Laboratory Procedure Manual

Analytes: **Zinc, Copper and Selenium**

Matrix: **Serum**

Method: **Serum Multi-Element ICP-DRC-MS**

Method No: DLS 3006.8-02

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

Contact: Dr. Kathleen L. Caldwell
Phone: 770-488-7990
Fax: 770-488-4097
Email: KCaldwell@cdc.gov

Dr. James L. Pirkle, M.D., PhD
Director, Division of Laboratory Sciences

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 items in the following table:

Data File Name	Variable Name	SAS Label
CUSEZN_I	LBXSCU	Copper(µg/dL)
	LBXSSE	Selenium (µg/L)
	LBXSZN	Zinc (µg/dL)

1) Clinical relevance and summary of test principle

a. Clinical Relevance:
This method is used to achieve rapid and accurate quantification of three elements of toxicological and nutritional interest including Zinc (Zn), Copper (Cu) and Selenium (Se). The method is useful to screen serum when people are suspected to be acutely exposed to these elements or to evaluate chronic environmental or other non-occupational exposure.

b. Test Principle:
Inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) is a multi-element analytical technique capable of trace level elemental analysis [1-4]. This ICP-DRC-MS method is used to measure the entire panel of 3 elements, or any subgroup. Liquid samples are introduced into the ICP through a nebulizer and spray chamber carried by a flowing argon stream. By coupling radio-frequency power into flowing argon, a plasma is created in which the predominant species are positive argon ions and electrons and has a temperature of 6,000-8,000 K. The sample passes through a region of the plasma and the thermal energy atomizes the sample and then ionizes the atoms. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP (at atmospheric pressure, ~760 torr) from the mass spectrometer (operating at a pressure of 10^{-5} torr). The ions pass through a focusing region, the dynamic reaction cell (DRC), the quadrupole mass filter, and finally are 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 molecular gas for the purpose of causing collisions and/or reactions between the molecular gas and the incoming ions. In general, collisions or reactions with the incoming ions selectively occur to eliminate an interfering ion by changing the ion of interest to a new mass, which is free from interference, or causing collisions between ions in the beam and the DRC gas, which can focus the ion beam to the middle of the cell thus increasing the ion signal. In this method, the instrument is operated in 'DRC' mode when measuring Zn, Cu and Se, and the cell is pressurized with 99.99+% ammonia gas, which collides or reacts with the incoming ions to eliminate interfering ions and leave the ion of interest to be detected.

After leaving the DRC cell, the ions are focused with ion optics into a quadrupole mass analyzer with a nominal mass resolution of 0.7 amu. The quadrupole is sequentially scanned to specific mass to charge ratio of each analyte and intensity is detected with a pulse detector. Electrical signals resulting from the detection of ions are processed into digital information that is used to indicate first the intensity of the ions and then the concentration of the element. This method was originally based on the methods by Piraner and Walters [5-8] and the DRC portions of the method are based on work published by Tanner et al. [2, 3]. The isotopes measured by this method are zinc (*m/z* 64), copper (*m/z* 65) and selenium (*m/z* 78) and the internal standard, gallium (*m/z* 71). Serum samples are diluted 1:1:28 with water and diluent containing gallium (Ga) for multi-internal standardization.

2) Limitations of method; interfering substances and conditions

a. Interferences addressed by this method

i. Correction and elimination of interferences (^{64}Ni, ^{36}Ar^{14}N$_2$) on zinc (^{64}Zn).

1. Mathematical correction for nickel (^{64}Ni) interference:
The correction equation (-0.035297* ^{60}Ni) is used in the "Equations" tab of the method to correct the counts observed as m/z 64 to exclude counts due to ^{64}Ni.

2. Elimination of ^{36}Ar^{14}N$_2$ interference using DRC: The dynamic reaction cell of the ELAN ICP-DRC-MS is used in this method to eliminate interference from ^{36}Ar^{14}N$_2$ onto zinc at m/z 64. See Section 1.b for an explanation of this process.

ii. Elimination of interferences (^{40}Ar^{25}Mg, ^{36}Ar^{14}N$_2$^{1}H) on copper (^{65}Cu) using DRC. The dynamic reaction cell of the ELAN ICP-DRC-MS is used in this method to eliminate the interference ^{40}Ar^{25}Mg, ^{36}Ar^{14}N$_2$^{1}H on copper at m/z 65. See Section 1.b for an explanation of this process.

iii. Correction and elimination of interferences (^{78}Kr, ^{38}Ar^{40}Ar, ^{38}Ar^{40}Ca) on selenium (^{78}Se).

1. Mathematical correction for krypton (^{78}Kr) interference:
The correction equation (-0.030461*^{83}Kr) is used in the "Equations" tab of the method to correct the counts observed as m/z 78 to exclude counts due to ^{78}Kr.

2. Elimination of ^{38}Ar^{40}Ar, ^{38}Ar^{40}Ca interference using DRC: The dynamic reaction cell of the ELAN ICP-DRC-MS is used in this method to eliminate interference from ^{38}Ar^{40}Ar, ^{38}Ar^{40}Ca onto selenium at m/z 78. See Section 1.b for an explanation of this process.

b. Limitations of method

i. ^{48}Ca^{16}O^{1}H interference on copper (^{65}Cu):
It has been determined that a small interference remains at m/z 65 when the serum matrix contains very high calcium levels. Even at extreme calcium levels, this interference has not been found to be significant (< 1%).

ii. Time between dilution of serum materials and analysis:
Selenium is not stable in the diluted sample for more than 7 hours. Diluted serum must be analyzed within 7 hours of preparation (see Appendix A, test 5 for details).

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 (DLS) Policies and Procedures Manual [5]. In general, if more than one vacutainer of blood is to be drawn from an individual, collect the trace metals

tube second or later. Draw the blood through a stainless steel needle into a pre-screened 7-mL vacutainer. Allow the blood in the stoppered vacutainer clot for 30-40 minutes, but not longer than 60 minutes. Without opening the vacutainer, centrifuge it for 10 minutes at 2400 rpm. Use a pre-screened serum separator to remove the serum from the clot. Under a laminar flow hood, pour the serum in the serum separator into pre-screened polyethylene vials.

   i. No fasting or special diets are required.

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

   iii. Transport serum specimens at $\leq 4^{\circ}C$.

   iv. Once received, store at $\leq -20^{\circ}C$ until time for analysis. Re-freeze remaining portions $\leq -20^{\circ}C$ after analytical aliquots are withdrawn. Thawing and refreezing samples has not been found to compromise sample results.

   v. Specimen stability for at least 5 years has been demonstrated at $< -20^{\circ}C$ storage conditions.

   vi. Acceptable containers for analytical aliquots include pre-screened polyethylene vials and pre-screened 7-mL vacutainers. A 3-mL vacutainer size will not produce the optimal volume of serum for this test. Externally threaded containers are preferred because they are less prone to contamination of the specimen and to leaks (internally threaded containers can develop leaks when biological material dries within the threads, compromising resealing).

b. Criteria for specimen rejection: Specimen characteristics that compromise test results are indicated above. Reasons for rejection of a sample for analysis include

   i. Low volume: Optimal amount of serum is 1 mL, minimum is 0.8 mL. The volume of serum used for one analysis is 0.15 mL.

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

In all cases, request a second serum specimen.

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

4) Safety precautions

a. General safety

i. Observe all safety regulations as detailed in the Division (DLS) Safety Manual. Additional information can be found in your lab's chemical hygiene plan.

ii. Observe Universal Precautions when working with serum.

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

iv. Exercise special care when handling and dispensing concentrated nitric acid. Add acid to water. Nitric acid is a caustic chemical that is capable of causing severe eye and skin damage. ***If nitric acid comes in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.***

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

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

vii. Training will be given before operating the ICP-DRC-MS, as there are many possible hazards including ultraviolet radiation, high voltages, radio-frequency radiation, and high temperatures. This information is also detailed in the PerkinElmer ELAN® ICP-DRC-MS System Safety Manual.

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

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

b. Waste disposal:

i. Autoclaving: All diluted biological specimens, original biological specimens being disposed, or consumables, which come into contact with biological specimens (even diluted or aerosolized). Use sharps containers or special autoclave pans for broken glass / quartz or items, which are puncture hazards (e.g. pipette tips). (see the "Autoclaving" section of the CDC safety policies and practices manual located in the laboratory).

ii. Other liquid waste

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

are handled according to DLS 3500 standard operating procedure for handling corrosive liquid laboratory waste.

