Title

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Authors

Zavorsky, Gerald S

van Wijk, Xander MR

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Gerald S. Zavorsky* and Xander M.R. van Wijk

The stability of blood gases and CO-oximetry under slushed ice and room temperature conditions

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Abstract

Objectives: Human blood gas stability data is limited to small sample sizes and questionable statistical techniques. We sought to determine the stability of blood gases under room temperature and slushed iced conditions in patients using survival analyses.

Methods: Whole blood samples from ~200 patients were stored in plastic syringes and kept at room temperature (22–24 °C) or in slushed ice (0.1–0.2 °C) before analysis. Arterial and venous pO_2 (15–150 mmHg), pCO_2 (16–72 mmHg), pH (6.73–7.52), and the CO-oximetry panel [total hemoglobin (5.4–19.3 g/dL), percentages of oxyhemoglobin (O_2Hb%, 20–99%), carboxyhemoglobin (COHb, 0.1–5.4%) and methemoglobin (MetHb, 0.2–4.6%)], were measured over 5-time points. The Royal College of Pathologists of Australasia's (RCPA's) criteria determined analyte instability. Survival analyses identified storage times at which 5% of the samples for various analytes became unstable.

Results: COHb and MetHb were stable up to 3 h in slushed ice and at room temperature; pCO_2, pH was stable at room temperature for about 60 min and 3 h in slushed ice. Slushed ice shortened the storage time before pO_2 became unstable (from 40 to 20 min), and the instability increased when baseline pO_2 was ≥60 mmHg. The storage time for pO_2, pCO_2, pH, and CO-oximetry, when measured together, were limited by the pO_2.

Conclusions: When assessing pO_2 in plastic syringes, samples kept in slushed ice harm their stability. For simplicity's sake, the data support storage times for blood gas and CO-oximetry panels of up to 40 min at room temperature if following RCPA guidelines.

***Corresponding author: Gerald S. Zavorsky**, PhD, RCP, RPFT, FACSM, Department of Physiology and Membrane Biology, University of California, Davis, Davis, California, USA, E-mail: gerryzavorsky@gmail.com. https://orcid.org/0000-0002-4473-1601

Xander M.R. van Wijk, Medical and Scientific Affairs, Beckman Coulter, Brea, CA, USA

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Introduction

The results of arterial blood gas analyses are used for many patient management decisions. As such, the samples analyzed must be free of preanalytical changes.

Several preanalytical changes can affect blood gas values. Blood gas values are affected by factors such as storage time [1–7] and temperature [1–3, 5, 8–11], presence of air bubbles in the syringe [8, 9, 12, 13], sample volume [14, 15], syringe composition [2–5], leukocytosis or thrombocytosis [16–26], and the initial oxygen pressure (pO_2) of the sample [1, 4, 13, 27].

Plastic syringes are used for virtually all blood gas sampling. The primary issue with plastic syringes used in blood gas analysis is that plastic syringes are gas permeable, unlike glass syringes. Nevertheless, plastic syringes are used everywhere today as they are inexpensive, less breakable, and disposable compared to glass syringes. Therefore, labs should define the maximal allowable storage times for blood gases/CO-oximetry at their institution based on up-to-date, sound research studies using plastic syringes.

Despite the relatively large number of studies that have determined the allowable storage time before blood gases become unstable (i.e., see online Supplementary Tables S1 and S2), there is no consensus. Indeed, there is a discrepancy among several studies. Some studies say that when blood is in plastic syringes, blood gases should be stored at room temperature. The analysis should be performed immediately [3, 28] or within 10 min [29], 15 min [7], 20 min [11, 12], 30 min [6, 13, 30, 31], or 60 min [8, 32] of the draw time. Furthermore, other studies recommend that samples be stored on ice if there is a delay in sampling time. Even in these studies, the storage time recommendations vary between "right away" and 95 min [1, 2, 5, 9, 10, 27, 31, 33, 34].

Some international guidelines [35–38] and manufacturer bulletins suggest analysing samples in a plastic syringe

within 30 min. If a delay in analysing samples is more than 30 min, storage in ice water should be considered [38]. Nevertheless, stability is worsened when the sample is placed on ice for some analytes (i.e., potassium [39] and pO_2 [15]), so if a combination of analytes are to be assessed together, then the storage time needs to be determined by the analyte with the shortest stability time of the group.

