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# Method validation for (ultra)-trace element concentrations in urine for small sample volumes in large epidemiological studies: application to the population-based epidemiological multi-ethnic study of atherosclerosis (MESA)<sup>†</sup>

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Analysis of essential and non-essential trace elements in urine has emerged as a valuable tool for assessing occupational and environmental exposures, diagnosing nutritional status and guiding public health and health care intervention. Our study focused on the analysis of trace elements in urine samples from the Multi-Ethnic Study of Atherosclerosis (MESA), a precious resource for health research with limited sample volumes. Here we provide a comprehensive and sensitive method for the analysis of 18 elements using only 100  $\mu\text{L}$  of urine. Method sensitivity, accuracy, and precision were assessed. The analysis by inductively coupled plasma mass spectrometry (ICP-MS) included the measurement of antimony (Sb), arsenic (As), barium (Ba), cadmium (Cd), cesium (Cs), cobalt (Co), copper (Cu), gadolinium (Gd), lead (Pb), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), strontium (Sr), thallium (Tl), tungsten (W), uranium (U), and zinc (Zn). Further, we reported urinary trace element concentrations by covariates including gender, ethnicity/race, smoking and location. The results showed good accuracy and sensitivity of the ICP-MS method with the limit of detections rangings between 0.001  $\mu\text{g L}^{-1}$  for U to 6.2  $\mu\text{g L}^{-1}$  for Zn. Intra-day precision for MESA urine analysis varied between 1.4% for Mo and 26% for Mn (average 6.4% for all elements). The average inter-day precision for most elements was <8.5% except for Gd (20%), U (16%) and Mn (19%) due to very low urinary concentrations. Urinary mean concentrations of non-essential elements followed the order of  $\text{Sr} > \text{As} > \text{Cs} > \text{Ni} > \text{Ba} > \text{Pb} > \text{Cd} > \text{Gd} > \text{Tl} > \text{W} > \text{U}$ . The order of urinary mean concentrations for essential trace elements was  $\text{Zn} > \text{Se} > \text{Mo} > \text{Cu} > \text{Co} > \text{Mn}$ . Non-adjusted mean concentration of non-essential trace elements in urine from MESA participants follow the order  $\text{Sr} > \text{As} > \text{Cs} > \text{Ni} > \text{Ba} > \text{Pb} > \text{Cd} > \text{Gd} > \text{Tl} > \text{W} > \text{U}$ . The unadjusted urinary mean concentrations of essential trace elements decrease from  $\text{Zn} > \text{Se} > \text{Mo} > \text{Cu} > \text{Co} > \text{Mn}$ .

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## 1 Introduction

Trace elements play essential roles in numerous physiological processes within the human body. Levels of trace elements in urine provide useful information about human metal exposure, nutritional status and related health conditions. Spot urine is

commonly used because it is a fast, simple and non-invasive collection method and because urine provides a wide array of biomarkers. Thus, the analysis of trace elements in urine has become an important method to quantify environmental contributors of chronic diseases.

In the United States, exposure to elevated levels of non-essential elements is prevalent due to contamination of food and drinking water from natural and/or anthropogenic sources or air pollution. Despite documented interactions between non-essential and essential trace elements enhancing or lowering each other's toxicity, few human population studies have determined exposure to "metal mixtures". Previous studies have mainly focused on urinary levels of individual trace elements which are of concern in specific cohorts, such as arsenic in Bangladesh and the USA.<sup>1–4</sup> More recent studies have examined

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trace element mixtures in urine from longitudinal cohorts in the USA, Spain, Bangladesh and China and have reported positive associations between specific trace elements and health conditions such as cardiovascular disease and diabetes.<sup>5–9</sup> Since 1999, sixteen specific urinary trace elements [antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), cesium (Cs), cobalt (Co), lead (Pb), manganese (Mn), molybdenum (Mo), platinum (Pt), strontium (Sr), thallium (Tl), tin (Sn), tungsten (W), and uranium (U)] have been surveyed across a large number of participants in the National Health and Nutrition Examination Survey (NHANES), which is a series of cross-sectional studies in the United States.<sup>10</sup> The NHANES urinary trace element panel has been selected based on toxicological and nutritional interest. However, the essential elements copper (Cu), zinc (Zn) and selenium (Se) are currently not measured in urine by NHANES, despite their potential protective, synergistic or antagonistic interactions with non-essential elements.<sup>11</sup> Cu, Zn and Se are tightly regulated in the body and being deficient or having too high concentrations of these essential elements can compromise immune, organ and metabolic functions. The U-shaped relationship of the deficiency and toxicity of Se can have synergistic or antagonistic health effects when humans are exposed to other toxic elements (e.g., As). Se can help mitigate the effects of As toxicity at low levels, but high levels can enhance As toxicity.<sup>12</sup> Thus, urinary Se can be used to assess nutritional and exposure status. For example, a study has shown that excess of Se is associated with higher risk of stroke.<sup>13</sup> Zn is an essential element that determines the catalytic, structural, and regulatory role of many proteins. Higher urinary Zn levels seems to be associated with Type 2 Diabetes Mellitus (T2DM) incidence and prediabetes prevalence.<sup>14</sup> Cu is tightly regulated because both too much and too little is associated with oxidative cell damage, compromised immune function and organ dysfunction.<sup>15,16</sup> Larger longitudinal studies, in general populations, are needed to evaluate the association of trace element mixtures with various diseases, particularly for chronic low exposure levels.

Urine samples collected for epidemiologic studies are precious resources for biomedical research, therefore, the sample volumes available for proposed laboratory analyses are often kept to a minimum. For our study we received urine volumes of <1 mL. Previous methods, however, often required >1 mL of urine for trace element analyses.<sup>8,17–20</sup> The Multi-Ethnic Study of Atherosclerosis (MESA) is a multiethnic cohort with low to moderate environmental exposure levels focusing on identifying risk factors for clinical and sub-clinical cardiovascular disease and with extensive genetic and phenotypic data and follow-up for over 20 years.<sup>21</sup>

The simultaneous measurement of trace elements in biological samples using a single method is highly challenging, as concentrations can vary over several orders of magnitude. More sensitive analytical methods, due to advances in technology, improve efficiency and allow the analysis for a broad concentration range (sub-ng L<sup>-1</sup> to µg L<sup>-1</sup> urine). Inductively coupled plasma mass spectrometry (ICP-MS) is the most commonly used instrumentation for trace element quantification in biological matrices. Advantages of using ICP-MS for biospecimen analyses

are its high sensitivity, wide linear range, broad elemental coverage, simultaneous multi-element capability, high sample throughput, and relatively simple sample preparation. Furthermore, collision and reaction cell technologies improve selectivity and helps to accurately measure elements with interferences.

To identify reliable and sensitive trace element biomarkers in urine, we need to optimize and validate the analytical and quality assurance methods for small urine volumes. We measured 18 elements including Sb, As, Ba, Cd, Cs, Co, Cu, Gd, Pb, Mn, Mo, nickel (Ni), Se, Sr, Tl, W, U, and Zn. We report detailed information on the quality control and quality assurance procedures. We compare the results of our updated analytical ICP-MS method with the previous ICP-MS methods and a method previously used at Columbia University. We analyzed 1200 urines including a subset of Exam 1 (pilot study, ~800 urines) and Exam 5 (~400 urines) using the “old” method and ~5800 urine samples from MESA Exam 1 and 543 urines from Exam 5 were analyzed with the advanced method.

## 2 Methods

### 2.1 Cohort, sample collection and storage

The MESA cohort comprises 6814 ethnically diverse men and women between 45 and 84 years of age at baseline and free from clinical cardiovascular disease.<sup>21</sup> The study was approved by the institutional review boards (IRB-AAAC945) at each site and all participants gave written informed consent. Between July 2000 and July 2002, participants were recruited from six urban areas in the United States including Baltimore MD, Chicago IL, Los Angeles CA, New York NY, St. Paul MN, and Winston Salem NC. Approximately 38% of the enrolled participants are White, 28% Black, 22% Hispanic/Latino, and 12% Asian-American, predominantly of Chinese descent. Six exams were conducted from 2000 through the end of 2018. For this study, we measured trace elements in the urine of 6618 participants from baseline (Exam 1 (2000–2002)) and 943 participants from Exam 5 (2010–2011).

Spot urine samples were collected during mid to late morning at Exam 1 and Exam 5 using urine cups, and then aliquoted into small vials. Urine samples were stored at –80 °C at the University of Vermont MESA Central Laboratory. Aliquots of 0.8 mL of urine from the participants were shipped on dry ice to Columbia University in 2019 where the samples were stored at –20 °C until trace element analysis.

