Laboratory Procedure Manual

Analytes: **Antimony, Arsenic, Barium, Beryllium, Cadmium, Cesium, Cobalt, Lead, Manganese, Molybdenum, Platinum, Strontium, Thallium, Tin, Tungsten, Uranium and Total Arsenic**

Matrix: **Urine**

Method: **Urine Multi-Element ICP-DRC-MS**
Renamed from "Inductively Coupled Plasma-Mass Spectrometry (ICP-DRC-MS)"

Method No: **3018.6-01 (15 element panel) and 3018A.4-01 (total arsenic)**

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As performed by: Inorganic and Radiation Analytical Toxicology
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Important Information for Users

The Centers for Disease Control and Prevention (CDC) periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information
This document details the Lab Protocol for testing the items listed in the following table:

This method file describes measurements of UM_H, UMS_H, UTAS_H, and UTASS_H. One method was used to measure the urinary metals, urinary metals for smokers, total arsenic and total arsenic for smokers. However, these results are released as 4 separate data files.

Data File Name	Variable Name	SAS Label
UTAS_H UTASS_H	URXUAS	Urinary Total Arsenic (µg/L)
UM_H UMS_H	URXUBA	Barium, urine (ng/mL)
	URDUCD	Cadmium, urine (ng/mL)
	URXUCO	Cobalt, urine (ng/mL)
	URXUCS	Cesium, urine (ng/mL)
	URXUMN	Manganese, urine (ng/mL)
	URXUMO	Molybdenum, urine (ng/mL)
	URXUPB	Lead, urine (ng/mL)
	URXUSN	Tin, urine (ng/mL)
	URXUSR	Strontium, urine (ng/mL)
	URXUSB	Antimony, urine (ng/mL)
	URXUTL	Thallium, urine (ng/mL)
	URXUTU	Tungsten, urine (ng/mL)
	URXUUR	Uranium, urine (ng/mL)

1) Clinical relevance & summary of test principle

a. Clinical relevance:

These methods are used to achieve rapid and accurate quantification of elements of toxicological and nutritional interest including Antimony (Sb), Barium (Ba), Beryllium (Be), Cadmium (Cd), Cesium (Cs), Cobalt (Co), Lead (Pb), Manganese (Mn), Molybdenum (Mo), Platinum (Pt), Strontium (Sr), Thallium (TI), Tin (Sn), Tungsten (W), and Uranium (U) or Arsenic (As). The methods may be used to screen urine when people are suspected to be acutely exposed to these elements or to evaluate chronic environmental or other non-occupational exposure. [1-4].

b. Test principle:

Inductively coupled plasma mass spectrometry (ICP-MS) is a multi-element analytical technique capable of trace level elemental analysis [1-4]. When used with dynamic reaction cell technology (DRC) the technique is referred to as ICP-DRC-MS. This ICP-DRC-MS method is used to measure either arsenic, a 15 element panel (antimony, barium, beryllium, cadmium, cesium, cobalt, lead, manganese, molybdenum, platinum, strontium, thallium, tin, tungsten, and uranium), or any subgroup of these.

Liquid samples are introduced into the ICP through a nebulizer and spray chamber carried by a flowing argon stream. By coupling radio-frequency power into flowing argon, plasma is created in which the predominant species are positive argon ions and electrons and has a temperature of 6000-8000 K. The sample passes through a region of the plasma and the thermal energy atomizes the sample and then ionizes the atoms. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP (at atmospheric pressure, ~760 torr) from the mass spectrometer (operating at a pressure of 10^{-5} torr). The ions pass through a focusing region, the dynamic reaction cell, the quadrupole mass filter, and finally are counted in rapid sequence at the detector allowing individual isotopes of an element to be determined. The dynamic reaction cell operates in one of two modes. In 'standard' mode the cell is not pressurized and ions pass through the cell to the quadrupole mass filter unaffected. In 'DRC' mode the cell is pressurized with a gas which will collide or react with the incoming ions to either eliminate an interfering ion or change the ion of interest to a new mass which is free from interference. In this method the instrument is operated in DRC mode when analyzing for cadmium, manganese and arsenic, but in standard mode when analyzing for all of the other analytes. For arsenic, the reaction cell is pressurized with a mixture of hydrogen (10%) and argon (90%) or 100% argon which causes the breakup of the $^{40}Ar^{35}Cl^{+}$ ion which would otherwise interfere with detection of ^{75}As at m/z 75. When analyzing for cadmium in biomonitoring applications, the reaction cell is pressurized with oxygen. The $^{98}Mo^{16}O^{+}$ ions which would normally interfere with detection of ^{114}Cd at m/z 114 react with the oxygen in the cell creating $^{98}Mo^{16}O_2^{+}$ and $^{98}Mo^{16}O_3^{+}$ at masses that no longer represent interference to low level ^{114}Cd analysis. When low level Cd analysis is not the principle purpose of the analysis, i.e. emergency response situations, Cd may be analyzed in vented

(standard) mode to save analytical time with the expectation that the $^{98}Mo^{16}O_2^+$ interference on ^{114}Cd will not significantly hinder the ability to identify elevated Cd levels. The DRC is also pressurized with oxygen gas when analyzing for ^{55}Mn. The $^{39}K^{16}O^+$ ions which would normally interfere with the detection of ^{55}Mn at m/z 55 react with the oxygen in the cell and no longer represent interference to ^{55}Mn analysis. In DRC mode, the voltage applied by the axial field technology (AFT) and the additional axial push from spectator ions also stable in the DRC bandpass serve to keep the ions moving axially through the pressurized DRC chamber where they would normally slow down due to collisions which result in loss of momentum. Gold is added to the diluent to normalize the spectator ion population in the DRC cell which could otherwise change significantly between low concentration and high concentration samples, a phenomenon called crosstalk[3, 5, 6]. Electrical signals resulting from the detection of ions are processed into digital information that is used to indicate first the intensity of the ions and then the concentration of the element. This method was originally based on the method by Mulligan et al. [7]. The DRC portions of the method are based on work published by Tanner et al. [2, 3]. Urine samples are diluted 1+ 9 with 2% (v/v) concentrated nitric acid (and 1.5% ethanol in the case of arsenic). The diluent for the 15 element panel contains iridium (Ir), rhodium (Rh) for multi-internal standardization. The diluent for arsenic contains gallium (Ga) for internal standardization. Nitric acid is used for the purpose of solubilizing and stabilizing metals in solution. Internal standards are a constant concentration in all blanks, calibrators and samples. Monitoring the instrument signal ratio of a metal to its internal standard allows correction for instrument noise and drift, and sample-to-sample matrix differences. Ethanol is used when analyzing for arsenic in biomonitoring situations for the purpose of providing a constant amount of signal enhancement (carbon effect) across all blanks, calibrators, and samples.

2) Limitations of method; interfering substances and conditions

a. Interferences addressed by this method

i. Argon chloride ($^{40}Ar^{35}Cl$) on arsenic (^{75}As): The dynamic reaction cell is used in this method to diminish the presence of the argon chloride ($^{40}Ar^{35}Cl$) interference on arsenic at m/z 75 [8] which is common to urine analysis by ICP-MS (see Section 1.b for an explanation of this process). The dynamic reaction cell gas used for this purpose is a mixture of hydrogen (10%) in argon; however 100% argon can also be used when the mixture is unavailable.

ii. Correction & elimination of interferences (^{114}Sn, $^{98}Mo^{16}O$) on cadmium (^{114}Cd).

1. Mathematical correction for tin (^{114}Sn) interference:
The correction equation (-0.026826*Sn118) is used in the “Equations” tab of the method to correct the counts observed as m/z 114 to exclude counts due to ^{114}Sn.

2. Elimination of molybdenum oxide ($^{98}Mo^{16}O$) interference using DRC:
The dynamic reaction cell is used in this method to eliminate interference from molybdenum oxide ($^{98}Mo^{16}O$) onto cadmium at m/z 114 [9]. Oxygen

(100%) is the gas used in the dynamic reaction cell for this purpose; however, when low level Cd analysis is not the principle purpose of the analysis, i.e. emergency response situations, Cd may be analyzed in vented (standard) mode to save analytical time with the expectation that the $^{98}Mo^{16}O_2^+$ interference on ^{114}Cd will not significantly hinder the ability to identify elevated Cd levels. See Section 1.b for an explanation of this process.

iii. Reduction of interference ($^{39}K^{16}O$) on manganese, ^{55}Mn, using DRC:
The dynamic reaction cell is used in this method to reduce the potassium oxide ($^{39}K^{16}O$) interference on manganese at m/z 55. See Section 1.b for an explanation of this process.

iv. Matrix enhancement of arsenic signal:
Matrix induced signal enhancement in ICP-MS analysis from carbon on arsenic has been previously reported in the literature [10, 11]. When arsenic is being determined by this method for normal biomonitoring purposes, ethanol (1.5% v/v) is added in the diluent and rinse solutions to "normalize" the arsenic signal enhancement in all blanks, calibrators, and samples. If arsenic is combined with multi-element analysis for emergency response situations where ethanol is not part of the diluent and rinse solutions, the positive bias potentially resulting is not anticipated to significantly hinder the identification of acute arsenic exposures.

b. Limitations of method (interferences remaining in method)

i. Calcium chloride ($^{40}Ca^{35}Cl$) interference on arsenic (^{75}As):
It has been determined that a small interference remains at m/z 75 when the urine matrix contains ***both*** high chloride ***and*** high calcium levels [8]. Even at extreme calcium and chloride levels, this interference is has not been found to be significant (approximately 0.4 μg/L).

ii. Gallium oxide ($^{71}Ga^{17}O$) interference on strontium (^{88}Sr):
Gallium at 10 μg/L in the method diluent or 100 μg/L in a urine specimen (extremely unlikely) will produce approximately a +2 μg/L bias on observed Sr concentration due to the formation of $^{71}Ga^{17}O^+$, which occurs at the same m/z as $^{88}Sr^+$. While this interference is not large in comparison to typical Sr concentrations in urine, ~150 μg/L, best urine Sr accuracy for biomonitoring purposes will be achieved when Ga is not present in the diluent as an internal standard. If As is combined with the multi-element method for emergency response purposes, the resulting bias on Sr from the $^{71}Ga^{17}O^+$ interference is not likely to hinder the ability to hinder the identification of acute Sr exposures.

3) Procedures for collecting, storing, and handling specimens; criteria for specimen rejection; specimen accountability and tracking

a. Procedures for collecting, storing, and handling specimens: Guidelines for receiving and shipping packages and an example case study specimen collection

protocol are presented in the laboratory Policies and Procedures Manual [12]. Special specimen handling conditions, requirements, and procedures for this method include:

i. No fasting or special diets are required before collection of urine.

ii. Use sterile, lot screened collectors for specimen acquisition.

iii. Transport urine specimens frozen (packed in dry ice during shipment is preferred when possible).

iv. Once received, store long term at ≤ -20 °C until time for analysis. Short-term storage at 2-8 °C is acceptable. Refreeze at ≤ -20 °C portions of the sample that remain after analytical aliquots are withdrawn. Thawing and refreezing samples has not been found to compromise sample results.

v. Acceptable containers for analytical aliquots include lot screened polypropylene (PP) cryovials or tubes (i.e. 2 to 5 mL cryogenic vial or 15mL centrifuge tube). Avoid colored plastics and containers containing o-rings when possible due to increased risk of trace element contamination from coloring agents or o-ring materials. Externally threaded containers are preferred because they are less prone to contamination of the specimen and to leaks (internally threaded containers can develop leaks when biological material dries within the threads, compromising resealing).

b. Criteria for specimen rejection: Specimen characteristics that may compromise test results are indicated above. Reasons for rejection of a sample for analysis include the following (in all cases, request a second urine specimen):

i. Low volume:

1. Method 3018 (15 element): Optimal amount of urine is 1.8^{+} mL. The volume of urine used for one analysis is 0.5 mL. If only a subset of the elements is required, smaller volumes may be required.

2. Method 3018A (As): Optimal amount of urine is 1^{+} mL. The volume of urine used for one analysis is 0.25 mL.

ii. Contamination: Improper collection procedures or collection devices can contaminate the urine by contact with dust, dirt, etc.

c. Transfer or referral of specimens; procedures for specimen accountability and tracking: Location, status, and final disposition of the specimens will be tracked and records are maintained according to the Division's Policies and Procedures Manual [12]. Use only numerical identifiers for samples within the laboratory (e.g., case ID numbers) in order to safeguard confidentiality. Only the medical supervisor (MS) or project coordinator (PC) i.e. non CDC personnel shall have access to the personal identifiers.

4) Safety precautions

a. General safety

i. Observe all safety regulations as detailed in the Laboratory Safety Manual and the Chemical Hygiene Plan.

ii. Wear gloves, lab coat, and safety glasses while handling reagents, prepared solutions, or urine specimens.

Stock calibration standard B contains 1% hydrofluoric acid (HF). Latex is not an appropriate protective barrier against HF. When handling these solutions nitrile gloves (or other glove material known to be an adequate protective barrier to HF) are required.

iii. Observe universal precautions when working with urine.

iv. Exercise special care when handling and dispensing concentrated nitric or hydrochloric acid. Use additional personal protective equipment which protects face, neck, and front of body. Add acid to water. Nitric and hydrochloric acids are caustic chemicals that are capable of causing severe eye and skin damage. ***If concentrated acids come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.***

v. Use secondary containment for containers of biological or corrosive liquids.

vi. The use of the foot pedal on the Micromedic Digiflex™ is recommended because it reduces analyst contact with work surfaces that have been in contact urine and also keeps the analyst's hands free to hold the specimen cups and autosampler tubes and to wipe off the tip of Micromedic Digiflex™.

vii. There are many potential hazards on an operating ICP-MS instrument including ultraviolet radiation, high voltages, radio-frequency radiation, and high temperatures. This information is detailed in the ICP-MS System Safety Manual.

viii. Transport and store compressed gas cylinders with proper securing harnesses. For compressed oxygen gas, use regulators which are oil-free and are equipped with a flash arrestor. Use of flash arrestors is recommended when working with compressed oxygen and compressed hydrogen at greater than a 7% composition.

ix. Wipe down all work surfaces at the end of the day with freshly prepared 10% (v/v) sodium-hypochlorite solution or comparable disinfectant.

b. Radiation safety: Calibration standards used in this method contain μg/L natural uranium. Staff performing this method in the CDC laboratory must maintain the status of Radiation Worker through the Radiation Safety Office and practice

appropriate radiation safety when handling these solutions in accordance to the CDC's license with the Nuclear Regulatory Commission (NRC).

c. Waste disposal:

i. Autoclaving: All diluted biological specimens, original biological specimens being disposed, or consumables which come into contact with biological specimens (even diluted or aerosolized). Use sharps containers or special autoclave pans for broken glass / quartz or items which may puncture autoclave bags (e.g. pipette tips).

ii. Other liquid waste

1. Waste discarded down sink: Only non-corrosive liquid waste (EPA defines as pH >2 and pH<12.5, 40CFR §261.22) from the ICP-DRC-MS instrument can be discarded at the sink. Flush the sink with copious amounts of water.

2. Waste to be picked up by the CDC radiation safety office: Solutions used in the CDC laboratory having uranium concentrations equal to that of the single element standard, intermediate stock standard, or intermediate working standards.

3. Waste to be picked up by CDC hazardous waste program: Submit request for hazardous waste removal of all other liquid waste generated in the CDC laboratory for this method.

5) Instrument & material sources

a. Sources for ICP-MS instrumentation

i. ICP-MS: Inductively coupled plasma mass spectrometer with dynamic reaction cell technology (ELAN® DRC II or NexION) (PerkinElmer Norwalk, CT, www.perkinelmer.com).

ii. Recirculating chiller / heat exchanger for ICP-MS: Refrigerated chiller (PolyScience 6105PE) or heat exchanger (PolyScience 3370) (PerkinElmer Norwalk, CT, www.perkinelmer.com).

iii. Autosampler: ESI SC4-DX autosampler (Elemental Scientific Inc., Omaha, NE) or equivalent.

iv. Computer: Computer controller provided or recommended by ICP-MS manufacturer is recommended to ensure proper communication between computer and ICP-MS. Recommend 1-2 Gb RAM and secondary internal hard disk for nightly backups (if network backups are not possible).

v. FAST sample introduction system (optional): Standard peristaltic pump on ICP-MS replaced by DXi-FAST micro-peristaltic pump / FAST actuator and valve combination unit. For NEXION, like part # DXI-54-P4-F6. If DXi-FAST upgrade on ICP-MS is not used, a separate FAST actuator (built-in option on ESI SC4-DX autosampler or stand-alone FAST actuator) will be necessary to complete the FAST sample introduction system.

b. Sources for ICP-MS parts & consumables

NOTE: The minimum number of spares recommended before reordering (if owning one instrument) are listed as "# *Spares* =" in the descriptions below.

i. Adapter, PEEK: Securely connects 1.6mm O.D. PFA tubing to 0.03" I.D. peristaltic tubing. Composed of three PEEK parts.

1. Female nut for 1.6mm O.D. (1/16") tubing. Like part P-420 (Upchurch Scientific, Oak Harbor, WA, www.upchurch.com).
2. PEEK ferrule. Like part P-260x (10pk SuperFlangeless ferrule, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com).
3. Conical Adapter Body. Like part P-692 (Upchurch Scientific, Oak Harbor, WA, www.upchurch.com).

ii. Bottles (for rinse solution): Four liter screw-cap polypropylene container with built-in luer connections (2) designed for use with FAST sample introduction system (like catalog# SC-0305-1, Elemental Scientific Inc., Omaha, NE.,.

iii. Carboy and cap assembly for waste collection: 10-15 L, polypropylene wide-mouth carboy (100 mm neck size) with handles and no spigot (Like part #7BE-25126, Lab Safety Supply, Janesville, WI, with cap assembly like part #

N0690271 (PerkinElmer, Norwalk, CT, with tubing connections built into the cap for addition of liquid waste.

iv. Coolant, for Polyscience chiller or heat exchanger: Only PerkinElmer part # WE01-6558 (PerkinElmer Norwalk, CT, www.perkinelmer.com) is approved for use by PerkinElmer. *# Spares = 6*.

v. Cone, hyperskimmer (NexION): PerkinElmer part # W1033995 (PerkinElmer Norwalk, CT, www.perkinelmer.com).

1. Screws (for hyper skimmer cone, NexION): PerkinElmer part # 09919737 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. *# Spares = 4 screws per instrument.*

vi. Cone, sampler (nickel/platinum):

1. ELAN ICP-MS: PerkinElmer part # WE021140 / WE027802 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Part # SC2011-Ni / SC2013-Pt (Testing has also found Spectron, Ventura, CA, www.spectronus.com cones to be comparable). *# Spares = 4*.

2. NexION ICP-MS: PerkinElmer part # W1033612 / W1033614 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Part # SC4011-Ni / SC4013-Pt (Testing has also found Spectron, Ventura, CA, www.spectronus.com cones to be comparable). *# Spares = 4*.

vii. Cone, skimmer (nickel / platinum):

1. ELAN ICP-MS: PerkinElmer part # WE021137 / WE027803 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Part # SC2012-Ni / SC2014-Pt (Testing has also found Spectron, Ventura, CA, www.spectronus.com cones to be comparable) *# Spares = 4*.

