Laboratory Procedure Manual

Analytes: **Iodine**

Matrix: **Urine**

Method: **Iodine in Urine by ICP-DRC-MS**

Method No: 3002.7-02

Revised:

As performed by: Inorganic and Radiation Analytical Toxicology Branch
Division of Laboratory Sciences
National Center for Environmental Health

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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:

Data File Name	Variable Name	SAS Label
U2IO_H_R	UR2IO	Iodine, Urine – 2nd Collection

1. Clinical relevance & summary of test principle

a. Clinical relevance:

Iodine (I), an essential element for thyroid function, is necessary for normal growth, development and functioning of the brain and body. Iodine-deficiency disorders (IDDs) are well-documented global health problems affecting over one billion people worldwide. Consequences of IDD include goiter, cretinism, intellectual impairment, brain damage, mental retardation, stillbirth, spontaneous abortions, miscarriages, congenital deformities, and increased perinatal mortality. Progress toward eliminating IDDs has been substantial; an estimated 70% of the world's edible salt is currently iodized. Most excess iodine is excreted, and typically people can tolerate fairly large amounts without experiencing problems. People with a tendency towards autoimmune thyroid disease are less tolerant of excess iodine. If a person has previously been iodine deficient, that person may be at risk for iodine-induced hyperthyroidism. Excessive iodine intake by a mother can pose a reproductive risk. Because urinary iodine values directly reflect dietary iodine intake, urinary iodine analysis is the recommended and most common method for biochemically assessing the iodine status of a population [1].

Mercury (Hg) is a toxic non-essential element that can affect various organ systems within the body, especially the central nervous system. The main sources of mercury intake in humans are fish, dental amalgams, and occupational exposure. The main organs affected by mercury are the brain and the kidneys [2]. Psychic and emotional disturbances are the initial signs of chronic intoxication by elemental mercury vapors or salts. Paresthesia, neuralgias, renal disease, digestive disturbances, and ocular lesions may develop [3]. Massive exposure over a longer period of time results in violent muscular spasms, hallucinations, delirium, and death [4]. The determination of total Hg in blood and urine are both used to assess the internal exposure. Since urine can be collected non-invasively, it is more commonly used to assess exposure to mercury, particularly in occupational health settings where biomonitoring of random spot urine samples is routinely practiced. Urine is the preferred matrix for assessing exposure to inorganic (metallic) mercury while blood is the preferred matrix for assessing exposure to organic forms of mercury.

b. Test principle:

This method directly measures the iodine and mercury content of urine specimens using mass spectrometry after a simple dilution sample preparation step.

During the sample dilution step, a small volume of urine is extracted from a larger urine patient specimen after the entire specimen is mixed (vortexed) to create a uniform distribution of the liquid and any particulates. This mixing step is important to provide a homogenous urine sample from which to sub-sample.

Dilution of the urine in the sample preparation step prior to analysis is a simple dilution of 1 part sample + 1 part water + 8 parts diluent. The effects of the dilution are to reduce ionization suppression of the biological matrix, prevent clogging of the sample introduction system pathways from high dissolved solids, and allow introduction of internal standards which will be utilized in the analysis step. Tetramethylammonium hydroxide (TMAH, 0.4% v/v) and Triton® X-100 (0.05%) in the sample diluent solubilizes organic components. Triton® X-100 also helps prevent biological deposits on internal surfaces of the instrument's sample introduction system and reduces collection of air bubbles in sample transport tubing. Ammonium pyrrolidine dithiocarbamate (APDC) in the sample diluent (0.01%) aids in solubilizing metals released from the biological matrix. Ethyl alcohol in the sample diluent (1%) aids solubility of organic components and aids in aerosol generation by reduction of surface tension of the solution. The internal standard, rhenium, is at a constant concentration in all blanks, calibrators, QC, 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.

Liquid samples are introduced into the mass spectrometer through the inductively coupled plasma (ICP) ionization source. The liquid diluted urine sample is forced through a nebulizer which converts the bulk liquid into small droplets in an argon aerosol. The smaller droplets from the aerosol are selectively passed through the spray chamber by a flowing argon stream into the ICP. By coupling radio-frequency power with flowing argon, plasma is created in which the predominant species are positive argon ions and electrons at a temperature of 6000 – 8000 K. The small aerosol droplets pass through a region of the plasma where the thermal energy vaporizes the liquid droplets, atomizes the molecules of the sample and then ionizes the atoms. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP (at atmospheric pressure, ~760 torr) from the mass spectrometer (operating at a pressure of 10^{-5} torr). The ions first pass through a focusing region, then the dynamic reaction cell (DRC), the quadrupole mass filter, and finally are selectively counted in rapid sequence at the detector allowing individual isotopes of an element to be determined.

Generally, the DRC operates in one of two modes. In 'vented' (or 'standard') mode the cell is not pressurized and ions pass through the cell to the quadrupole mass filter unaffected. In 'DRC' mode, the cell is pressurized with a gas for the purpose of causing collisions and/or reactions between the fill gas and the incoming ions. In general, collisions or reactions with the incoming ions selectively occur to either eliminate an interfering ion, change the ion of interest to a new mass, which is free from interference, or collisions between ions in the beam and the DRC gas can focus the ion beam to the middle of the cell and increase the ion signal. In this method, the instrument is operated in DRC mode (pressurized with oxygen gas, O_2, 99.999%) when analyzing for mercury. In the reaction cell, oxygen (O_2) molecules react with $^{186}W^{16}O^+$ ions, which would otherwise act as an interfering polyatomic ion to ^{202}Hg, to form $^{186}W^{16}O_2^+$ at m/z 218. Mercury ions pass relatively unaffected through the DRC on toward the analytical quadrupole and detector.

Some collisional focusing of the mercury ions occurs in the pressurized DRC, increasing the observed mercury signal at the detector by approximately a factor of two.

Once ions pass through the DRC cell and are electrically selected for passage through the analytical quadrupole, electrical signals resulting from the ions striking the discrete dynode detector are processed into digital information that is used to indicate the intensity of the ions. The intensity of ions detected while aspirating an unknown sample is correlated to an elemental concentration through comparison of the analyte:internal standard signal ratio with that obtained when aspirating calibration standards.

2) Limitations of Method; Interfering Substances and Conditions

a. Interferences addressed by this method

i. Reduction of tungsten oxide ($^{186}W^{16}O$) interference on mercury ($^{202}Hg^{+}$) using ICP-DRC-MS: $^{186}W^{16}O^{+}$ is a polyatomic ion formed in the plasma as a result of a reaction between the tungsten in the urine sample and oxygen from water molecules from the sample. The dynamic reaction cell of the ICP-MS is used to reduce ion signals from polyatomic ions via ion-molecule reaction chemistry [5, 6]. In the reaction cell, oxygen (O_2) molecules react with $^{186}W^{16}O^{+}$ ions to form $^{186}W^{16}O_2^{+}$, removing the $^{186}W^{16}O^{+}$ interference from m/z 202. Using these method conditions a urine matrix spiked to 100 µg/L tungsten yielded no observable difference from the urine blank, which when analyzed in vented mode conditions yielded a 1.4 - 3.2 µg/L apparent Hg concentration.

b. Limitations of Method (interferences remaining in method)

i. Rhenium use in radiotherapy for cancer: Radioactive rhenium-186 (^{186}Re) is used for pain palliation from cancerous metastases in bones [7], as well as antibody labeling for targeted radiotherapy [8]. The process to make ^{186}Re involves neutron capture on enriched ^{185}Re [9, 10], so the resulting ^{186}Re radiopharmaceutical would be expected to contain residual ^{185}Re. If a person was receiving treatment with this radiopharmaceutical, an unpredictable amount of residual ^{185}Re in the person's urine could interfere with the ^{185}Re used in this method as an internal standard in the diluent for sample preparation. Native ^{185}Re in the urine will affect the analyte:internal standard ratio by which iodine and mercury concentrations are determined, making observed I and Hg concentrations erroneously low.

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 [11]. In general,

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

ii. The specimen type is urine with preservative (for mercury). The preservative is a solution of 200 g/L sulfamic acid, 0.01% Triton® X-100 solution and it is added for the purpose of preventing loss of mercury from the urine before analysis. Mix urine with the preservative as soon as possible after initial collection in the proportion of 10 µL of preservative solution per 1 mL of urine (example: To a tube containing 50 µL of preservative, up to 5 mL of urine can be added for urine mercury analysis). Mix the urine well after addition of the preservative. See Section 6.a for details on preparation of the preservative solution.

iii. Optimal amount of specimen is 1.8 mL. Request a minimum volume of 1.0 mL. 250 µL is needed for one analytical measurement.

iv. Verify sample collection devices and containers are free of significant contamination ("pre-screened") before use. Acceptable containers for allotment of urine for this method include 15 mL PP centrifuge tubes (i.e., Becton, Dickinson and Company model number 352097) or other comparable container.

v. Specimen stability has been demonstrated for 1 year at ≤-20 °C.

vi. Specimen characteristics that may compromise test results include high storage temperature or no preservative.

b. Criteria for specimen rejection: The criteria for an unacceptable specimen include:

i. Contamination: Improper collection procedures, collection devices, or sample handling can contaminate the urine through contact with dust, dirt, etc.

ii. Low Volume (≤0.25 mL).

iii. Failure to add the proper preservative to urine to prevent the loss of mercury.

In all cases, request a second urine specimen.

c. Transfer or referral of specimens; procedures for specimen accountability and tracking: Location, status, and final disposition of the specimens will be tracked at least by paper document in the "Study Folder" (created before analysts receive the samples). Maintain records for a minimum of 2 years. Use only numerical identifiers for samples within the laboratory (i.e., case ID numbers) in order to safeguard confidentiality. Only the medical supervisor (MS) or project coordinator (PC) i.e. non CDC personnel shall have access to the personal identifiers.

4) Safety precautions

a. General safety

i. Observe all safety regulations as detailed in the Laboratory Safety Manual and the Chemical Hygiene Plan. Participate in training regarding urine-borne pathogens prior to performing this method.

ii. Observe Universal Precautions when working with urine.

iii. Wear appropriate gloves, lab coat, and safety glasses while handling all solutions.

iv. Take special care when handling and dispensing bases and concentrated acids. Use additional personal protective equipment which protects face, neck, and front of body. **If concentrated TMAH or concentrated hydrochloric or nitric acid comes in contact with any part of the body, quickly wash with copious quantities of water for at least 15 minutes.**

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

vi. The use of the foot pedal on the Digiflex™ 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 tip of Digiflex™.

