Laboratory Procedure Manual

Analytes: **Arsenic, Chromium, and Nickel**

Matrix: **Urine**

Method: **Arsenic, Chromium, and Nickel in Urine by ICP-MS**

Method No: **3031.1-01**

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As performed by: Inorganic and Radiation Analytical Toxicology Branch
Division of Laboratory Sciences
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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 UTAS_J, UCM_J, and UNI_J.

File Name	Variable Name	SAS Label
UTAS_J	URXUAS	Urinary Total Arsenic (µg/L)
UCM_J	URXUCM	Urinary Chromium (µg/L)
UNI_J	URXUNI	Urinary Nickel (µg/L)

1. Clinical relevance and summary of test principle

A. Clinical relevance

Arsenic (As) naturally occurs in both highly toxic (e.g. As III, As V, MMA, DMA) and less toxic forms (e.g. arsenobetaine, arsenocholine)[2]. Chromium (Cr) is considered both toxic (e.g. Cr^{6+}) and an essential element (e.g. Cr^{3+}, carbohydrate metabolism)[3]. Less is known about the biological importance of nickel (Ni) [4]. Essentiality of nickel has not been proven in humans, though it has been for animal studies, while toxicity in humans has been documented.

The principal route of human exposure to As is through ingestion of food and water; other routes are possible, depending on the environment and proximity to industrial sites. Both Cr and Ni can be introduced into the human body by way of ingestion of plants or supplements, degradation of metal-on-metal implants, and industrial exposure.

Inorganic As has been determined a carcinogen by the Department of Health and Human Services (DHHS) [2] and the Environmental Protection Agency (EPA) [5] and ingestion of large oral doses can result in death. Lower levels can irritate the stomach and intestines leading to nausea, vomiting, and diarrhea, and chronic exposure will lead to changes in skin appearance and perhaps skin cancer. Inhalation of inorganic arsenic will irritate the throat and lungs and poses an increased risk of lung cancer. Exposure and ingestion of organic arsenic may cause diarrhea and damage to the bladder and kidneys[2]. Inhalation of chromium(VI) can damage the nose and cause lung cancer, while chromium(VI) in drinking water has led to increased stomach ulcers [6]. Contact with nickel can cause allergic skin reactions; inhalation of nickel has been found to lead to asthma, chronic bronchitis, and lung cancer; drinking water containing high levels of nickel has been found to cause stomach, blood, and kidney issues [7, 8]. Comprehensive evaluation of exposure requires both total and speciated analysis of a biological sample. For example, total Cr measurements are necessary since Cr^{6+} and Cr^{3+} can be converted to the other species depending on the biological or environmental conditions.

This method is used to achieve rapid and accurate quantification of total As, Cr, and Ni in one analytical run. Use this method to screen urine when people are suspected to be acutely exposed to these elements or to evaluate chronic environmental or other non-occupational exposure (i.e. biomonitoring). [9-12].

B. Test principle

Inductively coupled plasma mass spectrometry (ICP-MS) is a multi-element analytical technique capable of trace level elemental analysis [11-13]. 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, a 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^{-7} torr). In the NexION ICP-MS, ions then pass through a focusing region, the Universal Cell (UCT), the quadrupole mass

filter, and finally are counted in rapid sequence at the detector allowing individual isotopes of an element to be determined.

The UCT is a pressurizable quadrupole that can be operated in one of three modes. In 'standard' mode (also called 'vented' mode) the cell is not pressurized and ions pass through the cell to the quadrupole mass filter unaffected. The remaining two modes are used to separate analyte ions from other interfering, often polyatomic, ions of the same mass-to-charge-ratio. In 'Dynamic Reaction Cell', or 'DRC', mode the cell is pressurized with a gas which will 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 'Kinetic Energy Discrimination', or 'KED', mode the cell is pressurized with a non-reactive gas which collides with the ions. Collisions will occur more frequently with larger polyatomic ions than smaller, atomic, analyte ions, thus lowering the kinetic energy of the polyatomic ions relative to the atomic ions. In this mode, the universal cell is maintained at a voltage more negative than the analyzing quadrupole, creating an energy barrier between the two. This barrier is the kinetic energy discriminator and the faster moving analyte ions are able to overcome it while the larger polyatomic ions are not, separating the analyte ions from the interference ions.

This ICP-MS method utilizes both DRC and KED modes. In DRC mode the cell is pressurized with ammonia (NH_3) gas to remove the polyatomic ions $^{40}Ar^{12}C^{+}$and $^{35}Cl^{16}O^{1}H^{+}$ at m/z 52 to allow for the measurement of $^{52}Cr^{+}$. In KED mode, the cell is pressurized with helium to measure arsenic and/or nickel. For arsenic, the helium collides with $^{40}Ar^{35}Cl^{+}$ and $^{40}Ca^{35}Cl^{+}$ ions, slowing these polyatomic interferences, which would otherwise interfere with detection of ^{75}As at m/z 75. For nickel, the helium collides with $^{44}Ca^{16}O^{+}$ ions, which would otherwise interfere with detection of ^{60}Ni at m/z 60.

Researchers have published a variety of approaches to removing interferences from ^{75}As, ^{52}Cr and ^{60}Ni using reaction cell or collision cell quadrupole ICP-MS instrumentation and gases. Arsenic methods have used a quadrupole-based cell with 10% hydrogen in argon [14], argon gas [15], or nitrogen gas [16]; a hexapole-based cell with helium [17] or helium and hydrogen [18]; and an octopole-based cell with helium [19] or reactive gases [20, 21]. Chromium analysis methods have used a quadrupole cell in reaction mode with ammonia gas [22-24], argon gas [15], or nitrogen gas [16] or in collision / KED mode with He [25]. Nickel analysis methods have used a quadrupole cell in reaction mode with ammonia [26, 27] or in KED mode with helium [25]. The method described in this write-up was validated to be comparable to using quadrupole-based cell with 10% hydrogen in argon [14] for arsenic analysis and a collision / KED mode with He [25] for chromium analysis. We selected the interference removal approaches used in this method to maximize efficiency of As, Cr, and Ni in urine analysis with a single method while maintaining selectivity necessary for biomonitoring.

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. Urine samples are diluted 1+9 with 2% v/v concentrated nitric acid. The diluent contains rhodium (Rh) for internal standardization. Nitric acid is used for the purpose of solubilizing and stabilizing metals in solution. Internal standards are at 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.

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 collision cell of the UCT is used in this method to diminish the presence of the argon chloride ($^{40}Ar^{35}Cl$) interference on arsenic at m/z 75 [14] which is common to urine analysis by ICP-MS (see Section 1.B for an explanation of this process).The collision gas used for this purpose is ultra-pure helium.

ii. Calcium chloride ($^{40}Ca^{35}Cl$) on arsenic (^{75}As): The collision cell of the UCT is used in this method to diminish the presence of the calcium chloride ($^{40}Ca^{35}Cl$) interference on arsenic at m/z 75 which can be present in urine analysis by ICP-MS (see Section 1.B for an explanation of this process).The collision gas used for this purpose is ultra-pure helium.

iii. Argon carbide ($^{40}Ar^{12}C$) and hypochlorous acid ($^{35}Cl^{16}O^{1}H$) on chromium (^{52}Cr): The reaction cell of the UCT is used in this method to remove the argon carbide ($^{40}Ar^{12}C$) and hypochlorous acid ($^{35}Cl^{16}O^{1}H$) interferences on chromium at m/z 52 which is common to urine analysis by ICP-MS (see Section 1.B for an explanation of this process). The collision gas used for this purpose is ultra-pure helium.

iv. Calcium oxide ($^{44}Ca^{16}O$) on nickel (^{60}Ni): The collision cell of the UCT is used in this method (see Section 1.B for an explanation of this process) to remove the calcium oxide ($^{44}Ca^{16}O$) interference on ^{60}Ni. The collision gas used for this purpose is ultra-pure helium.

v. Non-spectral, matrix enhancement of arsenic signal: Matrix induced signal enhancement in ICP-MS analysis from carbon on arsenic has been previously reported in the literature [28, 29]. 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.

B. Interferences not addressed by this method:

No remaining interferences are known.

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

A. Procedures for collecting, storing, and handling specimens

Specimen handling conditions, special requirements, and procedures for collection and transport are discussed in the Division of Laboratory Science’s (DLS) Policies and Procedures Manual [1]. In general:

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 (e.g. two weeks). 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 (e.g. 2 to 5 mL cryogenic vial or 15 mL 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 compromise test results are indicated above. Other reasons for rejecting a sample for analysis are listed below. In all cases, request a second urine specimen.

i. Low volume: Optimal amount of urine is ~1.0 mL. The volume of urine used for one analysis is 0.3 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 [1]. 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) will have access to the personal identifiers.

4. Safety precautions

A. General safety

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

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

iii. Observe "universal precautions" when working with urine.

iv. Exercise special care when handling and dispensing concentrated nitric acid. Use additional personal protective equipment which protects face, neck, and front of body. Add acid to water. Nitric acid is a caustic chemical 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 hazardous liquids.

vi. The use of the foot pedal on the benchtop automatic pipette is recommended because it reduces analyst contact with work surfaces that have been in contact with urine and also

keeps the analyst's hands free to hold the specimen cups and autosampler tubes and to wipe off the dispensing tip.

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.

ix. Place ammonia gas cylinders (either in use or in storage) in a cabinet which is well ventilated to the house exhaust. Do not place ammonia cylinders on their side while in use as the cylinder valve can become "frozen" in place as a result of the cooling capacity of expanding ammonia gas. Label the cabinet to indicate it contains ammonia.

x. Wipe down all work surfaces at the end of the day with disinfectant. Use either a daily remake of diluted bleach (1 part household bleach containing 5.25% sodium hypochlorite + 9 parts water) or an equivalent disinfectant.

B. Waste disposal

i. Autoclaving: Autoclave all diluted biological samples, original biological specimens being disposed, and non-metallic consumables which come into contact with biological samples (even after dilution or aerosolization). Use a plastic sharps container or labeled autoclave pan for broken glass / quartz and other puncture hazards (e.g. pipette tips).

ii. Other liquid waste

1) Waste discarded down sink: Do not discard solutions at the sink having a pH lower than 5.0 or higher than 11.5 (limits defined by Dekalb County, GA). Inactivate biological compounds and cellular constituents in mixed chemical and biological waste, such as the waste carboy of the ICP-MS, by adding an approved disinfectant (e.g. household bleach at a 1:100 dilution or equivalent) prior to drain disposal. Flush the sink with copious amounts of water.

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

5. Instrument and material sources

A. Sources for ICP-MS instrumentation

i. ICP-MS: Inductively coupled plasma mass spectrometer (NexION 300 D) (PerkinElmer Norwalk, CT, www.perkinelmer.com) with the universal cell updated to the bio-monitoring Universal Cell (Bio UCell), or equivalent.

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

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 NexION ICP-MS replaced by DXi-FAST micro-peristaltic pump / FAST actuator and valve combination unit. 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 and 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 1.6 mm 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-260 x (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 2 luer connections (catalog# SC-0305-1, Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com) or equivalent.

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, www.lss.com) with cap assembly like part # N0690271 (PerkinElmer, Norwalk, CT, www.perkinelmer.com) or equivalent.

iv. Cone, aluminum gasket for sampler cone: PerkinElmer part # WE012989 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or equivalent. # Spares = 4

v. Cone, hyperskimmer (aluminum): PerkinElmer part # W1033995 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or equivalent. # Spares = 1

vi. Cone, sampler (nickel/platinum): PerkinElmer part # W1033612**/**W1033614 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or cross-referenced part number manufactured by Spectron Inc. (Ventura, CA, www.spectronus.com) or Glass Expansion (Pocasset, MA, www.geicp.com). *# Spares = 4*.

vii. Cone, screws for hyperskimmer: PerkinElmer part # 09919737(PerkinElmer Norwalk, CT, www.perkinelmer.com) or equivalent. # Spares = 4

viii. Cone, skimmer (nickel/platinum): PerkinElmer part # W1026356**/**W1026907 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or cross-referenced part number

manufactured by Spectron Inc. (Ventura, CA, www.spectronus.com) or Glass Expansion (Pocasset, MA, www.geicp.com). *# Spares = 4*.

ix. 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*.

x. 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*.

xi. Detector, electron multiplier: Like part # N8140163 (PerkinElmer Norwalk, CT, www.perkinelmer.com). *# Spares = 1*.

xii. Enclosure for NexION roughing pump (optional): Reduces noise of mechanical roughing pump. Enclosure and rolling platform like part numbers N8121068 and N8121069 (PerkinElmer, Shelton, CT, www.perkinelmer.com), respectively.

xiii. Enclosure for SC-4 DX autosampler (autosampler): Protects samples on the autosampler waiting for analysis from contamination. Like part number SC-1407-DX (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). Setup with ventilation from either an exhaust duct or (preferred) an UPLA filter like part number SC-0602 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xiv. Hose, for connection to 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. Do not normally need spare hose (unless moving instrument into a new location).

xv. Hose, for exhaust of NexION: Available as part of NexION installation kit from Perkin Elmer (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available direct from manufacturer as part # S-LP-10 air connector (Thermaflex, Abbeville, SC, www.thermaflex.net), or equivalent. *# Spares = (2) 10 feet of 4" diameter hose.*

xvi. Injector, quartz with ball joint: I.D. = 2.0 mm. PerkinElmer part # WE023948 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or equivalent. Available direct from manufacturer as part # 400-30 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com) or from various distributors. *# Spares = 2.*

xvii. Loop, sample (FAST): 2 mL Teflon, white connector-nuts for high flow valve head, 1.6 mm i.d., ¼-28 fittings. Like part # SC-0315-20 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xviii. Loop, sample (FAST): 0.5 mL Teflon, white connector-nuts for high flow valve head, 1.6 mm i.d., ¼-28 fittings. Like part # SC-0315-05 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). Can be used if only one element is measured.

xix. 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., www.elementalscientific.com). *# Spares = 1*. Different nebulizers are acceptable, 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., www.elementalscientific.com). *# Spares = 1*.

b) Adapter kit: Plastic adapters to connect Teflon tubing (2.4 mm 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 like part # ES-2501-1000 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). # *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., www.elementalscientific.com). *# Spares = 2*.

xx. 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.*

xxi. 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.*

xxii. O-ring: (for hyperskimmer cone) PerkinElmer part #09902123 (pkg. of 5, PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. *# Spares = 20 O-rings.*

xxiii. Oil, Fomblin® GV80: Part #N8145003 (1 liter, PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. *# Spares = 4.*

xxiv. 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.*

xxv. 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.*

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

xxvii. 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).

xxviii. Screw, for torch mount: PerkinElmer part # WE011870. (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. *# Spares = 3*.

xxix. Spray chamber, quartz concentric: PerkinElmer part # N8145013 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares = 2.*

xxx. 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).

xxxi. Torch, quartz: PerkinElmer part # N812-2006 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. *# New Spares = 2.*

xxxii. Tubing and adapter, for SC autosampler rinse station drain: Tygon tubing and adapter to attach to back of SC autosampler for draining rinse station waste (like part # SC-0303-002, Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xxxiii. Tubing and adapters, for SC autosampler rinse station filling: 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).

xxxiv. Tubing, connects nebulizer to valve: See "Nebulizer"

xxxv. Tubing, FAST 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).

xxxvi. Tubing and nut, for FAST carrier solution: 0.5 mm 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).

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

xxxviii. Tubing, PFA: I.D. = 0.5 mm, O.D. = 1.59 mm (1/16"). Used to transfer liquid between rinse solution jug and peristaltic pump tubing. The Perfluoroalkoxy (PFA) copolymer is a form of Teflon®. Like part # 1548 (20ft length, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com) or equivalent.*# Spares = 20ft.*

xxxix. 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, www.spectronus.com) or equivalent. #Spares = 6 packs of 12 tubes. *Use this type of tubing with ESI DXi micro-peristaltic pump.*

xl. 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, www.perkinelmer.com) 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, www.spectronus.com) or equivalent. #Spares = 6 packs of 12 tubes. *Use this type of tubing with ESI DXi micro-peristaltic pump.*

xli. Tubing, stainless steel, o.d. = 1/8", wall thickness = 0.028": Used to connect gas cylinders to NexION UCT gas ports. Like part # SS-T2-S-028-20 (20ft, Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. *Spares = 20 ft.*

xlii. 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) or equivalent. *# Spares = 2.*

xliii. 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).

C. Sources for ICP-MS maintenance equipment and 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. Container, to hold acid baths for glassware: Polypropylene or polyethylene containers with lids (must be large enough for torch, injector, or spray chamber submersion). Available from laboratory or home kitchen supply companies. *# On hand = 4*.

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

iv. 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.

v. Electronic leak detector: To detect gas leaks in any of the gas lines and connections. Part # N9306089 ((PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent.

vi. 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).

vii. 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 Xenon 1902 cordless area-imaging scanner (Honeywell International Inc., Morristown, NJ, www.honeywellaidc.com). For scanning sample IDs during analysis setup. Any bar code scanner capable of reading Code 128 encoding at a 3 mil label density and 2D bar codes 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 or equivalent) and 4L polypropylene jugs (like catalog# 02-960-10A, Fisher Scientific, Pittsburgh, PA, www.fishersci.com or equivalent).

iv. Containers, secondary containment for rinse and waste: Polypropylene or polyethylene containers that rinse or waste bottles are placed into for secondary containment. Like part

14257 Polypropylene tray 16" L x 16" W x 8" H (United States Plastics Corp, Lima, Ohio, www.usplastics.com) or equivalent.

v. Cups for urine collection: Like polypropylene 4.5 oz cup, catalog # 354013 (Becton Dickinson Labware, Franklin Lakes, NJ, www.bd.com) or equivalent. Pre-screen and purchase tubes within specific manufacturing lots.

vi. Flask, volumetric:

1) 50mL volumetric flasks (like catalog # 40000050, Thermo Scientific, Fisher Scientific, Pittsburgh, PA., www.fishersci.com). Plastic or glass is acceptable.

2) 100mL volumetric flasks (like catalog # 40000100, Thermo Scientific, Fisher Scientific, Pittsburgh, PA., www.fishersci.com). Plastic or glass is acceptable.

3) 200mL volumetric flask (like catalog # 40000200, Thermo Scientific, Fisher Scientific, Pittsburgh, PA., www.fishersci.com). Plastic or glass is acceptable.

4) 500mL volumetric flask (like catalog # 40000500, Thermo Scientific, Fisher Scientific, Pittsburgh, PA., www.fishersci.com). Plastic or glass is acceptable.

5) 1L volumetric flask (like catalog # 40001000, Thermo Scientific, Fisher Scientific, Pittsburgh, PA., www.fishersci.com). Plastic or glass is acceptable.

6) 2L volumetric flask (like glass flask catalog # 92812G2000, DWK Life Sciences (Kimble), Fisher Scientific, Pittsburgh, PA., www.fishersci.com). Plastic or glass flask is acceptable.

vii. Gloves: Powder-free, low particulate nitrile (like Best CleaN-DEX™ 100% nitrile gloves, any vendor).

viii. 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, use a wipe designed for cleanrooms such as the Econowipe or Wetwipe (Liberty, East Berlin, CT, www.liberty-ind.com).

ix. Parafilm M ®: flexible plastic for general laboratory use (Bemis, Neenah, WI, www.bemis.com).

x. Pipette, benchtop automatic: For preparation of urine dilutions to be analyzed and intermediate working calibration standards. Like the Microlab 625 advanced dual syringe diluter (Hamilton, Reno, NV, http://www.hamilton.com/) equipped with a 10.0 mL left syringe, a 1.0 mL right syringe, a 12 gauge Concorde CT probe dispense tip, the Microlab cable management system and a foot pedal. PEEK valves like part # 60676-01 (left) and part # 60675-01 (right) may reduce metal background in prepared samples.

xi. Pipettes (for preparation of intermediate stock working standards & other reagents): Like Picus® NxT electronic, single-channel pipettes (Sartorius AG, Göttingen, Germany, www.sartorius.com). 5-120 µL (catalog # LH-745041), 10-300 µL (catalog #LH-745061), 50-1000 µL (catalog #LH-745081), 100-5000 µL (catalog #LH-745101).

xii. Tubes for sample analysis (for autosampler): Like polypropylene 15 mL conical tubes Greiner Bio-One part number 188261 (Greiner Bio-One North America Inc., Monroe, NC,

www.gbo.com), or equivalent. Pre-screen and purchase tubes within specific manufacturing lots.

xiii. Tubes for storage of intermediate working stock standards: Like polypropylene 50 mL conical tubes, Falcon model #352098 (Corning, Corning, NY, www.corning.com) or equivalent. For use in storage of intermediate working stock standards.

xiv. 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.

xv. Water purification system: Like NANOpureDIamond Ultrapure Water System (Barnstead International, Dubuque, Iowa, www.barnstead.com) or equivalent. For ultra-pure water used in reagent and dilution preparations.

