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Long-term urine biobanking: Storage stability of clinical chemical parameters under moderate freezing conditions without use of preservatives

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ABSTRACT

Objective: To examine the long-term stability and validity of analyte concentrations of 21 clinical biochemistry parameters in 24-h urine samples stored for 12 or 15 yr at −22 °C and preservative free.

Design and methods: Healthy children's 24-h urine samples in which the respective analytes had been measured shortly after sample collection (baseline) were reanalyzed. Second measurement was performed after 12 yr (organic acids) and 15 yr (creatinine, urea, osmolality, iodine, nitrogen, anions, cations, acid–base parameters) with the same analytical methodology. Paired comparisons and correlations between the baseline and repeated measurements were done. Recovery rates were calculated.

Results: More than half of the analytes (creatinine, urea, iodine, nitrogen, sodium, potassium, magnesium, calcium, ammonium, bicarbonate, citric & uric acid) showed measurement values after >10 yr of storage not significantly different from baseline. 15 of the 21 parameters were highly correlated ($r = 0.99$) between baseline and second measurement. Poorest correlation was $r = 0.77$ for oxalate. Recovery ranged from 73% (oxalate) to 105% (phosphate).

Conclusion: Our results suggest high long-term stability and measurement validity for numerous clinical chemistry parameters stored at −22 °C without addition of any urine preservative. Prospective storage of urine aliquots at −22 °C for periods even exceeding 10 yr, appears to be an acceptable and valid tool in epidemiological settings for later quantification of several urine analytes.

Introduction

Urinary measurement of metabolites is a common and widely used non-invasive tool for the assessment of health status in clinical [1] and epidemiological [2–5] settings. Prospective and longitudinal studies require a particular methodological attention regarding the stability of metabolites, because often not all analyses can be performed immediately after collection. The stability of analytes in urine is dependent on many factors, such as collection procedures of the samples and storage temperature [6–9]; addition of preservatives to keep the urines free of bacteria [10,11]; time between collection and analysis; number of thaw-cycles before analysis, among others. Despite the importance to know such methodological characteristics for certain key analytes in clinical and epidemiological research [2], studies that have examined the long-term stability of urinary analytes like creatinine, urea, osmolality, iodine, nitrogen, anions, cations, acid–base parameters, or organic acids are rare. Particularly the long-term influence on recovery under conditions of low temperature storage for urine samples collected and stored without preservatives is unknown.

The Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study, is an open cohort study initiated in 1985 to collect detailed data on diet, growth, development, and metabolism between infancy and adulthood in German healthy children [12]. 24-H urine collection (usually performed at intervals of one year), is one important research component of the study. To date the DONALD urine-biobank stores almost 8000 aliquots of 24-h urine samples from 3 to >18 yr old participants. Aliquots are stored at −22 °C without addition of preservatives [13]. In the present study, we aimed to examine the stability of select urinary analytes measured in the DONALD Study, and the validity of reanalyzing these parameters, after 12 or 15 yr of storage at −22 °C. This may provide valuable information for a number of current as well as future epidemiological and/or intervention studies. The examined analytes include: creatinine, urea, osmolality, iodine, nitrogen, chloride, phosphate, sulfate, sodium, potassium, calcium, magnesium,

Abbreviations: 24-h, 24-hour; yr, years; DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed [Study]; CV, coefficient of variance; SD, standard deviation; r, correlation coefficient; HCl, hydrochloric acid; $NaHCO_3$, bicarbonate.

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components of renal net acid excretion, pH, titratable acidity, ammonium, bicarbonate, and the organic acids citrate, uric acid, oxalate, and total (titrated) organic acids.

Materials and methods

Subjects

For baseline analysis, 10 urine samples (from 5 boys and 5 girls aged 4–10 yr old) collected between 1995 and 1998 were randomly selected from the DONALD urine-data bank. Repeated measurements were done after 12 yr for organic acids (total titrated organic acid, citrate and uric acid) and 15 yr for the rest of the metabolites. Mean age of the children was 6.7 yr (SD ± 1.8) at the time of collection. The DONALD Study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects, including regular 24-h urine sampling, were approved by the Ethics Committee of the University of Bonn (Germany). All assessments were carried out with parental and later on with children's written consent.

Urine collection and storage

24-H urine collections are performed using preservative-free, Extran-cleaned (Extran, MA03; Merck Darmstadt, Germany) 1 l plastic containers which are immediately stored between −12 °C and −20 °C, before transfer to the research lab. At the institute the containers are stored at −22 °C without the addition of any preservative or chemical until analyzed. Further details regarding 24-h urine collection have been presented elsewhere [13].

After thawing, combining urine contents of >1 containers, and thorough mixing, total urine volume is determined and a routine check performed using a commercial test strip (Combur 9, Roche Diagnostics GmbH, Mannheim, Germany). Dipping of the test strip into the original urine sample is strictly avoided since a considerable liberation of iodine from the strips occurs [14]. From each 24-h urine sample several aliquots of 20 mL are stored at −22 °C for further analyses and as a reserve in the DONALD urine bank. The clinical biochemical analyses at baseline were done as specified below without further treatment apart from short stirring before pipetting the sample for the particular measurement. For the repeat measurements (12 or 15 yr later), a separate aliquot was used that underwent the same procedure as at baseline. Urine aliquots used in the study went through one freeze–thaw cycle both before baseline and repeat measurement.