2. NexION ICP-MS: PerkinElmer part # W1026356 / W1026907 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Part # SC4012-Ni / SC4014-Pt (Testing has also found Spectron, Ventura, CA, www.spectronus.com cones to be comparable) *# Spares = 4*.

viii. Connector (for tubing): Use to connect 1/8" I.D. PVC tubing to 0.125" I.D peristaltic pump tubing. Use part # 3140715 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or equivalent. *# Spares = 4*.

ix. Detector, electron multiplier: Like part # N8125001 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available direct from manufacturer (part # 14210, SGE Incorporated, Austin, Texas, http://www.etpsci.com) or various distributors. *# Spares = 1*.

x. FAST / ESI SC4-DX autosampler accessories:

1. Valve: CTFE High-flow valve head for SC-FAST (uses ¼-28 fittings). Like part # SC-0599-1010 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

2. Stator: CTFE Stator for 6 port SC-FAST high flow valve (¼-28 fittings). Like part # SC-0599-1010-01 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

3. Rotor: Composite rotor for 6 port SC-FAST high flow valve (¼-28 fittings). Like part # SC-0599-1010-05 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

4. Sample loop:

   a. DLS 3018: 3 mL Teflon loop with white connector-nuts for high flow valve head, like part # SC-0315-30 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). Subsets of elements can be analyzed using different loop sizes to minimize sample consumption (e.g. 0.5 mL loop for a single element subset, or a 1.25 mL loop size for any three element subset).

   b. DLS 3018A: 0.5mL Teflon sample loop with white nut connectors for high flow valve head of FAST sample introduction system, like part # SC-0315-20 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

5. Probe, autosampler: Teflon, carbon fiber support, 0.8mm i.d., blue marker, 1/4-28 fittings. Like part number SC-5037-3751 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). *# Spares = 2.*

6. Probe, carrier solution: Teflon, carbon fiber support, 0.5mm i.d., orange marker, 1/4-28 fittings. Like part number SC-5037-3501 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). *# Spares = 2.*

7. Tubing, carrier solution: 0.5mm i.d. Teflon tubing (orange marker) with red ¼-28 male nut. Connects to high flow FAST valve head, port #2. Like part # SC-0316-0500 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

8. Tubing, nebulizer: See "Nebulizer, PolyPro-ST micro flow"

9. Tubing, rinse station: Teflon tubing and adapters (to attach to back of SC autosampler for filling rinse stations and to attach to rinse containers). Like part # SC-0302-0500, Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

10. Tubing, vacuum: Vacuum line for SC-FAST high flow valve, connects to port #6, black nut for connection to valve head, natural brown color nut on other end for connection to SC autosampler vacuum port. Like part # SC-0321 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xi. Hose, for connection to recirculator / chiller: Push on hose. I.D. = ½", O.D. = ¾". Use part # PB-8 (per inch, Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. No spares necessary.

xii. Hose, for exhaust of ICP-MS: Available as part of ICP-MS installation kit from PerkinElmer (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available direct from manufacturer as part # S-LP-10 air connector (Thermaflex, Abbeville, SC, www.thermaflex.net). Equivalent part may be substituted. *# Spares = 10 feet of 4" diameter (ELAN and NexION) and 10 feet of 6" diameter hose (ELAN).*

xiii. Injector, quartz with ball joint: I.D. = 2.0 mm. PerkinElmer part # WE023948 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available direct from

manufacturer as part # 400-30 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com) or from various distributors. # *Spares = 2.*

xiv. Ion lens (ELAN): PerkinElmer part # WE018034 (PerkinElmer Norwalk, CT, www.perkinelmer.com). # Spares = 1.

xv. Nebulizer: PolyPro-ST micro flow polypropylene nebulizer with external 1/4-28 threaded connector for liquid delivery, low pressure version or equivalent. Like part # ES-4040-7010 (Elemental Scientific Inc., Omaha, NE.. # *Spares = 1*. Different nebulizers may be used, however, the nebulizer gas flow rate, sample flush time, read delay time, loop fill time, loop size, urine sample dilution preparation volume, and sample-to-sample carry-over must be evaluated and optimized.

1. Gas connection:

   a. Teflon tubing: 4mm o.d., 2.4mm i.d. Teflon tubing (like part # ES-2502, Elemental Scientific Inc., Omaha, NE.. # *Spares = 1*.

   b. Adapter kit: Plastic adapters to connect Teflon tubing (2.4mm i.d.) to ¼" male Swagelok (compression) port on ICP-DRC-MS. Parts can be obtained as components in a "gas fittings kit for microflow nebulizer", kit part # ES-2501-1000, Elemental Scientific Inc., Omaha, NE. # *Spares = 1*.

2. Liquid connection: Connects nebulizer to port #3 of high flow FAST valve head with green, 1/4- 28 fitting. Like part # SC-0317-0250 (Elemental Scientific Inc., Omaha, NE.,. # *Spares = 2*.

xvi. Nut: (for flanged connections of 1.59mm (1/16") o.d. PFA tubing) Flanged, for 1/16" o.d. tubing, 1/4-28 threads. Use part # P-406x (pkg. of 10, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com) or equivalent. Use a Teflon-coated Viton o-ring with this nut instead of the stainless steel washer that comes with part # P-406x*)*. # *Spares = 10.*

xvii. Nut and ferrule set, 1/8" Swagelok: Such as part # SS-200-NFSET (stainless steel) or part # B-200-NFSET (brass) (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. For part numbers listed here a quantity of 1 means 1 nut, 1 front ferrule, and 1 back ferrule. *Spares = 20.*

xviii. Nut and ferrule set, 1/4" Swagelok: Such as part # SS-400-NFSET (stainless steel) or part # B-400-NFSET (brass) (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. For part numbers listed here a quantity of 1 means 1 nut, 1 front ferrule, and 1 back ferrule. *Spares = 20.*

xix. Oil for roughing pumps:

1. Welch Directorr Gold: For roughing pumps. Available direct from manufacturer as part # 8995G-15 (1 gallon, Welch Rietschle Thomas, Skokie, IL, www.welchvacuum.com) or from various distributors. Equivalent oil may be substituted. # *Spares = 4.*

2. Fomblin Y14/5 fluid: PerkinElmer part # N8122265 (1 kg bottle, PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. *# Spares =1 per instrument.*

xx. O-ring (for hyper skimmer cone, NexION): PerkinElmer part # 09902123 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. *# Spares = 20 o-rings.*

xxi. O-ring / gasket (for sampler cone):

1. ELAN (o-ring): PerkinElmer part # N8120511 (pkg. of 5, PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. *# Spares = 20 o-rings.*

2. NexION (aluminum gasket): PerkinElmer part # WE012989 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. *# Spares = 20 gaskets.*

xxii. O-ring (for skimmer cone):

1. ELAN: PerkinElmer part # N8120512 (pkg. of 5, PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. *# Spares = 20 o-rings.*

xxiii. O-ring: (for flanged connections of 1.59mm (1/16") o.d. PFA tubing) Teflon-coated Viton o-ring, i.d. = 1/16", thickness = 1/16", o.d. = 3/16". Such as part # V75-003 (O-rings West, Seattle, WA, www.oringswest.com) or equivalent. *# Spares = 20.*

xxiv. O-ring: (for injector support).

1. Internal o-rings: ID = ¼", OD = 3/8", thickness = 1/16". Need 2 o-rings per injector support setup. PerkinElmer part # N8122008 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent (such as part # V75-010, O-rings West, Seattle, WA, www.oringswest.com). *# Spares = 20.*

2. External o-rings: ID = 3/8", OD = 1/2", thickness = 1/16". Need 2 o-rings for each injector support setup. PerkinElmer part # N8122009 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent (such as part # V75-012, O-rings West, Seattle, WA, www.oringswest.com). *# Spares = 20.*

xxv. O-ring (for inside nebulizer port on standard PerkinElmer cyclonic quartz spray chamber for the ELAN): Such as part # 120-56 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com). Additional o-rings can sometimes be obtained free of charge or at reduced price when acquired while purchasing spray chambers. *# Spares = 20.*

xxvi. O-ring (for inside of ELAN bayonet torch mount): Part # WE017284 (PerkinElmer, Shelton, CT, www.perkinelmer.com). Do not substitute. The PerkinElmer o-ring is metal impregnated to minimize RF leakage though the torch mount. *# Spares = 2.*

xxvii. Photon Stop (ELAN): PerkinElmer part # WE018278 (PerkinElmer, Shelton, CT, www.perkinelmer.com). *# Spares = 1.*

xxviii. Plugs, quick change for roughing pump oil: These plugs will only work on the roughing pumps which come standard on ELAN DRC II and NexION ICP-MS instruments. These plugs will not fit the Leybold pumps which come standard on ELAN DRC Plus instruments. Part # W1011013 (PerkinElmer, Shelton, CT, www.perkinelmer.com). No spares typically needed.

xxix. RF coil. PerkinElmer part # WE02-1816 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares = 2.*

xxx. Spray chamber, quartz concentric:

1. ELAN: PerkinElmer part # WE025221 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. Available direct from manufacturer as part # 400-20 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com) or from various distributors. # *Spares = 2.*
2. NexION: PerkinElmer part # N8145013 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares = 2.*

xxxi. Torch, quartz: PerkinElmer part # N812-2006 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. Available direct from manufacturer as part # 400-10 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com) or various distributors. # *New Spares = 2.*

xxxii. Tubing, main argon delivery to instrument: I.D. = 1/8", O.D. = ¼". Such as part # C-06500-02 (pkg. of 100ft, polypropylene, Fisher Scientific International, Hampton, NH, www.fishersci.com) or equivalent. # *Spares = 50ft*.

xxxiii. Tubing, peristaltic, 0.03" i.d. (carrier solution for ESI autosampler): *use either*

1. Standard PVC, 2-stop (black / black) peristaltic pump tubing, i.d. = 0.03". PerkinElmer part # 09908587 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares = 6 packs of 12 tubes.*
2. Standard PVC, 3-stop (black/ black/black) peristaltic pump tubing, i.d. 0.76 mm. Spectron part # SC0056 (Spectron, Ventura, CA, or equivalent. #Spares = 6 packs of 12 tubes. *Use this type of tubing with ESI DXi micro-peristaltic pump.*

xxxiv. Tubing, peristaltic, 0.125" i.d. (spray chamber drain): *use either*

1. Standard PVC, 2-stop (black / white) peristaltic pump tubing, i.d. = 0.125" or equivalent. PerkinElmer part # N812-2012 (PerkinElmer, Shelton, CT,) or equivalent. # *Spares = 6 packs of 12 tubes.*
2. Standard Santoprene, 3-stop (grey/ grey/ grey) peristaltic pump tubing, i.d. 1.30 mm. Spectron part # SC0311 (Spectron, Ventura, CA,) or equivalent. #Spares = 6 packs of 12 tubes. *Use this type of tubing with ESI DXi micro-peristaltic pump.*

xxxv. Tubing, PVC, i.d. = 1/8", o.d. = 3/16". May be used to transfer liquid

1. between spray chamber waste port and peristaltic pump
2. between peristaltic pump and liquid waste jug

Like part # 14-169-7A (pkg. of 50ft, Fisher Scientific International, Hampton, NH,) or equivalent. # *Spares = 20ft*.

xxxvi. Tubing, stainless steel, o.d. = 1/8", wall thickness = 0.028": Used to connect DRC gas cylinders to ICP-MS gas ports. Like part # SS-T2-S-028-20 (20ft, Georgia Valve and Fitting, Atlanta, GA, or equivalent. *Spares = 20ft.*

xxxvii. Tubing, Teflon, corrugated, ¼" o.d.: Connects to the auxiliary and plasma gas side-arms of the torch. Part # WE015903 (PerkinElmer, Shelton, CT, www.perkinelmer.com). *# Spares = 2.*

xxxviii. Union elbow, PTFE ¼" Swagelok (ELAN bayonet mount): Connects argon tubing to torch auxiliary gas sidearm on bayonet mount NEXION ICP-MS instruments. Like part # T-400-9 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. *Spares = 2.*

xxxix. Union tee, PTFE, ¼" Swagelok (ELAN bayonet mount): Connects argon tubing to torch plasma gas sidearm and holds igniter inside torch sidearm on bayonet mount NEXION ICP-MS instruments. Like part # T-400-3 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. *Spares = 2.*

c. Sources for ICP-MS maintenance equipment & supplies

i. Anemometer: Like digital wind-vane anemometer (Model 840032, SPER Scientific LTD., Scottsdale, AZ, www.sperscientific.com) or equivalent. Use to verify adequate exhaust ventilation for ICP-MS (check with hoses fully disconnected).

ii. Pan, for changing roughing pump oil: Like part # 53216 (United States Plastics Corporation, Lima, OH, www.usplastic.com) or equivalent. # Spares = 1.

iii. Container, to hold acid baths for glassware: Polypropylene or polyethylene containers with lids (must be large enough for torch, injector, or spray chamber submersion). May be purchased from laboratory or home kitchen supply companies. *# Spares = 4*.

iv. Cotton swabs: Any vendor. For cleaning of cones and glassware.

v. Cutter (for 1/8" o.d. metal tubing): Terry tool with 3 replacement wheels. Like part # TT-1008 (Chrom Tech, Inc., Saint Paul, MN, www.chromtech.com) or equivalent.

vi. Getter regeneration kit: Part # WE023257 (PerkinElmer, Shelton, CT, www.perkinelmer.com). Use this as needed (at least annually) to clean the getter in the pathway of channel A DRC gas.

vii. Magnifying glass: Any $10x^{+}$ pocket loupe for inspection of cones and other ICP-MS parts. Plastic body is preferred for non-corrosion characteristics. Like part # 5BC-42813 (Lab Safety Supply, Janesville, WI, www.labsafety.com).

viii. Screw driver, for ion lens removal: Screw driver with long, flexible shaft, and 2mm ball-Allen end for removal of ion lens screws part # W1010620. Extra 2mm bits, part # W1010598 (PerkinElmer, Shelton, CT, www.perkinelmer.com). This is not necessary if the lens is mounted in a quick-release mount.

ix. Ultrasonic bath: Like ULTRAsonik™ Benchtop Cleaners (NEYTECH, Bloomfield, CT, www.neytech.com) or equivalent.

d. Sources for general laboratory consumable supplies

i. Bar code scanner: Like Code Reader 2.0 (Code Corporation, Draper, UT, www.codecorp.com) or equivalent. For scanning sample IDs during analysis setup. Any bar code scanner capable of reading Code 128 encoding at a 3 mil label density can be substituted.

ii. Carboy (for preparation of urine quality control pool and waste jug for ICP-MS sample introduction system): Polypropylene 10-L carboy (like catalog # 02-960-20C, Fisher Scientific, Pittsburgh, PA, www.fischersci.com) or equivalent. Carboys with spouts are not advised due to potential for leaking.

iii. Containers for diluent and rinse solution: Two liter Teflon™ containers (like catalog# 02-923-30E, Fisher Scientific, Pittsburgh, PA., www.fishersci.com) and 4L polypropylene jugs (like catalog# 02-960-10A, Fisher Scientific, Pittsburgh, PA, www.fishersci.com) have both been used. Acid rinse before use. Equivalent containers may be substituted.

iv. Cups for urine collection: Like polypropylene 4.5 oz cup, catalog # 354013 (Becton Dickinson Labware, Franklin Lakes, NJ, www.bd.com) or equivalent. Each lot of cups used must be lot screened (tested to be free of trace metal contamination). Colorless plastics tend to have lowest trace metal contamination.

v. Gloves: Powder-free, low particulate nitrile (like Best CleaN-DEX™ 100% nitrile gloves, any vendor). Equivalent nitrile or latex gloves may be substituted (nitrile only for handling calibration stock standard solution B which contains 1% HF).

vi. Paper towels: For general lab use, any low-lint paper wipes such as KIMWIPES®EX-L Delicate Task Wipers or KAYDRY®EX-L Delicate Task Wipers (Kimberly-Clark Professional, Atlanta, GA, www.kcprofessional.com). For sensitive applications in cleanrooms, a wipe designed for cleanroom use may be desired such as the Econowipe or Wetwipe (Liberty, East Berlin, CT, www.liberty-ind.com).

vii. Pipette (for preparation of urine dilutions to be analyzed): Micromedic Digiflex-CX Automatic™ pipette equipped with 10.0-mL dispensing syringe, 2 mL sampling syringe, 0.75-mm tip, and foot pedal (Titertek, Huntsville, AL, http://www.titertek.com/).

viii. Pipettes (for preparation of intermediate stock working standards & other reagents): Like Brinkmann Research Pro Electronic pipettes (Brinkmann Instruments, Inc., Westbury, NY, http://www.brinkmann.com/home/). 5-100 µL (catalog #4860 000.070), 20-300 µL (catalog #4860 000.089), 50-1000 µL (catalog #4860 000.097), 100-5000 µL (catalog #4860 000.100). Note: pipette catalog numbers are without individual chargers. Can purchase individual chargers (pipette catalog numbers will differ) or a charging stand that will hold four pipettes (catalog #4860 000.860). When purchasing pipette tips (epTips), purchase one or more boxes, then “reloads” for those boxes after that: 5-100 µL (box catalog # 22 49 133-4, reload catalog # 22 49 153-9), 20-300 µL (box catalog # 22 49 134-2, reload catalog # 22 49 154-7), 50-1000 µL (box catalog # 22 49 135-1, reload catalog # 22 49 155-5), 100-5000 µL (box catalog # 22 49 138-5, reload catalog # 22 49 198-9, bulk bag catalog # 22 49 208-0). Equivalent pipettes and tips can be substituted.

ix. Tubes for sample analysis (for autosampler): Like polypropylene 15-mL conical tubes, BD Falcon model #352097 (Becton Dickinson Labware, Franklin Lakes, NJ, www.bd.com). Equivalent tubes may be substituted which are shown by lot screening to be free of trace metal contamination. Clear plastics tend to have lowest trace metal contamination. Blue colored caps have also been used successfully for this method.

x. Tubes for storage of intermediate working stock standards: Like polypropylene 50-mL conical tubes, BD Falcon model #352098 (Becton Dickinson Labware, Franklin Lakes, NJ, www.bd.com). For use in storage of intermediate working stock standards. Equivalent tubes may be substituted which are shown by lot screening to be free of trace metal contamination. Clear plastics tend to have lowest trace metal contamination. Blue colored caps have also been used successfully for this method.

xi. Vortexer: Like MV-1 Mini Vortexer (VWR, West Chester, PA, www.vwr.com). Used for vortexing urine specimens before removing an aliquot for analysis. Equivalent item can be substituted.

xii. Water purification system: Like NANOpure DIamond Ultrapure Water System (Barnstead International, Dubuque, Iowa, www.barnstead.com). For ultra-pure water used in reagent and dilution preparations. An equivalent water purification unit capable of producing ≥18 Mega-ohm·cm water may be substituted.

e. Sources of chemicals, gases, and regulators

i. Acid, hydrochloric acid: Environmental Grade, 30-38% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com). This is referred to as “concentrated” hydrochloric acid in this method write-up. For use in preparation of intermediate working stock standards. An equivalent hydrochloric acid product may be substituted, but it must meet or exceed the purity specifications of this product for trace metals content.

ii. Acid, nitric acid: Environmental Grade, 70% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com). For use in diluent, rinse solution, intermediate working stock standards, and QC pool preparations. This is referred to as “concentrated” nitric acid in this method write-up. An equivalent nitric acid product may be substituted, but it must meet or exceed the purity specifications of this product for trace metals content.

iii. Ethanol (EtOH): USP dehydrated 200 proof (Pharmco Products, Inc.) or equivalent.

iv. Argon gas (for plasma & nebulizer) and Regulator: High purity argon (>99.999% purity, Specialty Gases Southeast, Atlanta, GA, www.sgsgas.com) for torch and nebulizer. Minimum tank source is a dewar of liquid argon (180-250L). Bulk tank (1500^{+}L is preferred).

1. Regulator for argon (at dewar): Stainless steel, single stage, specially cleaned regulator with 3000 psig max inlet, 0-200 outlet pressure range, CGA 580 cylinder connector, and needle valve shutoff on delivery side terminating in a ¼” Swagelok connector. Part number

"KPRCGRF415A2/AG10-AR1" (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com). An equivalent regulator from an alternate vendor may be substituted. # *Spares = 1*.

2. Regulator for argon (between bulk tank and PerkinElmer filter regulator): Single Stage 316SS Regulator, with 0-300 psi Inlet Gauge, 0-200 psi Outlet Gauge, Outlet Spring Range, 0-250 psi, ¼" Swagelok Inlet Connection, ¼ turn Shut off Valve on Outlet with ¼" Swagelok Connection and Teflon Seals. Part number KPR1GRF412A20000-AR1 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com). An equivalent regulator from an alternate vendor may be substituted. # *Spares = 1*.

3. Regulator for argon (filter regulator on back of ICP-MS):

   a. ELAN: Argon regulator filter kit. Catalog number N812-0508 (PerkinElmer, Shelton, CT, www.perkinelmer.com).

   b. NexION: Argon regulator filter kit. Catalog number N8145023 (PerkinElmer, Shelton, CT, www.perkinelmer.com).

v. Argon / hydrogen: Argon (90%) / hydrogen (10%) for DRC channel A. Initial purity of argon = 99.9997^{+}% ("Research grade 5.7"). Initial purity of hydrogen = 99.9999^{+}% ("Research Grade 6.0"). Mixture is typically purchased in cylinder size 35 (6"x24") (Airgas South, Atlanta, GA, www.airgas.com).