E. Sources of chemicals, gases, and regulators

i. Acid, nitric acid: Veritas™ double-distilled grade, 68–70% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com) or equivalent. For use in intermediate working stock standards, diluent, and QC pool preparations. This is referred to as "concentrated" nitric acid in this method write-up.

ii. Acid, nitric acid: Environmental grade, 68–70% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com) or equivalent. Allowed for use in rinse solutions only. This is referred to as "concentrated" nitric acid in this method write-up.

iii. Ammonia, anhydrous: Ammonia (99.99+%) for DRC channel A is typically purchased in cylinder size LB (2"x12") (Matheson Tri-Gas, Montgomeryville, PA, 18936. www.mathesontrigas.com).

1) Regulator for ammonia: Stainless steel, two stage, specially cleaned regulator with 3,000 psig max inlet, 2-30 outlet pressure range, cylinder connector CGA 180 or 660 (for lecture bottle cylinder, CGA connection will depend on vendor) or CGA 705 (for Airgas cylinder size 200), and needle valve shutoff on delivery side terminating in a ¼" Swagelok connector. Like part number 3813-180 or 3813-705 (Matheson Tri-Gas, Montgomeryville, PA, www.matheson-trigas.com). An equivalent regulator from an alternate vendor can be substituted. *# Spares = 1*.

2) Cabinet, for storage of ammonia lecture bottle: Small cabinet with air vent to connect to house exhaust. Like Sure-grip® EX countertop flammable safety cabinet part # 890400 (Justrite Manufacturing Company, Des Plaines, IL, justritemfg.com), or equivalent. You must drill a hole in the side wall and add a rubber grommet for passage of the stainless steel gas delivery tubing from the regulator to the ICP-MS instrument.

iv. Argon gas (for plasma and 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 (180-250 L). 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) or equivalent. *# 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) or equivalent*. # Spares = 1*.

3) Regulator for argon (PerkinElmer filter regulator on back of NexION): Argon regulator filter kit. Catalog number N812-0508 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent.

v. Helium: “Research grade” helium for UCT channel B. Gas is typically purchased in cylinder size 35 (6”x24”) (Airgas South, Atlanta, GA, www.airgas.com, part # HE R300).

1) Regulator for helium: 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) or equivalent. *# Spares = 1*.

2) He Filter, advanced filter system: Part # N9303963 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent; Replacement cartridge part #N9303964 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent.

vi. Disinfectant, for work surfaces: Diluted bleach (1 part household bleach containing 5.25% sodium hypochlorite + 9 parts water), remade daily, or equivalent disinfectant.

vii. Standard, As calibration standard: Like item number “10003-1” (High Purity Standards, Charleston, SC, http://www.hps.net/) or equivalent. See Table 3 in Appendix C for concentrations. This solution is 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. Note Cr/Ni standard must be purchased separately.

viii. Standard, Cr and Ni calibration standard: Item number “SM-2107-055” (High Purity Standards, Charleston, SC, http://www.hps.net/) or equivalent. See Table 3 in Appendix C for concentrations. This solution is 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. Note As standard must be purchased separately.

ix. Standard, rhodium: Like 1,000 mg/L, item # PLRH3-2Y. (SPEX Industries, Inc., Edison, NJ, www.spexcsp.com) or equivalent. Used as an internal standard in diluent. Standard must be traceable to the National Institute for Standards and Technology.

x. Standard, single element stock standards for preparation of urine quality control pools: Like National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs) 3103a (As), 3112a (Cr) and 3136 (Ni) (National Institute of Standards and Technology (NIST), Office of Standard Reference Materials, Gaithersburg, MD,

www.nist.gov). Standards must be traceable to the National Institute for Standards and Technology.

xi. Triton X-100™ surfactant: Like "Baker Analyzed" TritonX-100™ (J.T. Baker Chemical Co., www.jtbaker.com).

6. Preparation of reagent and materials

A. Internal standard intermediate mixture

i. Purpose: Preparation of a single intermediate solution containing the internal standard will simplify the addition of the internal standard into the final diluent solution. This solution can be purchased rather than prepared. Different volumes can be prepared by adjusting amounts accordingly.

ii. Preparation and storage: To prepare 50 mL of 40 µg/mL Rh in 2% v/v HNO_3:

1) If not previously dedicated to this purpose, acid wash a 50 mL volumetric flask (PP, PMP, or Teflon™). For example, with 2% v/v HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.

2) Partially fill the volumetric flask with ≥18 Mohm·cm water (approximately 40–45 mL).

3) Carefully add 1 mL of double-distilled, concentrated nitric acid. Mix into solution.

4) Add 2,000 µg of rhodium (e.g. 2 mL of 1,000 µg/mL Rh stock standard).

5) Fill to mark (50 mL) and mix thoroughly.

6) Label appropriately. Store at room temperature. Expiration date is 1 year from preparation date.

B. Intermediate Triton X-100™ solution

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 and storage: To prepare 2 L 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 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.

2) Partially fill the bottle with ≥18 Mohm·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) Label appropriately. Store at room temperature. Expiration date is 1 year from preparation date.

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 and storage: To prepare 2 L of 10 µg/L Rh in 2% v/v HNO_3 and 1.5% v/v ethanol:

NOTE: This solution does not have to be made up in a volumetric flask. The important thing about the concentration of the internal standards is that they be consistent within all samples in one run. To prepare different volumes of diluent, add proportionally larger or smaller volumes of the solution constituents.

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

2) Partially fill the 2 L container with ≥18 Mohm·cm water.

3) Carefully add 40 mL concentrated nitric acid and mix.

4) Carefully add 30 mL dehydrated 200 proof ethanol and mix.

5) Add 500 µL of the 40 µg/mL Rh internal standard solution. If other concentrations are used, adjusted the volume proportionally.

6) Make up to volume (2 L) with ≥18 Mohm·cm water.

7) Label appropriately. Store at room temperature. Expiration date is 1 year from preparation date.

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 and storage: To prepare 4L of a 0.002% Triton X-100™, 2% v/v nitric acid, and 1.5% v/v ethanol solution.

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

2) Partially fill the bottle with ≥18 Mohm·cm water (approximately 2–3 L). Use of volumetric flask is not required.

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

4) Carefully add 80 mL of double distilled or environmental grade concentrated nitric acid and mix well.

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

6) Fill to 4 L using ≥18 Mohm·cm water.

7) Label appropriately. Store at room temperature. Expiration date is 1 year from preparation date.

E. Base urine

i. 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.

ii. Preparation and storage: To prepare a mixture of multiple urine sources collected from anonymous donors (to approximate an average urine matrix).

1) 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 (complete details can be found in CDC protocol #3994).

2) Once the urine is collected from donors, ensure by analysis that concentrations are relatively low, so as to not interfere with the proper measurement of calibrators (see Table 2 in Appendix C for suggested maximum base urine concentrations).

3) Once screened, mix the urine collections together in a larger container (i.e. acid washed polypropylene (PP), polymethylpentene (PMP), or Teflon™) and stir for 30+ minutes on a large stir plate (acid wash large Teflon™ stir bar before use).

4) 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.

5) Label appropriately and store frozen (e.g. ≤ -20°C). Urine stored at ≤ -20°C has been used successfully for up to 5 years as quality control material.

F. Stock calibration standard

i. Purpose: This master solution will be diluted to prepare the intermediate stock calibration standard and intermediate working calibrators S4–S6.

ii. Purchase and storage: To obtain an aqueous solution containing As, Cr and Ni, concentrations listed in Table 3 of Appendix C, in a matrix of 2% v/v HNO_3:

1) Purchasing from vendors: Purchase NIST-traceable custom mixtures of Ni and Cr, and As, or prepare the mixture in-house from NIST-traceable, single element standards. See Section 5.E.vii and 5.E.viii.

2) Storage: Store at room temperature. Label appropriately. Expiration is 1 year from date of opening or as noted by the manufacturer, whichever comes first.

G. Intermediate calibration standard diluent (S0)

i. Purpose: This diluent will be used to fill the flasks during preparation of the intermediate stock and intermediate working calibration standards and can be used in cleaning flasks and checking container cleanliness.

ii. Preparation and storage: To prepare 2 L of 2% v/v HNO_3 in ≥18 Mohm·cm water.

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

2) Partially fill the bottle with ≥18 Mohm·cm water (approximately 1–1.5 L).

3) Carefully add 40 mL high purity, concentrated HNO_3 (double distilled).

4) Fill to the mark and mix thoroughly.

5) Once mixed, transfer to acid-cleaned, labeled containers (PP, PMP, or Teflon™) for storage.

6) Set aside a portion (3 x 50 mL suggested) to be used as the S0 intermediate working calibrator, used in preparing the S0 working calibrator.

7) Label appropriately. Store at room temperature. Expiration date is 1 year from preparation date.

H. Intermediate stock calibration standards

i. Purpose: This solution will be diluted to prepare the intermediate working calibrators S1–S3.

ii. Preparation and storage:

1) Store at room temperature. Label appropriately. Expiration is 1 year from date of opening or as noted by the manufacturer, whichever comes first.

2) If not previously dedicated to this purpose, acid wash two 100 mL volumetric flasks (PP, PMP, or Teflon™). For example, with 2% v/v HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Dedicate to purpose. Label one flask "As Int." and the other flask "Cr/Ni Int."

3) Partially fill (50–75% full) the 100 mL flask with the intermediate calibrator diluent (2% v/v HNO_3).

4) Using the volumes listed in Table 4 of Appendix C, pipette the appropriate volume of the intermediate stock calibration standard into each of the volumetric flasks.

5) Dilute each to the volumetric mark with the intermediate calibrator diluent (2% v/v HNO_3) using a pipette for the final drops. Mix each solution thoroughly.

6) Transfer to acid-cleaned, labeled, 50 mL containers (PP, PMP, or Teflon™) for storage.

7) Label appropriately. Store at room temperature. Expiration date is 1 year from preparation date. Final concentrations are listed in Table 4 of Appendix C.

I. Intermediate working calibration standards

i. Purpose: Use each day of analysis to prepare the final working calibrators that will be placed on the autosampler.

ii. Preparation and storage:

1) Store at room temperature. Label appropriately. Expiration is 1 year from date of opening or as noted by the manufacturer, whichever comes first.

2) If not previously dedicated to this purpose, acid wash two 100 mL, three 200 mL, and one 500 mL volumetric flasks (PP, PMP, or Teflon™). For example, with 2% v/v HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Dedicate to purpose.

3) Partially fill (50–75% full) the 100 mL, 200 mL, and 500 mL flasks with the intermediate calibrator diluent (2% v/v HNO_3).

4) Using the volumes listed in Table 5 of Appendix C, pipette the appropriate volume of the intermediate stock calibration standard into each of the volumetric flasks.

5) Dilute each to the volumetric mark with the intermediate calibrator diluent (2% v/v HNO_3) using a pipette for the final drops. Mix each solution thoroughly.

6) Transfer to acid-cleaned, labeled, 50 mL containers (PP, PMP, or Teflon™) for storage.

7) Label appropriately. Store at room temperature. Expiration date is 1 year from preparation date. Final concentrations are listed in Table 5 of Appendix C.

J. Working calibrators

i. Purpose: The working calibrators will be analyzed in each run to provide a signal-to-concentration response curve for each analyte in the method across appropriate concentration range. 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 calibrators.

ii. Preparation and use: To prepare dilutions (1:100) of the corresponding intermediate working calibration standards: see Table 8 of Appendix C for details of sample preparation. Make these immediately prior to analysis when the intermediate working calibration standards are mixed with base urine and diluent using a benchtop automatic pipette.

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

i. 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.

ii. 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 spiked, if necessary, to reach a desired concentration. The analyte concentrations are in the low-normal concentration range ("low QC"), high-normal concentration range ("high QC"), and elevated concentration range ("elev. QC").

1) Preparation and 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:

2) 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, high, and elevated bench QC.

3) 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).

a) Keep urine refrigerated whenever possible to minimize microbial growth.

b) 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.

c) Select urine samples for low QC and high QC levels to represent the low-normal and high-normal population ranges. See the National Report on Human Exposure to Environmental Chemicals for estimations of the normal population ranges for metals (http://www.cdc.gov/exposurereport/).

4) 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.

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

b) 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.

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

d) 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.

e) Settling out of solids:

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

g) 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.

h) Repeat steps f and g until minimal solids are left at the bottom of the carboy after sitting overnight.

5) Spiking of urine

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

b) Use these results to determine target analyte concentrations possible for the pools

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

d) 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).

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

f) Repeat steps d and e until all analytes reach target concentrations keeping track of the total volume of spiking solution added to each urine pool.

6) Dispensing and storage of urine

a) Container types: Dispense urine into lot screened containers (i.e. 2–15 mL polypropylene tubes). If possible, prepare tubes of QC which have only enough volume for one typical run + 1 repeat analysis (e.g. 1.8 mL). 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.

b) 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.

c) Dispensing: Use a benchtop automatic pipette in continuous cycling dispense mode in a clean environment (i.e. a class 100 cleanroom area or hood).

i) 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).

ii) Attach tubing to the syringe of the benchtop automatic pipette with a length of clean Teflon™ tubing long enough to reach into the bottom of the carboy while it is sitting on the stir plate.

iii) Check cleanliness of the benchtop automatic pipette before use by analyzing 1–2% v/v HNO_3 which has been flushed through the pipette with a portion of the same solution which has not been through the pipette.

iv) Approximately one hour before dispensing begins,

(1) With the large stir plate close to the left side of the pipette, begin stirring the urine pool to be dispensed.

(2) Also during this time, flush the pipette 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 M® so they will not come out during the flushing process.

(3) 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.

d) 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.

e) Storage: Store urine pools long term at ≤ -20°C. Short term storage (several days) at refrigerator temperature (~ 2–8°C) is acceptable.

L. Universal cell optimization solutions

i. Purpose: For as-needed verification / troubleshooting of the DRC and KED parameters for As, Cr and/or Ni. Procedure requires the following solutions: base urine blank (1), urine blank spiked with analyte (2), urine blank spiked with interference (3), and urine blank spiked with analyte and interference (4). Interferences are discussed in Sections 1.B and 2.A. Interference concentrations can be prepared higher as needed by adjusting the volume of spikes. Keep interference spike volumes small (<0.3 mL) using high concentration stock solutions (i.e. 1000 µg/mL). Analyte concentrations can be made higher if needed for sensitivity reasons by preparing a higher concentration calibrator. If elimination of the interference is difficult to verify, compare effect of DRC or KED

parameters on multiple isotopes of Cr and / or replace the use of urine in these preparations with ultrapure water to minimize trace amounts of the analyte in the preparation.

ii. Preparation and storage (Cl interferences on Cr and As)

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

2) Base urine in diluent (1 + 9) + 2 µg/L Cr + 6 µg/L As: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 3 as described in Table 8 of Appendix C (multiply volumes by 5).

3) Base urine in diluent (1 + 9) + 0.1% v/v HCl: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 0 as described in Table 8 of Appendix C (multiply volumes by 5). Add 0.05 mL of high purity, concentrated HCl.

4) Base urine in diluent (1 + 9) + 2 µg/L Cr + 6 µg/L As + 0.1% v/v HCl: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 3 as described in Table 8 of Appendix C (multiply volumes by 5). Add 0.05 mL of high purity, concentrated HCl.

5) Label appropriately. Store at room temperature. Expiration date is 1 year from preparation date.

iii. Preparation and storage (C interference on Cr)

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

2) Base urine in diluent (1 + 9) + 1 µg/L Cr: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 3 as described in Table 8 of Appendix C (multiply volumes by 5).

3) Base urine in diluent (1 + 9) + 0.1% ethanol: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 0 as described in Table 8 of Appendix C (multiply volumes by 5). Add 0.05 mL of 200 proof ethanol.

4) Base urine in diluent (1 + 9) + 1 µg/L Cr + 0.1% ethanol: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 3 as described in Table 8 of Appendix C (multiply volumes by 5). Add 0.05 mL of 200 proof ethanol.

5) Label appropriately. Store at room temperature. Expiration date is 1 year from preparation date.

iv. Preparation and storage (Ca interference on Ni)

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

2) Base urine in diluent (1 + 9) + 1 µg/L Ni: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 3 as described in Table 8 of Appendix C (multiply volumes by 5).

3) Base urine in diluent (1 + 9) + 20 µg/mL Ca: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 0 as described in Table 8 of Appendix C (multiply volumes by 5). Add 0.1 mL of 10,000 µg/mL Ca.

4) Base urine in diluent (1 + 9) + 1 µg/L Ni + 20 µg/mL Ca: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 3 as described in Table 8 of Appendix C (multiply volumes by 5). Add 0.1 mL of 10,000 µg/mL Ca.

5) Label appropriately. Store at room temperature. Expiration date is 1 year from preparation date.

7. Analytical instrumentation and equipment setup and parameters

See Section 5 for details on hardware used, including sources. See Tables and Figures in Appendix C for a complete listing of the instrument and method parameters and software screen shots.

A. Configuration of tubing for liquid handling

i. FAST valve setup: See Appendix C, Figure 1. Configuration of tubing and devices for liquid handling using FAST sample introduction.for diagram and Section 5.A “FAST / ESI SC4-DX autosampler accessories” for source information.

1) Port 1: sample loop (white nut).

2) Port 2: 0.5 mm ID “carrier in” line (red nut) for carrier solution.

3) Port 3: nebulizer line (green nut) for transfer of liquid to nebulizer.

4) Port 4: sample loop (white nut).

5) Port 5: 0.8 mm ID probe (blue nut) for diluted samples.

6) Port 6: vacuum line (black nut).

ii. Carrier solution uptake: Use peristaltic pump to control uptake flow rate of carrier solution to the SC-FAST valve. The carrier probe tubing can be connected directly to the peristaltic pump tubing. The other side of the peristaltic pump tubing connects directly to “carrier in” line with the red nut (see consumables descriptions in Section 5.B).

iii. Spray chamber waste removal: Use the peristaltic pump to control the removal of liquid waste from the spray chamber. The spray chamber drain tubing connects directly to the Santoprene™ peristaltic pump tubing. Connect the other end of the peristaltic pump tubing to 0.5 mm i.d. PFA tubing. Place the free end of the PFA tubing through the lid of the waste jug (be sure it is secure).

iv. Rinse solution for autosampler

1) Rinse solution jug: Leave the cap 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.

2) 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.

3) 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.

B. Gas delivery and regulation

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

1. 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.
2. 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 psi above the delivery pressure of the filter regulator on the ICP-MS.
3. Filter Regulator at NexION ICP-MS: Single stage "argon regulator filter kit" supplied with the ICP-MS. Set the delivery pressure to 90–100 psi when plasma is running.

ii. Ammonia for UCT channel A

1. Regulator for NH_3 gas: Set delivery pressure to 15 ± 2 psi. See Section 5.E.iii.1) for part numbers and details.

iii. Helium for UCT channel B

1. Regulator for He gas: Set delivery pressure to 15 ± 2 psi when using the helium filter system between the He gas tank and the NexION. See Section 5.E.v.1) for part numbers and details.
2. Filter system: Used to purify He gas. See Section 5.E.v.2) for part numbers and details.

C. Chiller / heat exchanger:

If using refrigerated chiller, set temperature control to approximately 18°C (typically 16 - 19°C) unless otherwise indicated by PerkinElmer service support.

D. Parameters for instrument and method:

See Tables and Figures in Appendix C for a complete listing of the instrument and method parameters and software screen shots.

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’), “high-normal” (‘High QC’) concentrations, and “above-normal” (‘Elevated QC’).

In each analytical run the analyst will test each of the three 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 40–50 patient samples total) 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. For example, the schemes shown in Table 6 in Appendix C are both acceptable ways to analyze multiple consecutive “runs”.

ii. Reference materials: Use standard reference material (SRM, e.g. SRM 2668 levels 1 and 2) from the National Institute of Standards and Technology (NIST) to verify method accuracy. Use previously characterized samples from proficiency testing program or commercially-produced reference materials when NIST SRMs are unavailable.

iii. Calibration verification: The test system is calibrated as part of each analytical run with NIST-traceable calibrators. 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: If the pump doesn’t automatically start, turn it on in the Devices tab. Make 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 UCT later in this procedure.

9) Daily performance check: Perform instrument optimizations (e.g. torch alignment, neb gas, detector voltages) if necessary and document a daily performance check. Save new parameters to the "default.tun" and "default.dac" files.

10) (Optional) Ready the instrument for priority samples: If priority samples are expected for analysis, the plasma can be started well in advance and left running to eliminate the need for an initial instrument warm-up period and / or a UCT 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 analysis with a 1700 s rinse time will take approximately 30 minutes to complete). Initial samples would be non-matrix, while final samples would be diluted matrix for conditioning. The NexION will remain in UCT pressurized mode for approximately 45 minutes after analysis of the last sample before automatically shutting off the UCT gas.

ii. Software setup for Analysis

1) Workspace (files and folders): Verify and set up the correct files and data directories for analysis (See Table 1 in Appendix C for defaults).

2) Samples / Batch window: Update the software to reflect the current sample set. Use a bar code scanner to input data whenever possible. See Table 1 in Appendix C for times and speeds.

3) Urine vs. aqueous method files

a) The difference: There are two method files for this one method (see Figure 6 in Appendix C). 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. See Table 7 in Appendix C.

i) The *"urblk" method file:* Use 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.

iii. Preparation of samples for analysis (See Table 8 in Appendix C)

1) Thaw urine samples; allow them to reach ambient temperature.