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

5) **Instrument and material sources**

a. Sources for ICP-MS instrumentation

i. ICP-MS: Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectrometer (ELAN® 6100 DRC^{Plus} or ELAN® DRC II) (PerkinElmer Norwalk, CT, www.perkinelmer.com).

ii.Recirculating chiller / heat exchanger for ICP-MS: Refrigerated chiller (PolyScience 6105PE for ELAN® 6100 DRC^{Plus} instruments) or heat exchanger (PolyScience 3370 for ELAN® DRC II instruments) (PerkinElmer Norwalk, CT, www.perkinelmer.com).

iii. Autosampler: ESI SC-4 autosampler (Elemental Scientific Inc., Omaha, NE) or equivalent.

b. Sources for ICP-MS parts and consumables

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

i. Adapter, plastic: 1/4-28 female threads on one side, 1.8mm barb adapter on the other. Connects ¼-28 nut at flanged tubing connection to 0.045" i.d. peristaltic pump tubing. Use part # B019-3342 ("Type A" adapter, PerkinElmer Norwalk, CT, www.perkinelmer.com) or equivalent. # *Spares = 4*.

ii. 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).

iii. 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*.

iv. Cone, sampler: Both platinum and nickel cones have been used successfully. For platinum cones, Spectron part # SC2013-Pt (Spectron, Ventura, CA, www.spectronus.com) or equivalent. For nickel cones, PerkinElmer part # WE021140 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or equivalent. # *Spares = 4*.

v. Cone, skimmer: Both platinum and nickel cones have been used successfully. For platinum cones, Spectron part # SC2014-Pt (Spectron, Ventura, CA, www.spectronus.com) or equivalent. For nickel cones, PerkinElmer part # WE021137 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or equivalent. *# 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. 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).

ix. Hose, for exhaust of ELAN: Available as part of ELAN 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 is acceptable. *# Spares = 10 feet of 4" diameter and 10 feet of 6" diameter hose.*

x. 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.*

xi. Injector support (for pass-through injector): PerkinElmer part # WE023951 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available direct from manufacturer as part # 400-37 (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, quartz concentric: Type C, 1 mL/min nebulizer with quick disconnects for liquid and gas ports such as part # 500-70QQDAC (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com). This nebulizer is designed to use quick disconnects part # 500-QD (liquid) and # 500-AC (argon).

xiv. Nebulizer connections (gas): (for nebulizer argon side-arm).

1. If *not* using quick disconnection fitting, insert nebulizer argon side-arm into the 1/8" i.d. vinyl tubing and secure the connection with a hose clamp for ¼" o.d tubing (like part # EW-06832-01, Cole Palmer Instrument Company, Vernon Hills, Illinois, www.colepalmer.com). *# Spares = 2.*

2. Quick disconnection fitting: Like part # 500-AC (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com). *# Spares = 2.*

xv. Nebulizer connections (liquid): (for nebulizer 4mm o.d. liquid sample backend). Can use quick disconnect or flangeless nut and ferrule assembly.

1. Quick disconnect: Like Part # 500-QD (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com). *# Spares = 2.*

2. Flangeless nut and ferrule assembly: An assembly such as part # FIT KIT 3 (Meinhard Glass Products, Golden, CO, www.meinhard.com) or equivalent. Individual pieces of FIT KIT #3 can be purchased as follows.

    a. Nut, flangeless, 1/16", ¼-28, Delrin® (Acetal), red. Part # P202x (10pk, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com). *# Spares = 10.*

    b. Ferrule, flangeless, 1/16", Tefzel® (ETFE), blue. Part # P-200x (10pk, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com). *# Spares = 10.*

    c. Adapter, 1/4-28 internal to 5/16-24 internal, PEEK™. Part # P-135 (Upchurch Scientific, Oak Harbor, WA, www.upchurch.com). *# Spares = 2.*

    d. Nut, 4mm ID PEEK. Part of fit kit 3 for concentric nebulizers. Part # S-1050 (Meinhard Glass Products, Golden, CO, www.meinhard.com). *# Spares = 2.*

    e. Ferrule, 4mm ID green Delrin. Part of fit kit 3 for concentric nebulizers. Part # S-1121 (Meinhard Glass Products, Golden, CO, www.meinhard.com). *# Spares = 2.*

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

xvii. Nut: (for bottom port of autosampler rinse station) 10-32 UMC threads for 1/16" tubing. Such as part # M653x (Upchurch Scientific, Oak Harbor, WA, www.upchurch.com) or equivalent. *# Spares = 2.*

xviii. 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.*

xix. 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.*

xx. Oil for roughing pumps:

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

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

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

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

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

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

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

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

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

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

xxvii. Photon stop: PerkinElmer part # WE018278 (PerkinElmer, Shelton, CT, www.perkinelmer.com). Alternate "snap in" lens assembly requires PerkinElmer part # W1013361 (PerkinElmer Norwalk, CT, www.perkinelmer.com). # *Spares = 1.*

xxviii. 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.

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

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

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

xxxii. 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.*

xxxiii. 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. Damaged torches can often be repaired for substantially lower cost than purchasing a new one by companies such as Wilmad LabGlass (Buena, NJ,

www.wilmad-labglass.com) or Precision Glass Blowing (Centennial, CO, www.precisionglassblowing.com). *# New Spares = 2.*

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

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

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

xxxvii. Tubing, peristaltic, 0.76 mm i.d. (sampling): Standard PVC, 2-stop (black/black) peristaltic pump tubing, i.d. = 0.76 mm. ESI part # MPP-076-F-PVC (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com) or equivalent. *# Spares = 6 packs of 12 tubes.*

xxxviii. Tubing, peristaltic, 1.3 mm i.d. (spray chamber drain): Santoprene, 2-stop (gray/gray) peristaltic pump tubing, i.d. = 1.3mm. ESI Part # MPP-130-PHR (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com) or equivalent. *# Spares = 6 packs of 12 tubes.*

xxxix. Tubing, PFA: I.D. = 0.5mm, O.D. = 1.59mm (1/16"). Used to transfer liquid

1. possibly used between nebulizer and peristaltic pump tubing (if quick connection is not used for liquid sample delivery)

The Perfluoroalkoxy (PFA) copolymer is a form of Teflon®. Such as part # 1548 (20ft length, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com) or equivalent. *# Spares = 20ft.*

xl. Tubing, PVC, i.d. = 1/8", o.d. = 3/16". Used to transfer liquid

1. between spray chamber waste port and peristaltic pump

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

xli. Tubing, stainless steel, o.d. = 1/8", wall thickness = 0.028": Used to connect DRC gas cylinders to ELAN DRC gas ports. Also used to replace plastic tubing in the DRC gas path within the ELAN. Like part # SS-T2-S-028-20 (20ft, Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. *Spares = 20ft.*

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

xliii. Tubing, Tygon, i.d. = 3/16", o.d. = 5/16": Used to transfer liquid between rinse station drain port and liquid waste jug. Like part # EW-06409-15 (50 ft, Cole Parmer, Vernon Hills, Illinois, www.coleparmer.com) or equivalent. *# Spares = 20ft.*

xliv. Tubing, vinyl (argon delivery to nebulizer): Vinyl Tubing, 1/8" ID x 1/4" OD. Like part # EW-06405-02 (Cole Parmer, Vernon Hills, Illinois, www.coleparmer.com) or equivalent. Equivalent tubing material is acceptable. *# Spares = 10ft.*

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

xlvi. Union tee, PTFE, ¼" Swagelok: Connects argon tubing to torch plasma gas sidearm and holds igniter inside torch sidearm. 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 and supplies

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

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

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

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

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

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

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

viii. Screw driver, for ion lens removal: Screw driver with long, flexible shaft, and 2mm ball-Allen end for removal of ion lens screws, part # W1010620. Extra 2mm bits, part # W1010598 (PerkinElmer, Shelton, CT, www.perkinelmer.com).

ix. Toothbrush: Any vendor. For cleaning ion lens and glassware.

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

d. Sources for general laboratory consumable supplies

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

ii. Carboy (for preparation of serum 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.

