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Short Communication

Is a discard tube necessary, when drawing blood for P-Ionized calcium analysis?

Vasilije Novakovic[a], Samire Abdija[a], Trine Borowik[a], Lene Gredal[b], Kirsten Vikkelsø Madsen[a], Katja Kemp Jacobsen[a,*]

[a] Department of Technology, Faculty of Health and Technology, University College Copenhagen, Denmark
[b] Department of Clinical Biochemistry, Hvidovre Hospital, Copenhagen University Hospital, Denmark

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ABSTRACT

Background: Deviation in blood collection procedures is a central source of preanalytical variation affecting overall analytical and diagnostic precision. The procedure of venous blood collection for ionized calcium is hypothesized to affect analytical results. Here, we evaluate the effect of blood collection with and without a discard tube, and storage duration on results of P-Ionized Calcium (pH adjusted = 7.4).

Methods: We collected 100 paired venous blood tubes from randomly selected outpatients using a winged blood collection. No discard tube was drawn before the first tube. The samples were divided in five subsamples, stored at 4°-6 °C at 24 (n = 20), 48 (n = 20), 72 (n = 20), 96 (n = 20) and 120 h (n = 20) after venipuncture, and analyzed for P-Ionized Calcium (pH adjusted = 7.4) on Konelab 60i (Thermo Scientific, Finland). Differences between first and second tubes were evaluated for all samples (n = 100) and for subsamples divided by storage duration, using Bland-Altman plot and Wilcoxon's rank-sum test.

Results: P-Ionized Calcium (pH adjusted = 7.4) results ranged from 1.13 to 1.37 mmol/L. We observed no statistical significant differences between the first and the second tube when comparing all samples. Dividing samples by storage duration, a statistically significant difference was found (p = .0068) after 120 h, but the difference of individual samples was not clinically relevant.

Conclusions: Our study has shown no significant difference between P-Ionized Calcium (pH adjusted = 7.4) values for the first and second tubes. Hence, the use of a discard tube is not required. A statistically significant difference was found on samples stored 120 h but was not considered clinically relevant.

1. Introduction

Calcium is one of the most important minerals in the human body. The majority of the calcium is sequestered in the skeleton, while the less abundant biologically active form, referred to as ionized calcium moves between extracellular and intracellular fluids. Out of the extracellular part, around 40–45% is bounded to plasma proteins. pH in blood is a determinant of the proportion of calcium bound to proteins, as hydrogen ions compete with calcium ions for protein binding sites [1]. For this reason, the International Federation of Clinical Chemistry (IFCC) recommend [2], that blood for ionized calcium measurement, must be drawn anaerobically to minimize the in vitro decrease in pH that might result from aerobic metabolism [2,3].

For P-Ionized Calcium (pH adjusted = 7.4), the pH in the patient sample need to be between 7.2 and 7.6, before the concentration can be adjusted relative to a serum pH of 7.4 [4,5]. By adjusting the P-Ionized Calcium, it is possible to avoid potential confounding effects of pH-change due to preanalytical factors [6]. When a winged blood collection set is used for venipuncture, the hose containing air is suspected to affect the pH in the patient sample [3]. This is solved by using a discard tube, when blood for P-Ionized Calcium (pH adjusted = 7.4), is the only tube requested.

However, there is a lack of evidence of the necessity of a discard tube. Therefore, the aim of this study was to evaluate the effect of drawing blood with versus without a discard tube on results of P-Ionized Calcium (pH adjusted = 7.4). Furthermore, the storage time of 120 h were investigated, due to the different recommendations in Danish Biochemical Departments.

2. Material and methods

Venous blood was drawn from 100 randomly selected outpatients.

* Corresponding author at: Department of Technology, Faculty of Health and Technology, University College Copenhagen, Copenhagen, Denmark.
E-mail address: katj@kp.dk (K.K. Jacobsen).

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Table 1
The P-Ionized Calcium (pH adjusted = 7.4) Minimum/Maximum range, Median, Mean difference, CV%, pH range and *p*-values.