2) Best analyte-to-internal standard ratio stability is obtained after 30-60 min of analysis of urine samples using the UCT KED/DRC method. Analyze enough "dummy" urine sample dilutions (e.g. standard 2 preparation) prior to any KED/DRC analysis run to fill 30-60 min of analysis time (~15 samples). See Figure 6 in Appendix C for example of setup in the Samples / Batch window.

3) Prepare the solutions into pre-labeled containers using the benchtop automatic pipette or other volumetric sample transfer device.

4) 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 calibrators (S0–S6). Prepare at least three S0 calibrators. 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 and QC Samples: Before taking an aliquot for analysis, homogenize the sample (e.g. vortex for 3-5 seconds, or invert 5-10 times).

5) After preparation, mix 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.

6) Room temperature storage of original samples for the work day is acceptable.

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

v. Monitor the analysis in real-time as much as possible. If necessary, leave the run 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) Verify proper operation of the instrument (proper loop filling, sample reaching nebulizer in correct timing, autosampler arm moving properly, etc . . .)

2) Verify that background signal from instrument and reagents are low. Helpful checks when diagnosing high background problems include:

a) Water to be used in Aq Blank Checks and dilutions.

b) Diluent before and after being flushed through the benchtop automatic pipette.

c) If contamination is observed from the pipette, flush the pipette with ≥500 mL of nitric acid solution (≤ 5% v/v HNO_3) and retest.

d) Comparison with other instruments.

3) Verify analyte / internal standard ratio stability. The net intensity (analyte / internal standard ratio) of the measurements made while stabilizing the UCT 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.

4) Verify calibration curves meet R^2 requirements (minimum of 0.98, typically 0.99 to 1.000).

5) Verify bench QC results are within the acceptable limits. If an analyte result for the beginning QC material(s) falls outside of the ± 3SD limits, then the following steps are recommended:

a) Evaluate the blank results.

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 calibrator 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 calibrator point per analyte between and including S2 and S5 can be removed from the curve. Follow up on 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.

i) 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.

6) Verify good precision among replicates of each measurement.

7) Verify consistent measured intensities of the internal standards. Some sample-to-sample variations are to be expected, however, intensities drifting continuously in one direction resulting in failing results for ending QC indicate the instrument needs additional pre-conditioning before the run or environmental conditions are changing too much around the instrument.

8) Verify elevated patient results. Refer to Figure 16 in Appendix C for flowchart.

a) Confirming an elevated concentration: Repeat for confirmation any sample having a concentration greater than the 1UB threshold. See Table 10 in Appendix C.

b) Dilution of a sample to within the calibration range: Repeat in duplicate with extra dilution any sample having a concentration greater than the highest calibrator to bring the observed result within the concentration range of the calibrators. See Table 9 of Appendix C for high calibrator concentrations and validated extra dilutions.

c) Confirming proper washout after an elevated sample: When monitoring the analysis in real-time, if a sample concentration is greater than the highest concentration validated for washout (see Table 10 of Appendix C), do the following to verify that the run is still in control for low concentration samples before proceeding with analysis.

i) Stop run following elevated sample.

ii) Verify that the run is still in control for lower concentration samples before proceeding with analysis. Analyze two urine blank checks followed by a low bench

QC washout check. If the low bench QC wash check is not in control (within ± 3SD limits), repeat these 3 check samples until washout is verified before proceeding with analysis. Example:
3031 UrBlkChk Wash1
3031 UrBlkChk Wash2
LUXXXXX Wash

iii) If the run is not verified in-control for low concentration samples before the next samples are analyzed, see Section 8.C.ii.1)b) for directions.

vi. Overnight operation or using auto stop: Ensure sufficient solution supply and waste collection during unattended operation. 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. The “Auto Stop” checkbox is at the top of the batch run list window.

vii. Records of results: Run results will be documented after each run.

1) Electronic file transfer to laboratory information system (LIMS): Transfer data electronically to the LIMS. 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. 2016-0714a_group55.txt). In the 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) Run summary records: Printed run sheets, or PDF equivalent, must be documented with

a) Analyst initials

b) Instrument ID

c) Date of analysis and run # for the day

C. Analyst evaluation of run results

i. 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[1]. 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.

1) Rules for bench quality control evaluation: The following are the CDC DLS QC rules for three QC pools per run with two or more QC results per pool.

a) If all 3 QC run means (low, high, and elevated bench QC) are within 2Sm limits and individual results are within 2Si limits, then accept the run.

b) If 1 of the 3 QC run means is outside a 2Sm limit - reject run if:

i) Extreme Outlier – Run mean is beyond the characterization mean +/- 4Sm

ii) 3S Rule - Run mean is outside a 3Sm limit

iii) 2S Rule – 2 or more of the 3 run means are outside the same 2Sm limit

iv) 10 X-bar Rule – Current and previous 9 run means are on same side of the characterization mean

c) If one of the QC individual results is outside a 2Si limit - reject run if:

i) Extreme Outlier – one individual result is beyond the characterization mean +/- 4Si

ii) R 4S Rule – 2 or more of the within-run ranges in the same run exceed 4Sw (i.e., 95% range limit). Note: Since runs have multiple results per pool for three pools, the R 4S rule is applied within runs only.

2) Abbreviations

a) S_i = Standard deviation of individual results.

b) S_m = Standard deviation of the run means.

c) S_w = Within-run standard deviation.

3) 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.

a) 3 elements in the run:

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

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

b) 1 – 2 elements in the run: ONLY the analyte which was "out of control" is invalid for reporting from the run.

ii. Patient results

1) Elevated concentrations: refer to Appendix C, Figure 16 for flowchart

a) Boundaries requiring confirmatory measurement:

i) 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 but default concentrations are in Table 10 in Appendix C.

(1) Results greater than the first upper boundary (1UB): Repeat for confirmation with a new sample preparation any sample with a concentration greater than the 1UB threshold. Report the first analytically valid result, as long as the confirmation is within 10%. Continue repeat analysis until a concentration is confirmed.

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

ii) Results greater than highest calibrator: Samples that exceed the high calibrator must be prepared with extra dilution in duplicate to bring the observed result within the calibration range (≤ S6). See Table 9 in Appendix C for permitted extra dilutions. 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.

b) Concentrations requiring verification of washout: Following a result greater than the highest concentrations tested for washout (see Table 10 of Appendix C) do the following:

i) If the run was determined to be in-control for low concentration samples before the next samples were analyzed, no further action is required.

ii) If the run was not determined to be in-control for low concentration samples before the next samples were analyzed confirm by re-analysis the results for the 2 samples immediately following the elevated sample. Report the results if they confirm the initial results within ±10% or ±3SD of the low bench QC, whichever is greater.

2) 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 listed in Table 10 in Appendix C, do not use the measurement for reporting. Repeat the analysis of the sample.

D. 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 usage of the instrument.

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

B. UCT optimizations

Use the UCT optimization solutions (see Section 6.L) to verify UCT gas flow rates and cell voltages as needed to ensure proper reduction of potential ICP-MS interferences. Additional optimization procedures are described in the user manual.

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 two 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, or network location, to prevent loss of data from failure of primary hard drive.

ii. Periodic backup: Backup relevant computer files periodically (e.g. 5 – 10 runs) 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 inside the lab.

10. Reporting thresholds

A. Reportable range

Urine arsenic, chromium and nickel values are reportable in the range between the method LOD and the high calibrator times the maximum permitted extra dilution (see Table 9 of Appendix C). Above the high calibrator, extra dilutions are made of the urine sample to bring the observed concentration within the calibration range.

B. Reference ranges (normal values)

In this method the 95% reference ranges (see Table 11 in Appendix C) 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 the elements determined with this method, there is no routine notification for elevated levels. 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)[1]. 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 [1].

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 (STARLIMS SAS QC program).

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

If the analytical system fails, setup analysis on other ICP-MS instrument, if available. If no other instrument is available, store the specimens at ≤ 20 °C until the analytical system can be restored to functionality.

13. Method performance documentation

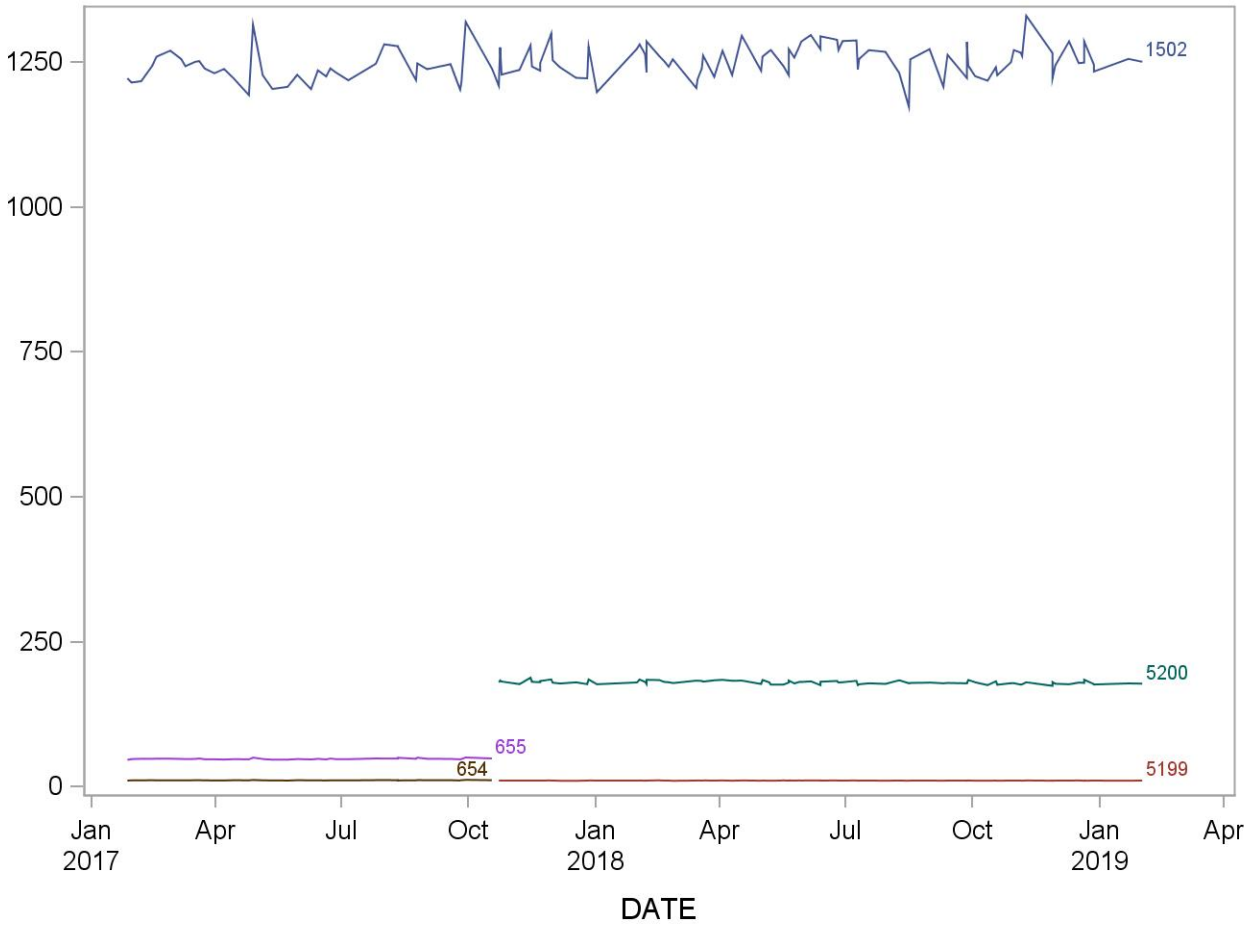
Method performance documentation for this method including accuracy, precision, sensitivity, specificity and stability is provided in Appendix A of this method documentation. **The signatures of the branch chief and director of the Division of Laboratory Sciences on the first page of this procedure denote that the method performance is fit for the intended use of the method.**

14. Summary Statistics and QC Charts

Please see following pages

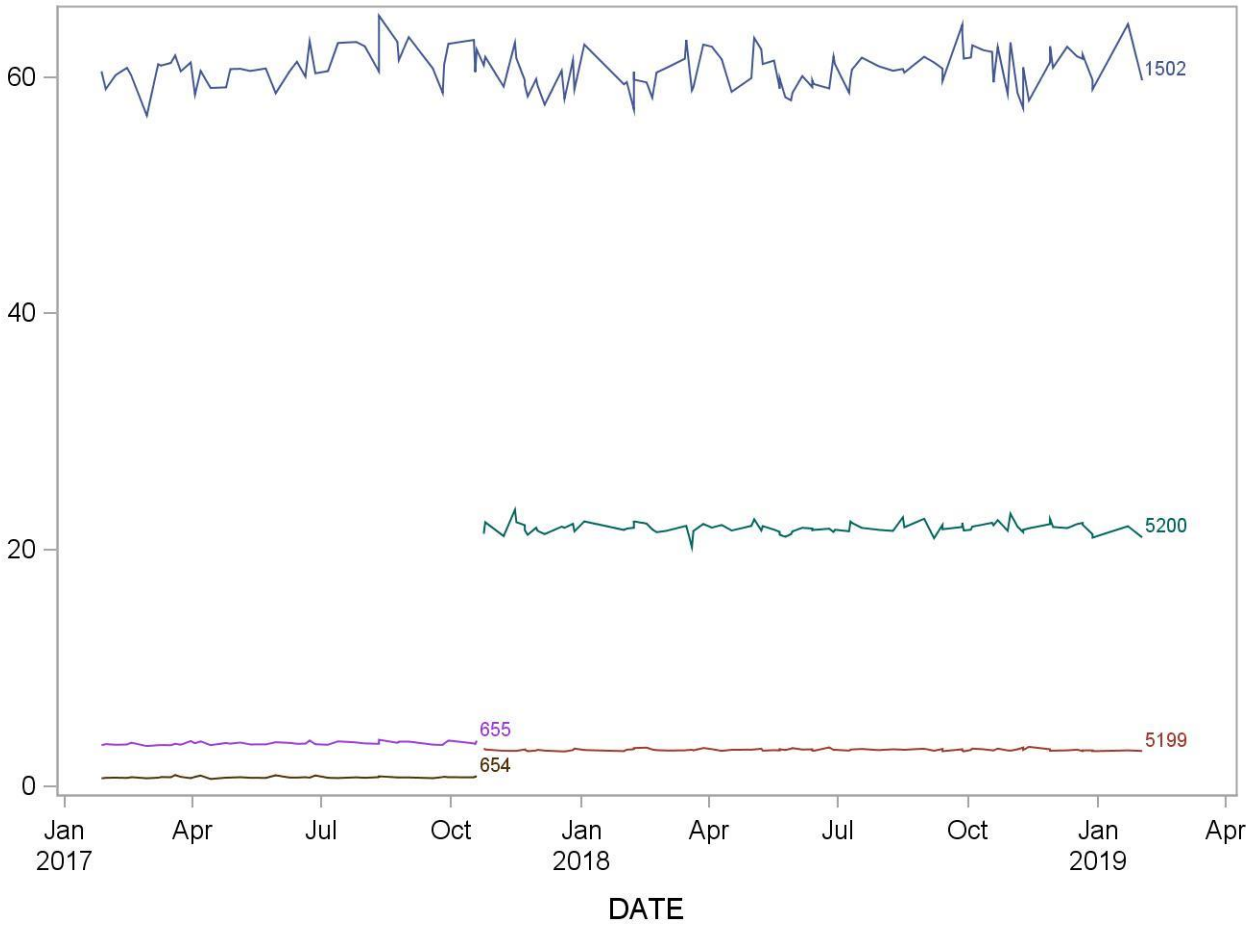
2017-2018 Summary Statistics and QC Chart for Urinary arsenic, total (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
1502	120	27JAN17	01FEB19	1249.591	28.115	2.2
654	38	27JAN17	18OCT17	10.694	0.248	2.3
655	38	27JAN17	18OCT17	47.683	0.972	2.0
5199	82	23OCT17	01FEB19	10.198	0.159	1.6
5200	82	23OCT17	01FEB19	179.731	2.925	1.6



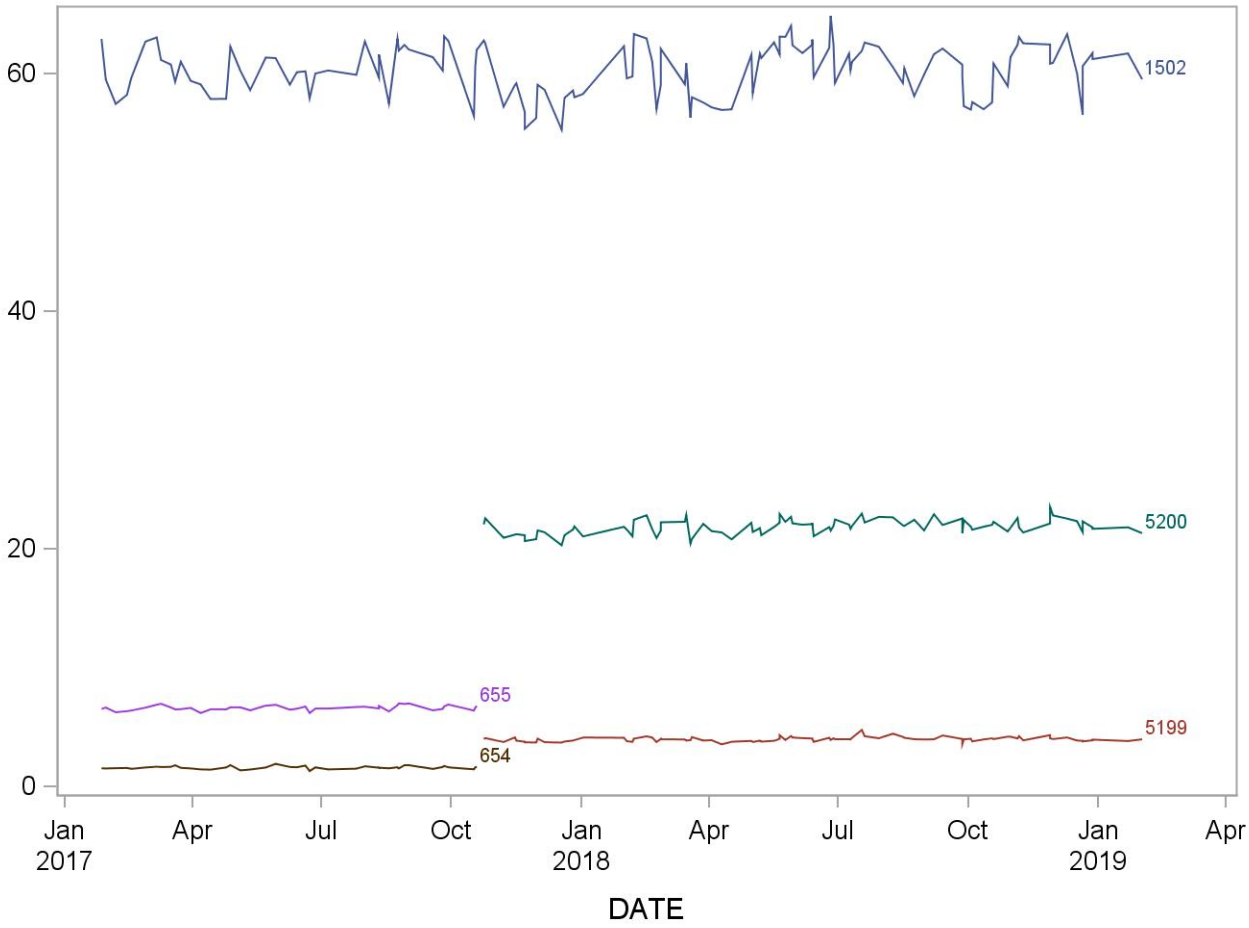
2017-2018 Summary Statistics and QC Chart for Urine Chromium (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
1502	131	27JAN17	01FEB19	60.670	1.645	2.7
654	43	27JAN17	19OCT17	0.778	0.069	8.9
655	43	27JAN17	19OCT17	3.654	0.137	3.7
5199	88	24OCT17	01FEB19	3.100	0.083	2.7
5200	88	24OCT17	01FEB19	21.877	0.471	2.2



2017-2018 Summary Statistics and QC Chart for Urinary Nickel (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
1502	129	27JAN17	01FEB19	60.253	2.121	3.5
654	42	27JAN17	19OCT17	1.591	0.128	8.0
655	42	27JAN17	19OCT17	6.612	0.215	3.3
5199	87	24OCT17	01FEB19	3.976	0.192	4.8
5200	87	24OCT17	01FEB19	21.847	0.630	2.9



15. Appendix A. Method performance documentation

A. Accuracy

i. Arsenic

Accuracy compared to Reference Material

Mean concentration should be within ±15% of the nominal value except at 3*LOD, where it should be within ± 20%

Method name:	Arsenic, Chromium and Nickel in Urine by ICP-MS
Method #:	3031
Matrix:	Urine
Units:	µg/L
Reference material:	**NIST SRM 2668 L1, NIST SRM 2668 L2 (2x dilution), NIST SRM 2668 L2**
Analyte:	**arsenic (total)**