1. Regulator for argon / hydrogen: Stainless steel, two stage, specially cleaned regulator with 3000 psig max inlet, 0-25 outlet pressure range, CGA 350 cylinder connector, and needle valve shutoff on delivery side terminating in a ¼" Swagelok connector. Like part number KCYADPF412A2AD10 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com). An equivalent regulator from an alternate vendor may be substituted. # *Spares = 1*.

2. Flash arrestor (stainless steel): Like part # 6104 (Matheson Tri Gas, Montgomeryville, PA, www.mathesontrigas.com) or equivalent.

vi. Disinfectant, for work surfaces: Bleach-rite spray (any distributor). On-site dilutions of bleach (1 part bleach + 9 parts water) may be substituted, but must be re-made daily.

vii. Oxygen: Oxygen ("Research Grade Research Grade 5.0", 99.9999% purity) for DRC channel B. Typically purchased in cylinder size 300 (9.5" x 54") (Airgas South, Atlanta, GA, www.airgas.com).

1. Regulator for oxygen: High purity brass body with monel trim, two stage regulator. Stainless steel is not used for this application due to safety concerns of working with oxygen at high pressure [13]. For one regulator, order the following parts, and ask that they be tested and assembled (Engineered Specialty Products, Kennesaw, GA, www.espgauges.com).

   a. Tescom part # 44-3410S24-555
   Regulator body: Brass bar stock, two stage, Monel trim, TFE seats, Elgiloy diaphragms, Cv=0.05, 3000 psig max inlet, 1-25 psig outlet range, 1/4 FNPT inlet / outlet / gauge ports, O_2 cleaned to ASTMG93 and CGA4.1.

b. Tescom part # 60500-3000N
Inlet pressure gauge: 2" diameter, 0-3000 psig range , O_2 cleaned, ¼" MNPT bottom, brass.

c. Tescom part # 60500-0015N
Delivery pressure gauge: 2" diameter, 0-15 psig range , O_2 cleaned, ¼" MNPT bottom, brass.

d. Tescom part # 63842-540-B
NPT to CGA Adaptor: ¼" NPT to CGA 540 adapter, brass.

e. Swagelok part # B-200-1-4:
Adapter: Brass male connector, ¼" MNPT to 1/8" Swagelok (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com).

An equivalent regulator from an alternate vendor may be substituted.
 # Spares = 1.

2. Flash arrestor (brass): Like part # 6103 (Matheson Tri Gas, Montgomeryville, PA, www.mathesontrigas.com) or equivalent.

viii. Standard, dual detector: Like item # SM-2107-052 (High Purity Standards, Charleston, SC, http://www.hps.net/).

ix. Standard, gallium: Like 1,000 mg/L, item # PLGA2-2Y. (SPEX Industries, Inc., Edison, NJ, www.spexcsp.com). Used as an internal standard in diluent. Any vendor whose standards are traceable to the National Institute for Standards and Technology may be substituted. The standard must have low trace metal contamination. Gallium is only used in the diluent for the measurement of arsenic (As).

x. Standard, iridium: Like 1,000 mg/L iridium, item # PLIR3-2Y (SPEX Industries, Inc., Edison, NJ, www.spexcsp.com). Used as an internal standard in diluent. Any vendor whose standards are traceable to the National Institute for Standards and Technology may be substituted. The standard must have low trace metal contamination.

xi. Standard, multi-element stock calibration standard: Item numbers "SM-2107-037 solution A" and "SM-2107-037 solution B" (High Purity Standards, Charleston, SC, http://www.hps.net/). This is a set of custom mixes for concentrations). Both are needed to cover all analytes of methods 3018 and 3018A. These solutions are diluted to prepare the intermediate stock working standards, which are in turn diluted to prepare the working calibrators. This solution can be prepared in-house from NIST traceable single element stock solutions if necessary.

xii. Standard, rhodium: Like 1,000 mg/L, item # PLRH3-2Y. (SPEX Industries, Inc., Edison, NJ, www.spexcsp.com). Used as an internal standard in diluent. Any vendor whose standards are traceable to the National Institute for Standards and Technology may be substituted. The standard must have low trace metal contamination.

xiii. Standard, single element stock standards for preparation of urine quality control pools: National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs) 3103a (As), 3105a (Be), 3113 (Co), 3132 (Mn), 3134 (Mo), 3108 (Cd), 3102a (Sb), 3111a (Cs), 3104a (Ba), 3163 (W), 3128 (Pb), 3140 (Pt), 3161a (Sn), 3156a (Sr), 3158 (Tl), and 3164 (U) (National Institute of Standards and Technology (NIST), Office of Standard Reference Materials, Gaithersburg, MD, www.nist.gov). Other sources of standards can be used if they are NIST traceable.

xiv. Triton X-100™ surfactant: Like "Baker Analyzed" TritonX-100™ (J.T. Baker Chemical Co., www.jtbaker.com). Another source may be substituted, but it must be free of trace-metal contamination.

xv. Standard, gold: Like 10,000 μg/mL, cat # 10M21-2. (High Purity Standards, Charleston, SC, www.highpuritystandards.com). Used in diluent and rinse solution. Any vendor whose standards are traceable to the National Institute for Standards and Technology may be substituted. The standard must have low trace metal contamination.

6) Preparation of reagent and materials

a. Intermediate internal standard mixture

i. Purpose: Preparation of single intermediate solution containing internal standards will simplify the addition of the internal standards into the final diluent solution. This solution can be purchased rather than prepared.

ii. Preparation:

1. For DLS 3018:

To prepare 200 mL of 2% v/v HNO_3, 40 µg/L Ir and Rh intermediate internal standard solution:

a. If not previously dedicated to this purpose, acid wash a 200 mL volumetric flask (PP, PMP, or Teflon™). For example, with 2% (v/v) HNO_3 and ≥18 Mega-ohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.

b. Partially fill the 200 mL volumetric flask with ≥18 Mega-ohm·cm water (approximately 100-150 mL).

c. Carefully add 4 mL of concentrated nitric acid. Mix into solution.

d. Add 0.8 mL of 10,000 μg/mL Rh standard. If initial Rh standard concentration is different, adjust volume proportionally.

e. Add 0.8 mL of 10,000 μg/mL Ir standard. If initial Ir standard concentration is different, adjust volume proportionally.

f. Fill to mark (200mL) and mix thoroughly.

g. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

2. For DLS 3018A:

   To prepare 200 mL of 2% v/v HNO_3, 40 µg/L Ga intermediate internal standard solution:

   a. If not previously dedicated to this purpose, acid wash a 200 mL volumetric flask (PP, PMP, or Teflon™). For example, with 2% (v/v) HNO_3 and ≥18 Mega-ohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.
   b. Partially fill the 200 mL volumetric flask with ≥18 Mega-ohm·cm water (approximately 100-150 mL).
   c. Carefully add 4 mL of concentrated nitric acid. Mix into solution.
   d. Add 0.8 mL of 10,000 µg/mL Ga standard. If initial Ga standard concentration is different, adjust volume proportionally.
   e. Fill to mark (200mL) and mix thoroughly.
   f. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

3. For emergency response combinations of 3018 and 3018A:

   To prepare 200 mL of 2% v/v HNO_3, 40 µg/L Ga, Rh and Ir intermediate internal standard solution:

   NOTE: Gallium at 10 µg/L in the method diluent or 100 µg/L in a urine specimen (extremely unlikely) will produce approximately a +2 µg/L bias on observed Sr concentration due to the formation of $^{71}Ga^{17}O+$, which occurs at the same m/z as $88Sr^+$. While this interference is not large in comparison to typical Sr concentrations in urine, ~150 µg/L, best urine Sr accuracy for biomonitoring purposes will be achieved when Ga is not present in the diluent as an internal standard. If As is combined with the multi-element method for emergency response purposes, the resulting bias on Sr from the $^{71}Ga^{17}O^+$ interference is not likely to hinder the ability to hinder the identification of acute Sr exposures.

   a. If not previously dedicated to this purpose, acid wash a 200 mL volumetric flask (PP, PMP, or Teflon™). For example, with 2% (v/v) HNO_3 and ≥18 Mega-ohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.
   b. Partially fill the 200 mL volumetric flask with ≥18 Mega-ohm·cm water (approximately 100-150 mL).
   c. Carefully add 4 mL of concentrated nitric acid. Mix into solution.
   d. Add 0.8 mL of 10,000 µg/mL Rh standard. If initial Rh standard concentration is different, adjust volume proportionally.
   e. Add 0.8 mL of 10,000 µg/mL Ir standard. If initial Ir standard concentration is different, adjust volume proportionally.

f. Add 0.8 mL of 10,000 μg/mL Ga standard. If initial Ga standard concentration is different, adjust volume proportionally.

g. Fill to mark (200mL) and mix thoroughly.

h. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

b. Intermediate Triton X-100™ solution (for DLS 3018 and DLS 3018A):

i. Purpose: To avoid the time-consuming process of dissolving Triton X-100 on a daily basis for use in rinse solution, prepare an intermediate solution for daily use.

ii. Preparation: To prepare 2L of 2% Triton X-100™ in 5% (v/v) HNO_3:

1. If not previously dedicated to this purpose, acid wash a 2 L bottle (PP, PMP, or Teflon™). For example, with 2% (v/v) HNO_3 and >18 Mega-ohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.
2. Partially fill the bottle with ≥18 Mega-ohm·cm water (approximately 1-1.5 L). Use of volumetric flask is not required.
3. Add 40 mL of Triton X-100™ and stir until completely dissolved. Use a Teflon™ stir bar and stir plate if necessary (acid wash stir bar before use).
4. Carefully add 100 mL of concentrated nitric acid.
5. Fill to 2 L and stir thoroughly.
6. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

c. Diluent and carrier

i. Purpose: All samples (blanks, calibrators, QC, or patient samples) are combined with the diluent during the sample preparation step before analysis. This is where the internal standards are added which during the analysis will compensate for instrumental variations on the analyte signal. If using the FAST sample introduction system, the diluent is also used as the carrier solution.

ii. Preparation:

1. For DLS 3018
and emergency response combinations of methods 3018 and 3018A:

To prepare 4L of an aqueous solution of 10 microgram/L internal standards and 500 μg/L gold in 2% (v/v) nitric acid:

a. If not previously dedicated to this purpose, acid wash a 4 L container (PP, PMP, or Teflon™). For example, with 2% (v/v) HNO_3 and >18

Mega-ohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.

b. Partially fill the 4 L container with ≥18 megaohm·cm water (~2/3 full).

c. Carefully add 80 mL concentrated nitric acid and mix.

d. Add spike of internal standard solution (to use other concentrations or volumes, adjust the volumes proportionally).

i. If for method 3018, add 1 mL of the 40 μg/mL Rh and Ir internal standard solution.

ii. If for emergency response combinations of methods 3018 and 3018A, add 1 mL of the 40 μg/mL Rh, Ir, and Ga internal standard solution.

e. Add 200 μL of the 10,000 μg/mL gold standard.

f. Make up to volume (4 L) with ≥18 megaohm·cm water.

g. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

2. For DLS 3018A

To prepare an 10 μg/L Ga in 2% (v/v) nitric acid and 1.5% (v/v) ethanol:

i. If not previously dedicated to this purpose, acid wash a 4 L container (PP, PMP, or Teflon™). For example, with 2% (v/v) HNO_3 and >18 Mega-ohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.

ii. Partially fill the 4 L container with ≥18 megaohm·cm water (~2/3 full).

iii. Carefully add 80 mL concentrated nitric acid and mix.

iv. Carefully add 60 mL dehydrated 200 proof ethanol and mix.

v. Add 1 mL of the 40 μg/mL Ga internal standard solution. To use other concentrations, adjust the volume proportionally.

vi. Make up to volume (4 L) with ≥18 megaohm·cm water.

vii. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

d. ICP-MS rinse solution

i. Purpose: Pump this solution into the sample introduction system between samples to prevent carry-over of the analytes of interest from one sample measurement to the next.

ii. Preparation:

1. For DLS 3018
and emergency response combinations of methods 3018 and 3018A

To Prepare 4 L of 0.002% Triton X-100™, 5% (v/v) nitric acid solution and 500 µg/L gold:

a. If not previously dedicated to this purpose, acid wash a 4 L bottle (PP, PMP, or Teflon™). For example, with 5% (v/v) HNO_3 and >18 Mega-ohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.

b. Partially fill the bottle with ≥18 Mega-ohm·cm water (approximately 2-3 L). Use of volumetric flask is not required, but the same diluent must be used for the entire analytical run on the ICP-MS.

c. Add 4 mL of the 2% Triton X-100™ / 5% (v/v) nitric-acid intermediate stock solution and mix well.

d. Carefully add 200 mL of concentrated nitric acid and mix well.

e. Add 200 µL of the 10,000 µg/mL gold.

f. Fill to 4 L using ≥18 Megaohm·cm water.

g. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

2. For DLS 3018A

To prepare 4 L of 0.002% Triton X-100™, 5% (v/v) HNO_3 solution and 1.5% (v/v) ethanol:

a. If not previously dedicated to this purpose, acid wash a 4 L bottle (PP, PMP, or Teflon™). For example, with 5% (v/v) HNO_3 and >18 Mega-ohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.

b. Partially fill the bottle with ≥18 Mega-ohm·cm water (approximately 2-3 L). Use of volumetric flask is not required, but the same diluent must be used for the entire analytical run on the ICP-MS.

c. Add 4 mL of the 2% Triton X-100™ / 5% (v/v) nitric-acid intermediate stock solution and mix well.

d. Carefully add 200 mL of concentrated nitric acid and mix well.

e. Carefully add 60 mL dehydrated 200 proof ethanol and mix well.

f. Fill to 4 L using ≥18 Megaohm·cm water.

g. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

e. Standards, calibrators, and QC

i. Multi-element stock calibration standard

1. Purpose: These two master solutions will be diluted to prepare intermediate working calibrators.

2. Preparation: These solutions may be purchased or prepared from NIST-traceable standards.

a. Solution A: 5% HNO_3 solution containing As, Ba, Be, Cd, Cs, Co, Pb, Mn, Sr, Tl, and U.

b. Solution B: 5% HNO_3, 1% HF, 0.5% HCl solution containing Sb, Mo, Pt, Sn, and W.

3. Storage: Store at room temperature and label appropriately. Expiration is determined by manufacturer or is 1 year after the container is opened (whichever comes first).

ii. Intermediate multi-element stock calibration standard

1. Purpose: The two stock standards are combined into a single intermediate stock calibration standard preparation.

2. Preparation: To prepare standards in 2% (v/v) nitric acid and 1% (v/v) hydrochloric acid.

a. If not previously dedicated to this purpose, acid wash a 100mL PP, PMP, or Teflon™ volumetric flask. For example, with a 2% (v/v) HNO_3 / 1% v/v HCl solution and >18 Mega-ohm·cm water (at least 3 times each) followed by verifying cleanliness through analysis of rinsate. Dedicate to purpose.

b. Partially fill the 100 mL flask with the 2% (v/v) nitric acid and 1% (v/v) hydrochloric acid prepared in Section 6.e.iii.b (50-75% full).

c. Pipette the appropriate volume of the multi-element stock calibration standard solutions (both A and B) into the volumetric flask. Dilute to the volumetric mark with the 2% (v/v) nitric acid and 1% (v/v) hydrochloric acid using a pipette for the final drops. Mix each solution thoroughly.

d. Once mixed, transfer to an acid-cleaned, labeled, 50 mL container (PP, PMP, or Teflon™) for storage.

e. Label appropriately and store at room temperature. Expiration is 1 year from the date of preparation.

iii. Multi-element intermediate working calibration standards

1. Purpose: Five multi-element standards (6 including S0) used each day of analysis to prepare the final working calibrators.

2. Preparation: To prepare multi-element standards S0-S5 in 2% (v/v) HNO_3, 1% (v/v) HCl:

a. Cleaning flasks: If not previously dedicated to this purpose, acid wash four 100-mL, one 200-mL, one 500-mL PP, and one 2 L PP (or PMP) volumetric flasks. For example, with a 2% (v/v) HNO3 / 1% v/v HCl solution and >18 Mega-ohm·cm water (at least 3 times each) followed by verifying cleanliness through analysis of rinsate. Mark each flask according to intended use. Dedicate to purpose.

b. HNO_3 & HCl diluent (S0) preparation: In the cleaned 2L flask, add 1-1.5L $\geq$18 Megaohm·cm water, 40 mL high purity concentrated HNO_3, and 20 mL high purity concentrated HCl. Fill to the mark and mix

thoroughly. Use this diluent to fill the remaining flasks during preparation of the intermediate working calibration standards.

c. Dilutions & storage:

i. Fill two acid-cleaned 50-mL containers (PP, PMP, or Teflon™) with the HNO_3 & HCl diluent. Label appropriately (including "S0") and store at room temperature. Expiration is 1 year from the date of preparation.

ii. Partially fill the 100 mL, 200 mL, and 500 mL flasks with the HNO_3 & HCl diluent (50-75% full).

iii. Pipette the appropriate volume of the multi-element stock calibration standard or the multi-element intermediate stock calibrator solutions (both A and B) into each of the volumetric flasks. Dilute each to the volumetric mark with the HNO_3 & HCl diluent using a pipette for the final drops. Mix each solution thoroughly. Once mixed, transfer to acid-cleaned, labeled, 50-mL containers (PP, PMP, or Teflon™) for storage.

iv. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

iv. Working multi-element calibrators

1. Purpose: The working multi-element calibrators will be analyzed in each run to provide a signal-to-concentration response curve for each analyte in the method. The concentration of an analyte in a patient urine sample dilution is determined by comparing the observed signal from the dilution of the patient urine sample to the response curve from the working multi-element calibrators.

2. Preparation: Make dilutions of the intermediate working calibration standards (S0-S5) immediately prior to analysis by combining with base urine (Section 6.e.v) and diluent (Section 6.c) using a Digiflex automatic pipettor. Expiration of capped dilutions is 3 days from preparation.

v. Base urine

1. Purpose: This urine pool material will be mixed with the intermediate working calibrators just prior to analysis to matrix-match the calibration curve to the urine matrix of the unknown samples.

2. Contents: A mixture of multiple urine sources collected from anonymous donors are used to approximate an average urine matrix.

3. Preparation & storage:

a. Collect urine anonymously by placing screened containers and collection cups in the restrooms with a sign stating the reason the specimens are being collected, the name of the investigator to contact for additional information, and requesting that people provide a urine specimen (see supervisor regarding potential Institutional Review Board, IRB, requirements).

b. Once collected, analyze to ensure that concentrations of the analytes in this method are relatively low, so as to not interfere with the proper measurement of calibrators.

c. Once screened, mix the urine collections together in a larger container (polypropylene (PP), polymethylpentene (PMP), or Teflon™) which as been acid washed. For example, with 2% (v/v) HNO_3 and >18 Mega-ohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Add large Teflon™ stir bar and stir for 30^+ minutes.

d. For short term storage, store at 2-8 °C. For long-term storage, dispense into smaller-volume tubes (i.e., 50-mL acid-washed or lot screened polypropylene tubes) and store at ≤ -20 °C.

e. Label appropriately, e.g. "Base Urine for Multi-element Method", "Store Long Term at ≤ -20 °C", "Store Short Term at 2-8 °C", preparation date, expiration date 3 years from prep date, and preparer's initials.

vi. Internal quality control materials ("Bench" QC)

1. Purpose: Internal (or "bench") quality control (QC) materials are used to evaluate the accuracy and precision of the analysis process, and to determine if the analytical system is "in control" (is producing results that are acceptably accurate and precise). They are included in the beginning and at the end of each analytical run.

2. Content: The internal (or "bench") quality control (QC) materials used in this method are pooled human urine, acidified to 1% (v/v) HNO_3, and may have been spiked to reach a desired concentration. The analyte concentrations are in the low-normal concentration range ("low QC"), high-normal concentration range ("high QC") and above-normal concentration range ("elevated QC").

