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Critically low sodium levels due to concentration gradients formed in patient samples after undergoing a freeze-thaw cycle

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ABSTRACT

Background: Concentrations gradients that form in plasma as a result of freezing and thawing is a well-known phenomenon. As the water fraction converts into ice, plasma constituents diffuse from the freezing front by natural convection in the liquid phase. This process can lead to erroneous lab results, if the sample is not thoroughly mixed prior to testing.

Methods: A series of patient samples received at the clinical chemistry core lab were found to have low sodium levels that normalized after tube inversion. We suspected that the samples may have frozen during shipping and therefore examined the effects of freezing and thawing on serum.

Results: Our investigation revealed that prior to arriving at the core lab, samples from one of our satellite clinics were undergoing a freeze-thaw cycle during shipping, which resulted in the formation of concentration gradients and spurious lab results on arrival.

Conclusions: Large hospitals that have a central core lab and receive patient samples from satellite clinics need to be aware of this phenomena, which can contribute to erroneous lab results being posted in a patient's electronic medical record, resulting in a misdiagnosis.

1. Introduction

In recent years the health care system has undergone tremendous change with the enactment of the Affordable Care Act, as small hospitals and clinics merge or affiliate with larger hospitals in an attempt to offset associated costs [1]. As such, like many other large hospital systems, the University of Texas Medical Branch (UTMB) at Galveston has acquired and built numerous primary clinics, specialty clinics, and hospitals in the surrounding communities [2,3]. To support this growth, the division of clinical chemistry recently installed 3 Vitros 5600 analyzers, coordinated by Ortho's *enGen*™ Laboratory Automation System for routine high-volume testing in the hospital's central core laboratory. The expansion of UTMB, the centralization of laboratory testing, and the introduction of automation to a rapidly growing hospital system, has led to unintended consequences. One such consequence was realized when our chemistry core lab noticed a series of critically low sodium values in samples arriving from one clinic, which corrected by inverting the tubes several times. The first case appeared in October 2016.

2. Case presentation

A 46 y old male with a medical history of type 2 diabetes mellitus presented to his primary care physician (PCP) at one of UTMB's satellite Family Medicine clinics for a routine diabetes follow-up visit, and medication refill. He denied any diabetic associated symptoms, and his only complaint was constant lower back stiffness, which had recently started after moving some furniture. His medication list included metformin, glyburide, lisinopril, and promethazine with dextromethorphan. The patient had been non-compliant in taking his diabetes medications for the 2 months prior to this visit. His vital signs were within normal limits and his physical exam was only remarkable for paravertebral pain and spasms in the lumbar area. His physician ordered some lab tests, and the patient arrived at the lab 2 weeks later for his blood to be drawn. Results are shown in Table 1 as Day 1. The day after his labs were drawn, the patient was advised by his PCP that he should go to the emergency department (ED) to be evaluated for hyponatremia.

Upon presentation to the ED, the patient was examined and found to be alert and oriented, with no complaints. His review of symptoms and

Abbreviations: ARUP, Associated Regional and University Pathologists, Inc; ALP, albumin, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CR, concentration ratio; ED, emergency department; HDL, high-density lipoprotein; PCP, primary care physician; SST, serum separator tubes; PST, plasma separator tubes

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Table 1
Patient's initial lab results (Day 1), and ED results (Day 2).

Analyte	Reference interval	Day 1	Day 2	Day 1/ Day 2
Sodium	135–145 mmol/l	116	139	0.83
Potassium	3.5–5.0 mmol/l	3.4	4.0	0.85
Chloride	98–108 mmol/l	87	99	0.88
Total CO_2	23–31 mmol/l	22	26	0.85
Urea	7–23 mg/dl (0.25–0.82 mmol/l)	12 (0.43 mmol/l)	12 (0.43 mmol/l)	1.00
Glucose	70–110 mg/dl (3.89–6.11 mmol/l)	166 (9.21 mmol/l)	191 (10.60 mmol/l)	0.87
Creatinine	0.60–1.25 mg/dl (45.78–95.38 μmol/l)	0.53 (40.44 μmol/l)	0.62 (47.31 μmol/l)	0.86
Total bilirubin	0.1–1.1 mg/dl (1.71–18.81 μmol/l)	0.7 (11.97 μmol/l)	1.3 (22.23 μmol/l)	0.54
Calcium	8.6–10.6 mg/dl (2.15–2.65 mmol/l)	7.3 (1.83 mmol/l)	9.3 (2.33 mmol/l)	0.78
Total protein	6.3–8.2 g/dl	5.8	7.2	0.81
Albumin	3.5–5.0 g/dl	3.0	4.1	0.73
ALP	34–122 U/l	49	56	0.88
ALT	9–51 U/l	31	30	1.00
AST	13–40 U/l (0.22–0.68 μKat/l)	13 (0.22 μKat/l)	17 (0.29 μKat/l)	0.76

physical exam were unremarkable, and his lab values were all within normal limits (Table 1, Day 2). In fact, the values for most of the laboratory tests in the initial run on day 1 were a fraction of the true values that resulted on day 2 in the ED, and ranged from 54% to 88%. Only BUN and ALT were relatively unchanged. The patient was discharged home with instructions to follow up with his PCP, since the ED's laboratory workup suggested that the results from the previous day were incorrect. In the months that followed, screening for low sodium levels at the same clinic revealed 17 more cases of spuriously low lab values, which corrected by inverting the tubes to mix the serum.