The discrepancies in the storage time recommendations for blood gases depend on several factors. Some of these factors include the different plastic syringes used (with their different permeabilities [5, 34]), the sample volume in each syringe [15], and the presence of bubbles in each syringe [10, 12]. Also, the chosen allowable error value permitted before samples are deemed unstable varies between studies. For example, one study deemed that pO_2 instability was defined as a change that is >9.3% from the initial value [1]; another study defined instability as a change in pO_2 that was ≥7.5 mmHg from the initial value [13]. These criteria differ from the analytical performance specifications from The College of American Pathologists (CAP) or the Royal College of Pathologists of Australasia (RCPA) [40, 41]. The initial pO_2 of the sample also affects storage time recommendations. The sample should be analysed immediately in studies where the initial pO_2 is >400 mmHg [2, 5]. However, if the sample pO_2 is about 100 mmHg, the recommendation for some studies has been 60 min [8, 32]. Furthermore, the storage time recommendations can change when different statistical analyses are used. If the recommendations are based on mean changes, then the storage time is roughly based on the instability of the average sample [32]. So about 50% of the samples would show a pO_2 change that is less than the mean change, and about 50% would show a change that is more than the mean change. Stability can also be defined as the storage time for which 5% of the samples exceed the stability threshold [29, 39]. Finally, the blood gas analyser's performance specifications also affect recommendations. Some of today's blood gas analyzers have a total analytical error of ≤5 mmHg for pO_2 in whole blood (when pO_2 is ≤145 mmHg) [42, 43], which is much lower than even 20 years ago.

Survival analyses have rarely been used for stability determination. This statistical technique has only been used twice before in blood/electrolyte stability studies [29, 39]. This study's premise is that this analysis will determine more accurate storage times for blood gases under room temperature and slushed ice conditions.

As such, the purpose of this study was to determine the storage time at which 5% of blood gas samples exceeded the performance specifications (total allowable error) as deemed by the Royal College of Pathologists of Australasia (RCPA) (Table 1) [40]. We specifically determined the storage time for individual analytes, as well as in combination with other analytes under slushed ice and room temperature conditions.

Table 1: Analytical performance specifications were used as the acceptable stability threshold.

Blood gas	Lower limit	Upper limit
pO_2	±5 mmHg for samples that have a pO_2 of ≤83 mmHg	±6% change for samples that have a pO_2 of >83 mmHg
pCO_2	±2.0 mmHg for samples that have pCO_2 of ≤34.0 mmHg	±6% change for samples that have pCO_2 of >34.0 mmHg
pH	±0.04	±0.04
Metabolites	**Lower limit**	**Upper limit**
Lactate	±0.5 mmol/L for samples that have a concentration of ≤5 mmol/L	±8.0% for samples that have a concentration of >5 mmol/L
Co-oximetry	**Lower limit**	**Upper limit**
Hemoglobin concentration	±5 g/L for samples that have a concentration of ≤100 g/L (±0.5 g/dL for samples that have a concentration of <10 g/dL)	±5% change for samples that have a concentration >100 g/L (±5% for samples that have a concentration of <10 g/dL)
Oxyhemoglobin percentage (O_2Hb%)	±3 units for samples ≤75.0%	±4 units for samples >75.0%
Carboxyhemoglobin percentage (COHb)	±1.0 units for samples ≤5.0%	±20% change for samples >5.0%
Methemoglobin percentage (MetHb)	±2.0 units for samples ≤20%	±10% change for samples >20%

August 2021. Royal College of Pathologists of Australasia [40].

Materials and methods

This study was completed along with two other companion studies examining whole blood lactate stability and electrolytes using identical specimens obtained for patient care [39, 44]. The UC Davis Institutional Review Board (IRB) administration reviewed the project (IRB ID 1469859-1). It determined that this research was IRB-exempt as it did not involve human subjects, and no patient-identifying information was obtained.

Whole blood venous and arterial patient specimens were obtained from the blood gas lab at the University of California, Davis Medical Center, in Sacramento, CA, between October 2019 and February 2020. The blood gas lab receives samples from all over the hospital, including

the emergency department and all the intensive care units, and neurosurgical units. The blood gas lab is accredited by the State of California Department of Public Health (Lab ID CDF0002547; CLIA Number: 05D0615654) and the College of American Pathologists (CAP Number 2422006). Both venous and arterial samples were included in the study if the pO_2 ranged from 10 to 150 mmHg. The high limit of 150 mmHg was selected to represent the maximum possible blood pO_2 at sea level when the sample is exposed to 21% oxygen and stored at room temperature. When blood samples are exposed to atmospheric pO_2, the movement of oxygen tends to go from high to low partial pressures. Thus, blood samples with an initial pO_2 higher than 150 mmHg would decrease when exposed to ambient air, while samples with a pO_2 lower than 150 mmHg would tend to increase when exposed to ambient air. Thus, we did not want conflicting results if the baseline pO_2 of the blood samples began at >150 mmHg. The samples obtained were sent to the lab via a pneumatic tubing system. All specimens were analyzed using the Radiometer ABL 90 Flex blood gas analyzer (Radiometer Medical, Brønshøj, Denmark). The within-analyzer precision of the analyzer is presented in Supplementary Table S3.

Samples were obtained from two different syringes: (A) 3 mL Portex Line Draw Arterial Blood Sample Syringes that contained 23.5 IU of dry lithium heparin neutralized for ionized calcium per mL (Ref: 4042-2, Smiths Medical, ASD, Inc.); (B) vented 3 mL Portex Pro-Vent® Arterial Sampling Kits that contained 23.5 IU of dry lithium heparin neutralized for ionized calcium per mL (Ref: 4598P-2, Smiths Medical, ASD, Inc.).