All 24–120 h (n = 100)

Tube	Range	Median (IQR[1]) (mmol/L)	Mean difference (mmol/L)	CV%	pH range	p-Value[2]
First	1.13–1.36	1.22 (1.21–1.25)	0.00	3.30	7.30–7.49	0.09
Second	1.13–1.37	1.22 (1.19–1.25)		3.30	7.29–7.51	

Samples divided by storage duration

	24 h (n = 20)		48 h (n = 20)		72 h (n = 20)		96 h (n = 20)		120 h (n = 20)	
Tube	First	Second	First	Second	First	Second	First	Second	First	Second
Range (mmol/L)	1.13–1.30	1.13–1.29	1.18–1.27	1.18–1.27	1.15–1.34	1.15–1.34	1.16–1.36	1.17–1.37	1.16–1.31	1.15–1.30
Median (IQR[1]) (mmol/L)	1.23 (1.21–1.25)	1.23 (1.21–1.25)	1.22 (1.20–1.24)	1.23 (1.20–1.24)	1.22 (1.19–1.25)	1.22 (1.18–1.25)	1.23 (1.19–1.26)	1.23 (1.19–1.25)	1.22 (1.21–1.25)	1.22 (1.20–1.24)
Mean difference (mmol/L)	0.00		−0.01		0.00		0.00		0.00	
CV%	3.30	3.40	2.30	2.20	3.60	3.50	4.40	4.50	2.60	2.70
pH range	7.32–7.44	7.36–7.44	7.32–7.44	7.33–7.47	7.33–7.49	7.34–7.50	7.35–7.47	7.36–7.51	7.30–7.41	7.29–7.46
p-value[2]	0.88		0.95		0.83		0.97		0.0068*	

n = number of samples.
[1] IQR = Interquartile range (p25 p75).
[2] p-*Values* derived from Wilcoxon's rank-sum test.
* Statistically significant difference (> 0,05).

Written consent was obtained from all patients. We used a winged blood collection set, with a 21-gauge, 0.8 × 19 mm needle (Greiner Bio-One GmbH), with a tubing length of 19 cm according to standard practice. Two 4 mL Clot Activator tubes with gel (Vacuette) were collected from each individual. No discard tube was drawn before the first tube. The samples were centrifuged at 3580*g* for 4 min at room temperature within 0.5 to 2 h after venipuncture. Afterwards, the samples were stored at 4°-6 °C for 24 h (n = 20), 48 h (n = 20), 72 h (n = 20), 96 h (n = 20) and 120 h (n = 20), respectively, before being analyzed pairwise with potentiometry on Konelab 60i (Thermo Scientific, Finland).

Results were compared using a Bland-Altman plot and with Wilcoxon's rank-sum test. A p-value of < 0.05 was considered to be statistically significant. The 95% limits of agreement were calculated using x ± 1.96*SD.

3. Results

The P-Ionized Calcium (pH adjusted = 7.4) results range from 1.13 mmol/L to 1.37 mmol/L (Table 1). The pH ranged from 7.29 to 7.50, thereby inside accepted limits. Both intern and extern controls were inside acceptable intervals.

The results showed no statistical significant differences between the first and the second tube within 120 h, when comparing all the samples (p = .09). When divided by storage time, a maximum mean difference of 0.01 mmol/L was observed after 48 h. A statistically significant difference (p = .0068) was found between the two tubes after 120 h. However, the maximum difference between individual tubes was 0.03 mmol/L. Bland-Altman plot showed acceptable agreement between the first tube and the second tube, with a mean difference of 0.0016 mmol/L (Fig. 1). All, except one sample, were within the 95% limit of agreement.

