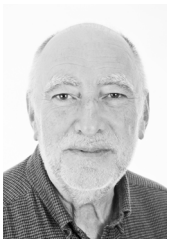
Patient Blood Management – the importance of hemoglobin measurement and minimizing phlebotomy-associated iatrogenic blood loss

July 2018



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Transfusion of donated (allogenic) red blood cells is of undoubted life-saving benefit to those suffering rapid and massive blood loss (hemorrhage) due to major trauma, or complications during surgery and childbirth. However, the procedure is not without risk, and accumulated evidence over the past 2 decades has confirmed that the benefit of red-cell transfusion is not as clear-cut in other, less dramatic clinical contexts associated with anemia.

Current conventional wisdom that red-cell transfusion may, in some circumstances, be non-beneficial or even harmful has been an important driver in developing the concept of Patient Blood Management (PBM) in recent years. PBM, which has been cited as one of 10 key advances in transfusion medicine over the past 50 years [1], is an internationally determined initiative, promoted by the World Health Organization (WHO) since 2010.

It is a multidisciplinary, evidenced-based approach intended to improve safety and outcomes for all patients who need or might need transfusion of donated blood products. Conservation of the patient's own blood, consideration of alternative anemia therapies and thereby avoidance of (or minimizing the use of) red-cell transfusion are central goals of PBM [2]. The patient-centered approach reflected in PBM-directed care is intended to ensure that red cells are only transfused after careful consideration of possible alternative treatments and assessment of the risk/benefit ratio for that particular patient.

The main purpose of this article is to highlight two narrow but important aspects of PBM so far as red-cell transfusion is concerned: measurement of hemoglobin concentration (*ct*Hb) (to diagnose anemia and help guide the decision to transfuse red cells); and minimizing blood loss associated with laboratory testing (to avoid iatrogenic anemia and thereby conserve patient's own blood). The article begins, however, with brief consideration of the risks of red-cell transfusion and some of the research evidence that underpins the rationale for the more restrictive use of red-cell transfusion that PBM protocols seek to ensure.

Risks of allogenic red-cell transfusion

The many potential risks associated with transfusion of donated blood products, including red cells (by far the most frequently prescribed), can be addressed under two headings: infectious risks and non-infectious risks.

Foremost among infectious risks is transmission of viruses that cause hepatitis (hepatitis B virus [HBV], hepatitis C virus [HCV] and hepatitis E virus [HEV]); and AIDS (human immunodeficiency virus [HIV]). Careful donor selection and highly effective testing of donated blood for the presence of these viruses has all but eliminated them from the blood supply in well-resourced developed countries, but in developing countries the risk of transfusion-transmitted HIV, HBV HCV and HEV infection remains significant [3].

Other infectious risks include: sepsis due to transfusion of bacteria-contaminated blood products; syphilis; and malaria. In addition, the safety of blood transfusion is threatened by a growing list of emerging blood-transmissible viruses, such as West Nile virus, Zika virus and dengue virus. There is expert assumption that other viruses that threaten the safety of the blood supply will continue to emerge in the future [4].

The most significant non-infectious risk is the potentially fatal acute immune hemolytic reaction that occurs during transfusion of ABO-incompatible blood [5]. Such incompatibility reactions are rare, entirely preventable and the result from human error at some point in the complex process of selecting and delivering the right donated blood product to each particular recipient patient.

Errors include: wrongly labelled blood sample tube; transfusion of blood to the wrong patient; and laboratory errors, both technical and administrative [6]. Two other serious non-infectious adverse effects of transfusion are currently less preventable and more common; they are transfusion-related lung injury (TRALI) and transfusion-related cardiac overload (TACO). These are now the two most common known causes of transfusion-related serious morbidity and mortality.

TRALI is caused by the presence of antibodies in donor plasma which bind antigens on the surface of recipient white cells [7]. Resulting agglutinated activated white cells, sequestered in the microvasculature of the lungs, release a range of toxic products that damage the endothelium, with resulting pulmonary edema. Consequent rapid onset (within a few hours) of severe acute respiratory distress can be fatal (mortality 5-25 %) or more usually, resolve without long-term effect within few days to a week.

Admission to intensive care and mechanical ventilation is usually necessary. TACO, which disproportionately affects the very young and elderly (particularly those with heart failure and/or chronic lung disease), shares features of TRALI but the pulmonary edema and acute respiratory distress in the case of TACO is due to circulatory overload induced by transfusion. Less significant, relatively common adverse effects of transfusion include mild, self-limiting febrile and allergic reactions.