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

viii. Transport and store compressed gas cylinders with proper securing harnesses. For compressed oxygen gas, use regulators which are oil-free and are equipped with a flash arrestor.

ix. Disinfect bench tops where urine samples are prepared daily. Use a 1:100 solution of 5.25% household bleach, or an EPA registered germicide (generally chlorine, phenolic, or iodophor based). Make diluted bleach solutions fresh daily or use sprayers which dilute bleach at time of use. Spray the surface with the disinfectant, allow 15 minutes contact time and then wipe up with clean water.

x. Read PerkinElmer provides safety information before operating the instrument. This information is found in the PerkinElmer ELAN ICP-DRC-MS System Safety Manual. Possible hazards include ultraviolet radiation, high voltages, radio-frequency radiation, and high temperatures.

b. Waste disposal:

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

ii. Other liquid waste

1. Waste discarded down sink: Only non-corrosive liquid waste can be discarded at the sink (EPA defines as pH >2 and pH<12.5, 40CFR §261.22, e.g. ICP-MS instrument waste, diluent for sample preparation, ICP-MS rinse solution and FAST carrier solution). 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 intermediate stock and stock standard solutions.

5) Instrument & material sources

a. Sources for ICP-MS instrumentation

i. ICP-MS: Inductively Coupled Plasma Mass Spectrometer with Dynamic Reaction Cell Technology (ELAN® DRC II) (PerkinElmer Norwalk, CT, www.perkinelmer.com), 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 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 & consumables

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

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

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

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

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) with tubing connections built into the cap for addition of liquid waste.

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

v. Cones: Platinum or Nickel cones have been used and tested to be comparable in performance from either PerkinElmer or Spectron. Platinum cones are more expensive, but will last longer, can be refurbished (often for free by the manufacturer), and will frequently yield higher sensitivity.

1. Sampler (nickel/platinum): PerkinElmer part # WE021140/WE027802 (PerkinElmer Norwalk, CT, www.perkinelmer.com). # *Spares* = *4*.
2. Skimmer (nickel/platinum): PerkinElmer part # WE021137/WE027803 (PerkinElmer Norwalk, CT, www.perkinelmer.com). # *Spares* = *4*.

vi. 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*.

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

viii. FAST accessories

1. Valve: CTFE High-flow valve head for SC-FAST (uses ¼-28 fittings). Like part # SC-0599-1010 (Elemental Scientific Inc., Omaha, NE., www.icpms.com).
2. Stator: CTFE Stator for 6 port SC-FAST high flow valve (¼-28 fittings). Like part # SC-0599-1010-01 (Elemental Scientific Inc., Omaha, NE., www.icpms.com).
3. Rotor: Composite rotor for 6 port SC-FAST high flow valve (¼-28 fittings). Like part # SC-0599-1010-05 (Elemental Scientific Inc., Omaha, NE., www.icpms.com).
4. Sample loop: 1 mL Teflon, white connector-nuts, 1.6 mm i.d. Like part # SC-0315-10 (Elemental Scientific Inc., Omaha, NE., www.icpms.com).
5. Probe, autosampler: Teflon, carbon fiber support, 0.8 mm i.d., blue marker, 1/4-28 fittings. Like part number SC-5037-3751 (Elemental Scientific Inc., Omaha, NE., www.icpms.com). *# Spares = 2.*
6. Probe, carrier solution: Teflon, carbon fiber support, 0.5 mm i.d., orange marker, 1/4-28 fittings. Like part number SC-5037-3501 (Elemental Scientific Inc., Omaha, NE., www.icpms.com). *# Spares = 2.*
7. Tubing, 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.icpms.com).
8. Tubing, nebulizer: See “Nebulizer, PolyPro-ST micro flow”
9. Tubing, rinse station: Teflon tubing and adapters (to attach to back of SC autosampler for filling rinse stations and to attach to rinse containers). Like part # SC-0302-0500, Elemental Scientific Inc., Omaha, NE., www.icpms.com).
10. Tubing, vacuum: Vacuum line for SC-FAST high flow valve, connects to port #6, black nut for connection to valve head, natural brown color nut on other end for connection to SC autosampler vacuum port. Like part # SC-0321 (Elemental Scientific Inc., Omaha, NE., www.icpms.com).

ix. 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).

x. Hose, for exhaust of ICP-MS: Available as part of ICP-MS 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). Equivalent part may be substituted. *# Spares = 10 feet of 4” diameter and 10 feet of 6” diameter hose.*

xi. Injector, quartz with ball joint: I.D. = 2.0 mm. PerkinElmer part # WE023948 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available direct from manufacturer as part # 400-30 (Precision Glass Blowing, Centennial,

CO, www.precisionglassblowing.com) or from various distributors. # *Spares = 2.*

xii. Ion lens: PerkinElmer part # WE018034 (PerkinElmer Norwalk, CT, www.perkinelmer.com). # Spares = 3.

xiii. 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.icpms.com). # *Spares = 1*. Different nebulizers may be used, however, the nebulizer gas flow rate, sample flush time, read delay time, loop fill time, loop size, urine sample dilution preparation volume, and sample-to-sample carry-over must be evaluated and optimized.

1. Gas connection:

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

   b. Adapter kit: Plastic adapters to conn*ect Teflon t*ubing (2.4 mm i.d.) to ¼" male Swagelok (compression) port on ICP-MS. Parts can be obtained as components in a "gas fittings kit for microflow nebulizer", kit part # ES-2501-1000, Elemental Scientific Inc., Omaha, NE., www.icpms.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.icpms.com). # *Spares = 2*.

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

xv. 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.*

xvi. 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.*

xvii. Oil for roughing pumps:

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

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

xviii. O-ring: (for sampler cone) PerkinElmer part # N8120511 (pkg. of 5, PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares = 20 o-rings.*

xix. O-ring: (for skimmer cone) PerkinElmer part # N8120512 (pkg. of 5, PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares = 20 o-rings.*

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

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

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

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

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

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

xxiv. Photon stop: PerkinElmer part # WE018278 (PerkinElmer, Shelton, CT, www.perkinelmer.com). # *Spares = 1.*

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

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

xxvii. Spray chamber, quartz concentric: PerkinElmer part # WE025221 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. Available direct from manufacturer as part # 400-20 (Precision Glass Blowing, Centennial,

CO, www.precisionglassblowing.com) or from various distributors. # *Spares = 2.*

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

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

xxx. 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. *For ESI DXi micro-peristaltic pump.*

xxxi. 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. *For ESI DXi micro-peristaltic pump.*

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

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

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

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

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

xxxv. Union elbow, PTFE ¼" Swagelok (ELAN bayonet mount): Connects argon tubing to torch auxiliary gas sidearm on bayonet mount NEXION ICP-MS instruments.

Like part # T-400-9 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. *Spares = 2.*

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

c. Sources for ICP-MS maintenance equipment & supplies

i. Anemometer: Digital wind-vane anemome*ter (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). May be purchased 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 (ChromTech, Inc., Saint Paul, MN, www.chromtech.com) or equivalent.

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

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

d. Sources for general laboratory equipment and consumables

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. Bottles, Glass: Like Qorpak 1oz model # 7981 (Fisher Scientific, Pittsburgh, PA, www.fischersci.com). For storage of intermediate stock and intermediate working standards.

iii. Carboy (for preparation of urine quality control pool and waste jug for ICPMS 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.

iv. Containers for diluent and rinse solution: Two liter Teflon™ containers (like catalog# 02-923-30E, Fisher Scientific, Pittsburgh, PA., www.fishersci.com) and

4 L polypropylene jugs (like catalog# 02-960-10A, Fisher Scientific, Pittsburgh, PA, www.fishersci.com) have both been used. Acid rinse before use. Equivalent containers may be substituted.

v. Gloves: Powder-free, low particulate nitrile (like Best CleaN-DEX™ 100% nitrile gloves, any vendor). Equivalent nitrile or latex gloves may be substituted.

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

vii. Pipette (for preparation of urine dilutions to be analyzed): Micromedic Digiflex-CX Automatic™ pipette equipped with 10.0 mL dispensing syringe, 2000 uL sampling syringe, 0.75-mm tip, and foot pedal or equivalent (Titertek, Huntsville, AL, http://www.titertek.com/) or equivalent automatic diluter and dispenser.

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

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

x. Tubes for storage of intermediate working stock standards: Like 16 x 125 mm glass tube with screw thread and polypropylene lined cap model # 14-962-26G (Fisher Scientific, Pittsburgh, PA, www.fischersci.com). For use in storage of intermediate working stock standards.

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

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

e. Sources of chemicals, gases, and regulators

i. Acid, hydrochloric acid: Veritas™ double-distilled grade, 30-35% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com). This is referred to as "concentrated" hydrochloric acid in this method write-up. For use in preparation of intermediate working stock standards. An equivalent hydrochloric acid product may be substituted, but it must meet or exceed the purity specifications of this product for trace metals content.

ii. Acid, nitric acid: Environmental grade, 68-70% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com). For use in cleaning any bottles, vials, tubes, and flasks. This is referred to as "concentrated" nitric acid in this method write-up. An equivalent nitric acid product may be substituted, but it must meet or exceed the purity specifications of this product for trace metals content.

iii. Ammonium pyrrolidine dithiocarbamate (APDC): Like laboratory grade (Fisher Scientific, Fairlawn, NJ) or equivalent.

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

1. Regulator for argon (at dewar): Stainless steel, single stage, specially cleaned regulator with 3000 psig max inlet, 0-200 outlet pressure range, CGA 580 cylinder connector, and needle valve shutoff on delivery side terminating in a ¼" Swagelok connector. Part number "KPRCGRF415A2/AG10-AR1" (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com). An equivalent regulator from an alternate vendor may be substituted. # *Spares = 1*.

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

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

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

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

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

1. Regulator for oxygen: High purity brass body with Monel trim, two stage regulator. Monel brass has a greater resistance to oxidation than stainless steel. For one regulator, order the following parts, and ask that they be tested and assembled (Engineered Specialty Products, Kennesaw, GA, www.espgauges.com).

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

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

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

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

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

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

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

viii. Standard, rhenium: Like 1,000 mg/L, item #CGRE1-1 (Inorganic Ventures, Christiansburg, VA http://www.inorganicventures.com). Used as an internal standard in diluent. Any vendor whose standards are traceable to the National Institute for Standards and Technology may be substituted. The standard must have low trace metal contamination.

ix. Standard, iodine: Like 1,000 mg/L, item #IC-II-M (High Purity Standards, Charleston, SC, http://www.hps.net/). Any vendor whose standards are traceable to the National Institute for Standards and Technology may be substituted. The standard must have low trace metal contamination.

x. Standard, mercury: Like 1,000 mg/L, item # 100033-1. (High Purity Standards, Charleston, SC, http://www.hps.net/). Any vendor whose standards are traceable to the National Institute for Standards and Technology may be substituted. The standard must have low trace metal contamination.

xi. Sulfamic acid: Like ACS grade, item # 825 (GFS Chemicals Inc., Columbus, OH 43265).

xii. Tetramethylammonium hydroxide 25% w/w: Like item # 20932 (AlfaAesar, 30 Bond St., Ward Hill, MA 01835).

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

6) Preparation of reagents and materials

a. Preservative for collected urine

i. Purpose: Sulfamic acid is added to urine specimens as close to point of collection as possible to prevent volatilization of mercury (ideally placed in the container before adding the urine aliquot).

ii. Preparation: To prepare 50 mL of 200 g/L sulfamic acid, 0.01% Triton® X-100.

