cerebral circulation. Heart, lung, and cerebrovascular diseases potentially limit adaptive responses. Age, severity of illness, and therapeutic interventions may also affect these

adaptive mechanisms. Hypovolemic shock is seen when vital organ systems such as the kidneys, central nervous system, and heart are affected [22].

Visual estimation is one of the most frequently used methods to determine blood loss, in addition to measuring hemoglobin and hematocrit. It has been demonstrated as inaccurate in repeated studies [23]. In a study assessing the accuracy of visual estimation of postpartum blood loss compared to a weight-based measurement, researchers at King Abdulaziz Medical City, Riyadh, Saudi Arabia found that attending physicians and obstetrics nurses had a tendency to underestimate blood loss by about 30% [24]. Other studies have found evidence of overestimation [23]. Whether over or underestimation, gross inaccuracies have been repeatedly documented. Objective data, such as vital signs and hematocrit changes, are helpful in the clinical management of patients with large blood loss over time [23].

2.6 PULSE OXIMETRY

Pulse oximetry is used to monitor for hypoxia, a condition in which the tissues are deprived of adequate oxygen. Pulse oximetry is used to measure peripheral saturation of oxygen (SpO_2) which can be used as an estimate for arterial oxygen saturation (SaO_2). The technology is capable of distinguishing between oxyhemoglobin and deoxyhemoglobin. It uses two different light emitting diodes (LEDs), one emitting red light at approximately 660 nm and one infrared light at approximately 940 nm. Due to the red color, oxyhemoglobin absorbs less red light than deoxyhemoglobin. This light passing from the LED through the finger is measured by the photodetector positioned opposite to the LED. Each LED is illuminated at a particular programmed frequency. The software of the oximeter assumes that all of the light reaching the photodetector has the wavelength of the illuminated LED.

In order to differentiate between venous blood and arterial blood, the saturation is based on the difference between absorption through systole and diastole. During systole there is an increase in light absorption that is assumed to be created by the influx of arterial blood. The software determines the difference between absorption during diastole and systole at both wavelengths. This absorption ratio is compared to *in vivo* data to compute the SpO_2 measurement.

Pulse oximeters measure tissue light transmission at two wavelengths to estimate arterial hemoglobin saturation. Using only two wavelengths, these pulse oximeters assume the presence of only two light absorbers in the blood: oxyhemoglobin and reduced hemoglobin, or deoxyhemoglobin. The dyshemoglobins been shown to introduce error into the calculation of the oxygen saturation (SpO_2) [25]. New technology using a multi-wavelength pulse technology is capable of differentiating between multiple species of hemoglobin. Masimo Corporation (Irvine, CA) has developed several devices, including the Rad-57 Pulse CO-oximeter and Rainbow SET technology, intended to measure SpO_2, as well as total hemoglobin concentration (SpHb), carboxyhemoglobin percentage (SpCO) and methemoglobin percentage (SpMet) [25]. As with pulse oximeters, pulse CO-oximeters are subject to error due to skin color and motion artifact.

3. MEASURING TOTAL HEMOGLOBIN

Several methods are used to measure total hemoglobin content in the blood. The most common methods utilize spectrophotometric analysis of light absorbencies based on the Beer-Lambert law. Other methods take advantage of the varying conductivities of blood at different concentrations of RBCs.

3.1 SPECTROPHOTOMETRY

Spectrophotometry is the basis for many clinical laboratory instruments, including those that measure hemoglobin. It is based on the fact that substances absorb or emit electromagnetic energy at different wavelengths. A basic spectrophotometer consists of a light source, wavelength selector, cuvette, and detector as seen in Figure 3. The detector is a radiation sensor capable of measuring the amount of power leaving the cuvette [26].

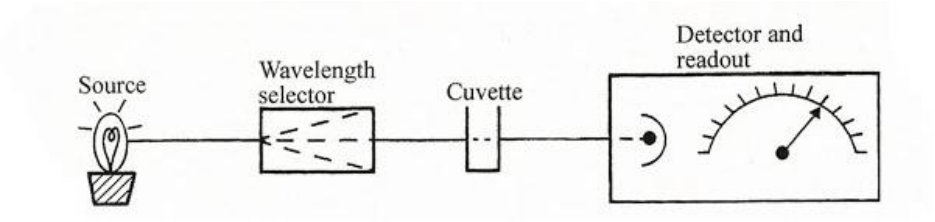

Figure 3: Block diagram of a spectrophotometer

Figure from Wheeler, Webster [26].

The cuvette holds the substance being analyzed. Substances within the cuvette sample absorb light selectively according to Beer's law. The cuvette's design must be so that it does not alter the spectral characteristics of the light as it enters or leaves the cuvette. The Beer's law relationship can be stated as follows:

$$P = P_0 10^{-aLC}$$

Where:
P_0 = radiant power arriving at the cuvette
P = radiant power leaving the cuvette
a = absorptivity of the sample (extinction coefficient)
L = length of the path through the sample
C = concentration of the absorbing substance

The percent transmittance ($\%T$) is a function of the radiant power arriving and leaving the cuvette. The absorptivity (a) and path length (L) are constant for an unknown.

Changes in P should reflect changes in the concentration of the absorbing substance. %T is defined as

$$\%T = 100\frac{P}{P_0} = \ 100\ 10^{-aLC}$$

Since the relationship between concentration and %T is logarithmic, absorbance can be defined as:

$$A = \log\ \frac{100}{\%T}\ = \log\ \frac{P_0}{P}$$

When simplified:

$$A = aLC$$

Finally, the absorbance of a standard A_s, of a known concentration C_s, is determined. The absorbance of the unknown A_u is determined by the relationship:

$$C_u = C_s\ \frac{A_u}{A_s}$$

Using the known extinction coefficients for the different forms of hemoglobin plotted in Figure 4, unknown concentrations of hemoglobin can be determined in whole blood samples. This is the basis for hemoglobin measurements is many laboratory analyzers and CO-oximeters.