Finally, there is accumulating evidence that donated red cells can have both pro-inflammatory and immunosuppressive adverse effect.

Compared with the well-defined and clearly understood risk factors outlined above, these pro-inflammatory, immunosuppressive adverse effects of red-cell transfusion – collectively called transfusion-related immunomodulation (TRIM) – are not yet fully understood. However, research is ongoing and current understanding of TRIM is recently reviewed [8]. TRIM has been linked to the increased morbidity and mortality associated with liberal use of red-cell transfusion revealed by some of the studies outlined below.

Evidence that red-cell transfusion can be non-beneficial or harmful

The evidence base for the restrictive use of red-cell transfusion reflected in Patient Blood Management (PBM) guidelines derives, at least in part, from the results of many clinical studies over the past 2 decades that challenge the traditional "10/30 rule" for triggering transfusion of red cells. The "10/30 rule", which dates back to the early 1940s when transfusion was in its infancy, long before the many risks of transfusion were fully appreciated, holds that red-cell transfusion is indicated to correct anemia when hemoglobin concentration (*c*tHb) falls below 10 g/dL (100 g/L) and hematocrit (Hct) falls below 30 %.

Despite apparently being based only on the clinical experience of two physicians who proposed the rule in 1942 [9], it remained a virtually unchallenged standard of care for close to 5 decades. Accumulated experience of Jehovah's Witnesses patients who refuse blood transfusion, suggested in a 1994 study [10] that patients could well tolerate *c*tHb <10 g/dL (<100 g/L) and that patients whose *c*tHb were in the range of 8-10 g/dL (80-100 g/L) might be exposed to the then known risks of transfusion without benefit.

This notion has been tested in a large number of randomized controlled trials conducted over the past 2 decades. Among the early studies, one focusing on critical care patients published in 1999 was particularly influential. For this landmark prospective study [11] investigators randomized 838 anemic critically ill patients with admission *c*tHb <9 g/dL to one of two red-cell treatment groups. The transfusion trigger for the control ("liberal strategy") group was 10 g/dL (100 g/L), the aim being to maintain *c*tHb in the range of 10-12 g/dL (100-120 g/L).

The transfusion trigger for the test ("restrictive strategy") group was 7 g/dL (70 g/L), with the aim of maintaining *c*tHb in the lower range of 7-9 g/dL (70-90 g/L). On average, those in the "liberal strategy" group received 5.6 red-cell units to maintain *c*tHb in the higher target, whereas those in the "restricted strategy" group needed, on average, only 2.6 red-cell units each; indeed, a third of these patients maintained *c*tHb within their target range (7-9 g/dL) without the need for any red cells.

In hospital mortality was significantly lower in the restrictive strategy group. Other outcome measures indicated either no statistical difference between the two groups or better outcome for those in the restrictive group. The authors were able to conclude that a restrictive strategy of red-cell transfusion is at least as effective and may be superior to liberal strategy among critically ill patients.

There followed many more similarly designed randomized trials comparing restrictive versus liberal use of red-cell transfusion in a broad range of clinical settings (trauma, orthopedic surgery, cardiac surgery, critical care, acute coronary care and hematological medicine). A Cochrane systematic analysis of 31 such trials involving 12,587 hospital patients is recently published [12].

This meta-analysis reveals that red-cell transfusion can be avoided in most patients whose *c*tHb is greater than 7-8 g/dL. This restrictive red-cell transfusion trigger range was shown to result in a 48 % reduction in the proportion of patients exposed to

red-cell transfusion (and its associated risk) than would be the case if all patients with *c*tHb <10 g/dL were given red cells.

The wealth of evidence supporting avoidance of red-cell transfusion when possible and judicious, restrictive use of red-cell transfusion when necessary, is now reflected in PBM guidelines written by experts representing all clinical specialties, in many countries around the world [13]. As can be implied from the above, PBM-directed care – so far as red-cell transfusion is concerned – depends now more than ever on timely and accurate monitoring of patient hemoglobin concentration (*c*tHb).