### 2.2 Certified reference materials (CRM)

Various certified reference materials (CRM), covering a wide range of trace element levels, were selected to obtain accurate element concentrations on a daily and long-term basis. We used QM-U-Q1822, 1823 and 1824 obtained from the Quebec Multi-element External Quality Assessment Scheme (QMEQAS, Quebec, Canada), SRM 2668 Level 1 and Level 2 from the National Institute of Standards & Technology (NIST, Gaithersburg, Maryland, USA) and lyophilized ClinChek Level 1 (Recipe, Munich, Germany). Due to the lack of CRMs for gadolinium

(Gd), in-house pooled urine was spiked with concentrations of 0.2, 0.5 and 1.0  $\mu\text{g L}^{-1}$  of Gd.

### 2.3 Reagents and calibration standards

All solutions were prepared using ultra-pure reagents. Ultra-pure water ( $18.2 \text{ M}\Omega \times \text{cm}$ , Hydro Picosystem) was used for reagents and standard solutions. Optima grade (Fisher Scientific) ultra-trace 67–70% nitric acid ( $\text{HNO}_3$ ), 1000  $\mu\text{g mL}^{-1}$  gold standard (in 10% HCl) and Triton X-100 (BP151-100, Fisher Scientific) was used for diluent preparation (aqueous 2% vol.  $\text{HNO}_3$  and 0.02%, v/v Triton X-100 solution + 500  $\mu\text{g L}^{-1}$  gold). A custom-made multi-element stock solution containing all elements (except W) in a dilute nitric and trace hydrofluoric acid matrix was purchased from Agilent for calibration standard preparation. For W, 1000  $\mu\text{g L}^{-1}$  stock was used for preparing the daily working calibrations. As an internal standard solution, we purchased another solution (5000 $\times$ ) from Agilent which contained  $50.1 \pm 0.3 \mu\text{g mL}^{-1}$  of each gallium (Ga), iridium (Ir) and rhodium (Rh) in 5%  $\text{HNO}_3$  with trace HCl in water. Both the calibration stock and internal standard stock solutions were prepared gravimetrically by Agilent in accordance with ISO 17034 and under the Agilent ISO 9001 registered quality system. The neat materials used for the calibration stock and internal standard stock were verified by an Agilent ISO 17025 laboratory and under the Agilent ISO 17034 accreditation.

Five-point and nine-point calibrations were tested using matrix matched standards (aqueous 2% vol.  $\text{HNO}_3$  and 0.02%, v/v Triton X-100 solution + 500  $\mu\text{g L}^{-1}$  gold). The concentration ranges for both five-point and nine-point calibration are given in Table S1.† The main goal of the different calibrations was to examine linearity and sensitivity at the very low end of the concentration distribution. Furthermore, since this was the first comprehensive study of urine samples from a population with low chronic trace element exposure and several of the trace elements (e.g., Gd, W and U) had not been previously measured in other cohort studies, the concentrations of these urinary trace elements were uncertain. After we analyzed  $\sim 1000$  samples using a five-point calibration (Table S1†), we decided to add additional calibration standards at low element concentrations (nine-point calibration) to attain more accurate quantitative concentrations at low-levels and near the limit of detection. Although it is technically true that ICP-MS provides calibration linearity over 10–11 orders of magnitude, this does not mean that calibrating over wide ranges will produce accurate results for the relevant concentration ranges. As accuracy at low trace element concentrations was the most important criteria in our study, the calibration curve was constructed so that sample concentrations fell within the calibrated range. The calibration solution for the advanced nine-point calibration was prepared daily by diluting 100  $\mu\text{L}$  of custom-made multi-element Agilent stock solution to 10 mL (2%  $\text{HNO}_3$  and 1% HCl matrix). Additionally, 100  $\mu\text{L}$  of a 1000  $\mu\text{g L}^{-1}$  W stock solution (in water) was added. We compared the calibration fits for different regression scenarios. The

simple calibration range includes a five-point calibration run and the advanced calibration includes a nine-point calibration run. We determined whether the nine-point calibration fit was dependent on the standard added at lower ends of the concentration range. We examined the variability between regression lines and the impact on slopes and intercepts when using different standard concentration values to fit the regression. The main goal was to examine linearity and sensitivity at the very low end of the concentration distribution.

For elemental analysis, samples were prepared in 15 mL metal-free centrifuge tubes (Labcon, Petaluma, CA, USA), pre-tested for contamination for all 18 elements. We mixed 0.1 mL of urine with the multi-element internal standard solution (gallium (Ga) iridium (Ir) and rhodium (Rh) each at 5  $\mu\text{g L}^{-1}$  final concentration). The resulting mixture was then diluted to 5 mL with diluent (aqueous 2% vol.  $\text{HNO}_3$  and 0.02%, v/v Triton X-100 solution + 500  $\mu\text{g L}^{-1}$  gold). Human urine contains a large proportion of total dissolved solids (TDS) and salt (2.5–37%). Usually, TDS content of less than  $<0.2\%$  ( $2 \text{ g L}^{-1}$ ) is recommended for ICP-MS analysis.<sup>22</sup> Thus, a 50-fold dilution is required to reduce the effects of polyatomic interferences, matrix-induced signal suppression and carbon-enhanced ionization effects in the argon plasma.

Method blanks were prepared in the same way as urine samples but substituted the volume of urine with diluent. Method blanks were analyzed before and after each set of ten MESA urines and were used for the calculation of detection limits and to check for cross-contamination between samples.

### 2.4 ICP-MS analysis

Urinary trace element concentrations for CRMs and cohort samples were measured using ICP-MS with dynamic reaction cell (DRC). The PerkinElmer NexION 350S (Waltham, MA, USA) ICP-MS was equipped with an Elemental Scientific (ESI) 4DX autosampler (Omaha, NE, USA). The ICP-MS was fitted with a platinum sampler and skimmer cones, a PFA-ST nebulizer, and a cyclonic quartz spray chamber. To increase sample throughput and fast residual sample washout, the ICP-MS sample introduction was controlled by a FAST ESI injection system with switching valve and 2 mL injection loop. Oxygen ( $\geq 99.999\%$ ) and ammonia ( $\geq 99.99\%$ ) were used as dynamic reaction cell gas in order to reduce polyatomic interferences on the analyte masses ( $m/z$ ). The instrumental operating parameters are listed in Table 1. Optimization of instrumental operation conditions were performed daily using the NexION tuning solution by reaching a minimum sensitivity (counts per second = cps) for Be ( $m/z = 9$ )  $>3000$  counts per seconds (cps), indium ( $m/z = 115$ )  $>40\,000$  cps, U ( $m/z = 238$ )  $>50\,000$  cps and oxide ratio (reported as cerium (Ce) ratios) of  $<2.5\%$  ( $^{140}\text{Ce}^{16}\text{O}^+/^{140}\text{Ce}^+$ ) and doubly charged ratio of  $<3\%$  ( $^{140}\text{Ce}^{2+}/^{140}\text{Ce}^+$ ).

Cobalt ( $m/z = 59$ ), Ni ( $m/z = 60$ ), Cu ( $m/z = 65$ ), Zn ( $m/z = 66$ ), Sr ( $m/z = 88$ ), Mo ( $m/z = 98$ ), Cs ( $m/z = 133$ ), Sb ( $m/z = 121$ ), Ba ( $m/z = 138$ ), Gd (as average of  $m/z = 155, 156, 157$ , and

**Table 1** Instrumental parameters for the ICP-MS (NexION 350S) analysis

Instrument parameter	Settings
RF power	1600 W
Plasma gas flow (Ar)	18 L min <sup>-1</sup>
Auxiliary gas flow (Ar)	1.2 L min <sup>-1</sup>
Nebulizer gas flow (Ar)	0.95–1.1 L min <sup>-1</sup>
Ammonia reaction cell flow	Mn: 0.8 L min <sup>-1</sup>
Oxygen reaction cell flow	Cd: 1.2 mL min <sup>-1</sup> ; Se, As: 0.7 mL min <sup>-1</sup>
Scan mode	Peak hopping
Sweeps/reading	20
Readings/replicate	1
Replicates	4
Quadrupole ion deflector (QID)	On; [STD/DRC] QID and [DRC] QID
Detector mode	Dual
Sweeps per reading	20
Replicates	4
Dwell time	50 ms
Calibration regression type	Linear through zero or simple linear
Sample flush	60 s
Read delay	40 s
Rinse time	90 s