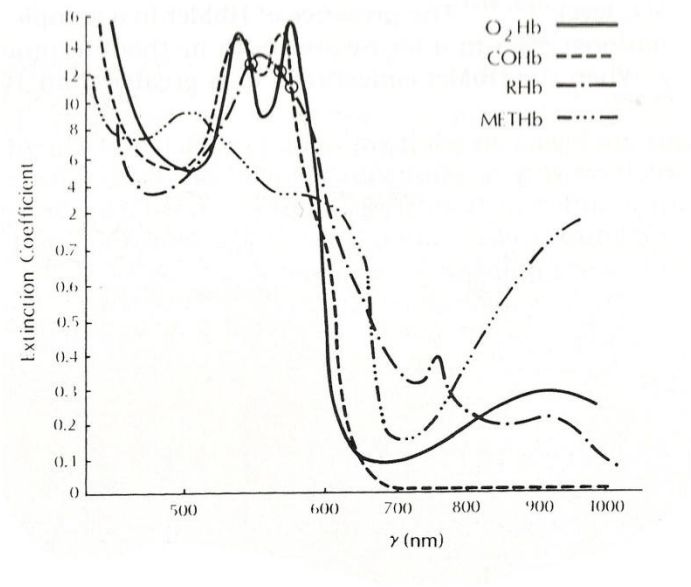

Figure 4: Extinction coefficients for hemoglobin moieties

Figure from Shapiro, Peruzzi, Templin [9]

3.2 HEMIGLOBINCYANIDE METHOD

The Hemiglobincyanide (HiCN) method chemically converts hemoglobin to HiCN, a form of hemoglobin that can be measured spectrophotometrically and has a relatively broad absorption maximum around a wavelength of 540 nm [27]. This technique is the most broadly used and appears in various technologies including: hematology analyzers, blood gas analyzers, stand-alone CO-oximeters and point of care testing devices.

In general, blood samples are sent through a lyzing chamber to prepare the specimen for measurement. The cell membranes rupture releasing the hemoglobin. After the sample is diluted by a lyzing agent, a second substance, Drabkin's reagent, is added. This converts the hemoglobin to cyanmethemoglobin or HiCN [26]. Drabkin's reagent contains iron, potassium, cyanide, and sodium bicarbonate [4]. This method using HiCN is the accepted standard for determining hemoglobin concentration; the advantage is that it includes essentially all forms of hemoglobin found in the blood [26].

Next, the absorbance at a particular wavelength is measured and related to the hemoglobin concentration as determined by the spectral analysis in Figure 4. The hemoglobin concentration is determined from the absorbance. The absorbance of azidemethemoglobin, as opposed to HiCN, is sometimes used to quantify the hemoglobin content of the blood. An azidemethemoglobin reagent contains a lyzing chemical such as sodium deoxycholate, an oxidizing chemical such as sodium nitrite, and an azide. The oxyhemoglobin in the blood containing ferrous iron is oxidized to form methemoglobin, containing ferric iron. The methemoglobin then combines with the azide to form azidemethemoglobin. This stable compound can also be measured spectrophotometrically [4].

3.2.1 HEMATOLOGY ANALYZERS

A Complete Blood Count (CBC) is a broad screening test used to check for certain disorders relating to the blood. Whenever a CBC is requested from the clinical laboratory, samples are processed on a hematology analyzer. A standard CBC includes: RBC count, WBC count, hemoglobin, HCT, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), and platelet count. Using the Coulter Principle, the analyzer can electronically count and size the red blood cells. In addition to electronic particle counting, hematology analyzers use HiCN or azidemethemoglobin methods to spectrophotometrically measure total hemoglobin and the dyshemoglobin content.

Blood samples are drawn by a phlebotomist, medical technologist or other qualified clinician in a collection tube containing an anticoagulant such as Ethylenediaminetetraacetic Acid (EDTA). The samples are labeled, bar-coded and processed in the laboratory. Once arriving at the hematology analyzer, the samples are

inserted into the analyzer and the automated measurement process is initialized by the lab technician.

Patient results are recorded in a Laboratory Information System (LIS), a database storing any laboratory data relating to a patient. Often, data is compared to previous data and abnormal variations, such as a drop in hemoglobin, are flagged. If the technician suspects an error, measurements on the analyzer may be repeated. The particle counting method used by the analyzers can only count mature RBCs. If a CBC results in a low MCHC, it may be flagged for a manual count. A low MCHC could be caused by either small RBCs or IV contamination. If venous blood is drawn distal to an Intravenous infusion, the sample would be diluted.

Modern analyzers have automated cleaning and Quality Control (QC) processes. Cleaning can take up to two hours to complete. QC is completed daily. Additional calibration is typically required after maintenance, as required by QC, or every 6 months depending on the manufacturer's specifications.

3.2.2 BLOOD GAS ANALYZERS

Blood gas analyzers are devices used for the determination of pO_2, pCO_2, pH, sodium (Na^+), potassium (K^+), ionized calcium (Ca^{++}), chloride (Cl^-), glucose, lactate, total hemoglobin (tHb) and the dyshemoglobins in arterial, venous, and capillary whole blood samples. Additional CO-oximetry features of some analyzers allow the measurement of tHb, Fraction of oxygenated Hemglobin (FO_2Hb), fraction of reduced hemoglobin (*F*HHb), fraction of methemoglobin (*F*MetHb), and fraction of carboxyhemoglobin (*F*COHb) [28].

The Bayer RapidLab 800 series of blood gas analyzers is a family of analyzers that provide different functionality. The base models can measure tHb and FO_2Hb, as well as

the standard blood gas measurements. More featured models, such as the RapidLab 865, have a separate CO-oximetry module that can measure the fractional components of the different hemoglobin derivatives. This CO-oximetry module spectrophotometrically measures hemoglobin. It contains an optics module and lamp, hemolyzer, and sample chamber. First the sample is pumped through the hemolyzer which uses ultrasonic sound vibrations to rupture the red blood cells and release the hemoglobin. The sample then enters the sample chamber for spectrophotometric measurement [28].

The RapidLab 865 has various reagent containers, cleanser solutions, and calibration solutions. The calibration procedure adjusts the electronic signal from the photometric sensor with the concentration of the known solution. One and two-point calibration procedures can be performed [28].

