Effect of blood collection tubes on the incidence of artifactual hyperkalemia on patient samples from an outreach clinic

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ABSTRACT

Background: An offsite satellite clinic of the University of Chicago Medical Center (UCMC) requested an investigation by the Clinical Chemistry Laboratory (CCL) into several cases of possible falsely elevated potassium (K+) values in their patients. Bloods for K+ and chemistry profiles are routinely collected in mint-green, heparinized plasma separator tubes (PST), centrifuged, and transported by courier from satellite clinic to CCL within several hours. Samples from on-site phlebotomy areas are similarly collected but sent uncentrifuged to CCL via a pneumatic tube system within minutes of collection.

Methods: Our investigations included extensive QC and QA review of UCMC onsite and offsite outpatient clinics, reference range studies using PST and serum separator tubes (SST), assessment of pre-analytic handling of specimens, including transportation simulation study, and comparison of K+ results for samples collected simultaneously using PST and SST tubes at an offsite clinic.

Results: Our transportation simulation demonstrated elevations in K+ concentrations following sample jostling and perturbations. We also observed RBC escape across the gel barrier further contributing to K+ elevations.

Conclusion: Serum is preferred sample type for an offsite clinic.

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1. Introduction

Potassium (K+) is one of the most frequently requested tests in a hospital laboratory. Our laboratory processes thousands of plasma K+ tests each year. Several factors can impact the accuracy of K+ measurements. These factors include (1) preanalytical variables such as hemolysis, phlebotomy, sample processing and transport to the central laboratory for testing, (2) analytical factors that could result from differences in performance of analytical modules and/or calibration differences, and finally, (3) post-analytical factors that include transcription errors, result upload errors and incorrect verbal communication of results.

We have recently been contacted by an offsite satellite clinic (OSC) of the University of Chicago Medical Center (UCMC) with an inquiry regarding a perception of increased numbers of patients with K+ results that were above our stated upper reference range of 4.7 mmol/l. The clinicians were concerned that majority of these elevated K+ values are likely to be artifactual increases. Patient samples for K+ and chemistry profiles are routinely collected in mint-green, heparinized plasma separator tubes (PST), centrifuged, and transported by courier from OSC to CCL within several hours. Specimens from UCMC on-site phlebotomy areas are similarly collected but sent uncentrifuged to CCL via a pneumatic tube system within minutes after collection. In this study we confirmed the difference in K+ values between the two clinics and performed detailed study of all the sample processing and handling effects to identify the root cause and to mitigate this problem.

2. Methods

2.1. Sample collection and processing

Venous blood samples were collected using standard venipuncture technique into BD Vacutainer®, 13 × 100 mm, plasma separator tubes (PST™) containing polymer gel and lithium heparin (BD Diagnostics, Franklin Lakes, NJ) and BD Vacutainer®, 13 × 75 mm serum separator tubes (SST™) with silica clot activator, polymer gel and silicone-coated interior (BD Diagnostics, Franklin Lakes, NJ).

The SST specimens were incubated for 10 min at room temperature to allow for a clot to form. Samples collected at the satellite clinic were centrifuged in a swing bucket rotor at 1800 × g for 10 min. Samples from UCMC were centrifuged in a Roche Modular Pre-Analytics system centrifuge at 1800 × g for 8 min.

As per our communication with the University of Chicago IRB office, this project is considered quality assurance/quality improvement and does not require a review by IRB.

2.2. Investigation of preanalytical processes at OSC vs onsite outpatient clinics

Potassium testing is performed at the UCMC Central Clinical Laboratory (CCL) on a Roche Modular Analytics System (Roche Diagnostics,
Indianapolis, IN) employing an indirect ion selective electrode (ISE) technology. We assessed the pre-analytical handling of specimens by visiting the OSC and observing the process. As a part of this process review we observed the type of specific collection devices utilized, tourniquet application techniques, sample mixing and centrifugation and finally, sample storage prior to transportation to CCL for analysis. Analytical accuracy and performance was evaluated by a review of QC performance and proficiency testing on all of the modules available.

2.3. Reference range study

This study was conducted by analyzing specimens collected concurrently into PST and serum separator tubes (SST) from apparently healthy volunteers. The total number of samples collected was 51 for each sample type and the reference ranges were computed using the Analyse-It for Excel software (http://www.analyse-it.com).

2.4. Transportation simulation study

Previously centrifuged UCMC plasma samples (n = 23) were removed from storage on the same day of clinical analysis and subjected to re-mixing and jostling to simulate the transport from an off-site location to UCMC. The specimens were transported in the upright position. The K+ levels were then re-assayed for all samples and compared to the originally reported results. Differences between these newly assayed K+ levels and previously reported values were analyzed by the paired t-test, using the GraphPad Prism software (San Diego, CA).

