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Authors

Zavorsky, Gerald S

van Wijk, Xander MR

Gasparyan, Samuel

et al.

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Stability of Whole Blood Electrolyte Specimens at Room Temperature vs. Slushed Ice Conditions

Gerald S. Zavorsky [a,*] **Xander M.R. van Wijk,**[b] **Samuel Gasparyan,**[c] **Nicholas S. Stollenwerk,**[d] **and Rebecca A. Brooks**[e]

Background: Data on the stability of whole blood electrolytes is limited to small sample sizes. We sought to determine the stability of whole blood electrolytes under room temperature and slushed iced conditions in human patients at a major hospital center.

Methods: Whole blood samples were obtained from 203 patients hospitalized for various pathophysiological conditions. Electrolyte concentrations of sodium, potassium [K^+], ionized calcium, and chloride were measured at 5 different timepoints spanning 3 h. Samples were stored at room temperature (22–24 °C) or under slushed ice conditions (0.1–0.2 °C) before analysis.

Results: Under both conditions, sodium, ionized calcium, and chloride did not show a measurable change up to 109 min compared to baseline; however, the mean increase in [K^+] over 138 min of storage in slushed ice was 0.0032 (0.0021 [5th percentile] to 0.0047 [95th percentile]) mmol/L/min (adjusted $R^2 = 0.62$, $P < 0.001$). Five percent of the specimens demonstrated a ≥0.3 mmol/L change in [K^+] from baseline after 67 min of storage in slushed ice. In contrast, 1% of the specimens stored at room temperature showed the same change at the same timepoint.

Conclusions: Whole blood sodium, [K^+], ionized calcium, and chloride concentrations remain stable for at least 109 min at room temperature. However, whole blood specimens stored in slushed ice for not more than 67 min exhibit a 5% probability that the [K^+] concentration will increase by at least 0.3 mmol/L compared to baseline. The other analytes do not destabilize for up to 178 min of slushed ice storage.

INTRODUCTION

The measurement of whole blood electrolytes can aid physicians in the diagnosis of various electrolyte disturbances. Electrolytes disturbances occur in several pathophysiological disorders including Addison disease (hyperkalemia, hyponatremia) (1, 2), Gitelman syndrome (hypokalemia) (3), cancer (hypercalcemia) (4, 5), chronic kidney disease or vitamin D deficiency (hypocalcemia) (6, 7), congestive heart failure (8–10), chemotherapy treatment (hypochloremia) (11), severe acute respiratory syndrome coronavirus 2–associated acute respiratory distress syndrome

[a]Pulmonary Services and Blood Gas Laboratories, University of California, Davis, Medical Center, Sacramento, CA, USA; [b]Department of Pathology, The University of Chicago, Chicago, IL, USA; [c]Respiratory Services, Stanford Healthcare, Palo Alto, CA, USA; [d]Department of Internal Medicine, Division of Pulmonary, Critical Care, and Sleep Medicine, University of California, Davis, Medical Center, Sacramento, CA, USA; [e]Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University of California, Davis, Medical Center, Sacramento, CA, USA.
*Address correspondence to this author at: Pulmonary Services, UC Davis Medical Center, 2315 Stockton Boulevard, Room 5703, Sacramento, CA 95817, USA. E-mail gszavorsky@ucdavis.edu.
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IMPACT STATEMENT

Measurements of whole blood electrolytes aid physicians to properly manage patients with various pathophysiological disorders. Several international organizations (CLSI, IFCC) state whole blood specimens kept at room temperature must be measured promptly, or ionized calcium results could be falsely elevated. Additionally, placing specimens on ice could falsely increase potassium concentration. We demonstrate that whole blood electrolyte specimens do not destabilize at room temperature or on ice for at least 109 min, except for potassium, in which 5% of the samples exceeded the total allowable error of 0.2 mmol/L (i.e., ≥0.3 mmol/L) after 67 min of slushed ice storage. Our data suggests that international recommendations should be revised.

(hypernatremia) (12), and acute kidney injury/kidney disease (hyperchloremia) (13, 14), among others. Electrolyte imbalances can also increase mortality in several patient populations (8, 9, 13, 15). Hence, accurate identification of electrolyte derangements is essential for patient care.

Early studies conducted over 50 years ago had shown that plasma/serum potassium [K^+] concentration could increase by approximately 1.3 mmol/L over a 5- to 6-h period when samples were stored in slushed ice or at 4 °C (16, 17). Cooling the sample inhibits red blood cell (RBC) glycolysis. Since RBCs are entirely dependent on glycolysis for the production of adenosine triphosphate (18), the lack of adenosine triphosphate production inside the red cell slows the sodium-[K^+] pump, which is needed to keep [K^+] contained within RBCs. Without energy, the pump activity slows, allowing [K^+] to leak out of the cells and into the plasma (19). As the [K^+] concentration inside RBC is approximately 25 times higher compared to the plasma [i.e., approximately 95 mmol/L inside an RBC (20) compared to approximately 4.0 mmol/L in plasma (21)], this large gradient allows for [K^+] to leak from the inside of the RBC to the plasma when stored on ice, causing erroneously high [K^+] measurements.