**Table 2** ICP-MS (NexION 350S) element operation conditions

Element	<i>m/z</i>	Instrument mode	Internal standard
Co	59	No gas	Ga [69]
Ni	60	No gas	Ga [69]
Zn	66	No gas	Ga [69]
Cu	65	No gas	Ga [69]
Sr	88	No gas	Rh [103]
Mo	98	No gas	Rh [103]
Cs	133	No gas	Rh [103]
Ba	138	No gas	Rh [103]
Gd	155, 156, 157, 158	No gas	Ir [193]
W	184	No gas	Ir [193]
Tl	205	No gas	Ir [193]
Pb	208	No gas	Ir [193]
U	238	No gas	Ir [193]
Mn	55	NH <sub>3</sub> mode	Ga [69]
Se	78	O <sub>2</sub> mode	Rh [103]
As	75 → 91	O <sub>2</sub> , mass shift	Rh [103]
Cd	111, 113	O <sub>2</sub> mode	Rh [103]

158), W (*m/z* = 184), Tl (*m/z* = 205), Pb (as sum of *m/z* = 206 + 207 + 208), U (*m/z* = 238), and the internal standards Ga (*m/z* = 69), Rh (*m/z* = 103) and Ir (*m/z* = 193) were measured without any reaction gas. Mn (*m/z* = 55) and internal standard Ga (*m/z* = 69) were measured in ammonia gas mode. Arsenic (*m/z* = 75 → 91 as oxygen-adduct), Se (*m/z* = 78), and Cd (average of *m/z* = 111 and 113) and the internal standard Rh (*m/z* = 103) were measured in the oxygen gas mode (summarized in Table 2). The internal standards were selected based on the conventional approach of matching the internal standard closest to

atomic mass of the analyte. Blanks bracketed the beginning and end of each set of 10 urine samples and at least one CRM was analyzed after each run sequence. Calibration standard (mainly Level 4 standard) was analyzed after each run sequence to correct for instrumental drift. A matrix-matched carrier solution (aqueous 2% vol. HNO<sub>3</sub> and 0.02%, v/v Triton X-100 + 500 µg L<sup>-1</sup> gold) was used to push the sample from the loop to the nebulizer during sample measurement.

The batch run of each analytical day included a calibration blank, calibration standards, front CRMs, blanks and CRMs as QCs. Every day, all six CRMs (QM-U-Q1822, QM-U-Q1823, QM-U-Q1824, NIST 2668 L1 and L2 and Clinchek L1) were analyzed after the calibration standards and prior to MESA urine samples to ensure method integrity. A standard analytical sequence routinely included a blank, 9 MESA urine samples, a replicate of one of the urine samples, and a second blank. After each sequence of 2 blanks and 9 samples, one CRM, alternating between QM-U-Q1822, QM-U-Q1823, QM-U-Q1824, and one intermediate standard were analyzed.

## 2.5 Normalization of urine metal concentration by hydration levels

Urine specific gravity (SG) was measured using a digital hand-held refractometer with automatic temperature compensation (ATAGO 4410 PAL-10S) and a measurement resolution of 0.001. The refractometer was calibrated to 1.000 with deionized water and checked periodically between sample measurement. 200 µL of urine was placed on the prism top of the refractometer and the SG reading was recorded with an accuracy of ±0.001. Urinary element concentrations (*C*<sub>norm</sub>) were normalized for hydration status using the Levin–Fahy equation:<sup>23</sup>

$$C_{\text{norm}} = C_{\text{measured}}(\text{SG}_{\text{median}} - 1)/(\text{SG}_{\text{measured}} - 1)$$

where *C*<sub>measured</sub> is the measured urine element concentration and *SG*<sub>measured</sub> is the urine specific gravity. *SG*<sub>median</sub> describes the median value of the MESA cohort. Urine creatinine was measured by the kinetic Jaffé method and uncorrected urine element concentrations were divided by urine creatinine (expressed in µg g<sup>-1</sup> creatinine).

## 2.6 Data acquisition, method validation and statistical analysis

Data acquisition for the trace element concentrations was performed using the Syngistix software package v2.5. provided for PerkinElmer NexION 350S. Urinary element concentrations were adjusted for internal standards by dividing the raw analyte cps by the internal standard cps and this net signal plotted against the calibration concentration for each element for external calibration. The cps of the calibration blank was subtracted from the intensity of measured method blank, CRM and sample urine for each element. The mean background concentration of all method blanks of an analytical day was subtracted from each analyzed sample after instrumental drift correction. Intra- and inter assay precisions were determined for CRMs and a subset of urine study samples. For intra-day



precision, 10% of urine samples were prepared separately on the same analytical day. For inter-day precision, urine samples were prepared separately on different analytical days. The coefficient of variation (CV) for intra- and inter-day precision was determined for each element of the CRMs and MESA urine samples. The limit of detection (LoD) was calculated by  $3.33 \times$  standard deviation of blank measurements ( $n_{\text{average}} = 1034$ ) and the method detection limit (MDL) using the LoD multiplied by a dilution factor of 50.

We used descriptive statistics to characterize the urine element concentrations overall, by exam, lab method, and sociodemographic characteristics (age, sex, race/ethnicity, areas, education), smoking status, and body mass index (BMI). Urinary elements are right skewed and log-transformed prior to statistical analysis to obtain normal distributions.

Median and interquartile range (IQR, 75th percentile to 25th percentile) were calculated for uncorrected, SG-adjusted and creatinine-adjusted urinary trace element concentrations. Intraclass correlation coefficient (ICC) was determined for two urine dilution/hydration correction approaches, SG and creatinine. Statistical calculations were performed using R software (version 3.6.1).

## 3 Results and discussion

### 3.1 Method validation and quality control

**3.1.1 Linearity.** We observed that the advanced calibration range with nine data points and covering the low concentration range shows less variability and shifts in the slope (Fig. 1). Good linearity was obtained for all trace elements for both five- and

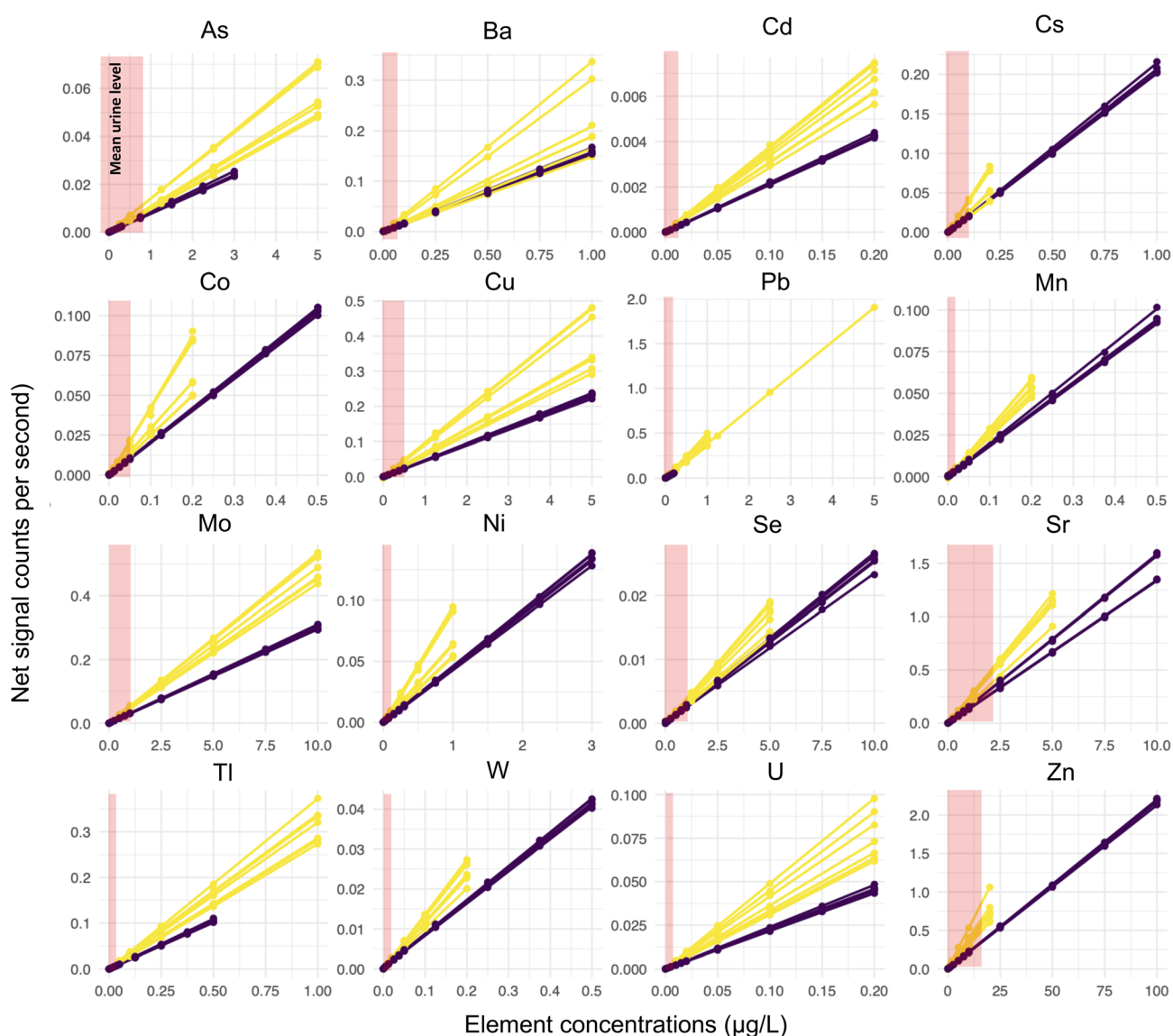


Fig. 1 Variability between regression lines, by element for selected calibration runs using a five-point calibration (yellow lines) or a nine-point-calibration (purple lines). Red area reflects the measured mean element concentration in the MESA urine accounting for the 50-fold dilution.