Samples were stored at room temperature (22–24 °C) or on slushed ice (0.1–0.2 °C) over an average of 80–90 min (and up to 3 h). The storage time was limited to two to 3 h as the lab rarely processed samples above 3 h. However, when the whole blood specimens were inserted into the analyzer, the analyzer measured all specimens at 37 °C. Measurements were obtained at five different time points: baseline (minute 0), then approximately 20–30, 40–60, 60–80, and 90–180 min after receiving the sample. Each blood sample at each timepoint was mixed thoroughly for 5 s in upright and inverted positions before inserting the sample into the analyzer. All bubbles were removed before analysis. If the specimen was in slushed ice, the syringe was placed vertically in a container containing slushed ice. The temperature of the slushed ice bath was measured via two thermometers of the same brand (Fisherbrand™ Traceable™ Refrigerator/Freezer Plus Thermometer, Thermo Fisher Scientific, Pittsburgh, PA), and the temperature of the two thermometers was averaged. The reported accuracy of the thermometers was ±0.5 °C.

Statistical analyses

A repeated-measures analysis of variance (rmANOVA) was used to identify if the average values for pO_2, pCO_2, and pH (measured at 37 °C) and CO-oximetry (total Hb, $\%O_2Hb$, COHb, MetHb) differed over five different timepoints and two conditions (room temperature and slushed ice). A rmANOVA was also used to compare the changes in each analyte at approximately 20, 40, 60, and 80 min post-draw time. For pO_2, we further compared stability in specimens with a pO_2<60 mmHg to those with a pO_2≥60 mmHg. The low and high pO_2 groups were split this way because the oxyhemoglobin dissociation curve begins to flatten out at a pO_2 of ~60 mmHg and is steeper when pO_2 is <60 mmHg [45], which in turn affects the oxygen buffering capacity of hemoglobin and the rate of change in pO_2 when samples are stored in ice-water [4]. Bonferroni correction was used to adjust for multiple comparisons and to determine *post-hoc* differences. If Mauchly's Test Sphericity was statistically significant, then a Greenhouse–Geisser adjustment was used.

Data screening was utilized to identify outliers from each rmANOVA. Any data point with a Cook's Distance dissimilar to the other data points via visualization was eliminated. Additionally, any studentized residual that was ≥±2.5 SD units during the screening was eliminated.

A Kaplan–Meier estimator (survival analysis) was also performed to estimate the "time to event" of only the analytes that show meaningful changes over time [46, 47]. In our case, the time to event involved computing the time at which 5% of the samples exceeded the performance specifications per the RCPA [40] (Table 1). We defined the storage time at which 5% of the samples exceeded performance specifications as the time of instability since there is general acceptance that 95% of samples must fall within the total allowable error [48, 49]. In addition, it is a conservative estimate used elsewhere in studies where survival analysis was performed [39, 50].

Results

After the elimination of samples with a baseline pO_2 of >150 mmHg, 99 samples (54 venous, 45 arterial) patient whole blood (heparinized) samples were used to examine the stability of pO_2, pCO_2, pH, and CO-oximetry over time under room temperature conditions (22–24 °C); another 93 (49 venous, 44 arterial) whole blood samples were used to examine the stability of those same analytes in slushed ice conditions (0.1–0.2 °C). From there, one outlier was removed for each of pCO_2, $\%O_2Hb$, COHb, and MetHb; six outliers were removed for pH, and 19 outliers were removed for pO_2 (primarily due to oxygen contamination). Baseline values are presented in Supplementary Table S4. About 17% of the arterial samples were considered "critical values" for pO_2 (Supplementary Table S5). About 7 and 34% of the samples were critical for pH and pCO_2 (Supplementary Table S5). None of the CO-oximetry values was considered critical at baseline in any specimen. When the groups were further subdivided based on pO_2, the pO_2 for the <60 mmHg group displayed a mean (SD) of 40 (10) mmHg, with a range of 15–59 mmHg. The pO_2≥60 mmHg group exhibited a mean (SD) of 85 (25) mmHg, with a range of 60–150 mmHg.

According to the RCPA (Table 1), the acceptable threshold for pO_2 is 5.0 mmHg when the baseline pO_2 is ≤83.0 mmHg and 6.0% when the baseline pO_2 is >83.0 mmHg. The survival analysis demonstrated that 5% of the samples stored at room temperature became unstable after 40 min, while samples stored in slushed ice became unstable after 20 min of storage; but this is when one considers the full range of oxygen pressures together (15–150 mmHg) (Figure 1).

However, when comparing the mean overall pO_2 between conditions, there was no statistical significance between samples stored in slushed ice vs. storing samples at room temperature. The mean difference was –3 mmHg (95% CI –10 to 3 mmHg) between the conditions overall (p=0.27).

