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

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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 commercial laboratory to help identify both populations and individuals with irregular elemental exposure. While large scale elemental analysis reveals important information about a population, 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 fast analysis time, sample stability, automation, ease and convenience of collection, and a small storage footprint. 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 10000 µg/L iodine, 100000 µg/L bromine, 1000 µg/L selenium, and 1000 µg/L arsenic in 0.1% ammonium hydroxide.
- Creatinine standard consists of 4 mg/mL creatinine in 0.1% ammonium hydroxide.

External Controls

- External trace element controls were obtained from NIST, SeroNorm, and ClinChek. 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 (6.5x5 cm).
- 682 deidentified patient urine samples pre-dried on filter paper with no exclusion criteria (male and female, average age 51 years old), 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 with 20 µm frit; Nunc) and extracted with 1000 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 (2.2 mL wells; VWR) to obtain the extract used for analysis.

Multi-Elemental 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 NexION 300D 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.2

Figure 1. NexION 300D ICP-DRC-MS

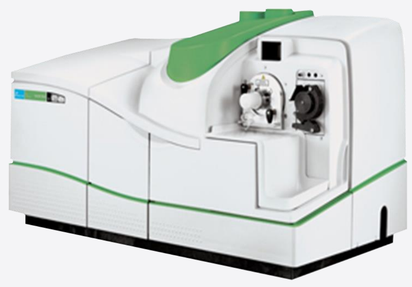

Table 1. ICP-DRC-MS Conditions.

Component/ Parameter	Type/Value/Mode
Nebulizer	ESI MicroFlow PolyPro ST
Spray Chamber	Perkin Elmer Glass Cyclonic
Peristaltic Pump	ESI MP-2
AutoSampler	ESI SC-2
Cones	Nickel
Plasma Gas Flow	18 L/min
Auxiliary Gas Flow	1.2 L/min
Nebulizer Gas Flow	1.04 L/min
RF Power	1600 W
KED Cell Gas	Helium (3.5 mL/min)
Sample Flow Rate	37.6 µL/min
Replicates per Sample	5
Sample Flush	60 sec
Read Delay	15 sec
Wash	45 sec
Dwell Time	50 ms

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, quantiles 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% (median), and 80% quantiles were established for informational purposes. [Table 2] Remarkably elevated iodine results are most likely a result of high dose iodine supplementation, 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.

Table 2. Population Reference Ranges With and Without Creatinine Correction

	Iodine (µg/L)	Iodine per Creatinine (µg/g)	Bromine (µg/L)	Bromine per Creatinine (µg/g)	Selenium (µg/L)	Selenium per Creatinine (µg/g)	Arsenic (µg/L)	Arsenic per Creatinine (µg/g)	Creatinine (mg/mL)
N:	682	682	682	682	682	682	682	682	682
Average	962.6	1222.4	2384.1	3427.3	68.9	73.2	40.2	41.4	1.02
5%:	48.5	64.2	643.4	893.5	17.4	26.9	2.7	3.3	0.33
20%:	92.7	105.0	1180.9	1403.9	31.0	40.3	6.1	6.6	0.58
Median:	186.3	200.8	2059.5	2137.2	55.4	57.6	13.3	15.2	0.95
80%:	473.9	469.6	3320.0	3427.8	98.1	90.1	39.6	42.7	1.39
95%:	6069.8	6361.8	5058.5	5696.3	167.9	174.5	152.5	138.9	1.98

Discussion and Conclusion

The Problem

- Nearly two billion individuals around the world are at risk of IDDs (iodine deficiency disorders).1
- Of the 32 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) with 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.4-6
- Arsenic and other heavy metals such as mercury bind tightly to selenium, reducing the amount of selenium available for incorporation into selenium-containing antioxidants (e.g. glutathione peroxidase and thioredoxin reductase) and deiodinases necessary for the activation/deactivation of thyroid hormones.7-11
- Bromine can bind to tyrosine residues 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.

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References

1) Andersson M, Karumbunathan V, Zimmermann MB. Global iodine status in 2011 and trends over the past decade. J Nutr. 2012 Apr;142(4):744-50.
2) Jaffé M. Ueber den Niederschlag welchen Pikrinsaure in normalen Harn erzeugt und uber eine neue Reaction des Kreatinins. Z Physiol Chem 1886;10:391-400.
3) Zimmermann MB, Andersson M. Update on iodine status worldwide. Curr Opin Endocrinol Diabetes Obes. 2012 Oct;19(5):382-7.
4) Arthur JR, Beckett GJ, Mitchell JH. The interactions between selenium and iodine deficiencies in man and animals. Nutr Res Rev. 1999 Jun;12(1):55-73.
5) Liu Y, Huang H, Zeng J, Sun C. Thyroid volume, goiter prevalence, and selenium levels in an iodine-sufficient area: a cross-sectional study. BMC Public Health. 2013 Dec 10;13:1153.
6) Zimmermann MB, Köhrle J. The impact of iron and selenium deficiencies on iodine and thyroid metabolism: biochemistry and relevance to public health. Thyroid. 2002 Oct;12(10):867-78.
7) Raie RM. Regional variation in As, Cu, Hg, and Se and interaction between them. Ecotoxicol Environ Saf. 1996 Dec;35(3):248-52.
8) Wu MM, Chiou HY, Wang TW, Hsueh YM, Wang IH, Chen CJ, Lee TC. Association of blood arsenic levels with increased reactive oxidants and decreased antioxidant capacity in a human population of northeastern Taiwan. Environ Health Perspect. 2001 Oct;109(10):1011-7.
9) Chouchane S, Snow ET. In vitro effect of arsenical compounds on glutathione-related enzymes. Chem Res Toxicol. 2001 May;14(5):517-22.
10) Kotyzová D, Eybl V, Mihaljevic M, Glattre E. Effect of long-term administration of arsenic (III) and bromine with and without selenium and iodine supplementation on the element level in the thyroid of rat. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2005 Dec;149(2):329-33.
11) Yang DY, Chen YW, Gunn J, Belzile N. Selenium and mercury in organisms: Interactions and mechanisms. Environ Rev. 2008;16:71-92.
12) Pavelka S, Vobecky M, Babicky A. Halogen speciation in the rat thyroid: Simultaneous determination of bromine and iodine by short-term INAA. J Radioanal Nucl Chem. 2008;278:575-9.
13) Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect. 2005 Feb;113(2):192-200.
14) Vejbjerg P, Knudsen N, Perrild H, Laurberg P, Andersen S, Rasmussen LB, Ovesen L, Jørgensen T. Estimation of iodine intake from various urinary iodine measurements in population studies. Thyroid. 2009 Nov;19(11):1281-6.
15) WHO/UNICEF/ICCIDD. Assessment of Assessment of iodine deficiency disorders and monitoring their elimination A guide for programme managers. 3rd ed. Geneva, Switzerland: World Health Organization; 2007.