At our major academic hospital, whole blood electrolyte samples are processed through our dedicated blood gas lab, which is separate from the main clinical lab. The blood gas lab is staffed by respiratory care practitioners who have license to practice respiratory care in the state of California. In 2020, our blood gas lab received approximately 13 800 blood gas specimens in syringes from roughly 2300 patients processed for whole blood electrolytes to facilitate patent management. Our current protocol mandates that all whole blood electrolyte specimens should be transported to the lab at room temperature via the pneumatic tubing system since cooling the sample may cause pseudohyperkalemia (19). Samples processed for whole blood electrolytes are rejected if received on ice, which results in additional resources, including patient blood, syringes, and staff time. Moreover, obtaining multiple blood samples from a patient can increase infection risk by frequently accessing the arterial or intravenous lines. If it could be demonstrated that electrolyte samples sent to the lab on ice would not affect its stability if processed in a timely fashion, patient care could be improved.

Data are limited, and the time course over which various whole blood electrolyte concentrations increased over time under different temperature conditions has not been well defined in a large patient population. As presented in online Supplemental Table 1, previous human studies

evaluating electrolyte parameters in whole blood specimens have been limited to less than 60 subjects. We therefore sought to define the rate of change in whole blood sodium, [K^+], ionized calcium, and chloride concentration over time and to determine the maximum length of time a specimen can be kept under room temperature and slushed ice conditions before exceeding a clinically measurable change (i.e., total allowable error).

METHODS

This was an observational, prospective cohort study done in conjunction with another study that examined whole blood lactate stability using the same patient samples (22). The UC Davis Institutional Review Board administration re viewed the project (ID no. 1469859-1) and determined that this research was exempt from institutional review 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 is accredited by the State of California Department of Public Health (Lab ID CDF0002547; CLIA no: 05D06 15654) and by the College of American Pathologists (CAP no: 2422006). The samples obtained were sent to the lab via a pneumatic tubing system. All samples were analyzed using the Radiometer ABL 90 Flex blood gas analyzer (Radiometer Medical). The analyzer reports the results to the nearest whole number for sodium and chloride, a first decimal place for [K^+], and 2 decimal places for ionized calcium. The samples were analyzed first for patient care and then deidentified for this study. Only samples within the instrument's analytical measurement range were used for this study (i.e., sodium, 85–178 mmol/L; [K^+], 1.5–12.0 mmol/L; ionized calcium, 0.25–1.90 mmol/L; chloride, 65–130 mmol/L). The within-analyzer precision for electrolyte concentrations are presented in Supplemental Table 2.

Most samples were obtained from 3 mL Portex Line Draw Arterial Blood Sample Syringes that contained 23.5 IU of dry lithium heparin neutralized for ionized calcium per milliliter (Ref: 4042-2, Smiths Medical, ASD, Inc.). The vented 3 mL Portex Pro-Vent® Arterial Sampling Kits, which also contained 23.5 IU of dry lithium heparin neutralized for ionized calcium per mL (Ref: 4598P-2, Smiths Medical, ASD, Inc.), were also used for several samples.

Samples were stored at room temperature (22–24 °C) or on slushed ice (0.1–0.2 °C) over an average of 80 to 90 min (and up to 3 h). We limited the study to a 2- to 3-hour storage time as we rarely process samples above 3 h. Measurements were obtained at 5 different time points: baseline (minute 0), then approximately 20 to 30, 40 to 60, 60 to 80, and 90 to 180 min after receiving the sample. Each blood sample at each timepoint was mixed thoroughly for 5 s in both upright and inverted positions before inserting the sample into the analyzer. All bubbles were removed prior to analysis. If the sample was a slushed ice sample, the syringe was placed vertically in a container containing slushed ice. The temperature of the slushed ice bath was measured via 2 thermometers of the same brand (Fisherbrand™ Traceable™ Refrigerator/Freezer Plus Thermometer, Thermo Fisher Scientific), and the temperature of the 2 thermometers was averaged. The reported accuracy of the thermometers was ±0.5 °C.

The total allowable error for [K^+], sodium, ionized calcium, and chloride concentrations are ±0.2, ±3.0, ±0.04, and ±3.0 mmol/L, respectively, per the Royal College of Pathologists of Australasia (RCPA) Quality Assurance Program standards for blood gases (23). As such, any

change that exceeds the total allowable error is considered clinically measureable.

Statistical Analyses

A repeated-measures analysis of variance was used to identify changes in whole blood electrolyte concentration (venous vs. arterial samples) over the 5 different timepoints and 2 different conditions (room temperature and slushed ice). A Bonferroni correction was used to adjust for multiple comparisons and to determine post hoc differences. If Mauchly's test of sphericity was statistically significant, then a Greenhouse-Geisser adjustment was used.

