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Short Communication

Solving a myth: Does boric acid stabilize aldosterone in urine at typical clinical laboratory storage conditions?

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ABSTRACT

Aldosterone is produced by the adrenal gland and plays an important role in blood pressure regulation and electrolyte hemostasis. Clinically, measurement of urine aldosterone provides evidence for the diagnosis of hyper- and hypo-aldosteronism. Urine specimen that is collected in consecutive 24 h is preferred, which mitigates the risk of misdiagnosis due to large diurnal variation in aldosterone secretion. Preservatives such as boric acid are routinely added to the collection containers prior to urine collection. However, little is known of the effectiveness of these preservatives on stabilizing aldosterone in urine. In the current study, we examined the stability of urine aldosterone under typical clinical laboratory storage conditions with and without the supplementation of boric acid. Our result demonstrated that the addition of boric acid is unnecessary.

1. Introduction

Aldosterone (ALDO) is measured in the investigation of different types of aldosteronism together with other biomarkers such as renin when patients present with uncontrolled high blood pressure [1–3]. Specimen types for measuring ALDO can be serum, plasma, or urine. Measurement of ALDO in 24 h urine is preferred and routinely performed in clinical labs as it eliminates the diurnal variation; although a random urine sampling with correction for creatinine might provide comparable diagnosis accuracy for primary aldosteronism [4]. Preservatives such as boric acid are routinely added prior to urine collection and are often required by the assay manufacturers and/or professional guidelines [5]. Despite the requirement of preservative addition at the beginning of 24 h urine collection, this is often not happening because of low patient adherence to the proper pre-analytical sample handling [6,7].

We reviewed sample pretreatment requirements for 24 h urine ALDO listed in the test directories of 4 major reference laboratories in the United States. Beside boric acid, some other types of preservatives used are 6 M HCl, 50% acetic acid, toluene, and thymol. Type and amount of preservatives required are not always in agreement by different laboratories. Only one laboratory required “plastic container with no preservative”. In addition, boric acid is now classified as being toxic to the reproduction system by the Globally Harmonized System of Classification and Labelling of Chemicals. Thus, the use of boric acid should be minimized or prevented to reduce potential harm to the lab personnel and environment.

Our laboratory previously required addition of boric acid to urine specimens for stabilizing ALDO. However, the effectiveness of boric acid stabilizing ALDO in urine is not fully understood to the best of our knowledge. The objective of this study was to determine the stabilizing effects of boric acid on urine ALDO measurement at various storage conditions typically encountered in a clinical laboratory (ambient, 4 °C, and −20 °C).

2. Materials and methods

Fresh urine samples (within 4 h of collection) were obtained from 10 individuals using sterile containers (mean ALDO at 40.13 ng/dL, ranging from 5.76 to 138.17 ng/dL). Each urine specimen was split into three portions: one without preservative and two with boric acid added at 1 g/100 mL of urine and 0.5 g/100 mL of urine, respectively. Each portion was measured for pH using a pH paper (Micro Essential Lab, Brooklyn, New York). One aliquot of each specimen was analyzed for ALDO immediately (time 0) and another was placed in the −70 °C freezer and remained there to the end of the study to test for ALDO

Abbreviations: ALDO, aldosterone; QC, quality control; HCl, hydrochloric acid; CV, coefficient of variation; ANOVA, analysis of variance

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stability at −70 °C. The remaining samples were aliquoted and placed at three different storage conditions (ambient, 4 °C, and −20 °C) for various times: 24, 48 h and 4 days at ambient; 4 and 7 days at 4 °C; 30 and 90 days at −20 °C. Once the specified storage time was reached, the samples were removed and placed in a − 70 °C freezer before batched analysis. All of these samples were analyzed on a LIAISON XL (DiaSorin, Saluggia, Italy) analyzer in triplicate, which included an acid hydrolysis step. The assay had an analytical measuring range of 3–100 ng/dL. Quality control (QC) data at two ALDO levels were collected during the entire study period (~3 months), and the overall coefficient of variation (CV) was used for the statistical analysis of significant change of ALDO measurements. Analysis of variance (ANOVA) was used to analyze the ALDO data across all preservative conditions at each time point. Given no group differences, we then estimated the time course using a repeated-measured ANOVA with time as the factor. Equivalence test was used to determine whether the ALDO % change was within the range from −2 CV to 2 CV. All analyses were performed using R (version 3.6.1, cran.r-project.org). The use of residual patient urine samples in this study was approved by the Cleveland Clinic Institutional Review Board (#10-297).

3. Results

The pH of the 10 original urine samples was determined to be in the ranges of 4–5, 5–6, or 6–7 by pH paper. The pH of each urine specimen remained in the same range after addition of boric acid at the two concentrations tested.