1. Partially fill a pre-screened or pre-acid-washed 50 mL polypropylene tube with ≥18 MΩ·cm water.
2. Add 10.0 g of sulfamic acid and 0.5 mL of 1% Triton® X-100 intermediate solution.
3. Fill to the 50 mL mark with ≥18 MΩ·cm water.
4. Dissolve the sulfamic acid by mixing well (use of a vortexer, sonicator, or warm water bath is helpful in this process).
5. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

b. Internal standard intermediate:

i. Purpose: Preparation of an internal standard solution at an intermediate concentration allows spiking into the final diluent solution with a spiking volume large enough to reduce pipetting error.

ii. Preparation: To prepare 50 mL of the intermediate internal standard solution:

1. Partially fill a pre-screened or pre-acid washed 50 mL volumetric flask or graduated 50mL polypropylene centrifuge tube with ≥18 MΩ·cm water (approximately 25–40 mL).
2. Add 1.5 mL of concentrated nitric acid. Mix well.
3. Add 5 mL of 1,000 μg/mL Re standard. If initial Re stock standard concentration is different, adjust volume proportionally.
4. Fill to mark (50 mL) with ≥18 MΩ·cm water and mix thoroughly.
5. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

c. Intermediate Triton® X-100 solution:

i. Purpose: To ease daily preparation of the diluent and rinse solutions by first preparing an intermediate Triton® X-100® solution.

ii. Preparation: To prepare 1 L of 20% Triton® X-100

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

2. Add 200 mL of Triton® X-100 to the 1 L container that is partially filled with ≥18 MΩ·cm water.
3. Fill to 1 L with ≥18 MΩ water and mix until the Triton® X-100 has completely dissolved into solution (overnight). A magnetic stirring plate can be used to assist mixing by adding an acid-washed Teflon® coated stirring bar to the bottle.
4. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

d. Sample diluent and carrier

i. Purpose: This solution will be used in the preparation of all samples and calibrators during the dilution process prior to analysis. It is important that all samples, standards, blanks, QC, etc., in a run be made from the same diluent solution so that the concentration of the internal standard will be the same among all calibrators and samples in the run. When using a flow-injection component in the sample introduction system (i.e. the Elemental Scientific SC4-FAST autosampler), use the same solution for sample diluent and the FAST carrier.

ii. Preparation: To prepare 2 L of 5 μg/L Re, 0.4% v/v TMAH, 1% ethyl alcohol, 0.01% APDC, and 0.05% Triton® X-100:

1. If not previously dedicated to this purpose, acid wash a 2 L container (PP, PMP, or Teflon™) with 1% v/v HNO_3 and ≥18 MΩ·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Dedicate to purpose.
2. Partially fill the 2 L container with ≥18 MΩ·cm water.
3. Add 0.2 g of APDC.
4. Add 8 mL of 25% v/v TMAH.
5. Add 20 mL of ethanol.
6. Add 5 mL of 20% Triton® X-100 (See Section 6.b for details on preparation).
7. Fill to 2 L using ≥18 MΩ·cm water.
8. Spike 100 μL of 100 mg/L Re (Internal Standard Intermediate) to the final diluent.
9. Invert bottle a few times to insure thorough mixing. Allow to sit for several hours or overnight before using.
10. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

e. ICP-MS rinse solution

i. Purpose: This solution will be pumped through the autosampler rinse station, probe, and sample loop between sample analyses to prevent carry-over of analytes from one sample measurement to the next.

ii. Preparation: To Prepare 4 L of 0.4% v/v TMAH, 1% ethyl alcohol, 0.01% APDC, and 0.05% Triton® X-100:

1. If not previously dedicated to this purpose, acid wash a 4 L container (PP, PMP, or Teflon™) with 1% v/v HNO_3 and ≥18 MΩ·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Dedicate to purpose.
2. Partially fill the 4 L container with ≥18 MΩ·cm water.
3. Add 0.4 g of APDC.
4. Add 16 mL of TMAH.
5. Add 40 mL of ethyl alcohol.
6. Add 10 mL of 20% Triton® X-100, (See Section 6.b for details on preparation).
7. Fill to 4 L using ≥18 MΩ·cm water.
8. Invert container a few times to ensure thorough mixing. Allow to sit for several hours or overnight before using.
9. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

f. Standards, calibrators, base urine and QC

I and Hg stock standards are purchased and intermediate stock calibration standards are prepared separately to permit larger spiking volumes for preparation of low concentration mercury intermediate working standards without the complicating factor of the presence of iodine, permit the use of acid matrix for optimum solubility of Hg in intermediate solutions without volatilizing iodine (I^- easily oxidizes to the volatile form I_2 in acidic solutions) and to prevent concerns about insolubility of Hg and I at high concentrations. HgI_2 is soluble in water up to around 55 mg/L [12], though, so combinations of Hg and I at the concentrations of this method's intermediate calibrator solutions do not present a solubility problem.

i. Stock calibration standards

1. Purpose: The single element stock standard will be used to prepare the intermediate working calibration standards.
2. Contents: A 2% v/v HNO_3 solution containing Hg and an aqueous solution containing I. Concentrations are typically 1000 mg/L.
3. Purchase & Storage:
   a. Purchasing from vendors: Whether purchased or prepared in-house, the starting materials must be NIST-traceable.
   b. Storage: Store at room temperature and label appropriately. Expiration is determined by manufacturer or is 1 year after the container is opened (whichever comes first).

ii. Diluent for mercury intermediate stock calibration standard preparations:

1. Purpose: This diluent is used to dilute mercury intermediate stock calibration standards, not to prepare working standards or urine samples for analysis.
2. Preparation: To prepare 1L of 10% v/v HCl:
   a. If not previously dedicated to this purpose, acid wash a 1 L volumetric flask (glass, PP, PMP, or Teflon™) with 3% v/v HCl and ≥18 MΩ·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Dedicate to purpose.
   b. Into the 1 L flask, add 500 – 750 mL ≥18 MΩ·cm water.
   c. Add 100 mL high purity concentrated HCl.
   d. Fill to the mark and mix thoroughly.
   e. Store at room temperature and label appropriately. Expiration is 1 year from the date of preparation.

iii. Mercury (Hg) intermediate stock calibration standards A and B

1. Purpose: The Hg intermediate stock standards A and B will be used to prepare the two Hg intermediate working calibration standards.
2. Preparation & storage: To prepare 100 mL of 10% v/v HCl solutions containing Hg concentrations listed in Table 4 of Appendix B:
   a. If not previously dedicated to this purpose acid-rinse two 100 mL, glass volumetric flasks. For example, with 3% v/v HCl and ≥18 MΩ·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Mark flask according to intended use. Dedicate to purpose.
   b. Partially fill (50–75% full) two 100 mL flasks with the 10% v/v HCl diluent prepared in Section 6.e.ii.
   c. Using the volume listed in Table 4 of Appendix B, pipette the appropriate volume of the mercury stock standard solution into the volumetric flasks. Dilute to the volumetric mark with the 10% v/v HCl diluent using a pipette for the final drops. Mix each solution thoroughly. Final concentrations are listed in Table 4 of Appendix B.
   d. Once mixed, transfer to a labeled, lot tested or cleaned glass vial (e.g. 15 mL). Store at refrigerated temperatures (~2–8ºC). Expiration is 6 months from the date of preparation.

iv. Iodine (I) intermediate stock calibration standard A

1. Purpose: I intermediate stock standard A will be used to prepare the intermediate working calibration standards.
2. Preparation & storage: To prepare 100 mL of an aqueous solution containing I at concentrations listed in Table 4 of Appendix B:
   a. If not previously dedicated to this purpose acid-rinse one 100 mL glass volumetric flask. For example, with 3% v/v HCl and ≥18 MΩ·cm water

(at least 3 times each) and verify cleanliness through analysis of rinsate. Mark flask according to intended use. Dedicate to purpose.

b. Partially fill (50–75% full) one 100 mL flask with ≥18 MΩ·cm water.

c. Using the volume listed in Table 4 of Appendix B, pipette the appropriate volume of the iodine stock standard solution into the volumetric flask. Dilute to the volumetric mark with ≥18 MΩ·cm water. Mix each solution thoroughly. Final concentrations are listed in Table 4 of Appendix B.

d. Once mixed, transfer to a labeled, lot tested or cleaned glass vial (i.e. 15 mL). Store solutions at refrigerated temperatures (~2–8 ºC). Expiration is 6 months from the date of preparation.

v. Diluent for intermediate working calibration standards preparations:

1. Purpose: This diluent is used to dilute I and Hg intermediate stock calibration standards. Not to be used to prepare working standards or urine samples for analysis.

2. Preparation: To prepare 2 L of an aqueous solution of 2 g/L sulfamic acid:

a. If not previously dedicated to this purpose, acid wash a 2 L container (PP, PMP, or Teflon™) with 5% v/v HNO_3 and ≥18 MΩ·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Mark each flask according to intended use. Dedicate to purpose.

b. In the 2 L container, add 1–1.5 L ≥18 MΩ·cm water.

c. Add 4 g sulfamic acid to the container.

d. Fill to the mark and mix thoroughly.

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

vi. Intermediate working calibration standards

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

2. Preparation & storage: To prepare 100 mL volumes of dilutions of Hg intermediate stock calibration standard solutions A and B, iodine stock calibration standard, and I intermediate stock calibration standard A in a 2 g/L (w/v) sulfamic acid matrix:

a. If not previously dedicated to this purpose acid-rinse eight 100 mL glass volumetric flasks with 3% v/v HCl and ≥18 MΩ·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Mark each flask according to intended use. Dedicate to purpose.

b. Fill each 100 mL flask 50–75% with the 2 g/L sulfamic acid diluent prepared in Section 6.f.v.

c. Using the volumes listed in Table 4 of Appendix B, pipette the appropriate volume of each of the three intermediate stock calibration standard solutions and the iodine stock calibration solution into each of

the volumetric flasks. Dilute each to the volumetric mark with the 2 g/L sulfamic acid diluent using a pipette for the final drops. Mix each solution thoroughly. Final concentrations are listed in Table 4 of Appendix B.

d. Once mixed, transfer to labeled, lot tested or cleaned glass containers for storage (e.g. 15mL for daily use). Store at refrigerated temperatures (~2–8 ºC). Expiration is 6 months from the date of preparation.

vii. Working calibration standards

1. Purpose: The working calibration standards will be analyzed in each run to provide a signal-to-concentration response curve for each analyte in the method. The concentration of the analyte of interest in a patient urine sample dilution is determined by comparing the observed signal ratio (element/internal standard) from the dilution of the patient urine sample to the signal ratio response curve from the working calibrators.
2. Preparation & use: To make dilutions of the corresponding eight intermediate working calibration standards, use a Digiflex automatic pipette and follow the volumetric directions in Table 5 of Appendix B along with directions in Section 8.b.ii. Expiration of capped dilutions is 3 days from preparation (see Appendix A, test for time between preparation and analysis).

viii. Base urine

1. Purpose: This urine pool material will be mixed with the intermediate working calibrators just prior to analysis to matrix-match the calibration curve to the urine matrix of the unknown samples.
2. Preparation & storage: To make a mixture of multiple urine sources collected from anonymous donors (mixture approximates an 'average' urine matrix):

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

b. Once collected, analyze to ensure that concentrations of the analytes in this method are relatively low, so as to not interfere with the proper measurement of calibrators (see Table 2 in Appendix B for suggested maximum base urine concentrations).

c. Once screened, mix the urine collections together in a larger container (polypropylene (PP), polymethylpentene (PMP), or Teflon™) which has been acid washed. For example, with 1% v/v HNO_3 and ≥18 MΩ·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Add large Teflon™ stir bar and stir for 30+ minutes.

d. Label appropriately.

e. For short term storage, store at 2–8 °C. For long-term storage, dispense into smaller-volume tubes (i.e., 50 mL labeled and acid-washed or lot screened polypropylene tubes) and store at ≤ -20 °C. Expiration date is 2 years from date of collection.

ix. Internal quality control materials ("bench" QC)

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

2. Content: The internal (or "bench") quality control (QC) materials used in this method are pooled human urine, acidified to 2 g/L sulfamic acid and 0.0001% Triton® X-100 (added to the urine in the proportion of 10 µL of preservative solution per 1 mL of urine) and then spiked, if necessary, with NIST traceable stock standards to reach a desired elemental concentration. The analyte concentrations are in the low-normal ("low QC"), high-normal ("high QC") and above-normal ("elevated QC") concentration ranges.