Initially, at the beginning of our investigation when we first noticed the spurious results, we did a review of patient medical records, which did not provide any clues to a possible inhibitor that might have interfered with test methods performed on the Vitros analyzer. Representatives from Ortho Clinical Diagnostics felt that the problem was preanalytical. Laboratory directors, supervisors, and a phlebotomist from the central core lab visited the satellite clinic to observe the workflow for several days. They found no deviation from the standard operating procedures in the processing of patient samples.

3. Materials and methods

3.1. Examination of patient samples with critically low sodium levels

We suspected that concentration gradients may have been forming sometime during storage or transportation; therefore, we examined the difference in constituent concentrations at the top and bottom of 3 serum samples, identified as having critically low sodium levels, from the same satellite clinic that the other samples with spurious results had arrived from. Carefully, a 200 μL aliquot was pipetted from the top layer of each SST tube, and 200 μL from the bottom layer, just above the gel separator, then analyzed on the Vitros 5600. The remaining serum in the tubes were then mixed by inverting at least 6 times and re-analyzed. The analytes measured included: sodium, chloride, potassium, total CO_2, BUN, creatinine, calcium, and glucose. We then divided the individual analyte concentrations from the top layer and the bottom layer by their respective concentrations after mixing to obtain the concentration ratios (CR) for each analyte (Fig. 1).

3.2. Study to examine the effects of freezing and thawing on serum

Additionally, we suspected that the concentration gradients may have formed as a result of freezing during storage or transportation; therefore, we examined the effects of one freeze-thaw cycle on constituent concentrations for six expired patient samples collected in serum separator tubes (product number: 367986 – 13 × 100 mm × 5.0 mL BD Vacutainer® Plus plastic serum tube). After baseline measurements (C0) were obtained, 3 tubes were placed upright in a −20 °C freezer, and 3 tubes in a − 70 °C freezer. The tubes were then removed and allowed to thaw at room temperature (22.1 °C). Carefully, without inversion, serum constituent concentrations were measured on a Vitros 5600 analyzer after 1 (C1), and 3 h (C3) of thawing. Immediately after the 3-h analysis, the tubes were inverted at least 6 times to thoroughly mix the serum, then re-analyzed (Cm). The analytes measured included: sodium, potassium, chloride, total CO_2, BUN, glucose, creatinine, total bilirubin, calcium, total protein, albumin, ALP, ALT, AST, cholesterol, triglycerides, and HDL. CRs were determined by dividing analyte concentrations at the various time points (C1, C2, and Cm) by the baseline concentrations (C0) (Fig. 2) [4].

3.3. Examination of sample workflow

To investigate any temperature fluctuations that may occur during storage and transportation, we placed two temperature probes (Veriteq Temperature Data Recorder 1000 VL-1000-21N) in each tube rack used for the transport of patient samples from the satellite clinic. One probe was placed in a 4.5 mL tube with water and the other probe is exposed to the ambient air, alongside the other samples collected for that day. Temperature measurements were taken every minute throughout the day (Fig. 3). The satellite clinic typically stores their specimen tubes in a 5 °C refrigerator, then places them in a Lock Box outside at about 5:00 p.m. with ice packs (ThermoSafe Polar Pack). Based on previous validation studies, it was determined that the number of ice packs used would be dependent on the outdoor temperature at the time the samples are placed in the Lock Box. If the outdoor temperature was 50 to 70 °F (10 to 21.1 °C), 4 ice packs would be used. If the temperature was > 70 °F (21.1 °C), then 9 ice packs would be used. The day we performed the study, the outdoor temperature at 5:00 p.m. was 77 °F (25 °C); therefore 9 ice packs were used. The samples were then switched to the courier's cooler at about 8:30 p.m., and transported to the main core lab, arriving at around 11:00 p.m.

4. Results

4.1. Examination of patient samples with critically low sodium levels

All of the analytes that were re-measured from the 3 samples, which had arrived from the satellite clinic, had similar CRs, except for total CO_2. Therefore, we excluded total CO_2, and then calculated the average CRs for each individual tube at the upper layer and lower layer, and generated a bar graph (Fig. 1). The 3 samples demonstrated a concentration gradient for all the analytes tested, with the upper serum layer in each tube being more dilute and becoming more concentrated as it approached the gel separator. The CR ranged from 0.62 to 0.84 at the serum's surface, and 1.11 to 1.37 near the gel.

4.2. Study to examine the effects of freezing and thawing on serum

Our freezing study also demonstrated similar CRs for most of the analytes in their respective experimental conditions; therefore, we took the average CR at each time point and freezing temperature, and generated a bar graph (Fig. 2) [4]. Total bilirubin and ALT, however, were both excluded from our calculations. Since bilirubin is normally in very low concentrations in serum, any small changes as a result of freezing