Based on the results from the repeated-measures analysis of variance, a Kaplan–Meier estimator was used to estimate the "time to event" of only the analytes that show measureable changes over time (24–26). In our case, it involved computing the probabilities of an analyte exceeding the total allowable error at a certain point in time. Thus, the estimator calculates the proportion of samples that have not exceeded the total allowable error at a given timepoint.

A forward multiple linear regression was conducted to determine which 2 independent variables (sample type: arterial vs. venous; time in minutes from baseline measurement) were predictors of the change in electrolyte concentration from baseline. Changes in electrolyte concentration are usually skewed, so depending on the skewness, a transformation of the dependent variable was necessary (i.e., square root of the change, the natural logarithm of the change, or the inverse of the change). For the natural logarithm [LG10(Change + 1)] or the inverse transformation (1/Change + 1), an addition of 1 was used for every specimen to account for specimens with a 0 change. When a full model accounted for <25% of the total variance, it was not included. As the changes within each specimen may be correlated with each other, only 1 of the 4 changes were used for each specimen in the linear regression model. That way, it was possible to control for nonindependence among repeated observations in each specimen. A pseudorandom number generator (www.randomizer.org) selected 1 of the 4 changes per specimen so that only 1 change per specimen was reported.

Data screening was utilized to identify influential cases in the linear regression analyses. Any data point that had a Cook's distance that was dissimilar to the remaining cases was eliminated. Additionally, any standardized residual that was $\geq \pm 3.0$ SD units during the screening was eliminated. To cross-validate the model for prediction accuracy, another randomized set of unused cases was used to fit the prediction model. A Spearman's rho correlation coefficient was obtained between each of the predicted values and actual values for those specimens. Since we wanted to determine which pair of electrolytes had the strongest association with each other at baseline, several Pearson product moment correlations were also performed.

Sample size calculation was based on changes in $[K^+]$ concentration estimated from an amalgamation of studies in Supplemental Table 1. At the last timepoint, an estimated change of +0.2 (SD 0.3) mmol/L was expected for samples stored on ice vs. 0.0 (SD 0.3) mmol/L for samples stored at room temperature. From this estimation, the calculated effect size was 0.67 and would result an approximate sample size of 57 specimens per group for an alpha probability of 0.01 and a statistical power of 80% (Wilcoxon–Mann–Whitney test of means, 2 groups, G*Power 3.1.9.2 statistical software). As we had the ability to collect more specimens than the required 57 specimens per group, our goal was to collect at least 100 specimens per group.

Data were analyzed with statistical software (IBM SPSS Statistics, Version 26.0, IBM. Corporation). An a priori *P*-value of 0.01 was used to signify statistical significance.

Table 1. Changes in whole blood electrolyte concentrations at room temperature (22–24 °C) and in slushed ice (0.1–0.2 °C) compared to baseline.

	Storage time								
	Room temperature (min)				Slushed ice (min)				
Analyte	22 (3)	42 (4)	62 (5)	82 (4)	24 (6)	45 (11)	67 (12)	89 (15)
Change in whole blood sodium (n = 101), mmol/L	0.3 (0.5) [0.2 to 0.5]	0.4 (0.7) [0.3 to 0.5]	0.4 (0.7) [0.2 to 0.5]	0.4 (0.7) [0.2 to 0.5]	0.1 (0.9) [−0.1 to 0.2]	0.1 (0.4) [−0.1 to 0.1]	−0.1 (0.5) [−0.2 to 0.0]	−0.3 (0.6) [−0.4 to −0.1]
Change in whole blood potassium, (n = 100), mmol/L	(0.0) [0.0 to 0.0]	0.0 (0.1) [−0.1 to 0.0]	−0.1 (0.1) [−0.1 to 0.0]	0.0 (0.1) [−0.1 to 0.0]	0.0 (0.1) [0.0 to 0.1]	0.1 (0.1) [0.1 to 0.1]	0.2 (0.1) [0.1 to 0.2]	0.2 (0.1) [0.2 to 0.3]
Change in whole blood ionized calcium (n = 101), mmol/L	0.01 (0.02) [0.00 to 0.01]	0.00 (0.01) [0.00 to 0.01]	0.01 (0.02) [0.00 to 0.01]	0.00 (0.01) [0.00 to 0.01]	0.00 (0.01) [0.00 to 0.00]	0.00 (0.02) [0.00 to 0.00]	0.00 (0.02) [0.00 to 0.00]	0.00 (0.01) [−0.01 to 0.00]
Change in whole blood chloride (n = 101), mmol/L	−0.1 (0.5) [−0.2 to 0.0]	−0.2 (0.5) [−0.3 to −0.1]	−0.2 (0.5) [−0.3 to −0.1]	−0.2 (0.6) [−0.3 to −0.1]	0.0 (0.5) [−0.1 to 0.1]	0.1 (0.4) [0.0 to 0.1]	0.1 (0.4) [0.0 to 0.1]	0.1 (0.4) [0.0 to 0.2]

Data are given as mean (SD) [95% bootstrapped CI of the mean change]. Changes in sodium, [K^+], and chloride concentration are rounded to the first decimal place. Arterial and venous samples are combined since the changes were similar between the sample types.

