

Dry Electrolyte-Balanced Heparinized Syringes Evaluated for Determining Ionized Calcium and Other Electrolytes in Whole Blood

John Toffaletti,¹ Pam Ernst, Paula Hunt, and Billy Abrams

By analyzing whole blood containing no anticoagulants (uncoagulated whole blood) immediately after collection, we evaluated the relative changes in the concentrations of ionized calcium and other electrolytes in whole blood collected in dry heparinized syringes and in serum prepared from blood collected in evacuated blood-collection tubes. Using these dry heparinized syringes, we collected and analyzed whole blood that contained either 33 or 13 int. units of lithium heparin or 40 int. units of electrolyte-balanced heparin per milliliter of blood. We evaluated the effects both of these heparins at different concentrations of ionized calcium and of the incomplete filling of the syringes. We conclude that: (a) when analyzed within 2-3 min after collection, uncoagulated whole blood provides ionized calcium results unaffected by anticoagulants or cellular metabolism; (b) the preparation of serum unpredictably changes ionized calcium; (c) the use of dry electrolyte-balanced heparin virtually eliminates the interference in ionized calcium concentrations between 0.9 and 1.6 mmol/L; and (d) incomplete filling of electrolyte-balanced heparinized syringes produces no effect in syringes two-thirds full (60 int. units/mL heparin concentration) and a small effect in syringes one-third full (120 int. units/mL heparin).

Additional Keyphrases: *variation, source of · sample preparation*

A frequent and persistent problem in measuring ionized calcium accurately is the interference by heparin, which is used as an anticoagulant in blood-collection devices (1-5). Although serum prepared from blood collected in sealed, evacuated tubes encounters no interference from heparin, the time required for clotting and centrifugation is too long for critical-care monitoring and allows glycolytic production of acids by blood cells, which may alter the concentration of ionized calcium (6). Because heparinized whole blood may be analyzed immediately after collection and allows use of the entire sample, preparations of calcium-titrated or electrolyte-balanced heparin have been developed to minimize or eliminate interference by heparin. A solution of heparin titrated with calcium to an ionized calcium concentration of ~1.25 mmol/L is available (1) that effectively eliminates the interference over a wide range of ionized calcium concentrations. However, it is not practical for

routine use in hospitals: only a limited number of syringes can be produced, and microbial contamination of the syringes may be a problem. This solution also dilutes any other constituents not contained in the solution, e.g., potassium and albumin. Therefore, the optimal heparinized syringe should: contain a dry anticoagulant, not alter the concentration of any constituent that will be analyzed in the blood, and be produced commercially and aseptically in sufficient quantity to be the only blood-drawing syringe used at a hospital.

A commercially produced syringe containing a dry electrolyte-balanced heparin is now available. Although this product may possess several of the desired properties noted above, an earlier study, in which both aqueous solutions and serum were added to syringes containing a calcium-heparin solution, found the following effects of 35 int. units of calcium heparin per milliliter of blood: for ionized calcium at 0.75 mmol/L, the calcium heparin increased ionized calcium in serum by 2.3%; at 2.50 mmol/L, the ionized calcium in serum was decreased by 2.2% (7). Therefore, we have evaluated a syringe containing a dry electrolyte-balanced heparin by studying the effect of this heparin over a range of ionized calcium concentrations and in situations when the syringes were not completely filled with blood.

Materials and Methods

Syringes: We used the following types of heparinized syringes: Quik ABG 3-mL plastic venting syringe containing ~100 int. units of dry lithium heparin derived from porcine intestinal mucosa (prod. no. P-4022; Marquest Medical Products, Inc., Englewood, CO 80112); Marksman 3-mL arterial blood-sampling syringe containing ~37.5 int. units of dry lithium heparin derived from porcine intestinal mucosa (prod. no. 335 LH; Martell Medical Products, Inc., Riverside, CA 92504); and QS 90 3-mL arterial blood sampler containing 120 int. units of dry heparin balanced with calcium, sodium, and potassium (prod. no. 956-335; Radiometer America Inc., Westlake, OH 44145). The calcium added is intended to balance samples at an ionized calcium concentration of 1.25 mmol/L.