Analytical methods

A standardized laboratory protocol was used for each analyte at the initial (first) and repeated (second) measurements, in almost all cases even by the same lab personnel (for specific description of the respective analyte methodology, see below). The protocol includes use of the same chemicals, calibrator materials, and quality-control parameters (mostly from identical suppliers), and if possible use of the same (original) measurement instruments. In case that certain reagents or chemicals were no longer available (or were non-purchasable for a certain time) from the standard supplier – as was the case for perchloric acid (iodine analytics) or high purity acids and bases (acid–base titration) – identical chemicals with the same analytical purity were obtained from alternative producers. To ensure analytical reliability also with respect to reagents' stability, test kits (like that for uric acid or citrate) were regularly ordered only few weeks in advance of scheduled measurements or in case of long-term stable assays or reagents, substances were rigorously discarded when they reached their expiration date. Working solutions, buffers, and substrate stock solutions were stored at room temperature (in the case of certain diluting reagents or pure acid and base solutions) or were stored at 4 °C in dark bottles if specified in the respective analytical instructions (*e.g.*, for uric acid, urea, citrate analyses). Buffers for anion chromatography, however, were freshly prepared for each analysis. Reagents' stability was periodically indirectly tested by measuring single urine samples (together with the *per se* analyzed controls) two times, namely in one analytical run with longer-term stored reagents and again during the following analytical run with freshly reconstituted reagents.

Long-term assay drift (an important aspect of analytical reliability) was checked and excluded with the use of (i) self-prepared in-house control urines for sulfate, NAE-parameters, citrate, iodine, and nitrogen; (ii) an aqueous standard prepared from a volumetric solution of oxalic acid (Fluka/Sigma-Aldrich, Steinheim, Germany) for oxalate; and (iii) a quantitative urine control (Lyphochek, Bio-Rad, Marnes-la-Coquette, France) for creatinine, urea, osmolality, chloride, phosphate, sodium, potassium, calcium, magnesium, and uric acid. For all actually measured latter analytes, the minus and plus deviations from the mean values, as provided by the urine-control producer, were consistently below 10% (mostly below 5%) over the whole observation period. The corresponding oxalate deviations (from the specific lab-prepared oxalic acid standard) lay below 15%. Sulfate, titratable acid, ammonium, citrate, iodine, and nitrogen deviated by not more than 5–10% from the mean values as determined initially for the self-prepared in-house control urines (in use for few years each).

The same analytical equipment (photometer, anion chromatograph, flame atomic absorption spectrometer, and Kjeldahl apparatus, see below) could be used over the 15-yr period and even beyond. This has been probably due (i) to stringent and systematic maintenance, repair and overhaul not only by technical company service, but especially by our highly mechanically and electronically specialized in-house technician, (ii) to the fact that average sample throughput in the DONALD Study lab is rather moderate (usually not more than 50 twenty-four-hour urine samples per month and a similar number of spot samples), and (iii) to our experienced and thorough lab technicians who have continuously done the analyses for more than 20 yr. Two machines (osmometer and titrator, see below) had to be replaced by newer models with an identical function principle.

Measurements of the 21 analytes under study were performed by the following methodology: creatinine (mmol/L) was measured by the kinetic Jaffe procedure [15] on a creatinine analyzer (Beckmann-2; Beckman Instruments Inc., Fullerton, CA). Photometric analysis (photometer PM2DL Zeiss, Oberkochen, Germany) was used to measure urea (mmol/L) with the urease–Berthelot method (Randox Laboratories Ltd, Crumlin, United Kingdom), uric acid (mmol/L) by the uricase method with the Uric Acid plus kit (Roche Diagnostics GmbH, Mannheim; Germany), and citrate (mmol/L) by enzymatic conversion of citrate *via* oxaloacetate to L-lactate by a citrate kit from Boehringer Mannheim according to the principle described by Moellering and Gruber [16]. Osmolality (mosm/kg) was calculated from the freezing point depression measured by an osmometer (OM 802-D Vogel, Giessen, Germany). The anions, chloride (mmol/L), phosphate (mmol/L), sulfate (mmol/L), and oxalate (μmol/L) were quantified by Dionex 2000i/SP ion chromatography with an ion Pac AS4A column (Dionex GmbH, Idstein, Germany); the cations, sodium (mmol/L), potassium (mmol/L), magnesium (mmol/L), and calcium (mmol/L) by flame atomic absorption spectrometry on a Perkin-Elmer 1100 Spectrometer (Perkin-Elmer GmbH, Ueberlingen Germany). Acid–base analytes, *i.e.* 24-h pH, titratable acidity (mEq/L), ammonium (mmol/L), and bicarbonate (mmol/L) were quantified by the three phase acid–base titration method [17] using a Mettler Toledo endpoint titrator (Mettler Toledo, Giessen, Germany). Renal net acid excretion (mEq/L) was then calculated as the sum of titratable acid plus ammonium minus bicarbonate [18]. Titration of total organic acids (mmol/L) was done according to van Slyke and Palmer [19]. Total nitrogen (mmol/L) was measured by the Kjeldahl technique (Buechi 430 Digestor and Buechi Distillation unit B-324), and iodine concentration (μg/dL) by a modified Sandell–Kolthoff method after acid wet-ashing of the samples [20].