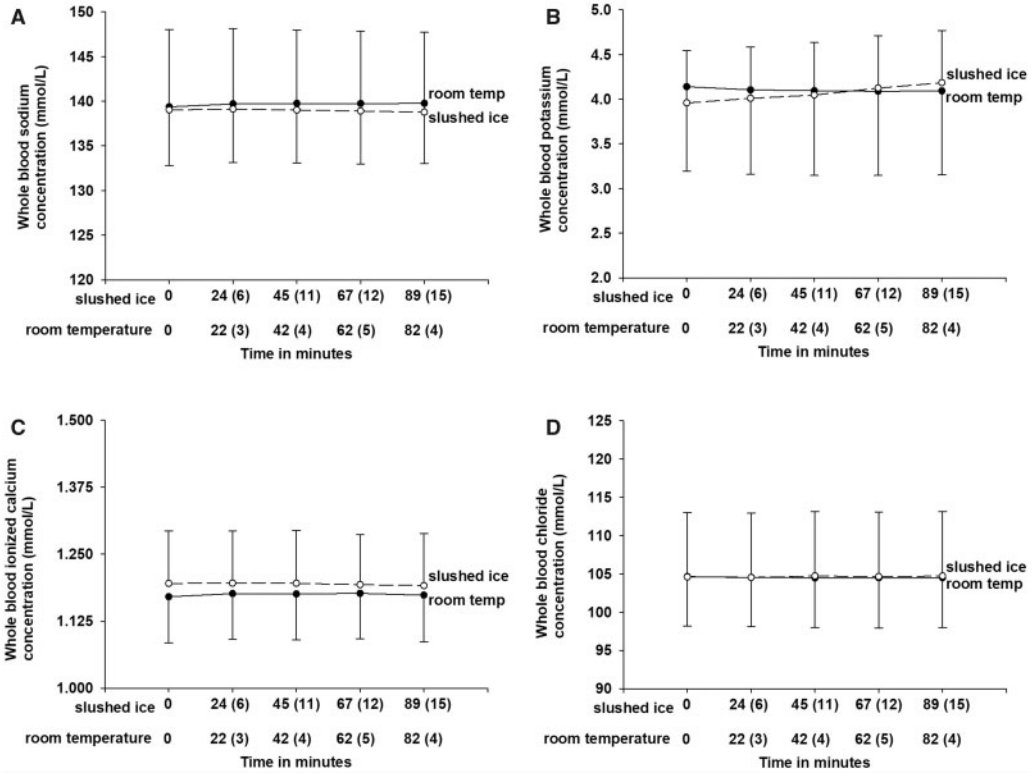

Fig. 1. **(A) Whole blood sodium values at room temperature vs. slushed ice over time. There was a statistically significant main effect of time (partial eta squared = 0.029, $P = 0.001$, observed power = 96%) and a significant interaction effect between time and condition (partial eta squared = 0.062, $P < 0.001$, observed power = 100%). Only $\leq$3% of the samples in either condition changed by more than the total allowable error by the study's endpoint. The resolution of measurement for sodium is in whole numbers. Error bars represent SD of the sample. (B) Whole blood potassium values at room temperature vs. slushed ice over time. There was a statistically significant main effect of time (partial eta squared = 0.35, $P < 0.001$, observed power = 100%) and a significant time by condition interaction effect (partial eta squared = 0.50, $P < 0.001$, observed power = 100%). Storing specimens on ice tends to increase $[K^+]$ concentration by 0.0032 mmol/L per minute but storing it a room temperature keeps it stable. By the study's endpoint, 34% of the samples stored on ice exceeded the total allowable error, as opposed to 1% of samples stored at room temperature. Please note that the resolution of measurement for potassium is to the first decimal place. Error bars represent SD of the sample. (C) Whole blood ionized calcium values at room temperature vs. slushed ice over time. There was a statistically significant main effect of time (partial eta squared = 0.021, $P = 0.003$, observed power = 91%) and a significant time by condition interaction effect (partial eta squared = 0.020, $P = 0.004$, observed power = 89%). However, while statistically significant, there was no meaningful changes for either of the 2 conditions across all timepoints. Only 5% of the samples in either condition exceeded the total allowable error by the study's endpoint. The resolution of measurement for ionized calcium is to the second decimal place. Error bars represent SD of the sample. (D) Whole blood chloride values at room temperature vs. slushed ice over time. There was a statistically significant time by condition interaction effect (partial eta squared = 0.03, $P = 0.001$, observed power = 98%). However, while statistically significant, not 1 sample from either condition exceeded the total allowable error by the study's endpoint. The resolution of measurement for chloride is to the whole number. Error bars represent SD of the sample.**

Table 2. Cumulative percentage of whole blood electrolyte samples that exceeded the total allowable error limits at room temperature (22–24 °C) and on slushed ice (0.1–0.2 °C) conditions.