2.5. Collection tube studies

This study was conducted in 2 stages. Initially, PST and SST samples were compared for each patient. All the specimens were collected in parallel at the satellite clinic. In the second phase of the study 2 SST samples were collected and the staff was instructed to transfer the serum from one of the tubes into a new tube (aliquot SST). We then compared K+ results for the following: (1) PST vs SST and (2) SST vs SST aliquot tubes to determine the optimal collection tube type and sample processing protocol. Comparisons were analyzed by the paired t-test using the GraphPad Prism software (San Diego, CA).

3. Results

3.1. Distribution of K+ results in the clinics (UCMC vs OSC)

The histogram of K+ results at the UCMC primary care clinic and the offsite clinic is shown in Fig. 1. During a one-month period, we received almost 1700 K+ requests from the UCMC primary care clinics. The results showed a distribution with 5.2% of patients falling below reference range and 4.4% of patients above the reference range. In contrast, the review of K+ results of 201 patients who visited the satellite clinic revealed a highly skewed distribution toward the higher K+ values with 14.4% results above the upper reference limit and only 0.5% below the lower reference limit. These results appear to be consistent with our clinicians’ perception in which they observe higher incidence of elevated K+ values when practicing in the satellite clinic versus the UCMC primary care clinic. To determine the cause of the higher incidence of K+ elevations, we observed the phlebotomy sample processing and handling at the satellite clinic. The overall impression was that, other than the frequent use of “butterfly” venipuncture sets for sample collection, the entire collection process appeared acceptable. The transport and storage conditions seemed acceptable although different than the procedure used for sending specimens to a typical reference lab, where processed samples are usually separated from primary tube and aliquoted into specific transport tubes. The OSC samples were transported in a cooled container and the transport pick-up was generally executed 2–3 h apart and took at least 30 min to arrive from the offsite clinic to the central laboratory. During the transport, however, the specimens were not kept upright and therefore were subjected to various degrees of jostling which could potentially contribute to false elevation of K+ due to cell lysis during transportation.

Review of QC and CAP proficiency samples revealed acceptable accuracy and precision on all modules for the period examined (data not shown). Both quality controls (level 1 at 2.2 mmol/l and level 2 at 7.1 mmol/l) demonstrated excellent precision with low QC levels showing the overall precision of <4.0% CV between all 4 modules, while high QC demonstrated CVs <2.0%.

3.2. Reference range verification study

The analysis of a total of 51 specimens of each serum and plasma collected concurrently from 51 apparently healthy volunteers is shown in Fig. 2. Our results verified the K+ reference range of 3.5–4.7 mmol/l for plasma (Fig. 2A) and 3.8–5.0 mmol/l for serum (Fig. 2B) and showed that the serum K+ values were, on average, 0.30 mmol/l higher than the plasma K+, with 95% CI of 0.21–0.32. This is the expected difference between serum and plasma specimens due to release of intracellular K+ during the clotting process [1].

3.3. Transportation simulation study

Analysis of 23 plasma samples over a two-day period revealed a consistently positive elevation in K+ over originally reported clinical result, with the mean difference of 0.20 mmol/l [95% CI 0.13–0.27] (paired t-test, p < 0.0001) as shown in Fig. 3. The increase in K+ levels
in repeated results ranged from 0.10 to 0.50 mmol/l. The results of this study indicated that perturbations of centrifuged primary PST during sample handling and transportation caused increased plasma K⁺ levels.

3.4. Effect of transportation on sample collection tubes

To determine if different sample types are affected differently by the transportation process, serum (SST) and plasma (PST) samples were collected concurrently at our satellite clinic for 85 patients and sent in the original collection tube via courier to UCMC CCL for K⁺ measurement. The results of this study are summarized in Fig. 4. As shown in Fig. 4A, there was no statistically significant difference between the PST and SST K⁺ (p = 0.364, paired t test). This confirmed our observation that the transportation is most likely the cause of spuriously elevated K⁺ levels in plasma samples, since it is well known and expected that K⁺ results are higher (on average, ~0.36 mmol/l) in serum than in plasma samples [1–3]. In addition, to test the permeability of gel barrier, we requested that a number of PST samples be collected in duplicate, where the second tube would be centrifuged immediately and plasma aliquoted into a secondary tube. These studies demonstrated a very interesting phenomenon of the “RBC escape” through the inert gel barrier and into the separated plasma (Fig. 5). We identified this phenomenon after observing the settling of the RBCs at the bottom of the secondary tube that contained the aliquoted (“pour-off”) PST specimen. Since some RBCs do escape through the PST gel into the plasma, they could

Fig. 2. Reference range (RR) studies. Plasma RR verified as 3.5–4.7 mmol/l; serum RR verified as 3.8–5 mmol/l. On average SST K⁺ was 0.3 mmol/l higher than PST K⁺ in these reference samples.