3.2.3 POINT OF CARE TESTING

Several devices available for use are point of care testing (POCT) instruments based on HiCN or azidemethemoglobin spectrophotometric measurements. These devices are useful in critical care areas as well as during surgical procedures in the operating room. The HemoCue Hb 201^{+} and 201DM Analyzers, manufactured by HemoCue AB (Ängelholm, Sweden) are widely used for discrete, instant total hemoglobin measurements. The HemoCue uses a modified azidemethemoglobin reaction to spectrophotometrically measure total hemoglobin.

The two models are similar; however, the HemoCue Hb 201DM System incorporates additional data management capabilities. The system consists of the HemoCue Hb 201 DM Analyzer, HemoCue Hb 201 Microcuvettes and the HemoCue DM Docking Station. The HemoCue 201 DM Analyzer spectrophotometrically measures the hemoglobin in the form of azidemethemoglobin as previously described. A whole blood

sample from the patient is required. Capillary, venous or arterial blood can be used. Since capillary blood can be used, a sample can also be obtained through a minimally invasive finger-prick. When the microcuvette is brought into contact with the exposed blood, approximately 10µL is drawn into the cavity of the microcuvette through capillary action [29]. If a venous or arterial sample is obtained, a small amount of blood should be dispensed onto a hydrophobic plastic or glass slide. The sample should be well-mixed. The microcuvette can then be applied to the aliquot of well-mixed blood.

The microcuvette contains sodium deoxycholate which acts initially to lyze the red blood cells and expose the hemoglobin. The hemoglobin is released and converted to methemoglobin by sodium nitrite. The methemoglobin combines with azide to generate azidemethemoglobin. Using an optical based method, the absorbance is measured at dual wavelengths: 570 nm to quantify azidemethemoglobin and 880 nm to compensate for sample turbidity [30].

The HemoCue DM Docking Station serves as a base station for charging and data upload. The HemoCue DM Analyzer can be customized to prompt for patient ID information, user ID or microcuvette lot through use of the barcode scanner. It can also prompt for quality control to meet regulatory requirements.

3.3 CONDUCTIVITY-BASED METHOD

The conductivity-based method measures the conductivity of the blood sample between two electrodes to determine the hematocrit. The measured conductivity is inversely related to the blood hematocrit. The hemoglobin concentration is calculated by the assumption that hemoglobin is approximately one-third of the total hematocrit.

Blood has a high temperature coefficient and it is essential to maintain a constant temperature during measurement. Conductivity-based devices have built-in thermostat-

regulated temperature chambers to regulate the sample temperature. The most abundant electrolyte in plasma is sodium. Increases or decreases in sodium concentration will affect RBC volume and ultimately, the hematocrit measurement. Additionally, decreases or increases in the protein concentration of plasma can also alter results [31].

3.3.1 I-STAT

The i-STAT portable handheld is produced by Abbott Laboratories (Abbott Park, IL). It is capable of an array of patient-side tests including hematocrit and hemoglobin as well as additional tests of chemistries, electrolytes, blood gases, coagulation and cardiac markers. Various tests are available depending on the single-use test cartridge used.

The i-STAT is similar to the HemoCue in that it is cartridge-based. First, whole blood is introduced by capillary action into the single-use micro-fabricated biosensor cartridge. Capillary, venous or arterial blood can be used. The conductivity is measured by the i-STAT device and corrected for electrolyte concentration. The i-STAT provides a hematocrit result within 90 seconds.

3.4 MULTI-WAVELENGTH PULSE CO-OXIMETERS

Masimo's noninvasive pulse CO-oximetry technology works similar to pulse oximetry but uses multiple wavelengths of light to discern the dyshemoglobins. Pulse oximeters are not capable of measuring carboxyhemoglobin and methemoglobin; the presence of either of the dyshemoglobins induces error in the SpO_2 measurement. The first device to use the pulse CO-oximetry technology was the Rad-57 handheld.

3.4.1 RAD-57 HANDHELD PULSE CO-OXIMETER

The Rad-57 is a continuous noninvasive CO-oximeter, measuring arterial oxygen saturation of oxygenated and deoxygenated hemoglobin (SpO_2), pulse rate and carboxyhemoglobin saturation (SpCO). The measurement is taken by placing a sensor on the patient, similar in appearance to the sensor used in traditional pulse oximetry technology, and connecting it to the device. The oxyhemoglobin, deoxyhemoglobin and carboxyhemoglobin species differ in their absorption of visible and infrared light. The sensor passes various visible and infrared light through a capillary bed, such as the fingertip. The photodetector receives the light and converts it to an electronic signal. The amount of arterial blood in tissue changes with your pulse. The varying quantities of arterial blood changes allow the device to determine the pulse. The device is indicated for use on neonate, infant, pediatric and adult patients [32]. The operating range accuracy for the Masimo Rad-57 is listed below in Table 4.

Table 4
Specifications and Operating Ranges for Masimo Rad-57

Range	
Oxygen Saturation (%SpO_2)	1-100%
Carboxyhemoglobin Saturation (%SpCO)	1-99%
Accuracy	
Oxygen Saturation During Motion and No Motion Conditions	
Adults, Pediatrics	70-100% ± 2 digits 0-69% unspecified
Neonates	70-100% ± 3 digits 0-69% unspecified
Carboxyhemoglobin Saturation(%SpCO)	0-40% ± 3 digits
Resolution	
Oxygen Saturation (%SpO_2)	1%
Carboxyhemoglobin Saturation (%SpCO)	1%

Data from Food and Drug Administration [32].

4. ACCURACY OF TOTAL HEMOGLOBIN MEASUREMENTS

To validate the use of pulse CO-oximeters to laboratory and point of care methods, multiple correlation studies have been completed. There is already significant variation between the various laboratory methods, as well as the point of care methods as previously discussed. Laboratory analyzers are particularly susceptible to errors due to sample storage and EDTA.