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

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

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

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

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

      iii. Spike analyte concentrations for the low bench QC pool in the low-normal population range. Spike analyte concentrations for the high or elevated bench QC pools less than some preselected target

concentration values in the high normal population range. See the National Report on Human Exposure to Environmental Chemicals for estimations of the normal population ranges for metals (http://www.cdc.gov/exposurereport/).

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

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

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

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

   iv. Acidify each urine pool to 0.2% (w/v) sulfamic acid by adding the appropriate mass of solid. Stir for 30+ min on large stir plates.

d. Settling out of solids

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

   ii. For each urine pool, decant the urine into another of the acid washed10 L carboys to remove the urine from the solids settled out on the bottom of the carboy.

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

e. Spiking of urine

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

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

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

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

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

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

a. Dispensing and storage of urine

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

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

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

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

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

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

4. Approximately one hour before dispensing begins:

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

b. Also during this time, flush the Digiflex with urine from the pool to be dispensed. Place the ends of the tubing attached to both the sample and dispensing syringes into the carboy of urine so that urine won't be used up during this process. Be sure to secure both ends of tubing in the carboy with Parafilm so they will not come out during the flushing process.

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

iv. Homogeneity test: Check homogeneity of analyte concentrations in pool aliquots. Keep samples pulled for homogeneity analysis in the sequence that they were dispensed for the purpose of looking for trends in concentrations. Once dispensed and homogeneity has been shown to be good throughout the tubes of a pool, store tubes at ≤ -20°C and pull tubes out as needed for analysis.

v. Storage: Urine pools shall be stored long term at ≤-20 °C or short term (several days) at refrigerator temperature (~2–8 °C). Expiration date is determined by evaluating QC results in each run.

g. Optimization solutions

i. DRC optimization:

1. Purpose: For periodic testing the elimination of $^{186}W^{16}O^{+}$ interference on ^{202}Hg using DRC.

2. Preparation & storage:

a. W spiking solution: A small volume of 50 mg/mL W solution will be needed and can be made for this purpose ahead of time in a test tube by diluting 0.1 mL of 1000 mg/mL W with 1.9 mL 2% v/v HNO_3. Keep interference spike volume small (<0.3 mL) using a high concentration stock solution (i.e. 1000 mg/mL).

b. Base urine + S0 (0.2% sulfamic acid) + diluent (1+1+8)
   i. 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 (multiply volumes by 20).

c. Base urine + S2 (0.3 μg/L Hg) + diluent (1+1+8)
   i. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 2 as described in Table 8 (multiply volumes by 20). Analyte concentrations can be made higher if needed for sensitivity reasons by preparing a higher concentration calibrator.

d. Base urine + S0 (0.2% sulfamic acid) + diluent (1+1+8) + 100 μg/L W
   i. 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 (multiply volumes by 20).
   ii. Add 0.1 mL of 50 mg/mL W

e. Base urine + S2 (0.3 μg/L Hg) + diluent (1 +1+8) + 100 μg/L W

i. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 2 as described in Table 8 (multiply volumes by 20). Analyte concentrations can be made higher if needed for sensitivity reasons by preparing a higher concentration calibrator.

ii. Add 0.1 mL of 50 mg/mL W

3. Label appropriately and store at room temperature. Expiration date is 3 days after preparation (see Appendix A, time past sample preparation test).

ii. Dual detector calibration:

1. Purpose: Use as necessary to perform the dual detector calibration.
2. Preparation & storage: To prepare a 50 mL aqueous solution of 300 µg/L I:
   a. Partially fill a pre-screened or pre-acid-washed 50 mL polypropylene tube with >18 MΩ·cm water.
   b. Spike in 0.015 mL of 1000 mg/L I.
   c. Dilute to the 50 mL mark with ≥18 MΩ·cm water.
   d. Label appropriately and store at room temperature. Expiration date is 1 year from date of preparation.

7) Analytical instrumentation setup

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

a. Instrumentation and equipment setup:

i. Configuration for liquid handling

1. FAST valve setup: See Appendix B, Figure 1 for diagram and Section 5.b "FAST/ESI SC4-DX autosampler accessories" for source information.
   a. Port 1: 1 mL sample loop (white nut).
   b. Port 2: 0.5 mm ID probe (red nut) for carrier solution.
   c. Port 3: nebulizer line (green nut) for transfer of liquid to nebulizer.
   d. Port 4: 1 mL sample loop (white nut).
   e. Port 5: 0.8 mm ID probe (blue nut) for diluted samples.
   f. Port 6: vacuum line (black nut).

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

3. Spray chamber waste removal

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

   a. Between spray chamber and peristaltic tubing:
      i. Spray chambers with threaded connection: Use vendor-supplied threaded connector on base of chamber, connecting tubing directly to peristaltic pump tubing through a PEEK adapter or directly.
      ii. Spray chambers without threaded connection: Use of specialized push-on connectors available from various vendors (like UFT-075 from Glass Expansion, Pocasset, MA) are preferred for safety reasons to direct connection of PVC tubing (i.e. 1/8" i.d. x ¼" o.d.).
   b. Between peristaltic pump tubing and waste container: Connect 1/8" i.d. x ¼" o.d. PVC tubing to the white/black peristaltic pump tubing using a tubing connector (PerkinElmer item # B3140715). Place the free end of the PVC tubing through the lid of the waste jug (be sure it is secure). In case of overflow, place the waste jug in a secondary containment tray (>110% the volume of the waste jug).

4. Rinse solution for autosampler:

   a. Rinse solution jug: Leave one of the caps on the top of the rinse jug loose to allow air venting into the jug as liquid is removed. Otherwise

the jug will collapse on itself as the liquid is removed and a vacuum is created inside. Use secondary containment tray.

b. Rinse solution uptake to autosampler rinse station: Use tubing of different lengths and inner diameters between the rinse solution container and the autosampler rinse station to control uptake rate of rinse solution. These can be obtained from the autosampler manufacturer, their distributors, or custom built in the lab. Optimize these factors along with fill time in the software so that waste of rinse solution is minimized and rinse station does not go empty.

c. Autosampler rinse station waste removal: Gravity drain of waste to the waste container will be sufficient. Use minimum drain tubing to make this connection. If this tube is too long, the rinse station will not drain properly.

ii. Gas delivery and regulation

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

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

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

3. Oxygen (99.999±%) gas: Used for dynamic reaction cell interference removal from manganese isotopes.
   a. Connect to DRC channel B.
   b. Set the delivery pressure of regulator to 5–7 psig when gas is flowing. See Section 5.e for part numbers and details.
   c. Use a brass flash arrestor on outlet side of regulator. See Section 5.e for part numbers and details.

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

b. Instrument and method parameters: See Tables and Figures in Appendix B 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'), and "above-normal" ('Elevated QC') concentrations.

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 40–60 patient samples total when analyzing for all elements in the method) to ensure analytical performance has not degraded across the time of the run. If more patient samples are analyzed on the same calibration curve after the second run of the bench QC, all bench QC must be reanalyzed before and after the additional samples. For example, the schemes shown in Table 6 in Appendix B are both acceptable ways to analyze multiple consecutive "runs".

ii. Reference materials: Standard Reference Materials (SRM) from the National Institute of Standards and Technology (NIST) (i.e. SRM 3668 Levels 1 and 2) can be used to verify method accuracy. Secondarily, historical challenge samples from proficiency testing programs or commercially-produced reference materials may be useful when NIST SRMs are unavailable.

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

b. Perform, evaluate and report a run

i. Starting the equipment for a run

1. Power on the computer, printer, and autosampler, and instrument computer controller.
2. Peristaltic pump: Set proper tension on peristaltic pump tubing.
3. Software: Start software for the ICP-MS and autosampler control.
4. Daily pre-ignition maintenance checks: Perform and document daily maintenance checks (i.e., Ar supply pressure, interface components cleanliness and positioning, interface pump oil condition, vacuum pressure, etc.).
5. Start the plasma
6. Start the peristaltic pump: *S*tart the pump running slowly, making sure that the rotational direction is correct for the way the tubing is set up.
7. Place probe in adequate volume of liquid (i.e. carrier, rinse solution): If using an ESI FAST, manually place carrier probe into liquid which will be aspirated during the warm-up time prior to the daily performance test. If not using an ESI FAST, send the autosampler probe to that solution.
8. Warm-up time: Allow warm-up time suggested by the manufacturer for the ICP-MS (i.e. RF generator) after igniting the plasma. There will be another warm-up time (or “stability time”) for the DRC later in this procedure.
9. Daily performance check: Perform and document a daily performance check and any optimizations necessary.

   Save new parameters to the “default.tun” and “default.dac” files.
10. 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 (i.e. autosampler rinse station).
11. DRC stability time: Typically, DRC mode analysis requires a period of repeated measurement of urine matrix samples prior to the analytical run to achieve a stable analyte-to-internal standard ratio. This phenomenon is not observed for the vented (standard) mode analysis of I. When analyzing Hg in DRC mode or Hg and I in mixed mode (DRC and vented mode, respectively), analyze a bulk preparation (≥50 mL) of a urine matrix calibration standard (i.e. standard 2) repeatedly before beginning the run to achieve a stable analyte-to-internal standard ratio. Time to reach stability is instrument-specific but 1–1.5 hours is typical (15–25 measurements of the combined Hg and I mixed-mode method). This stability time can be run while other sample preparation is on-going. Stability can be verified before analysis begins by evaluating the measurement-to-measurement stability

of Hg/Re in the stability time analyses. See Table 7 in Appendix B for example of setup in the Samples/Batch window and Table 8 in Appendix B for volume ratios to use for preparation of the calibration standard.