Reference material	Replicate	Nominal value	Measured concentration					Mean	SD	CV (%)	Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5				
Level 1	1	10.81	10	10	11	11	10	10.46	0.27	2.56	-3.3
	2		11	10	11	11	10				
Level 2	1	106.55	102	108	113	108	102	106.6	3.87	3.63	0.1
	2		102	108	112	107	105				
Level 3	1	213.1	210	218	221	222	212	217.5	4.78	2.20	2.1
	2		213	217	222	224	215				

ii. Chromium

Accuracy compared to Reference Material

Mean concentration should be within ±15% of the nominal value except at 3*LOD, where it should be within ± 20%

Method name:	Arsenic, Chromium and Nickel in Urine by ICP-MS
Method #:	3031
Matrix:	Urine
Units:	µg/L
Reference material:	**NIST SRM 2668 L1, NIST SRM 2668 L2 (2x dilution), NIST SRM 2668 L2**
Analyte:	**chromium**

Reference material	Replicate	Nominal value	Measured concentration					Mean	SD	CV (%)	Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5				
Level 1	1	1.08	1.2	1.1	1.5	1.0	1.0	1.21	0.22	18.30	12.1
	2		1.3	1.2	1.7	1.0	1.0				
Level 2	1	13.9	13	15	15	14	14	14.32	0.72	5.05	3.4
	2		13	15	15	14	15				
Level 3	1	27.7	27	28	28	28	29	28.20	0.86	3.05	1.8
	2		27	28	28	28	30				

iii. Nickel

Accuracy compared to Reference Material

Mean concentration should be within ±15% of the nominal value except at 3*LOD, where it should be within ± 20%

Method name:	Arsenic, Chromium and Nickel in Urine by ICP-MS
Method #:	3031
Matrix:	Urine
Units:	µg/L
Reference material:	**NIST SRM 2668 L1, NIST SRM 2668 L2 (2x dilution), NIST SRM 2668 L2**
Analyte:	**nickel**

Reference material	Replicate	Nominal value	Measured concentration					Mean	SD	CV (%)	Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5				
Level 1	1	2.31	2.2	2.4	2.3	2.1	2.2	2.22	0.08	3.8	-3.9
	2		2.1	2.3	2.3	2.1	2.2				
Level 2	1	57.5	53	62	60	57	56	57.5	3.12	5.4	0.0
	2		53	62	60	57	56				
Level 3	1	115	108	126	119	115	112	116	6	5.3	1.0
	2		108	125	120	115	113				

B. Precision

i. Arsenic

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Arsenic, Chromium and Nickel in Urine by ICP-MS
Method #:	3031
Matrix:	Urine
Units:	µg/L
Analyte:	**arsenic (total)**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	183	180	181.45	1.34281744	1.34281744	65847.55178
2	187	186	186.45	0.56791296	0.56791296	69527.65248
3	184	180	181.77	3.78574849	3.78574849	66083.9377
4	177	176	176.37	0.08398404	0.08398404	62211.97777
5	181	181	181.25	0.014532302	0.014532303	65702.94375
6	178	176	177.43	0.902785022	0.902785023	62963.41306
7	183	177	180.04	8.260738223	8.260738222	64827.61494
8	182	179	180.76	2.755102023	2.755102022	65349.69283
9	175	173	174.02	1.36235584	1.36235584	60565.22472
10	176	175	175.36	0.045262562	0.045262562	61499.76911

Grand sum	3589.7987	**Grand mean**	179.489935

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	38.24247781	3.824247781	1.955568403	1.09
Between Run	247.0428322	27.44920358	3.436928556	1.91
Total	285.28531		3.954329991	**2.20**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1286	1270	1277.87	63.504961	63.504961	3265883.539
2	1248	1304	1276.36	784.8206146	784.8206146	3258169.533
3	1193	1172	1182.86	111.457639	111.457639	2798292.139
4	1198	1177	1187.44	110.4348774	110.4348774	2820045.556
5	1199	1202	1200.51	1.995297503	1.995297502	2882465.567
6	1204	1235	1219.29	231.4794888	231.4794888	2973340.842
7	1237	1260	1248.16	130.3672986	130.3672986	3115794.04
8	1242	1215	1228.21	182.3256078	182.3256078	3017013.855
9	1180	1165	1172.08	56.76567649	56.76567649	2747545.866
10	1219	1206	1212.56	42.64416506	42.64416506	2940586.289

Grand sum	24410.6658	**Grand mean**	1220.53329

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	3431.591253	343.1591253	18.52455466	1.52
Between Run	25106.98585	2789.665094	34.97503373	2.87
Total	28538.5771		39.57792452	**3.24**

ii. Chromium

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Arsenic, Chromium and Nickel in Urine by ICP-MS
Method #:	3031
Matrix:	Urine
Units:	µg/L
Analyte:	**chromium**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	22	22	21.99	0.051324902	0.051324902	967.0190486
2	23	23	22.64	0.002347402	0.002347403	1025.279573
3	21	21	20.71	0.014896203	0.014896202	858.0857364
4	22	22	21.96	0.004699102	0.004699103	964.1011338
5	22	22	22.11	0.004283703	0.004283702	977.5848096
6	23	22	22.49	0.06290064	0.06290064	1011.816116
7	22	21	21.85	0.155748623	0.155748622	955.1552952
8	22	21	21.66	0.02886601	0.02886601	938.0166471
9	22	22	21.90	0.023485563	0.023485563	958.8564944
10	21	21	21.19	0.03290596	0.03290596	897.8711632

Grand sum 436.9891 **Grand mean** 21.849455

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.762916215	0.076291622	0.27620938	1.26
Between Run	5.812340954	0.645815662	0.533630977	2.44
Total	6.575257169		0.600877393	**2.75**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	61	60	60.44	0.62758084	0.62758084	7306.639967
2	63	63	63.06	0.00636804	0.00636804	7952.900186
3	57	57	56.97	0.026001563	0.026001563	6490.831378
4	61	62	61.49	0.004128062	0.004128063	7561.216256
5	61	60	60.53	0.008836	0.008836	7327.689164
6	64	62	62.95	0.615675622	0.615675622	7926.424823
7	62	61	61.42	0.39879225	0.39879225	7544.267747
8	63	61	61.98	0.764662802	0.764662803	7683.028404
9	60	61	60.88	0.194436902	0.194436903	7412.760976
10	60	59	59.39	0.473412802	0.473412803	7053.334606

Grand sum 1218.2084 **Grand mean** 60.91042

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	6.23978977	0.623978977	0.789923399	1.30
Between Run	57.50821502	6.389801669	1.697913822	2.79
Total	63.74800479		1.872669304	**3.07**

iii. Nickel

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Arsenic, Chromium and Nickel in Urine by ICP-MS
Method #:	3031
Matrix:	Urine
Units:	µg/L
Analyte:	**nickel**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	21	21	21.23	0.04309776	0.04309776	901.2474768
2	22	22	21.95	0.01708249	0.01708249	963.3065031
3	21	21	21.12	0.004563003	0.004563003	892.2059546
4	23	23	22.71	0.002209	0.002209	1031.860678
5	22	22	22.34	0.00090601	0.00090601	998.1512
6	23	22	22.50	0.089670303	0.089670303	1012.135533
7	23	22	22.35	0.095481	0.095481	998.9019651
8	22	23	22.34	0.080287222	0.080287222	997.941215
9	20	20	19.99	0.01542564	0.01542564	799.4001125
10	21	21	21.03	0.061281003	0.061281003	884.8877598

Grand sum 435.1172 **Grand mean** 21.75586

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.82000686	0.082000686	0.286357619	1.32
Between Run	13.68951073	1.521056748	0.848249981	3.90
Total	14.50951759		0.895281362	**4.12**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	61	58	59.61	3.052533123	3.052533122	7105.667024
2	62	62	61.59	0.002093062	0.002093062	7585.584572
3	58	57	57.45	0.00835396	0.00835396	6601.211822
4	63	63	63.28	0.008714223	0.008714223	8007.894181
5	61	61	61.07	0.015264603	0.015264602	7458.955447
6	64	62	62.92	0.949747703	0.949747703	7916.858695
7	62	62	61.92	0.16662724	0.16662724	7667.850819
8	62	63	62.39	0.23648769	0.23648769	7785.698027
9	55	57	56.33	1.005908703	1.005908703	6345.157696
10	61	60	60.63	0.585225	0.585225	7352.842645

Grand sum 1214.35 **Grand mean** 60.7175

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	12.06191061	1.206191061	1.098267299	1.81
Between Run	95.42480155	10.60275573	2.167552152	3.57
Total	107.4867122		2.429912219	**4.00**

C. Stability

i. Arsenic

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Three times frozen at -70°C and then thawed (3 freeze-thaw cycles) to room temperature.
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Original samples stored capped at room temperature for 1 day prior to preparation and analysis.
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Processed samples (ready for instrument analysis) stored capped at room temperature for 1 day prior to analysis.
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Samples stored at -70°C for 1.5 years.

All stability sample results should be within ±15% of nominal concentration

Method name:	Arsenic, Chromium and Nickel in Urine by ICP-MS
Method #:	3031
Matrix:	Urine
Units:	µg/L
Analyte:	**arsenic (total)**

Quality material 1

	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample	Initial measurement	Long-term stability
Replicate 1	180	177	182	182	174	178	3.5	3.4
Replicate 2	181	176	183	181	177	180	3.7	3.6
Replicate 3	182	177	183	181	177	181	3.6	4.1
Mean	180.8216867	176.5683497	182.4981403	181.4	176.1442683	179.395324	3.6311	3.7
% difference from initial measurement	--	**-2.4**	--	**-0.6**	--	**1.8**	--	**2.1**

Quality material 2

	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample	Initial measurement	Long-term stability
Replicate 1	1236	1221	1261	1232	1240	1244	61	60
Replicate 2	1242	1215	1243	1241	1214	1240	64	62
Replicate 3	1237	1216	1242	1225	1208	1256	59	62
Mean	1238.41289	1217.494579	1248.384481	1232.8	1220.551005	1246.715833	61.36096667	61.6
% difference from initial measurement	--	**-1.7**	--	**-1.2**	--	**2.1**	--	**0.3**

ii. Chromium

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Three times frozen at -70°C and then thawed (3 freeze-thaw cycles) to room temperature.
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Original samples stored capped at room temperature for 1 day prior to preparation and analysis.
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Processed samples (ready for instrument analysis) stored capped at room temperature for 1 day prior to analysis.
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Samples stored at -70°C for 1.5 years.

All stability sample results should be within ±15% of nominal concentration

Method name:	Arsenic, Chromium and Nickel in Urine by ICP-MS
Method #:	3031
Matrix:	Urine
Units:	µg/L
Analyte:	**chromium**

Quality material 1								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	25	22	23	22	25	23	3.5	3.4
Replicate 2	23	22	23	22	23	23	3.7	3.6
Replicate 3	23	23	24	22	23	23	3.6	4.1
Mean	23.81513767	22.44271333	23.32008167	22.2	23.68631133	22.905012	3.6311	3.7
% difference from initial measurement	--	**-5.8**	--	**-4.8**	--	**-3.3**	--	**2.1**

Quality material 2								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	64	62	64	60	65	63	61	60
Replicate 2	65	64	65	60	67	63	64	62
Replicate 3	63	63	63	61	63	63	59	62
Mean	64.12642767	62.821311	64.353982	60.4	64.99773333	62.80420833	61.36096667	61.6
% difference from initial measurement	--	**-2.0**	--	**-6.2**	--	**-3.4**	--	**0.3**

iii. Nickel

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Three times frozen at -70°C and then thawed (3 freeze-thaw cycles) to room temperature.
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Original samples stored capped at room temperature for 1 day prior to preparation and analysis.
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Processed samples (ready for instrument analysis) stored capped at room temperature for 1 day prior to analysis.
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Samples stored at -70°C for 1.5 years.

All stability sample results should be within ±15% of nominal concentration

Method name:	Arsenic, Chromium and Nickel in Urine by ICP-MS
Method #:	3031
Matrix:	Urine
Units:	µg/L
Analyte:	**nickel**

Quality material 1								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample**	**Initial measurement**	**Long-term stability**
Replicate 1	25	23	24	21	24	22	3.5	3.4
Replicate 2	24	23	23	22	23	22	3.7	3.6
Replicate 3	23	23	32	30	23	22	3.6	4.1
Mean	23.859838	22.78171333	26.16285933	24.3	23.63805067	22.29668833	3.6311	3.7082333
% difference from initial measurement	--	**-4.5**	--	**-7.1**	--	**-5.7**	--	**2.1**

Quality material 2								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample**	**Initial measurement**	**Long-term stability**
Replicate 1	65	62	64	58	65	60	61	61
Replicate 2	65	64	66	59	66	60	64	62
Replicate 3	64	62	63	59	63	61	61	62
Mean	64.55760967	62.76430767	64.391128	58.8	64.90837867	60.14322567	62.16716667	61.8075
% difference from initial measurement	--	**-2.8**	--	**-8.7**	--	**-7.3**	--	**-0.6**

D. Analytical Sensitivity and Specificity

LOD, specificity and fit for intended use

Method name:	Arsenic, Chromium and Nickel in Urine by ICP-MS
Method #:	3031
Matrix:	Urine
Units:	µg/L

Analytes	Limit of Detection (LOD)	Interferences successfully checked in at least 50 human samples	Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use
arsenic, total (UTAS)	0.23	Yes	Yes
chromium (UCM)	0.19	Yes	Yes
nickel (UNI)	0.31	Yes	Yes

16. Appendix B. Ruggedness Testing Results

A. Ruggedness parameter test #1: impact of extra dilutions

i. Test details: We used a total of six urine samples from proficiency testing programs (CTQ and the Wadsworth Center), National Institute of Standards and Technology Standard Reference Material 2668 level 2, and the elevated bench QC as samples for this experiment. Each sample was diluted 1X, 2X, 5X, 10X, and 20X and analyzed by the method.

ii. Results:See Ruggedness Table 1.

iii. Conclusion: Limit extra dilutions to ≤ 5x for all analytes to achieve the best accuracy and reproducibility. Clinical treatment is likely the same at concentrations greater than these dilutions permit for reporting.

Ruggedness Table 1. Effect of extra sample dilutions on As, Cr and Ni. Normalized concentration ± 1 standard deviation.

Extra Dilution factor	As	Cr	Ni
No extra dilution	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
2X	0.97 ± 0.01	0.99 ± 0.02	0.98 ± 0.02
5X	0.95 ± 0.01	1.02 ± 0.03	0.97 ± 0.04
10X	0.92 ± 0.01	1.03 ± 0.06	0.93 ± 0.02
20X	0.91 ± 0.02	0.90 ± 0.06	0.91 ± 0.07

B. Ruggedness parameter test #2: stability of prepared samples

i. Test details: Evaluate the impact a delay in analysis start time (24 or 48 hours) after sample preparation has on measured concentrations of As, Cr, and Ni. "Junk urine" samples (20) were analyzed between the beginning and ending QC of each run. All other method parameters were kept per method.

1) Day 1 – a total of five sets of blanks, calibrators, and QC were prepared. One set was analyzed immediately. Two sets were stored in the refrigerator and two sets were stored on the laboratory bench top.

2) Day 2 – one set of blanks, calibrators, and QC were prepared and analyzed immediately. One set from both the refrigerator and laboratory bench top were analyzed (24 hours).

3) Day 3 – one set of blanks, calibrators, and QC were prepared and analyzed immediately. One set from both the refrigerator and laboratory bench top were analyzed (48 hours).

ii. Results: See Ruggedness Table 2 and Ruggedness Table 3.

iii. Conclusion: Prepared samples, capped, and stored for up to 48 hours at room temperature, or in the refrigerator at 4 °C, are still valid for analysis without degrading normal method accuracy and precision.

Ruggedness Table 2. Stability of sample preparations (bench quality control samples).

Sample ID	TPSP	^{75}As (µg/L)		^{60}Ni (µg/L)		^{52}Cr (µg/L)	
LU10709	± 2SD range[1]	9.94 – 11.7		1.22 – 1.99		0.597 – 0.975	
	0 hr, Day 1	11.1		1.50		0.766	
	0 hr, Day 2	11.5		1.61		0.665	
	0 hr, Day 3	11.2		1.12		0.759	
	Storage temp.	**4 °C**	**room**	**4 °C**	**room**	**4 °C**	**room**
	24 hr	11.2	10.7$^{\delta}$	1.51	1.45$^{\delta}$	0.697	0.627$^{\delta}$
	48 hr	11.2	11.2	1.93	1.58	0.924	0.662
HU10710	± 2SD range[1]	45.1 – 51.1		5.98 – 7.15		3.26 – 4.02	
	0 hr, Day 1	49.8		6.45		3.72	
	0 hr, Day 2	50.9		6.35		3.38	
	0 hr, Day 3	50.1		6.16		3.79	
	Storage temp.	**4 °C**	**room**	**4 °C**	**room**	**4 °C**	**room**
	24 hr	50.2	46.6$^{\delta}$	6.55	6.06$^{\delta}$	3.55	3.38$^{\delta}$
	48 hr	50.5	50.4	7.24	6.48	4.44	3.51
EU10713	± 2SD range[1]	1148 – 1332		54.6 – 65.0		54.4 – 64.9	
	0 hr, Day 1	1299		59.0		64.0	
	0 hr, Day 2	1324		57.6		54.7	
	0 hr, Day 3	1242		55.6		58.4	
	Storage temp.	**4 °C**	**room**	**4 °C**	**room**	**4 °C**	**room**
	24 hr	1301	1249$^{\delta}$	60.5	57.6$^{\delta}$	58.4	58.9$^{\delta}$
	48 hr	1320	1318	62.3	60.4	61.8	59.8

[1]from characterization results calculated on 2016-0707
$^{\delta}$24 hr Room temperature data is beginning QC only

Ruggedness Table 3. Stability of sample preparations (standard reference material samples).

Sample ID	Time past sample preparation	^{75}As (µg/L)		^{60}Ni (µg/L)		^{52}Cr (µg/L)	
NIST 2668 L1	Evaluation criteria[2]	9.53 – 12.1[¥]		1.73 – 2.89[¥]		0.635 – 1.52 [α]	
	Target ± U	10.81 ± 0.54		2.31 ± 0.32		1.08 ± 0.31	
	0 hr, Day 1	10.7		2.21		1.27	
	0 hr, Day 2	11.1		2.43		1.19	
	0 hr, Day 3	10.8		2.31		1.29	
	Storage temp.	**4 °C**	**room**	**4 °C**	**room**	**4 °C**	**room**
	24 hr	11.0	10.6	2.66	2.18	1.18	1.18
	48 hr	11.3	11.2	2.32	2.27	0.939	1.48
NIST 2668 L2	Evaluation criteria [2]	191.8 – 234.4[β]		103.8 – 126.8 [β]		24.4 – 31.0 [α]	
	Target ± U	213.1 ± 44		115.3 ± 5.2		27.7 ± 2.1	
	0 hr, Day 1	219		116		30.0	
	0 hr, Day 2	228		111		26.6*	
	0 hr, Day 3	220		111		28.7	
	Storage temp.	**4 °C**	**room**	**4 °C**	**room**	**4 °C**	**room**
	24 hr	224	213	119	111	29.4	28.3
	48 hr	227	224	120	116	29.5	28.4

[2]Evaluation criteria were the greater of the following:
[α] NIST cert mean ± U
[β] NIST cert mean ± 10%
[¥] NIST cert mean ± bench QC limits of similar concentration material
* The first replicate of this measurement (0.447 µg/L) was deleted due to an analytical instrument anomaly. The remaining replicates (26.5 and 26.7 µg/L) were used.

C. Ruggedness parameter test #3: KED and DRC mode cell gas flow rate

i. Test details

1) KED mode: Evaluate the impact of changes in gas flow rates in KED modes have on measured concentrations of As and Ni.

a) Run #1 (method default, 2.5 mL min^{-1} (As) and 4.5 mL min^{-1} (Ni) helium)

b) Run #2 (decreased flow rates, 2.0 mL min^{-1} (As) and 3.6 mL min^{-1} (Ni) helium)

c) Run #3 (increased flow rates, 3.0 mL min^{-1} (As) and 5.4 mL min^{-1} (Ni) helium)

2) DRC mode: Evaluate the impact ammonia gas flow rate has on measured concentrations of Cr.

a) Run #1 (method default, 0.7 mL min-1 ammonia)

b) Run #2 (decreased flow rates, 0.48 mL min-1 ammonia)

c) Run #3 (method default, 0.7 mL min-1 ammonia)

d) Run #4 (increased flow rates, 0.84 mL min-1 ammonia)

ii. Results: See Ruggedness Table 4 and Ruggedness Table 5.

iii. Conclusions:

1) *KED mode (As and Ni)*: The variation tested in the He gas flow rates did not affect the accuracy of the method for As and Ni outside of expected uncertainties. Ni results outside of the evaluation criteria were traced to other factors, not the cell gas flow rate.

2) *DRC mode (Cr)*: The variation tested in the ammonia gas flow rate did not affect the accuracy of the method for Cr outside of expected uncertainties.

Ruggedness Table 4 (As and Ni). Impact of cell gas flow rate changes on observed results.