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

iv. Gloves: Powder-free, low particulate nitrile (like Best CleaN-DEX™ 100% nitrile gloves, any vendor). Equivalent nitrile or latex gloves are acceptable.

v. 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, wipes designed for cleanroom use are available such as the Econowipe or Wetwipe (Liberty, East Berlin, CT, www.liberty-ind.com).

vi. Pipette, benchtop automatic (for preparation of serum dilutions to be analyzed): Like the Microlab 625 advanced dual syringe diluter (Hamilton, Reno, NV, http://www.hamilton.com/) equipped with a 5.0 mL left syringe, a 2.5 mL right syringe, a 12 gauge Concorde CT probe dispense tip, the Microlab cable management system and a foot pedal. Alternatives are acceptable, including the Micromedic Digiflex™ (Titertek, Huntsville, AL, http://www.titertek.com/) equipped with 10.0-mL dispensing syringe, 2 mL sampling syringe, 0.75-mm tip, and foot pedal.

vii. Pipettes (for preparation of intermediate working standards and 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), 50-1,000 μL (catalog #4860 000.097), 100-5,000 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), 50-1,000 μL (box catalog # 22 49 135-1, reload catalog # 22 49 155-5), 100-5,000 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.

viii. 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 are acceptable 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.

ix. Tubes for storage of intermediate working stock standards: Like polypropylene 50-mL centrifuge tubes, Corning Incorporated #430290 (Corning, NJ, 14831. www.scienceproduct.corning.com). For use in storage of intermediate working stock standards. Equivalent tubes are acceptable which are shown by lot screening to be free of trace metal contamination. Clear plastics tend to have lowest trace metal contamination. Orange colored caps have also been used successfully for this method.

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

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

e. Sources of chemicals, gases, and regulators

i. Acid, hydrochloric acid: Veritas™ environmental grade, 30-35% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com). This is referred to as "concentrated" hydrochloric acid in this method write-up. It is approximately 12 molar in concentration. For use in preparation of intermediate working stock standards. Equivalent products must meet or exceed the purity specifications of this product for trace metals content.

ii. Acid, nitric acid: Veritas™ environmental grade, 68-70% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com). For use in diluent, rinse solution, intermediate working stock standards, and QC pool preparations. This is referred to as "concentrated" nitric acid in this method write-up. It is approximately 16 molar in concentration. Equivalent nitric acid must meet or exceed the purity specifications of this product for trace metals content.

iii. Ethyl alcohol (C_2H_5OH), USP dehydrated 200 proof (Pharmco Products, Inc.) or equivalent.

iv. Triton X-100™ ("Baker Analyzed," J.T. Baker Chemical Co. [www.jtbaker.com], or any source whose product is low in trace-metal contamination).

v. Argon gas (for plasma and nebulizer) and regulator: High purity argon (>99.999% purity, Specialty Gases Southeast, Atlanta, GA, www.sgsgas.com) for torch and nebulizer. Minimum tank source is a dewar of liquid argon (180-250L) but bulk tank for total building needs is preferred.

1. Regulator for argon (at dewar, if used): Stainless steel, single stage, specially cleaned regulator with 3,000 psig max inlet, 0-100 outlet pressure range, CGA 580 cylinder connector, and needle valve shutoff on delivery side terminating in a ¼" Swagelok connector. Part number KPRAFPF415A2AG10 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com), or equivalent. *# Spares = 1*.

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

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

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

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

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

viii. Standard, Gallium: Like 1,000 mg/L, item # PLGA2-2Y. (SPEX Industries, Inc., Edison, NJ, www.spexcsp.com), or equivalent. Used as an internal standard in diluent. Standards must be traceable to the National Institute for Standards and Technology and have low trace metal contamination.

ix. Standard, multi-element stock standard: Item number SM-2107-013 (High Purity Standards, Charleston, SC, http://www.hps.net/). This is a custom mix solution (see Table 3, Appendix B for concentrations). This solution is diluted to prepare the intermediate working standards, which are in turn diluted to prepare the working calibrators. This solution can be prepared in-house from NIST traceable single element stock solutions if necessary.

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

6) Preparation of reagents and materials.

a. Intermediate Ga internal standard solution:

    i. Purpose: Internal standards solution is prepared to be added to the sample diluent. During analysis, the internal standard will compensate for instrumental variations on the analyte signal.

    ii. Preparation: To prepare 2 L of 2% v/v HNO_3, 20 µg/mL Ga solution:

        1. If not previously dedicated to this purpose, acid wash a 2 L container (PP, PMP, or Teflon™) with dilute HNO_3 (e.g. 1% v/v HNO_3) and ≥18 MΩ·cm water (at least 3 times each). Verify cleanliness through analysis of rinsate. Dedicate to purpose, if possible.

        2. Partially fill the 100-mL volumetric flask with >18 MΩ·cm water.

        3. Carefully add 2 mL of concentrated nitric acid. Mix into solution.

        4. Add 0.2 mL of 10,000 µg/mL Ga standard. If initial Ga concentration is different adjust volume proportionally.

        5. Fill to mark (100 mL) and mix thoroughly.

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

b. Intermediate Triton X-100 solution

i. Purpose: Use of the intermediate solution reduces the need to frequently dissolve pure Triton X-100 (frequently an over-night process).

ii. Preparation: To prepare 2 L of 2% Triton X-100™ solution:

   1. If not previously dedicated to this purpose, acid wash a 2 L PP, PMP, or Teflon™ container with dilute HNO_3 (e.g. 1% v/v) and ≥18 MΩ·cm water (at least 3 times each). Verify cleanliness through analysis of rinsate. Dedicate to purpose, if possible.

   2. Partially fill the pre-cleaned 2 L bottle with >18 MΩ·cm water (approximately 1-1.5 L).

   3. Add 40 mL of Triton X-100™ and stir until completely dissolved. Use a pre-cleaned Teflon™ stir bar and stir plate if necessary.

      i. Carefully add 100 mL of double-distilled, concentrated nitric acid to the partially filled 2L bottle.

      ii. Fill to 2 L and mix thoroughly.

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

c. Diluent

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

   ii. Preparation: To prepare 2L of 10 μg/L Ga in 2% v/v concentrated nitric acid, 5% Ethyl Alcohol and 0.01% Triton X-100™:

      1. If not previously dedicated to this purpose, acid wash a 2 L container (PP, PMP, or Teflon™) with dilute HNO_3 (e.g. 1% v/v) and ≥18 MΩ·cm water (at least 3 times each). Verify cleanliness through analysis of rinsate. Dedicate to purpose, if possible.

      2. Partially fill (i.e. 70-80% full) the 2 L container with >18 MΩ·cm water.

      3. Add 40 mL concentrated nitric acid and mix.

      4. Add 100 mL Ethyl Alcohol and mix.

      5. Add 1 mL of 20 μg/mL Ga internal standard intermediate solution.

      6. Add 10 mL of the intermediate 2% Triton X-100™ solution and mix.

      7. Make up to 2 L with >18 MΩ·cm water.

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

d. ICP-DRC-MS rinse solution

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

ii. Preparation: To prepare 4L of 0.01% Triton X-100™, 2% (v/v) HNO_3, 5% ethyl alcohol and 0.5% v/v HCl:

   1. If not previously dedicated to this purpose, acid wash a 4 L container (PP, PMP, or Teflon™) with dilute HNO_3 (e.g. 1% v/v) and ≥18 MΩ·cm water (at least 3 times each). Verify cleanliness through analysis of rinsate. Dedicate to purpose, if possible.

   2. Partially fill the pre-cleaned 4 L bottle with >18 MΩ·cm water (approximately 2-3 L).

   3. Add 80 mL of concentrated HNO_3 and mix well.

   4. Add 200 mL ethyl alcohol and mix well.

   5. Add 20 mL of concentrated HCl and mix well.

   6. Add 20 mL of the 2% Triton X-100™/5% (v/v) nitric-acid intermediate stock solution and mix well.

   7. Fill to 4 L using >18 MΩ·cm water and mix well.

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

e. Standards, calibrators, and QC

  i. Multi-element stock calibration standard

   1. Purpose: All working intermediate calibrators are prepared by dilution of this stock standard, which contains all 3 elements of interest for this method, per the concentrations listed in Table 3 of Appendix B.

   2. Purchasing from vendors: The multi-element stock standard is typically purchased as a custom mixture (e.g. part number SM-2107-013 from High Purity Standards (Charleston, SC)). The vendor must provide documentation of traceability to the National Institute for Standards and Technology (NIST). Details of the HPS preparation of the multi-element stock standard is as follows (per statement on their literature):

   3. Storage: Store the solution at room temperature. Expiration date is as defined by vendor or 1 year from date of opening.

  ii. Diluent for intermediate working calibration standards

   1. Purpose: This diluent is used to dilute stock calibration standards down to the intermediate working calibration standard concentrations.