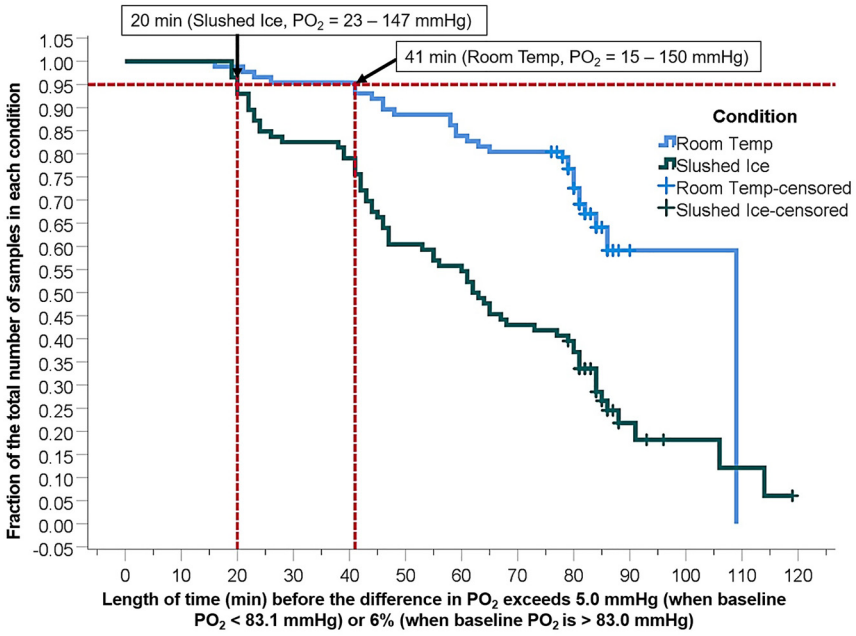

Figure 1: The time before the change in pO_2 exceeds the threshold of acceptability for stability. When samples are stored at room temperature in a plastic syringe (no air bubbles), about 5% exceed the acceptable threshold after 41 min. Comparison between survival curves was statistically significant [Log Rank (Mantel–Cox)=Chi-square=25.5, df=1, p<0.001]. There were 86 samples stored on ice (range 23–147 mmHg) and another 87 samples stored at room temperature (range=15–150 mmHg).

Nevertheless, when examining the mean *change* in pO_2 over ~85 min, there were significant differences between room temperature and slushed ice conditions, exacerbated when the initial pO_2 was ≥60 mmHg (Figure 2). In Figure 2, it can be seen that storing samples in slushed ice worsens stability compared to room temperature conditions [mean difference in the increase in pO_2 compared to room temperature when averaging all time points=+10 mmHg (95% CI=8–12 mmHg)], which is more exacerbated when the initial PO_2 is ≥60 mmHg (+18 mmHg) compared to when the initial pO_2 is <60 mmHg (+2 mmHg) and the sample is stored for extended periods (Figure 2). Main effects present: time, condition, and group (p<0.001) (Figure 2). There were also interaction effects (condition × group; time × condition; and time × group, all p<0.001) (Figure 2). There were 55 samples stored at room temperature with the initial pO_2<60.0 mmHg, there were 32 samples stored at room temperature with the initial pO_2≥60.0 mmHg, there were 42 samples stored in slushed ice with the initial pO_2<60.0 mmHg, and there were 44 samples stored in slushed ice with the initial pO_2≥60.0 mmHg (Figure 2). As well, 15 out of 32 samples that had an initial mean pO_2 of 109 (SD 18 mmHg) increased to 166 (SD 14) mmHg when stored in slushed ice for 86 (SD 6) min.

According to the RCPA (Table 1), the acceptable threshold for $\%O_2Hb$ is 3.0 units for samples ≤75.0% and 4.0 units for samples >75.0%. The survival analysis revealed that stability for $\%O_2Hb$ was not significantly different between conditions, at about 40–45 min, when considering the full range of $\%O_2Hb$ values together (20–98.8%) (Figure 3). The mean difference in $\%O_2Hb$ was −1.3% (95% CI= −4.8 to 2.1%) between the conditions overall (p=0.46).

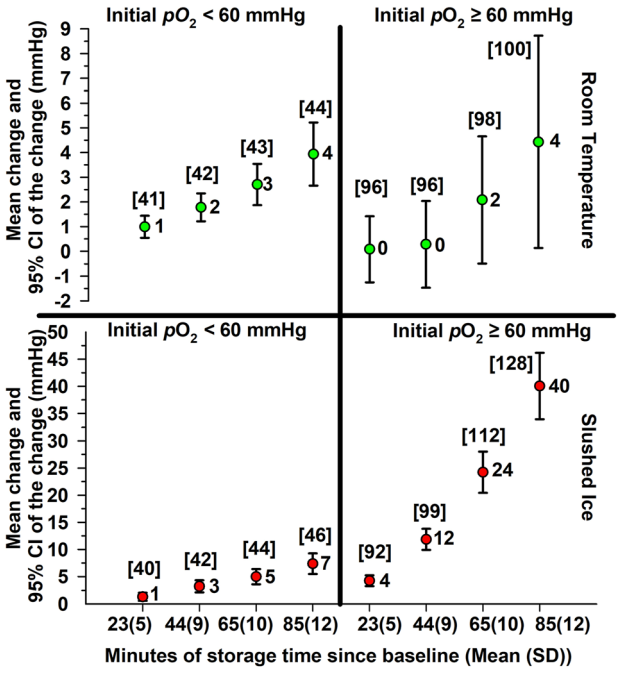

Figure 2: The mean change in pO_2 (and 95% CI of the mean change) compared to baseline at each time point under different conditions (room temperature vs. slushed ice) and varying initial oxygen partial pressures (<60 mmHg or ≥60 mmHg). The numbers within the brackets represent the mean pO_2 at that time point. The numbers to the right of the green and red-filled circles represent the mean change value.