Subjects: We collected blood from >75 apparently healthy volunteers and 15 patients undergoing major surgical procedures. All subjects read and signed a consent form to participate in the study. Both the protocol and consent form were approved by the Review Board for Clinical Investigations at Duke Medical Center. The healthy volunteers ranged in age from 21 to 52 years; the patients, from 46 to 68 years.

Protocol for blood collection: We collected blood into a 20-mL syringe containing no anticoagulant. While be-

Blood Gas Laboratory, Department of Pathology, Duke University Medical Center, Durham, NC.

¹ Address for correspondence: P.O. Box 3015, 119 Carl Bldg., Duke Medical Center, Durham, NC 27710.

Received January 19, 1991; accepted July 10, 1991.

ing mixed continually by gentle inversion, the blood was dispensed into the three heparinized syringes described above by use of a three-way plastic stopcock (prod. no. K 75P; Baxter Healthcare Corp., Valencia, CA 91355-8900). When serum was needed, blood was injected into an empty, open blood-collection tube, which we then quickly sealed by replacing the original stopper. We generally let blood clot at room temperature, although occasionally we warmed samples in a 37 °C water bath for 5 min before centrifugation in a non-temperature-controlled centrifuge. After centrifugation, we collected and sealed serum in plain syringes for analysis.

Immediately after filling the syringes with whole blood, we injected the uncoagulated whole blood into the analyzers to measure ionized calcium, pH, sodium, and potassium (always ≤ 3 min after collection). This blood containing no anticoagulant did not cause any apparent clogging of the analyzers during the six months of the study. Next, the heparinized blood samples were analyzed as soon as possible.

Instruments: Ionized calcium and pH were measured with a Radiometer ICA-1 and sodium and potassium with a Radiometer KNa-2. Both analyzers make use of ion-selective electrodes that measure undiluted samples. Lactate was measured with a Model 2300 Lactate Analyzer from Yellow Springs Instrument Co. (Yellow Springs, OH 45387).

Results

In preliminary comparisons, we analyzed aqueous solutions containing calcium, sodium, and potassium, collected in the balanced-heparin syringes, without effect on ionized calcium. However, for about 15 pairs of samples, results for ionized calcium in serum compared with those for balanced-heparinized whole blood differed by -0.07 to 0.06 mmol/L. We suspected that cell metabolism during serum preparation had altered the ionized calcium concentration. Therefore, we analyzed whole blood containing no heparin within 2 min after collection. Because this type of sample should have minimal or no effect on ionized calcium concentration attributable to either heparin content or cellular metabolism, we used results from uncoagulated whole blood as controls in the comparisons.

To study the effect of cellular metabolism, we measured lactate in three types of samples, collected from each of five healthy donors, with the following results: in serum prepared from blood clotted for 20 min at room temperature and centrifuged for 5 min (lactate = 1.64 ± 0.30 mmol/L), in whole blood collected in balanced-heparinized syringes analyzed within 5 min (lactate = 0.88 ± 0.23 mmol/L), and in uncoagulated whole blood analyzed within 2 min (lactate = 0.74 ± 0.18 mmol/L). These results confirm that cells produce lactate during the clotting and centrifugation, causing variable effects on the ionized calcium concentration (8).

Next we compared ionized calcium results for 22 samples from which we prepared and analyzed serum, uncoagulated whole blood, whole blood with balanced heparin at 40 int. units/mL, and whole blood with

lithium heparin at 13 or 33 int. units/mL. With uncoagulated whole blood as the control, the average difference between serum and uncoagulated whole blood was very small (-0.008 mmol/L), whereas the variation was large: the SD of differences = 0.026 mmol/L and the range of differences = -0.06 to 0.05 mmol/L. Both the SD and range of differences were more than threefold greater than in the comparison between uncoagulated whole blood and whole blood with balanced heparin (SD of differences = 0.008 mmol/L, range of differences: -0.01 to 0.02 mmol/L). The whole blood with either 13 or 33 int. units of lithium heparin per milliliter showed the expected decreases in the SD of the differences of ionized calcium relative to uncoagulated whole blood: -0.039 and -0.076 mmol/L, respectively.