12. Readying the instrument for quick-start analysis: The plasma may be left running to eliminate the need for an initial instrument warm-up period and/or a DRC stabilization period as long as appropriate planning is made for sufficient solution supply and waste collection. Analysis of conditioning samples (diluted urine matrix) can also be scheduled to occur at roughly a predetermined time. Accomplish this by setting up multiple sample analyses with extended rinse times (i.e. one analysis with a 1500s rinse time will take approximately 30 minutes to complete). Initial samples would be non-matrix, while final samples would be diluted matrix for conditioning. If running a DRC-only method during these scheduled analyses, the ICP-MS will remain in DRC-mode for approximately 45 minutes without depressurizing the cell after the last analysis of the sample batch is performed.

13. Software setup for analysis:

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

    b. 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 B for times and speeds.

        1. Urine vs. aqueous method files:

            a. The difference: There are two method files for this one method (see Table 1 in Appendix B). 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-based 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 B.

                i. The *“urblk” method file:* *U*se to analyze the initial urine blank (blank for the calibration curve), the urine

calibrators, and the urine blank checks at the very beginning of the run. The urine blank method defines the autosampler location of the urine blank and the urine calibration standards.

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

ii. Preparation of samples for analysis (See Table 6 in Appendix B)

1. Thaw urine samples; allow them to reach ambient temperature.
2. If instrument stability in DRC mode requires it, prepare 50 mL^+ of a calibration standard (i.e. standard 2) to be analyzed repeatedly before the beginning of the run to achieve a stable analyte-to-internal standard ratio (see Appendix C, DRC Stability Test Solution for bulk preparation instructions). It is most efficient to start the DRC stability time analyses before preparing the rest of the dilutions to be analyzed.
3. Prepare the following solutions into pre Labeled containers using the Micromedic Digiflex™ or other volumetric sample transfer device. See Table 8 in Appendix B for a summary.

   a. *Aqueous Blank*: Prepare a minimum of two aqueous blanks. One will be the actual aqueous blank and the other will be a backup ("Aqueous Blank Check") in case the original aqueous blank is unusable.

   b. *Calibrators*: Prepare the working calibration standards (S0–S8). Eight preparations of the S0 calibrator will be needed. One of these S0 preparations will be the zero standard (urine blank) for the calibration standards; another will be the S0 check sample analyzed prior to S0 (in case of a problem with the initial S0); four will be analyzed after the last calibrator to perform washout after the calibration standards (these may be prepared in one tube); and the remaining two will collect run blank data that can be used in calculating method LOD.

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

   After preparation, cover and mix the diluted samples then uncover and place them on the ICP-MS autosampler in the order corresponding to the sequence setup in the ICP-MS software. ***Diluted samples must be analyzed within 48 hours of preparation***. See critical parameter test results in Appendix A for details.

   Original samples are not compromised by being at room temperature for the work day, going through multiple freeze-thaw cycles, or being refrigerated short term (a few days). Store long term at ≤-20 °C.

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

iv. Monitor or evaluate the analysis to verify and ensure the quality of the run:

1. Verify proper operation of the instrument (e.g. loop filling, sample introduction and measurement timing, 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 Digiflex.

      If contamination is observed from the Digiflex, flush the Digiflex with nitric acid solution no greater than 5% v/v HNO_3 and retest (≥500 mL may be required).

   c. Comparison with other instruments.

3. Verify analyte/internal standard ratio stability (esp. DRC measurements)

   The net intensity (analyte / internal standard ratio) of the measurements made while stabilizing the DRC gives indication of 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 R2 requirements (minimum of 0.98, typically 0.99 to 1.000).
5. Verify bench QC results are within 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 calibration standard is obviously in error, it can be re-analyzed as a sample (old or new dilution) and incorporated into the curve through data reprocessing as a calibrator. As a last resort, a single calibration point per analyte between or including S2 and S7 can be removed from the curve (Do not drop S0, S1 or S8). If repeated problems are observed with calibration standards, follow up with appropriate corrective actions (i.e. 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 or instrument.

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 4 in Appendix B for flowchart.

   a. Confirming an elevated concentration: Any sample having a concentration greater than the 1UB shall be repeated for confirmation. See Section 8.b.vii.2.a for details.

   b. Dilution of a sample to within the calibration range: Any sample having a concentration greater than calibration standard 8 shall be re-prepared with extra dilution to bring the observed result within the reportable range (see Section 8.b.vii.2.a for details).

   c. Confirming proper washout after an elevated sample: When monitoring the analysis in real-time, if sample concentrations following an elevated sample are greater than standard 8 for either analyte (see Appendix B, Table 5 for concentrations).

      i. Stop run following elevated sample

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

      Example:
      3002 UrBlkChk Wash1
      3002 UrBlkChk Wash2
      LUXXXXX Wash

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

v. Overnight operation or using auto stop: The run may be left to complete itself unattended using the AutoStop feature of the ICP-MS software. Delay the shutdown at least 5 minutes to rinse the sample introduction system of urine matrix before the plasma is shut off. Verify there is sufficient rinse solution for the remainder of the run. Sample peristaltic tubing must be replaced when clamped shut overnight.

vi. 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. 2014-0714a_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.

2. Run summary records: Printed run sheets, or PDF equivalent, must be documented with

   i. Analyst initials

   ii. Instrument ID

   iii. Date of analysis and run # for the day

vi. Analyst evaluation of run results:

1. Bench quality control: After completing a run, and importing the results into the laboratory information system, evaluate the run bench QC according to laboratory QC rules. The QC limits are based on the average and standard deviation of the beginning and ending analyses of each of the bench QC pools, so it will not be possible to know if the run is in control until statistically reviewed.

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

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

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

         1. Extreme Outlier – Run mean is beyond the characterization mean ± $4S_m$

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

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

1. Extreme Outlier – One individual result is beyond the characterization mean ± $4S_m$
2. R 4S Rule – 2 or more of the within-run ranges in the same run exceed $4S_w$ (i.e., 95% range limit)

   Note: Since runs have multiple results per pool for 3 pools, the R 4S rule is applied within runs only.

Abbreviations:

S_i = Standard deviation of individual results.

S_m = Standard deviation of the run means.

S_w = Within-run standard deviation.

b. Implications of QC failures: If the DLS SAS program declares the run "out of control" for an analyte, only the analyte which was "out of control" is invalid for reporting from the run.

2. Patient results:

a. Elevated concentrations: Refer to Figure 5 in Appendix B for flowchart.

i. Boundaries requiring confirmatory measurement:

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

   The concentrations assigned to 1UB and 2UB for an element is determined by study protocol but default concentrations are in Table 9 in Appendix B.

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

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

2. Results greater than highest calibrator: Samples that exceed the high calibrator must be prepared with minimum extra dilution in duplicate to bring the observed result within the calibration range (≤ S8). 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. To calculate the minimum extra dilution needed, find the smallest dilution factor where the observed result divided by the dilution factor is still less than S8 for the analyte.

ii. Concentrations requiring verification of washout: following observation of a result greater than calibration standard 8

1. If return to standard zero (urine blank) levels were verified before subsequent sample analysis, no further action is required.
2. If return to standard zero (urine blank) levels were not verified before subsequent sample analysis, consult a supervisor regarding the reportability of results subsequent to the elevated result in the run.

b. Unacceptable reproducibility: If the range of the three replicate readings (maximum replicate concentration value - minimum replicate concentration value) for a single sample analysis is greater than the range maximum criteria listed in Table 9 in Appendix B ***and*** the range of the three replicate readings is greater than 10% of the observed concentration, do not use the measurement for reporting. Repeat the analysis of the sample.

vii. 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 (i.e. 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 (i.e. autosampler, exhaust, compressed gases, and coolant). Frequency of equipment maintenance will be dependent on instrument throughput.

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

i.Dual detector calibration: Perform dual detector calibration regularly as instrument stability requires (e.g. weekly or monthly) for any element exceeding

1,000,000 cps for calibration standard 8. This is typically only I. Dual detector calibration solution is described in Section 6.g.ii.

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

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

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

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

10) Reporting Thresholds

a. Reportable range: Urine mercury and iodine values are reportable in the range between the method LOD and the highest calibrator. Above the highest concentration verified, extra dilutions are made of the urine sample to bring it within the reportable range.

b. Reference ranges (normal values): In this method the 95% reference ranges (see Appendix B, Table 10) for these elements in urine fall within the range of the calibrators.

c. Action levels: Concentrations observed greater than the “second upper boundary” (defined in the laboratory database as the “2UB”) shall be reported to the QC reviewer as an “elevated result”. The concentration assigned to the 2UB for an element is determined by study protocol but default concentrations are listed in Table 9 in Appendix B.

11) Method Calculations

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

b. Method limit of quantitation (LOQ): The Division of Laboratory Sciences does not currently utilize limits of quantitation in regards to reporting limits [11].

c. QC Limits: Quality control limits are calculated based on concentration results obtained in at least 20 separate runs. It is preferable to perform separate analyses on separate days and using multiple calibrator lot numbers, instruments, and analysts to best mimic real life variability. The statistical calculations are performed

using the SAS program developed for the Division of Laboratory Sciences (DLS_QC_compute_char_stats.sas).