4.1 CYANMETHEMOGLOBIN/AZIDEMETHEMOGLOBIN

Gamma-Dynacare, a Canadian-based community laboratory partnership, sought to standardize their laboratory analyzers across their network of laboratories. A 4-way evaluation was developed by Bourner *et al.* to compare the Abbott Cell-Dyn 3500, their currently used analyzer, to the Beckman Coulter LH 750, Bayer Advia 120, and Sysmex XE2100 [33]. Using the same samples, the evaluation determined precision, within-run and run-to-run, linearity, and sample stability. Table 5 summarizes the coefficient of variation for stability and precision, as well as the average systematic error calculated during the linearity test. The coefficient of variation, the ratio of the standard deviation to arithmetic mean, is useful in comparing the reproducibility of the different variables.

Table 5
Laboratory Analyzer Comparison of Stability, Precision and Average Error

	Long Term Stability Testing at RT - Hgb	Long Term Stability Testing at RT - HCT	Within Run Precision - Hgb	Between Day Precision - Hgb	Average Systematic Error
Lab Analyzer	%CV	%CV	%CV	%CV	%
LH 750	0.80	0.77	0.4	0.7	1.9
Advia 120	1.20	1.34	0.6	0.7	3.0
XE 2100	0.90	0.85	0.7	0.8	2.1

Data from Bourner, Dhaliwal and Sumner [33].

Within run precision for total Hemoglobin was assessed on each analyzer by running a quality control material a total of 10 times. The run-to-run precision or between day precision was intended to look at calibration drift over time; each quality control

material was analyzed on a daily basis. Sample stability was also measured to determine the effects of sample storage on the measurement. The LH750 had the lowest failure rate with only three occurrences outside of the total allowable error set for each parameter. This compares to a failure rate of 10 or more for the other analyzers. The LH750 was also the least affected by aged samples as shown by the stability testing with minimal changes in MCV and HCT. The study concluded with the selection of the LH750 as their standard laboratory analyzer.

Torp *et al.* tested the correlation between the Beckman Coulter Ac-T diff2 laboratory analyzer and the Nova Biomedical pHOx laboratory CO-Oximeter [34]. Using arterial blood samples from 33 liver transplant patients, Torp *et al.* found the correlation between the two devices to be 0.93 with a bias of 0.97 g/dL. The precision was found to be 0.5 g/dL. The results are summarized in Table 6.

Table 6
Correlation of Hematology Analyzer and CO-Oximeter

Study	Hematology Analyzer	CO-Oximeter	Corr	Bias	Precision	Arms
Torp *et al.* n=471	Coulter Ac-T diff2 (Beckman Coulter)	pHOx CO-Ox (Nova Biomedical)	r=0.93	-0.97 g/dL (Coulter-pHOx)	0.58 g/dL	1.13 g/dL

Data from Torp,K [34].

4.2 CONDUCTIVITY BASED METHODS

While the conductivity-based method used by the i-STAT provides a hematocrit result within 90 seconds, the reliability of the measurement is debatable. A preliminary search on the FDA's Manufacturer and User Facility Device Experience (MAUDE) database returned several reports of discrepancies between multiple readings on the i-STAT as well as in comparison to the lab CO-oximetry device. One report, posted September 24, 2007 by the manufacturer, presented a case where the measured hemoglobin was 7.5 g/dL and hematocrit 22%. An hour and a half later the measured hemoglobin was 6.8 g/dL and hematocrit of 20%. The same sample was sent to the

laboratory yielding a result of hemoglobin 7.9 g/dL and hematocrit of 22.5%. According to the report, an unnecessary transfusion was performed based on the i-STAT result of 6.8 g/dL [35].

Another report, dated July 24, 2007, detailed a hematocrit measurement of 22% followed by another sample 10 minutes later at 25%. Since the patient didn't present with any symptoms related to low hematocrit, the samples were retested on a different device. The hematocrit results were 36% [35]. A study of reconstituted whole blood samples with varying hematocrit and hemoglobin levels compared the accuracy and precision of the i-STAT with the HemoCue as well as a GenS Laboratory Analyzer [36]. Hemodilution was simulated by diluting samples with saline or lactated Ringer's solution. The HemoCue correlated well with a constant bias of 0.3 g/dL. The discrepancy of the i-STAT increased with the solutions of lower protein content and lower hematocrit and hemoglobin levels. To correct for these discrepancies, the manufacturer added a Cardiopulmonary Bypass (CPB) mode that automatically corrects hematocrit for the decreased plasma protein levels typically associated with hemodilution.

4.3 MULTIWAVELENGTH PULSE CO-OXIMETERS

In comparing the pulse CO-oximeters to laboratory analyzers, most clinical studies use the Bland-Altman method for assessing agreement between two methods of clinical measurements [37]. To measure the agreement and determine interchangeability of two different methods, the Bland-Altman statistical approach compares the measured difference to the average of the two measurements since neither method is considered a "gold standard". Similar to a t-test, the measurement correlation, bias, and precision are reported.

Allard *et al.* compared hemoglobin measurements from the Masimo Radical 7 to the Radiometer ABL820 laboratory CO-oximeter [38]. The test was composed of 20 patients undergoing a hemodilution protocol as approved by the internal review board. Macknet *et al.* completed a study with 49 surgical patients and 18 volunteers undergoing hemodilution [39]. A prototype pulse CO-oximeter from Masimo was compared to the Radiometer ABL 735 laboratory CO-Oximeter. Macknet *et al.* also reported on a single kidney transplantation case study where measurements of arterial samples show good correlation during rapidly changing concentrations of hemoglobin [40]. The precision of the laboratory CO-Oximeter was 0.4 g/dL compared to the 0.74 g/dL of the pulse CO-Oximeter.

The correlation for noninvasive pulse CO-Oximeters, as seen in Table 7, ranges from 0.83 to 0.88. These correlations are lower than the correlation of 0.93 seen in the study by Torp *et al.* comparing the Coulter Ac-T Diff2 laboratory analyzer and pHOx CO-Oximeter. The studies also show that the pulse CO-Oximeter may not be as precise; however, the required precision for measuring hemoglobin is undetermined. The added value from the pulse CO-oximeters is that the continuous monitoring allows for trending, providing feedback for instantaneous hemoglobin changes.