However, when separating the $\%O_2Hb$ into high and low initial baseline values (<90% or ≥90%), ice shortened stability compared to room temperature conditions [mean difference in the increase in $\%O_2Hb$ compared to room

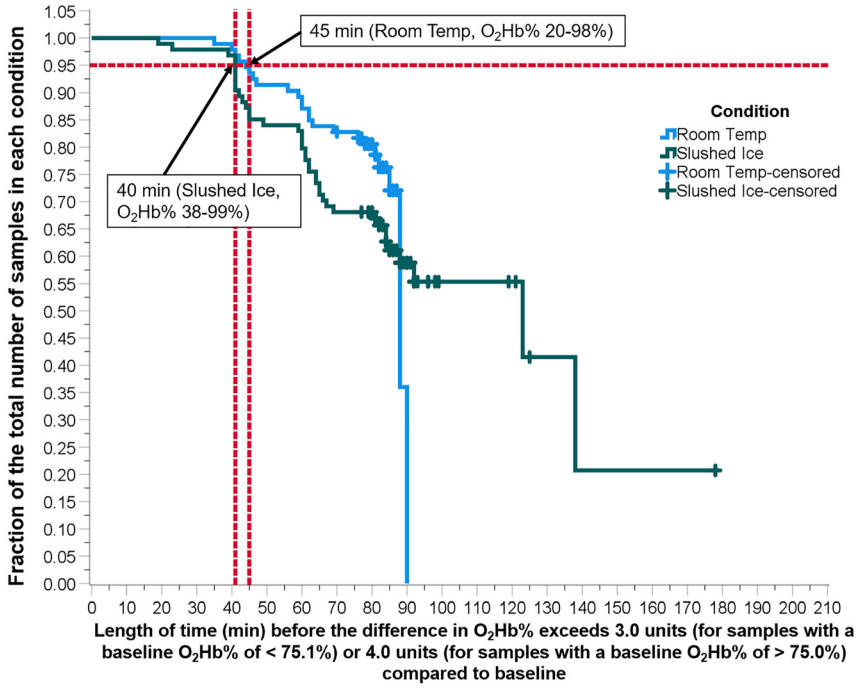

Figure 3: The time before the change in oxyhemoglobin percentage (O_2Hb%) exceeds the threshold of acceptability for stability. When samples are stored at room temperature, about 5% exceed the acceptable threshold after 45 min. When samples are stored on ice in a plastic syringe (no air bubbles), about 5% of the examples exceed this threshold by 40 min. The comparison between conditions was not statistically significant [Log Rank (Mantel–Cox)=Chi-square=1.8, df=1, p=0.18]. There were 94 samples stored on ice [mean 83.6 (SD) (17.1%), range 37.9–98.8%] and another 93 samples stored at room temperature [mean 80.2 (SD) (19.2%), range=20.0–98.2%].

temperature when averaging all time points=+1.3% (95% CI=0.9–1.7%), which is more exacerbated when the initial O_2Hb is <90% (+1.8%) compared to when the initial %O_2Hb is <90% (+0.8%) and the sample is stored for extended periods (Figure 4). Main effects present: time, condition, and group ($p<0.001$) (Figure 4). There were also interaction effects (condition × group; time × condition; and time × group, all $p<0.001$) (Figure 4). There were 51 samples stored at room temperature with the initial O_2Hb%<90%, there were 42 samples stored at room temperature with the initial O_2Hb%≥90%, there were 41 samples stored in slushed ice with the initial O_2Hb%<90%, and there were 53 samples stored in slushed ice with the initial O_2Hb%≥90% (Figure 4). When O_2Hb% was less than 90%, stability was reduced regardless of whether the sample was stored on ice (Figure 4, Supplementary Figure S2).

The mean differences in Hb concentration did not clinically change over ~85 min. There was a mean increase of +0.1 (95% bootstrapped CI=0.0 to 0.2) g/dL after ~85 min of storage under room temperature conditions and +0.1 (95% bootstrapped CI=0.1 to 0.2) g/dL after ~85 min stored in slushed ice. The survival analysis demonstrated stability was ~40 min under slushed ice conditions and 46 min when stored at room temperature. There was no statistical difference between conditions (Supplementary Figure S3).