	Percentage of specimens that exceeded the total allowable error limits				
	≥15–29 min	By 59 min	By 89 min	By 109 min	By 125 min
Room temperature (101 samples)					
Whole blood sodium samples that exceeded total allowable error limits of ±3.0 mmol/L	0	1	2	2	
Whole blood potassium samples that exceeded total allowable error limits of ±0.2 mmol/L	0	2	2	2	
Whole blood ionized calcium samples that exceeded total allowable error limits of ±0.04 mmol/L	4	4	5	5	
Whole blood chloride samples that exceeded total allowable error limits of ±3 mmol/L	0	0	0	0	
Slushed ice (102 samples)					
Whole blood sodium samples that exceeded total allowable error limits of ±3.0 mmol/L	2	2	2		3
Whole blood potassium samples that exceeded total allowable error limits of ±0.2 mmol/L	2	3	26		34
Whole blood ionized calcium samples that exceeded total allowable error limits of ±0.04 mmol/L	2	3	4		5
Whole blood chloride samples that exceeded total allowable error limits of ±3 mmol/L	0	0	0		0

The total allowable errors limits are obtained from the RCPA.

RESULTS

A total of 101 (53 venous, 48 arterial) whole blood samples were used to examine the stability of electrolytes (from whole blood) over time under room temperature conditions (22–24 °C). Another 102 (52 venous, 50 arterial) whole blood samples were used to examine the stability of electrolytes over time in slushed ice conditions (0.1–0.2 °C). As baseline venous and arterial concentrations for all electrolytes were not meaningfully different, they were grouped together (Supplemental Table 3). The concentration of all electrolytes in whole blood remained stable under room temperature conditions up to 109 min (Table 1; Fig. 1, A–D). The same is true for slushed ice conditions except for $[K^+]$. Under slushed ice conditions, $[K^+]$ concentration significantly increased with time (Fig. 1B). In fact, 26% of the specimens had a change of ≥0.3 mmol/L by 89 min of slushed ice storage (Table 2). In contrast, 2% of the $[K^+]$ specimens stored at room temperature showed a change of ≥0.3 mmol/L by 89 min (Table 2). Five percent of the specimens demonstrated a ≥0.3 mmol/L change in $[K^+]$ from baseline after being stored in slushed ice for 67 min. In contrast, 1% of the specimens stored at room temperature showed the same change at the same timepoint.

Forward multiple regression results indicated an overall model of one predictor (time, in

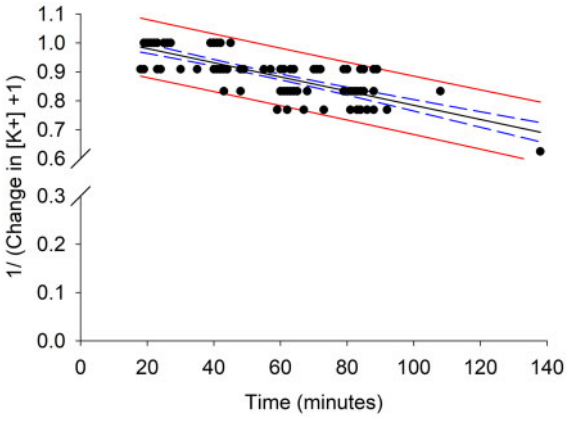

Fig. 2. Inverse change in potassium concentration over time when stored on slushed ice. 1/(change in [K^+] +1) = 1.029 − 0.00245·(time in minutes), r^2 = 0.62, standard error of the estimate = 0.05, $F(1,99)=167.8$, $P < 0.001$, n = 101 specimens, 95% CI of the slope = −0.0028 to −0.0020. This equation is valid up to 138 min. Normality test (Shapiro–Wilk) passed (P = 0.111); constant variance test (Spearman rank correlation) passed (P = 0.21). For a mean change of [K^+] of 0.25 mmol/L, which approximates an average rate of change of [K^+] of 0.0027 mmol/L/min, the storage time is 93 min. The resolution of measurement for [K^+] is 0.1 mmol/L on the Radiometer ABL 90 Flex analyzers, so after 60 min of slushed ice storage, the upper limit of the change would be 0.25 mmol/L, which would round to 0.3 mmol/L. The 95% prediction intervals are represented by the solid red lines. The 95% CIs are represented by the dashed blue lines.

minutes) that significantly predicted the change in whole blood [K^+] concentration stored under slushed ice conditions (Fig. 2). One hundred two specimens were kept on ice, and each sample had 4 measurement changes compared to its baseline value. The changes within each specimen were correlated with each other (Spearman's rho = 0.50–0.54), so only 1 of the 4 changes were used for each specimen in the linear regression model. That way, we controlled for nonindependence among repeated observations in each specimen. The pseudorandom number generator chose 1 change out of the 4 possible options in each specimen. Thus, 102 measured changes (observations) were selected.