Torp *et al.* also presented a study on 5 patients undergoing liver transplantation [41]. Arterial measurements on a Nova Biomedical pHOx Plus laboratory CO-oximeter were compared to the Masimo pulse CO-oximeter. The study concluded that the measurements were comparable in accuracy and that inherent device and physiologic variation should also be considered. Lamhaut *et al.* studied 20 patients undergoing urologic surgical procedures to compare the Masimo CO-Oximeter to a laboratory CO-Oximeter [42]. This study concluded that the correlation and standard deviation are acceptable, but should improve with continued sensor development. Lamhaut *et al.* also

concluded that further studies to define indications and limits of the technology are necessary.

Table 7
Summary of Studies Comparing Noninvasive Pulse CO-Oximeters to Laboratory CO-Oximeters

Study	Sample	Device Tested	Standard	Correlation	Bias	Precision	Arms
Allard [A]	n=335	Masimo Radical 7	Radiometer ABL 820	r=0.84 (p<0.001)	-0.15	0.92	
Macknet [B]	n=1207	Prototype pulse CO-Oximeter	Radiometer ABL735	r=0.827	-0.11 g/dL	1.28 g/dL	1.28 g/dL
Macknet [C], case study	n=17	Prototype pulse CO-Oximeter	Radiometer ABL735		0.146g/dL	0.740 g/dL	
Torp [D]	n=55	Masimo Pulse CO-Oximeter	Nova Biomedical pHOx Plus		0.2 g/dL	0.8 g/dL	0.8 g/dL
Lamhaut [E]	n=54	Masimo Radical 7	Hb Lab - unknown	r=0.88	0.26 g/dL	1.11 g/dL	

[A] Data from Allard, M [38]; [B] Data from Macknet, Norton, Kimball-Jones, Applegate, Martin, Allard [39]; [C] Data from Macknet, Kimball-Jones, Applegate, Martin, Allard [40]; [D] Data from Torp,K [41]; [E] Data from Lamhaut, L [42].

Since the pulse CO-oximeter has the advantage of instantaneous continuous monitoring, it could be proposed as a replacement for POCT devices. Jou *et al.* directly compared the Abbott Laboratories i-STAT and Masimo Pulse CO-Oximeter to the Abbott Diagnostics Cell-Dyn Sapphire Differential Cell Counter within a single study [43]. The final study was composed of 15 pediatric patients undergoing a variety of surgical cases. An average of 2.7 arterial blood samples were collected per patient. The Bias, precision and root mean square are summarized in Table 8.

Table 8
Evaluation of Pulse CO-oximeter, POC, and Lab Analyzer

Sample	Device Tested	Bias	Precision	Arms
n=46	i-STAT	-0.26 g/dL (POC-Lab Hb)	0.46 g/dL	0.53 g/dL
n=92	Masimo Pulse CO-Oximeter	0.18 g/dL (SpHb-LabHb)	1.10 g/dL	1.12 g/dL

Data from Jou, C [43].

The study concluded that the directional changes provided by the continuous monitoring were earlier indications than the intermittent hemoglobin values provided by POCT and lab sampling.

In addition to measuring total hemoglobin, Masimo's new noninvasive pulse CO-Oximeter technology can measure carboxyhemoglobin and methemoglobin. The majority of the research surrounds the diagnosis of carbon monoxide poisoning (COP) and early triage of COP in the ED. Coulange *et al.* evaluated the reliability of pulse CO-oximetry technology for noninvasive real-time measurement of COHb levels in victims of COP [11]. An investigation led by Suner *et al.* also concentrated on screening and identifying occult COP. Taking place at Rhode Island Hospital, Suner *et al.* put a policy in place requiring noninvasive SpO_2 measurements being taken during the triage of all patients with few exceptions [10].

According to an article submitted for publication March 9, 2006, Barker *et al.* performed a study on 20 healthy volunteers in affiliation with the University of Arizona College of Medicine [25]. This study aimed at evaluating the accuracy and reliability of Masimo's pulse CO-oximetry technology in measuring COHb and MetHb compared to lab CO-oximetry devices. As noted in the article, Touger *et al.* led one of the most recent studies of 120 emergency department (ED) patients, between January 19, 2008 and April 9, 2009 [39]. Dr. Michael Touger has also published research assessing the accuracy of using venous blood to estimate arterial COHb levels [44].

The study by Coulange *et al.* used Masimo Rad-57 CO-oximeters to diagnose patients admitted to the ED suspected of COP [11]. The noninvasive measurement was obtained in combination with the standard work-up procedures without changing therapeutic strategies. A venous blood sample was obtained for analysis with an IL 682 CO-oximeter (Instrumentation Laboratory, Barcelona, Spain) at the same time as a measurement using a Rad-57 CO-oximeter. The sensor of the Rad-57 was placed on the patient's middle or ring finger.

Suner *et al.*, working with Rhode Island Hospital, replaced all of the standard pulse oximeters in the public triage area as well as in the ambulances with Masimo Rad-57 pulse CO-oximeters [10]. This allowed them to create a large sample potentially including any patient presenting to the ED by ambulance, personal vehicle, or walk-in. Nurses and technicians in the ED were trained in the use of the CO-oximeters as well as any factors that might affect the SpCO reading, including false nails, slender fingers, and inappropriate sensor positioning. Research assistants reviewed patient charts daily, recording SpCO as well as several other details generally recorded. This study was not only interested in the accuracy of the Masimo technology compared to lab CO-oximetry, but also the ability of the device to diagnose occult COP. Nursing staff were provided with questions to ask at triage about possible environmental CO sources. These questions also established a smoking history, the identification on non-conventional heating sources, or use of equipment with combustion engines. Standard triage information was also recorded, such as age, gender, venous COHb, SpO_2 heart rate, etc.

In addition to obtaining patient data, the research assistants also interviewed clinicians working in the Rhode Island Hospital ED the day after any diagnosis of COP to

ascertain what, if any, role the SpCO measurement had in reaching the diagnosis and if COP was occult and detected as a result of standard SpCO triage screening.