The means of the QC results during the entire study were 14.36 ng/dL and 51.56 ng/dL with an overall CV of 13.05%. ALDO % change from the time 0 value was calculated for each storage conditions (24, 48 h and 4 days at ambient; 4 and 7 days at 4 °C; 30 and 90 days at −20 °C) for each patient, and the mean % change of all 10 patient specimens for each storage condition was calculated. The mean % change with no preservative, with 1 g/100 mL, and with 0.5 g/100 mL of boric acid was in the range of −8.64% to 1.50%, −12.56% to −1.39% and − 12.29% to 0.62% respectively for all storage conditions. Percent change from time 0 within 2 analytical CV was deemed stable for that specific storage condition (Fig. 1). The *p*-values were all smaller than 0.001 for the equivalence test. Overall, 95% of ALDO measurements have passed 2 CV and 100% passed 3 CV criteria for the samples across all individuals and conditions tested. All measured values of the time 0 samples stored at −70 °C passed 2 CV, indicating that ALDO is also stable at −70 °C for the entire study period. By performing ANOVA, we observed no difference among the groups with no preservative and with boric acid added at 1 g/100 mL and 0.5 g/100 mL of urine at all storage conditions; the *p*-values are 0.654, 0.286, 0.436, 0.902, 0.872, 0.969 and 0.995 ($p < .05$ is statistically significant) for 24, 48 h and 4 days at ambient; 4 and 7 days at 4 °C; 30 and 90 days at −20 °C (Fig. 1). The mean percent changes from time 0 calculated from ANOVA were −11.2%, −3.3%, −5.1%, −10.2%, −1.3%, −3.1% and − 3.5% at 24, 48 h and 4 days at ambient; 4 and 7 days at 4 °C; 30 and 90 days at −20 °C, respectively; the decrease is only significant for 24 h and 4 days at ambient, 4 days at 4 °C (*p*-values < 0.05).

4. Discussion

In the literature, there is very limited information available addressing urine ALDO stability in a clinical laboratory setting. In one report, the researchers found that omitting boric acid for urine samples kept at ambient for 3, 6, 24 and 48 h did not have negative impact on ALDO stability [8]. However, the stability at different conditions was not investigated. Besides stability, addition of certain preservatives might adversely affect assay performance per another investigation [9].

Our data demonstrated that ALDO in the original urine specimens without boric acid was stable for the entire study periods: 4 days at ambient, 7 days at 4 °C, 90 days at −20 °C and 90 days at −70 °C. Addition of preservative boric acid at the two concentration levels (1 g/100 mL of urine and 0.5 g/100 mL of urine) did not have significant impact on ALDO stability in these urine specimens.

In general, preservative is added to biological samples to prevent overgrowth of bacteria and the undesired chemical changes/modifications of urine due to ongoing microbial metabolism [10,11]. In this study, the 10 individual urine samples were collected from the outpatient urology clinic and included patients with diverse medical conditions such as kidney stones, chronic renal failures, transplant, and urinary tract infections. Therefore, these samples represent most clinically relevant scenarios for urine collection to measure ALDO.

In conclusion, we found addition of boric acid as preservative to urine for ALDO measurement is not necessary using LIAISON XL analyzer. The difference among the groups with and without boric acid added at each storage conditions examined were statistically insignificant. To ease urine specimen collection and to avoid unnecessary rejection of urine specimens, we have changed the specimen requirement in our laboratory to accept urine samples with and without boric acid. We recommend that laboratories using other instruments to measure ALDO should perform a similar study to confirm our findings.

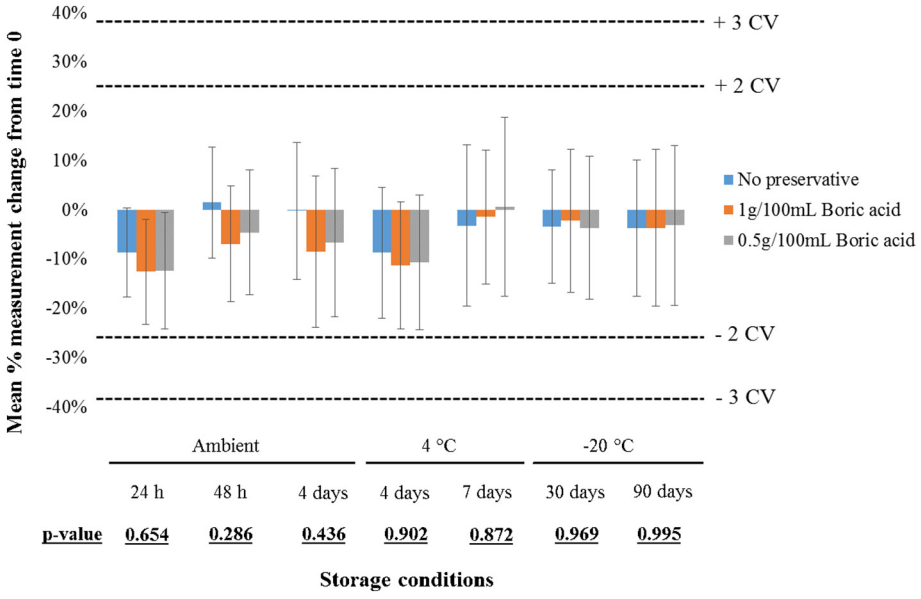

Fig. 1. Overall urine aldosterone stability across all storage conditions tested. Mean percent ALDO concentration change from time 0 (averaged from the 10 urine specimen) were plotted for the different storage conditions tested. CV was calculated from the two levels of QC data collected during the entire study period. Error bar represents the standard deviation of the % ALDO concentration change from time 0 of the 10 urine specimens. *P*-values from ANOVA were indicated on each storage conditions ($p < 0.05$ is statistically significant).

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