Sample ID	Cell gas flow rate (mL/min)	Evaluation criteria	^{75}As (µg/L)	Cell gas flow rate (mL/min)	^{60}Ni (µg/L)
LU10709		± 2SD range[1]	9.94 – 11.7		1.22 – 1.99
	Low (2.0)		10.6	Low (3.6)	1.86
	Normal (2.5)		10.5	Normal (4.5)	1.42
	High (3.0)		10.9	High (5.4)	1.72
HU10710		± 2SD range[1]	45.1 – 51.1		5.98 – 7.15
	Low (2.0)		48.0	Low (3.6)	6.08
	Normal (2.5)		47.7	Normal (4.5)	6.67
	High (3.0)		48.9	High (5.4)	6.69
EU10713		± 2SD range[1]	1148 – 1332		54.6 – 65.0
	Low (2.0)		1245	Low (3.6)	57.1
	Normal (2.5)		1231	Normal (4.5)	64.3
	High (3.0)		1281	High (5.4)	157$^{\delta}$
NIST 2668 L1		Evaluation criteria[2] Target ± U	9.53 – 12.1$^{¥}$ 10.81 ± 0.54		1.73 – 2.89$^{¥}$ 2.31 ± 0.32
	Low (2.0)		10.7	Low (3.6)	2.38
	Normal (2.5)		10.1	Normal (4.5)	2.22
	High (3.0)		10.3	High (5.4)	2.24
NIST 2668 L2		Evaluation criteria[2] Target ± U	191.8 – 234.4$^{\beta}$ 213.1 ± 44		103.8 – 126.8$^{\beta}$ 115.3 ± 5.2
	Low (2.0)		208	Low (3.6)	107
	Normal (2.5)		216	Normal (4.5)	126*
	High (3.0)		215	High (5.4)	119

[1]from characterization results calculated on 2016-0707
[2]Evaluation criteria were the greater of the following:
$^{\alpha}$ NIST cert mean ± U $^{\beta}$ NIST cert mean ± 10%
$^{¥}$ NIST cert mean ± bench QC limits of similar concentration material
$^{\delta}$ Sample was determined to be contaminated
*Sample QC fails; > highest calibrator for ^{60}Ni (125 µg/L)

Ruggedness Table 5 (Cr). Impact of cell gas flow rate changes on observed results.

Sample ID	Cell gas flow rate (mL/min)	Evaluation criteria	^{52}Cr (µg/L)
LU10709		± 2SD range[1]	0.597 – 0.975
	Low (0.48)		0.930
	Normal (0.7)		0.845 (0.685*)
	High (0.84)		0.881
HU10710		± 2SD range[1]	3.26 – 4.02
	Low (0.48)		3.52
	Normal (0.7)		3.59 (3.62*)
	High (0.84)		3.68
EU10713		± 2SD range[1]	54.4 – 64.9
	Low (0.48)		57.1
	Normal (0.7)		60.5 (61.5*)
	High (0.84)		58.9
NIST 2668 L1		Evaluation criteria[2] Target ± U	0.635 – 1.52 [α] 1.08 ± 0.31
	Low (0.48)		1.24
	Normal (0.7)		1.31 (1.07*)
	High (0.84)		1.37
NIST 2668 L2		Evaluation criteria[2] Target ± U	24.4 – 31.0 [α] 27.7 ± 2.1
	Low (0.48)		26.5
	Normal (0.7)		29.3 (29.3*)
	High (0.84)		29.8

[1]from characterization results calculated on 2016-0707
[2]Evaluation criteria were the greater of the following:
[α] NIST cert mean ± U [β] NIST cert mean ± 10%
[¥] NIST cert mean ± bench QC limits of similar concentration material
*normal cell gas flow rate Run # 3

D. Ruggedness parameter test #4: axial field voltage (AFV)

i. Test details: Evaluate the impact of Axial Field Voltage (AFV) changes has on observed Cr concentrations. AFV values were tested in separately prepared, consecutive runs of 20 "junk urine" samples on the instrument without turning off the plasma.

1) Run #1 (normal method, 275 V)

2) Run #2 (decreased AFV, 220 V)

3) Run #3 (increased AFV, 330 V)

ii. Results: See Ruggedness Table 6.

iii. Conclusion: The variation tested in the DRC mode AFV did not affect the accuracy of the method for Cr outside of expected uncertainties.

Ruggedness Table 6. Impact of axial field voltage changes on observed results.

Sample ID	AFV	^{52}Cr (µg/L)
LU10709	± 2SD range[1]	0.597 – 0.975
	Reduced (220 V)	0.751
	Normal (275 V)	0.749
	Elevated (330 V)	0.697
HU10710	± 2SD range[1]	3.26 – 4.02
	Reduced (220 V)	3.69
	Normal (275 V)	3.64
	Elevated (330 V)	3.48
EU10713	± 2SD range[1]	54.4 – 64.9
	Reduced (220 V)	54.5
	Normal (275 V)	59.0
	Elevated (330 V)	51.3
NIST 2668 L1	Evaluation criteria [2] Target ± U	0.635 – 1.52 [α] 1.08 ± 0.31
	Reduced (220 V)	1.03
	Normal (275 V)	1.04
	Elevated (330 V)	0.915
NIST 2668 L2	Evaluation criteria [2] Target ± U	24.4 – 31.0 [α] 27.7 ± 2.1
	Reduced (220 V)	27.8
	Normal (275 V)	28.4
	Elevated (330 V)	27.0

[1]from characterization results calculated on 2016-0707
[2]Evaluation criteria were the greater of the following:
[α] NIST cert mean ± U
[β] NIST cert mean ± 10%
[¥] NIST cert mean ± bench QC limits of similar concentration material

E. Ruggedness parameter test #5: kinetic energy discrimination (KED) value

i. Test details: Evaluate the impact the KED value has on measured concentrations of As and Ni. Values were tested in separately prepared, consecutive runs of 20 "junk urine" samples on the instrument without turning off the plasma.

1) Run #1 (normal method, 3 V)

2) Run #2 (decreased KED, 2 V)

3) Run #3 (increased KED, 4 V)

ii. Results: See Ruggedness Table 7.

iii. Conclusion: Keep the KED value, a global parameter, at 3V. The variations in the KED value from 2V to 4V did not affect the accuracy or precision for ^{75}As analysis, but the low KED setting did result in elevated results for ^{60}Ni.

Ruggedness Table 7. Impact of KED voltage changes on observed results.

Sample ID	KED	^{75}As (µg/L)	^{60}Ni (µg/L)
LU10709	± 2SD range[1]	9.94 – 11.7	1.22 – 1.99
	Reduced (2 V)	10.5	1.65
	Normal (3 V)	10.7	1.64
	Elevated (4 V)	10.7	1.61
HU10710	± 2SD range[1]	45.1 – 51.1	5.98 – 7.15
	Reduced (2 V)	47.6	6.95
	Normal (3 V)	48.0	6.72
	Elevated (4 V)	48.2	6.85
EU10713	± 2SD range[1]	1148 – 1332	54.6 – 65.0
	Reduced (2 V)	1212	66.2
	Normal (3 V)	1206	57.0
	Elevated (4 V)	1238	61.4
NIST 2668 L1	Evaluation criteria [2] Target ± U	9.53 – 12.1[¥] 10.81 ± 0.54	1.73 – 2.89[¥] 2.31 ± 0.32
	Reduced (2 V)	10.3	2.19
	Normal (3 V)	10.1	2.24
	Elevated (4 V)	10.4	2.34
NIST 2668 L2	Evaluation criteria [2] Target ± U	191.8 – 234.4[β] 213.1 ± 44	103.8 – 126.8 [β] 115.3 ± 5.2
	Reduced (2 V)	207.5	128*
	Normal (3 V)	210.1	112
	Elevated (4 V)	210.2	119

[1]from characterization results calculated on 2016-0707
[2]Evaluation criteria were the greater of the following:
[α] NIST cert mean ± U [β] NIST cert mean ± 10%
[¥] NIST cert mean ± bench QC limits of similar concentration material
*Sample QC fails; > highest calibrator for ^{60}Ni (125 µg/L)

F. Ruggedness parameter test #6: kinetic energy discrimination (KED) cell exit voltage

i. Test details: Evaluate the impact the KED mode cell exit voltage has on As and Ni. Three different KED mode cell exit values were tested in separately prepared, consecutive runs on the instrument without turning off the plasma. "Junk urine" samples (20) were analyzed between the beginning and ending QC of each run. All other method parameters were kept per method.

1) Run #1 (normal value NexION_P, -39 V)

2) Run #2 (decreased value NexION_P, -34 V)

3) Run #3 (increased value NexION_P, -44V)

ii. Results: See Ruggedness Table 8.

iii. Conclusion: The accuracy of ^{75}As and ^{60}Ni results were not compromised by varying the KED mode cell exit voltage by the settings tested.

Ruggedness Table 8. Effect of KED mode cell exit voltage on observed concentrations.

Sample ID	KED exit voltage	^{75}As (µg/L)	^{60}Ni (µg/L)
LU10709	± 2SD range$^{\alpha}$	9.94 – 11.7	1.22 – 1.99
	Reduced (-34 V)	10.6	1.70
	Normal (-39 V)	10.9	1.59
	Elevated (-44 V)	10.7	1.53
HU10710	± 2SD range$^{\alpha}$	45.1 – 51.1	5.98 – 7.15
	Reduced (-34 V)	48.2	6.91
	Normal (-39 V)	49.4	6.23
	Elevated (-44 V)	47.8	6.22
EU10713	± 2SD range$^{\alpha}$	1148 – 1332	54.6 – 65.0
	Reduced (-34 V)	1246	60.9
	Normal (-39 V)	1279	59.7
	Elevated (-44 V)	1246	57.9
NIST 2668 L1	Evaluation criteria [2] Target ± U	9.53 – 12.1$^{¥}$ 10.81 ± 0.54	1.73 – 2.89$^{¥}$ 2.31 ± 0.32
	Reduced (-34 V)	10.5	2.55
	Normal (-39 V)	10.7	2.11
	Elevated (-44 V)	10.6	2.22
NIST 2668 L2	Evaluation criteria [2] Target ± U	191.8 – 234.4$^{\beta}$ 213.1 ± 44	103.8 – 126.8 $^{\beta}$ 115.3 ± 5.2
	Reduced (-34 V)	212	115
	Normal (-39 V)	216	114
	Elevated (-44 V)	219	112

$^{\alpha}$from characterization results calculated on 2016-0707
[2]Evaluation criteria were the greater of the following:
$^{\alpha}$ NIST cert mean ± U $^{\beta}$ NIST cert mean ± 10%
$^{¥}$ NIST cert mean ± bench QC limits of similar concentration material

17. Appendix C: Tables and figures

Table 1. Instrument and method parameters.

Instrument: NexION 300D ICP-MS with biomonitoring universal cell (Bio UCell) upgrade	
Autosampler: ESI SC4 autosampler with FAST sample introduction system	
Optimization window parameters	
RF power	1.60 kW
Plasma gas flow (Ar)	18 L/min
Auxiliary gas flow (Ar)	1.2 L/min
Nebulizer gas flow (Ar)	~0.80 – 1.1 L/min (optimized as needed for sensitivity)
QID voltage(s)	Standard/DRC mode and KED mode optimized daily
QRO, CRO, CPV, discriminator threshold	Optimized per instrument by service engineer, or advanced user.
Parameters of torch position, nebulizer gas flow, QID voltages, mass calibration, and detector voltages are optimized regularly. Optimization file name = default.dac.	
Configurations window parameters	
Cell gas changes pause times	Pressurize Delay (From Standard to UCT) = 40 Exhaust Delay (From UCT to Standard mode) = 40 Flow Delay (Gas changes while in UCT mode) = 15 Channel Delay (channel change in UCT mode) = 40
File Names and Directories	
Method file names	CDC_DLS3031_urblk.mth and CDC_DLS3031_aqblk.mth
Dataset	Create a new dataset subfolder each day. Name as "2016-0121" for all work done on January 21, 2016
Sample File	Create for each day's work
Report file name	*For sample results printouts* cdc_quant comprehensive.rop *For calibration curve information* CDC_Quant Comprehensive (6 calib curve info).rop (see Figure 8 in Appendix C)
Tuning	Default.tun
Optimization	Default.dac
Calibration	N/A
Polyatomic	polylatomic.ply
Report options template (transferring results to the database)	CDC_Database Output.rop *Report Format Options: select only "Use Separator"* *File Write Option: Append* *Report File name: include date, instrument, and group being analyzed in file name (i.e. 2016-0704b_NexP_HM-0364.txt)*
Method parameters	
Method parameters: Timing page (see Figure 2 in Appendix C)	

Sweeps/reading	70
Readings/replicate	1
Replicates	3
Enable QC checking	On
Isotopes monitored and internal standard associations (exact mass)	^{52}Cr (51.9405),^{60}Ni (59.9308), ^{75}As (74.9216) ^{103}Rh (102.905) (internal standard)
Dwell times	30 ms^{103}Rh in KED mode 150 ms ^{60}Ni and 100 ms ^{75}As KED in mode 30 ms ^{103}Rh-1 in DRC mode 70 ms for ^{52}Cr in DRC mode
Scan mode	Peak Hopping for all isotopes (1 MCA channel)
UCT channel A gas flow rate	Anhydrous ammonia (128-130 psig) Typically 0.7 mL/min* (optimized instrument, and periodically verified)
UCT channel B gas flow rate	Helium (13-17 psig delivery pressure) Typically 2.5 (As) and 4.5 (Ni) mL/min * * (optimized instrument, and periodically verified)
RPa	0 for all isotopes
RPq	0.25 for ^{60}Ni, ^{75}As, ^{103}Rh KED mode 0.6 for ^{52}Cr and ^{103}Rh-1 in DRC mode* (* Optimize per instrument, and periodically verified)
KED (QRO – CRO)	typically 3V (see ruggedness test #5 in Appendix B)
Method parameters: Processing page (see Figure 3 in Appendix C)	
Detector mode	Dual
Process spectral peak	N/A
QID	On
Isotope ratio mode	Off
Enable short settling time	Off
Blank subtraction	After internal standard
Measurement units	Cps
Process signal profile	N/A
Method parameters: Equations page (see Figure 4 in Appendix C)	
Equations	Not used
Method parameters: Calibration page (see Figure 5 in Appendix C)	
Calibration type	External std.
Curve type	Weighted linear
Sample units	" µg/L" or "ppb"
Spiked calibrator concentrations (µg/L)	Cr: 0.1, 0.4, 2, 8, 30, 125 Ni: 0.1, 0.4, 2, 8, 30, 125 As: 0.2,1, 6, 30, 250, 1500
Method parameters: Sampling page (see Figure 6 in Appendix C)	
"Peristaltic pump under computer control"	On

Autosampler Tray Port Sampling device	***If using ESI autosampler*** Autosampler type: ESI Tray name: esi\esi.try (use shortcut in C:\users\public\public documents\PerkinElmer Syngistix\ICPMS\Autosampler\ESI\esi.try) Port: COM4 Sampling device: None ***If using other autosampler*** Refer to autosampler user guide.
Sample flush	***FAST defaults*** 9 s at 3 rpm (with ESI DXi peristaltic pump) Can be optimized as needed to adequately fill the FAST loop. As a matter of lab practice, set this time to equal the loop fill time in the ESI FAST program. As long as the combined time of sample flush + read delay is equal to the time required for signal to reach stability, analytical measurement will be good.
Read delay	***Default*** 45 s at 3 rpm (with ESI DXi peristaltic pump) Can be optimized as needed to reach signal stability before beginning analysis. As a matter of lab practice, set this time equal to the total time required for the signal to reach stability minus the loop fill time. As long as the combined time of sample flush + read delay is equal to the time required for signal to reach stability, analytical measurement will be good.
Wash	***Default*** 30 s at 3 rpm (with ESI DXi peristaltic pump) Can be optimized to allow for changes in FAST loop rinsing (must be greater than total time of steps in FAST program after the initial “on rinse” command).
Autosampler locations of blanks and standards	***Default*** *For calibration curve (points to urine blank)* CDC_DLS3031_urblk.mth Urine Blank and spiked calibrators 1–6 in autosampler positions 101–107 by default, but can be customized. *For QC and patient samples (points to aqueous blank)* CDC_DLS3031_aqblk.mth Aqueous Blank in autosampler position 110, but can be customized.

<table>
<tr><th colspan="2">Method parameters: QC page, sample tab (see Figure 7 in Appendix C)</th></tr>
<tr><td>extended wash</td><td>For sample concentrations greater than these, setup the ICP-MS software's 'QC checking' feature to "Wash for X and continue."

Analyte | Extended Rinse Trigger Conc. | Extended Rinse Time
Cr | >125 µg/L | 200 s
Ni | >125 µg/L | 200 s
As | >1,500 µg/L | 200 s</td></tr>
<tr><th colspan="2">FAST parameters: See Figure 9 through Figure 15 in Appendix C</th></tr>
<tr><td>Configuration file</td><td>default.sc
(saved at C:\Program Files\ESI\ESI-SC\)</td></tr>
<tr><td>FAST program</td><td>2 mL loop 8 sec fill 2016-0126.txt</td></tr>
<tr><td><u>Non-FAST sample introduction system</u>:</td><td>If the FAST sample introduction system is not available on any instruments, the method can still be implemented, but the sample flush, read delay and rinse times will all need to be optimized.</td></tr>
<tr><th colspan="2">Potential emergency response modifications:</th></tr>
<tr><td><u>Arsenic</u></td><td>• Arsenic can be analyzed in urine using pure argon as the DRC gas to remove the $^{40}Ar^{35}Cl^{+}$ interference. Small interferences ($^{40}Ca^{35}Cl^{+}$, $^{59}Co^{16}O^{+}$) are expected, but are not anticipated to be significant for emergency response applications. A tee can be setup on the main argon delivery line for the ICP-MS to provide this argon for the DRC. Suggested DRC settings are 0.7 mL/min and RPq=0.7, however these need to be optimized [15].

• Arsenic can be analyzed with no ethanol in the diluent and rinse solutions. This could bias As results 1-5% high, likely requiring revised QC limits.</td></tr>
</table>

Table 2. Suggested maximum analyte concentrations for base urine

Analyte	Concentration (µg/L)
Cr	0.9
Ni	1.5
As	5

Table 3. Stock standard concentrations

Analyte	Stock Calibration Standard Conc. (µg/mL) such as High Purity Standards Item # 10003-1	Stock Calibration Standard Conc. (µg/mL) such as High Purity Standards Item # SM-2107-055
Cr		100

Ni		100
As	1000	

Table 4. Preparation of As Int. Stock Calib Std and Cr/Ni Int. Stock Calib. Std.

Name	As Int. Stock Calib Std	Cr/Ni Int. Stock Calib Std
Volume of flask (mL)	100	100
As stock Calib Std (mL)	1	
Cr/Ni Stock Calib Std (mL)		1
Concentration (µg /mL)		
As	10	
Cr/Ni		1
Prepare two separate Int. Stock Calib Standards. One for As, and one for Cr/Ni		

Table 5. Preparation of multi-element intermediate working calibrators

Standard #	0	1	2	3	4	5	6
Volume of Flask (mL)	-	500	200	100	200	200	100
Volume Spike of Stock Std. As (mL)	0				0.06	0.5	1.5
Volume Spike of Stock Std. Cr/Ni (mL)	0				0.16	0.6	1.25
Volume Spike of As Int. Stock Std. (mL) [$]	0	0.10	0.2	0.6			
Volume Spike of Cr/Ni Int. Stock Std. (mL) [$]	0	0.5	0.8	2			
Concentrations (µg/L)							
Cr*	0	1 (0.1) [‡]	4 (0.4) [‡]	20 (2.0) [‡]	80 (8.0) [‡]	300 (30.0) [‡]	1250 (125) [‡]
Ni*	0	1 (0.1) [‡]	4 (0.4) [‡]	20 (2.0) [‡]	80 (8.0) [‡]	300 (30.0) [‡]	1250 (125) [‡]
As*	0	2.0 (0.2) [‡]	10 (1.0) [‡]	60 (6.0) [‡]	300 (30) [‡]	2500 (250) [‡]	15000 (1500) [‡]

[‡] A further 1:10 dilution occurs when added to base urine. Enter concentrations in parentheses into the NexION software (method window, calibration page).
* Rh-103 used as internal standard
[$] Int. Stock Std. = see Table 4 for preparation

Table 6. Acceptable ways to perform two consecutive analytical runs, bracketing with bench quality control samples

Setup 1	**Setup 2** (typical)
Run #1 calibrators low bench QC high bench QC elevated bench QC patient samples low bench QC high bench QC elevated bench QC *Run #2* low bench QC high bench QC elevated bench QC patient samples low bench QC high bench QC elevated bench QC	*Run #1* calibrators low bench QC high bench QC elevated bench QC patient samples low bench QC high bench QC elevated bench QC *Run #2* calibrators low bench QC high bench QC elevated bench QC patient samples low bench QC high bench QC elevated bench QC

Table 7. A typical SAMPLE/BATCH window

AS Location*	Sample ID	Measurements Action	Method

231	KED/DRCstability1	Run sample	Urblank
231	KED/DRCstability2	Run sample	Urblank
231	KED/DRC stability3	Run sample	Urblank
231	KED/DRC stability4	Run sample	Urblank
Continue KED/DRC stability samples . . .			
231	KED/DRC stability18	Run sample	Urblank
231	KED/DRC stability19	Run sample	Urblank
108	UrBlkChk1	Run blank, standards, and sample **	Urblank
109	UrBlkChk2	Run sample	Urblank
111	AqBlkChk1	Run blank and sample ¥	Aqblank
112	AqBlkChk2	Run sample	Aqblank
136	L Bench QC	Run sample	Aqblank
148	H Bench QC	Run sample	Aqblank
160	E Bench QC	Run sample	Aqblank
301	Sample 1	Run sample	Aqblank
302	Sample 2	Run sample	Aqblank
303	Sample 3	Run sample	Aqblank
136	L Bench QC	Run sample	Aqblank
148	H Bench QC	Run sample	Aqblank
160	E Bench QC	Run sample	Aqblank

* The exact autosampler positions of QCs and patient samples do not have to be those shown above.