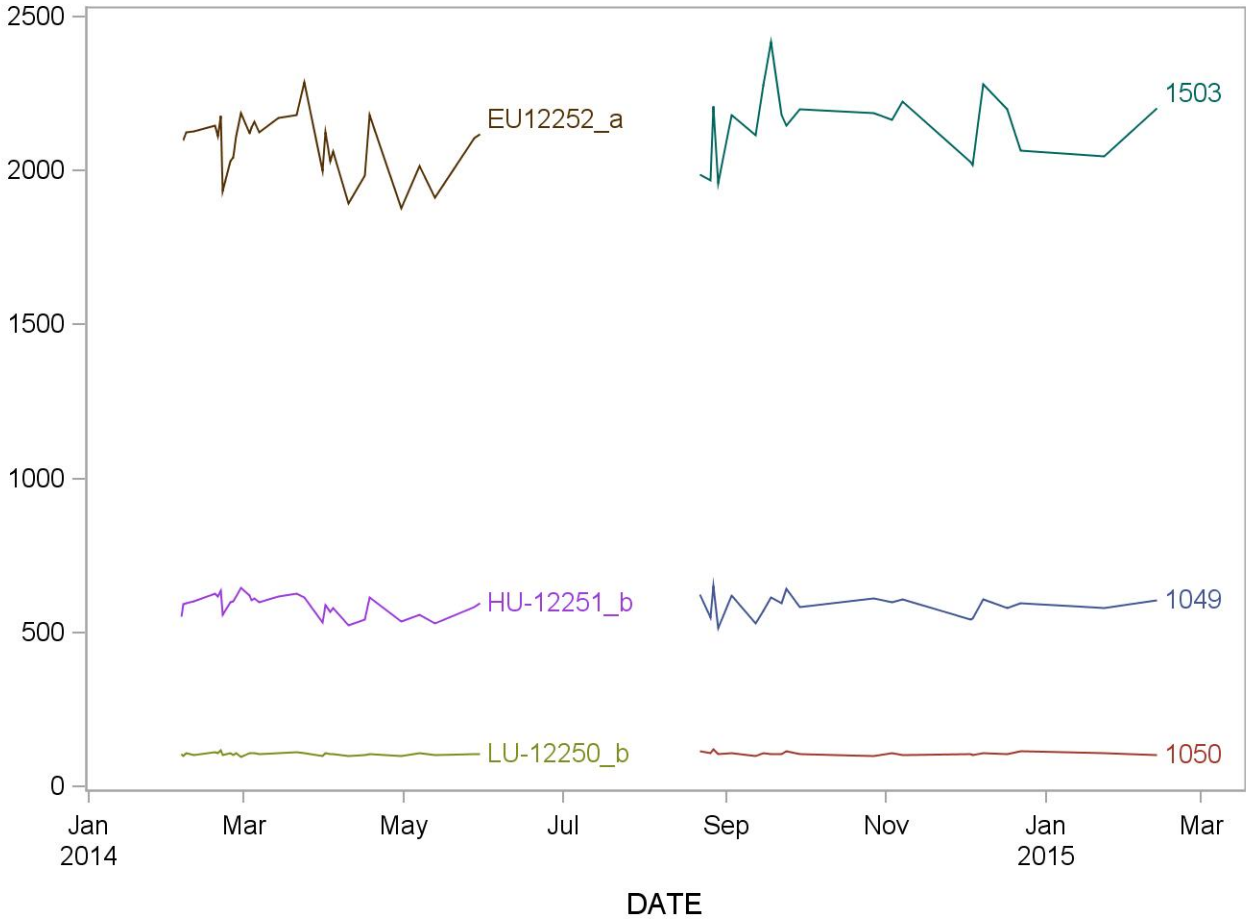
12) Alternate methods for performing test and storing specimens if test system fails:

If the analytical system fails, the analysis may be setup on other ICP-MS instruments in the laboratory. If no other instrument is immediately available, the samples will be stored according to Section 3 until a test system is available.

13) Summary Statistics and QC Graphs

See following page

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU-12251_b	44	05FEB14	30MAY14	594.71	30.37	5.1
LU-12250_b	44	05FEB14	30MAY14	106.82	4.59	4.3
EU12252_a	43	06FEB14	30MAY14	2090.08	86.49	4.1
1049	24	22AUG14	12FEB15	590.36	35.55	6.0
1050	24	22AUG14	12FEB15	108.31	5.13	4.7
1503	24	22AUG14	12FEB15	2155.65	119.18	5.5



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Appendix A: Critical parameter test results

Critical parameter test #1: Evaluating the significance of time from preparation to analysis. Verifies accurate results are attainable if samples are not analyzed the same day of preparation. New preparations (days 2 and 3) permit comparison to results from same day preparations.

Test details:
All analytical runs had approximately 40 samples between beginning and ending QC.
Day 1: Prepare samples for analysis in triplicate (calibrators, blanks, QC and reference materials in three separate sets of tubes). Analyze set #1 immediately. Cap sets #2 and #3 and leave at room temperature.
Day 2: Prepare a new run (set #4). Analyze set #4 then set #2.
Day 3: Prepare a new run (set #5). Analyze set #5 then set #3.

Appendix A, Table 1.
Time past preparation results (11/20-22/13, ELAN DRC2-F, Denise Tevis).

ID	Time, prep to analysis	Hg (µg/L)	I (µg /L)
LU12250	*Char. mean (± 2SD range)*	0.31 (0.14 – 0.48)	104 (93.4 – 113.6)
	Same day	0.31	115
	Prep day +1	0.36	116 (119)*
	Prep day +2	0.27	113 (113)*
HU12251	*Char. mean (± 2SD range)*	3.02 (2.39 – 3.65)	594 (518 – 670)
	Same day	2.85	657
	Prep day +1	2.83	656
	Prep day +2	2.88	647
EU12252	*Char. mean (± 2SD range)*	57.3 (48.4 – 66.2)	2108 (1891 – 2324)
	Same day	48.9	2270
	Prep day +1	51.1	2350 (2380)*
	Prep day +2	57.7	2325 (2330)*
NIST SRM 3668 L1	*target mean (± 10% range)*	0.91 (0.82 – 1.00)	143 (129 – 157)
	Same day	0.85	149
	Prep day +1	1.17 (0.91)*	158
	Prep day +2	1.01 (0.91)*	149
NIST SRM 3668 L2	*target mean (± 10% range)*	6.38 (5.74 – 7.02)	279 (251 – 307)
	Same day	6.68	295
	Prep day +1	7.43 (7.86)*	301
	Prep day +2	6.72 (8.56)*	293

* Results for sets #4 and #5, prepared same day as analysis for comparison.

Results: Samples are usable up to 2 days hours past preparation. Not all results are within expected ranges, however differences across days do not form a consistent pattern for all samples analyzed and results from samples prepared same day are similar to those from held-over samples.

Appendix A: Critical parameter test results (continued)

Critical parameter test #2: Evaluating how changes in the dynamic reaction cell gas flow rate (oxygen) affects observed Hg concentrations.

Test details:

1) Prepare a run (calibrators, blanks, QC, reference materials, dummy samples) for analysis in triplicate (three separate sets of tubes).
2) Analyze them in three separate runs on the same day using the same instrument with different cell gas flow rates in each run.

Appendix A, Table 2. Evaluating effect of changing cell gas flow rate on observed Hg concentration (11/20/13, ELAN DRC2-G, Katie Vance).

ID	Cell Gas Flow Rate, mL/min	Hg (µg/L)
LU12250	*Char. mean (± 2SD range)*	0.31 (0.14 – 0.48)
	1.04 mL/min (decreased)	0.551 *
	1.3 mL/min (normal)	0.493 *
	1.56 mL/min (increased)	0.344
HU12251	*Char. mean (± 2SD range)*	3.02 (2.39 – 3.65)
	1.04 mL/min (decreased)	2.71
	1.3 mL/min (normal)	3.04
	1.56 mL/min (increased)	2.04 *
EU12252	*Char. mean (± 2SD range)*	57.3 (48.4 – 66.2)
	1.04 mL/min (decreased)	53.7
	1.3 mL/min (normal)	49.4
	1.56 mL/min (increased)	56.6
NIST SRM 2668 L1	*target mean (± 10% range)*	0.91 (0.82 – 1.00)
	1.04 mL/min (decreased)	0.927
	1.3 mL/min (normal)	0.926
	1.56 mL/min (increased)	0.813 *
NIST SRM 2668 L2	*target mean (± 10% range)*	6.38 (5.74 – 7.02)
	1.04 mL/min (decreased)	7.84*
	1.3 mL/min (normal)	6.42
	1.56 mL/min (increased)	6.98

* Not all results are within expected ranges, however these deviations do not form a consistent pattern for all samples analyzed which relates to the changing of the cell gas flow rate.

Results: Method ruggedness appears to be acceptable across the cell gas flow rate range of 1.04 to 1.56 mL/min. Not all results are within expected ranges, deviations from the expected ranges is not consistent for all samples analyzed at the gas flow rates tested.

Appendix A: Critical parameter test results (continued)

Critical parameter test #3: Evaluating how changes in the RPq setting for DRC mode (O_2) affects observed Hg concentrations.

Test details:

1) Prepare a run (calibrators, blanks, QC, reference materials, dummy samples) for analysis in triplicate (three separate sets of tubes).

2) Analyze them in three separate runs on the same day using the same instrument with different RPq settings in each run.

Appendix A, Table 3. Evaluating effect of changing cell gas flow rate on observed Hg concentration (11/22-25/13, ELAN DRC2-B, Brandi Heath).

ID	RPQ	Hg (µg/L)
LU12250	*Char. mean (± 2SD range)*	0.31 (0.14 – 0.48)
	0.32 RPq (decreased)	0.25
	0.4 RPq (normal)	0.32
	0.48 RPq (increased)	0.33
HU12251	*Char. mean (± 2SD range)*	3.02 (2.39 – 3.65)
	0.32 RPq (decreased)	3.25
	0.4 RPq (normal)	3.13
	0.48 RPq (increased)	3.18
EU12252	*Char. mean (± 2SD range)*	57.3 (48.4 – 66.2)
	0.32 RPq (decreased)	56.8
	0.4 RPq (normal)	56.5
	0.48 RPq (increased)	54.1
NIST SRM 2668 L1	*target mean (± 10% range)*	0.91 (0.82 – 1.00)
	0.32 RPq (decreased)	1.37*
	0.4 RPq (normal)	0.734
	0.48 RPq (increased)	0.755
NIST SRM 2668 L2	*target mean (± 10% range)*	6.38 (5.74 – 7.02)
	0.32 RPq (decreased)	6.47
	0.4 RPq (normal)	6.03
	0.48 RPq (increased)	6.42

* Not all results are within expected ranges, however these deviations do not form a consistent pattern for all samples analyzed which relates to the changing of the RPq setting.

Results: Method ruggedness appears to be acceptable across the RPq range of 0.32 to 0.48. Not all results are within expected ranges, however, deviations from the expected ranges is not consistent for all samples analyzed at the RPq settings tested.

Appendix A: Critical parameter test results (continued)

Critical parameter test #4: Evaluating how changes in the axial field voltage (AFV) affects observed Hg concentrations (DRC mode, O_2).

Test details:

1) Prepare a run (calibrators, blanks, QC, reference materials, dummy samples) for analysis in triplicate (three separate sets of tubes).

2) Analyze them in three separate runs on the same day using the same instrument with different axial field voltage settings in each run.

Appendix A, Table 4. Evaluating effect of changing the axial field voltage on observed Hg concentration (12/3/2013, ELAN DRC II, Katie Vance).

ID	AFV	Mean UHG *	Replicate Averages **	%RSD	Re cps S8/S0
LU12250	Char. mean (± 2SD range) = 0.31 (0.14 – 0.48)				
	300 V	0.42	0.41, 0.44, 0.40	11%	1.01
	375 V	0.38	0.37, 0.43, 0.35	12%	0.99
	450 V	0.28	0.32, 0.23, 0.30	21%	0.99
HU12251	Char. mean (± 2SD range) = 3.02 (2.39 – 3.65)				See LU12250
	300 V	3.28	3.31, 3.23, 3.31	4%	
	375 V	3.10	2.98, 3.24, 3.07	5%	
	450 V	2.73	2.71, 2.90, 2.57	6%	
EU12252	Char. mean (± 2SD range) = 57.3 (48.4 – 66.2)				
	300 V	58.1	57.5, 57.4, 59.4	2%	
	375 V	59.7	59.0, 61.1, 59.0	2%	
	450 V	56.1	57.8, 55.4, 55.1	4%	
NIST SRM 3668L1	target mean (± 10% range) = 0.91 (0.82 – 1.00)				
	300 V	0.83	0.95, 0.73, 0.82	13%	
	375 V	0.94	0.90, 1.00, 0.92	5%	
	450 V	0.80 *	1.02, 0.72, 0.66	24%	
NIST SRM 3668L2	target mean (± 10% range) = 6.38 (5.74 – 7.02)				
	300 V	5.51 *	5.52, 5.50, 5.52	0%	
	375 V	8.02 *	8.17, 7.95, 7.93	2%	
	450 V	6.71	6.78, 6.44, 6.92	4%	

* Mean UHG is avg of beginning and ending QC results and rep avg are of beginning and ending rep 1 results, beginning and ending rep 2 results, etc . . .