Table 3 Quality performance for urine CRMs from the Quebec Multi-element External Quality Assessment Scheme (QMEQAS), National Institute of Technology (NIST) and ClinChek (RECIPe)<sup>a</sup>

Certified reference material	Analyte	Co	Ni	Zn	Cu	Sr	Mo	Cs	Ba	W	Tl	Pb	U	Mn	Se	As	Cd
QM-U-Q1822 (N = 412)	Certified ( $\mu\text{g L}^{-1}$ )	5.06	5.2	467	459	181	373	9.09	8.49	*	6.32	84.1	0.77	1.6	100	36.2	9.43
	mean $\pm$ SD	( $\pm 0.8$ )	( $\pm 2.0$ )	( $\pm 87$ )	( $\pm 62$ )	( $\pm 73$ )	( $\pm 51$ )	( $\pm 3.7$ )	( $\pm 1.3$ )		( $\pm 0.9$ )	( $\pm 13$ )	( $\pm 0.2$ )	( $\pm 1.2$ )	( $\pm 38$ )	( $\pm 9$ )	( $\pm 1.4$ )
	Measured ( $\mu\text{g L}^{-1}$ )	5.57	10.3	504	459	190	398	9.48	8.51		5.81	77.2	0.76	1.61	89.2	37.0	9.44
	mean $\pm$ SD	( $\pm 0.2$ )	( $\pm 2.6$ )	( $\pm 102$ )	( $\pm 17$ )	( $\pm 79$ )	( $\pm 25$ )	( $\pm 0.4$ )	( $\pm 0.7$ )		( $\pm 0.3$ )	( $\pm 5.9$ )	( $\pm 0.1$ )	( $\pm 0.6$ )	( $\pm 6.8$ )	( $\pm 2.5$ )	( $\pm 0.5$ )
QM-U-Q1823 (N = 390)	Recovery (%)	110	198	108	100	105	107	104	100		92	92	99	100	89	102	100
	Certified ( $\mu\text{g L}^{-1}$ )	1.63	45.4	281	18	289	778	15 ( $\pm 6$ )	19.4	*	20.2	34	3.69	4.63	320	92.9	1.61
	mean $\pm$ SD	( $\pm 0.3$ )	( $\pm 8.3$ )	( $\pm 59$ )	( $\pm 4.2$ )	( $\pm 116$ )	( $\pm 105$ )		( $\pm 2.6$ )		( $\pm 2.7$ )	( $\pm 5.8$ )	( $\pm 0.8$ )	( $\pm 1.7$ )	( $\pm 50$ )	( $\pm 16$ )	( $\pm 0.6$ )
	Measured ( $\mu\text{g L}^{-1}$ )	1.88	50.9	300	19.9	302	837	15.9	19.7		18.8	31.5	3.64	4.37	315	95.9	1.87
QM-U-Q1824 (N = 367)	mean $\pm$ SD	( $\pm 0.1$ )	( $\pm 0.75$ )	( $\pm 30$ )	( $\pm 1.6$ )	( $\pm 12$ )	( $\pm 36$ )	( $\pm 0.6$ )	( $\pm 0.8$ )		( $\pm 0.6$ )	( $\pm 2.2$ )	( $\pm 0.1$ )	( $\pm 0.2$ )	( $\pm 14$ )	( $\pm 4.2$ )	( $\pm 0.1$ )
	Recovery (%)	115	112	107	111	104	108	106	101		93	93	99	94	99	103	116
	Certified ( $\mu\text{g L}^{-1}$ )	7.66	17.4	592	255	240	181	6.90	11.5	*	41.5	140	1.74	3.19	157	361	4.71
	mean $\pm$ SD	( $\pm 1.1$ )	( $\pm 3.8$ )	( $\pm 106$ )	( $\pm 34$ )	( $\pm 96$ )	( $\pm 25$ )	( $\pm 2.8$ )	( $\pm 0.75$ )		( $\pm 5.5$ )	( $\pm 21$ )	( $\pm 0.4$ )	( $\pm 1.5$ )	( $\pm 47$ )	( $\pm 52$ )	( $\pm 0.8$ )
NIST 2668 L1 (N = 155)	Measured ( $\mu\text{g L}^{-1}$ )	8.19	21.6	626	254	251	195	7.36	11.7		37.6	131	1.71	2.95	144	385	4.71
	mean $\pm$ SD	( $\pm 0.3$ )	( $\pm 2.1$ )	( $\pm 39$ )	( $\pm 11$ )	( $\pm 11$ )	( $\pm 7.8$ )	( $\pm 0.3$ )	( $\pm 0.5$ )		( $\pm 3.1$ )	( $\pm 8.7$ )	( $\pm 0.1$ )	( $\pm 0.2$ )	( $\pm 6.7$ )	( $\pm 18$ )	( $\pm 0.2$ )
	Recovery (%)	107	124	106	100	105	108	107	101		91	93	98	92	92	107	100
	Certified ( $\mu\text{g L}^{-1}$ )	0.82	2.31	*	28.1	*	51.6	4.90	1.96	1.3	0.72	1.23	0.03	1.08	*	10.8	1.06
NIST 2668 Level 2 (N = 136)	mean $\pm$ SD	( $\pm 0.06$ )	( $\pm 0.3$ )		( $\pm 2$ )		( $\pm 1.8$ )	( $\pm 0.3$ )	( $\pm 0.1$ )	( $\pm 0.08$ )	( $\pm 0.03$ )	( $\pm 0.06$ )	( $\pm 0.002$ )	( $\pm 0.2$ )		( $\pm 0.5$ )	( $\pm 0.05$ )
	Measured ( $\mu\text{g L}^{-1}$ )	0.85	4.44	27.2	27.2	27.2	51.3	4.97	1.98	1.1	0.61	1.11	0.03	1.24		11.1	1.09
	mean $\pm$ SD	( $\pm 0.1$ )	( $\pm 1.1$ )	( $\pm 2.7$ )	( $\pm 2.7$ )	( $\pm 2.4$ )	( $\pm 2.4$ )	( $\pm 0.3$ )	( $\pm 0.4$ )	( $\pm 0.2$ )	( $\pm 0.04$ )	( $\pm 0.2$ )	( $\pm 0.01$ )	( $\pm 0.3$ )	( $\pm 0.3$ )	( $\pm 0.7$ )	( $\pm 0.1$ )
	Recovery (%)	105	192	97	134	*	99	101	101	90	85	90	88	115	103	103	103
RECIPe Clinchek Level 1 (N = 160)	Certified ( $\mu\text{g L}^{-1}$ )	51.8	115	*	134	*	1687	221	255	62.5	115	138	13.4	47.6	*	213	16.4
	mean $\pm$ SD	( $\pm 1.7$ )	( $\pm 5.2$ )		( $\pm 5.4$ )		( $\pm 58$ )	( $\pm 12$ )	( $\pm 3.2$ )	( $\pm 1.0$ )	( $\pm 2.8$ )	( $\pm 3.6$ )	( $\pm 0.5$ )	( $\pm 3.4$ )		( $\pm 4.4$ )	( $\pm 0.3$ )
	Measured ( $\mu\text{g L}^{-1}$ )	50.0	114	120	120	1777	1777	228	250	58.5	97.1	117	12.3	46.7		224	16.2
	mean $\pm$ SD	( $\pm 2.7$ )	( $\pm 0.6$ )	( $\pm 9.5$ )	( $\pm 9.5$ )	( $\pm 111$ )	( $\pm 111$ )	( $\pm 16$ )	( $\pm 17$ )	( $\pm 3.8$ )	( $\pm 6.1$ )	( $\pm 17$ )	( $\pm 0.8$ )	( $\pm 2.9$ )	( $\pm 15$ )	( $\pm 1.0$ )	( $\pm 0.1$ )
RECIPe Clinchek Level 1 (N = 160)	Recovery (%)	97	99	90	90	105	105	103	98	94	84	85	92	98	105	99	99
	Certified ( $\mu\text{g L}^{-1}$ )	2.05	3.25	195	58.2	*	20.2	*	11.0		5.16	26.4	*	4.09	29.0	17.0	2.56
	mean $\pm$ SD	( $\pm 0.4$ )	( $\pm 0.7$ )	( $\pm 39$ )	( $\pm 12$ )		( $\pm 4.0$ )		( $\pm 2.2$ )		( $\pm 1.5$ )	( $\pm 5.3$ )		( $\pm 0.8$ )	( $\pm 7.2$ )	( $\pm 3.4$ )	( $\pm 0.5$ )
	Measured ( $\mu\text{g L}^{-1}$ )	2.01	3.66	222	58.1	20.9	20.9	10.2	10.2		6.63	14.4		4.29	23.6	17.2	2.35
RECIPe Clinchek Level 1 (N = 160)	mean $\pm$ SD	( $\pm 0.1$ )	( $\pm 0.6$ )	( $\pm 39$ )	( $\pm 2.5$ )	( $\pm 1.3$ )	( $\pm 1.3$ )	( $\pm 0.5$ )	( $\pm 0.5$ )		( $\pm 0.3$ )	( $\pm 3.6$ )		( $\pm 0.8$ )	( $\pm 1.6$ )	( $\pm 0.9$ )	( $\pm 0.1$ )
	Recovery (%)	98	113	114	100	104	104	92	92		128	55		105	82	101	92