3. Preparation & storage: Quality control materials can be either prepared by and purchased from an external laboratory or prepared within the CDC laboratories. Quality control must always be traceable to the National Institute for Standards and Technology (NIST). The CDC laboratory currently prepares its own bench QC materials using the following procedures:

a. Collection of urine: Collect urine anonymously by placing screened containers and / or collection cups in the restrooms with a sign stating the reason the specimens are being collected, the name of the investigator to contact for additional information, and requesting that people provide a urine specimen. Volume of urine to collect is dependent on the desired pool size. This write-up will assume a 10-L pool size for both the low and high bench QC.

b. Screening urine: Screen collected samples for metal content before mixing together to make separate pools that will be spiked to low, high, and elevated levels. Samples can be screened individually or after combining several together (reduces number of analyses).

i. Keep urine refrigerated whenever possible to minimize microbial growth.

ii. Because this is only a quick screen of the metal content, the number of replicates in the urine method can be reduced to one in order to reduce analysis time.

iii. Spike analyte concentrations for the low bench QC pool in the low-normal population range. Spike analyte concentrations for the high bench QC pool less than some preselected target concentration values in the high normal population range. See the National Report on Human Exposure to Environmental Chemicals for estimations of the normal population ranges for metals (http://www.cdc.gov/exposurereport/).

c. Combining collected urine: Be attentive not to combine only diluted matrix urine samples into the low pool and only concentrated matrix urine samples into the pool for high and elevated QC. The goal is for combining samples is to approach an 'average' matrix for each pool.

i. Graduate four acid-washed 10-L carboys (PP or PMP) in 0.5 L increments (two will be used for decanting into).

ii. Combine collected urine samples into separate acid-washed 10-L carboys (PP or PMP), according to their concentrations, for the low high, and elevated bench QC pools.

iii. Mix each urine pool using large acid washed, Teflon™ coated stir bars and large stir plates. Keep urine refrigerated whenever possible.

iv. Acidify each urine pool to 1% (v/v) HNO_3 by adding the appropriate volume of concentrated HNO_3. Stir for 30+ min on large stir plates.

d. Settling out of solids:

i. Refrigerate the urine (no stirring) for 1-3 days to allow for settling out of solids.

ii. For each urine pool, decant the urine into another of the acid-washed 10-L carboys to remove the urine from the solids settled out on the bottom of the carboy.

iii. Repeat steps (i) and (ii) until minimal solids are left at the bottom of the carboy after sitting overnight.

e. Spiking of urine

i. Analyze a sample of each urine pool. Record these results for future recovery calculations.

ii. Use these results to determine target analyte concentrations possible for the pools

iii. Calculate the volume of single element standards needed to spike each pool to the desired concentrations.

iv. While stirring the pools on large stir plates, spike each pool with calculated volumes of single element standards (all spiking standards used must be traceable to NIST).

v. Continue to stir pools for 30+ minutes after spiking, then reanalyze.

vi. Repeat steps 4 and 5 until all analytes reach target concentrations keeping track of the total volume of spiking solution added to each urine pool.

f. Dispensing and storage of urine

i. Container types: Dispense urine into lot screened containers (i.e. – 2 to 15 mL polypropylene tubes). If possible, prepare tubes of QC which have only enough volume for one typical run + 1 repeat analysis (e.g. 1.8mL). This allows for one vial of QC to be used per day of analysis, reducing chances of contamination of QC materials due to multi-day use.

ii. Labels: Place labels on vials after dispensing and capping if the vials are originally bagged separately from the caps. This minimizes the chance for contamination during the process. Include at least the name of QC pool (text and bar code), date of preparation, and a vial number on the labels.

iii. Dispensing: Dispensing can be accomplished most easily using a Digiflex automatic pipettor in continuous cycling dispense mode. Complete this process in a clean environment (i.e., a class 100 cleanroom area or hood).

1. Allow urine pool to reach room temperature before dispensing (to prevent temperature gradients possibly causing concentration gradients across the large number of vials being dispensed and to prevent condensation problems during labeling of vials). This may require leaving the carboy of urine at room temperature overnight before dispensing.

2. Replace the tubing attached to the dispensing syringe (left when looking at front of Digiflex) with a length of clean Teflon™ tubing long enough to reach into the bottom of the 10L carboy while it is sitting on the stir plate.

3. Check cleanliness of Digiflex before use by analyzing 1-2% (v/v) HNO_3 which has been flushed through the Digiflex with a portion of the same solution which has not been through the Digiflex.

4. Approximately one hour before dispensing begins,

a. With the large stir plate close to the left side of the Digiflex, begin stirring the urine pool to be dispensed.

b. Also during this time, flush the Digiflex with urine from the pool to be dispensed. Place the ends of the tubing attached to both the sample and dispensing syringes into the carboy

of urine so that urine won't be used up during this process. Be sure to secure both ends of tubing in the carboy with Parafilm so they will not come out during the flushing process.

5. After dispensing the urine into the vials, cap the vials and label them. Placing labels on vials after capping minimizes the chance for contamination during the process.

iv. Homogeneity testing: After dispensing, check homogeneity of analyte concentrations in pool aliquots by analysis of vials selected from across those dispensed. Seek guidance from a statistician regarding the number of vials needed for homogeneity analysis.

v. Storage: Store long-term as smaller portions for daily use (e.g. 2 mL cryovials) according the same storing and handling criteria described in Section 3.

f. Optimization solutions

i. DRC optimization (cell gas flow rate and RPq):

1. Purpose: For periodic testing of the DRC cell parameters. Procedure requires at a minimum a blank (i), an analyte solution (ii), a blank with interference (iii), and an analyte and interference containing solution (iv).

2. Content:

Diluent in this section refers to sample diluent (10 µg/L internal standards and 500 µg/L gold in 2% v/v nitric acid) described in Section 6c.

a. Solutions for testing elimination of $^{98}Mo^{16}O$ interference on ^{114}Cd:

i. Base urine in diluent (1 + 9)
ii. Base urine in diluent (1 + 9) + 0.24 µg/L Cd
iii. Base urine in diluent (1 + 9) + 300 µg/L Mo
iv. Base urine in diluent (1 + 9) + 0.24 µg/L Cd + 300 µg/L Mo

b. Solutions for testing elimination of $^{39}K^{16}O$ interference on ^{55}Mn:

i. Base urine in diluent (1 + 9)
ii. Base urine in diluent (1 + 9) + 0.3 µg/L Mn
iii. Base urine in diluent (1 + 9) + 400 µg/L K
iv. Base urine in diluent (1 + 9) + 0.3 µg/L Mn + 400 µg/L K

3. Preparation & storage: Different volumes may be prepared by adding proportionally larger or smaller volumes of solution constituents. Interference concentrations can be prepared higher as needed by adjusting the volume of this spike. Keep interference spike volume small (<0.3 mL) using a high concentration stock solution (i.e. 1000 mg/L). Analyte concentrations can be made higher if needed for sensitivity reasons by preparing a higher concentration calibrator.

a. Solutions for testing elimination of $^{98}Mo^{16}O$ interference on ^{114}Cd:

i. Base urine in diluent (1 + 9)
   1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 0 as described in Table 8a (multiply volumes by 5).

ii. Base urine in diluent (1 + 9) + 0.24 μg/L Cd
   1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 2 as described in Table 8a (multiply volumes by 5).

iii. Base urine in diluent (1 + 9) + 300 μg/L Mo
   1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 0 as described in Table 8a (multiply volumes by 5).
   2. Add 0.015 mL of 1000 mg/L Mo.

iv. Base urine in diluent (1 + 9) + 0.24 μg/L Cd + 300 μg/L Mo
   1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 2 as described in Table 8a (multiply volumes by 5).
   2. Add 0.015 mL of 1000 mg/L Mo.

b. Solutions for testing elimination of $^{39}K^{16}O$ interference on ^{55}Mn:

i. Base urine in diluent (1 + 9)
   1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 0 as described in Table 8a (multiply volumes by 5).

ii. Base urine in diluent (1 + 9) + 0.3 μg/L Mn
   1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 2 as described in Table 8a (multiply volumes by 5).

iii. Base urine in diluent (1 + 9) + 400 μg/L K
   1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 0 as described in Table 8a (multiply volumes by 5).
   2. Add 0.02 mL of 1000 mg/L K

iv. Base urine in diluent (1 + 9) + 0.3 μg/L Mn + 400 μg/L K
   1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 2 as described in Table 8a (multiply volumes by 5).
   2. Add 0.02 mL of 1000 mg/L K.

c. Store at room temperature and prepare as needed.
d. Label appropriately, i.e. “Store at room temperature”, preparation date, expiration date one year from preparation date, and preparer’s initials.

ii. DRC optimization (axial field voltage):

1. Purpose: Use as necessary to verify the elimination of crosstalk phenomenon. The crosstalk phenomenon will present itself as increased sensitivity for an ion of interest in the presence of greater population of spectator ions in the DRC cell. Elimination is verified when the ratio of the intensity for iridium in standard 5 versus standard 0 is the same (1.00 ± 0.05).
2. Content: Working calibration standards 0 and 5 (see Section 6.e.iv).
3. Preparation and Storage: See section 6.e.iv.

iii. For dual detector calibration:

1. Purpose: Use as necessary to perform the dual detector calibration if any element exceeds 1,000,000 cps for calibration standard 5 (typically Sr).
2. Content: Dilutions of single element or special mix stock standards in 2% (v/v) HNO_3. Recommended elements include: As, Ba, Cs, Co, Pb, Mo, Sr, Sn. Other elements can be added as required for optimal instrument performance (esp. if measured intensities approach 500,000 cps in highest working calibrator).
3. Preparation & storage: To prepare elements of interest at 200 µg/L in 2% (v/v) HNO_3:
   a. Partially fill a 50 mL lot screened or acid-washed polypropylene tube with 2% (v/v) HNO_3,
   b. Add a 0.1 mL of 100 ug/mL special mix standard.
   c. Add 0.01 mL of any additional 1,000 ug/mL single element stock standard desired to be added.
   d. Dilute to the 50 mL mark with 2% (v/v) HNO_3.
   e. Label appropriately and store at room temperature. Expiration date is one year from preparation date.

7) Analytical instrumentation setup

(see Section 5 for details on hardware used, including sources)

a. Instrumentation & equipment setup:

i. Configuration for liquid handling

1. FAST valve setup: See Section 5.b “FAST / ESI SC4-DX autosampler accessories” for source information.

a. Port 1: sample loop (white nut).
b. Port 2: 0.5 mm ID probe (red nut) for carrier solution.
c. Port 3: nebulizer line (green nut) for transfer of liquid to nebulizer.
d. Port 4: sample loop (white nut).
e. Port 5: 0.8 mm ID probe (blue nut) for diluted samples.
f. Port 6: vacuum line (black nut).

2. Carrier solution uptake: Use peristaltic pump to control uptake flow rate of carrier solution to the SC-FAST valve. Use of a 'peristaltic to Teflon tubing adapter' for prevents damage to small i.d. tubing when making connections (see consumables descriptions in Section 5.b).

3. Spray chamber waste removal

Use of a 'peristaltic to Teflon tubing adapter' for prevents damage to small i.d. tubing when making connections (see consumables descriptions in Section 5.b).

a. Between spray chamber and peristaltic tubing:
   i. Spray chambers with threaded connection: Use vendor-supplied threaded connector on base of chamber, connecting tubing directly to peristaltic pump tubing through a PEEK adapter or directly.
   ii. Spray chambers without threaded connection: Use of specialized push-on connectors available from various vendors (like UFT-075 from Glass Expansion, Pocasset, MA) are preferred for safety reasons to direct connection of PVC tubing (e.g. 1/8" i.d. x ¼" o.d.).
b. Between peristaltic pump tubing and waste container: Connect 1/8" i.d. x ¼" o.d. PVC tubing to the white / black peristaltic pump tubing using a tubing connector (PerkinElmer item # B3140715). Place the free end of the PVC tubing through the lid of the waste jug (be sure it is secure). Place the waste container in a deep secondary containment tray in case of overflow.

4. Rinse solution for autosampler:

a. Rinse solution jug: Leave one of the caps on the top of the rinse jug loose to allow air venting into the jug as liquid is removed. Otherwise the jug will collapse on itself as the liquid is removed and a vacuum is created inside. Use secondary containment tray.
b. Rinse solution uptake to autosampler rinse station: Use tubing of different lengths and inner diameters between the rinse solution container and the autosampler rinse station to control uptake rate of rinse solution. These can be obtained from the autosampler manufacturer, their distributors, or custom built in the lab. Optimize these factors along with fill time in the software so that waste of rinse solution is minimized and rinse station does not go empty.
c. Autosampler rinse station waste removal: Gravity drain of waste to the waste container is sufficient. Use minimum drain tubing to make this

connection. If this tube is too long, the rinse station will not drain properly.

ii. Gas delivery and regulation

1. ICP-MS modifications:
   a. Plastic tubing between mass flow controllers and dynamic reaction cell have been replaced with stainless steel. Stainless steel tubing is preferred between the reaction gas cylinder / regulator and the back of the ICP-MS instrument.
   b. A second mass flow controller will be needed (channel B) that does not send the DRC gas through a 'getter'.

2. Argon gas: Used for various ICP-MS functions including plasma and nebulizer.

   a. Regulator for argon source (if a dewar): Set delivery pressure of this regulator at least 10 psi higher than the delivery pressure of the step-down regulator to allow for pressure drop across tubing that stretches to the instrument.
   b. Step down regulator (if source of argon is a bulk tank): Place this single stage regulator in the lab so that incoming argon pressure can be monitored and adjusted. Set delivery pressure to 10 psig above the delivery pressure of the filter regulator on the ICP-MS.
   c. Filter Regulator at ICP-MS: Single stage "argon regulator filter kit" supplied with the ICP-DRC-MS. Set the delivery pressure depending on the instrument setup:
      i. ELAN with a 0-60psi gauge on the filter regulator: 52±1 psi when plasma is running (need 0-150 psi regulator if using a PolyPro or PFA nebulizer made by Elemental Scientific Inc).
      ii. ELAN or NexION with a 0-150psi gauge on the filter regulator: 90-100 psi when plasma is running.

3. Argon (90%) / hydrogen (10%) gas mixture: Used for dynamic reaction cell interference removal from arsenic isotopes.
   a. Connect to DRC channel A.
   b. Set the delivery pressure of regulator to 5-7 psig when gas is flowing.
   c. Use a flash arrestor is on the outlet side of the regulator.
   d. This gas can be replaced by 100% argon. Argon from the ICP-MS main argon supply can be split off for this purpose by placing a tee in the delivery tubing on the low pressure side of the filter regulator.

4. Oxygen (99.999±%) gas: Used for dynamic reaction cell interference removal from cadmium and manganese isotopes.
   a. Connect to DRC channel B.
   b. Set the delivery pressure of regulator to 5-7 psig when gas is flowing.
   c. Use a flash arrestor is on the outlet side of the regulator.

iii. Chiller / Heat Exchanger: If using refrigerated chiller, set temperature control to approximately 18 °C.

8) The run: quality, execution, evaluation, and reporting

a. Bench QC, reference materials and calibration verification:

i. Bench "QC": Analysis of bench QC permits assessment of methodological imprecision, determination of whether the analytical system is 'in control' during the run, and assessment of time-associated trends. Before QC materials can be used in the QC process, they must be characterized by at least twenty (20) analytical runs to determine appropriate QC parameters.

Bench QC pool analyte concentrations in this method span the analyte concentration range of the calibrators including "low-normal" ('Low QC') and "high-normal" ('High QC') concentrations.

In each analytical run the analyst will test each of the two bench QC samples two times, subjecting them to the complete analytical process. Bench QC pool samples are analyzed first in the run after the calibration standards but before any patient samples are analyzed. This permits making judgments on calibration linearity and blank levels prior to analysis of patient samples. The second analysis of the bench QC pools is done after analysis of all patient samples in the run (typically 20-30 patient samples total when analyzing for all elements in the method) to ensure analytical performance has not degraded across the time of the run. If more patient samples are analyzed on the same calibration curve after the second run of the bench QC, all bench QC must be reanalyzed before and after the additional samples.

ii. Reference materials: Standard Reference Materials (SRM) from the National Institute of Standards and Technology (NIST) (i.e. SRM 2668 Level 1 & 2668 Level 2) can be used to verify method accuracy. Secondarily, historical challenge samples from proficiency testing programs or commercially-produced reference materials may be useful when NIST SRMs are unavailable.

iii. Calibration verification: The test system is calibrated as part of each analytical run with NIST-traceable calibration standards. These calibrators, along with the QCs and blanks, are used to verify that the test system is performing properly.

b. Perform, evaluate and report a run

i. Starting the equipment for a run

1. Power on the computer, printer, and autosampler, and instrument computer controller.
2. Peristaltic pump: Set proper tension on peristaltic pump tubing.
3. Software: Start software for the ICP-MS and autosampler control.
4. Daily pre-ignition maintenance checks: Perform and document daily maintenance checks (e.g., Ar supply pressure, interface components cleanliness and positioning, interface pump oil condition, vacuum pressure, etc.).

5. Place probe in adequate volume of carrier or rinse solution: If using an ESI FAST, manually place carrier probe into carrier solution. If not, send the autosampler probe to a rinse solution (e.g. autosampler rinse station).
6. Start the plasma
7. Start the peristaltic pump: *S*tart the pump running slowly, making sure that the rotational direction is correct for the way the tubing is set up.
8. Warm-up time: Allow warm-up time suggested by the manufacturer for the ICP-MS (e.g. RF generator) after igniting the plasma. There will be another warm-up time (or "stability time") for the DRC later in this procedure.
9. Daily performance check: Perform and document a daily performance check and any optimizations necessary.

   Save new parameters to the "default.tun" and "default.dac" files.
10. DRC Stability time: Best analyte-to-internal standard ratio stability is typically observed after 1-1.5 hours of analysis of urine samples using the DRC mode method (~12 measurements of the 15 element panel, or 50 measurements of the total arsenic method can be made in 1 hour). Prepare 50mL^{+} of a calibration standard (e.g. standard 2) to be analyzed repeatedly before the beginning of the run to achieve a stable analyte-to-internal standard ratio. Time to reach stability is instrument-specific and learned from performance of runs.
11. Readying the instrument for quick-start analysis: The plasma may be left running to eliminate the need for an initial instrument warm-up period and / or a DRC stabilization period as long as appropriate planning is made for sufficient solution supply and waste collection. Analysis of conditioning samples (diluted urine matrix) can also be scheduled to occur at roughly a predetermined time. Accomplish this by setting up multiple sample analyses with extended rinse times (e.g. one 15 element analysis with a 1400s rinse time will take approximately 30 minutes to complete). Initial samples would be non-matrix, while final samples would be diluted matrix for conditioning. If running a DRC-only method during these scheduled analyses, the ICP-MS will remain in DRC-mode for approximately 45 minutes without depressurizing the cell.
12. Software setup for Analysis:
    a. Workspace (files & folders): Verify & set up the correct files and data directories for your analysis
    b. Samples / Batch Window: Update the software to reflect the current sample set. Use a bar code scanner to input data whenever possible.

        1. Urine vs. Aqueous Method Files:
            a. The difference: There are two method files for this one method. It is necessary to use both to accomplish each run because the current PerkinElmer software will not allow for

more than one blank per method file. The ONLY DIFFERENCE between these two files is on the Sampling tab where one lists the autosampler positions of the urine blank and urine calibrators (the "urblk" method file) and the other lists the autosampler position of the aqueous blank (the "aqblk" method file).

b. Use: The ONLY TIME when it matters which of these files is used is when the measurement action *includes* "Run blank" or "Run standards". When the measurement action is only 'run sample', it does not matter whether the "urblk" or "aqblk" method file is used. Analysts typically follow the pattern below, however, for the sake of consistency and as a reminder of which blank must be used for which type of sample.

   i. The *"urblk" method file:* *U*se to analyze the initial urine blank (blank for the calibration curve), the urine calibrators, and the urine blank checks at the very beginning of the run. The urine blank method defines the autosampler location of the urine blank and the urine calibration standards.

   ii. The "*aqblk*" method file must be used to analyze all QC materials and patient samples. The aqueous blank method defines the aqueous blank in autosampler location.

ii. Preparation of samples for analysis

1. Thaw urine samples; allow them to reach ambient temperature.
2. Prepare the following solutions into pre-labeled containers using the Micromedic Digiflex™ or other volumetric sample transfer device.