Oxygen delivery and hemoglobin – some physiological considerations

Life depends on continuous delivery of oxygen to all tissue cells. Aerobic metabolism, which cannot proceed in the absence of oxygen, is the means by which cells generate the energy (in the form of ATP) required to survive and function. Cell death (necrosis), organ dysfunction and organ failure follow in turn if tissues are deprived of oxygen for too long. Hypoxia is the term used to describe the state in which tissues have insufficient oxygen to support aerobic cell metabolism; hypoxia is a consequence of inadequate oxygen supply and/or increased oxygen demand.

Our only source of oxygen is that present in inspired air which is delivered to tissue cells from the lungs via the cardiovascular system in arterial blood. Due to the low solubility of oxygen, only a very small (<2 %) amount of the total oxygen delivered in arterial blood is simply dissolved in blood plasma. Most (98 - 99 %) is transported bound to the oxygen-carrying protein hemoglobin contained in red blood cells.

The oxygen-combining property of hemoglobin depends on a single atom of iron present at the center of four heme groups within the hemoglobin molecule. An oxygen molecule forms a weak (reversible) ionic link with each of these four atoms of iron in turn; the product is oxyhemoglobin. Each molecule of hemoglobin can thus bind a maximum of four molecules of oxygen; 1 g of hemoglobin can effectively carry a maximum of 1.34 mL oxygen.

In the microvasculature of the lungs, local conditions determine that hemoglobin has highest affinity for oxygen so that hemoglobin rapidly becomes fully saturated with the inspired oxygen that has diffused to blood from lung alveoli. By contrast, conditions in the microvasculature of the tissues determine that affinity of hemoglobin for oxygen is much reduced, enabling oxygen release from oxyhemoglobin, and subsequent diffusion from blood to tissue cells. Resulting deoxygenated hemoglobin leaves the microvasculature of the tissues and returns via the venous system and right side of the heart to the lungs for renewed oxygenation.

Global oxygen delivery (DO_2) is the total volume of oxygen delivered to the whole body from the lungs every minute [14]. It is the product of total blood flow or cardiac output (CO), normally ~5 liters/min [15] and the total oxygen content of arterial blood (C_aO_2), normally around ~200 mL/L [15].

Thus:

DO_2 (mL/min) = CO × C_aO_2 = 1000 Eqtn 1 [14, 15]

C_aO_2 is the total volume of oxygen in one liter of arterial blood. It is the sum of the volume of oxygen bound to hemoglobin in red cells (normally ~197 mL/L) and the volume of oxygen dissolved in the plasma of arterial blood (normally around just ~3 mL/L). The volume of oxygen bound to hemoglobin is a function of hemoglobin concentration (*c*tHb) and the % of hemoglobin that is saturated with oxygen (sO_2(aB)). The volume of oxygen dissolved in arterial blood plasma is a function of partial pressure of oxygen of arterial blood (pO_2(aB)) and the solubility coefficient of oxygen (0.003).

Thus:
C_aO_2 (mL/L) = (*c*tHb × 1.34 × *s*O_2(aB)) + (*p*O_2(aB) × 0.003) = 200 Eqtn 2 [14]

Global oxygen consumption (VO_2) is the total volume of oxygen consumed by all tissues per minute and is the product of cardiac output and the difference between the oxygen in arterial blood (C_aO_2) and oxygen in venous blood (C_vO_2), normally around ~135 mL/L.

Thus:
VO_2(mL/min) = CO × (C_aO_2 – C_vO_2) = 325 Eqtn 3 [14]

Oxygen extraction ratio (O_2ER) is the ratio of total oxygen consumed by tissues (VO_2) to total oxygen delivered to tissues (DO_2).

Thus:
$O_2ER = VO_2 / DO_2$ Eqtn 4 [14]

The parameters and relationships outlined above [14, 15] help to explain the physiological compensation that normally ensures continued oxygenation of tissues during mild to moderate chronic anemia.

Anemia, physiological compensation and *c*tHb transfusion thresholds

Anemia is diagnosed, in accordance with WHO criteria, when hemoglobin concentration (*c*tHb) is <12.0 g/dL (120 g/L) in women, and <13.0 g/dL (130 g/L) in men [16]. Since hemoglobin is the principal means of oxygen delivery, reduction in *c*tHb (i.e. anemia) causes reduction in C_aO_2 (Eqtn 2) and thereby reduction in DO_2 (Eqtn 1) with consequent increased risk of hypoxia. The rationale for red-cell transfusion is to increase the oxygen-carrying capacity and thereby DO_2 in order to avoid hypoxia. Physiological compensation for anemia, however, ensures continued delivery of oxygen to tissues (avoidance of hypoxia) during mild to moderate anemia.