** When executing this row, the NexION will first analyze the urine blank at AS position 101, then standards 1-6 at autosampler positions 102-107, then the “UrBlkChk1” at A/S position 108. The sampling information about AS positions 101-107 are stored in the “urblank” method file and can be customized.

¥ When executing this row, the NexION will first analyze the aqueous blank at AS position 110, then the “AqBlkChk1” at AS position 112. The sampling information about AS positions 110 is stored in the “aqblank” method file and can be customized.

Table 8. Preparation of samples, working standards, and QC materials for analysis

If a different total volume is prepared, adjust the volumes for each component proportionally.

These directions are written with the expectation of a 10,000 µL syringe on the left side and a 1,000 µL syringe on the right side of the benchtop automatic pipette.

Dilution ID	Water (µL)	Base Urine (µL)	AQ Intermediate Working Standard (µL)	Patient or QC urine sample (µL)	Diluent (µL)*	Total volume (µL)
Table 9Working Calibrators (S0-S6) and UrineBlkChk (S0)	-	540 (540 x 1)	60 (60 x 1)	-	5,400 (2,700 x 2)	6,000
AQ Blank and AqBlkChk	300 (300 x 1)	-	-	-	2,700 (2,700 x 1)	3,000
Patient Urine or Urine-Based QC	-	-	-	300 (300 x 1)	2,700 (2,700 x 1)	3,000
Patient Urine *2x Extra Dilution* [H]	300 (300 x 1)	-	-	300 (300 x 1)	5,400 (2,700 x 2)	6,000
Patient Urine *5x Extra Dilution* [H]	480 (480 x 1)	-	-	120 (120 x 1)	5,400 (2,700 x 2)	6,000

* By splitting the dispense step of diluent into two or more portions, liquids pulled up into the right pipette tip are flushed out more completely. For example, when preparing a working calibrator dilution, do the preparation in two steps: in step 1, dispense 2700 µL diluent + 60 µL int. working standard; in step 2, dispense 2700 µL diluent + 540 µL base urine to prepare a 6 mL total volume dilution.

[H] Extra dilution is performed on urine samples whose concentration is greater than the concentration of the highest calibrator listed in Table 9 of Appendix C. Any extra dilution within these limits can be prepared as long as the 9:10 ratio of diluent to total dilution volume is maintained. Use of the lowest possible dilution level is preferred to minimize differences between the calibrators and the samples (i.e. 2x dilution is preferred over 5x if 2x is sufficient to dilute analyte into the documented linearity range).

Table 9. Reportable range concentrations (µg/L).

Analyte	Limit of Detection (LOD)*	High Calibrator	Maximum Extra Dilution **	Reportable Range Upper Boundary
As	0.23	125	5	625
Cr	0.19	125	5	625
Ni	0.31	1,500	5	7,500

* Re-evaluated periodically (2^{+} years) or at significant method changes. LODs shown were calculated 07/11/2016.
** See ruggedness test 1 results in Appendix B for supporting validation data.

Table 10. Boundary concentrations for urine concentrations (µg/L)

Analyte	1st Upper Boundary ("1UB") *	2nd Upper Boundary ("2UB") **	Range Maximum ("Lim Rep Delta") †	Highest Concentration Validated for Washout
As	100	200	2 µg/L for values <20 10% of value at ≥20	1500
Cr	2	4	0.4 µg/L for values <4 10% of value at ≥4	125
Ni	6	12	0.5 µg/L for values <5 10% of value at ≥5	125

* Typically, the 1UB threshold is based on percentiles of non-weighted, non-creatinine corrected concentration results from NHANES. In the absence of that data, these boundaries can be based on normal ranges reported in the literature. The concentrations assigned to these boundaries is determined by study protocol but default concentrations are listed in this table.

**Typically the 2nd upper boundary (2UB) is set to 2x the 1UB. The concentrations is determined by study protocol but default concentrations are listed in this table.

† Range maximum is the maximum permitted concentration range (max – min) of the three replicate readings for a single sample measurement. This is a two-phase intra-measurement precision limit permitting a fixed limit at lower concentrations and a percentage-based limit at higher concentrations. This value is also called the "Lim RepDelta" in the database which handles data for the Inorganic and Radiation Analytical Toxicology Branch.

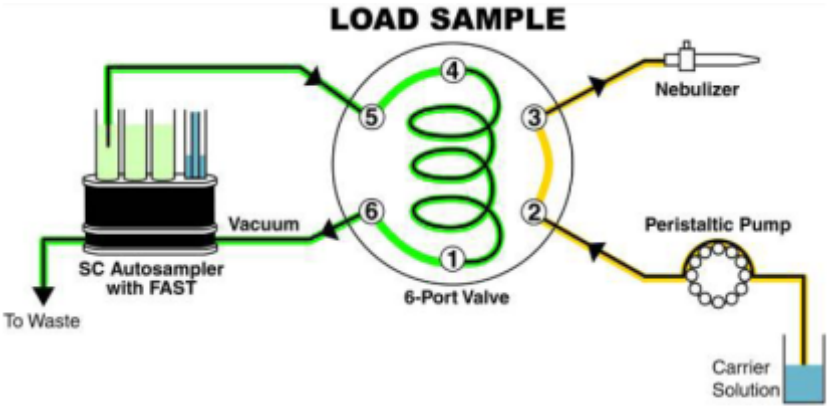
Table 11. References to total urine concentrations

Element	Concentration (µg/L)	N	References
Cr	0.06 – 40.0 *	-	Burtis[30]
	0.18	-	ATSDR[6]
	0.9 –14.0	74	Sarmiento-Gonzalez[31]
	0.06 – 0.26	19	Rodushkin[32]
	0 – 3.5	1045	Komaromy-Hiller[33]
	0.1-2.0	-	Tietz [30]
Ni	0.06 – 20.0 *	-	Burtis[30]
	1.1 – 32.1	74	Sarmiento-Gonzalez[31]
	0.27 – 3.68	19	Rodushkin[32]
	0 – 12.0	2035	Komaromy-Hiller[33]
	1.4 – 4.8[β]	5737	Health Canada[34]
As	5.82 – 46.0[¥]	2662	Fourth National Report of Exposures to Environmental Chemicals [35]

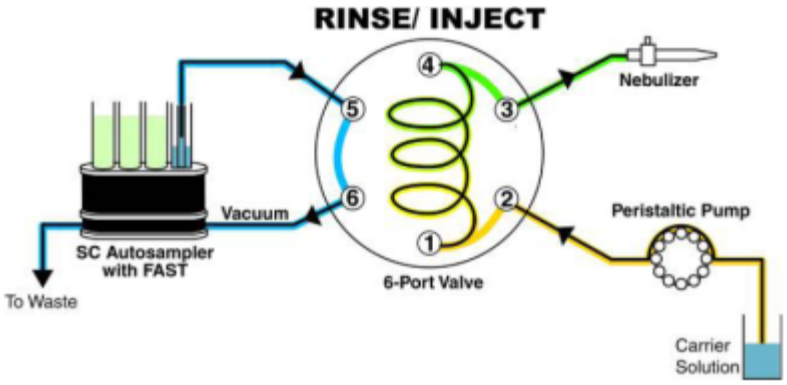
* Urine volume used for determining concentration ranges: 500 – 1800 mL d^{-1} (500 – 1400 mL d^{-1} child and 600 – 1800 mL d^{-1} adult)[30].
[β] 50^{th} – 95^{th} percentile from cycle 2 (2009 – 2011)
[¥] 50^{th} – 95^{th} from cycle 2013-2014

Figure 1. Configuration of tubing and devices for liquid handling using FAST sample introduction.

Below shows the correct connections to the 6-port FAST valve. The two diagrams show the differences in liquid flow directions when the valve changes from “Load” to “Inject” This change is internal to the valve. The shift of the valve cannot be seen, but it can be heard, and felt (with hand on the valve). The light indicators on the actuator body also indicate the valve position.



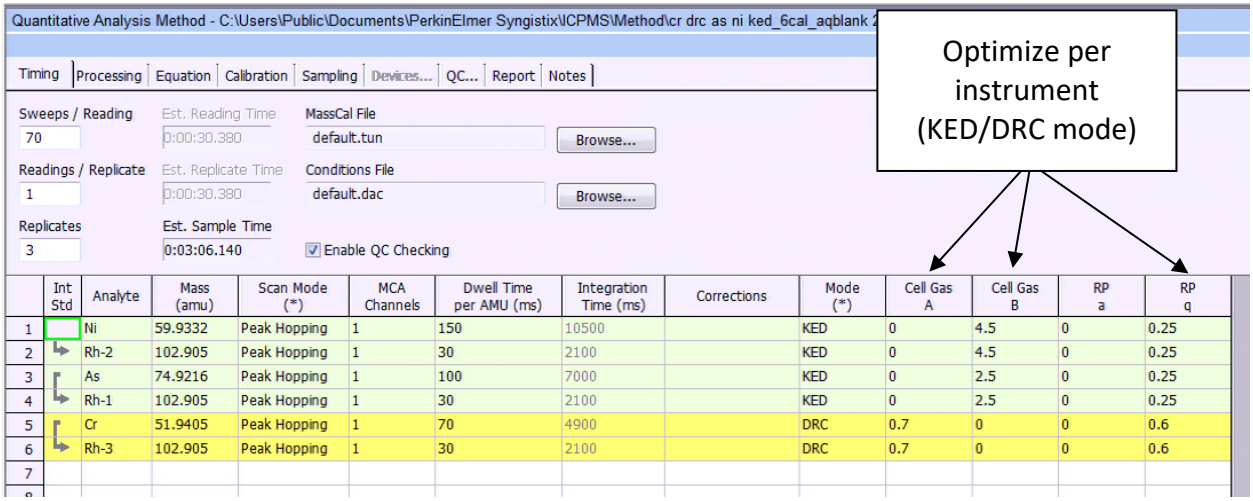
Teflon vacuum pump loads sample into loop while carrier solution is nebulized



Carrier solution pushes sample into nebulizer at the same time sample line is rinsed

Enable the FAST program in the ESI software before running the method, but optimizations can be done in either FAST or non-FAST mode.

Figure 2. NexION ICP-MS method screen shots (timing page) from Syngistix v1.1



	Int Std	Analyte	Mass (amu)	Scan Mode (*)	MCA Channels	Dwell Time per AMU (ms)	Integration Time (ms)	Corrections	Mode (*)	Cell Gas A	Cell Gas B	RP a	RP q
1		Ni	59.9332	Peak Hopping	1	150	10500		KED	0	4.5	0	0.25
2		Rh-2	102.905	Peak Hopping	1	30	2100		KED	0	4.5	0	0.25
3		As	74.9216	Peak Hopping	1	100	7000		KED	0	2.5	0	0.25
4		Rh-1	102.905	Peak Hopping	1	30	2100		KED	0	2.5	0	0.25
5		Cr	51.9405	Peak Hopping	1	70	4900		DRC	0.7	0	0	0.6
6		Rh-3	102.905	Peak Hopping	1	30	2100		DRC	0.7	0	0	0.6
7													

Figure 3. NexION ICP-MS method screen shots (processing page) from Syngistix v1.1.

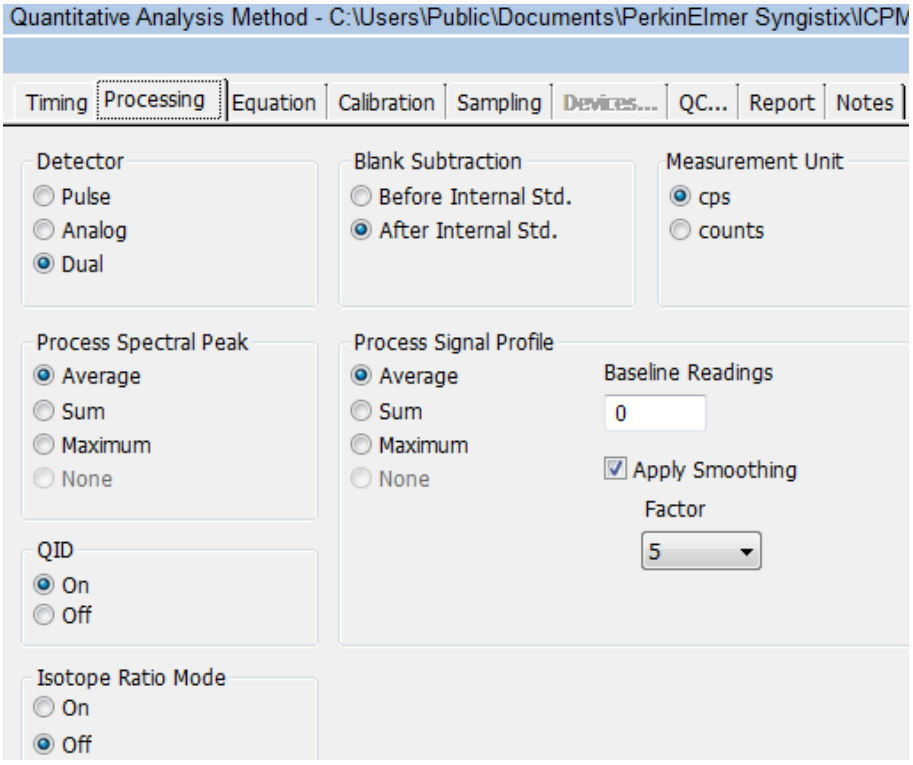


Figure 4. NexION ICP-MS method screen shots (equation page) from Syngistix v1.1.

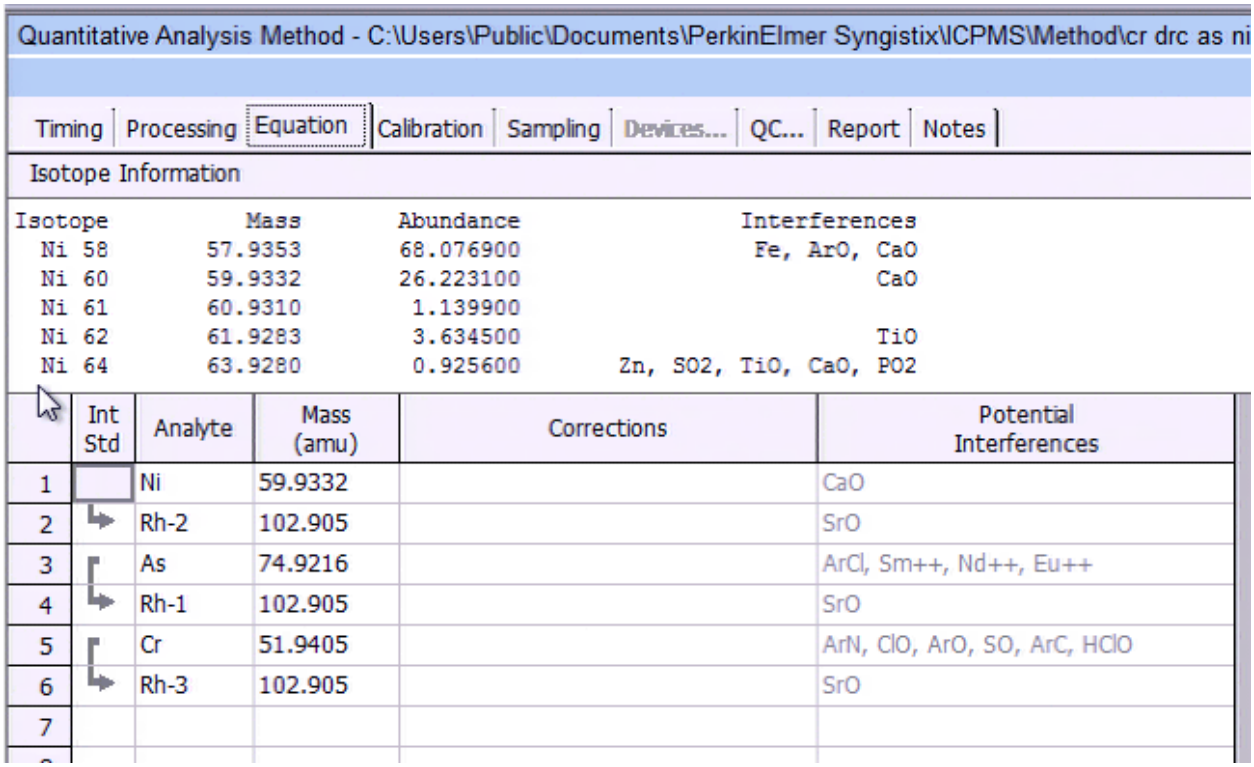
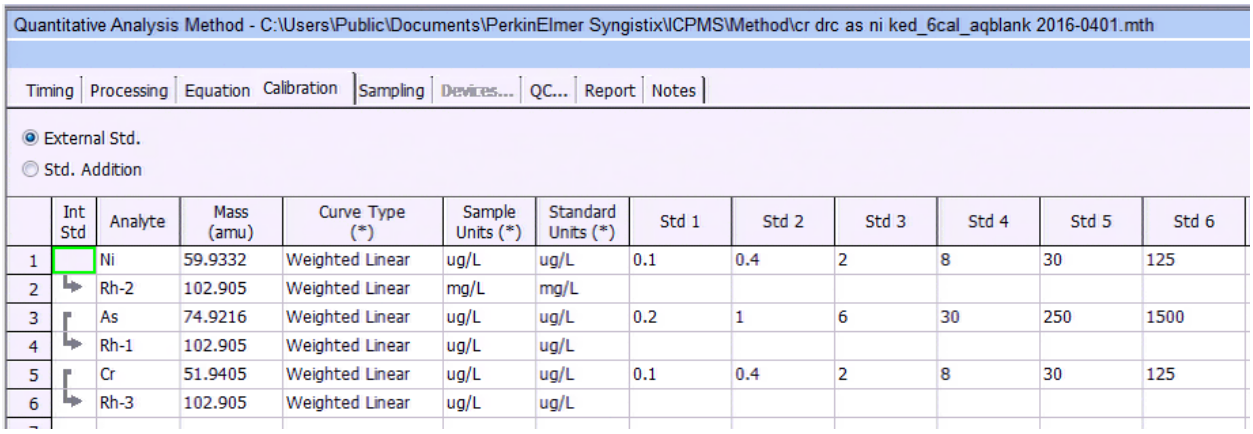


Figure 5. NexION ICP-MS method screen shots (calibration page) from Syngistix v1.1.



