Reproducibility of Serum Potassium Values in Serum From Blood Samples Stored for Increasing Times Prior to Centrifugation and Analysis

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Goals: The goal of this work was to determine if immediate versus postponed centrifugation of samples affects the levels of serum potassium. **Methods:** Twenty participants donated normal venous blood that was collected in four serum separator tubes per donor, each of which was analyzed at 0, 1, 2, or 4 hr on the Siemens Advia 1800 autoanalyzer. **Results:** Coefficients of variation (CVs) for potassium levels ranged from 0% to 7.6% with a mean of $3 \pm 2\%$. ANOVA testing of the means for all 20 samples showed a *P*-value of 0.72 (>0.05) indicating that there was no statistically significant difference between the means of the samples at the four time points. Sixteen samples

were found to have CVs that were $\leq 5\%$. Two samples showed increases of potassium from the reference range to levels higher than the upper reference limit, one of which had a 4-hr value that was within the reference or normal range (3.5–5 mEq/l). Overall, most samples were found to have reproducible levels of serum potassium. **Conclusions:** Serum potassium levels from stored whole blood collected in serum separator tubes are, for the most part, stable at room temperature for at least 4 hr prior to analysis. However, some samples can exhibit significant fluctuations of values. J. Clin. Lab. Anal. 30:244–247, 2016. © 2015 Wiley Periodicals, Inc.

Key words: potassium; serum level; storage; reproducibility

INTRODUCTION

Serum potassium ion concentration is among the most tightly regulated of all analytes. Even slight increases above or below the reference or normal range (at our medical center 3.5–5.0 mEq/l) can cause serious cardiac arrhythmias and neuromuscular dysfunction. Since potassium is the chief intracellular ion, tissue necrosis or hemolysis results in hyperkalemia that can have serious physiological consequences. It is therefore important to obtain accurate and reproducible levels of this analyte on patients.

In several prior studies (1–3), we determined the reproducibility of critical serum analyte values, that is, electrolytes (sodium, potassium, chloride, calcium), BUN, creatinine, and glucose from patients or donors. In these studies, stored sera were assayed once daily for at least 1 week; with the exception of one sample, involving

creatinine, values were found to have low coefficients of variation (CVs), indicating excellent reproducibility.

The above studies were performed on serum from whole blood samples, all of which had been centrifuged prior to analysis. The question arises as to whether the time of storage of samples prior to centrifugation can affect the analytical results. This question is particularly poignant for potassium levels for the following reason.

During hurricane Sandy, the main chemistry analyzers at the Manhattan Campus of the New York Harbor VA

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Medical Center were nonfunctional. Due to the urgent need for emergency services at this site, blood samples were analyzed for critical analytes on blood gas analyzers that were functional. In order to validate the results on these analyzers, split samples were analyzed on the blood gas analyzers in New York and on main Chemistry analyzers at the Brooklyn Campus. This required a delay of about 2 hr for transport time. Two sets of studies were performed: one in which the samples were transported to the Brooklyn site uncentrifuged and one in which they were immediately centrifuged after collection (Lu et al., unpublished observations).

We found that both correlation results for potassium were not satisfactory, but the correlations improved for the samples that were centrifuged immediately, suggesting that potassium values might depend on the storage time prior to centrifugation and the separation of cells from plasma or serum. This consideration was heightened by our finding that all of the samples sent to the Brooklyn site were found to have potassium levels that were higher than the corresponding values for the samples analyzed immediately on the blood gas analyzers and that the LDH level was elevated in a significant number of these samples. Both of these findings suggested hemolysis as one possible cause of the observed discrepancies.

If time of centrifugation affects potassium values, there is the possibility that routine blood samples, which may be stored for several hours prior to analysis, may be found to have falsely elevated values for this analyte. If indeed this is found to occur, measures would have to be instituted to prevent the development of factitious hyperkalemia. In this article, we have designed a quality assurance study to determine whether time of centrifugation affects potassium values in blood samples.

METHODS

Blood samples were collected from 20 staff members at the Brooklyn Campus of the VA New York Harbor Health Care System in four (4) serum separator tubes. In our procedure, the first tube was centrifuged immediately after collection and analyzed for potassium using ion selective electrode methodology on an Advia 1800 Chemistry Auto-Analyzer (Siemens, Tarrytown, NY). The remaining tubes were stored at room temperature for 1 hr after which the second tube was centrifuged and analyzed. The remaining tubes were stored at room temperature for a total of 2 and 4 (in one case, 3) hr, respectively, at which times they were centrifuged and analyzed. The mean, standard deviation, and CV for the four potassium levels were calculated for each set of samples. The maximum time limit for sample storage was chosen as 4 hr since this is actually beyond any normal storage time for patient samples from the time of collection to the time of analysis.

Fig. 1. Plots of serum potassium values for each donor as a function of time. Each plot has its own unique color and symbol shown on the right side of the plots so that the time course can be followed. For clarity, the plots are divided into two sections, each of which contains the results for ten donors, or a total of 20 plots between the two sections. The numbers are those referred to in the text.

RESULTS

The results of the potassium analysis are shown in Figure 1, which are plots of the potassium values from the sera of the four blood tubes collected from each of the 20 donors. The figure is divided into two sets of plots, each plot showing the results for ten donors. As can be seen from these plots, most, that is, 15, potassium values appear not to change dramatically; six samples were found to fluctuate significantly, namely samples 5, 9, 14, 16, 17, and 20. ANOVA testing of the means for the 20 samples at all time points showed a *P*-value of 0.72 that is greater than 0.05. Thus, there is no significant difference in the means for these samples at any of the time points. The means for the four time points are shown in Figure 2; the standard deviations are given in the legend to this figure.