One hundred percent of the samples remained stable for pH, pCO_2, COHb, and MetHb, in slushed ice conditions, even up to ~3 h (Supplementary Figures S4–S8). As with pO_2, the average pCO_2 did not differ between conditions, but the change in pCO_2 did (Supplementary Figure S6). Storing

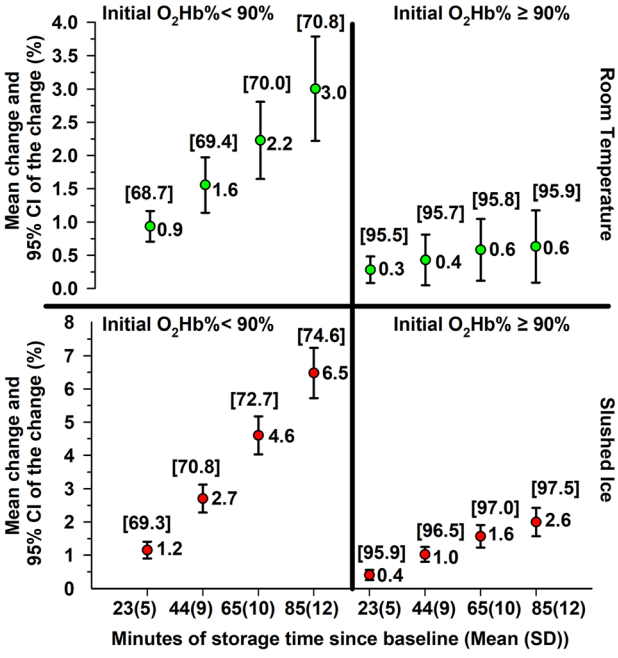

Figure 4: The mean change in O_2Hb% (and 95% CI of the mean change) compared to baseline at each time point, including the initial measurement under different conditions (room temperature vs. slushed ice) and varying initial oxyhemoglobin percentages (<90% or ≥90%). The numbers within the brackets represent the mean O_2Hb% at that time point. The numbers to the right of the green and red-filled circles represent the mean change value.

samples in slushed ice is similar to storing samples at room temperature: the mean difference was –1 mmHg (95% CI –4 to 3 mmHg) between the conditions overall (p=0.76). There was a main effect of time, with time 0 significantly different

from 23-, 44-, 65-, and 85-min post-storage ($p<0.001$). There was also a time × condition interaction effect, $p<0.001$). Furthermore, under room temperature conditions, 5% of the samples became unstable by ~60 min (Supplementary Figure S4). Regarding pH, 5% of the samples became unstable at room temperature after 65 min of storage (Supplementary Figure S5). Specifically, the mean pH decreased by 0.02 pH units (95% CI=−0.03 to −0.02 pH unit change, $p<0.01$) after a mean storage time of 82 min at room temperature and by −0.01 pH units (95% CI=−0.01 to − 0.01 pH unit change, $p<0.01$) after a mean storage time of 90 min in slushed ice. No specimen stored in slushed ice ever exceeded a change in pH of more than 0.04 pH units.

Based on these findings, summary tables are presented. Table 2 displays the recommended storage time when a combination of analytes is measured, and Table S6 displays the recommended storage time when only one analyte is measured.

Discussion

The purpose of this study was to determine the storage time at which 5% of blood gas samples exceeded the performance specifications (total allowable error) as deemed by the RCPA (Table 1) [40]. We determined the storage time for individual analytes and a combination of analytes under slushed ice and room temperature conditions using Kaplan–Meier survival analysis, a technique rarely used in stability studies. This study demonstrated that when the complete blood gas and CO-oximetry panels were analyzed, the acceptable storage time was 25 min at room temperature, but only when the pO_2 was >60 mmHg. If the pO_2 was ≤60 mmHg, the storage time for blood gases and CO-oximetry panels was 45 min. If the complete range of pO_2's is considered together (15–150 mmHg) with no consideration for initial pO_2, then a storage time of up to 40 min can be acceptable at room temperature, depending on the laboratory's total allowable error and RCPA criteria (Table 1). Storing specimens on ice worsened the stability of pO_2 compared to samples stored at room temperature.

When blood gas and CO-oximetry analytes were measured individually, pCO_2, pH, COHb, and MetHb remained stable in plastic syringes stored in slushed ice for 3 h. The high stability of pH and pCO_2 stored under cold temperatures is confirmed in other studies [1, 3, 11, 12, 27].

The confusion in interpreting stability from various studies (i.e., studies presented in Tables S1 and S2) can be partly due to the statistical analysis performed and the pO_2 ranges evaluated together or separately. We believe that presenting data in terms of mean values and standard deviation of the mean values at various storage time points, as so many studies have done (i.e. [3, 6, 7, 15, 32, 51]), does not tell the whole story. For example, we have shown no statistically significant difference between conditions for pO_2 if presenting the mean value (and 95% CI of the mean value) over five different time points). However, if the mean *change* in pO_2 is compared to the baseline, there is a statistical difference between conditions (Figure 2). Nevertheless, even when presenting the figure this way, there are some misleading numbers. For example, one could think that a mean increase in pO_2 of 4 mmHg over 85 min of storage time at room temperature would suggest that the pO_2 is stable over 85 min since the analyte performance specifications for pO_2 are within ±5 mmHg or 6% [40]. However, this would be incorrect as the survival analysis shows that 5% of the samples exceed this threshold by ~40 min post-draw time when looking at specimens with oxygen pressures varying from 15 to 150 mmHg (Figure 1). Nonetheless, in Figure 1, the initial pO_2 was not considered. If the initial pO_2 was considered by separating the groups into low (≤60 mmHg) and high baseline pO_2 values (>60 mmHg), the pO_2 stability increases to ~60 min when considering samples with low oxygen pressures and decreases to ~25 min when considering specimens with high oxygen pressures (Supplementary Figure S1). Thus, the various findings between studies presented in Supplementary Tables S1 and S2 can be due to the type of statistical analysis and the range of oxygen pressures lumped together in the analyses.