Results: Method ruggedness appears to be acceptable across the AFV range of 300 – 450 V. Not all results are within expected ranges, however, deviations from the expected ranges is not consistent for all samples analyzed at the AFV settings tested. No consistent trends across the replicates are observed within the measurements and

no large deviations from unity in the S8/S0 ratio are observed at any AFV tested, which would be symptomatic of a non-optimized AFV.

Appendix A: Critical parameter test results (continued)
Parameter test #5: Evaluate the impact on observed concentration if an extra dilution is performed on the sample relative to the calibration standards.
Test details:

1. Spike a volume of urine (10 to 100 mL) with I and Hg to concentrations approximating that of calibration standard 8.
2. Mix it well.
3. In at least 4 separate runs prepare the dilutions detailed below (2x, 5x, 10x, 20x, and 100x). Best precision is obtained when >10% of the digiflex's 2.0 mL syringe capacity is used. Analyze each as an unknown sample (i.e. subtract the aqueous blank).
    a. No extra dilution (5 mL total):
       500 µL urine sample + 500 µL water + 4000 µL diluent
    b. 2x extra dilution (5 mL total):
       250 µL urine sample + 750 µL water + 4000 µL diluent
    c. 5x extra dilution (10 mL total):
       200 µL urine sample + 1800 µL water + 8000 µL diluent
    d. 10x extra dilution (20 mL total):
       200 µL urine sample + 3800 µL water + 16000 µL diluent
    e. 20x extra dilution (40 mL total):
       200 µL urine sample + 7800 µL water + 32000 µL diluent
    f. 100x extra dilution (50 mL total)
    g. 50 µL urine sample + 9950 µL water + 40000 µL diluent
4. Keep the spiked urine sample frozen (≤-20 ºC) between experiments and mix it well before each sampling.

Appendix A, Table 5. Ruggedness testing results: Evaluating the effect of matrix changes due to additional dilution. Test performed 11/4/13 – 11/5/13 by Denise Tevis, Brandi Heath and Katie Vance, instruments ELAN DRC II B, F, and G. Results are shown as the concentration normalized to that observed with no additional dilution (± 1SD).

Dilution level	Hg	I
No Extra Dilution	1.00 ± 0.00	1.00 ± 0.00
2x dilution (n=5)	1.04 ± 0.05	1.04 ± 0.02
5x dilution (n=5)	1.04 ± 0.06	1.07 ± 0.04
10x dilution (n=5)	0.97 ± 0.07	0.98 ± 0.10
20x dilution (n=4)	0.97 ± 0.08	0.97 ± 0.11
100x dilution (n=5)	0.97 ± 0.06	1.02 ± 0.08

Results: Method ruggedness appears to be acceptable across the extra dilution levels of 2x up to 100x.

Appendix B

Table 1. Instrument and method parameters.	
Instrument: PerkinElmer ELAN DRC II ICP-MS, ESI SC4 autosampler, ESI FAST sample introduction system and ESI DXi micro peristaltic pump	
Optimization window parameters	
RF power	1450 W
Plasma Gas Flow (Ar)	15 L/min
Auxiliary Gas Flow (Ar)	1.2 L/min
Nebulizer Gas Flow (Ar)	~0.90 – 1.0 L/min (optimized as needed for sensitivity)
Ion Lens Voltage(s)	AutoLens (optimized as needed for sensitivity)
AFV, QRO, CRO, CPV, Discriminator Threshold	Optimized per instrument by service engineer, or advanced user.
Parameters of x-y alignment, nebulizer gas flow, AutoLens voltages, mass calibration, dual detector 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 DRC mode) = 30 Exhaust Delay (From DRC to Standard mode) = 30 Flow Delay (Gas changes while in DRC mode) = 30 Channel Delay (Gas channel change in DRC mode) = 30
File names & directories	
method file names	*calibration curve (programmed for urine blank)* CDC_DLS3002_urblk.mth *For QC & patient sample analysis (programmed for aqueous blank)* CDC_DLS3002_aqblk.mth
dataset	Create a new dataset subfolder each day. Name as “2013-0820” for all work done on August 20, 2013
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 (calib curve info).rop
tuning	Default.tun
optimization	Default.dac
calibration	N/A
polyatomic	elan.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: make descriptive including date (i.e. 2013-0311b_DRC2B_group1.txt)*
Method Parameters	

Table 1. Instrument and method parameters.	
Method Parameters: Timing Page (see Figures 1a, 2a and 2d in Appendix B)	
sweeps/reading	30
readings/replicate	1
replicates	3
enable qc checking	On
isotopes monitored and internal standard associations (exact mass)	^{185}Re as internal standard for ^{127}I (126.9) and ^{202}Hg (201.971)
dwell times	100 ms ^{202}Hg 30 ms for ^{185}Re, and ^{127}I
scan mode	Peak Hopping for all isotopes (1 MCA channel)
DRC channel A gas flow rate	None
DRC channel B gas flow rate	99.99% oxygen (5-7 psig delivery pressure) typically 1.3 L/min (1.04 – 1.56) * *optimized per instrument, and periodically verified
RPa	0 for all isotopes
RPq	Typically* 0.4 (0.32 – 0.48) for ^{185}Re and ^{202}Hg 0.25 for ^{185}Re and ^{127}I Use the same RPQ for each analyte and its IS. (* Optimize per instrument, and periodically verified)
Method parameters: processing page (see Figures 1b in Appendix B)	
detector mode	Dual
process spectral peak	N/A
AutoLens	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 1c in Appendix B)	
equations	None
Method parameters: calibration page (see Figures 1d in Appendix B)	
calibration type	external std.
curve type	weighted linear
sample units	“μg/L” or “ppb”

Table 1. Instrument and method parameters.	
calibration standard concentrations (μg/L)	Hg: 0.08, 0.3, 1, 5, 20, 80, 150, 300 I: 8, 20, 60, 160, 400, 1200, 1500, 3000
Method parameters: sampling page (see Figures 1e and 1f in Appendix B)	
"peristaltic pump under computer control"	On
autosampler tray port sampling device	***If using ESI autosampler*** Autosampler Type: AS-93plus Tray Name: esi.try Sampling Device: None If using other autosampler, refer to user guide.
sample flush	default is 3 s at 3 rpm (~320 μL/min, ESI DXi peristaltic pump, FAST sample introduction system) Time can be optimized as needed to adequately fill the FAST loop. Time and rpm can be optimized as needed to using a different style peristaltic pump (maintaining approximate liquid flow rate). 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	37 s at 3 rpm (~320 μL/min, ESI DXi peristaltic pump, FAST sample introduction system) Time can be optimized as needed to reach signal stability before beginning analysis. Time and rpm can be optimized as needed to using a different style peristaltic pump (maintaining approximate liquid flow rate). 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	100 s at 10 rpm (~160 μL/min, ESI DXi peristaltic pump, FAST sample introduction system) Time 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). Time and rpm can be optimized as needed to using a different style peristaltic pump (maintaining approximate liquid flow rate).

Table 1. Instrument and method parameters.	
extended wash (via ICP-MS software QC checking)	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** Hg / >80 µg/L / 400 s I / >3000 µg/L / 400 s NOTE: When samples subsequent to the elevated trigger concentrations are also known to be elevated (≥S5 concentrations), the default method rinse time (100 s) may be used to save time by disabling the 'QC checking feature'. However, verify that standard zero (urine blank) levels have been re-achieved before proceeding with analysis of unknowns or the ending QC of run (see Section 8.b.iv.7.c).
autosampler locations of blanks and standards	*For calibration curve (points to urine blank)* CDC_DLS3002_urblk.mth Calibration Stds 0 – 8 in autosampler positions 101 – 109 by default, but can be customized. *For QC & patient sample analysis (points to aqueous blank)* CDC_DLS3002_aqblk.mth Aqueous Blank in autosampler position 117 by default, but can be customized.
FAST parameters: See Figures 4a through 4g in Appendix B for details	
configuration file	default.sc (saved at C:\Program Files\ESI\ESI-SC\)
FAST program	cdc_1mL loop_UIHG_dls3002.txt
Potential Emergency Response Modifications:	
mercury:	Analyze Hg in standard (vented) mode. Set all DRC gas flows to 0 and RPq values to 0.25. See Section 2.a for details of potential interferences that may be incurred by not using DRC conditions for Hg.

Table 2. Suggested concentrations for base urine

analyte (units)	suggested concentration
Hg (µg/L)	≤ 0.5
I (µg/L)	≤ 150

Table 3. Stock calibration standards

Element	Concentration
Mercury (Hg)	1,000 µg/mL
Iodine (I)	1,000 µg/mL

Table 4. Preparation of intermediate stock calibration standards			
Mercury	**Hg Stock Calibration Standard**	**Hg Intermediate Stock Calibration Standard A**	**Hg Intermediate Stock Calibration Standard B**
Flask Vol. (mL)	purchased	100	100
Hg Stock Calib Std Spike Vol. (mL)		2	0.05
Concentration (µg/mL)	1,000	20	0.5
Iodine	**I Stock Calibration Standard**	**I Intermediate Stock Calibration Standard A**	
Flask Vol. (mL)	purchased	100	
I Stock Calib Std Spike Vol. (mL)		1	
Concentration (µg/mL)	1,000	10	

Table 5. Preparation of multi-element intermediate working standards								
Standard #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Flask volume (mL)	100	100	100	100	100	100	100	100
Hg Int. Stock Std. A (mL)					0.1	0.4	0.75	1.5
Hg Int. Stock Std. B (mL)	0.016	0.06	0.20	1.0				
I Stock Std. (mL)					0.04	0.12	0.15	0.30
I Int. Stock Std. A (mL)	0.08	0.20	0.60	1.6				
	concentrations (µg **/L)**							
Hg	0.08	0.3	1	5	20	80	150	300
I	8	20	60	160	400	1200	1500	3000

Appendix B (continued)

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

setup 1	setup 2
Run #1	*Run #1*
calibration standards	**calibration standards**
low bench QC	low bench QC
high bench QC	high bench QC
elevated bench QC	elevated bench QC
patient samples	patient samples
low bench QC	low bench QC
high bench QC	high bench QC
elevated bench QC	elevated bench QC
Run #2	*Run #2*
calibration standards	low bench QC
low bench QC	high bench QC
high bench QC	elevated bench QC
elevated bench QC	patient samples
patient samples	low bench QC
low bench QC	high bench QC
high bench QC	elevated bench QC
elevated bench QC	

Appendix B (continued)