Part of this compensation is due to the body's capacity to increase O_2ER (Eqtn 4). In the resting state, global O_2ER is normally around 0.3, indicating that only a third of the oxygen delivered to the tissue microcirculation is actually extracted and consumed by tissue cells; the rest stays bound to hemoglobin during venous return (% saturation of hemoglobin in venous blood (SvO_2) is normally ~70 %). This spare capacity allows for increased oxygen extraction; in fact, global O_2ER can rise to a maximum of around 0.7, allowing the body to maintain adequate oxygenation of tissues if the oxygen supply (DO_2) decreases as in, for example, anemia, or oxygen consumption (VO_2) increases as in, for example, vigorous exercise.

Demand for oxygen varies between tissues, so there is quite normal deviation from global O_2ER in specific tissues. Heart and brain, for example, have high oxygen demand: O_2ER 0.6 and 0.3, respectively. By contrast some tissues, including skin and kidney, require less oxygen; here O_2ER is <0.1. Anemia provokes selective vasodilatation and redistribution of blood flow from areas of low oxygen demand, such as skin and kidneys, to areas of high oxygen demand, such as the heart and brain.

As Eqtn 1 above highlights, oxygen delivery (DO_2) can be maintained when the oxygen content of blood is reduced (i.e. when anemia occurs) by increasing cardiac output, i.e. by increasing heart rate and/or stroke volume. Increased heart rate (tachycardia) is a common sign in anemia and reflects this physiological mechanism of anemia compensation.

The ability to compensate for reduced *c*tHb and preserve tissue oxygenation is limited. However, the critical *c*tHb below which hypoxia inevitably occurs is not defined. Experimental study suggests that for young healthy adults, compensatory mechanisms ensure adequate tissue oxygenation so long as *c*tHb is >5 g/dL (50 g/L) [17], but there are individual case reports [18, 19] of patients

Hemoglobin concentration (*c*tHb) **Ref range:** Male 13.0 - 18.0 g/dL (130 - 180 g/L) Female 12.0 - 16.5 g/dL (120 - 165 g/L)	**Transfusion guidance**
*c*tHb >10 g/dL (>100 g/L)	**Transfusion generally NOT indicated** except in exceptional circumstances
*c*tHb 8 - 10 g/dL (80 - 100 g/L)	**Transfusion generally NOT indicated**, but should be considered for some populations, e.g.: • those with symptomatic anemia • those with ongoing bleeding • those suffering acute coronary syndrome with ischemia • hematology/oncology patients with severe thrombocytopenia, who are at risk of bleeding
*c*tHb 7 - 8 g/dL (70 - 80 g/L)	**Transfusion may be appropriate** in patients undergoing orthopedic or cardiac surgery and in those with stable cardiovascular, after evaluating the patient's clinical status
*c*tHb 6 - 7 g/dL (60 - 70 g/L)	**Transfusion generally likely to be indicated**
*c*tHb <6 g/dL (<60 g/L)	**Transfusion recommended** except in exceptional circumstances

TABLE I: *c*tHb thresholds for red-cell transfusion [20]

surviving without critical signs of hypoxia despite *c*tHb as low as 1.7-2.0 g/dL (17-20 g/L). Cardiovascular disease limits physiological compensation for anemia.

PBM guidelines reflect the lack of a reliable single *c*tHb value to trigger red-cell transfusion for the treatment of anemia. Guidance is clear that the decision to prescribe red cells should be informed by patient *c*tHb, but should never be based on *c*tHb alone. In broad terms, current guidance suggests that red-cell transfusion is generally not beneficial when *c*tHb is >10 g/dL (>100 g/L), but is usually indicated when it is <6 g/dL (60 g/L).

Patient-related factors (e.g. age, comorbidities, risk or evidence of ischemia, continuing blood loss) determine the likely benefit of transfusion for those whose *c*tHb is in the range of 6-10 g/dL. The expert evidence-based recommendations [20] from a US authority, AABB (formerly known as American Association of Blood Banks), contained in Table I below, broadly reflects the guidance in all current PBM protocols.

In what other ways does measurement of *c*tHb support PBM?