As the change in [K^+] concentration was positively skewed (+1.14), it was transformed by obtaining the inverse of the change in [K^+] concentration and adding 1 to it [1/(change in [K^+] concentration since time zero + 1)]. The sample type (venous vs. arterial) did not influence the model.

Based on Cook's distance and the examination of standardized residuals, data screening led to eliminating 1 out of 102 specimens, and 101 specimens remained in the model. Approximately 62% of the variance in the change in whole blood [K+] concentration was shared by the amount of time a sample remained under slushed ice conditions before analysis (Fig. 2). Performing back-transformation of the data, [K^+] concentration increased by a mean of 0.0032 mmol/L (0.0021 [5th percentile] to 0.0047 mmol/L/min [95th percentile]) over 138 min when stored in slushed ice (adjusted R^2 = 0.62, $P < 0.001$). Since an increase of ≥0.3 mmol/L is considered significant based on the RCPA, the regression determined that the mean time for a specimen stored in slushed ice to reach a change of 0.25 mmol/L (compared to baseline values) was 93 min. It is worth noting that the resolution of the measurement is 0.1 mmol/L for [K^+] on the ABL 90 Flex analyzer, so a change in [K^+] by 0.25 mmol/L is reported as 0.3 mmol/L, which is an average rate of change of 0.0027 mmol/L per minute between baseline and 93 min of storage. However, over 60 min of slushed ice storage time, the regression model predicts that 5% of the samples would change by 0.25 mmol/L (0.0042 mmol/L per minute), but if using 0.30 mmol/L in the model (and not 0.25 mmol/L), then a change of exactly 0.30 mmol/L would be at 72 min of storage time. In terms of the linear model's prediction accuracy, the Spearman's rho correlation coefficient was 0.70

(95% bootstrapped CI = 0.57–0.80, $P < 0.001$, n = 101 specimens) between the measured values and the predicted values, validating the accuracy of the model.

A secondary analysis using the Kaplan–Meier estimate was used to compare findings of the linear regression model. Thirty-four specimens (33%) had [K^+] change of ≥0.3 mmol/L compared to baseline when stored in slushed ice by the study's end. The median time to reach a change of at least +0.3 mmol/L was 96 min (Fig. 3). There was a 95% probability than the change in [K^+] did not increase by ≥0.3 mmol/L if specimens were stored on slushed ice for not more than 67 min (Fig. 3). There is a 90% probability than the change in [K^+] does not increase by ≥0.3 mmol/L if specimens were stored on slushed ice for not more than 79 min (Fig. 3). Since the percentage of censored events[1] was different between the specimens stored in slushed ice compared to room temperature (67% vs. 99%, respectively), no comparison could be made between the 2 survival curves. That is, 99% of the samples stored at room temperature was censored because the study ended before a change of at least +0.3 mmol/L in [K^+] occurred. Thus, the room temperature survival curve is not shown in Fig. 3.

When multiple linear regressions were performed for all other electrolytes (sodium, ionized calcium, chloride) using samples stored on ice, the full models accounted for less than 25% of the total variance, so they were omitted. Furthermore, no statistically significant multiple linear regression equations were found for any electrolyte concentration stored at room temperature for the study duration. This is because there was no considerable slope (no statistically significant change in mmol/L per minute) during the times observed. Thus, Kaplan–Meier estimates were not determined for sodium, ionized calcium, and chloride.

The pair of electrolytes that had the strongest correlation at baseline was sodium and chloride (Spearman's rho = 0.54, 95% bootstrapped CI = 0.42 to 0.65, $P < 0.001$, n = 203). That is, 30% of the variance in sodium concentration was accounted for by differences in chloride concentration. All other paired electrolyte combinations shared no more than 4% variance with each other.

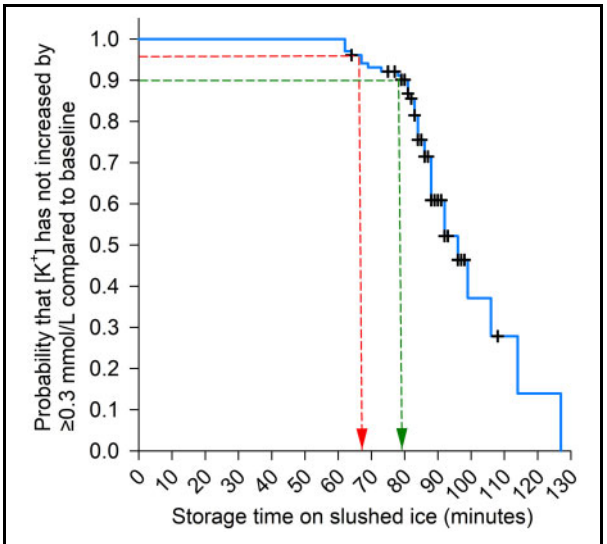

Fig. 3. A Kaplan–Meier estimator for [K^+] under slushed ice conditions. The probability that the change in [K^+] has not increased by at least 0.3 mmol/L is presented in the figure. As can be seen, there is a 50% chance that the specimen has not increased by at least 0.3 mmol/L after 96 min of being stored on slushed ice; as well, there is a 95% probability that the change in [K^+] has not increased by ≥0.3 mmol/L if specimens are stored in slushed ice for not more than 67 min (red dotted lines). Also, there is a 90% probability than the change in [K^+] will not increase by ≥0.3 mmol/L if specimens are stored on slushed ice for not more than 79 min (green dotted lines). The black crosshairs are censored data, showing that in some samples, the event did not occur by study's end (n = 102 total samples).