Table 1 summarizes the overall comparisons between 83 heparinized whole-blood and uncoagulated whole-blood samples. This comparison shows the expected negative bias that is proportional to the heparin concentration in whole blood containing either 13 or 33 int. units of heparin per milliliter. Table 1 also shows that, whereas the whole-blood samples containing balanced heparin have virtually no mean bias compared with the uncoagulated whole blood, the range of differences (-0.07 to 0.03 mmol/L) indicates that some samples may have a significant bias. Only two samples differed by >0.04 mmol/L from uncoagulated whole blood. Although these samples were obvious outliers, with differences of -0.06 and -0.07 mmol/L at ionized calcium concentrations of about 1.20 mmol/L, we did not exclude them from our data. Also, using syringes containing balanced heparin, we collected 15 samples from patients undergoing major surgical operations. The ionized calcium concentrations, which ranged from 1.05 to 1.25 mmol/L, never deviated by >0.02 mmol/L from results of the uncoagulated whole blood (data not shown).

We investigated the effect of balanced heparin, 40 int. units/mL, in blood samples having a range of ionized calcium concentrations. In Figure 1, the plot of the deviations vs ionized calcium concentration shows a large bias, ranging from -0.07 to -0.09 mmol/L in samples with heparin at 33 int. units/mL, and a negative bias of -0.03 to -0.06 mmol/L with 13 int. units/mL heparin content. The use of balanced heparin reduced interference at nearly all ionized calcium concentrations to <0.03 mmol/L. For ionized calcium in the

Table 1. Summary of Ionized Calcium Results in Heparinized Whole Blood Compared with Those in Uncoagulated Whole Blood

Comparison with UWB*	Differences in Ca^{2+} , $\mu\text{mol/L}$		
	Mean	SD	Range
FHWB	-76	21	-130 to 0
RHWB	-37	22	-120 to 0
BHWB	-3	18	-70 to 30

n = 22.

* UWB, uncoagulated whole blood (no anticoagulant); FHWB, fully heparinized (33 int. units/mL) whole blood; RHWB, reduced-heparinized (13 int. units/mL) whole blood; BHWB, balanced-heparinized whole blood.

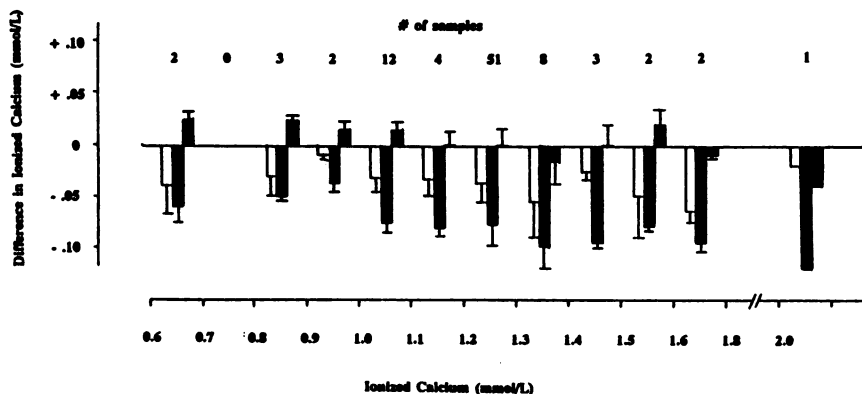


Fig. 1. Differences in ionized calcium between heparinized whole blood and uncoagulated whole blood plotted against the mean of ionized calcium concentrations

Bars represent the mean differences (and SD) of samples within a concentration range of 0.1 mmol/L, e.g., 12 uncoagulated whole-blood samples with ionized calcium concentrations from 1.00 to 1.09 mmol/L. Samples were collected in lithium heparin, 13 (□) or 33 (■) int. units/mL, or in balanced heparin, 40 int. units/mL (▨)

range of 0.6 to 0.9 mmol/L, the maximum bias for any sample was 0.03 mmol/L (mean 0.025). For a sample with an ionized calcium concentration of 2.09 mmol/L, we observed a bias of -0.04 mmol/L.

We also studied the effect on ionized calcium of incompletely filling the 3-mL syringes with blood. From each blood sample collected from 19 donors, we filled three syringes with 3, 2, and 1 mL of blood. As Figure 2 shows, whereas the syringe containing lithium heparin had the expected pronounced effect, the syringe containing balanced heparin had virtually no effect on the mean ionized calcium concentration. For syringes one-third full (120 int. units of balanced heparin per milliliter), two high-calcium samples (1.62 and 2.03 mmol/L) had biases of -0.05 mmol/L and a low-calcium sample (0.62 mmol/L) had a bias of 0.06 mmol/L. For all other samples, the bias ranged from -0.03 to 0.03 mmol/L in

syringes one-third full.