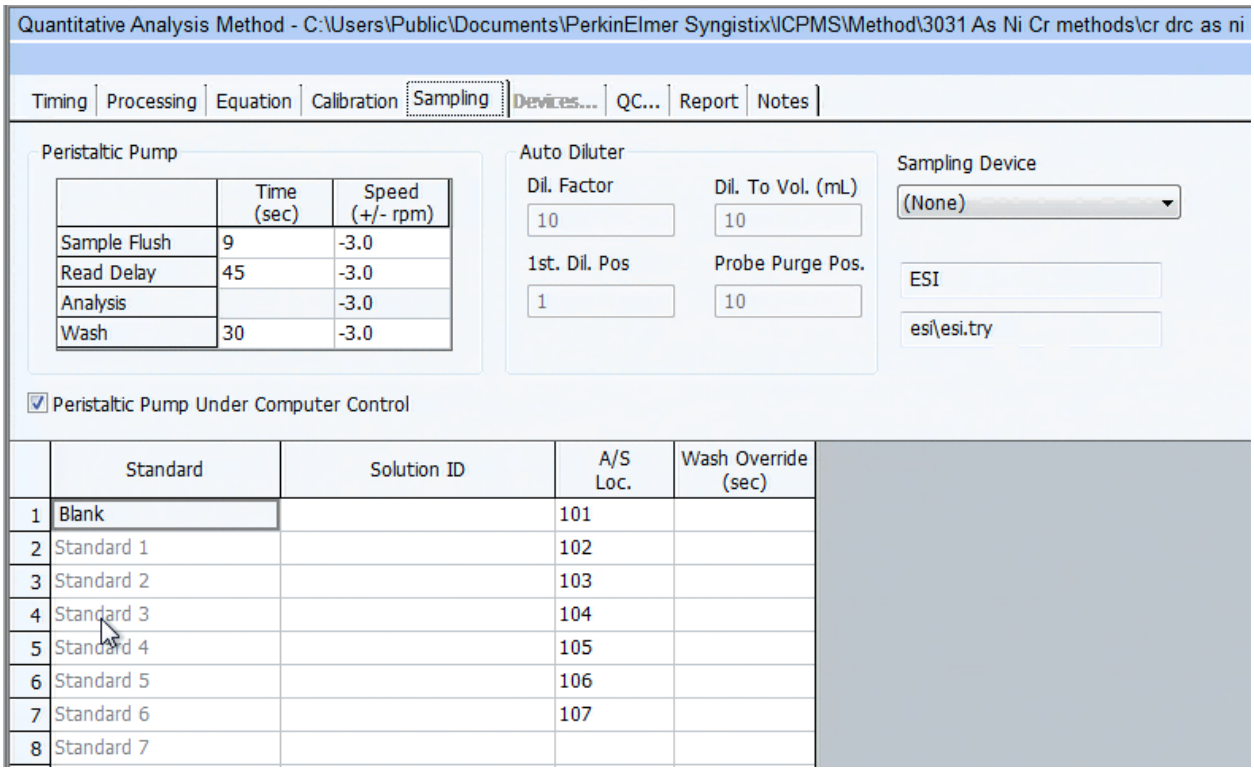
Quantitative Analysis Method - C:\Users\Public\Documents\PerkinElmer Syngistix\ICPMS\Method\cr drc as ni ked_6cal_aqblank 2016-0401.mth

Timing | Processing | Equation | Calibration | Sampling | Devices... | QC... | Report | Notes

(•) External Std.
( ) Std. Addition

	Int Std	Analyte	Mass (amu)	Curve Type (*)	Sample Units (*)	Standard Units (*)	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
1		Ni	59.9332	Weighted Linear	ug/L	ug/L	0.1	0.4	2	8	30	125
2	↳	Rh-2	102.905	Weighted Linear	mg/L	mg/L						
3		As	74.9216	Weighted Linear	ug/L	ug/L	0.2	1	6	30	250	1500
4	↳	Rh-1	102.905	Weighted Linear	ug/L	ug/L						
5		Cr	51.9405	Weighted Linear	ug/L	ug/L	0.1	0.4	2	8	30	125
6	↳	Rh-3	102.905	Weighted Linear	ug/L	ug/L						

Figure 6. NexION ICP-MS method screen shots (sampling page) from Syngistix v1.1; Urine blank method (top), Aqueous blank method (bottom).



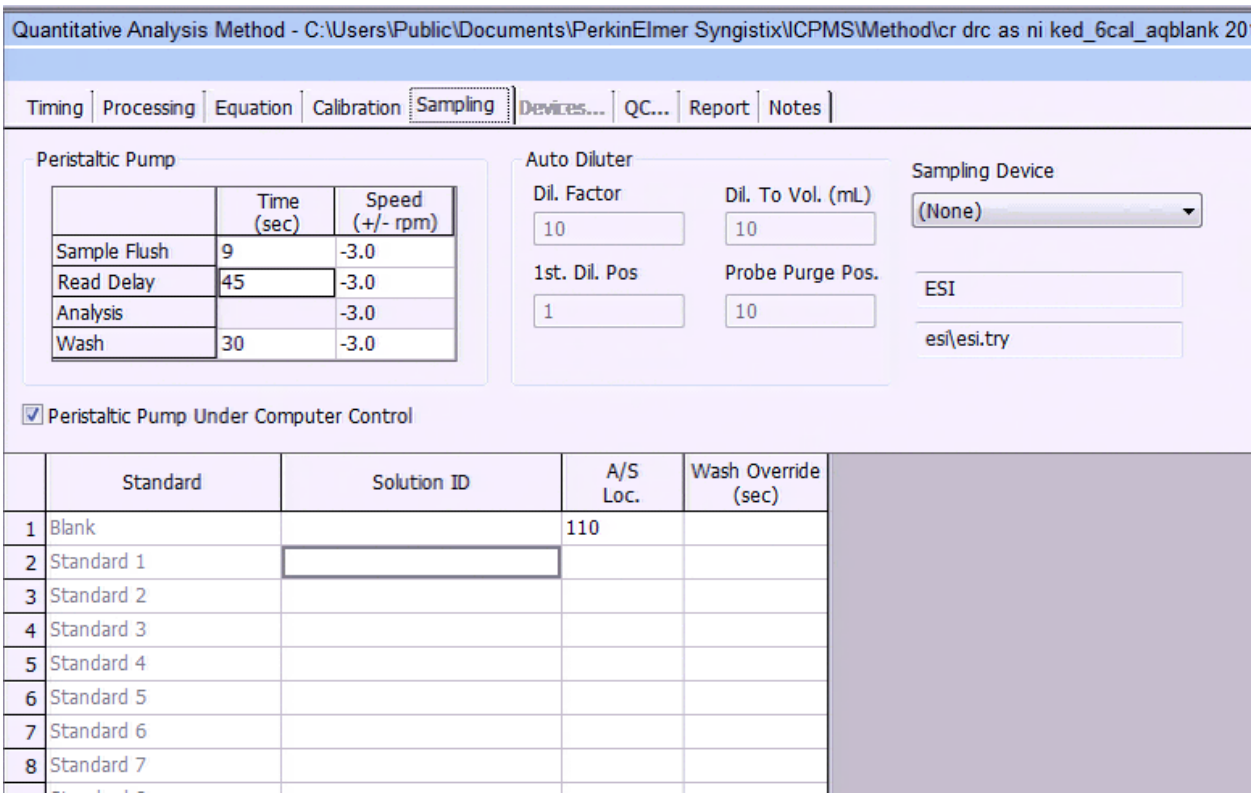


Figure 7. NexION ICP-MS method screen shots (QC page, Sample tab) from Syngistix v1.1.

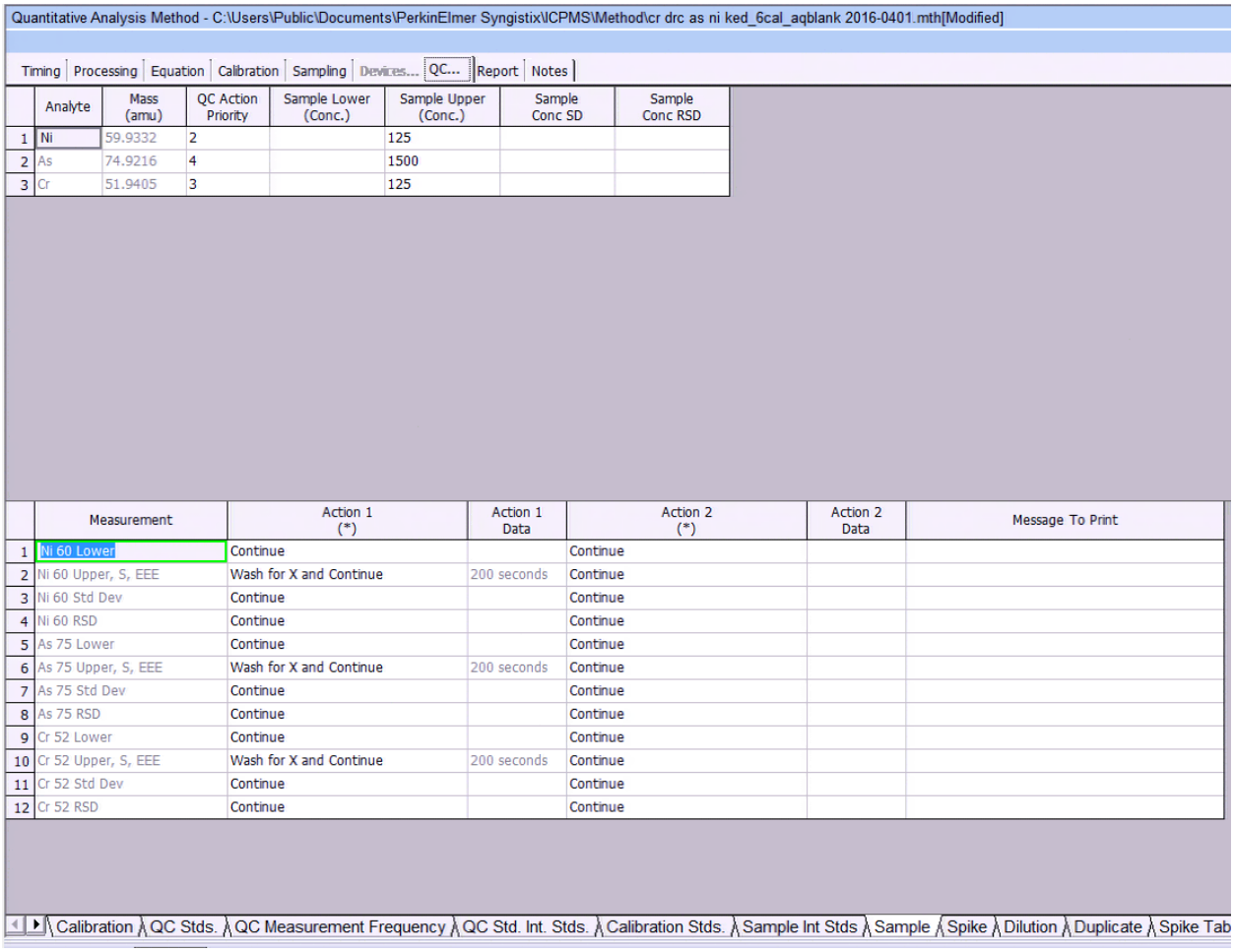


Figure 8. NexION ICP-MS method screen shots (Report page) from NexION v1.2.

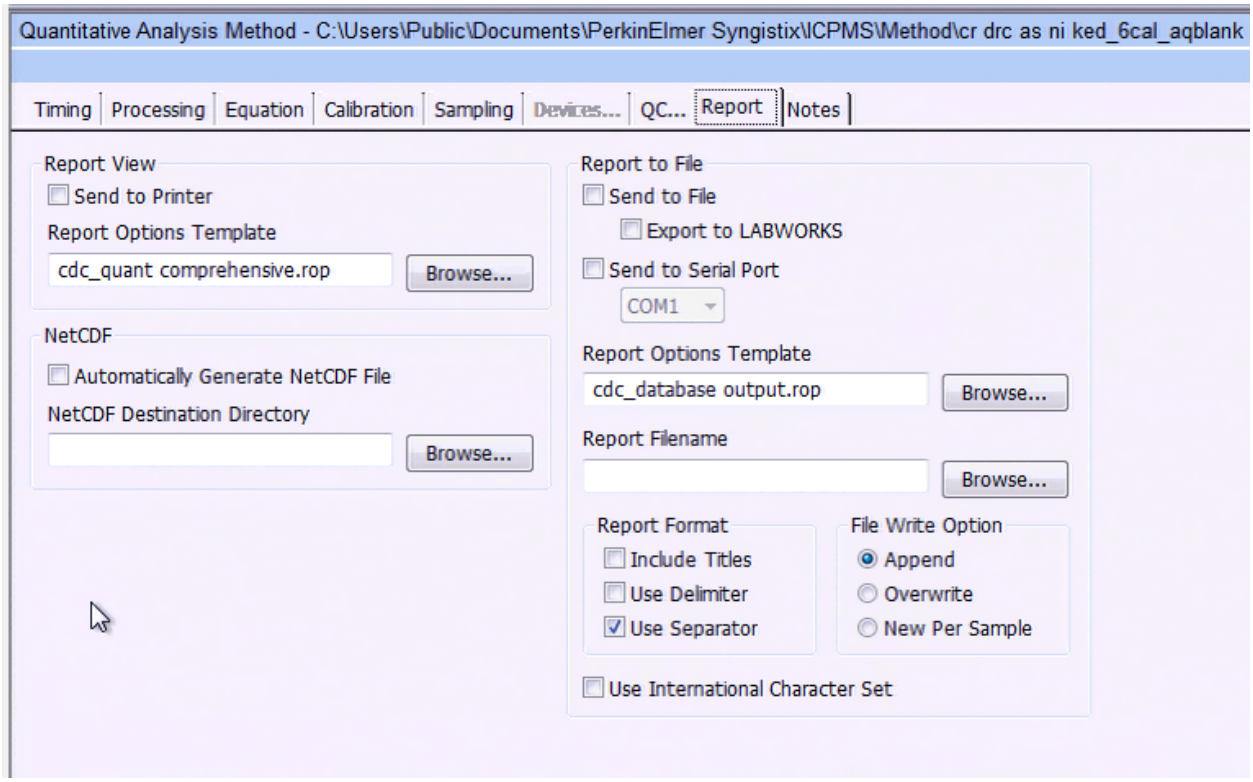
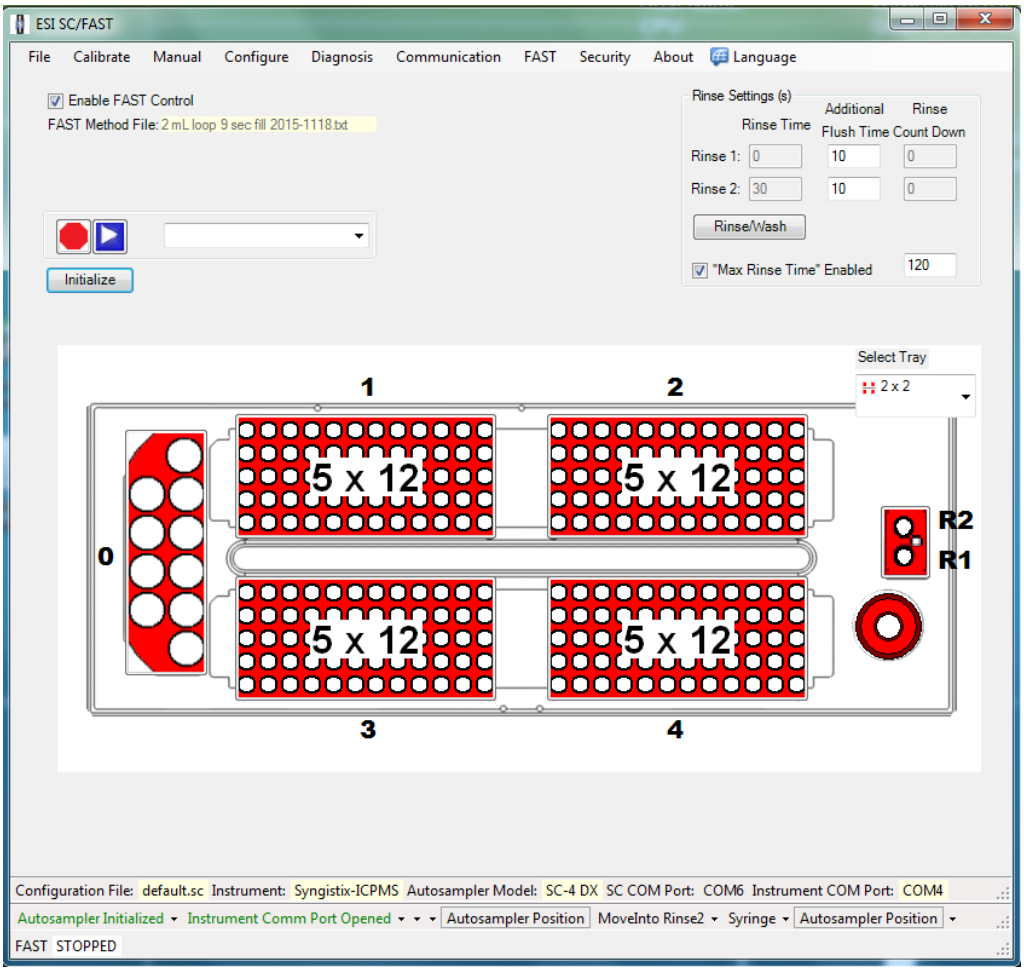


Figure 9. ESI SC4 autosampler screen shots used (main page) ESI SC v2.9.0.4.

Additional flush times and “Max Rinse Time” are default, but can be optimized for best reduction of elemental carry-over between samples. Tray types can be changed to allow for different volumes of diluted sample digests. ‘FAST control’ must be enabled before start of method, but does not need to be used in instrument optimization (pre-analysis) steps. Rinse and additional flush times for eliminating carry-over from one sample to the next while using the minimum amount of rinse solution.



A rinse time of 0 causes the probe to only dip into the station, but spends no time there. A rinse time of -1 will result in the probe skipping that rinse station. Additional flush times can be optimized to keep the rinse station full while not using too much rinse solution. The inner diameter size of the tubing providing the rinse solution to the rinse station determines how quickly the station will fill. Various sizes are available for purchase or can be made in the laboratory.

Figure 10. ESI SC4 autosampler screen shots used ("Configure>Autosampler" page) ESI SC v2.9.0.4

"High Speed" option is to only be used for 'High Speed' models of the SC4 (look for "HS" in serial number). Speeds, accel / decel values, and RAF can be optimized per analyst preference and to minimize droplet splatter off of probe.

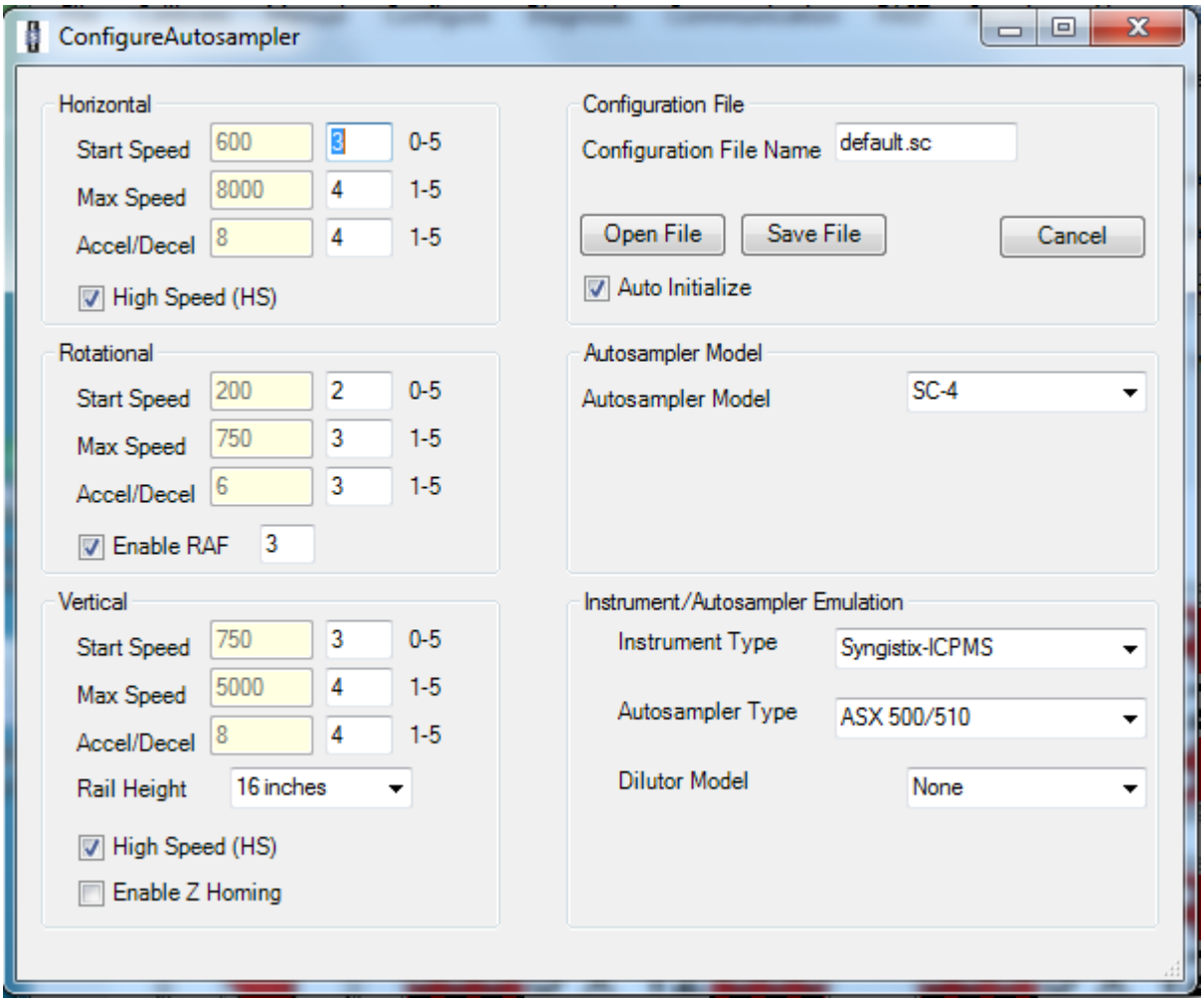


Figure 11. ESI SC4 autosampler screen shots used ("Communication" page) ESI SC v2.9.0.4

Communication ports will differ depending on available ports on instrument control computer. The NexION ICP-MS uses a "Virtual COM Port".