Table 7. A typical SAMPLE/BATCH window.			
AS Location*	Sample ID	Measurements Action	Method
233	DRCstability1	Run sample	…DLS3002_urblk.mth
233	DRCstability2	Run sample	…DLS3002_urblk.mth
Continue DRC stability samples . . .			
233	DRCstability14	Run sample	…DLS3002_urblk.mth
233	DRCstability15	Run sample	…DLS3002_urblk.mth
110	3002 S0 check	Run sample	…DLS3002_urblk.mth
301	3002 UrblkChk Wash1	Run blank, standards, and sample **	…DLS3002_urblk.mth
301	3002 UrblkChk Wash2	Run sample	…DLS3002_urblk.mth
301	3002 UrblkChk Wash3	Run sample	…DLS3002_urblk.mth
301	3002 UrblkChk Wash4	Run sample	…DLS3002_urblk.mth
302	3002 UrblkChk1	Run sample	…DLS3002_urblk.mth
303	3002 UrblkChk2	Run sample	…DLS3002_urblk.mth
112	3002 AQBLK	Run blank and sample ¥	…DLS3002_aqblk.mth
125	L Bench QC	Run sample	…DLS3002_aqblk.mth
126	H Bench QC	Run sample	…DLS3002_aqblk.mth
127	E Bench QC	Run sample	…DLS3002_aqblk.mth
137	Sample 1	Run sample	…DLS3002_aqblk.mth
138	Sample 2	Run sample	…DLS3002_aqblk.mth
125	L Bench QC	Run sample	…DLS3002_aqblk.mth
126	H Bench QC	Run sample	…DLS3002_aqblk.mth
127	E Bench QC	Run sample	…DLS3002_aqblk.mth

* The exact autosampler positions of QCs and patient samples do not have to be those shown above. QC samples do not have to be run in the order of low, then high, then elevated.

** When executing this row, the ELAN will first analyze the standard 0 (urine blank) at AS position 101, then standards 1–8 at autosampler positions 102–109, then the “3002 UrblkChk Wash1” sample at A/S position 301. The sampling information about AS positions 101-109 are stored in the “urblk” method file.

¥ When executing this row, the ELAN will first analyze the aqueous blank at AS position 111, then the “Aq blank ” at AS position 112. The sampling information about AS position 111 is stored in the “aqblk” method file.

Appendix B (continued)

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

Total volume of prepared sample may be changed, from what is presented here. However, adjust volumes for each component proportionally.

Description	Water (µL)	Base Urine (µL)	AQ Int Working Std (µL)	Patient or QC urine sample (µL)	Diluent (µL)**	Total Vol (µL)
AQ Blank	500 x 1	-	-	-	2,000 (1,000 x 2)	2,500
Urine Blank and UrBlkChk	250 x 1	250 x 1	-	-	2,000 (1,000 x 2)	2,500
Working Calibration Standards	-	250 x 1	250 x 1	-	2,000 (1,000 x 2)	2,500
Patient urine or Urine-Based QC	250 x 1	-	-	250 x 1	2,000 (1,000 x 2)	2,500
Patient Urine *2x Dilution* [H]	750 x 1	-	-	250 x 1	4,000 (2,000 x 2)	5,000
Patient Urine *5x Dilution* [H]	1,800 (600 x 3)	-	-	200 x 1	8,000 (2,000 x 4)	10,000
Patient Urine *10x Dilution* [H]	3,800 (1,600 x 3)	-	-	200 x 1	16,000 (4,000 x 4)	20,000
Patient Urine *20x Dilution* [H]	7,800 (2,600 x 3)	-	-	200 x 1	32,000 (8000 x 4)	40,000
Patient Urine *100x Dilution* [H]	9,950 (1,990 x 5)	-	-	50 x 1	40,000 (7,000 x 5 + 1 x 5,000)	50,000

* These directions are written with the expectation of a 10,000 µL dispensing syringe (left side) and a 2000 µL sampling syringe (right side) on a Digiflex automatic pipettor.

** Best volumetric transfers can be achieved by dispensing diluent concurrently with (behind) other liquid portions. Dispense diluent volume in two equal portions is the typical practice. For example, when preparing a working calibration standard dilution, dispense 1000 µL diluent + 250 µL standard in one cycle of Digiflex™, then 1000 µL diluent + 250 µL base urine in the next cycle of the Digiflex™ to prepare a 2.5 mL total volume dilution.

[H] Extra dilution is performed on urine samples whose concentration is greater than standard 8 concentrations. Any extra level of dilution up to 100x (see Appendix A, Experiment 6) can be prepared as long as the ratio of 1 parts (water + urine matrix) to 4 parts diluent is maintained. Better precision is obtained when the volume is ≥10% of the digiflex syringe volume. Volumes in parenthesis are suggested volumes to dispense so that diluent is dispensed concurrently with water and/patient sample. For example, when preparing a 20x dilution, dispense 8000 µL diluent + 200 µL in one cycle of the Digiflex, then 8000 µL diluent and 2600 µL water for three cycles for a total volume of 40 mL.

Appendix B (continued)

Table 9. Boundary concentrations for urine concentrations

analyte (units)	2nd lower boundary ("2 LB")	1st lower boundary ("1 LB")	1st upper boundary ("1UB") *	2nd upper boundary ("2UB") **	range maximum ("Lim Rep Delta") †
Hg (μg/L)	-	-	5	10	1
I (μg/L)	10	10	800	2000	30

* Typically, the 1st upper boundary (1UB) is the 99th percentile of non-weighted concentration results from the NHANES 1999-2000 subset groups, a concentration significant to public health, or a concentration defined by study protocol. The default 1UB concentrations are listed in this table. Concentrations observed greater than the 1UB shall be confirmed by repeat analysis of a new sample preparation. Report the original result, as long as the confirmation is within 10% of the original. Continue repeat analysis until a concentration can be confirmed.

** The 2nd upper boundary (2UB) may be 2x the 1UB, a concentration significant to public health, or defined by study protocol. Report patient results confirmed to be greater than the 2UB to the QC reviewer as an "elevated result".

† Range maximum (Lim Rep Delta) is the allowed limit to the range of the three replicate readings for a single sample analysis. If the range of replicate readings for analysis of an unknown sample is greater than the range maximum, and represents greater than a 10% relative standard deviation for the measurement, do not use the measurement for reporting.

Appendix B (continued)

Table 10. Reference ranges for urine concentrations. [13]

analyte (units)	survey years	geometric mean	50th	75th	95th	N
Hg (μg/L)	05–06	.468 (.426–.514)	.460 (.410–.510)	1.03 (.900–1.12)	2.94 (2.58–3.26)	2578
	07–08	.443 (.408–.482)	.440 (.400-.470)	.880 (.760-1.00)	2.66 (2.29–3.08)	2634
	09–10	*	.400 (.360–.450)	.850 (.770–.910)	2.42 (2.07–2.72)	2865
I (μg/L)	03–06	156 (148 – 163)	162 (154 – 170)	-	603 (565 – 676)	5,175

* Not calculated: proportion of results below limit of detection was too high to provide a valid result.

Appendix C: Help Sheets

Reagent Preparation (page 1 of 3)

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

Rinse solution
(0.4% v/v TMAH, 1% ethyl alcohol, 0.01% APDC, and 0.05% Triton® X-100)

1) Partially fill a 4 L bottle with ≥18 MΩ·cm water.
2) Add 0.4 grams of APDC.
3) Add 16 mL of TMAH (Tetramethylammonium hydroxide, 25% w/w ($(CH_3)_4NOH$).
4) Add 40 mL of ethyl alcohol (C_2H_5OH, 200 proof)
5) Add 10 mL of 20%Triton X-100.
6) Add enough ≥18 MΩ·cm water to bring to 4 L mark.
7) Mix well by gently inverting several times.
8) Label appropriately.

Sample diluent
(5 µg/L Re, 0.4% v/v TMAH, 1% ethyl alcohol, 0.01% APDC, and 0.05% Triton® X-100)

1) Partially fill a 2 L bottle with ≥18 MΩ·cm water.
2) Add 0.2 gram of APDC.
3) Add 8 mL of TMAH.
4) Add 20 mL of ethyl alcohol.
5) Add 5 mL of 20% Triton X-100 solution.
6) Add 100 µL of a 100 mg/L stock solution of Re.
7) Add enough ≥18 MΩ·cm water to bring to 2 L mark.
8) Mix well by gently inverting several times.
9) Label appropriately.

Appendix C: Help Sheets (continued)

Reagent Preparation (page 2 of 3)

1% v/v HNO3 (for acid washing containers)

1) Partially fill a 2 L Teflon or polypropylene container with ≥18 MΩ·cm water (> 50% full).
2) Add 20 mL of concentrated HNO_3.
3) Add enough ≥18 MΩ·cm water to bring to 2 L mark.
4) Mix well by inverting and swirling.
5) Label appropriately.

5% v/v HNO3 (for soaking quartz and glass components)

1) Partially fill a 2 L Teflon or polypropylene container with ≥18 MΩ·cm water (> 50% full).
2) Add 100 mL of concentrated HNO_3.
3) Add enough ≥18 MΩ·cm water to bring to 2 L mark.
4) Mix well by inverting and swirling.
5) Label appropriately.

1% Triton X-100

1) 1. Partially fill a 1 L bottle with ≥18 M-ohm water.
2) 2. Add 10 mL of Triton X-100.
3) 3. Add enough ≥18 M-ohm water to bring to 1 L mark.
4) 4. Allow to dissolve overnight (or add a Teflon magnetic stirring bar and stir on stirrer until dissolved).
5) 5. Mix well by gently inverting several times.

20% Triton X-100

1) Partially fill a 1 L bottle with ≥18 MΩ·cm water.
2) Add 200 mL of Triton® X-100.
3) Add enough ≥18 MΩ·cm water to bring to 1 L mark.
4) Allow to dissolve overnight (or add a Teflon magnetic stirring bar and stir on stirrer until dissolved). Mix well by gently inverting several times.
5) Label appropriately.

100 mg/L (ppm) Re internal standard intermediate spiking solution

1) Partially fill an acid rinsed, 50 mL flask with 1% v/v HNO_3.
2) Add 5 mL of Re from 1000 mg/L stock standard.
3) Add 1.5 mL of concentrated HNO_3.
4) Add enough water to fill to 50 mL mark.
5) Mix well by gently inverting several times.
6) Pour the standard solution over into a 50 mL tube.
7) Label appropriately.

Appendix C: Help Sheets (continued)

Reagent Preparation (page 3 of 3)

1 µg/L (ppb) Daily Performance Test Solution in 2% v/v HNO3

1) Partially fill a 1 L volumetric flask with ≥18 MΩ·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 MΩ water to bring to 1 L mark.
5) Mix well by gently inverting several times.

DRC stability test solution (1 liter bulk prep)

1. Use a 1 L bottle dedicated to stability test solution preparation.
2. Add 800 mL of sample diluent.
3. Add 100 mL of “junk” urine
4. Add 100 mL of an Intermediate Working Calibration Standard (i.e. S2)
5. Mix well by gently inverting several times.
6. Store in the refrigerator (when not using).