The study by Barker *et al.* was the only study to assess the accuracy of the Masimo Rad-57 device in diagnosing methemoglobinemia through the measurement of methemoglobin (MetHb); it was also the only study composed of volunteers with induced symptoms [25]. Twenty subjects were used for the study, 10 for COHb and 10 for MetHb. Each subject had peripheral venous and radial arterial cannulas inserted, monitoring by three-lead ECG and automated sphygmomanometer. The Masimo Rad-57 Rainbow sensors were placed on digits 2, 3 and 4 on both hands. Blood was sampled periodically from the radial arterial cannula for analysis by three calibrated lab CO-oximeters: one ABL-730 (Radiometer America, Copenhagen, Denmark) and two Radiometer OSM-3s (Radiometer America).

Carboxyhemoglobinemia was induced in the first group of 10 subjects by the inspiration of CO at 0.3% delivered by a Drager-2A anesthesia machine. The CO was adjusted to 500 ppm which is designated by the US Occupational Safety and Health Administration as the maximum safe level for 15-minute exposure. Methemoglobinemia was induced in the second group of 10 subjects by the infusion of sodium nitrite, approved by the FDA for treatment of cyanide toxicity. The sodium nitrite was infused at a rate of 6mg/min for a total dose of 300 mg.

Touger *et al.* assessed the agreement between the Masimo Rad-57 and the Siemens RapidLab 1200 blood gas analyzer [45]. Patients presenting to the Jacobi Medical Center suspected of COP were eligible for inclusion, with the exception of patients with burns involving the fingers. In a sample of 120 ED patients, clinicians were asked to place the finger probe on the patient's digit for 15 seconds, read the

measurement, and remove the probe. The probe was replaced on the same digit, allowed to recalibrate and another measurement was taken. Arterial or venous blood was taken with the first measurement and sent to the laboratory for measurement of whole blood COHb by the Siemens RapidLab 1200.

The first study, led by Macknet *et al.*, investigating the ability of Masimo's pulse CO-oximetry device in measuring hemoglobin concentration, included 49 patients scheduled for surgery and 18 healthy volunteers [39]. The subjects were monitored and a radial artery cannula was used for arterial blood samples. Three prototype Masimo SpCO sensors were used. The 18 healthy volunteers went through a hemodilution protocol, consisting of the withdrawal of one unit of blood and replacement with 30 ml\kg of saline, while the patient was monitored using the Masimo sensors. Blood samples were collected during each surgery or hemodilution procedure through the arterial cannula and analyzed by an ABL-735 CO-oximeter (Radiometer). These results were compared with the SpCO readings from the prototype Masimo device. The second documentation of this study was very similar; however, it only included 30 surgery patients and 18 healthy volunteers [46]. The same test procedure and test devices were used.

The results in all studies were reported using Bland and Altman methods or plots, used to measure agreement between two methods using bias (mean error) and precision (standard deviation of error) [37].The results from all studies are summarized in Table 9. Barker *et al.* showed very good agreement when measuring Methemoglobin with the Masimo Rad-57. The average mean difference of the pooled data was 0.00 [25]. Since this was the only study available measuring methemoglobin, additional research would need to be produced to validate this error.

Table 9
Summary of Results: Linear Regression and Bland and Altman

Study	Sample	Range	Correlation	Bias	Precision
Carboxyhemoglobin					
Touger *et al.* [A] n=120	Arterial/ venous	(0-38%)		1.4%	1.96%
Barker *et al.*[B] n=530	Arterial	(0-15%)		-1.22%	2.19%
Suner *et al.*[C] n=64	Venous	(0-33%)	r=0.72	-4.2%	4.2%
Coulange *et al.*[D] n=12	Venous	(1.2 – 31.6%)		-1.5%	2.5%
Methemoglobin					
Barker *et al.*[B] n=970	Arterial	(0-12%)		0.00%	0.45%

[A] Data from Touger, Birnbaum, Wang, Chou, Pearson [45]; [B] Data from Barker, Curry, Redford, Morgan [25]; [C] Suner, Partridge, Sucov, Valente, Chee et al [10]; [D] Coulange, Barthelemy, Hug, Thierry, DeHaro [11].

Overall, the studies show that the device error is within that expected from the manufacturer specifications. The difficult part is determining what clinical values are acceptable. Various values were assumed for the acceptable limit of SpCO in diagnosing COP. Touger *et al.* used 15% as the cutoff for diagnosing COP [45]. Suner *et al.* left the diagnosis completely up to the clinician. In retrospect, they found that this cutoff was 9% for non-smokers and 13% for smokers [10]. COP is difficult to diagnose since symptoms are not always present. Misdiagnosis however could lead to unnecessary and costly hyperbaric oxygen treatment or possible other complications.

5. FACTORS CONTRIBUTING TO MEASUREMENT UNCERTAINTY AND ERROR

Various factors can contribute to the expanded measurement uncertainty of the total hemoglobin concentrations. Each method and device has unique contributors adding to this potential error. In POCT devices, the majority of the factors have been eliminated due to engineering controls; however, some sources still exist, mostly related to use error. Some methods also have intrinsic error which doesn't contribute to measurement uncertainty, but will potentially affect the outcome of the measurement. Since

noninvasive CO-oximeters are likely to compete against POCT devices, the measurement uncertainty is compared between those devices.

The HemoCue is classified as a CLIA waved device by the FDA; however, use error has not been completely eliminated. The following can have an impact on the hemoglobin measurement:

- Air bubbles within the microcuvette
- Sampling duration
- Improper capillary collection technique

If a microcuvette is filled and contains air bubbles in the optical eye of the microcuvette, the portion through which the spectrophotometric measurement is taken in the HemoCue, erroneously low readings could be produced. Readings must be made within 10 minutes of filling the microcuvette; otherwise false results may also be obtained. Finally, when obtaining blood samples from a finger or heel stick, the first drop of blood should never be used to avoid the hemolysis of blood cells mixing with any alcohol on the prepared skin surface. Measurement errors due to improper handling of the device are omitted. It is assumed that the required quality control and electronics check would capture any failure of the device.