It is an important tenet of PBM-driven care that only one unit of red cells should normally be transfused at a time to patients who are not actively bleeding [21]. Each subsequent unit should only be transfused after patient reassessment to determine if further transfusion is indicated. This reassessment may include measurement of *c*tHb. Evidence suggests that the single-unit transfusion policy (as opposed to routine transfusion of two units), significantly limits red-cell use and thereby the associated patient risk [22].

Avoidance of red-cell transfusion, wherever possible, is of course central to PBM-directed care.

To this end PBM guidelines relating to surgical care of patients [23] advocate early (preferably 4-8 weeks before surgery) preoperative assessment of patient *c*tHb to identify those who are anemic so that the cause of anemia can be identified and treated (without recourse to red-cell transfusion) before elective surgery. Study [24] confirms that some degree of anemia is very common in patients requiring surgery (prevalence 39 % in this study).

Successful preoperative treatment of anemia significantly reduces the necessity for transfusion in the event of perioperative blood loss. Irrespective of the need for transfusion, correction of anemia preoperatively is associated with better outcome (reduced morbidity and mortality) for surgical patients [24, 25].

The most common cause of anemia is iron deficiency. Slowly progressive depletion of iron stores occurs before anemia becomes evident, so that it is quite possible to be iron depleted, but not yet anemic (i.e. *c*tHb is still within the normal range). PBM perioperative guidance [23, 25] makes clear that it is important to not only identify patients destined for surgery who have iron-deficiency anemia (IDA), but also those who are at high risk of IDA because of reduced iron stores.

Both groups benefit by being at reduced risk of perioperative transfusion if replacement iron (either oral or i.v.) is administered before surgery. So, in addition to measurement of *c*tHb, PBM guidelines advise preoperative assessment of patient's iron stores by measurement of serum ferritin. In addition to preoperative treatment, the underlying cause of iron deficiency (there are many) should also be determined by further investigation prior to planned surgery.

Although the most common cause, iron deficiency is by no means the only cause of preoperative anemia, so that other causes (e.g. deficiency of vitamins B6, B12, and folate, and anemia of chronic disease, etc.) should be sought and treated appropriately, wherever possible, before surgery, particularly important, of course, if significant perioperative blood loss is anticipated [26].

Conservation of patient's own blood – PBM is supported by minimizing iatrogenic blood loss

Conservation of the patient's own blood (i.e. minimizing blood loss) is a central PBM strategy for reducing the need for red-cell transfusion. Minimizing patient blood loss during surgery by cell salvage techniques [27] and acute normovolemic hemodilution (ANH) [28], are just two examples of many methods advocated in PBM guidelines to conserve patient's blood during the perioperative period.

One particular PBM strategy for conserving the patient's own blood – a focus of this article – is to minimize the volume of blood that is taken by phlebotomy for diagnostic testing and monitoring, i.e. iatrogenic blood loss. This has particular relevance for avoiding or minimizing the need for red-cell transfusion among critically ill patients being cared for in intensive care units.

Anemia is highly prevalent among the critically ill; around two thirds of patients are already anemic at the time of admission to intensive care unit (ICU) and almost all (97 %) are anemic by the end of the first week in ICU [29]. Given this high prevalence of anemia it is not surprising that red-cell transfusion is common in ICU; a 2004 survey of 284 ICUs across the US suggests that close to half of all ICU patients receive red-cell transfusion [30].

The cause of anemia in critical illness is multifactorial and relates in part to the underlying disease or condition that rendered the patient critically ill. A significant contributory factor is the iatrogenic blood loss that results from the frequent and regular blood testing that critically ill patients require. This can amount to an estimated mean daily blood loss of 40-70 mL [29]. Five days in ICU

thus represents an inevitable blood loss of 200-350 mL (around 4-7 % of total blood volume). So, this hospital-acquired or iatrogenic anemia is significant among a population already predisposed to anemia, and becomes increasingly more significant as the length of ICU stay extends.

The impact of blood loss due to laboratory testing is greatest for premature very-low-birthweight (<1500 g) babies being cared for in neonatal intensive care units. These very vulnerable neonates may have a total blood volume as low as 50 mL. Sampling just 1 mL of blood for testing may thus be equivalent to sampling 100 mL from an adult with normal total blood volume ~5000 mL. Laboratory testing has been acknowledged as the primary factor leading to anemia and the need for transfusion during the first weeks of life of these critically sick babies, already predisposed to so-called anemia of prematurity [31].