   2. Preparation: To prepare 2 L of 2% v/v HNO_3:

     a. If not previously dedicated to this purpose, acid wash a 2 L glass, PP, PMP, or Teflon™ volumetric flask with dilute HNO_3 (e.g. 1% v/v) and ≥18 MΩ·cm water (at least 3 times each). Verify cleanliness through analysis of rinsate. Dedicate to purpose, if possible.

b. Partially fill the 2 L volumetric flask with >18 MΩ·cm water (approximately 50% to 75% full).

c. Add 40 mL concentrated HNO_3.

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. Multi-element intermediate working calibration standards

1. Purpose: Use the intermediate working standard solutions 1-5 each day of analysis to prepare the final working calibrators that will be placed on the autosampler of the ELAN® ICP-DRC-MS.

2. Preparation: To prepare the volumes and concentrations of intermediate working standards per Table 4 in Appendix B:

   a. If not previously dedicated to this purpose, acid wash PP, PMP, or Teflon™ volumetric flasks with dilute HNO_3 (e.g. 1% v/v) and ≥18 MΩ·cm water (at least 3 times each). Verify cleanliness through analysis of rinsate. Label and dedicate to purpose, if possible.

   b. Partially fill the volumetric flasks with the 2% v/v HNO_3 diluent (approximately 50-75% full).

   c. Pipette the volumes of the multi-element stock standard listed in Table 4 of Appendix B into each of the labelled volumetric flasks.

   d. Dilute each volumetric flask to the mark with the 2% v/v HNO_3 diluent using a pipette for the final drops. Mix each solution thoroughly.

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

   f. Store at room temperature and label appropriately. Expiration is 1 year from the date of preparation. The final concentrations of the 3 elements are listed in Table 4, Appendix B.

iv. Working multi-element calibrators

1. Purpose: The working multi-element calibrators are dilutions of the intermediate working standards. Analysis of these calibrators provides each run with a signal to concentration response curve for each analyte in the method. The concentration of an analyte in a patient serum sample dilution is determined by comparing the observed signal from the dilution of the patient serum sample to the response curve from the working multi-element calibrators.

2. Preparation: Prepare the volumes and concentrations of the matrix-matched working standards per Table 7 in Appendix B immediately prior to analysis.

v. Base serum

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

2. Collection of serum: A mixture of multiple human serum sources purchased from Tennessee Blood Services, 807 Poplar Ave., Memphis, TN 38105. These serum were collected from different anonymous donors are used to approximate an average serum matrix.

3. Screening serum: Screen serum sources for metal content and choose sources, which reflect the low-normal population range (see Table 2, Appendix B for maximum suggested concentrations).

4. Preparation and storage:

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

   b. Dispense into smaller-volume, pre-screened tubes for use in the lab.

   c. Label appropriately and store frozen (e.g. ≤ -20° C). Serum stored at ≤ -20° C has been used successfully for up to 10 years as quality control material.

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

1. Purpose: Internal (or "bench") quality control (QC) materials are used to evaluate the accuracy and precision of the analysis process, and to determine if the analytical system is "in control" (is producing results that are acceptably accurate and precise). They are included in the beginning and at the end of each analytical run. These pools will need to be prepared periodically, as supply indicates, by spiking base serum. Prepare new pools far enough in advance so that both old and new pools can be analytes together for a period time (preferably at least 20 runs) before switching to the new quality control materials.

2. Content: The internal (or "bench") quality control (QC) materials used in this method are pooled human serum. The serum is spiked, when necessary, with inorganic, NIST-traceable standards to achieve desired concentrations. The analyte concentrations in the "low QC" are in the low-normal concentration range. The analyte concentrations in the "high QC" are in the high-normal concentration range.

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

   a. Collection of serum: Human serum can be purchased from blood services companies such as Tennessee Blood Services, 807 Poplar Ave., Memphis, TN 38105.

   b. Screening serum: Screen different bottles for metal content before mixing together to make 2 separate base serum pools (for preparing the low and high bench QC materials).

      i.   Keep serum at ≤ -20C whenever possible to minimize microbial growth.

    ii.   Choose base serum with concentrations in the low-normal population range (see Table 9, Appendix B) for low QC. Choose base serum with concentrations below the targeted high-normal population concentration for high QC.

c. Spiking of serum

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

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

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

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

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

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

d. Dispensing and storage of serum

    i.   Container types: Dispense serum into lot screened containers (i.e. 2 mL polypropylene cryovials). 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 benchtop automatic pipette in continuous cycling dispense mode. Carry out this process in a clean environment (e.g. a class 100 cleanroom area or hood is preferred to avoid contamination).

        1. Allow serum pool to reach room temperature before dispensing (to prevent temperature gradients possibly causing concentration gradients across the large number of vials being dispensed and to prevent condensation problems during labeling of vials).

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

        3. Check cleanliness of benchtop automatic pipette before use by analyzing 1-2% (v/v) HNO_3 which has been flushed through the

pipette with a portion of the same solution, which has not been through the pipette.

4. Approximately one hour before dispensing begins,

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

b. Also during this time, flush the pipette syringe(s) with serum from the pool to be dispensed. Place the ends of the tubing attached to both the sample and dispensing syringes into the carboy of serum so that serum won't be used up during this process. Secure both ends of tubing in the carboy with Parafilm so they will not come out during the flushing process.

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

iv. Homogeneity testing: After dispensing, check homogeneity of analyte concentrations in pool aliquots by analysis of every Nth sample dispensed (where N ~ 20 - 50 depending on the pool size). Sample more heavily from the beginning and the ending portions of the tubes dispensed (these are the regions where most homogeneity problems occur). 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: Store serum pools long term at ≤ -20°C. Short-term storage (up to several days) is permitted at refrigerated temperatures (~ 2-8°C).

f. Optimization solutions

i. DRC optimization:

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

a. Solutions for testing elimination of $^{36}Ar^{14}N_2$ plasma interference on ^{64}Zn.

i. Base serum in diluent (1+29)

ii. Base serum in diluent (1+29) + 90 µg/dL Zn

b. Solutions for testing elimination of $^{40}Ar^{25}Mg$ interference on ^{65}Cu:

i. Base serum in diluent (1+29)

ii. Base serum in diluent (1+29) + 90 µg/dL Cu

iii. Base serum in diluent + 3 mg/L Mg

iv. Base serum in diluent + 90 µg/dL Cu + 3 mg/L Mg

c. Solutions for testing elimination of $^{38}Ar^{40}Ca$ interference on ^{78}Se:

i. Base serum in diluent (1+29)

ii. Base serum in diluent (1+29) + 90 µg/L Se

iii. Base serum in diluent (1+29) + 100 mg/L Ca

iv. Base serum in diluent (1+29) + 90 µg/L Se + 100 mg/L Ca

2. Preparation: To prepare these DRC optimization solutions, use the 10 µg/L Ga, 2% v/v nitric acid, 5% Ethyl Alcohol, 0.01% Trion X-100^{TM} diluent as described in section 6 (same as used to prepare serum samples for analysis). Prepare different volumes by adding proportionally larger or smaller volumes of solution constituents. Interference concentrations can be prepared higher as needed by adjusting the volume of this spike. Keep interference spike volume small (<0.3 mL) using a high concentration stock solution (i.e. 1000 mg/L). The Ca spike has to be 0.5 mL because a stock solution at a concentration higher than 10,000 mg/L is not available. In this case, 0.5 mL of >18.2 M-Ohm-cm water is added to the non-spike solutions as well. Analyte concentrations can be made higher if needed for sensitivity reasons by preparing a higher concentration calibrator.

a. Solutions testing elimination of $^{36}Ar^{14}N_2$ plasma interference on ^{64}Zn.

i. Base serum in diluent (1 + 29)

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 0 as described in Table 7 (multiply volumes by 11).

ii. Base serum in diluent (1+29) + 90 µg/dL Zn

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 3 as described in Table 7 (multiply volumes by 11).

Store at room temperature and label appropriately. Expiration is 8 hours from preparation.

b. Solutions for testing elimination of $^{40}Ar^{25}Mg$ interference on ^{65}Cu:

i. Base serum in diluent (1 + 29)

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 0 as described in Table 7 (multiply volumes by 11).

ii. Base serum in diluent (1+29) + 90 µg/dL Cu

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 3 as described in Table 7 (multiply volumes by 11).

iii. Base serum in diluent + 3 mg/L Mg

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 0 as described in Table 7 (multiply volumes by 11).

2. Add 0.15 mL of 1000 mg/L Mg standard

iv. Base serum in diluent + 90 µg/dL Cu + 3 mg/L Mg

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 3 as described in Table 7 (multiply volumes by 11).