<sup>a</sup> N = number of preparation and analyses of the certified reference material over the course of the study. SD = standard deviation. \*Elemental values not certified.

nine-point calibration ( $r^2 > 0.999$ ). Both the mean and variance of intercepts and slopes changes noticeably across calibration phases. We conclude that the added lower concentration points used in the calibration range with nine data points are helpful in two ways (1) by stabilizing estimates, and (2) confirming that there is a strong linear correlation, even at concentrations close to LoDs. Since all trace element concentrations fall within the lower range of both calibration types, the larger variance of intercepts and slopes for the five-point calibration has only a small effect at low values. Assuming the linear relationship for five-point calibration data is plausible, it thus gives a clear justification to extrapolate below the previous lower calibration levels included in the advanced 9-point calibration approach.

**3.1.2 Accuracy and sensitivity.** Our ICP-MS method for small volumes of urine was validated using urine CRMs. Five CRMs were analyzed repeatedly over the analytical period of the MESA study, and the measured average values and accuracies are reported in Table 3. The measured values of the target urinary

elements of these CRMs were reliable and satisfactory except for Ni. Although CRMs with higher certified Ni levels showed satisfactory accuracy (99–115% for QM-U-Q1823 and NIST 2668 L2), Ni accuracy was  $\sim 200\%$  for most CRMs within a certified concentration range between 2.3 and 17.4  $\mu\text{g L}^{-1}$  urine. Due to the falsely high Ni levels at low concentrations, covering the measured Ni level in MESA, urinary Ni data were excluded from further analysis.

The MDL was calculated from over 1000 method blank measurements from 130 analytical days. Values for MDL are given in Table 4 and 5. While MDL accounts for each method step including the dilution factors, other studies often only report elemental LoDs. In order to compare the detection limits of different study, we also report the LoDs in Table 6. Our LoDs fall within range published in other studies and often surpass them by being lower. The minimum element concentrations that could be reliably detected (reported as MDL) ranged from 0.001  $\mu\text{g L}^{-1}$  urine for U to 6.2  $\mu\text{g L}^{-1}$  urine for Zn (Tables 4 and 5). For 7677

**Table 4** Summary of urinary non-essential elements ( $\mu\text{g L}^{-1}$ ) at the MESA cohort (Exam 1 and subset of Exam 5)

	As	Ba	Cd	Cs	Gd <sup>a</sup>	Ni <sup>a</sup>	Pb	Sr	Tl	U	W
<i>N</i>	7677	7677	7677	7677	6367	6367	7677	7677	7677	7677	7677
Minimum	0.3	0.075	0.008	0.01	0.002	0.214	0.034	1.71	0.002	0.001	0.025
Percentile 10th	2.95	0.32	0.17	1.77	0.002	0.91	0.31	26.6	0.06	0.001	0.025
Percentile 25th	6.2	0.59	0.3	2.99	0.002	1.59	0.52	48.4	0.09	0.003	0.025
Percentile 50th	14.3	1.11	0.51	4.96	0.002	2.81	0.89	87.2	0.15	0.005	0.055
Percentile 75th	34.4	2.09	0.89	7.51	0.006	4.57	1.43	142	0.23	0.011	0.110
Percentile 90th	78.2	3.77	1.42	10.6	0.04	6.94	2.17	210	0.33	0.026	0.202
Maximum	2509	408	10.8	119	1555	109	42.3	787	7.2	1.0	11.6
Mean	35.4	1.94	0.7	5.91	0.59	3.55	1.16	107	0.18	0.011	0.112
SD	80.5	5.88	0.67	4.68	23.1	3.26	1.31	83.2	0.16	0.024	0.288
Method detection limit (MDL) <sup>b</sup>	0.14	0.11	0.01	0.007	0.003	0.30	0.05	0.14	0.003	0.001	0.04
Samples below the MDL <sup>b</sup> (no.)	0	51	4	0	3747	42	6	0	3	894	2507
Samples below MDL <sup>b</sup> (%)	0	0.66	0.05	0	58.9	0.66	0.08	0	0.04	11.7	32.7
Coefficient of variation (CV)											
Intra-day (%; <i>n</i> = 706)	1.7	9.0	3.5	1.4	13	7.7	4.3	1.6	3.2	14	11
Inter-day (%; <i>n</i> = 1000)	3.8	9.7	5.8	3.2	20	12	6.8	3.2	4.9	16	13

<sup>a</sup> Elements have not been analyzed for pilot study. <sup>b</sup> Considering a dilution factor of 50.

**Table 5** Summary of urinary essential elements ( $\mu\text{g L}^{-1}$ ) at the MESA cohort (Exam 1 and subset of Exam 5)

	Co	Cu	Mn	Mo	Se	Zn
<i>N</i>	7677	7677	7677	7677	7677	7677
Minimum	0.015	0.603	0.098	0.629	1.219	4.38
Percentile 10th	0.16	4.98	0.1	12.3	14.8	152
Percentile 25th	0.26	8.21	0.15	22.6	26.6	294
Percentile 50th	0.41	13.0	0.25	41.2	46.6	568
Percentile 75th	0.59	18.7	0.38	67.6	71.2	981
Percentile 90th	0.82	25.3	0.61	103	99.2	1554
Maximum	12 747	31 496	504	878	540	7611
Mean	2.38	19.2	0.55	52.8	53.7	758
SD	146	360	6.54	47.9	38.2	709
Limit of detection (LOD)	0.02	0.85	0.14	0.53	1.72	6.20
Number of samples below the MDL	6	1	1635	0	1	2
Samples below MDL (%)	0.08	0.01	21.3	0	0.01	0.03
Coefficient of variation (CV)						
Intra-day (%; <i>n</i> = 706)	4.0	2.9	26	1.4	2.2	2.5
Inter-day (%; <i>n</i> = 1000)	6.3	6.3	19	3.3	5.4	6.5

**Table 6** Comparison of limit of detection (LoD) of trace elements from different studies investigating urinary trace element profiles using ICP-MS

	Element LoDs																
	Non-essential											Essential					
	As	Ba	Cd	Cs	Gd	Ni	Pb	Sr	Tl	U	W	Co	Cu	Mn	Mo	Se	Zn
<b>Our study</b>	<b>0.003</b>	<b>0.002</b>	<b>0.0002</b>	<b>0.0001</b>	<b>0.0001</b>	<b>0.006</b>	<b>0.001</b>	<b>0.003</b>	<b>0.0001</b>	<b>0.00002</b>	<b>0.0008</b>	<b>0.0004</b>	<b>0.02</b>	<b>0.003</b>	<b>0.01</b>	<b>0.03</b>	<b>0.1</b>
Schramel <i>et al.</i> 1997 (ref. 17)	—	—	0.02	—	—	—	0.03	—	0.05	—	0.02	—	—	—	—	—	—
Bocca <i>et al.</i> 2004 (ref. 18)	—	—	0.001	—	—	0.002	0.006	—	—	—	—	0.002	—	0.004	—	—	—
Heitland and Köster, 2006 <sup>a</sup> (ref. 19)	0.09	0.009	0.009	0.01	0.002	0.01		0.02	0.003	0.001		0.005	0.14	0.024	0.03	0.13	0.1
Burton <i>et al.</i> 2016 <sup>a</sup> (ref. 20)																	
Schmied <i>et al.</i> , 2021 <sup>a</sup> (ref. 24)	0.01		0.002			0.05	0.001		0.001			0.001		0.02	0.003		0.2
Zhang <i>et al.</i> , 2023 <sup>b</sup> (ref. 25)	0.001		0.001			0.03	0.18	0.002	0.004				0.02			0.08	0.04

<sup>a</sup> Studies report LoQ converted values to LoD. <sup>b</sup> Study reported MDL converted to LoD by dividing with dilution factor ( $\times 10$ ).