DISCUSSION

The purpose of the study was to demonstrate the effects of time on the concentration of whole

blood electrolytes and to determine how long these specimens can be stored in slushed ice or at room temperature before values exceed the total allowable error as deemed by the RCPA.

Based on 102 patient samples evaluated at five different time points over a maximum duration of 109 min, all electrolyte concentrations remained stable at room temperature with no significant changes. Even when the samples were kept in slushed ice, all electrolyte concentrations remained stable for up to 178 min except for [K^+]. A substantial increase in [K^+] of at least +0.3 mmol/L occurred in 5% of the samples stored on ice by 67 min of storage.

When samples were stored in slushed ice for the entire length of the study (138 min), [K^+] concentration increased at a mean rate of 0.0032 mmol/L per minute, similar to the Kaplan–Meier median estimate rate of change. The Kaplan–Meier estimate conveyed a 5% probability that [K^+] increases by ≥0.3 mmol/L by 67 min of ice storage. This is similar with the linear regression prediction interval at the 95th percentile, showing that 5% of the samples will show a change of ≥0.28 mmol/L at 67 min of ice storage. Thus, both different statistical methods provided similar results.

One of the earliest studies from more a half a century ago determined that whole blood (plasma) or serum [K^+] concentration increases by approximately 0.0033 to 0.0043 mmol/L per minute under cold conditions (4 °C or ice) (16, 17); however, these older studies were limited by small sample sizes (≤25 patients) and lack of any regression analysis. Nevertheless, our mean rate of change is consistent with these studies. Later work demonstrated that whole blood [K^+] concentration increased between 0.0023 and 0.0025 mmol/L per minute under cold conditions (Supplemental Table 1; 0.0022 [n = 50, 8 °C] (27); 0.0022 [n = 25–34, ice water] (28); 0.0024 [n = 10, 4 °C] (29); and 0.0029 [n = 50, 4 °C] (30) mmol/L per min). In animal studies, the average increase in [K^+] concentration was 0.0019 to 0.0077 mmol/L per minute (31–33). If centrifugation occurs before storage at 4 °C, then the rise in [K^+] concentration per minute slows by at least 10-fold (34).

As for the changes in the other electrolytes (sodium, ionized calcium, chloride), our data support the literature demonstrating no real change above the total allowable error limits established by the RCPA for up to 178 min on ice. Samples being stored at room temperature do not show any change in any of the 4 electrolytes up to 109 min. However, previous data demonstrate that all 4 electrolyte concentrations would remain stable for at least 3 h when stored at room temperature (Supplemental Table 1). Whole blood electrolytes are stable for a longer period when stored at room temperature compared to colder conditions (Supplemental Table 1).

The CLSI has stated that sodium and [K^+] samples should be kept a room temperature and that plasma should be separated from the cells within 60 min after collection (35). Our data demonstrate that whole blood specimens could be placed on ice for at least 67 min before significant changes occur in [K^+] concentration. In addition, the CLSI has also recommended that room temperature heparinized whole blood specimens should be analyzed within 30 min when determining ionized calcium concentration, as whole blood does not store as well as serum (36). This is similar to the recommendation of the IFCC, which, in 1991, said that room temperature whole blood specimens used for ionized calcium concentrations should be analyzed within 15 min (37). Yet, we did not notice any changes on ice or at room temperature during the study duration (178 min). Thus, it would be worth considering updating these guidelines, some of which are over 20 to 30 years old.

Protocols vary between labs for processing of whole blood electrolyte specimens. A cursory

search for clinical lab policies online found a wide variation in acceptable draw to analyze time across institutions: (e.g., [K^+], 60–120 min at room temperature; ionized calcium, 30–120 min room temperature). There may be a benefit to using data-driven protocols to standardize practices as more studies like this are published. Although samples are usually processed quickly in clinical practice, real-life delays can occur in which case this may not be possible. For example, travel delays to the lab (i.e., tube systems being down), processing delays where electrolytes are not prioritized, or situations where electrolyte specimens may be mixed with many other lab specimens if there is no dedicated blood gas lab in the hospital. Also, there may be an insufficient number of analyzers in some hospitals to process the specimens quickly. Our data support being able to use these samples.

Our study adds to the literature in many ways. First, we believe that our findings are more accurate and precise compared to the literature as we had a larger patient population. Second, we provide accurate statistical analyses and cross-validated our model for prediction accuracy. This study provides a useful regression equation tested for prediction accuracy (of up to 138 min) and is now helpful for clinical practice. The Kaplan–Meier estimate (survival analysis) is another useful way to estimate the time samples can be stored before significant changes occur.