2. Add 0.15 mL of 1000 mg/L Mg standard

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

c. Solutions for testing elimination of $^{38}Ar^{40}Ca$ interference on ^{78}Se:

   i. Base serum in diluent (1+29)

      1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 0 as described in Table 7 (multiply volumes by 11).

      2. Add 0.5 mL of >18.2 MOhm water.

   ii. Base serum in diluent (1+29) + 90 µg/L Se

      1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 3 as described in Table 7 (multiply volumes by 11).

      2. Add 0.5 mL of >18.2 MOhm water.

   iii. Base serum in diluent (1+29) + 100 mg/L Ca

      1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 0 as described in Table 7 (multiply volumes by 11).

      2. Add 0.5 mL of 10,000 mg/L Ca

   iv. Base serum in diluent (1+29) + 90 µg/L Se + 100 mg/L Ca

      1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 3 as described in Table 7 (multiply volumes by 11).

      2. Add 0.5 mL of 10,000 mg/L Ca

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

7) Analytical instrumentation and parameters
(See Section 5 for details on hardware used, including sources)

a. Instrumentation and equipment setup:

   i. Configuration for liquid handling

   See Figure 1 in Appendix B for an example setup.

      1. Tubing for liquid sample uptake:

    a. Probe-to-peristaltic pump tubing: Use of a 'peristaltic to Teflon tubing adapter' is recommended to prevent damage to small i.d. tubing when making connections.

    b. Nebulizer-to-peristaltic pump tubing: It is recommended to use quick connection fittings on either end of the PFA tubing: a plug which pushes inside the liquid port of the nebulizer and a 'peristaltic to Teflon tubing adapter' to prevent damage to small i.d. tubing when making connections.

2. Spray chamber waste removal

Use of a 'peristaltic to Teflon tubing adapter' is recommended to prevent damage to small i.d. tubing when making connections.

    a. Between spray chamber and peristaltic tubing:
       i. Spray chambers with threaded connection: Use vendor-supplied threaded connector on base of chamber, connecting tubing directly to peristaltic pump tubing through a PEEK adapter or directly.
      ii. Spray chambers without threaded connection: Use of specialized push-on connectors available from various vendors (like UFT-075 from Glass Expansion, Pocasset, MA) are preferred for safety reasons to direct connection of PVC tubing (e.g. 1/8" i.d. x ¼" o.d.).

    b. Between peristaltic pump tubing and waste container: Connect 1/8" i.d. x ¼" o.d. PVC tubing to the white/black peristaltic pump tubing using a tubing connector (PerkinElmer item # B3140715). Connect the free end of the PVC tubing to the lid of the waste jug. The waste jug must be in a deep secondary containment tray in case of overflow (large enough to hold 110% of waste container volume).

3. Rinse solution for autosampler:

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

  ii. Gas delivery and regulation

1. ICP-MS modifications:
   a. Plastic tubing between mass flow controllers and dynamic reaction cell have been replaced with stainless steel. Stainless steel tubing is preferred between the reaction gas cylinder/regulator and the back of the ICP-MS instrument.

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

   a. Regulator for argon source (if a dewar): Set delivery pressure of this regulator at least 10 psi higher than the delivery pressure of the step-down regulator to allow for pressure drop across tubing that stretches to the instrument.
   b. Step down regulator (if source of argon is a bulk tank): Place this single stage regulator in the lab so that incoming argon pressure can be monitored and adjusted. Set delivery pressure to 10 psig above the delivery pressure of the filter regulator on the ICP-MS.
   c. Filter Regulator at ICP-MS: Single stage "argon regulator filter kit" supplied with the ICP-DRC-MS. Set the delivery pressure depending on the instrument setup:
      i. ELAN with a 0-60psi gauge on the filter regulator: 52±1 psi when plasma is running (need 0-150 psi regulator if using a PolyPro or PFA nebulizer made by Elemental Scientific Inc).
      ii. ELAN with a 0-150psi gauge on the filter regulator: 90-100 psi when plasma is running.
   iii. Chiller / heat exchanger: Refrigerated chiller (for ELAN® 6100 DRC^Plus instruments) or heat exchanger (for ELAN® DRC II instruments). For refrigerated chiller, set temperature control to 18°C.

b. Parameters for instrument and method: 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') and "high-normal" ('High QC') concentrations.

   In each analytical run, the analyst will test each of the two bench QC samples two times, subjecting them to the complete analytical process. Bench QC pool samples are analyzed first in the run after the calibration standards but before any patient samples are analyzed. This permits making judgments on calibration linearity and blank levels prior to analysis of patient samples. The second analysis of the bench QC pools is done after analysis of all patient samples in the run (typically 20-30

patient samples total when analyzing for all elements in the method) to ensure analytical performance has not degraded across the time of the run. If more patient samples are analyzed on the same calibration curve after the second run of the bench QC, all bench QC must be reanalyzed before and after the additional samples. For example, the schemes shown in Table 5 in Appendix B are both acceptable ways to analyze multiple consecutive "runs".

  ii. Reference materials: Use standard reference material (SRM, e.g. SRM 1598A) from the National Institute of Standards and Technology (NIST) to verify method accuracy. Use previously characterized samples from proficiency testing program or commercially-produced reference materials when NIST SRMs are unavailable.

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

b. Perform, evaluate, and report a run

  i. Starting the equipment for a run

1. Power on the computer, printer, and autosampler, and instrument computer controller.

2. Peristaltic pump: Set proper tension on peristaltic pump tubing.

3. Software: Start software for the ICP-MS and autosampler control.

4. Daily pre-ignition maintenance checks: Perform and document daily maintenance checks (e.g., Ar supply pressure, interface components cleanliness and positioning, interface pump oil condition, vacuum pressure, etc.).

5. Place probe in adequate volume of rinse solution: Send the autosampler probe to a rinse solution (e.g. autosampler rinse station).

6. Start the plasma

7. Start the peristaltic pump: Start the pump running slowly, making sure that the rotational direction is correct for the way the tubing is set up.

8. Warm-up time: Allow warm-up time suggested by the manufacturer for the ICP-MS (e.g. RF generator) after igniting the plasma. There will be another warm-up time (or "stability time") for the DRC later in this procedure.

9. Daily performance check: Perform and document a daily performance check and any optimizations necessary.

    Save new parameters to the "default.tun" and "default.dac" files.

10. Readying the instrument for quick-start analysis: Leave the plasma 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 serum matrix) can also be scheduled to occur at roughly a predetermined time. Accomplish this by setting up multiple sample analyses with extended rinse times (e.g. one analysis with a 1600s 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. Prepare working dilutions of serum materials close in time to analysis so that they are not more than 7 hours old when analyzed (see Appendix A, ruggedness test 5).

11. Software setup for analysis:

   a. Workspace (files and folders): Verify and 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. Serum 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 serum blank and serum calibrators (the "sblk" 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 "sblk" 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 6 in Appendix B.

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

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

   ii. Preparation of samples for analysis (See Tables 8a and 8b in Appendix B)

      1. Thaw serum samples; allow them to reach ambient temperature.

      2. If instrument stability in DRC mode requires it, prepare 50mL[+] of a junk serum sample to be analyzed repeatedly before the beginning of the run to achieve a stable analyte-to-internal standard ratio. Time to reach stability is instrument-specific but 1-1.5 hours is typical (~18 measurements of the 3 element serum method can be made in 1 hour). See Table 6 in Appendix B for example of setup in the Samples/Batch window.

NOTE: Selenium is not stable in the diluted sample for more than 7 hours. Diluted serum must be analyzed within 7 hours of preparation (see Appendix A, test 5 for details)

3. Prepare the following solutions into pre-labeled containers using the benchtop automatic pipette. See Table 7 for a summary.

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

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

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

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

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

    Original serum samples are not compromised by staying at room temperature during the workday. However, store long-term at ≤ -20 °C.

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

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

Monitor the analysis for the following:

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

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

    a. Water to be used in Aq Blank Checks and dilutions.
    b. Diluent before and after being flushed through the benchtop automatic pipette.

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

    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 can be evaluated to determine the readiness of the

system to begin analysis. Continual trending in this ratio indicates that unwanted instrument drift will occur within the run.