MESA urine samples (6367 for Gd and Ni), Gd was <MDL in 3747 (59%; MDL = 0.003  $\mu\text{g L}^{-1}$  urine), W in 2507 (33%; MDL = 0.04  $\mu\text{g L}^{-1}$  urine), U in 894 (12%; MDL = 0.001  $\mu\text{g L}^{-1}$  urine), and Mn in 1635 (21%; MDL = 0.14  $\mu\text{g L}^{-1}$  urine) samples. The other trace elements were detectable in all, or the majority (>99%) of urine samples (Tables 4 and 5).

Overall, the method for the simultaneous analysis of trace elements in small volumes of urine (100  $\mu\text{L}$ ) is satisfactory for the tested elements. Values for the intra-day precision ranged from 1.4% for Mo and Cs to 26% for Mn (Tables 4 and 5). The inter-day precision showed slightly higher CV for Gd (20%), U (16%) and Mn (19%) which can be explained by the very low concentration of U and Gd in MESA urine. For Mn, most

urinary concentrations in MESA ranged between the MDL and MQI which explains the higher inter-day and intra-day variability compared to other elements. Antimony was only analyzed for 17% of urine samples ( $n = 1310$ ) because of the poor inter-day (45%) and intra-day (60%) precision. For Sb, 16% of the MESA analyzed samples were <MDL of 0.03  $\mu\text{g L}^{-1}$  urine and 65% were <MQI (not included in Table 4).

One potential limitation of the study pertains to the sensitivity of the analytical method to quantify urinary concentrations for elements (*e.g.*, Gd, W, U) near the MDL. This is because of their very low concentrations and the 50-fold dilution of the small 100  $\mu\text{L}$  sample volume, which affect confidence levels of the measurements for these elements. Despite the poorer

**Table 7** Unadjusted ( $\mu\text{g L}^{-1}$ ), SG-adjusted ( $\mu\text{g L}^{-1}$ ) and creatinine-adjusted ( $\mu\text{g g}^{-1}$  creatinine) (ultra)-trace elements in MESA spot urine samples from Exam 1 and 5<sup>a</sup>

Elements	Unadjusted	SG-adjusted ( $\mu\text{g L}^{-1}$ )	Creatinine-adjusted ( $\mu\text{g g}^{-1}$ )	Unadjusted	SG-adjusted ( $\mu\text{g L}^{-1}$ )	Creatinine-adjusted ( $\mu\text{g g}^{-1}$ )	ICC (creatinine)	ICC (SG)
<b>Non-essential</b>								
As	35.4 (80.5)	36 (143.9)	32.9 (72.8)	14.3 (6.2, 34.4)	14.9 (7.4, 33.9)	13.7 (6.7, 32.2)	0.83	0.44
Ba	1.9 (5.9)	2 (4.7)	2 (5.6)	1.1 (0.6, 2.1)	1.2 (0.7, 2.1)	1.2 (0.7, 2.1)	0.65	0.87
Cd	0.7 (0.7)	0.7 (0.6)	0.6 (0.6)	0.5 (0.3, 0.9)	0.6 (0.4, 0.8)	0.5 (0.3, 0.8)	0.59	0.72
Cs	5.9 (4.7)	6 (5.2)	5.6 (3.5)	5 (3, 7.5)	5.1 (4, 6.8)	4.8 (3.6, 6.5)	0.54	0.57
Gd	0.6 (23.1)	0.6 (20.8)	0.6 (22.6)	0.002 (0.002, 0.006)	0.003 (0.002, 0.007)	0.003 (0.002, 0.008)	0.80	0.92
Ni	3.5 (3.3)	3.5 (3.1)	3.5 (3.7)	2.8 (1.6, 4.6)	2.9 (2, 4.3)	2.8 (1.8, 4.3)	0.68	0.81
Pb	1.2 (1.3)	1.2 (1.4)	1.1 (1.2)	0.9 (0.5, 1.4)	0.9 (0.7, 1.4)	0.9 (0.6, 1.3)	0.61	0.69
Sr	107 (83.2)	109 (84.5)	106 (74.1)	87.2 (48.4, 142)	93.8 (63.5, 135)	88.8 (56.1, 136)	0.53	0.60
Tl	0.2 (0.2)	0.2 (0.2)	0.2 (0.2)	0.2 (0.1, 0.2)	0.2 (0.1, 0.2)	0.1 (0.1, 0.2)	0.64	0.65
U	0.01 (0.02)	0.01 (0.06)	0.01 (0.08)	0.005 (0.003, 0.01)	0.005 (0.003, 0.01)	0.005 (0.003, 0.01)	0.33	0.49
W	0.1 (0.3)	0.1 (0.3)	0.1 (0.3)	0.06 (0.03, 0.1)	0.06 (0.04, 0.1)	0.062 (0.04, 0.1)	0.76	0.86
<b>Essential</b>								
Co	2.4 (146.1)	3.1 (202)	3 (185.5)	0.4 (0.3, 0.6)	0.4 (0.3, 0.5)	0.4 (0.3, 0.6)	0.97	0.95
Cu	19.2 (360)	21.1 (457)	21.9 (619)	13 (8.2, 18.7)	13.2 (10.8, 16.5)	12.3 (10, 15.8)	0.87	0.97
Mn	0.6 (6.5)	0.6 (5.6)	0.6 (5.3)	0.2 (0.2, 0.4)	0.3 (0.2, 0.4)	0.2 (0.2, 0.4)	0.83	0.91
Mo	52.8 (47.9)	52.6 (48.4)	49.3 (44.7)	41.2 (22.6, 67.6)	43.3 (30.8, 60.9)	40.5 (28.6, 58.0)	0.60	0.71
Se	53.7 (38.2)	52.4 (32.1)	48.4 (22.7)	46.5 (26.6, 71.2)	47.1 (37, 60.2)	43.8 (34.4, 56.1)	0.41	0.58
Zn	758 (709)	761 (702)	705 (654)	568 (294, 981)	590 (380, 902)	544 (363, 819)	0.60	0.72

<sup>a</sup> SD, standard deviation; IQR, interquartile range; ICC, intra class correlation.



precision for these elements marginally above the MDL (*e.g.*, Gd, W, U), our method can be used to investigate the trace element profiles in small volumes of urine within a longitudinal cohort, for which good precision is crucial to avoid false associations. Furthermore, a single urine specimen is a more convenient approach for large cohort studies, but the urinary element variability within participant has to be considered.

### 3.2 Urinary trace element concentrations in the MESA cohort

Normalization of urine trace element concentrations has been performed using both correction approaches, creatinine and SG. Scatter plots of correlations between creatinine-corrected and SG-corrected trace element concentrations in urine are presented in Fig. 4 and Table 7. Both methods are widely used to adjust for urine element concentrations in spot urines and can improve the correlation in groups (*e.g.*, smoker, women/men) and reduce intra-individual variation.<sup>26</sup> Table 7 shows the effect of adjustment by creatinine ( $\mu\text{g g}^{-1}$ ) and SG on urine trace element concentrations. It shows that intra-class coefficients (ICC) are 10–25% (ICC = 0.75–0.90) less variable between the uncorrected and creatinine-corrected concentrations for As, Gd, W, Co, Cu, and Mn. The variability is moderate for Ba, Cd, Cs, Ni, Pb, Sr, Tl, Cu, Mo, Ni, Se and Zn (ICC 0.35–0.75) and the

highest for U (ICC = 0.33). The large variability between creatinine-adjusted and unadjusted urinary U concentrations can be explained by the potential association of low creatinine clearance with high U concentrations, an element which is nephrotoxic.<sup>27</sup>

Comparing unadjusted and SG-adjusted urinary trace element concentrations, low variability (ICC 0.75–0.9) occurs for Ba, Gd, Ni, W, Co, Cu, and Mn. The variability is moderate for all other elements including As, Cd, Cs, Pb, Sr, Tl, Mo, Se, U and Zn (ICC 0.35–0.75). Adjusting for creatinine showed overall lower ICCs (except for As) than SG-adjusted urinary trace element concentrations (Table 7). However, limitations for both adjustment approaches have been reported. Creatinine is expected to vary with body composition and activity and some trace elements might not be excreted in urine *via* the same pathway as creatinine.<sup>28,29</sup> SG correction may not be appropriate for individuals with diabetes mellitus and kidney dysfunction.<sup>30</sup> For instance, adjustment of urinary Cd levels using SG has been suggested as the better approach since it seems to be less controlled by age and sex (Suwazono *et al.*, 2005).<sup>31</sup> Future studies using the published dataset will adopt the appropriate element normalization approach according to element sensitivity, to avoid any bias to health outcome.