The following strategies have been advocated to minimize phlebotomy-associated (iatrogenic) blood loss [32]:

- The use of small-volume (pediatric-sized) phlebotomy tubes is a strategy made possible by advances in laboratory analyzer technology that have allowed analysis on ever smaller sample volumes. The use of conventional sample tubes now results in considerable wastage of patient's blood. A number of studies [33 - 36] comparing the use of conventional and small-volume tubes (SVT) in the clinical setting have demonstrated significant reduction in iatrogenic blood loss with the use of SVTs (74 % reduction in one study [33]).

- The use of closed inline sampling devices is a strategy aimed at combating the blood wastage associated with sampling blood via indwelling catheters, a common practice in critical care settings. Traditionally, this mode of blood sampling requires that an initial volume of blood (typically ~5 mL) is discarded to clear the line before collecting the sample for analysis. Closed online devices allow this discarded blood to be reinfused to the patient, thereby avoiding blood wastage. Mukhopadhyaya *et al* [37] showed that the use of such a device was associated with reduction in red-cell transfusion requirements in critically ill adults. Macisaac *et al* [38] demonstrated the device reduced iatrogenic blood loss in critically ill adult patients by on average around 20 mL/day; however, this relatively small reduction in blood loss was found not sufficient to materially affect *c*tHb.

- Frequent evaluation of routine sampling orders is a strategy recommended to reduce unnecessary testing and consequent unnecessary blood loss. As Raad *et al* demonstrated [39], a program of sustained clinical staff education and more rationed approach to test ordering (not based on routine orders) can lead to sustained reduction in laboratory test utilization in intensive care units, without affecting patient safety.

- Bundled scheduling of blood sampling is a strategy that applies in ICUs that do not employ closed line-sampling devices. Under this circumstance, reducing the number of blood draws from indwelling catheters by bundled scheduling would reduce the volume of discarded blood wasted in clearing the line.

- The use of point-of-care (POC) analyzers represents a strategy for minimizing iatrogenic blood loss by virtue of the very small sample volume required when compared with central laboratory testing. Madan *et al* [40] demonstrated a 30 % reduction in the volume of blood taken for analysis following introduction of a POC analyzer to their neonatal intensive care unit, despite no change in the number of tests performed. This reduction in iatrogenic blood loss was associated with a 48 % reduction in transfusion requirement among

the low-birthweight premature infants in their care. Mahieu *et al* [41] report similar findings after introducing a POC analyzer to another neonatal intensive care unit (23 % reduction in blood taken for analysis and 48 % reduction in transfusion requirement).

- Charting of cumulative daily phlebotomy loss of all intensive care patients has been proposed as a means for clinical staff to stay aware of the significance of iatrogenic blood loss and provide an audit of success in minimizing it [42].

Point-of-care hemoglobin measurement supports PBM

As discussed above measurement of *c*tHb is essential to delivery of PBM-directed care and point-of-care (POC) testing reduces iatrogenic blood loss, a strategic goal of PBM. It follows that POC *c*tHb measurement is supportive of PBM.

Blood gas and other POC analyzer platforms, that are now commonplace in intensive care units, often have the capacity for *c*tHb measurement on the same low-volume (<2 mL) whole-blood sample used to provide a range of parameters (blood gases, pH, electrolytes lactate glucose, etc.) essential to monitoring critically ill patients.

The results of a number of studies [43 - 46] have allowed the conclusion that *c*tHb measured by POC analyzers reflects *c*tHb measured by reference methods in the central laboratory with sufficient accuracy for clinical purposes.

Inadequate mixing of blood samples immediately prior to analysis is, however, a potential source of preanalytical error in POC *c*tHb measurement [47, 48]. A homogeneous blood sample is essential to accurate measurement of hemoglobin concentration; any degree of red-cell sedimentation is associated with risk of spurious *c*tHb results. In the central laboratory, roller mixers are used to maintain blood samples destined for *c*tHb in a constant homogenous state.

At the point of care, samples for *c*tHb analysis on blood gas analyzers are contained in syringes with all air expelled, and mixed by hand (gentle rotation of the syringe for 2 minutes is the recommended procedure). This may not be sufficiently prolonged or effective to render all samples truly homogenous.

Three studies [47 - 49] highlight this potential source of error in POC *c*tHb measurement and demonstrate that it can be eliminated by a more efficient semi-automatic mixing technique involving a metal ball within the blood containing syringe.

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