Because calcium is added to the balanced-heparin syringes, we measured total calcium in several samples collected in these syringes. We found that the added calcium is detectable as a mean (\pm SD) increase in total calcium of 0.06 ± 0.04 mmol/L, with a range of difference of 0 to 0.11. Thus, these samples are not well suited for analysis of total calcium.

Our comparison of results for sodium, potassium, and pH confirms that neither the ordinary lithium heparin, the reduced heparin, nor the balanced heparin affected these analyses as compared with results for uncoagulated whole blood. We also found that incomplete filling of the syringes had virtually no effect on these analyses. Except for one difference of 2 mmol/L, no comparisons for sodium differed by >1 mmol/L; for potassium, none differed by >0.1 mmol/L; and for pH, the maximum difference of incomplete filling was 0.02. In 22 comparisons between serum and whole blood, sodium differed by ≤ 1 mmol/L and potassium by ≤ 0.2 mmol/L. However, as indicated by the changes in ionized calcium in serum, the pH differed substantially in some serum samples compared with that in uncoagulated whole blood, with differences ranging from 0.01 to 0.10 (mean \pm SD: 0.04 ± 0.02).

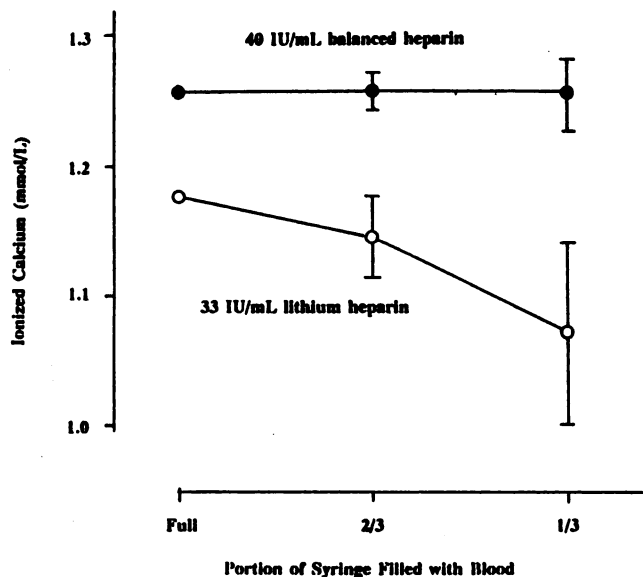


Fig. 2. Effects on ionized calcium of incomplete filling of syringes with blood

Vertical bars represent 1 SD of 19 samples collected in 3-mL syringes containing balanced heparin (●) or lithium heparin (○). The concentrations of heparin listed (int. units/mL) refer to those in syringes filled with 3 mL of blood

Discussion

To evaluate the effects of different blood-collection products on laboratory results, one must establish which type of sample, by virtue of having the fewest interferences or biases, can be used as a control in the comparisons. Although properly collected and processed serum is sometimes believed to give the most nearly accurate ionized calcium result, our study reconfirms that serum may have metabolic artifacts from accumulation of hydrogen and lactate ions (8). Because uncoagulated whole blood has no additives and virtually no artifacts due to metabolism, we believe that this type of sample, analyzed within 3 min after collection, represents a nearly ideal control sample to determine the effects of additives, sample-handling procedures, and

storage conditions on electrolyte measurements. Perhaps the only disadvantage is that the analyzer must be close to the subject providing blood.

Another key factor in studies comparing relatively small differences among samples is the precision of the analyzer. The Radiometer ICA-1 ionized calcium analyzer used in our study consistently gives within-run precision (CV) of 0.4–0.7%. We believe that use of an ionized calcium analyzer with, e.g., a CV of 2%, would at least partly obscure the true differences between samples. Our study shows that use of the dry balanced-heparin syringes causes no clinically significant bias to measurements of sodium, potassium, or pH at balanced-heparin concentrations of ≤ 120 int. units/mL in blood.