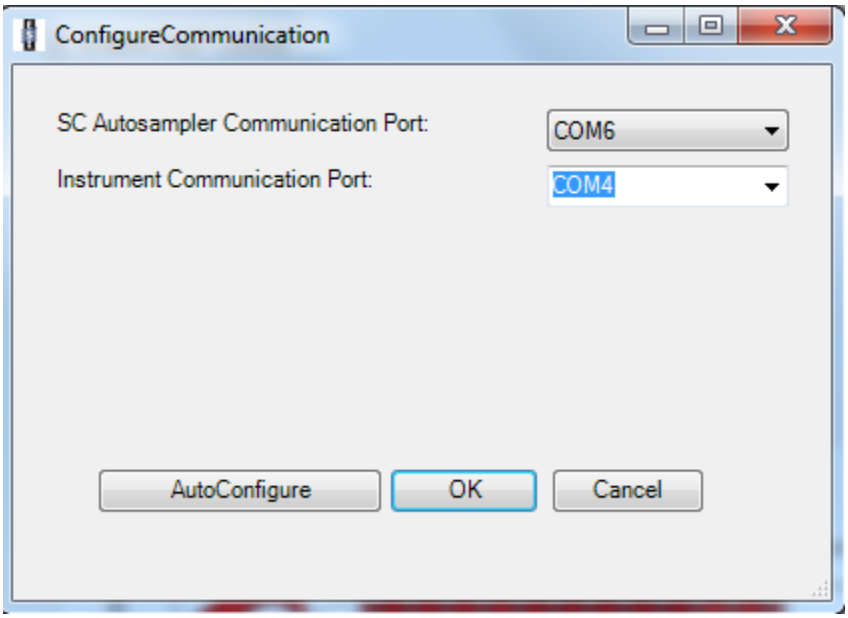
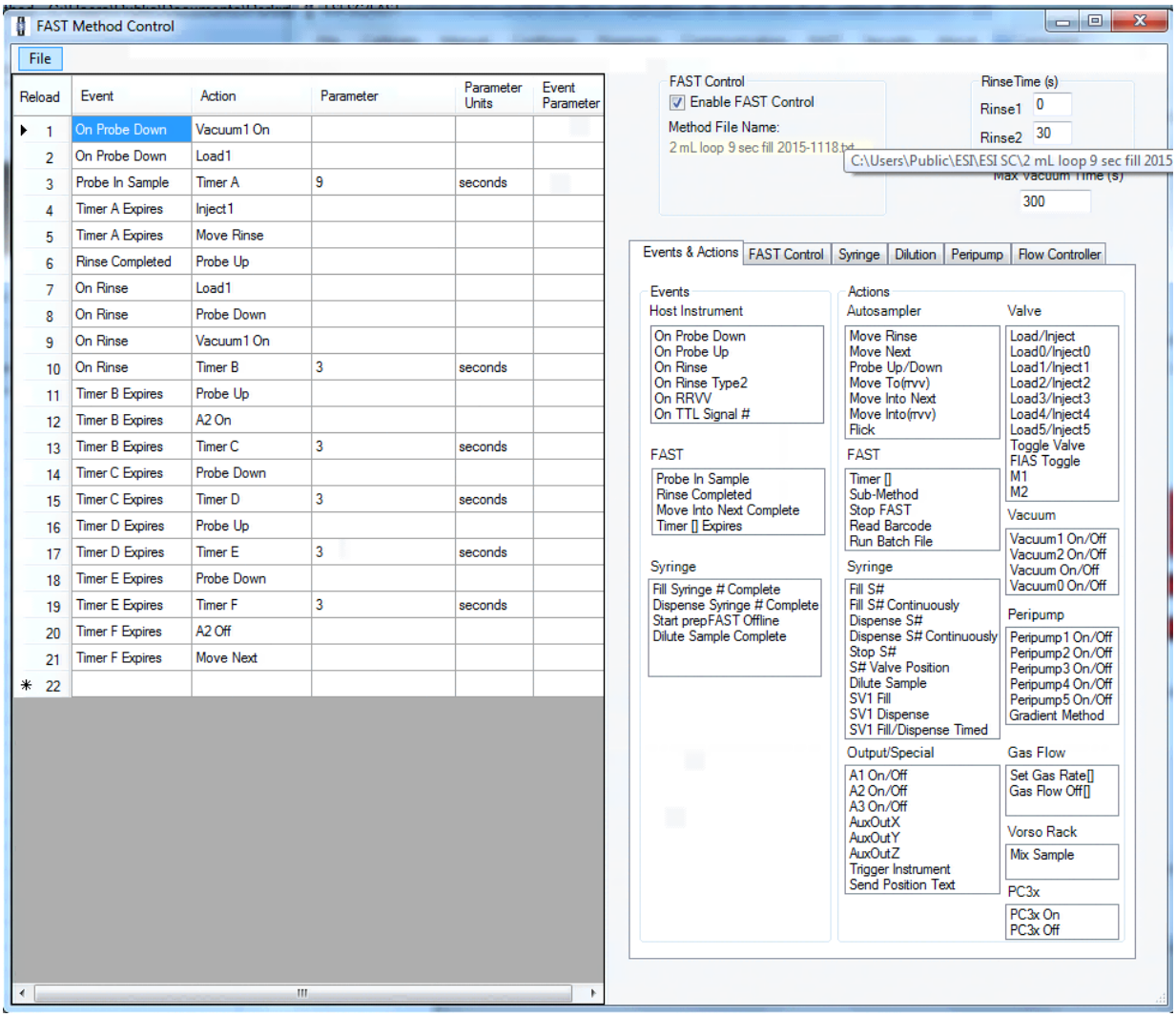


Figure 12. ESI SC4 autosampler screen shots ("FAST" page) ESI SC v2.9.0.4

Timer A can be optimized to achieve proper filling of loop with diluted sample digestate. Timers B, C, D, E, and F control rinsing the loop after analysis and can be optimized for eliminating carry-over from one sample to the next while using the minimum amount of rinse solution. File can be found in the directory C:\Users\Public\ESI\ESI-SC\.

Manually clicking the "Load" button prior to starting analysis will ensure the position of the actuator is always the same at the beginning of the analysis.

Manually clicking the "Vacuum On" button prior to starting the analysis will help initial sample uptake to be consistent.



NOTE: "Probe in Sample" time will need to be optimized per instrument (sample probe length from probe to 6-port valve will dictate time needed) to allow for the sample loop to be filled completely and be repeatable throughout a run.

Figure 13. ESI SC4 autosampler screen shots (5x12 Rack Setup window) ESI SC v2.9.0.4

Settings are approximate. To be sure the loop is filled, position the probe close to the bottom of the cup, but not touching. Optimize retraction speed for least droplet splatter.

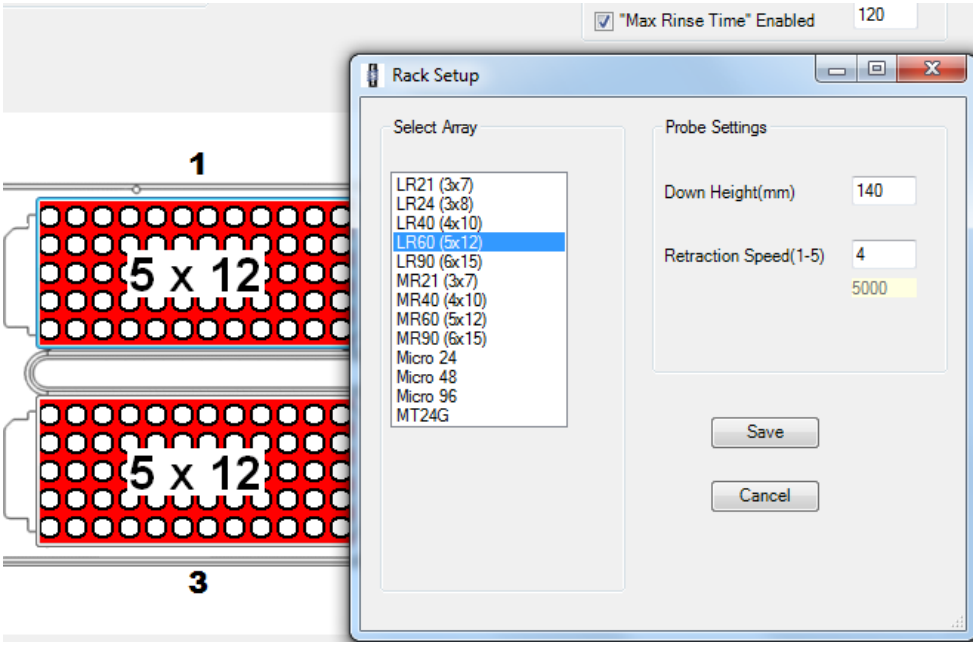


Figure 14. ESI SC4 autosampler screen shots (50mL Tube Rack Setup window) ESI SC v2.9.0.4

Settings are approximate. To be sure the loop is filled, position the probe down close to the bottom of the cup, but not touching. Optimize retraction speed for least droplet splatter.

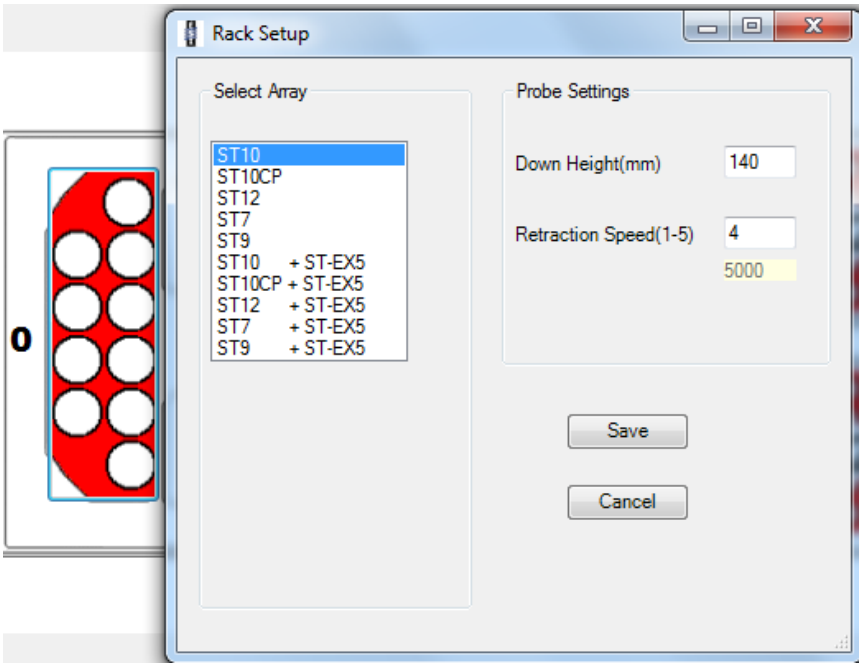


Figure 15. ESI SC4 autosampler screen shots (Rinse Station Rack Setup Window) ESI SC v2.9.0.4

Settings are approximate. Optimize down height for best probe cleaning, and retraction speed for least droplet splatter.

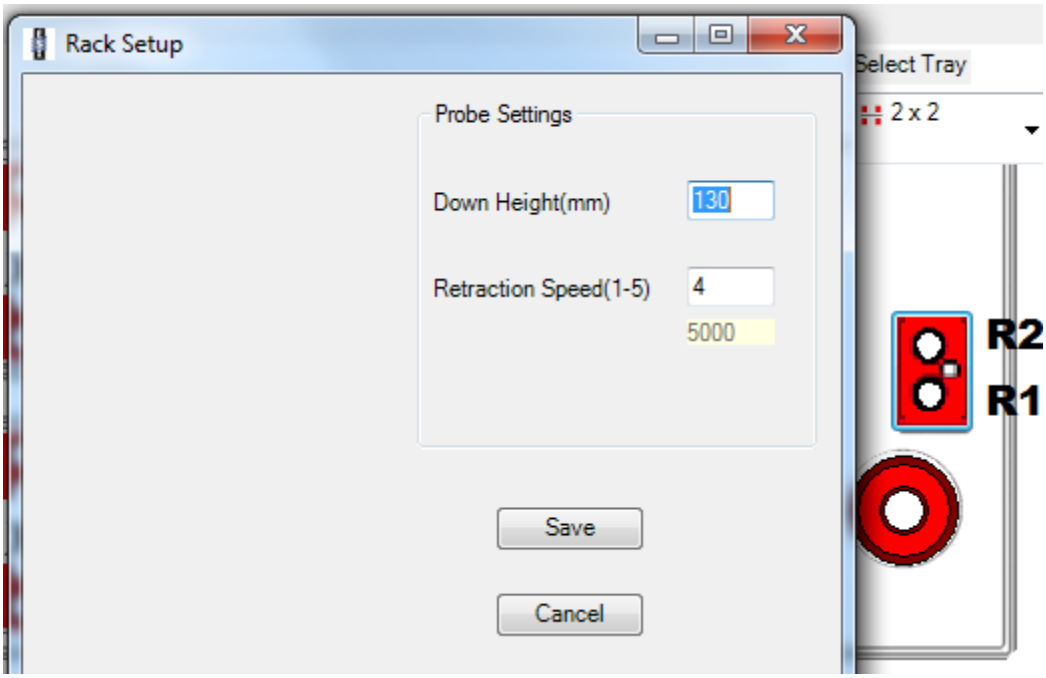
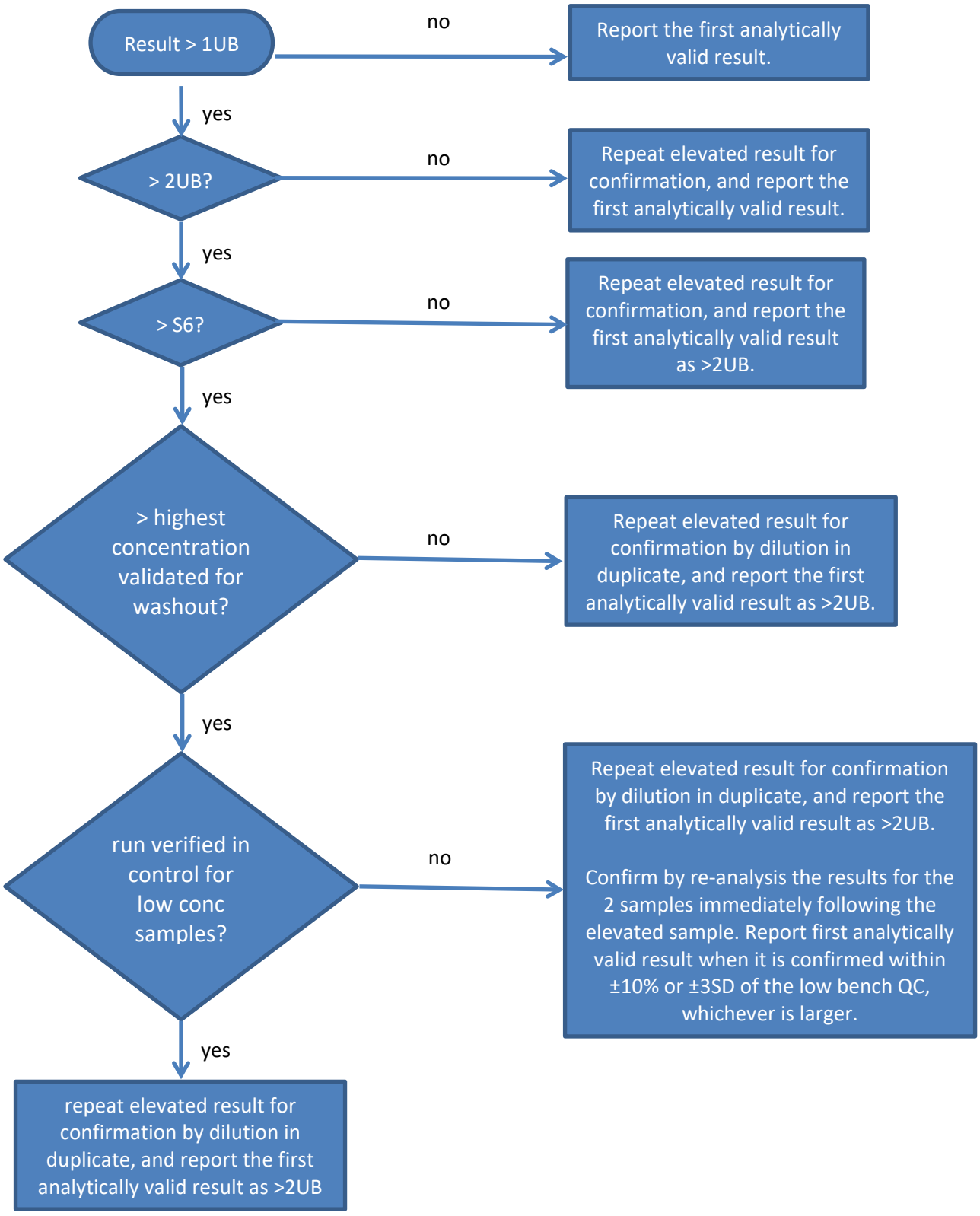


Figure 16. Flow chart for handling an elevated result



18. Appendix D: Help sheets

Reagent preparation

NOTE:
mg/L = ppm
µg/L = ppb
µg/mL = ppm

Rinse solution – 4 L

(2.0% v/v HNO_3, 1.5% ethanol, 0.002% Triton X-100)

1. Partially fill a 4 liter bottle with ≥18 Mohm·cm water.
2. Add 4 mL of the 2% Triton X-100™ / 2% v/v nitric-acid intermediate stock solution.
3. Carefully add 80 mL of environmental grade (or equivalent), concentrated HNO_3.
4. Carefully add 60 mL of dehydrated 200 proof ethanol and mix well.
5. Add enough ≥18 Mohm·cm water to bring to 4 liter mark.
6. Mix well by gently inverting several times.
7. Label appropriately and store at room temperature. Expiration date is 1 year from preparation date.

Sample diluent/carrier solution – 2 L

(2.0% v/v HNO_3, 1.5% ethanol, and 10 µg/L Rh)

1. Partially fill a 2 liter bottle with ≥18 Mohm·cm water.
2. Add 40 mL of double-distilled, concentrated HNO_3.
3. Add 30 mL of dehydrated 200 proof ethanol and mix well.
4. Add 500 µL of the 40 µg/mL Rh internal standard solution.
5. Add enough ≥18 Mohm·cm water to bring to 2 liter mark.
6. Mix well by gently inverting several times.
7. Label appropriately and store at room temperature. Expiration date is 1 year from preparation date.

Intermediate internal standard solution – 50 mL

(2.0% v/v HNO_3, 40 µg/mL Rh)

1. Partially fill a 50 mL volumetric flask with ≥18 Mohm·cm water.
2. Add 1 mL of double-distilled, concentrated HNO_3.
3. Add 2 mL of 1,000 µg/mL Rh standard.
4. Add enough ≥18 Mohm·cm water to bring to 50 mL mark.
5. Mix well by gently inverting several times.
6. Label appropriately and store at room temperature. Expiration date is 1 year from preparation date.

2% Triton X-100 in 5% v/v HNO_3 – 2 L

1. Partially fill a 2 liter bottle with ≥18 Mohm·cm water.
2. Add 40 mL of Triton X-100.
3. Add 100 mL of concentrated HNO_3.
4. Add enough ≥18 Mohm·cm water to bring to 2 liter mark.
5. Add a Teflon magnetic stirring bar (acid wash stir bar before use) and stir on stirrer until dissolved.
6. Label appropriately and store at room temperature. Expiration date is 1 year from preparation date.

0.5% v/v HNO_3

1. Partially fill a 2 liter bottle with ≥18 Mohm·cm water.
2. Add 10 mL of concentrated HNO_3.
3. Add enough ≥18 Mohm·cm water to bring to 2 liter mark.
4. Mix well by gently inverting several times.
5. Label appropriately and store at room temperature. Expiration date is 1 year from preparation date.

2% v/v HNO_3

1. Partially fill a 2 liter bottle with ≥18 Mohm·cm water.
2. Add 40 mL of concentrated HNO_3.
3. Add enough ≥18 Mohm·cm water to bring to 2 liter mark.
4. Mix well by gently inverting several times.
5. Label appropriately and store at room temperature. Expiration date is 1 year from preparation date.

5% v/v HNO_3

1. Partially fill a 2 liter bottle with ≥18 Mohm·cm water.
2. Add 100 mL of concentrated HNO_3.
3. Add enough ≥18 Mohm·cm water to bring to 2 liter mark.
4. Mix well by gently inverting several times.
5. Label appropriately and store at room temperature. Expiration date is 1 year from preparation date.

KED/DRC stability solution–200 mL

1. Add 180 mL of diluent to a 250 mL bottle.
2. Add 18 mL of "junk" human urine to bottle.
3. Add 2 mL of an intermediate working calibration standard (Standard 2) to bottle.
4. Mix well by gently inverting several times.
5. Label appropriately and store at room temperature. Expiration date is 1 year from preparation date.

Daily performance solution (1 µg/L) in 2% v/v HNO_3

1. Partially fill a 1 liter volumetric flask with ≥18 Mohm·cm water.
2. Add 1 mL of High Purity Standard: SM-2107-018.
3. Add 20 mL of concentrated HNO_3.
4. Add enough ≥18 Mohm·cm water to bring to 1 liter mark.
5. Mix well by gently inverting several times.
6. Label appropriately and store at room temperature. Expiration date is 1 year from preparation date.

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