DRIED URINE MULTI-ELEMENT ANALYSIS TO AID IN THE PREVENTION AND TREATMENT OF IODINE DEFICIENCY DISORDERS: MEASUREMENT OF IODINE, BROMINE, SELENIUM AND ARSENIC BY ICP-DRC-MS, WITH AND WITHOUT CREATININE CORRECTION

Theodore T Zava, PhD
ZRT Laboratory, Beaverton, Oregon, USA

Introduction

Iodine deficiency is a worldwide health problem, affecting an estimated 1.88 billion people in both underdeveloped and developed countries.1 Typically urine iodine is measured alone to assess the risk of developing IDDs (iodine deficiency disorders); however, often overlooked elements such as selenium, arsenic and bromine play a large part in the progression of IDDs. We developed a method using inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) to analyze iodine, bromine, selenium and arsenic in urine dried on filter paper. Creatinine normalization was used to adjust for an individual’s hydration status and references ranges for each element were established for our laboratory to help identify both populations and individuals with irregular elemental exposure. While large scale elemental analysis results reveal important information about the health of populations or communities, subgroups and individuals may be at risk of deficiencies or excesses not recognized when assessing the population as a whole. ICP-DRC-MS analysis of iodine, bromine, selenium and arsenic from urine dried on filter paper allows for faster analysis time, sample stability, automation and ease of storage with minimal waste. This methodology is therefore ideal for assessment of individuals, subgroups and populations, and aiding in the ultimate prevention of IDDs.

Methods and Materials

Multi-Element and Creatinine Standards
- Multi-element standard consists of 1000 µg/L iodine, 10000 µg/L bromine, 1000 µg/L selenium, and 1000 µg arsenic in 0.1% ammonium hydroxide.
- Creatinine standard consists of 4 mg/mL creatinine in 0.1% ammonium hydroxide.

External Controls
- External trace elements were obtained from NIST, Serionorm, and CinnChek. Creatinine controls were obtained from BioRad and Audit.

Sample Preparation
- Liquid standards, controls, and 0.1% ammonium hydroxide blank solution were dried on Ahlstrom/Perkin Elmer grade 226 filter paper (6.5xcm).
- 682 deidentified patient urine samples pre-dried on filter paper with no exclusion criteria (male and female, average age 51 years and up), representative of our commercial laboratory’s sampling population, were selected for analysis. Six 6.0 mm diameter disks of dried standards, controls, blanks and patient urine samples were punched (Wallac DBS Puncher; Perkin Elmer) into a 96-well filter block (1 mL wells, 20 µm frit; Nunc) and extracted with 300 µL of 0.1% ammonium hydroxide containing germanium as an internal standard.
- The fritted block was then centrifuged at 3000 rpm into a deep 96-well plate (2.1 mL wells, VWR) to obtain the extract used for analysis.

Multi-Element Assay
- Extracted multi-element standard was serially diluted with extracted blank solution to create the standard curve.
- Multi-element analysis was performed on a Perkin Elmer NeoXN 3000 inductively coupled plasma mass spectrometer (ICP-MS) with Dynamic Reaction Cell (DRC) technology. (Figure 1) (Table 1)
- Iodine, bromine, selenium, arsenic and germanium (used as an internal standard) were measured at mass 127, 79, 78, 75 and 72 respectively.

Creatinine Assay
- Extracted creatinine standard was serially diluted with extracted blank solution to create the standard curve.
- Creatinine analysis was completed using a modified version of Jaffe’s reaction in a 96-well microtiter plate.

Results

External controls, intra- and inter-assay percent variations, spike recoveries, detection limits and limits of linearity were used to check the validity of the iodine, bromine, selenium and arsenic assay and creatinine assay and were all acceptable (data not shown).

Iodine, bromine, selenium and arsenic results in µg/L were divided by their respective creatinine results in mg/mL to correct for patient hydration status. Reference ranges were established for each element, with and without creatinine correction. Due to the non-gaussian distribution of patient sample results, quantities and subgroups were used to establish reference ranges. The 95% and 5% quantiles were used as the upper and lower confidence limits of our reference range, while 20%, 50%, and 80% quantiles were established for informational purposes. (Table 2) Remarkably elevated iodine results are most likely a result of high-dose iodine suppletion, which can drastically affect the population average and upper quantiles. Ideally these data points should be removed if the target population does not consume high iodine containing supplements.

Discussion and Conclusion

The Problem
- Nearly two billion individuals around the world are at risk of IDDs (iodine deficiency disorders).1
- Of the 123 countries identified as at risk for IDDs in 2011, 11 are in Europe.3
- Individuals and subgroups (e.g. vegans, athletes, children, pregnant women, isolated communities, well water users) have a higher risk for developing IDDs are commonly grouped together into a larger population that may show iodine sufficiency, indicating no need for prophylaxis.
- Intake of iodine, bromine, selenium and arsenic differs from person to person, with diet and environment contributing to exposure. Selenium, arsenic and bromine can affect the progression or correction of IDDs, but are not commonly tested.4
- Bromine can bind to thyroglobulin on thyroglobulin in place of iodine during periods of iodine deficiency, interfering with thyroid hormone production.10 12

Our Solution
- We developed a simple and convenient method to collect, ship, store, and measure elements in urine dried on filter paper.
- Identify individuals and subgroups at risk for development of IDDs and thyroid disorders by establishing reference ranges for iodine, bromine, selenium and arsenic based on the sampled population.
- Median population values with and without creatinine corrections are very close, indicating that creatinine correction, while it is important for individual analysis, is not necessary when testing large populations.13-15

Conclusion
- Dried urine sampling and ICP-DRC-MS technology can be used to establish reference ranges for the synergistic elements iodine and selenium and their antagonists bromine and arsenic to help identify individuals, subgroups and populations at risk of developing IDDs.

Contact

Theodore Zava
ZRT Laboratory

Email: ttzava@zrtlab.com
Website: www.zrtlab.com
Phone: 503-466-2445

References