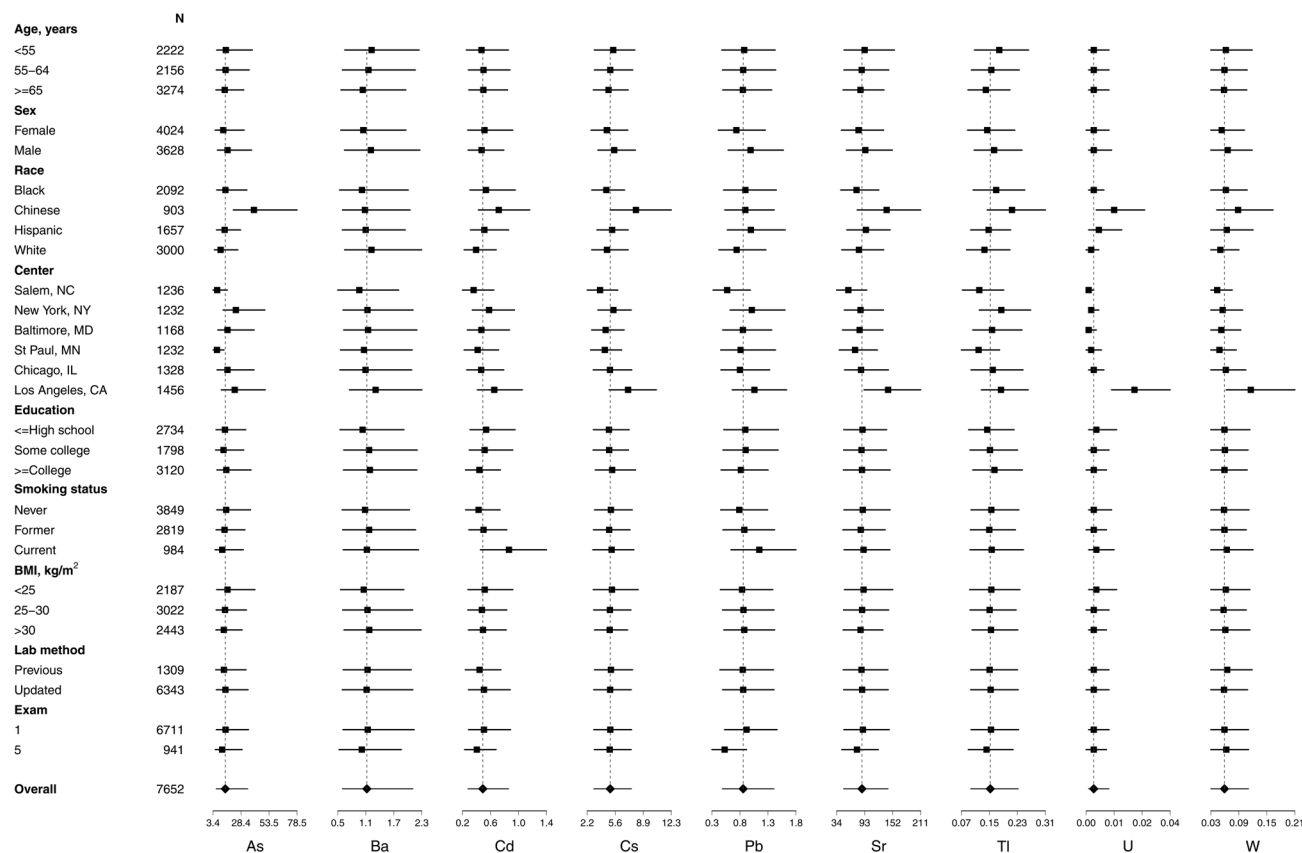


Fig. 2 Median and interquartile range of urinary non-essential trace elements ( $\mu\text{g L}^{-1}$ ) at MESA Exam 1 and 5 by participants' characteristics. Diamond-shaped points represent the unadjusted median urine concentration of the non-essential elements and lines correspond to the interquartile range overall and for each subgroup. The dotted line represents the overall unadjusted median urine concentrations of the non-essential elements.

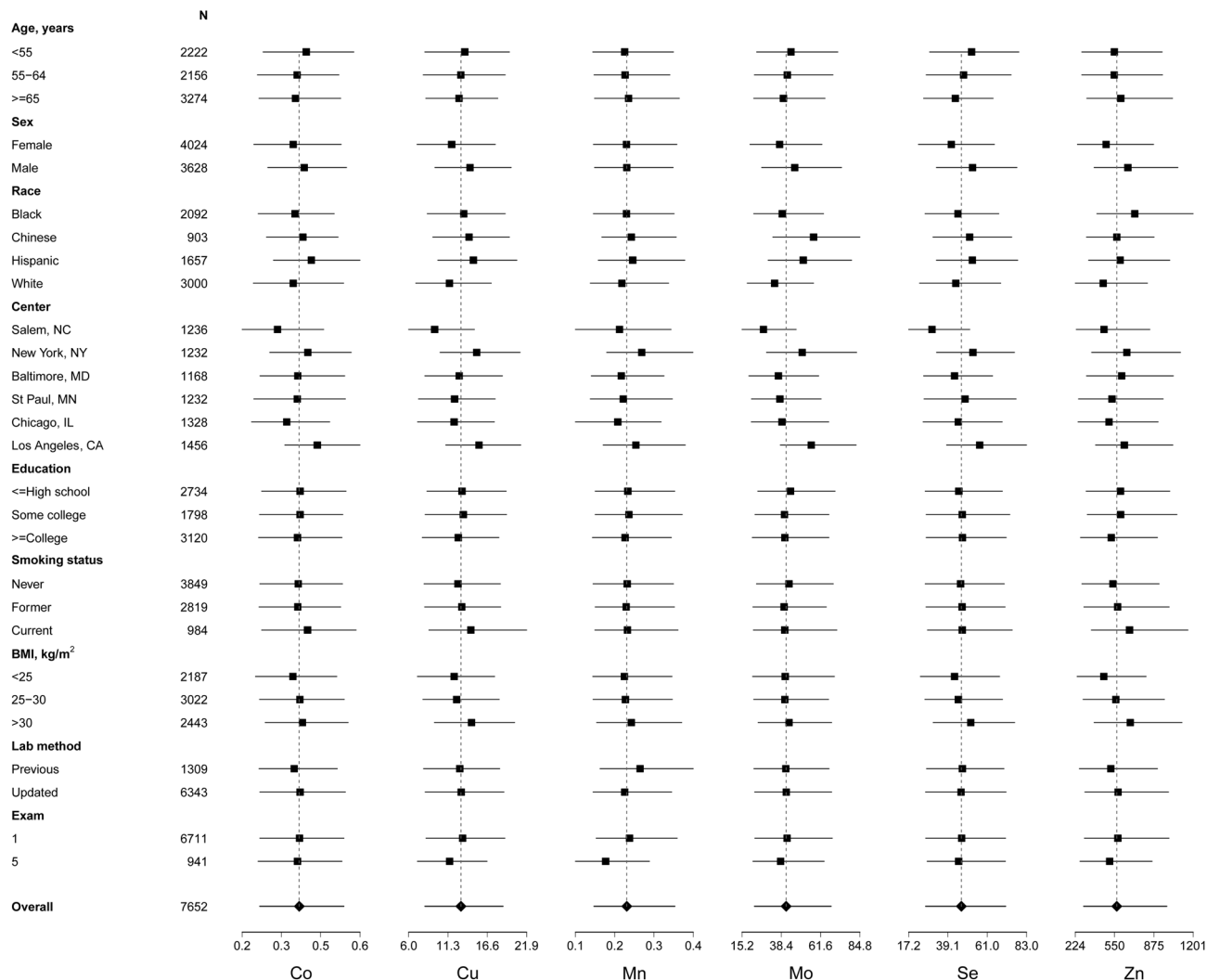


Fig. 3 Median and interquartile range of urinary essential trace elements ( $\mu\text{g L}^{-1}$ ) at Exam 1 and 5 by participants' characteristics. Diamond-shaped points represent the unadjusted median urine concentrations of the essential trace elements and lines correspond to the interquartile range overall and for each subgroup. The dotted line represents the overall unadjusted median urine concentrations of the essential trace elements.