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

5. *Verify bench QC results within the acceptable limits.*

   If an analyte result for the beginning QC material(s) falls outside of the ± 3SD limits, then the following steps are recommended:

   a. Evaluate the blank results.

   b. Evaluate the reproducibility of the 3 replicates within the measurements.

   c. Evaluate the consistency of the internal standard across the measurements (esp. the calibrators).

   d. Evaluate calibration curves. If a particular 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 S4 can be removed from the curve. Follow-up repeated problems with calibration standards with appropriate corrective actions (e.g. re-preparation of intermediate working standards or troubleshooting instrument parameters).

   e. Prepare a fresh dilution of the failing QC material (same vial) and reanalyze it to see if the QC dilution was not properly made.

   f. Prepare a fresh dilution of the failing QC material (unused vial) and analyze it to see if the QC vial had become compromised.

   g. Prepare and analyze new working calibrators.

   h. Test a different preparation of intermediate working calibration standards.

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

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: Repeat for confirmation any sample having a concentration greater than the 1UB threshold (see Appendix B, Table 8).

   b. Dilution of a sample to within the calibration range: Repeat in duplicate with extra dilution any sample having a concentration greater than the highest calibration standard to bring the observed result within the concentration range of the calibrators (see Appendix B, Table 7).

c. Confirming proper washout after an elevated sample: When monitoring the analysis in real-time, if a sample concentration is greater than standard 5 + 10% (see Appendix B, Table 4), do the following to verify that the run is still in control for low concentration samples before proceeding with analysis.

    i. Stop run following elevated sample

    ii. Verify that the run is still in control for lower concentration samples before proceeding with analysis. Analyze 2 serum blank checks followed by a low bench QC washout check. If the low bench QC wash check is not in control (within ± 3SD limits), repeat these 3 check samples until washout is verified before proceeding with analysis.

        Example:
        3006 sblkchk Wash1
        3006 sblkchk Wash2
        LSXXXXX Wash

    iii. If the run is not verified in-contol for low concentration samples before the next samples are analyzed, see Section 8.b.vii.2. for directions.

v. Instrument cleaning between consecutive runs: In between consecutive runs, aspirate >18 Mohm·cm water through the sample introduction system for approximately 30 minutes at peristaltic pump speed similar to that used in the analysis. This assists cleaning out the sample introduction system to prevent clogging.

vi. Overnight operation or using auto stop: Ensure sufficient solution supply and waste collection during unattended operation. Turn on the AutoStop feature of the ICP-MS software. Delay the shutdown at least 10 minutes (use peristaltic pump speed approximately that of the method wash) to rinse the sample introduction system of serum matrix before turning off the plasma. It will be necessary to replace the sample peristaltic pump tubing the next day since it will have been clamped shut overnight. Enable "Auto Start/Stop" is on the "AutoStop" tab of the Instrument window.

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

    1. Electronic records: Transfer data electronically to the laboratory information system. When keyboard entry must be used, proofread transcribed data after entry.

        a. Export data from the ICP-MS software using "original conditions" or files and folders used during the analysis. Use descriptive report filenames (e.g. 2005-0714a_group55.txt). In the ICP-MS software under "Report Format" (METHOD window, REPORT tab) choose the "Use Separator" option, and under the "File Write" Section choose "Append."

        b. Move the generated .TXT data file to the appropriate subdirectory on the network drive where exported data are stored prior to import to the laboratory information management system.

        c. Import the instrument file into the laboratory information system with appropriate documentation (e.g. instrument ID, analyst, calibration standards lot number, and run or sample specific comments).

2. Paper records: Printed run sheets must be documented with

    i.   Analyst initials

    ii. Instrument ID

    iii. Date of analysis and run # for the day

viii. Analyst evaluation of run results:

1. Bench quality control: After completing a run, and importing the results into the laboratory information system, evaluate the run bench QC according to laboratory QC rules. 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. Quality Control Rules: The SAS program applies the division QC rules to the data as follows:

    i.   If both QC run means (low and high bench QC) are within 2Sm limits and individual results are within 2Si limits, then accept the run.

    ii. If 1 of the 2 QC run means is outside a 2Sm limit - reject run if:

        1. Extreme Outlier – Run mean is beyond the characterization mean +/- 4Sm

        2. 1 3S Rule - Run mean is outside a 3Sm limit

        3. 2 2S Rule - Both run means are outside the same 2Sm limit

        4. 10 X-bar Rule – Current and previous 9 run means are on same side of the characterization mean

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

        1. R 4S Rule – Within-run ranges for all pools in the same run exceed 4Sw (i.e., 95% range limit)

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

Abbreviations:

Si = Standard deviation of individual results (the limits are not shown on the chart unless run results are actually single measurements).

Sm = Standard deviation of the run means (the limits are shown on the chart).

Sw = Within-run standard deviation (the limits are not shown on the chart).

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

    i.   If only one analyte of the three fails bench QC, then report results for the other two, which passed bench QC.

    ii. If two analytes of the three fail bench QC, then no results are reportable from the run. Investigate the cause of QC failures and repeat the run with the appropriate corrective action.

2. Patient results:

    a. Concentrations outside of the normal range (refer to Appendix B, Figure 4 for flowchart for elevated concentration samples):

        i. Boundaries requiring confirmatory measurement:

            1. Results outside of the first (1LB or 1UB) or second (2LB or 2UB) boundaries.

               The concentrations assigned to 2LB, 1LB, 1UB and 2UB for an element is determined by study protocol but default concentrations are in Table 8 in Appendix B.

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

               b. Analyst reporting of results outside of the normal range: Report any patient results confirmed to be less than the second lower boundary (2LB) as an "unusually low result" or greater than the second upper boundary (2UB) as an "elevated result".

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

        ii. Concentrations requiring verification of washout: Following observation of a result greater than calibration standard 5 + 10%:

            1. If the run was verified to be in control for lower concentration samples before subsequent sample analysis was performed, no further action is required.

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

    b. Unacceptable measurement 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 8 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.

ix. Submitting final work for review: All analyses must undergo quality control and quality assurance review. After appropriately documenting the run in the laboratory

information system (e.g. sample and run QC, and run and sample comments), inform the first level reviewer of the completed work and submit any printed documentation.

9) Routine equipment maintenance and data backups

Maintenance activities will be documented in the instrument logbook.

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

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

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

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

i. Daily backups to secondary hard drive: Program automatic backups of the relevant computer files to occur each night onto a secondary hard drive 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: Serum multi-element values are reportable in the range between the method LOD and the highest calibrator times the maximum validated extra dilution. Above the highest calibrator, extra dilutions are made of the serum sample to bring the observed concentration within the calibration range.

b. Reference ranges (normal values): In this method, the 95% reference ranges (see Appendix B, Table 9) for these elements in serum fall within the range of the calibrators.

c. Action levels: There is no routine notification for levels of every analyte determined with this method. The protocol for supervisors reporting elevated results to medical personnel is defined according to the study protocol.

11) Method calculations

a. Method limit of detection (LOD): The method detection limits for elements in serum 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 serum 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 [6].