Despite these strengths, we acknowledge potential limitations to our data. We assert that a clinically measurable change of $> \pm 0.2$ mmol/L for [K^+] and $> \pm 3.0$ mmol/L for sodium is appropriate; it is above those concentrations that the RCPA has determined to exceed the total allowable error. However, other organizations have different thresholds. The College of American Pathologists has determined the acceptable peer group (same instrument) acceptability across laboratories in the United States should not be more than ± 0.5 mmol/L for [K^+] and not more than ± 4 mmol/L for sodium (38). In 2019, the Centers for Medicare and Medicaid had requested to update proficiency testing regulations under the CLIA of 1988 (39). In this proposed rule (regulation: CMS-3355-P), the standard for acceptable performance for [K^+] is suggested to be reduced from ± 0.5 to ± 0.3 mmol/L due to the advancements of technology (39).

The differences between using a total allowable error of ± 3 or ± 4 mmol/L for sodium does not affect the results of this study. But due to the wide range of acceptability for the total allowable error for [K^+] in the literature (± 0.2 to ± 0.5 mmol/L), we also provide time estimates for samples stored on ice before these limits are met. That is, based on the upper(faster) rate of change of 0.0047 mmol/L per minute, it would take an estimated 42, 85, and 106 min to cause changes in [K^+] of 0.2, 0.4, and 0.5 mmol/L, respectively (compared to baseline), in about 5% of the specimens stored on ice.

It may be suggested that the rate of change for [K^+] differs by the starting baseline [K^+] values. However, this was not the case. We examined patients with the 20 highest baseline [K^+] values (4.9 [SD 0.3] mmol/L) and compared this to patients with the 20 lowest values (3.2 [SD 0.3] mmol/L). A repeated-measures analysis of variance demonstrated that the mean difference in the change in [K^+] between the highs (0.12 mmol/L) and lows (0.17 mmol/L) across all timepoints was 0.05 mmol/L and not statistically significant ($P = 0.11$).

Since the Kaplan–Meier estimator and linear regression demonstrated similar results for [K^+] stability when stored on ice, we recommend that the storage time for whole blood, uncentrifuged specimens be no more than 70 min if storing the sample on ice, as the [K^+] is the limiting factor in terms of stability. We selected 70 minutes for the sake of simplicity. Sometimes, ionized calcium is

the only concern, and thus storing ionized calcium on ice for up to 178 min has no effect on stability.

One possible limitation to our study is that we did not measure white blood cell counts in conjunction with electrolyte measurements. Significant increases in whole blood [K^+] concentration over time have been reported in samples with extreme leukocytosis (40, 41). While initial whole blood [K^+] measurement is not affected (in contrast to serum or plasma samples) (42), Colussi et al. reported a 0.4 mmol/L increase in [K^+] after 1 h of storage of whole blood in a heparinized syringe at room temperature and found significant changes in glucose, lactate, and carbon dioxide partial pressure, suggesting a shortage of energy production as the likely explanation of [K^+] release from leukocytes (41). We found 1 outlier in our data for [K^+] rate of change that would indicate the presence of samples with significant leukocytosis, and that led to the elimination of that specimen in the regression model. Indeed, leukocytosis (white blood cell count $>50 \times 10^9$/L) is rare (<1%) (42), and thus, it is highly unlikely that we had more than 1 specimen with abnormally high white blood cell counts.

The literature suggests that whole blood electrolytes can be stored (if measured together all at once, uncentrifuged) for at least 3 h at room temperature (Supplemental Table 1). There are some cases where only whole blood ionized calcium is of interest to the physician and not the entire electrolyte panel. In that case, placing the sample on ice does not affect ionized calcium stability for at least 3 h.

In 2020, the mean (SD) draw to analysis time for all approximately 13 800 whole blood electrolyte specimens received at our lab was 11 (6) min, and only approximately 1% of the electrolyte samples exceeded a draw-to-analyze time of >33 min. This is like the draw-to-analyze times of our blood gases (pH, oxygen partial pressure, carbon dioxide partial pressure) and suggests any electrolyte specimen that arrived on ice and measured within 67 min of the draw time would be appropriate. Our data support a recommendation that laboratories not automatically reject a whole blood specimen for analysis of the complete electrolyte panel if it was received on ice and the draw-to-analyze time is no more than 67 min. Furthermore, we prefer that these samples be sent to us at room temperature to increase the length of time the sample remains stable.

We conclude that whole blood sodium, [K^+], ionized calcium, and chloride concentrations remain stable for at least 109 min under room temperature conditions. However, if a whole blood specimen is stored in slushed ice for 67 min or less, there is no more than a 5% chance that [K^+] concentration would increase by at least ≥0.3 mmol/L. Slushed ice conditions did not destabilize the other analytes for up to 178 min.

SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

Nonstandard Abbreviations: K^+, potassium; RBC, red blood cell; RCPA, Royal College of Pathologists of Australasia.

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