Furthermore, the use of ice in stability studies is also confusing to interpret. When one uses plastic syringes, the oxygen can move from the slushed ice through the plastic syringe and into the blood due to the syringe's permeability. As blood is cooled, the oxyhemoglobin dissociation curve shifts left, resulting in hemoglobin having an increased affinity for oxygen [45]. At the same time, the solubility of oxygen increases when blood is cooled [52]. The oxygen solubility in the blood and plasma approximately doubles when blood is cooled from 37 to about 0.2 °C [52, 53]. In combination, these two phenomena lead to an influx of exogenous oxygen in the sample when stored in a semi-permeable container such as a plastic syringe [4]. This explains why in the current study, nearly 50% of the samples with an initial pO_2>60 mmHg ended up with a final pO_2 of >150 mmHg after nearly 90 min of slushed ice storage. Moreover, when the sample is reheated to 37 °C in a blood gas analyzer, the exogenous oxygen added to the blood and plasma while cooled results in a falsely increased pO_2 [54]. We have shown this to be true (Figure 2, bottom panel), confirming other reports [4, 27]. Interestingly, we observed that the magnitude of this change is dependent on the initial pO_2 of the sample, as was also shown by Mahoney et al. [4].

Table 2: The recommended allowable storage time (min) for analytes grouped in various combinations.

Analytes analyzed and reported in combinations	Room temperature	Slushed ice
Blood-gases only pO_2 (15–150 mmHg) pCO_2 (16–72 mmHg) pH (6.73–7.52)	40 min [storage time limited by the pO_2]	20 min [storage time limited by the pO_2]
Blood-gases only pO_2 (<60 mmHg) pCO_2 (16–72 mmHg) pH (6.73–7.52)	60 min [all analytes had similar storages times, separately]	40 min [storage time limited by the pO_2]
Blood-gases only pO_2 (≥60 mmHg) pCO_2 (16–72 mmHg) pH (6.73–7.52)	25 min [storage time limited by the pO_2]	20 min [storage time limited by the pO_2]
CO-oximetry panel only Total Hb (5.4–19.3 g/dL) O_2Hb% (20.0–98.8%) COHb (90.1–5.4%) MetHb (0.2–4.6%)	45 min [storage time limited by the O_2Hb%]	40 min
Metabolite panel only Lactate (0.6–13.5 mmol/L) Glucose (2–17 mmol/L)	40 min [storage time limited by lactate]	~180 min
Electrolyte panel only K^+ (2.2–11.7 mmol/L) Ca^{2+} (0.73–1.50 mmol/L) Cl^- (77–130 mmol/L) Na^+ (97–167 mmol/L)	120 min	~70 min [storage time limited by potassium concentration]
Blood gas panel and CO-oximetry panel together, with or without the metabolite panel (pO_2<150 mmHg)	40 min [storage time limited by the pO_2 and lactate]	20 min [storage time limited by the pO_2]
Blood gas panel and CO-oximetry panel together (pO_2<60 mmHg)	45 min [storage time limited by the O_2Hb%]	40 min [storage time limited by the pO_2]
Blood gas panel and CO-oximetry panel together, including the metabolite panel (pO_2<60 mmHg)	40 min [storage time limited by lactate]	40 min [storage time limited by the pO_2]
Blood gas panel and CO-oximetry panel, together with or without the metabolite panel (pO_2≥60 mmHg)	25 min [storage time limited by the pO_2]	20 min [storage time limited by the pO_2]
pH values in pleural fluid (pH 7.00–7.61)	60 min	135 min

The recommended allowable storage time (min) is rounded to the nearest 5-min value before 5% of the specimens become "unstable." Each specimen was kept inside a plastic syringe with no air bubbles. Unstable means that >5% of samples for the analyte have exceeded the performance specifications per the Royal College of Pathologists of Australasia [40]. The numbers within parentheses are the ranges under which each analyte was assessed. Most labs report either the blood gas panel or the combination of blood gases and CO-oximetry. As such, storing samples in ice shortens the stability considering that most analytes are measured together. In other words, avoid ice when analyzing the blood gas panel or the combination of blood gases and CO-oximetry. This table was created from the current study and two other companion studies that used identical specimens [39, 44]. In addition, another study published by the same group developed storage times for pleural fluid pH [50].

They attributed this to hemoglobin's loss of oxygen buffering capacity at a higher initial pO_2.