Fig. 3. Transportation simulation study.

Fig. 4. Effect of tube types on K⁺ results.
However, we suspected that there may still be some residual bias in K⁺ result distribution toward the higher levels. To examine whether this residual bias originates from the sample processing, we evaluated K⁺ levels on 134 serum samples collected at our satellite clinic and provided to us in the original collection tube, as well as a secondary tube that contained poured-off serum. As shown in Fig. 4B, there is a statistically significant mean difference of 0.09 mmol/l, with 95% CI ranging from 0.02 to 0.16, between SST and SST aliquots (paired t-test, p = 0.0132). Interestingly, one extreme K⁺ outlier of 8.5 mmol/l was observed in the SST and not in the SST aliquot group, which resulted in normal K⁺ of 4.7 mmol/l.

4. Discussion

It is well known that pseudohyperkalemia is mostly present in patients with thrombocytosis where K⁺ is released from platelets or leukocytosis where K⁺ is released from WBCs [1,4–8]. Furthermore, since K⁺ can also be released from platelets during clotting process, serum samples tend to have higher K⁺, by as much as 0.36 mmol/l [1] than plasma K⁺. Our reference range study is in agreement with these observations and showed that serum K⁺ concentration is, on average, 0.30 mmol/l higher than plasma K⁺. Pseudohyperkalemia can also be caused by sample perturbations during transport and or centrifugation of bloods collected in PST, particularly in patients with extreme leukocytosis [7] where WBC counts are >100,000/ml [5] as well as by fist clenching action during phlebotomy [9]. In the study conducted by Seimiya et al. [9], the authors report that fist clenching for only 1 min could cause erroneously elevated serum K⁺ concentrations. Interestingly, these authors found that tourniquet application had no significant effect on serum K⁺ concentrations in their patient population. Recent study by Streichert et al. [10] demonstrated that the hospital pneumatic tube speed settings can have significant impact on K⁺ results.

However, to our knowledge, there are no literature reports on the effects of transportation on different sample type collections and potassium concentrations. We directly compared paired SST and PST K⁺ results and confirmed our clinicians’ observations of higher frequency of patients with K⁺ elevations in PST versus SST sample types, when their respective reference ranges are applied. In fact, we observed no statistically significant differences in K⁺ values between samples collected into PST versus SST. Based on these observations, we hypothesized that spurious K⁺ elevations in PST samples are the result of K⁺ leakage from the cells during the transport process. Although SST does seem like a better sample type choice for satellite clinic, we did observe one high K⁺ SST outlier, compared to serum aliquoted from the original SST. We could not identify any pre-analytical or analytical causes for this outlier other than the possibility of cells escaping through the gel due to sheer force exerted during transportation and leaking K⁺ into serum. Taking into consideration all these observations, we recommended to our outreach client that they change sample type requirement from PST to SST aliquot tubes. We outlined the optimal specimen collection and transport process as follows: specimen should be collected into SST, centrifuged immediately and separated serum should be aliquoted into the secondary tube. This secondary tube should then be labeled and sent to the central laboratory for analysis. Since in the aliquot tubes the serum is separated from the cells, the incidence of spuriously elevated K⁺ results will be eliminated. As Fig. 6 shows, our post-intervention data show normal distribution of K⁺ values in the OSC compared to our primary care clinic.

In addition to transportation effects study, we decided to perform concurrent reference range study to confirm our existing reference ranges and show that the shift in K⁺ concentrations in outreach population is not simply an effect of reference range shift. We looked at both serum and plasma sample types and concluded that not only our reference range still holds but also that although serum samples do run higher than plasma, the difference between the two is not significant enough to warrant separate reference ranges. Therefore, since we...
have changed our sample requirements to accept both PST and SST, we also extended our upper limit of reference range from 4.7 to 5.0 mmol/L.

Finally, our observation that the forces exerted on RBCs during transport could cause them to cross the gel barrier and “contaminate” the separated plasma (Fig. 5) must not be overlooked since it can potentially cause the higher incidence of pseudohyperkalemia in both PST and SST samples and lead to unnecessary alarm and clinical interventions, or, what is even more dangerous, potential hypokalemic patients might be missed. The choice of aliquoted serum specimens will help circumvent all the pre-analytical problems described above. Our overall conclusion is, therefore, that for offsite clinics, SST pour-off tube is the preferred tube type to use in order to minimize incidence of pseudohyperkalemia.

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References