Fig. 2. Plot of the mean potassium value for the 20 samples for each of the four time points (0, 1, 2, and 4 hr). Standard deviations are 0.36, 0.40, 0.42, and 0.40 at hours 0, 1, 2, and 4 hr, respectively.

The range for the CVs in this study was observed to be 0% (sample 12) to 7.6% (sample 16). The mean CV for all samples was $3.0 \pm 2.0\%$. Overall, 15 sample values exhibited CVs of 5% or less. Of these, nine sample values had CVs that were less than 2% and 12 sample values were found to have CVs less than 3%. Five sample values (5, 14, 16, 17, and 20) were found to have CVs $>5\%$. The potassium values for two of these samples (14 and 16) remained well within the reference range. Two of these samples (5 and 17) showed increases of potassium above the upper limit of the reference range (mEq/L). One sample (Fig. 1, no. 20) underwent a steady decrease in potassium levels with a CV of 5.5%.

Sample 5 was found to have an elevated potassium level in all measurements except for the initial measurement suggesting hemolysis or the possibility that the initial value may have been spuriously low. Values for sample 17 were somewhat erratic, beginning with a normal value of 4.6 mEq/L that decreased to 4.4 mEq/L at 1 hr. The value for the 2-hr sample spiked to 5.2 mEq/L, an elevated value that then decreased in the 4-hr sample to a value of 4.9 mEq/L, within the reference range.

Potassium levels for two samples, 5 and 16, were found to increase over the time period of 4 hr, and those for two other samples, 9 and 14, increased over a 2-hr period but then decreased at 4 hr. Thus the potassium levels of only two samples, 5 and 16, were found to increase consistently. It is possible that the increase of potassium in these two samples was caused by a low level of hemolysis of red blood cells that may have resulted from damage to red cells during the collection process.

DISCUSSION

Our results suggest that potassium values of serum from whole blood stored at room temperature are reproducible as evidenced by the observations that potassium values changed little for most samples, the mean CVs being 3%, and that the means for the potassium values at all time

points did not differ statistically from one another in the ANOVA test. However, some samples, such as 5, 14, 16, 17, and 20, exhibited fluctuations in potassium values. Some of these, such as samples 5 and 17, resulted in values that exceeded the upper limit of the reference range. The reason for these fluctuations is not clear and deserves further investigation. We have observed a similar phenomenon for stored samples subject to daily assays for other analytes such as creatinine (2).

Our results are compatible with the results of several other studies. In one study using a smaller sample size (10 vs. 20 as in this study), on the stability of many analytes commonly assayed for, whole blood from ten donors, collected in lithium-heparin and serum separator tubes and stored at room temperature, was analyzed at time points from 0 to 56 hr (4). The mean values for serum potassium were statistically insignificantly different from one another at 0, 0.5, and 4 hr although the mean plasma value became statistically significantly different at 4 hr.

Another study found that assays for the serum levels of a variety of chemical analytes were reproducible over time at 8 and 22°C. The one exception to this result was potassium, which was not reproducible after 6 hr at 8°C but was reproducible at 22°C (5), suggesting the effect of temperature on serum potassium levels. As noted above, low temperatures are thought to cause factitious hyperkalemia due to decreased activity of the sodium-potassium dependent ATPase in the red cell membrane that enables potassium transport into the cells.

This phenomenon may have played a role in causing our prior findings (discussed in the Introduction) that motivated this study, that potassium values in blood samples transported on ice from one site to the other over about a 2-hr period did not correlate with the values obtained immediately on blood gas analyzers and were all significantly higher. While low temperature may have contributed to the differences, hemolysis was also found to occur. Our present results suggest that hemolysis is not significant although it may have occurred in two samples (5 and 16 in Fig. 1 in which there was a steady increase in potassium levels. If hemolysis did occur in these samples, the cause is not clear although factors such as sample withdrawal and/or differences in rates of transfer of blood samples from vacutainer to sample tubes may have contributed.

It is of interest to compare our present results with those in a prior study on the reproducibility of potassium and other electrolyte values in sera from blood samples collected from ten patients, which were stored over a 9-day period at 4°C (1). The highest CV for potassium in this prior study was 1.46% (range 0.76–1.46%), and the mean was $0.93 \pm 0.37\%$. In this present study, we find the highest CV was 7.6% (range 0–7.6%), and the mean CV was $3 \pm 2\%$. Our results in this study therefore suggest that storage of whole blood samples at room temperature may

increase the CV for serum potassium values. One difference between the two studies is the temperature: the serum samples in the prior study were stored at 4°C while the whole blood samples in this current study were stored at room temperature. Another difference between the studies is that, in the present study, four separate tubes of blood collected at the same time were assayed for potassium versus one sample that was repeatedly analyzed.

The CV value for potassium listed in the package insert for the Siemens Advia 1800 analyzer on which our potassium determinations were carried out is 1.83%, higher than in our previous study but lower than in this present study. The Siemens' CV was determined for samples on which analysis was performed repeatedly on the same serum, that is, the samples were all centrifuged and the CVs were determined for repeated determinations on these serum samples. In contradistinction, our CVs were computed for the same *whole blood* samples that were centrifuged after different storage times.

CONCLUSION

There is no statistically significant change in serum potassium levels from whole blood stored over a 4-hr period at room temperature although significant fluctuations occur for a number of samples. Overall, normal

delays between collection time and time of centrifugation and analysis of whole blood samples do not affect serum potassium levels.

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