The i-STAT requires the use of an electronically-based cartridge for each sample measurement. Due to the nature of conductivity-based measurements, the device has several unique contributors to errors to error including:

- Use of an EDTA tube
- Decreased protein concentration in sample
- Incorrect collection tube or technique
- Extensive sampling duration

The use of an EDTA tube will cause a clinically significant error in hematocrit results. Venous whole blood samples collected in sodium or lithium heparin evacuated tubes is required. The i-STAT is primarily electronic and contains a fluid sensor to electronically verify the flow of fluids within the cartridges and ensure the sample is free of air segments. As opposed to using a “wet” quality control procedure, the i-STAT’s electronic simulator can verify the conductivity circuitry used for the hematocrit test at multiple levels.

As previously discussed in Section 3.3, sodium and protein concentrations can also affect the hematocrit measurements on the i-STAT. To address this problem, a CPB option was implemented for use with samples with abnormally low protein levels. The instrument automatically corrects hematocrit for the decreased protein levels typically associated with hemodilution by approximately 3% [36].

Similar to pulse oximetry, pulse CO-oximetry is susceptible to measurement error from the following sources [47]:

- Ambient light interference
- Low peripheral perfusion
- Motion artifact
- Incorrect sensor positioning
- Nail polish

Ambient light can be absorbed by the photodetector of a pulse CO-oximeter finger probe and interpreted as a pulsatile absorbance signal form the patient. Shielding around the finger probe or photodetector helps to minimize this interference. If there is no detectable peripheral pulsation, the pulse CO-oximeter cannot function. Hypotension, cold extremities and sever vascular disease are all factors that reduce peripheral pulsations.

A pulse CO-oximeter cannot be used during CPB since pulsatile blood flow is typically not present. Patient motion can also cause error in the readings; however, advances in pulse oximetry and CO-oximetry technology have reduced this. Finally, nail polish can also affect the measurement and should be removed before probe placement.

6. REGULATION AND MANAGEMENT OF CO-OXIMETRY DEVICES

Laboratory medicine is responsible for managing the Hematology analyzers, blood gas analyzers, and POCT devices that measure hemoglobin concentration and must assure their compliance with regulations. The Clinical Laboratory Improvement Act (CLIA) of 1988 establishes quality standards for laboratory testing based on complexity of test method. The Center for Medicare and Medicaid Services (CMS) administers the CLIA laboratory certification program in conjunction with the Food and Drug Administration (FDA) and the Center for Disease Control and Prevention (CDC). The FDA is responsible for test categorization [48]. Test methods or devices are categorized into three levels of complexity: waived, moderate and high. The more complicated the test, the more stringent the requirements. The classification of a selection of devices is shown in Table 10. Most of the analyzers are classified as moderately complex by the FDA.

Laboratories can apply for various certificates for accreditation. A Certificate of Waiver permits a laboratory to perform only waved tests. Waved tests have been determined to be so simple and accurate that there is little risk of error if the test is performed incorrectly. CLIA waved instruments are designed to be used by individuals not trained in laboratory science. A Certificate of Compliance is issued to laboratories to perform moderate or high complexity testing. For laboratories conducting moderate or high complexity testing, CMS conducts surveys to determine a laboratory's regulatory compliance. Additionally, by law, these labs must also participate in proficiency testing

three times per year to evaluate whether the laboratory's results are accurate and consistent with peer laboratories. Biennial inspections are completed by an accreditation program approved by CMS; this allows the laboratory to apply a Certificate of Accreditation [48].

Table 10
CLIA Test Complexity for a Selection of tHb Instruments

Manufacturer	Model	Classification
Hematology/Chemistry Analyzers		
Beckman-Coulter	LH-750	Moderate
Siemens	Advia 1200	Moderate
Sysmex	XE-2100	Moderate
CO-Oximeter		
Instrumentation Laboratory	IL682	Moderate
Nova	CO-Oximeter	Moderate
Blood Gas Analyzer		
Radiometer	ABL90	Moderate
Siemens	Rapidlab1200	Moderate
Point of Care Testing		
Abbott Laboratories	i-STAT	Waved/Moderate*
A-VOX Systems	AVOXimeter 4000	Moderate
HemoCue	HB 201 System	Waved

*i-Stat classification dependent on specific cartridge analytes
Data from CLIA Test Complexity Database [49].

The HemoCue has been classified as a waved test by the FDA. Engineering controls are built in that require completion of appropriate quality control before the device can be unlocked for use. Quality control requires several control standards of varying hemoglobin levels. Multiple levels of quality control are carried out throughout the day as indicated by the manufacturer, licensure organizations, and good lab practices. Patient information may be entered; information on the microcuvette or cartridge is also entered to ensure the disposables have not expired. In addition to CMS accreditation, The College of American Pathologists (CAP) also oversees laboratory medicine. These agencies review the POCT programs during inspection to ensure the users are trained,

proper procedures for quality control are being followed, and the devices are being maintained in accordance with the manufacturer's recommendations.

Hematology analyzers and blood gas analyzers are all classified as moderately complex due to the staff knowledge and training required, use of reagents, complicated operational steps and calibration and quality control procedures. The instruments measure several other analytes in addition to hematocrit and hemoglobin.

Noninvasive pulse CO-oximeters are classified as monitoring devices by the FDA and subsequently do not fall under the CAP and CMS accreditation of laboratory medicine. Like pulse oximeters, these devices use either a disposable or reusable finger probe. The reusable or "reposable" finger probes are guaranteed for approximately 500 uses. Preventative maintenance is carried out annually using a simulator to verify performance. Routine quality control is not necessary. The device can be operated by a respiratory therapist, registered nurse, certified nursing assistant, or doctor.