4. Discussion

This study reveals no significant difference between P-Ionized calcium (pH adjusted = 7.4) values for the first and second tubes. Hence, the use of a discard tube is not required. This applies to samples stored at 4°-6 °C up until 120 h in the range from 1.13 to 1.37 mmol/L. Results for samples stored 120 h showed a statistically significant difference

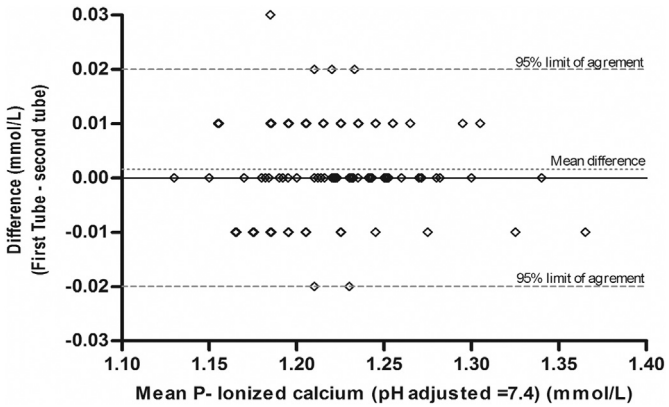

Fig. 1. Bland-Altman plot for the P-Ionized Calcium (pH adjusted = 7.4) results. Solid line indicates zero bias; The thicker squares, the more samples are on the same spot. Difference 0.03 mmol/L (n = 1), difference 0.02 mmol/L (n = 3), difference 0.01 (n = 30), difference 0.00 mmol/L (n = 45), difference − 0.01 (n = 19), difference − 0.02 (n = 2), difference − 0.03 (n = 0).

(p = .0068). However, the maximum difference between individual samples was 0.03 mmol/L, which was not considered clinically relevant.

Therefore, based on our results, there is no indication that blood for ionized calcium measurements, must be drawn with a discard tube, to ensure accuracy of P-Ionized Calcium (pH adjusted = 7.4) results. Nor does 120 h storage influence P-Ionized Calcium (pH adjusted = 7.4).

It is important to point out, that our findings indicate no need of a discard tube, because pH is not affected enough to go outside 7.2–7.6, when drawing blood with a winged blood collection set. Thus, this study is limited to the use of pH adjusted calcium analysis. This means that our findings cannot be related to other P-Ionized Calcium methods [6].

To our knowledge this is the first study that has evaluated the effect of drawing blood with versus without a discard tube on results of P-Ionized Calcium (pH adjusted = 7.4). However, our results can be applied only to samples influenced by air from a winged blood collection set. It is still unknown how more air would influence the accuracy of the results, when tubes are only partially filled. Another limitation is the

small number of samples within each group of storage duration. Furthermore, only a few samples were outside the normal range of P-Ionized Calcium, limiting this study to be representative for the normal range. To ensure that the findings in this study applies to all samples, a new study with more samples outside the normal range is necessary.

Hopefully, this study can help reducing deviation in blood collection practices, by simplifying the procedure.

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References

[1] D.A. Bushinsky, R.D. Monk, Calcium, Lancet 352 (9124) (1998) 305–311.
[2] A.B. Boink, B.M. Buckley, T.F. Christensen, A.K. Covington, A.H. Maas, O. Müller-Plathe, et al., IFCC recommendation on sampling, transport and storage for the determination of the concentration of ionized calcium in whole blood, plasma and serum, J. Autom. Chem. 13 (5) (1991) 235–239.
[3] Z.L. Bandi, Estimation, prevention, and quality control of carbon dioxide loss during aerobic sample processing, Clin. Chem. 27 (10) (1981) 1676–1681.
[4] N. Fogh-Andersen, T.F. Christensen, L. Komarmy, O. Siggard-Andersen, Measurment of free calcium ion in capillary blood and serum, Clin. Chem. 24 (9) (1978) 1545–1552.
[5] N. Fogh-Andersen, Ionized calcium analyzer with a built-in pH correction, Clin. Chem. 27 (7) (1981) 1264–1267.
[6] V. Lam, S.S. Dhaliwal, J.C. Mamo, Adjustment of ionized calcium concentration for serum pH is not a valid marker of calcium homeostasis: implications for identifying individuals at risk of calcium metabolic disorders, Ann. Clin. Biochem. 50 (3) (2013) 224–229.