   Prepare samples in the cleanest environment available to prevent trace element contamination and an environment which provides personnel protection (e.g. Class II, Type A/B3 biological safety cabinet).

   a. *Aqueous Blank*: Prepare at least two aqueous blanks. One will be the actual reagent blank for patient and QC samples and the other will be a backup ("Aqueous Blank Check") in case the original aqueous blank is unusable.

   b. *Calibrators*: Prepare the working calibration standards (S0-S5). Prepare S0 in triplicate. One of these S0 preparations will be the zero standard (urine blank) for the calibration standards; the other two will be analyzed twice after the last calibrator to collect run blank data that can be used in periodically evaluating the method LOD.

   c. *Patient & QC Samples*: Before taking an aliquot for analysis, homogenize the sample.

After preparation, mixed and cover diluted samples. Place prepared dilutions on the autosampler of the ICP-MS in the order corresponding to the sequence setup in the ICP-MS software.

Original samples may be at room temperature for the work day and go through repeated freeze-thaw cycles without being compromised.

iii. Start the analysis using the ICP-MS software.

iv. Monitor the analysis in real-time as much as possible. If necessary, the run may be left to complete itself unattended as long as appropriate planning is made for either overnight operation or Auto Stop (see below).

v. Monitor the analysis in real-time as much as possible. If necessary, the run may be left to complete itself unattended as long as appropriate planning is made for either overnight operation or Auto Stop (see below).

Monitor the analysis for the following:

1. *Proper operation of the instrument (proper loop filling, sample reaching nebulizer in correct timing, autosampler arm moving properly, etc . . .).*

2. *Evaluate reagents used in sample preparation for contamination.*

   Before the start of any sample, QC, or working calibrator preparation, checking reagents for contamination by monitoring the measured intensities (cps) of the analytes of interest in the following three reagent checks provides valuable troubleshooting information.

   a. Water to be used in Aq Blank Checks and dilutions.
      If contamination is observed in the water then allow the DI system to run for a few minutes to flush any impurities from the line, or use water from a different DI system.

   b. Diluent before being flushed through the Digiflex.
      If contamination is observed in all diluent, then re-preparing the diluent is recommended.

   c. Diluent after being flushed through the Digiflex.
      Compare the measured intensities (cps) of the diluent before and after being pipetted through the Digiflex to determine if contamination is coming from the Digiflex.

      If contamination is observed from the Digiflex, flush the Digiflex with dilute nitric acid solution (e.g. 2% v/v HNO_3). Run the diluent reagent check to confirm the Digiflex measured intensities (cps) are consistent.

3. *DRC stability (analyte / internal standard ratio stability)*

   The net intensity (analyte / internal standard ratio) of the measurements made while stabilizing the DRC can be evaluated to determine the readiness of the system to begin analysis. Continual trending in this ratio indicates that unwanted instrument drift will occur within the run. Additional measurements can be inserted at regular intervals throughout the run if drift is suspected.

4. *Evaluate the Axial Field Voltage (AFV) optimized value (recommended daily when using the PerkinElmer NexION series ICP-MS.)*

   Verify the optimal AFV value by running a working calibrator preparation of S0 immediately followed by S5. Monitor the change between S0 and S5 for measured intensities (cps) of the internal standard iridium in DRC mode. If the percent difference between the iridium intensities is greater than 5% (especially if greater in S5), then run the axial field voltage optimization. See Section 6.f.ii for preparation of optimization solutions.

   Evaluate the AFV near the end of, or after, the DRC mode stability analysis. Be sure to run 3-4 junk urine or UrBlkChk samples to verify washout of S5 concentrations before beginning analysis of the blank for the calibration curve.

5. Low background levels of analytes in the blanks.

   Compare measured intensities for analytes in the urine (calibration) blank and reagent (QC and sample) blank against historical values and other blank checks within the run. It may be necessary to use an alternate blank (blank check) for the run. Trace contamination in blanks to the source and take corrective action(s).

6. *Linear calibration curves* (minimum of 0.98, typically 0.99 to 1.000).

7. *Bench QC results within the acceptable limits*.

   If an analyte result for the beginning QC material(s) falls outside of the ± 2SD limits, then the following steps are recommended:

   a. Evaluate the blank results if QC results are abnormally low.
   b. Evaluate the reproducibility of the 3 replicates within the measurements.
   c. Evaluate the consistency of the internal standard across the measurements (esp. the calibrators).
   d. Evaluate calibration curves. If a particular calibration standard is obviously in error, it can be re-analyzed as a sample (old or new dilution) and incorporated into the curve through data reprocessing as a calibrator. As a last resort, a single calibration point per analyte including or between S2 and S4 can be removed from the curve. Follow-up repeated problems with calibration standards with appropriate corrective actions (e.g. re-preparation of intermediate working standards or troubleshooting instrument parameters).
   e. Prepare a fresh dilution of the failing QC material (same vial) and reanalyze it to see if the QC dilution was not properly made.
   f. Prepare a fresh dilution of the failing QC material (unused vial) and analyze it to see if the QC vial had become compromised.
   g. Prepare and analyze new working calibrators.
   h. Test a different preparation of intermediate working calibration standards.

If these steps do not result in correction of the out-of-control values for QC materials, consult the supervisor for other appropriate corrective actions.

8. *Good precision among replicates*.

   Pay close attention to the within-sample, percent relative standard deviation (% RSD) of internal standards for all analyses in the run.

9. *Consistent measured intensities of the internal standards*.

   Some sample-to-sample variations are to be expected. However, expect the intensities to be within a few percent of one another and to fluctuate around an average value (not drift continuously in one direction). If a one-direction drift in the measured intensities for the internal standards is observed in DRC mode, additional DRC stability time may be necessary before performing a run. See Section 8.b.ii.2 for details about running DRC stability measurements. Observing an increase of the measured intensities for the DRC mode internal standard(s) as the calibration standards increase in concentration which drops back down immediately after the calibration standards may indicate an improperly optimized axial field voltage. See Section 6.f.ii for preparation of optimization solutions.

10. *Elevated patient results.*

    a. <u>Confirming an elevated concentration</u>: Repeat for confirmation any sample having a concentration greater than the 1UB. See Section 8.b.viii.2.a for details.

    b. <u>Dilution of a sample to within the calibration range:</u> Any sample having a concentration greater than calibration standard 5 will need to be diluted with a minimal extra dilution. See Section 8.b.viii.2.a for details.

    c. <u>Confirming proper washout after an elevated sample</u>: If possible (e.g. monitoring analysis in real-time) following observation of a result greater than the concentrations listed in Table 1 “extended wash”,

       i. Stop run following elevated sample

       ii. Verify that standard zero (urine blank) levels have been re-achieved before proceeding with analysis. Typically, analysis of 2 urine blank checks followed by a low bench QC, identified as a washout check, will be sufficient. If not, repeat the 3 check samples until washout is verified before proceeding with analysis.

           Example:
           3018 UrBlkChk Wash1
           3018 UrBlkChk Wash2
           LUXXXXX Wash

           If return to standard zero (urine blank) levels is not verified prior to subsequent sample analysis, see Section 8.b.viii.2.a for details.

vi. Overnight operation or using auto stop: The run may be left to complete itself unattended as long as appropriate planning is made (e.g. sufficient solution supply and waste collection). Turn on the AutoStop feature of the ICP-MS software. Delay the shutdown at least 10 minutes (use peristaltic pump speed approximately that of the method wash) to rinse the sample introduction system of urine matrix before turning off the plasma. It will be necessary to replace the sample peristaltic pump tubing the next day since it will have been clamped shut overnight.

1. ELAN specifics: Enable "Auto Start / Stop" is on the "AutoStop" tab of the Instrument window.
2. NexION specifics: Enable AutoStop in on the Run List window. Select "Batch Completed" for Stop Criteria.

vii. Records of results: Run results will be documented after each run in both electronic and paper form.

1. Electronic records: Transfer data electronically to the laboratory information system. When keyboard entry must be used, proofread transcribed data after entry.
   a. Export data from the ICP-MS software using "original conditions" or files and folders used during the analysis. Use descriptive report filenames (e.g. 2005-0714a_group55.txt). In the ELAN or NexION software under "Report Format" (METHOD window, REPORT tab) choose the "Use Separator" option, and under the "File Write" Section choose "Append."
   b. Move the generated .TXT data file to the appropriate subdirectory on the network drive where exported data are stored prior to import to the laboratory information management system.
   c. Import the instrument file into the laboratory information system with appropriate documentation (e.g. instrument ID, analyst, calibration standards lot number, and run or sample specific comments).
2. Paper records: Run sheets must be documented with
   i. Analyst initials
   ii. Instrument ID
   iii. Date of analysis and run # for the day

viii. Analyst evaluation of run results:

1. Bench quality control: After completing a run, and importing the results into the laboratory information system, evaluate the run bench QC according to laboratory QC rules [12]. The QC limits are based on the average and standard deviation of the beginning and ending analyses of each of the bench QC pools, so it will not be possible to know if the run is in control until statistically reviewed.
   a. Rules for bench quality control evaluation: The following are the CDC DLS QC rules for two QC pools per run with two or more QC results per pool.

i. If both QC run means are within $2S_m$ limits and individual results are within $2S_i$ limits, then accept the run.

ii. If one of the two QC run means is outside a $2S_m$ limit - reject run if:

1. Extreme Outlier – Run mean is beyond the characterization mean ± $4S_m$
2. 3S Rule - Run mean is outside a $3S_m$ limit
3. 2S Rule – Two or more of the run means are outside the same $2S_m$ limit
4. 10 X-bar Rule – Current and previous 9 run means are on same side of the characterization mean

iii. If one of the 4 QC individual results is outside a $2S_i$ limit - reject run if:

1. Extreme Outlier – One individual result is beyond the characterization mean ± $4S_i$
2. R 4S Rule – Within-run ranges for all pools in the same run exceed $4S_w$ (i.e., 95% range limit)

   Note: Since runs have multiple results per pool for 2 pools, the R 4S rule is applied within runs only.

Abbreviations:

S_i = Standard deviation of individual results.

S_m = Standard deviation of the run means.

S_w = Within-run standard deviation.

b. Implications of QC failures: If the DLS SAS program declares the run "out of control" for any analyte, use the following to determine the implications on usability of the data from the run.

i. 13 – 15 elements in the run

1. 1, 2 or 3 analytes "out of control": ONLY the analytes which were "out of control" are invalid for reporting from the run.
2. 4 or more analytes "out of control": All results, regardless of analyte, are invalid for reporting from the run.

ii. 4 – 12 elements in the run

1. 1 or 2 analytes "out of control": ONLY the analytes which were "out of control" are invalid for reporting from the run.
2. 3 or more analytes "out of control": All results, regardless of analyte, are invalid for reporting from the run.

iii. 3 elements in the run

1. 1 analyte "out of control": ONLY the analyte which was "out of control" is invalid for reporting from the run.

2. 2 or more analytes "out of control": All results, regardless of analyte, are invalid for reporting from the run.

iv. 1 – 2 elements in the run

ONLY the analyte which was "out of control" is invalid for reporting from the run.

2. Patient results:

a. Elevated concentrations:

i. Boundaries requiring confirmatory measurement:

1. Results greater than the first (1UB) or second (2UB) upper boundaries.

The concentrations assigned to 1UB and 2UB for an element is determined by study protocol.

a. Results greater than the first upper boundary (1UB): Confirm by repeat analysis of a new sample preparation concentrations observed greater than the "first upper boundary" (defined in the laboratory database as the "1UB"). Report the first analytically valid result, as long as the confirmation is within 10%. Continue repeat analysis until a concentration can be confirmed.

b. Analyst reporting of elevated results: Report any patient results confirmed to be greater than the second upper boundary (2UB) as an "elevated result".

2. Results greater than highest calibrator: Samples that exceed the high calibrator must be prepared with minimum extra dilution in duplicate to bring the observed result within the calibration range (≤ S5). Report the first analytically valid result (i.e. the first one within the calibration range), as long as the confirmation is within 10%. Continue repeat analysis until a concentration can be confirmed.

ii. Concentrations requiring verification of washout (see Table 1, extended washout concentration thresholds).

1. If return to standard zero (urine blank) levels was verified before subsequent sample analysis, no further action is required.

2. If return to standard zero (urine blank) levels was not verified before subsequent sample analysis, consult a supervisor regarding the reportability of results subsequent to the elevated result in the run.

b. Unacceptable reproducibility: If the range of the three replicate readings (maximum replicate concentration value - minimum replicate concentration value) for a single sample analysis is greater than the range maximum criteria ***and*** the range of the three replicate readings is greater than 10% of the observed concentration, do not use the measurement for reporting. Repeat the analysis of the sample.

ix. Submitting final work for review: All analyses must undergo quality control and quality assurance review. After appropriately documenting the run in the laboratory information system (e.g. sample and run QC, and run and sample comments), inform the first level reviewer of the completed work and submit any printed documentation.

9) Routine equipment maintenance and data backups

Maintenance activities will be documented in the instrument logbook.

a. Equipment maintenance: Analysts are expected to regularly evaluate the need for, and when necessary perform, cleaning, replacement, or re-positioning of components in ICP-MS the sample introduction system, interface, ion optics region, and equipment required resources (e.g. autosampler, exhaust, compressed gases, and coolant). Frequency of equipment maintenance will be dependent on instrument throughput.

   i. Parameter optimizations: Analysts are expected to optimize instrument parameters.

   ii. Dual detector calibration: Perform dual detector calibration regularly (weekly or monthly) for any element exceeding 1,000,000 cps for calibration standard 5. This is typically only Sr. The dual detector calibration solution is described in Section 6.f.iii.

b. DRC optimizations: DRC conditions (cell gas flow rate and RPq value) can be verified by analyzing the DRC optimization solutions (see Section 6.f.i) as needed to ensure proper reduction of potential ICP-MS interferences.

c. Data backup: Data on the instrument computer will be backed up via two backup routines. Files used and produced by the ICP-MS in analyzing samples will be backed up and kept a minimum of three years after analysis.

   i. Daily backups to secondary hard drive: Program automatic backups of the relevant computer files to occur each night onto a secondary hard drive to prevent loss of data from failure of primary hard drive.

   ii. Weekly backup: Backup relevant computer files weekly either to secondary hard drive which is remote to the laboratory or to removable media which will be placed remote to the laboratory for retrieval in the case of catastrophic data loss elsewhere.

10) Reporting thresholds

a. Reportable range: Urine multi-element values are reportable in the range between the method LOD and the highest calibrator. Above the highest calibrator, extra dilutions are made of the urine sample to bring the concentration within the reportable range. If extra dilution has been necessary, the reported value will exceed the upper end of the reportable range.

b. Reference ranges (normal values): In this method the 95% reference ranges for these elements in urine fall within the range of the calibrators.

c. Action levels: Due to the uncertainty of the health implications of elevated concentrations of many of the elements determined with this method, there is no routine notification for elevated levels of every analyte determined with this

method. The present NRC standard for workplace removal is 15 μg/L of U in urine [14]. Other action levels for reporting to supervising physicians are determined on a study-by-study basis.

11) Method calculations

a. Method limit of detection (LOD): The method detection limits for elements in urine specimens are defined as 3 times s_0, where s_0 is the estimate of the standard deviation at zero analyte concentration. S_0 is taken as the y-intercept of a linear or 2nd order polynomial regression of standard deviation versus concentration (4 concentration levels of the analytes in urine each measured 60 times across at least a 2-month timeframe). Method LODs are re-evaluated periodically.

b. Method limit of quantitation (LOQ): The Division of Laboratory Sciences does not currently utilize limits of quantitation in regards to reporting limits [12].

c. QC limits: Quality control limits are calculated based on concentration results obtained in at least 20 separate runs. It is preferable to perform separate analyses on separate days and using multiple calibrator lot numbers, instruments, and analysts to best mimic real-life variability. The statistical calculations are performed using the SAS program developed for the Division of Laboratory Sciences (DLS_QC_compute_char_stats.sas).

12) Alternate methods for performing test and storing specimens if test system fails:

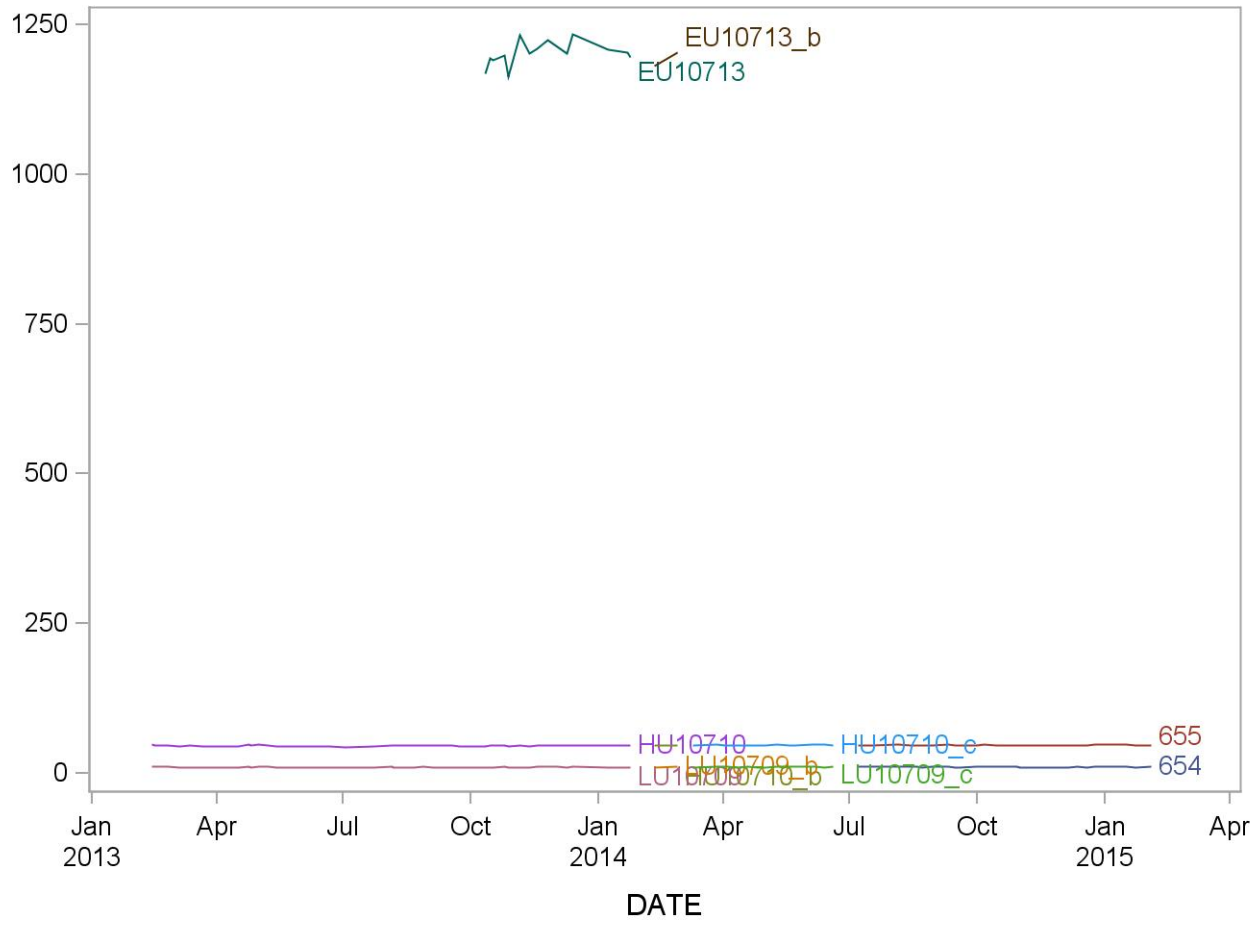
If the analytical system fails, the analysis may be setup on other ICP-MS instruments in the laboratory. If no other instrument is available, store the specimens at ~4 °C until the analytical system can be restored to functionality. If interruption longer than 4 weeks in anticipated, then store urine specimens at ≤ -20 °C.