The recommendations for storage times in plastic syringes are presented in Table 2 (combined analytes) and Supplementary Table S5 (individual analytes). Those tables are based on the decision limits for stability by determining when 5% of the samples exceed the thresholds specified by the RCPA [40]. It is also understood that laboratories may wish to have a straightforward recommendation that works for all analytes without being perfectly precise. There would be no need to partition pO_2 into low and high categories. When specimens are stored without air inside plastic syringes, a storage time of up to 40 min at room temperature would seem acceptable "enough" for all analytes, depending on the total allowable error and RCPA's performance criteria (Table 1).

In two companion studies, whole blood lactate and electrolyte stability were determined from identical specimens [39, 44], but the lactate study did not use a survival analysis. We have now updated the stability of lactate based on survival analysis (Supplementary Figure S9). Nevertheless, Supplementary Figure S9 shows similar results to the original paper [44], i.e., whole blood lactate stored at room temperature in a blood gas syringe for less than 45 min remains stable. Indeed, blood-gas analyzers can assess blood gases, CO-oximetry, metabolites, and electrolytes. Therefore, Table 2 provides the precise storage times when metabolites and electrolytes are included in the analyses, along with blood gases, CO-oximetry, and pleural fluid. When the combination of analytes is measured, the stability depends on the most unstable analyte. For example, suppose the complete electrolyte panel is assessed, and the sample is stored on ice. In that case, potassium's storage time is limited as it is the first electrolyte to exceed the stability criteria (Supplementary Table S5 and Zavorsky et al. [39]).

There may be some concern that white blood cell or platelet counts were not simultaneously measured along with the blood gases in this study. A high white blood cell (WBC) or platelet count in syringes could consume enough oxygen *in vitro* to artificially lower the pO_2 in the syringe, resulting in "spurious hypoxemia" [24]. In patients with leukocytosis or thrombocytosis, the decline in pO_2 is greater when stored at room temperature compared to on ice [21, 23, 24, 26], but regardless of the storage medium, the pO_2 decline occurs almost immediately when blood is placed in a syringe [21–23, 26, 55]. At room temperature, pO_2 declines by ~10–50 mmHg within ~0–15 min of the draw-time in patients with leukocytosis [21–23, 25, 55] vs. 0–11 mmHg when stored on ice [16, 21, 23]. However, the WBC or platelet counts must be sufficiently high for a rapid decline in pO_2 to occur. There was extreme leukocytosis in nine case reports totaling 12 patients (median leukocyte count was 276,000 cells per mm^3) when falsely low pO_2 was manifested [16–23, 26]. Extreme thrombocytosis (>500,000 cells per mm^3) also must be observed before spurious hypoxemia appears [24]. Specifically, the WBC count needs to be ≥~50,000 cells per mm^3 or the platelet count needs to be >500,000 cells per mm^3 for any chance of spurious hypoxemia in blood gas samples [18, 23–26, 56]. Spurious hypoxemia and pseudo hyperkalemia are rare since only ~1% of patients admitted to a major hospital has either >500,000 platelets per mm^3 or >50,000 leukocytes per mm^3 or both [57]. Finally, only one of our samples had a drop in pO_2 of 10 mmHg within the first 20 min of storage at room temperature, and another sample stored on ice decreased by one mmHg over the same period. Thus, we are confident that spurious hypoxemia was unlikely to occur in more than 1% of our samples.

In conclusion, this study provides a novel method to determine appropriate storage times of blood in plastic syringes using survival analysis. Another strength of this study was the large sample size (~200 human blood specimens measured over five different time points under room temperature and slushed ice conditions). We show that 95% of all whole blood specimens obtained from humans remain stable at room temperature when the complete CO-oximetry panel or the combination of the CO-oximetry panel and pO_2, pCO_2, and pH are assessed together within 45 min of the draw time, but only when the baseline pO_2<60 mmHg. If the baseline pO_2 is ≥60 mmHg, 95% of the samples remain stable within 25 min of the draw time. When baseline pO_2≥60 mmHg, the reduction in stability time can be due to the decreased buffering capacity of hemoglobin since hemoglobin is more saturated at higher oxygen pressures, reducing its buffering capacity. When assessing multiple analytes together, ice usually worsens stability and shortens the storage time before 5% of the samples become unstable. Nevertheless, if a laboratory wishes to have one practical, simple recommendation on blood gas stability, which includes most analytes obtained from a blood-gas analyzer, 30 min of storage time at room temperature can be recommended. However, a storage time of up to 40 min is acceptable under room temperature conditions, depending on the lab's total allowable error and stability criteria used.

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data analysis. Manuscript preparation was performed by XVW, and GZ. Those responsible for drafting the work and revising it critically were GZ and XVW. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: GSZ is the Vice-Chair of the Clinical & Laboratory Standards Institute (CLSI) Document Development Committee (DDC) for Blood Gas and pH Analysis and Related Measurements (C46). XVW is a Member of CLSI DDC for Blood Gas and pH Analysis and Related Measurements (C46) and is a Board member of the American Association of Clinical Chemistry Industry Division. XVW is also an employee of Beckman Coulter (part of Danaher).

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