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

13) Summary Statistics and QC Graphs

See following pages

Summary Statistics and QC Chart for Serum Copper (ug/dL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
1504	174	24FEB15	06FEB17	102.68	4.56	4.4
1505	174	24FEB15	06FEB17	253.01	10.68	4.2
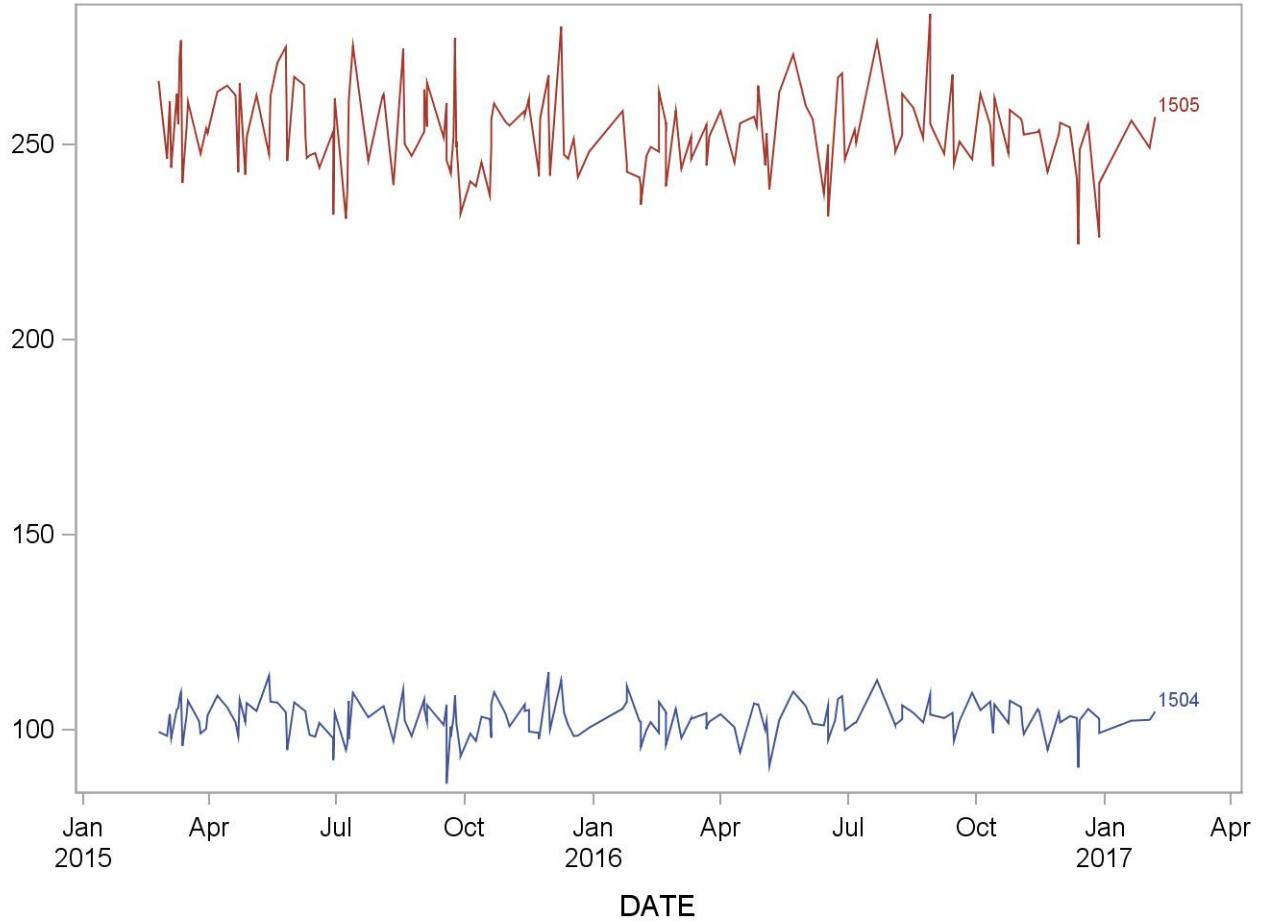
Summary Statistics and QC Chart for Serum Selenium (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
1504	177	24FEB15	06FEB17	128.81	4.82	3.7
1505	177	24FEB15	06FEB17	259.21	9.56	3.7
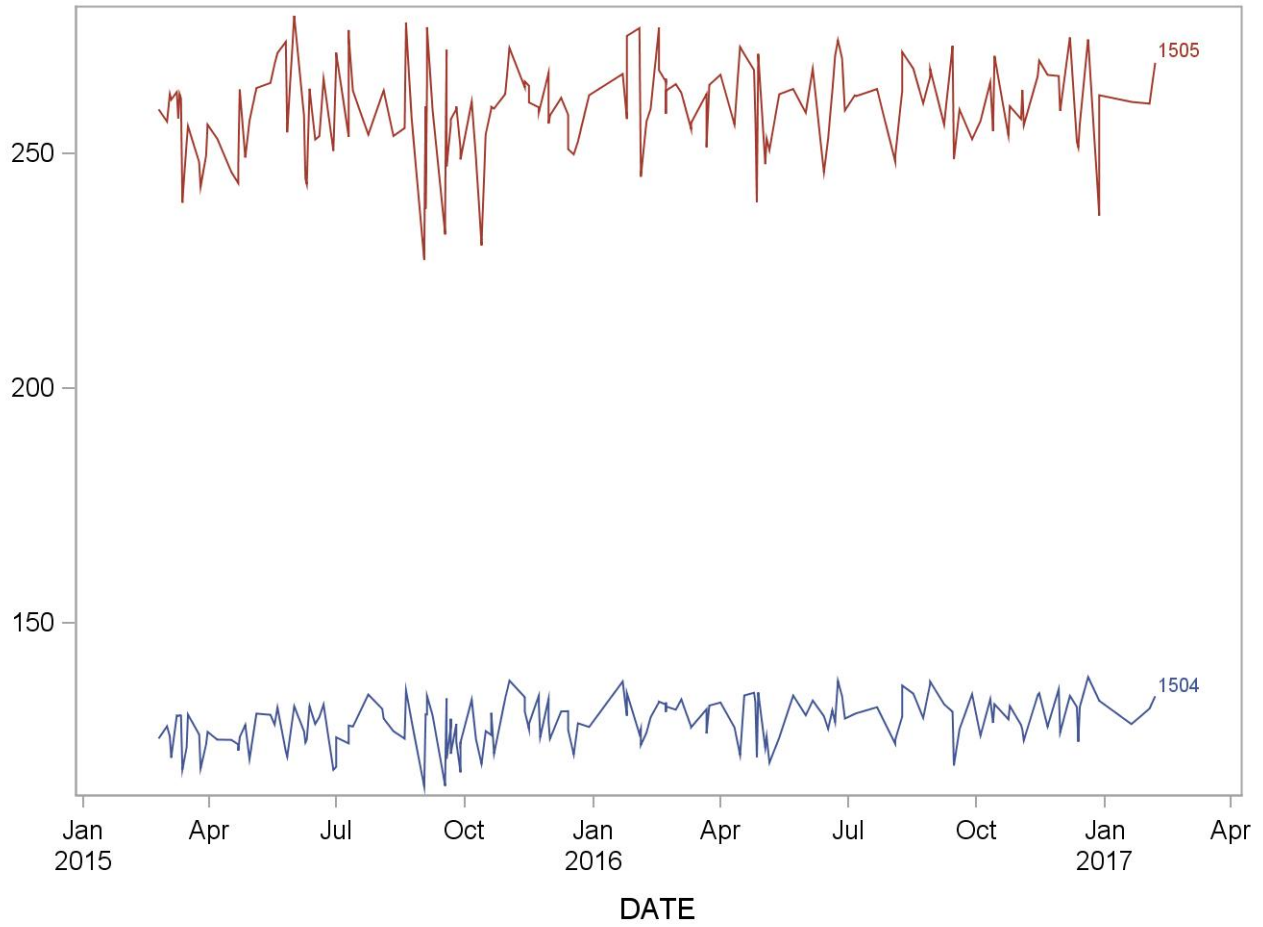
Summary Statistics and QC Chart for Serum Zinc (ug/dL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
1504	181	24FEB15	06FEB17	69.94	3.75	5.4
1505	181	24FEB15	06FEB17	254.42	13.02	5.1

References

1.   Thomas, R., Practical Guide to ICP-MS (Practical Spectroscopy). 2003, New York, NY: Marcel Dekker. 336.

2.   Tanner, S.D., Baranov, Vladimir I, Theory, Design, and Operation of a Dynamic Reaction Cell for ICP-MS. Atomic Spectroscopy, 1999. 20(2): p. 45-52.

3.   Tanner, S.D., V.I. Baranov, and D.R. Bandura, Reaction cells and collision cells for ICP-MS: a tutorial review. Spectrochimica Acta Part B-Atomic Spectroscopy, 2002. 57(9): p. 1361-1452.