13) Summary Statistics and QC Graphs

See following pages

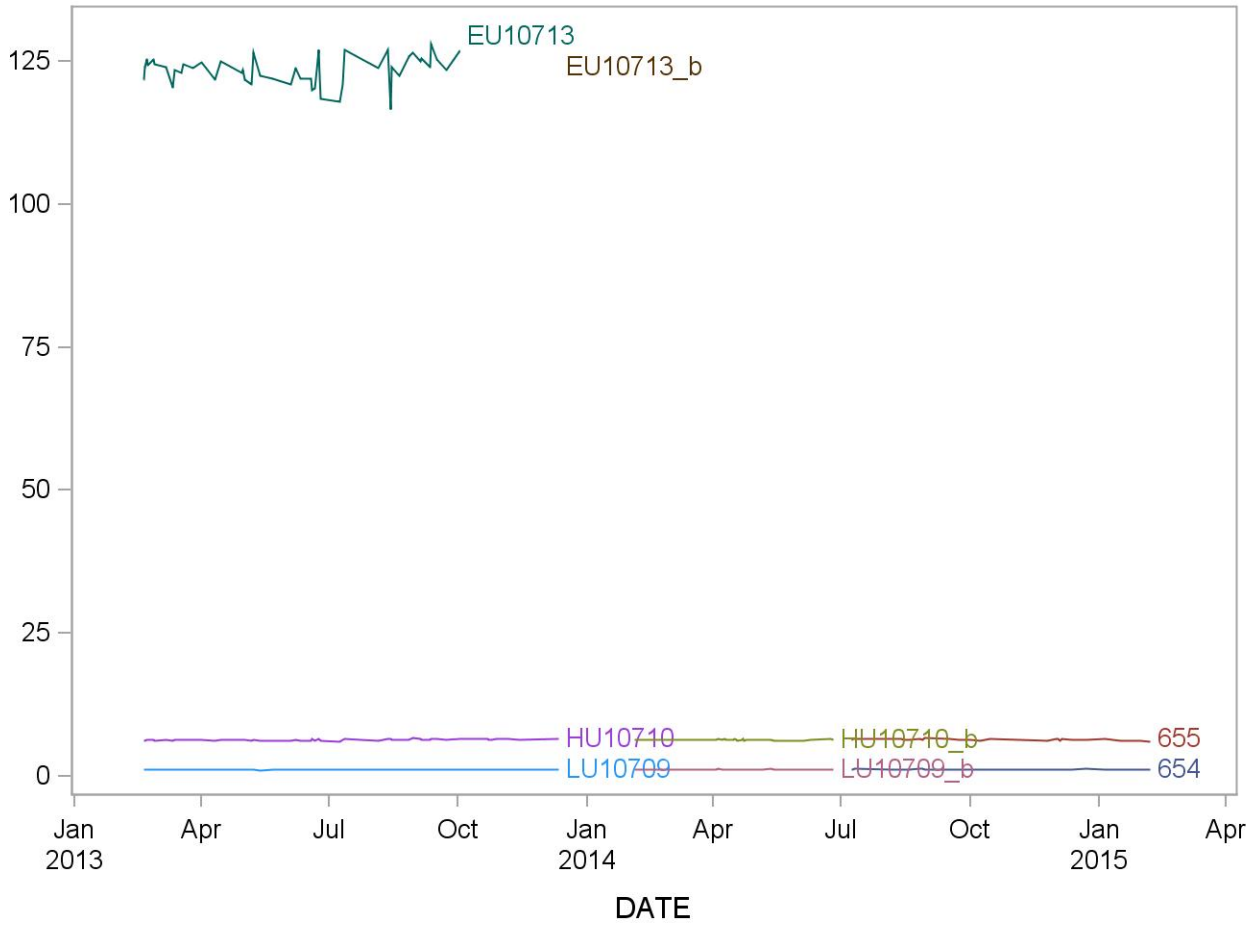
2013-2014 Summary Statistics and QC Chart for Urinary arsenic, total (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU10710	48	13FEB13	24JAN14	46.158	0.945	2.0
LU10709	48	13FEB13	24JAN14	10.568	0.235	2.2
EU10713	15	11OCT13	24JAN14	1202.577	20.112	1.7
HU10710_b	3	10FEB14	27FEB14	46.747	0.499	1.1
LU10709_b	3	10FEB14	27FEB14	10.730	0.075	0.7
EU10713_b	3	10FEB14	27FEB14	1195.900	16.394	1.4
HU10710_c	16	10MAR14	19JUN14	47.309	0.578	1.2
LU10709_c	16	10MAR14	19JUN14	10.785	0.180	1.7
655	32	07JUL14	03FEB15	47.239	0.494	1.0
654	32	07JUL14	03FEB15	10.713	0.168	1.6



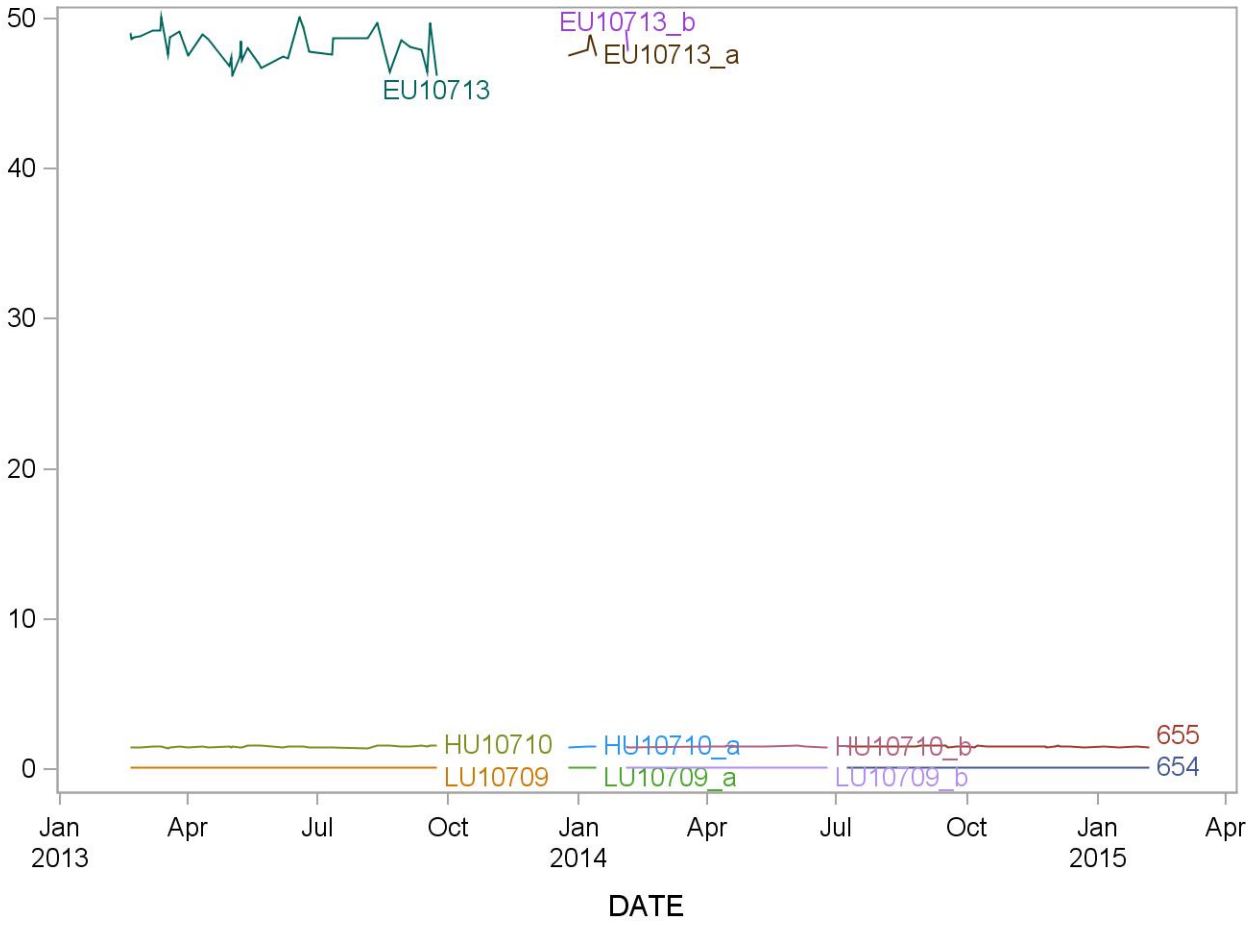
2013-2014 Summary Statistics and QC Chart for Barium, urine (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU10710	86	19FEB13	12DEC13	6.404	0.128	2.0
LU10709	86	19FEB13	12DEC13	1.214	0.037	3.0
EU10713	72	19FEB13	02OCT13	123.410	2.340	1.9
HU10710_b	48	04FEB14	25JUN14	6.450	0.121	1.9
LU10709_b	48	04FEB14	25JUN14	1.235	0.041	3.3
EU10713_b	4	04FEB14	04FEB14	122.875	2.056	1.7
655	53	08JUL14	06FEB15	6.442	0.170	2.6
654	53	08JUL14	06FEB15	1.228	0.044	3.6



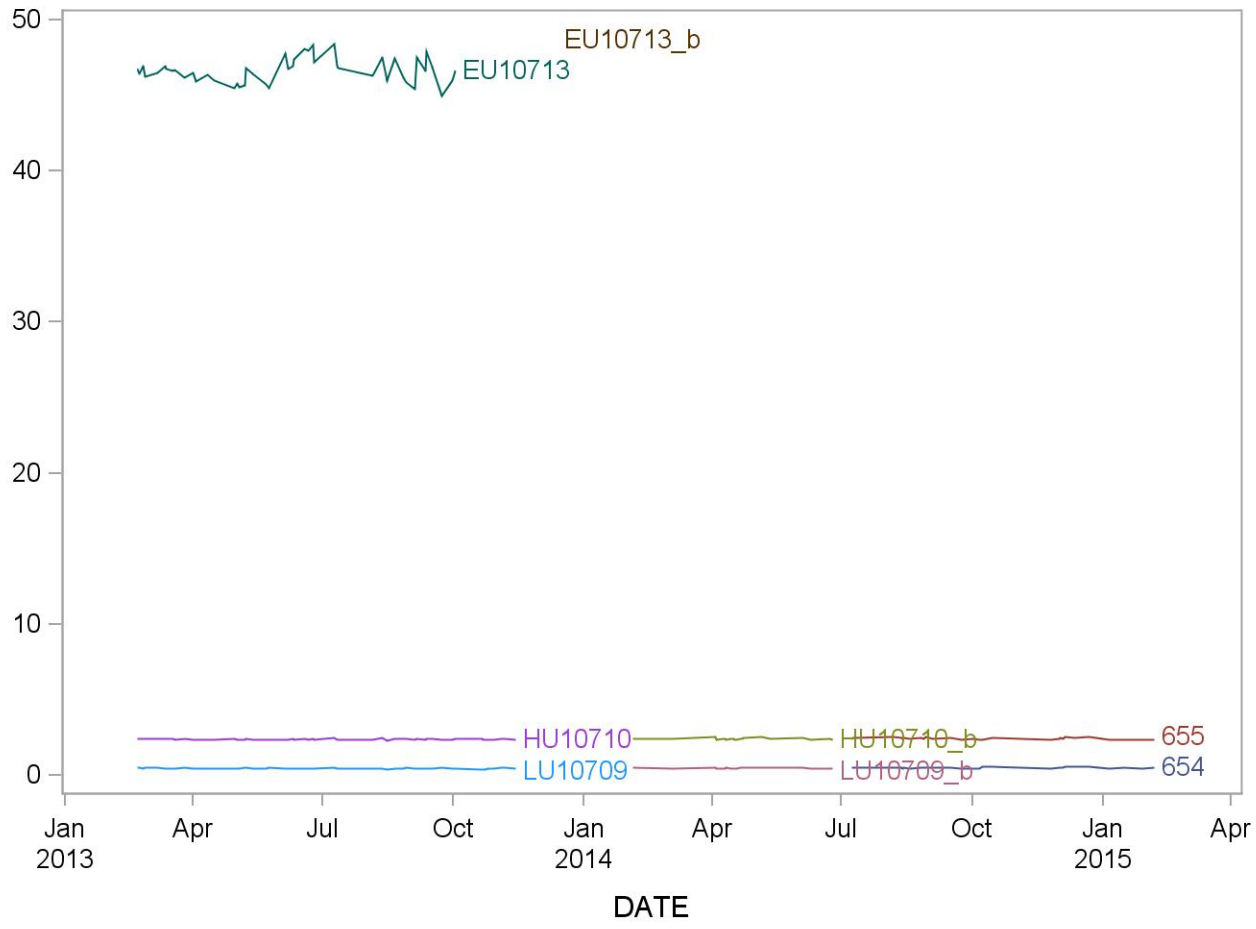
2013-2014 Summary Statistics and QC Chart for Cadmium, urine (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU10710	65	19FEB13	23SEP13	1.5334	0.0493	3.2
LU10709	65	19FEB13	23SEP13	0.1428	0.0136	9.6
EU10713	65	19FEB13	23SEP13	48.2938	1.2398	2.6
HU10710_a	10	24DEC13	13JAN14	1.5280	0.0264	1.7
LU10709_a	10	24DEC13	13JAN14	0.1440	0.0115	8.0
EU10713_a	10	24DEC13	13JAN14	48.1650	0.7623	1.6
HU10710_b	48	03FEB14	25JUN14	1.5475	0.0405	2.6
LU10709_b	48	03FEB14	25JUN14	0.1484	0.0102	6.9
EU10713_b	5	03FEB14	04FEB14	48.1500	1.7667	3.7
655	51	08JUL14	06FEB15	1.5408	0.0417	2.7
654	51	08JUL14	06FEB15	0.1469	0.0100	6.8



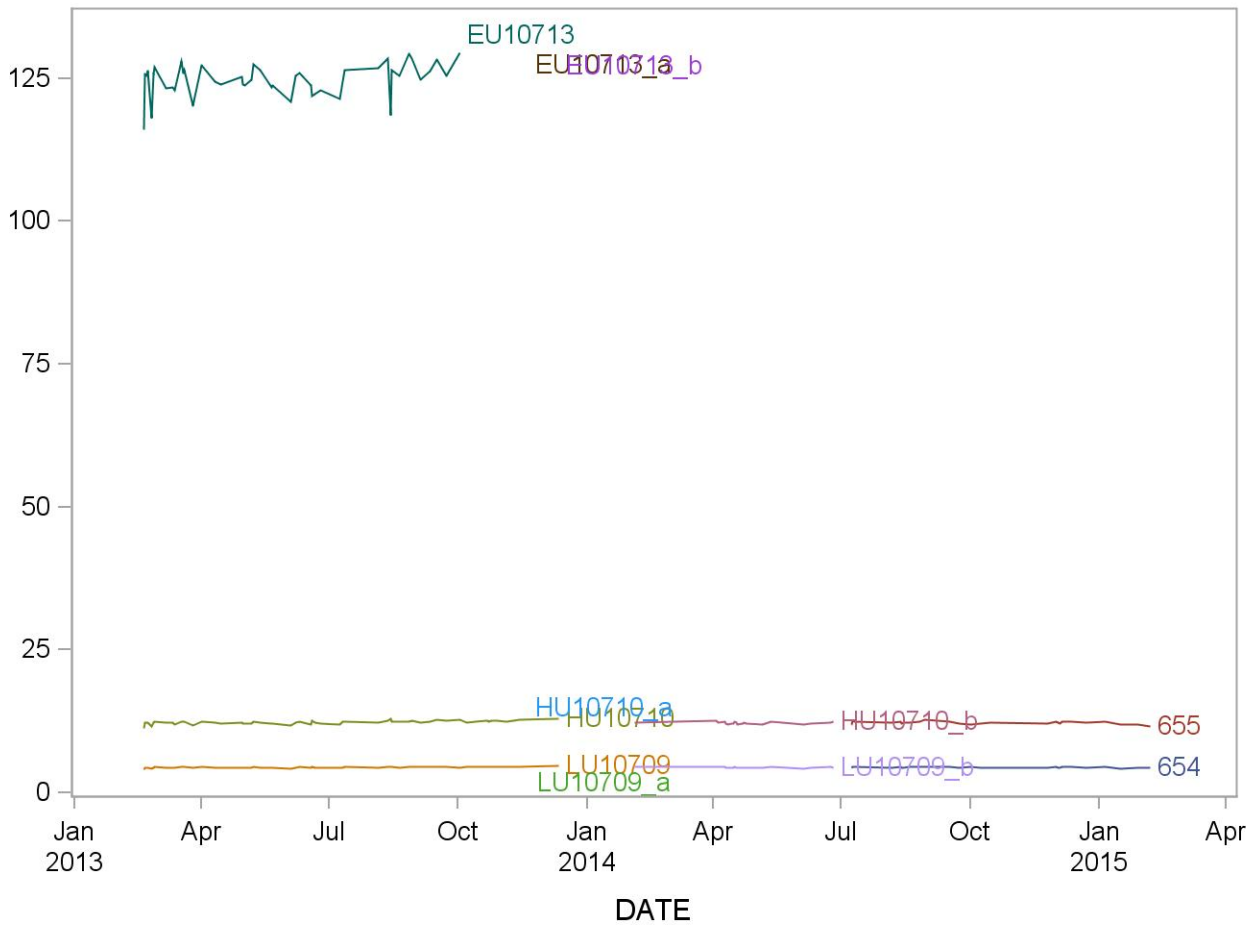
2013-2014 Summary Statistics and QC Chart for Cobalt, urine (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU10710	83	21FEB13	14NOV13	2.3923	0.0379	1.6
LU10709	83	21FEB13	14NOV13	0.4720	0.0286	6.1
EU10713	69	21FEB13	02OCT13	46.5986	0.7995	1.7
HU10710_b	50	04FEB14	25JUN14	2.4246	0.0586	2.4
LU10709_b	50	04FEB14	25JUN14	0.4821	0.0428	8.9
EU10713_b	4	04FEB14	04FEB14	47.1625	0.8673	1.8
655	54	08JUL14	06FEB15	2.4481	0.0595	2.4
654	54	08JUL14	06FEB15	0.5045	0.0346	6.9