7. DISCUSSION

The previously discussed studies provided a comparison of total hemoglobin measurements between various hematology analyzers, POCT devices, and noninvasive pulse CO-oximeters. Significant variation was found by Bourner *et al.* when comparing multiple hematology analyzers. The average error of these instruments when compared to the lab's existing analyzer ranged from 1.9% to 3.0%. Even at the low end of the reference range, 7 g/dL, an error of 3% on a total hemoglobin concentration would be 0.21 g/dL. This is minimal error considering if a value in the range of 7 g/dL was obtained, the measurement would likely be retested.

Correlation studies comparing pulse CO-oximeters to laboratory analyzers found the correlation of the pulse oximeters to range from 0.83 to 0.88. When comparing blood gas

analyzers to laboratory analyzers, correlations as high as 0.93 were found. Pulse CO-oximeters do not correlate as well to laboratory analyzers as do blood gas analyzers or other table top CO-oximetry devices.

Other studies looked at the precision of the pulse CO-oximeter compared to laboratory analyzers. The precision found ranged from 0.74 to 1.28 g/dL between several studies. One study in particular compared the precision of an i-STAT and pulse CO-oximeter to a laboratory analyzer. The precision of the i-STAT and Pulse CO-oximeter was found to be 0.46 g/dL and 1.10 g/dL. Assuming the confidence interval is 65% for a normal distribution, the precision could be too low for obtaining a confident measurement of total hemoglobin. If the actual value of hemoglobin was 7 g/dL, the measured value could lie between 5.9 and 8.10 g/dL. If 7 g/dL was being used as a restrictive transfusion indicator, the patient may be unnecessarily transfused at a hemoglobin concentration of 5.9 g/dL when using a SpHb monitor.

As previously indicated, pulse CO-oximeters are most likely to replace POCT devices since hematology and blood gas analyzers are still going to be required for measuring other analytes. POCT devices are subject to much more use error than pulse CO-oximeters due to the sample collection that is necessary for measurement. Specimens or tubes may be mislabeled causing a turn-back and another sample collection from the patient. The wrong tube may be used for the wrong method. An incorrect sample type could be obtained, i.e. venous as opposed to arterial, leading to invalid results that may not be discovered. If a questionable measurement is obtained, the typical protocol is to run QC on the POCT device to validate the performance and then rerun the test. Significant opportunity for human error or use error is still inherent in the POCT devices.

Since the pulse CO-oximeter is classified as a monitoring device, it is much less regulated than the POCT devices. POCT devices require extensive maintenance, QC and training which must be maintained constantly to meet the requirements of licensure organizations. CLIA waved devices still require extensive management of the device by laboratory medicine.

Finally, the costs associated with POCT are likely significantly higher. Cartridges for the hemoglobin test on the i-STAT can cost hundreds of dollars. The microcuvettes used with the HemoCue are much less expensive, but require closer monitoring to maintain storage requirements and guarantee expired lots of microcuvettes are not used. Additional savings in reduced transfusion costs can also be argued.

One of the limitations of this research is the lack of detailed research. A number of the studies cited in this paper are summaries published through conferences. An additional limitation to the studies is the difficulty of testing the instrument at physiological extreme levels of hemoglobin concentration. Volunteer testing using hemodilution methods cannot explore the upper limitations of the hemoglobin concentrations. In the studies using surgical patients, extensive variability between subjects may exist. There are many controls to consider such as blood sample for azidemethemoglobin and conductivity-based measurements or minimizing motion artifact with SpHb probes.

Another limitation to the analysis of error and uncertainty of SpHb monitoring compared to POCT is that POCT instruments are not calibrated using control samples. While measurement uncertainty in the device exists, the control samples and test cartridges are only used for QC which does not actually alter the methods of

measurement. Expanded measurement uncertainty from a calibration process cannot be derived.

8. CONCLUSION

To summarize the research discussed, noninvasive pulse CO-oximetry provides a clear advantage of measuring carboxyhemoglobin and methemoglobin as well as continuous monitoring compared to current POCT and laboratory analyzers. While Torp *et al.* concluded that hemoglobin measurements were comparable in accuracy, it was suggested that inherent device and physiologic variation should also be considered. It has been shown that arterial and venous blood samples are not rheologically comparable; hematocrit is higher in venous blood than in arterial blood [50]. Additionally, variation can be introduced depending on how the patient is positioned. Education of clinicians will be necessary to better understand how the values obtained from pulse CO-oximeters relate to the health and diagnosis of the patient.

While noninvasive pulse CO-oximetry already provides an improvement to patient care through the noninvasive and trending capabilities, the technology also provides potential cost savings to the hospital and a reduced effort in maintenance and regulation of the devices in comparison to current POCT devices. Considering the proven reduction in transfusion frequency when using SpHb-guided blood and the total costs per unit of blood ranging between $522 and $1,183, significant savings could be realized. This reduction in blood transfusions would also reduce the risks to the patients. It could be assumed that the noninvasive nature of the instrument would induce less stress on the patient since blood samples would not be necessary. As the market for SpHb monitoring continues to grow, it will continue to be the responsibility of each hospital to perform correlation studies as is already the case with new devices in laboratory medicine.

APPENDIX I: ACRONYMS

AAH	Acute Hypervolemic Hemodilution
ANH	Acute Normovolemic Hemodilution
CAP	College of American Pathologists
CBC	Complete Blood Count
CDC	Center for Disease Control and Prevention
CLIA	Clinical Laboratory Improvement Act
CMS	Center for Medicare and Medicaid Services
CO	Carbon Monoxide
COHb	Carboxyhemoglobin
COP	Carbon Monoxide Poisoning
CPB	Cardiopulmonary Bypass
ED	Emergency Department
EDTA	Ethylenediaminetetraacetic Acid
FDA	Food and Drug Administration
HCT	Hematocrit
Hgb/Hb	Hemoglobin
ICU	Intensive Care Unit
MAUDE	Manufacturer and User Facility Device Experience
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MetHb	Methemoglobin
PCV	Packed Cell Volume
POCT	Point of Care Testing
PPH	Postpartum Hemorrhage
RBC	Red Blood Cell

SpCO	Carboxyhemoglobin Saturation
SpO_2	Oxygen Saturation
SulfHb	Sulfhemoglobin
tHb	Total hemoglobin

APPENDIX II: REFERENCES

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