We identified some differences in levels of unadjusted urinary trace element features by covariate levels (Fig. 2 and 3), although the purpose of this comparison is descriptive, not inferential, as a more complex analysis adjusting for covariates would be needed to formally evaluate differences in trace element levels by subgroups. Among non-essential elements, the order of mean concentration in urine follows the order of  $\text{Sr} > \text{As} > \text{Cs} > \text{Ni} > \text{Ba} > \text{Pb} > \text{Cd} > \text{Gd} > \text{Tl} > \text{W} > \text{U}$ . Men tend to have higher concentrations compared to women for most elements, except for Cd, which was higher in women. Chinese Americans tend to have higher urinary levels of non-essential elements compared to other ethnic groups. Increased Cd and Pb concentrations were associated with smoking status. Pb concentrations are 1.2 [0.67, 1.8  $\mu\text{g L}^{-1}$ ] for current smokers compared to 0.82 [0.49, 1.3  $\mu\text{g L}^{-1}$ ] among non-smokers. Likewise, median (IQR) Cd is 0.89 [0.48, 1.44  $\mu\text{g L}^{-1}$ ] for participants who are current smokers compared to 0.45 [0.26, 0.76  $\mu\text{g L}^{-1}$ ]

among non-smokers. Also, most non-essential elements are higher in urine from MESA participants from Los Angeles, CA compared to other centers. Other covariates such as education, sex and BMI were not associated with non-essential element concentrations in urine among all MESA participants in these analyses unadjusted for other factors.

For essential elements, the order of urinary mean concentrations decreases from  $\text{Zn} > \text{Se} > \text{Mo} > \text{Cu} > \text{Co} > \text{Mn}$ . White participants tend to have the lowest concentrations of essential elements compared to all other ethnic groups (Fig. 3). For instance, median (IQR) Zn and Cu concentrations for white are 455 [224, 821  $\mu\text{g L}^{-1}$ ] and 11.5 [7.0, 17.1  $\mu\text{g L}^{-1}$ ] compared to Black (Zn 717 [402, 1201  $\mu\text{g L}^{-1}$ ], Cu 13.4 [8.5, 19.0  $\mu\text{g L}^{-1}$ ]), Chinese (Zn 568 [317, 877  $\mu\text{g L}^{-1}$ ], Cu 14.1 [9.3, 19.6  $\mu\text{g L}^{-1}$ ]) and Hispanic Zn 596 [334, 1007  $\mu\text{g L}^{-1}$ ], Cu 14.7 [10, 20.6  $\mu\text{g L}^{-1}$ ] participants. Higher levels of essential trace elements in urine are not necessarily a positive finding, as it can reflect loss of essential trace

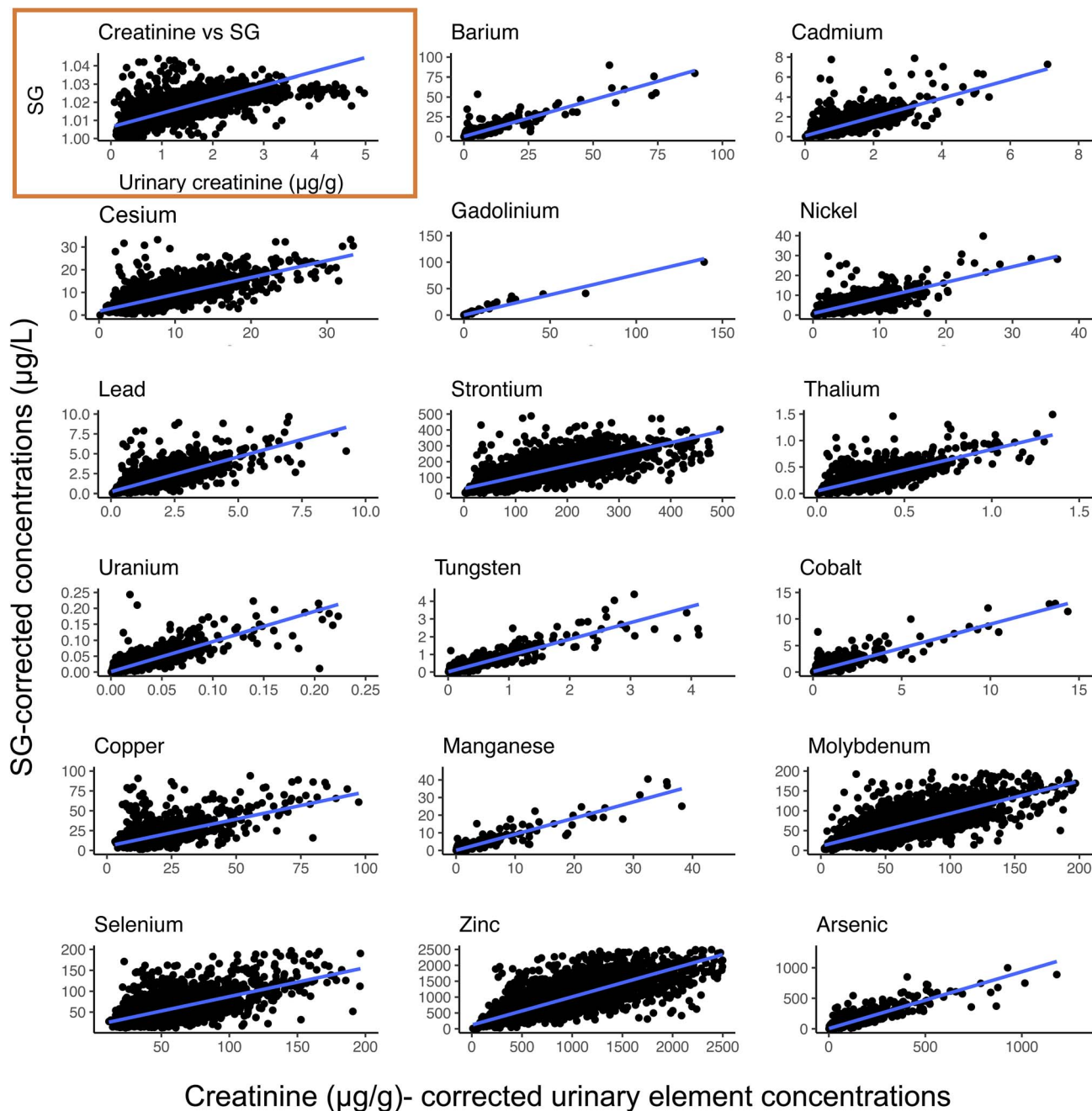


Fig. 4 Scatter plots of urinary trace element concentrations corrected for specific gravity ( $\mu\text{g L}^{-1}$ ) and urine creatinine ( $\mu\text{g g}^{-1}$ ). Solid blue line = line of agreement.

elements through the urine and metal dyshomeostasis. The concentrations of essential elements seem to be slightly higher in men (Zn 660 [379, 1075  $\mu\text{g L}^{-1}$ ], Cu 14.3 [9.6, 19.8  $\mu\text{g L}^{-1}$ , Se 52.8 [32.7, 77.6]) than women (Zn 479 [241, 873  $\mu\text{g L}^{-1}$ ], Cu 11.8 [7.1, 17.7  $\mu\text{g L}^{-1}$ , Se 41 [22.7, 65  $\mu\text{g L}^{-1}$ ]), except for Mn (women 0.25 [0.15, 0.39  $\mu\text{g L}^{-1}$ ], men 0.25 [0.15, 0.38  $\mu\text{g L}^{-1}$ ]). Participants from Salem, NC tended to have the lowest concentrations of essential elements in urine, while participants from Los Angeles, CA tended to have the highest. Sex, age, BMI and education are not clearly associated with urinary non-essential element concentrations among all MESA participants in unadjusted analyses.

The main focus of this work was on the analytical method including accuracy and precision of the urinary trace element data with some general descriptive presentations of the results. Future studies will focus on more specific comparisons that require additional adjustments and statistical tests for the dataset.

## 4 Conclusion

Our ICP-MS performance study confirmed that the sensitivity necessary for the analysis of 18 trace elements in very small

volumes of urine (100 µL) is satisfactory for analysis and routine biomonitoring in large epidemiological studies in populations exposed to low to moderate levels of elements in the environment. In conclusion, the analysis of urinary trace elements in MESA serves as a crucial tool that will allow us to assess sources of exposure to non-essential elements, status of essential elements, evaluate genetic and environmental determinants of trace elements in human populations, and monitor health conditions. By providing quantitative data on trace element levels, data from MESA will aid in identifying potential health risks of trace element exposures, guiding intervention, and promoting overall well-being.

## Conflicts of interest

There are no conflicts to declare.

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