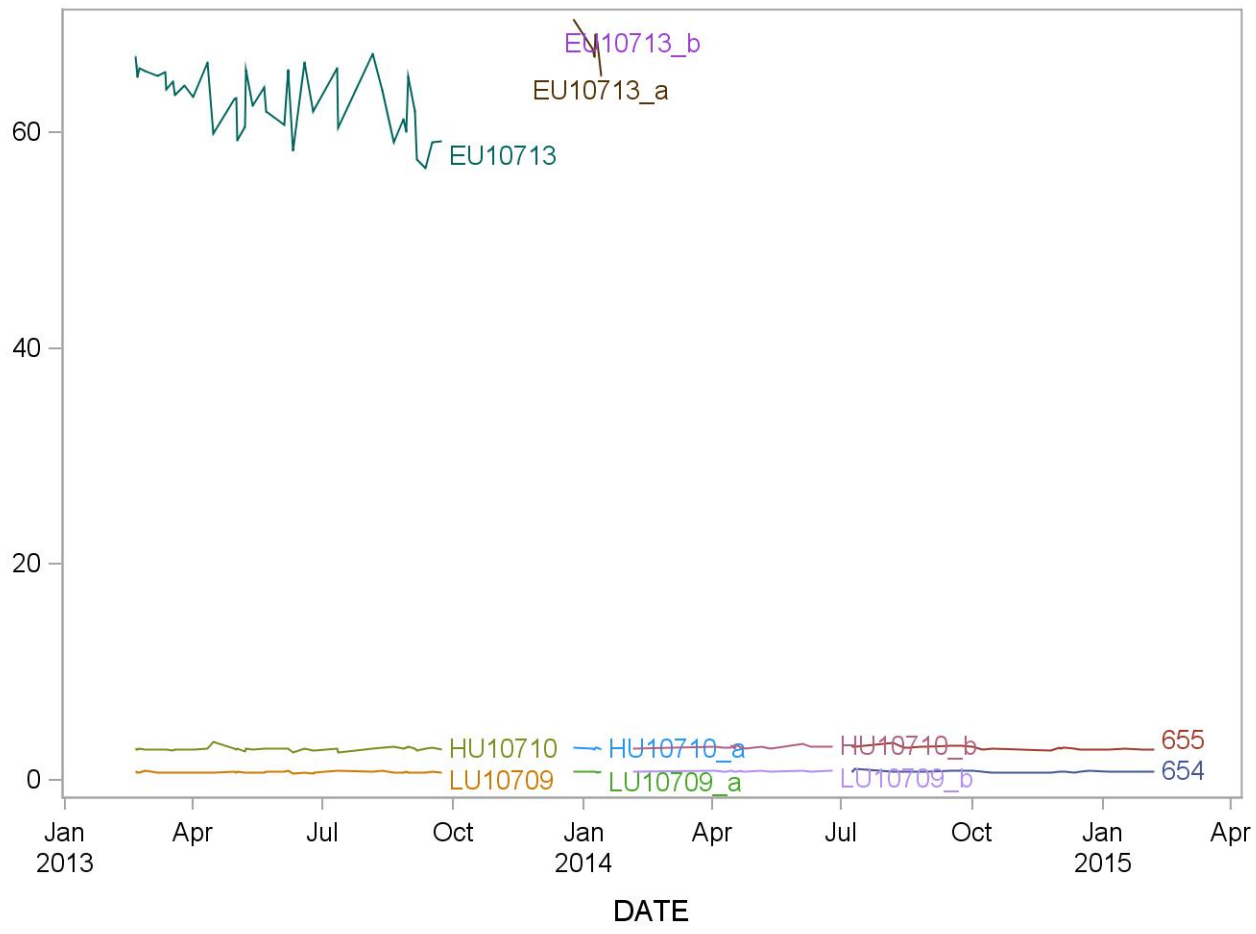
2013-2014 Summary Statistics and QC Chart for Cesium, urine (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU10710	86	19FEB13	12DEC13	12.3570	0.2760	2.2
LU10709	86	19FEB13	12DEC13	4.4655	0.1017	2.3
EU10713	71	19FEB13	02OCT13	124.8944	2.6725	2.1
HU10710_a	2	13JAN14	13JAN14	12.3000	0.0707	0.6
LU10709_a	2	13JAN14	13JAN14	4.4550	0.0354	0.8
EU10713_a	2	13JAN14	13JAN14	124.7500	3.1820	2.6
HU10710_b	46	04FEB14	25JUN14	12.2957	0.2558	2.1
LU10709_b	46	04FEB14	25JUN14	4.4647	0.0734	1.6
EU10713_b	4	04FEB14	04FEB14	124.6250	2.8687	2.3
655	50	08JUL14	06FEB15	12.2662	0.2983	2.4
654	50	08JUL14	06FEB15	4.4489	0.0933	2.1



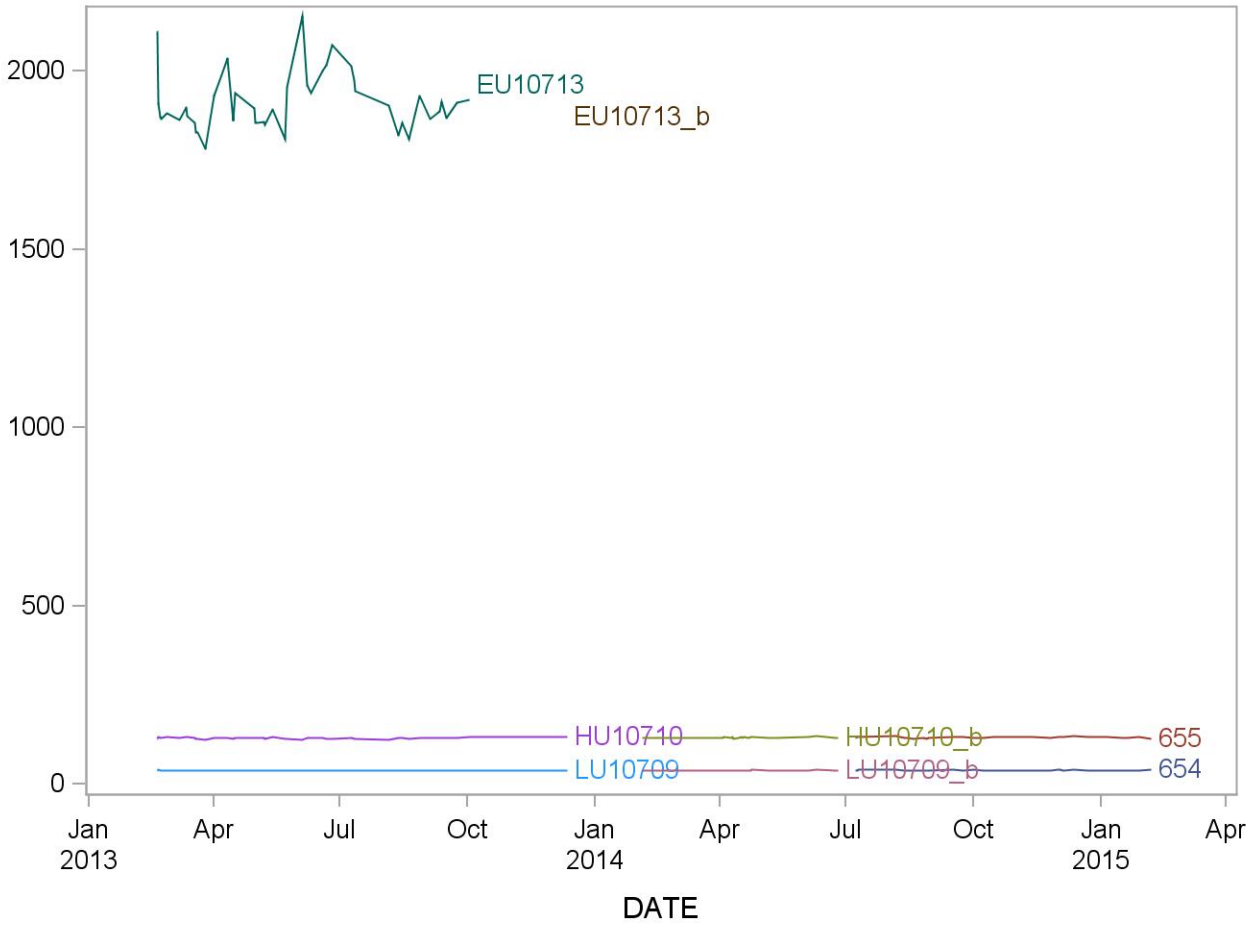
2013-2014 Summary Statistics and QC Chart for Manganese, urine (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU10710	68	19FEB13	23SEP13	2.910	0.156	5.3
LU10709	68	19FEB13	23SEP13	0.768	0.076	9.8
EU10713	68	19FEB13	23SEP13	63.122	3.372	5.3
HU10710_a	10	24DEC13	13JAN14	2.950	0.078	2.6
LU10709_a	10	24DEC13	13JAN14	0.784	0.039	5.0
EU10713_a	10	24DEC13	13JAN14	67.662	2.136	3.2
HU10710_b	47	04FEB14	25JUN14	3.063	0.154	5.0
LU10709_b	47	04FEB14	25JUN14	0.851	0.076	8.9
EU10713_b	4	04FEB14	04FEB14	66.861	4.021	6.0
655	51	08JUL14	06FEB15	3.031	0.181	6.0
654	51	08JUL14	06FEB15	0.818	0.071	8.7



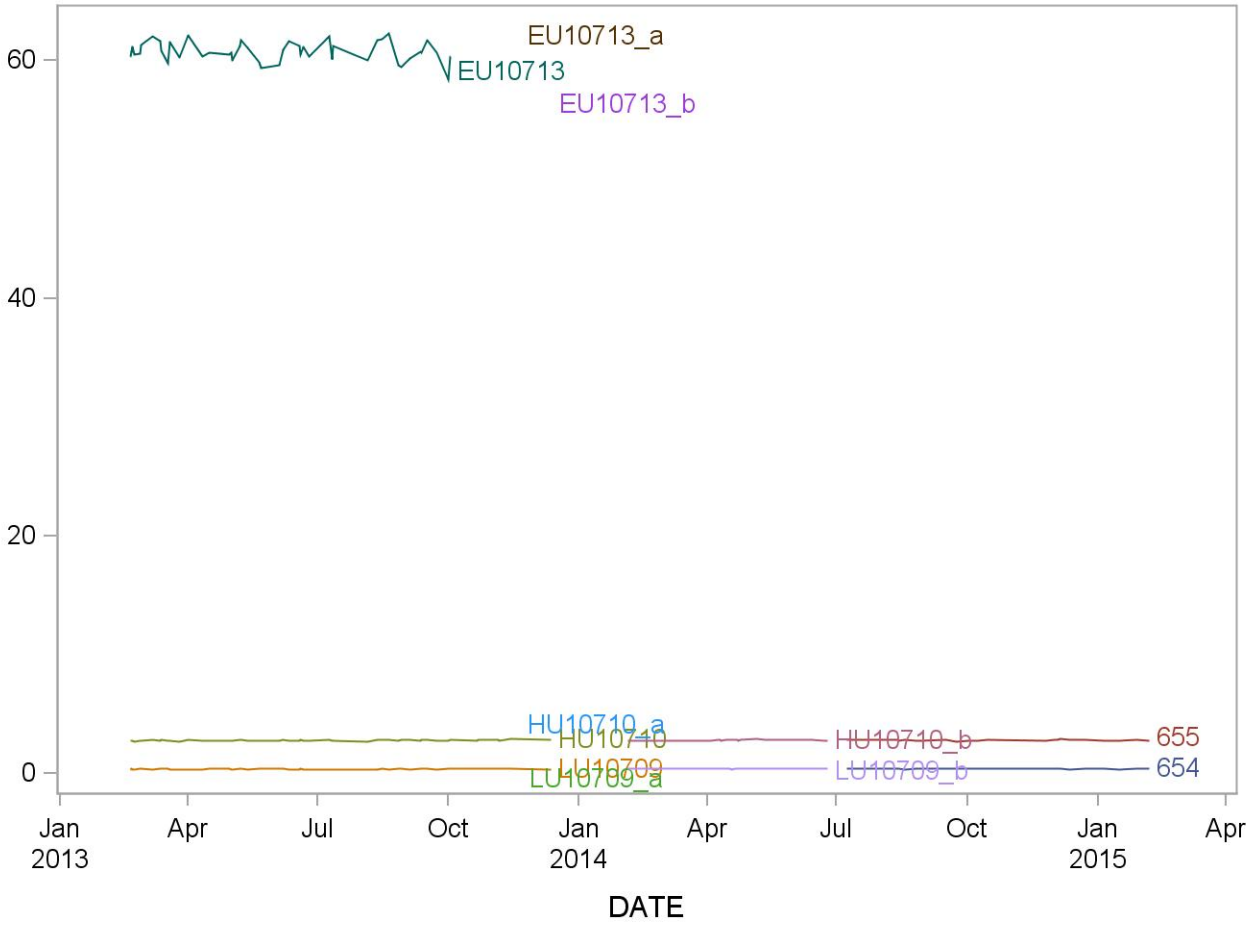
2013-2014 Summary Statistics and QC Chart for Molybdenum, urine (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU10710	86	19FEB13	12DEC13	129.936	2.204	1.7
LU10709	86	19FEB13	12DEC13	38.323	0.648	1.7
EU10713	71	19FEB13	02OCT13	1909.718	77.643	4.1
HU10710_b	47	04FEB14	25JUN14	130.840	1.785	1.4
LU10709_b	47	04FEB14	25JUN14	38.951	0.514	1.3
EU10713_b	4	04FEB14	04FEB14	1872.500	104.363	5.6
655	55	08JUL14	06FEB15	131.375	2.211	1.7
654	55	08JUL14	06FEB15	39.079	0.603	1.5



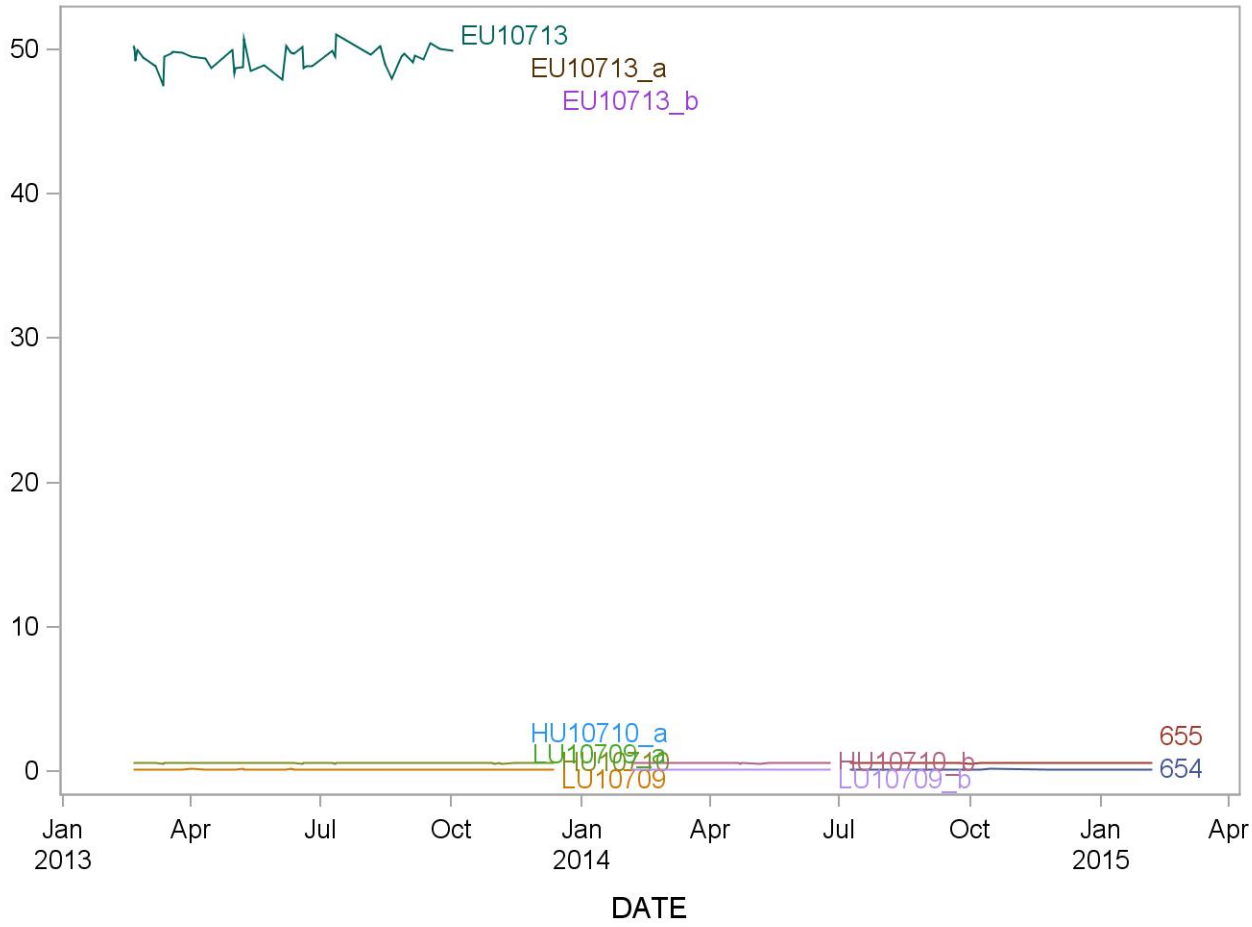
2013-2014 Summary Statistics and QC Chart for Lead, urine (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU10710	86	19FEB13	12DEC13	2.793	0.043	1.5
LU10709	86	19FEB13	12DEC13	0.380	0.022	5.8
EU10713	72	19FEB13	02OCT13	60.788	0.873	1.4
HU10710_a	2	13JAN14	13JAN14	2.875	0.007	0.2
LU10709_a	2	13JAN14	13JAN14	0.395	0.007	1.8
EU10713_a	2	13JAN14	13JAN14	60.875	1.025	1.7
HU10710_b	45	04FEB14	25JUN14	2.826	0.048	1.7
LU10709_b	45	04FEB14	25JUN14	0.397	0.016	3.9
EU10713_b	4	04FEB14	04FEB14	59.488	0.407	0.7
655	51	08JUL14	06FEB15	2.810	0.047	1.7
654	51	08JUL14	06FEB15	0.390	0.014	3.6



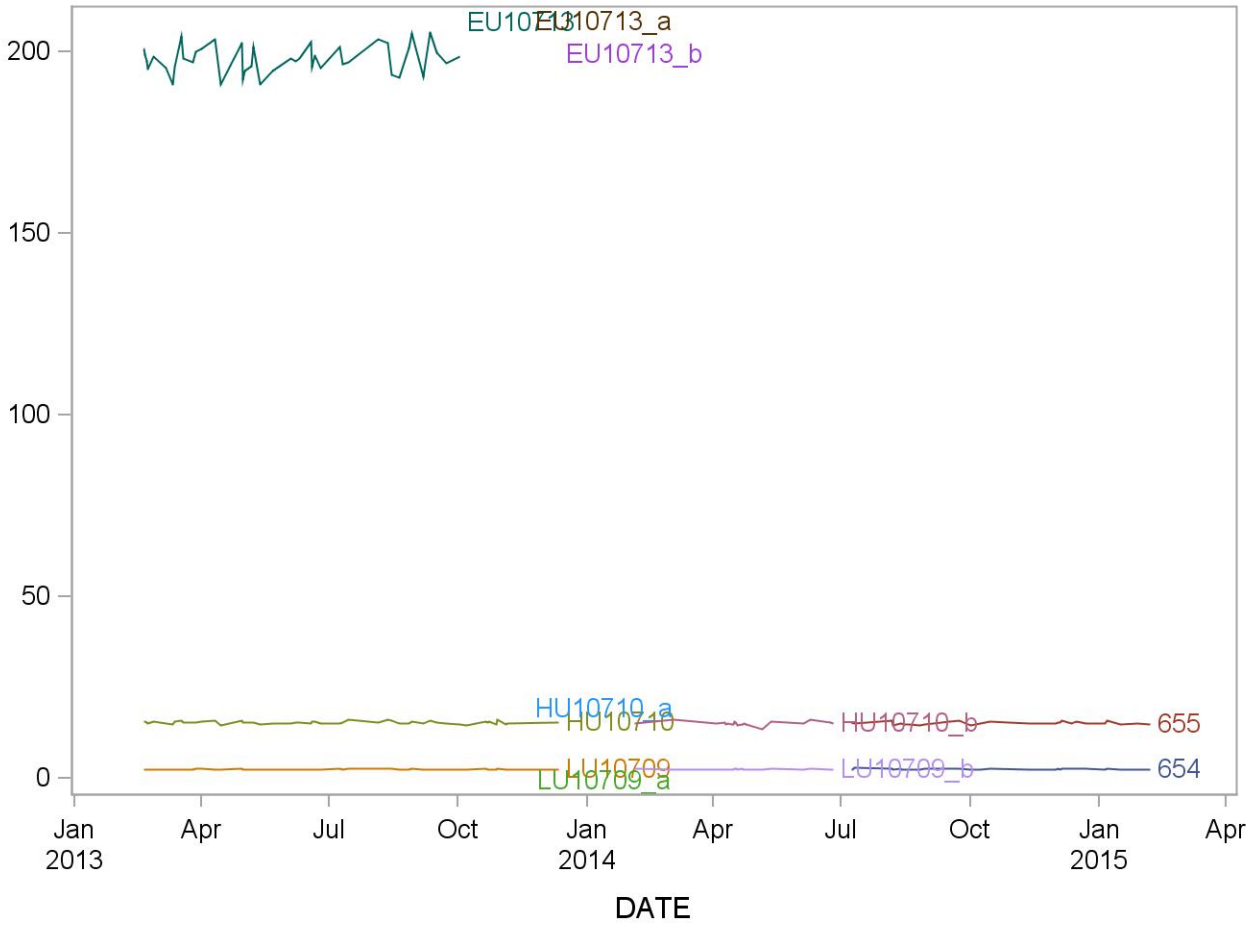
2013-2014 Summary Statistics and QC Chart for Antimony, urine (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU10710	83	19FEB13	12DEC13	0.6001	0.0192	3.2
LU10709	83	19FEB13	12DEC13	0.1550	0.0153	9.9
EU10713	68	19FEB13	02OCT13	49.4368	0.7702	1.6
HU10710_a	2	13JAN14	13JAN14	0.6118	0.0011	0.2
LU10709_a	2	13JAN14	13JAN14	0.1518	0.0032	2.1
EU10713_a	2	13JAN14	13JAN14	49.2500	1.4849	3.0
HU10710_b	46	04FEB14	25JUN14	0.5968	0.0185	3.1
LU10709_b	46	04FEB14	25JUN14	0.1612	0.0104	6.5
EU10713_b	4	04FEB14	04FEB14	47.7625	0.8864	1.9
655	50	08JUL14	06FEB15	0.5962	0.0142	2.4
654	50	08JUL14	06FEB15	0.1595	0.0075	4.7