Although dry lithium heparin negatively biases measurements of ionized calcium, the use of balanced heparin decreases the bias to ≤ 0.02 mmol/L for ionized calcium concentrations between 0.9 and 1.8 mmol/L. This range represents perhaps 90% to 95% of all ionized calcium results, even in critically ill patients, with most other results being < 0.9 mmol/L. For five samples ranging in concentration from 0.65 to 0.85 mmol/L, ionized calcium concentrations were 0.02 or 0.03 mmol/L greater than in uncoagulated whole blood. Although this is analytically troublesome, we believe this bias is clinically insignificant. With an ionized calcium concentration of ≥ 2.0 mmol/L, the negative bias of 0.05 mmol/L would become more negative as the ionized calcium concentration increased. However, the lack of clinical significance of a 0.05 mmol/L bias at this concentration would be only a minor concern in actual use.

Even though ordinary heparin has been known for years to lower ionized calcium results, this effect can be compensated for somewhat both by using only one type of heparinized syringe within a hospital and by adjusting reference intervals appropriately for heparinized whole blood. However, neither of these procedures can compensate for incompletely filled heparinized syringes, which, by increasing the heparin concentration, will proportionally lower the ionized calcium concentration even further. Our study of incompletely filled syringes shows that the use of balanced heparin minimizes this effect. In syringes two-thirds full, containing balanced heparin at 60 int. units/mL, the maximal effect was 0.03 mmol/L through the entire ionized calcium concentration range of 0.65 to 2.05 mmol/L. In syringes one-third full, containing balanced heparin at 120 int. units/mL,

results for ionized calcium were seldom > 0.03 mmol/L different from those for the filled syringe, differing by 0.05 to 0.06 mmol/L only at the very lowest and highest ionized calcium concentrations. Therefore, in an ionized calcium range of 0.7 to 1.8 mmol/L, the balanced-heparin syringes reduce the effect of incomplete filling to 0.03 mmol/L, even at a balanced-heparin concentration of 120 int. units/mL.

A disadvantage of balanced heparin over ordinary heparin is that, because of the calcium added to balance the heparin binding, one should not measure total calcium on plasma obtained from these syringes. Although this is an unlikely request in critical-care monitoring, it would be a nuisance when analysis for total calcium is requested for this type of sample.

Clearly, the dry balanced-heparin syringes represent a significant advance over ordinary lithium-heparinized samples for measurement of ionized calcium. However, the user should be aware of small to moderate biases when a syringe is about one-third full or less, because the bias may be noticeable, especially at very low or high concentrations of ionized calcium. Also, total calcium should not be measured in plasma from these syringes.

We acknowledge the financial support of Radiometer America, Inc., in this study.

References

1. Urban P, Buchmann B, Scheidegger D. Facilitated determination of ionized calcium. *Clin Chem* 1985;31:264–6.
2. Burns J, Law RWG, Paterson CR. Measurement of plasma ionised calcium: comparison of calcium-titrated sodium heparin and lithium heparin as anticoagulants. *Med Lab Sci* 1987;44:187–9.
3. Biswas CK, Ramos JM, Kerr DNS. Heparin effect on ionised calcium concentration. *Clin Chim Acta* 1981;116:343–7.
4. Heining MPD, Jordan WS. Heparinization of samples for plasma ionized calcium measurement. *Crit Care Med* 1988;16:67–8.
5. Nelson N, Ohman S, Larsson L. Effect of hematocrit and added heparin on ionized calcium in capillary blood samples from neonates. *Clin Chem* 1989;35:486–8.
6. Toffaletti J, Blosser N, Kirvan K. Effects of storage temperature and time before centrifugation on ionized calcium in blood collected in plain Vacutainer Tubes and Silicone-Separator (SST) Tubes. *Clin Chem* 1984;30:553–6.
7. Sachs C, Rabouine P, Chaneac M, Kindermans C, Dechaux M. Anticoagulant-induced preanalytical errors in ionized calcium determination on blood. *Scand J Clin Lab Invest* 1989;49:647–51.
8. Toffaletti J, Abrams B. Effects of in vivo and in vitro production of lactic acid on ionized, protein-bound, and complex-bound calcium in blood. *Clin Chem* 1989;35:935–8.