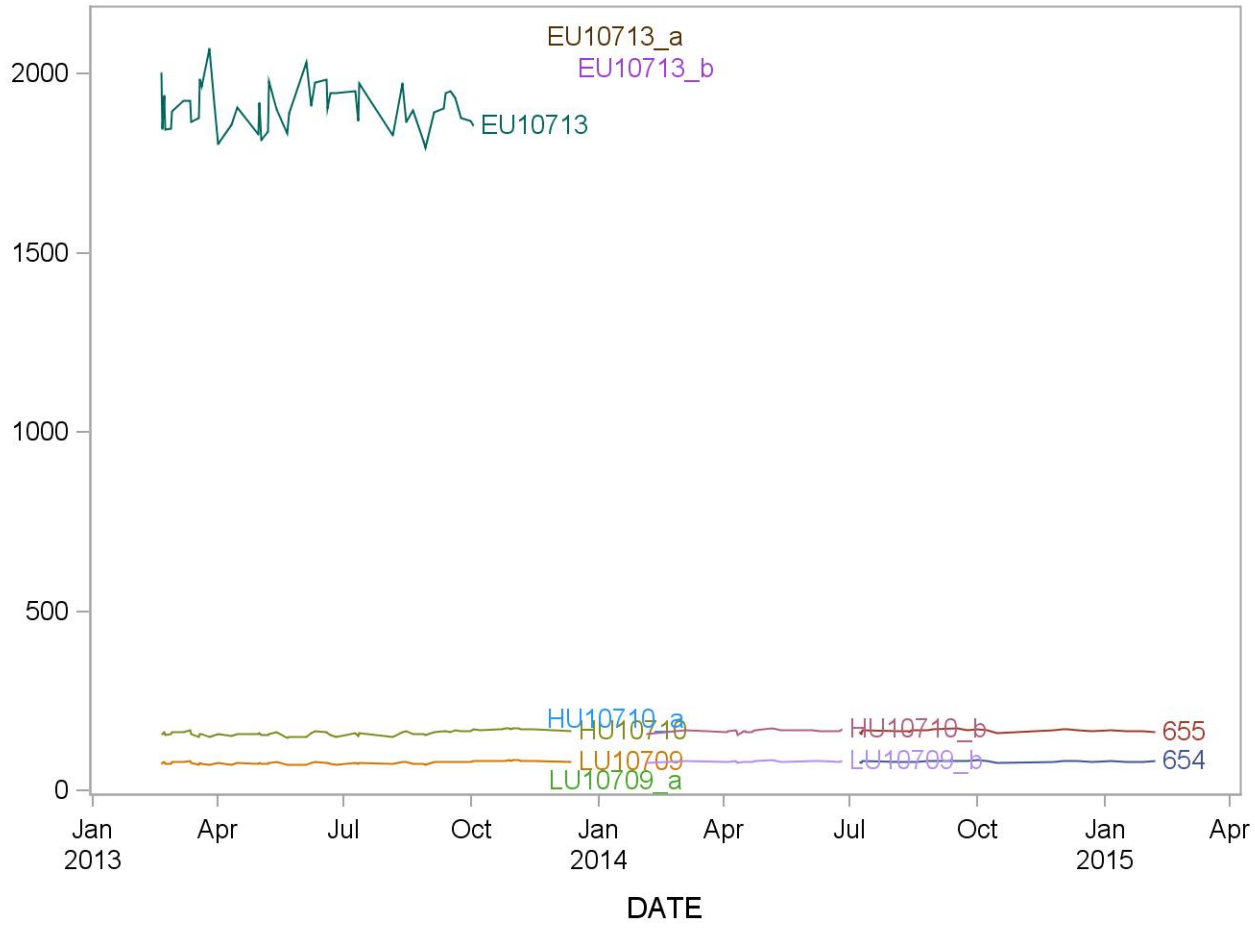
2013-2014 Summary Statistics and QC Chart for Tin, urine (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU10710	81	19FEB13	12DEC13	15.403	0.409	2.7
LU10709	81	19FEB13	12DEC13	2.479	0.088	3.6
EU10713	67	19FEB13	02OCT13	197.975	4.091	2.1
HU10710_a	2	13JAN14	13JAN14	15.228	0.350	2.3
LU10709_a	2	13JAN14	13JAN14	2.405	0.134	5.6
EU10713_a	2	13JAN14	13JAN14	200.145	4.391	2.2
HU10710_b	47	04FEB14	25JUN14	15.150	0.480	3.2
LU10709_b	47	04FEB14	25JUN14	2.536	0.099	3.9
EU10713_b	3	04FEB14	04FEB14	195.227	3.685	1.9
655	53	08JUL14	06FEB15	15.211	0.410	2.7
654	53	08JUL14	06FEB15	2.531	0.108	4.3



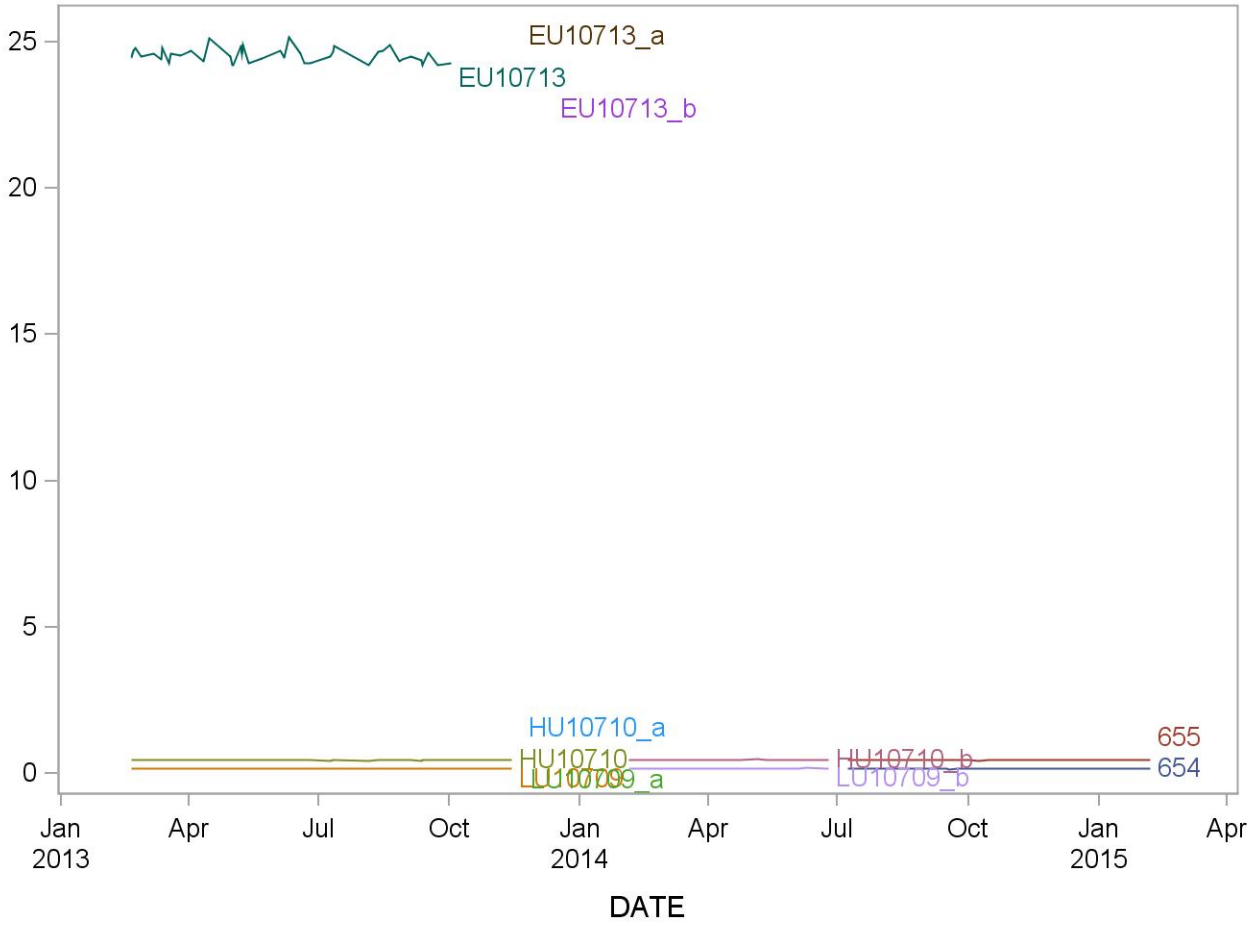
2013-2014 Summary Statistics and QC Chart for Strontium, urine (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU10710	93	19FEB13	12DEC13	162.413	7.287	4.5
LU10709	93	19FEB13	12DEC13	78.751	3.500	4.4
EU10713	76	19FEB13	02OCT13	1903.214	64.981	3.4
HU10710_a	2	13JAN14	13JAN14	159.340	2.857	1.8
LU10709_a	2	13JAN14	13JAN14	78.153	1.057	1.4
EU10713_a	2	13JAN14	13JAN14	2062.443	49.430	2.4
HU10710_b	51	04FEB14	25JUN14	166.171	4.405	2.7
LU10709_b	51	04FEB14	25JUN14	81.067	2.138	2.6
EU10713_b	4	04FEB14	04FEB14	1990.868	45.978	2.3
655	51	08JUL14	06FEB15	168.507	3.557	2.1
654	51	08JUL14	06FEB15	81.972	1.717	2.1



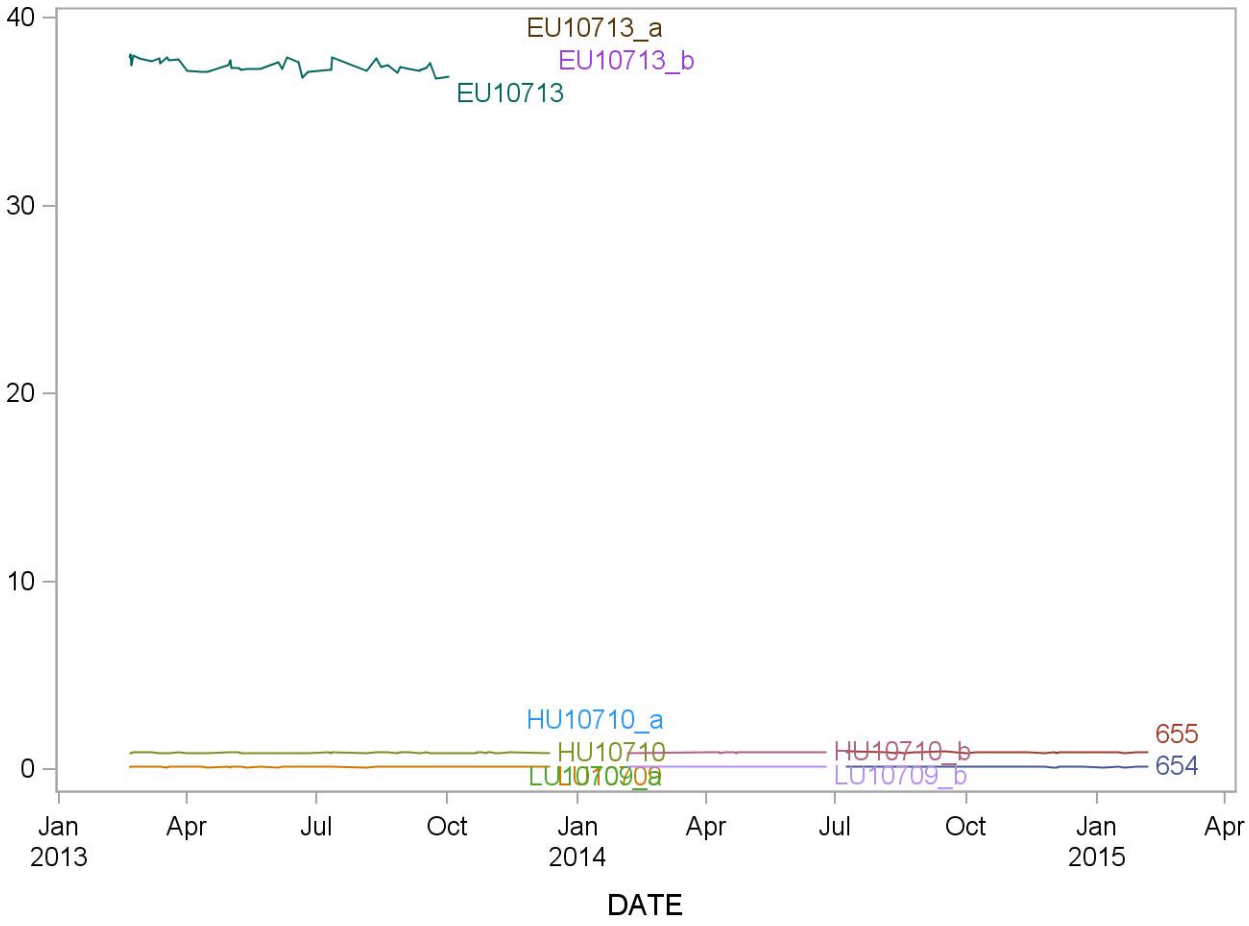
2013-2014 Summary Statistics and QC Chart for Thallium, urine (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU10710	85	19FEB13	14NOV13	0.4584	0.0081	1.8
LU10709	85	19FEB13	14NOV13	0.1701	0.0063	3.7
EU10713	72	19FEB13	02OCT13	24.5132	0.2733	1.1
HU10710_a	2	13JAN14	13JAN14	0.4710	0.0049	1.1
LU10709_a	2	13JAN14	13JAN14	0.1760	0.0007	0.4
EU10713_a	2	13JAN14	13JAN14	24.7000	0.2121	0.9
HU10710_b	47	04FEB14	25JUN14	0.4661	0.0114	2.4
LU10709_b	47	04FEB14	25JUN14	0.1740	0.0093	5.3
EU10713_b	4	04FEB14	04FEB14	23.9625	0.1436	0.6
655	51	08JUL14	06FEB15	0.4656	0.0117	2.5
654	51	08JUL14	06FEB15	0.1712	0.0089	5.2



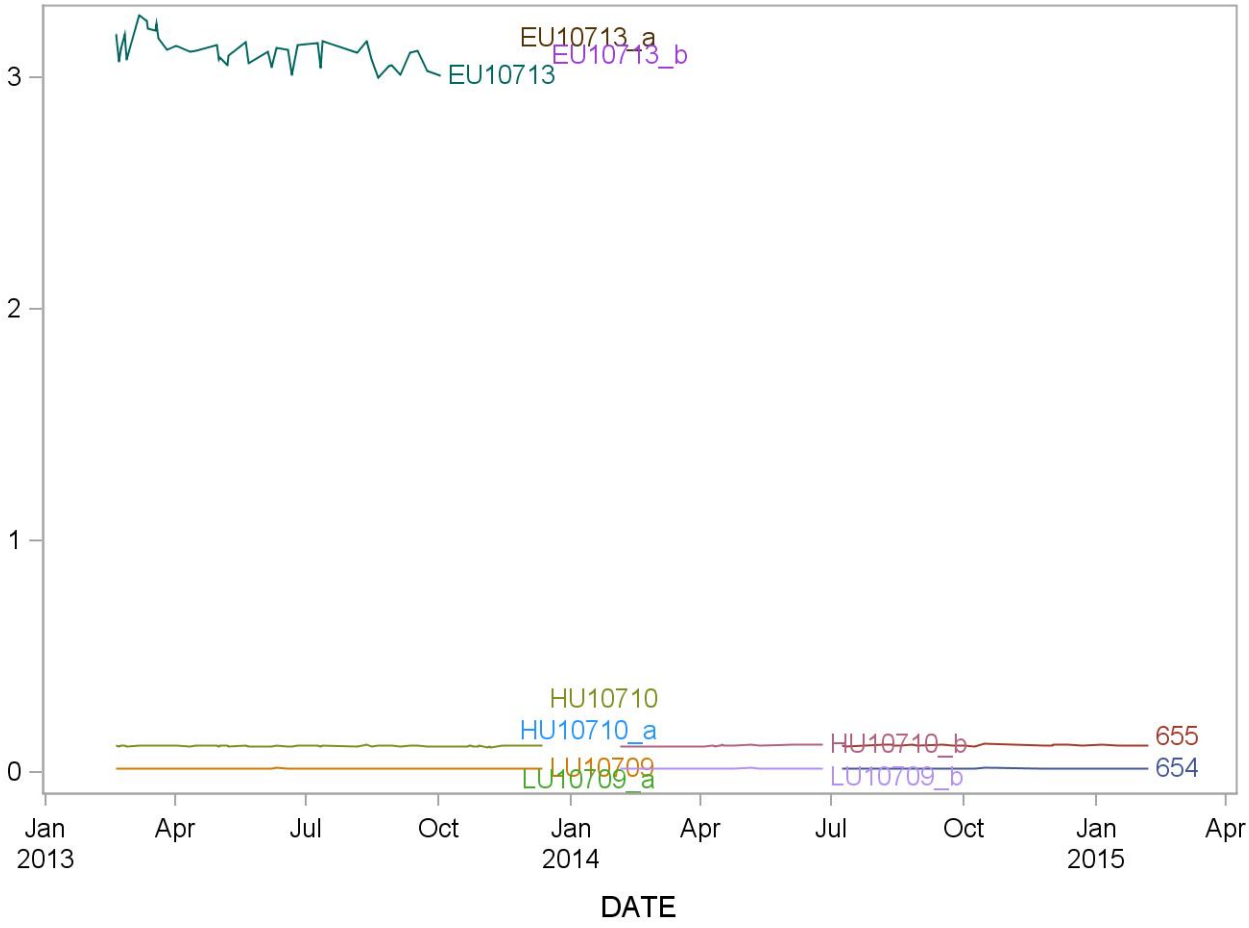
2013-2014 Summary Statistics and QC Chart for Tungsten, urine (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU10710	89	19FEB13	12DEC13	0.9001	0.0162	1.8
LU10709	89	19FEB13	12DEC13	0.1455	0.0114	7.9
EU10713	72	19FEB13	02OCT13	37.4508	0.3993	1.1
HU10710_a	2	13JAN14	13JAN14	0.9123	0.0011	0.1
LU10709_a	2	13JAN14	13JAN14	0.1435	0.0021	1.5
EU10713_a	2	13JAN14	13JAN14	37.4425	0.1025	0.3
HU10710_b	43	04FEB14	25JUN14	0.9139	0.0199	2.2
LU10709_b	43	04FEB14	25JUN14	0.1564	0.0128	8.2
EU10713_b	2	04FEB14	04FEB14	36.9225	0.1732	0.5
655	53	08JUL14	06FEB15	0.9148	0.0232	2.5
654	53	08JUL14	06FEB15	0.1462	0.0147	10.0



2013-2014 Summary Statistics and QC Chart for Uranium, urinary (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU10710	82	19FEB13	12DEC13	0.11504	0.00243	2.1
LU10709	82	19FEB13	12DEC13	0.01738	0.00079	4.6
EU10713	67	19FEB13	02OCT13	3.10824	0.07044	2.3
HU10710_a	2	13JAN14	13JAN14	0.11508	0.00392	3.4
LU10709_a	2	13JAN14	13JAN14	0.01783	0.00011	0.6
EU10713_a	2	13JAN14	13JAN14	3.10450	0.07283	2.3
HU10710_b	45	04FEB14	25JUN14	0.11765	0.00349	3.0
LU10709_b	45	04FEB14	25JUN14	0.01767	0.00093	5.2
EU10713_b	4	04FEB14	04FEB14	3.03325	0.05459	1.8
655	50	08JUL14	06FEB15	0.11850	0.00260	2.2
654	50	08JUL14	06FEB15	0.01799	0.00078	4.3



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