4.   PerkinElmer SCIEX Instruments, ELAN DRC II Hardware Guide. 2001, Canada.
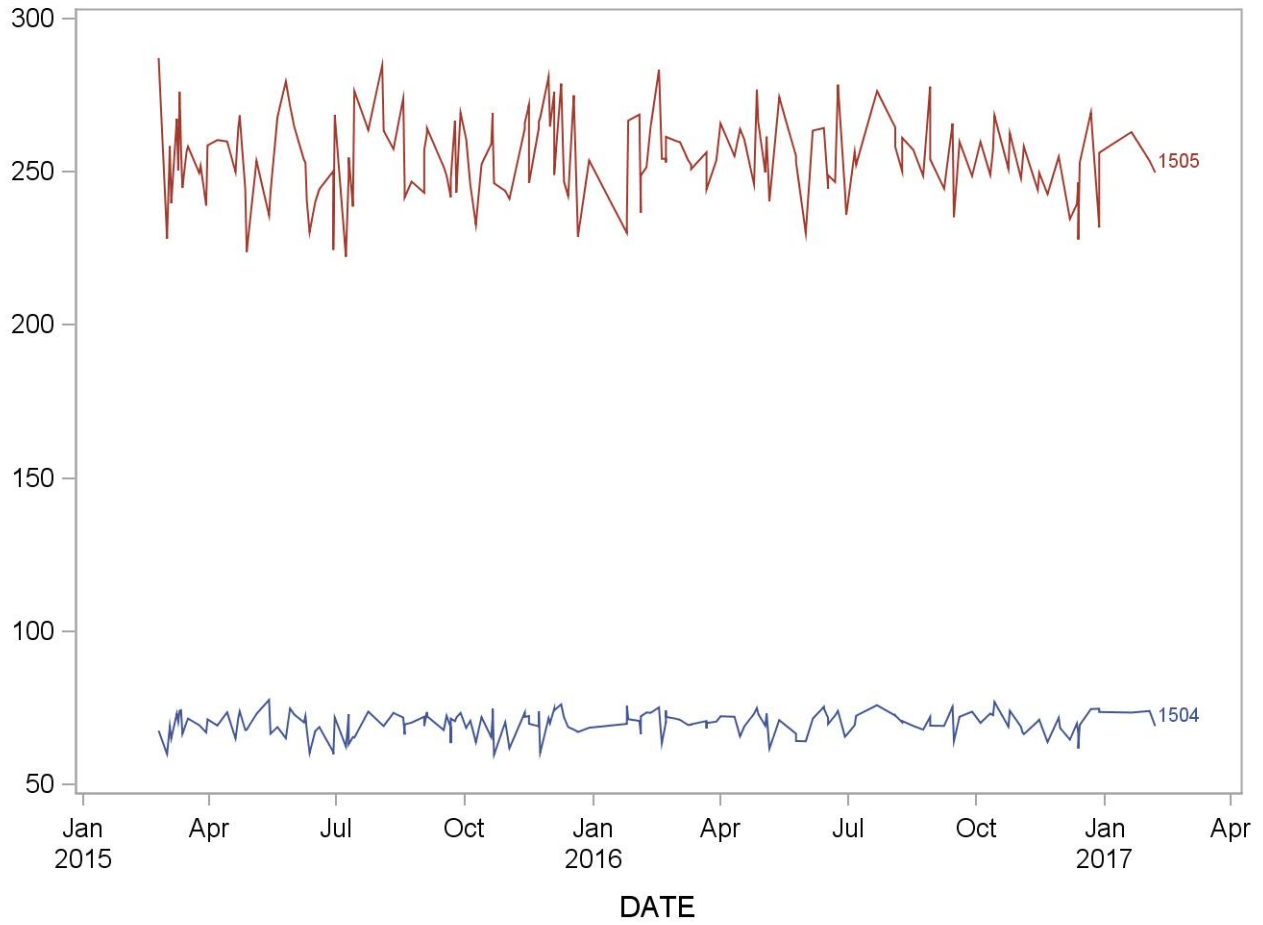
5.   Piraner, O., Serum vanadium ICPDRCMS_ITS004A. 2003, Centers for Disease Control and Prevention.

6.   Piraner, O., Serum manganese ICPDRCMS_ITS003A. 2003, Centers for Disease Control and Prevention.

7.   Piraner, O., Serum selenium ICPDRCMS_ITS002A. 2004, Centers for Disease Control and Prevention.

8.   Walters, P. J., Serum Copper Zinc ICP-DRC-MS_ITS001A. 2004, Centers for Disease Control and Prevention.

9.   Office of Health and Safety in the Division of Laboratory Sciences, Policies and Procedures Manual. 2015, Division of Laboratory Sciences (DLS), National Center for Environmental Health, Centers for Disease Control and Prevention, Public Health Service, Department of Health and Human Services, Centers for Disease Control and Prevention.

10.   Centers for Disease Control and Prevention (CDC) Radiation Safety Committee, CDC/ATSDR Occupational Health and Safety Manual (Radiation Safety chapter). Centers for Disease Control and Prevention, Public Health Service, Department of Health and Human ServicesCenters for Disease Control and Prevention.

11.   Tietz Textbook of Clinnical Chemistry, Third Edition, edited by Burtis C. A., Ashwood E. R., 1999

12.   Agency for Toxic Substance and Disease Regidtry (2000). Toxicological Profile for Selenium. Atlanta, GA: U. S. Department of Health and Human Services, Public Health Service.

13.   Lauwerys R.R. Chapter II: Biological monitoring of exposure to inorganic and organometallic substances, In: Industrial Chemical Exposure: Guidelines for Biological Monitoring, Biomedical Publications, pp. 9-50, 1983

14.   Handbook on Metals in Clinical and Analytical Chemistry, edited by Seiler H.G., Sigel A., Sigel H., Marcel Dekker, Inc, 1994

15.   Niskar AS, Paschal DC, Kieszak SM, Flegal KM, Bowman B, Gunter EW, Pirkle JL, Rubin C, Sampson EJ, McGeehin M. Serum selenium levels in the US population: Third National Health and Nutrition Examination Survey, 1988-1994. Biol Trace Elem Res 2003 Jan; 91(1): 1-10

Appendix A. Ruggedness testing results.

Parameter test#1: Evaluate the impact on analysis results if the set RF power is increased to 1600W (instrument maximum) or decreased to 1150W (by 20%).

Test details:

1. Three different PF power settings were tested in separately prepared, consecutive runs on the instrument without turning off the plasma. At least 15 minutes stabilization time was allowed between each run after the RF power was changed. "Junk serum" samples (20) were analyzed between the beginning and ending QC of each run. All other method parameters were kept per method.

2. Run #1 (method default, 1450W); Run #2 (decreased RF power by 20% to 1150W); Run #3 (increased RF power to instrument maximum, 1600W); Run #4 (increased RF power to instrument maximum, 1525W).

Ruggedness parameter #1 test results				
QC pool ID	RF power tested	Zn (µg/dL)	Cu (µg/dL)	Se (µg/L)
LS-03601b	characterized mean 2SD range	50.7 41.9 - 59.5	64.9 61.9 – 67.9	75.0 66.7 – 83.3
	1150w (reduced)	52.5	63.1	75.6
	1450w (per method)	49.0	62.7	70.1
	1525w (increased)	43.3	63.4	75.7
	1600w (increased)	54.1	63.9	75.6
HS-03601b	characterized mean 2SD range	175 142 – 209	203 191 – 215	144 130 – 157
	1150w (reduced)	178	197	145
	1450w (per method)	168	196	146
	1525w (increased)	157	201	145
	1600w (increased)	178	199	149

Conclusion: Results are not compromised by changes in RF power within the range of 1150W to 1600W.

Appendix A. Ruggedness testing results (continued).

Parameter test#2: Evaluate the impact on analysis results if the Cell Gas Flow Rate is increased or decreased by 20% for the analytical run.

Test details:
1. Three different Cell Gas Flow Rates were tested in separately prepared, consecutive runs on the instrument without turning off the plasma. At least 15 minutes stabilization time was allowed between each run after the axial field voltage was changed. "Junk serum" samples (20) were analyzed between the beginning and ending QC of each run. All other method parameters were kept per method.
2. Run #1 (method default = 0.5mL/min); Run #2 (decreased Cell Gas Flow Rate by 20% to 0.4mL/min); Run #3 (increased Cell Gas Flow Rate by 20% to 0.6mL/min).

Ruggedness parameter #2 test results				
QC pool ID	cell gas flow rate tested	Zn (µg/dL)	Cu (µg/dL)	Se (µg/L)
LS-03601b	characterized mean 2SD range	50.7 41.9 - 59.5	64.9 61.9 – 67.9	75.0 66.7 – 83.3
	0.40 ml/min (reduced)	49.5	65.3	80.7
	0.50 ml/min (per method)	49.5	67.6	73.8
	0.60 ml/min (increased)	47.8	63.4	75.6
HS-03601b	characterized mean 2SD range	175 142 – 209	203 191 – 215	144 130 – 157
	0.40 ml/min (reduced)	167	204	152
	0.50 ml/min (per method)	169	205	146
	0.60 ml/min (increased)	171	203	147

Conclusion: Results are not compromised by changes in cell gas flow rate within the range tested (0.40-0.60 mL/min).

Appendix A. Ruggedness testing results (continued).

Parameter test#3: Evaluate the impact on analysis results if the RPq is increased or decreased by 20% for the analytical run.

Test details:
1. Three different RPq settings were tested in separately prepared, consecutive runs on the instrument without turning off the plasma. At least 15 minutes stabilization time was allowed between each run after the axial field voltage was changed. "Junk serum" samples (20) were analyzed between the beginning and ending QC of each run. All other method parameters were kept per method.
2. Run #1 (method default DRC RPq: 0.56); Run #2 (decreased DRC RPq 20%: 0.70); Run #3 (increased DRC RPq 20%: 0.84).

Ruggedness parameter #3 test results				
QC pool ID	RPq tested	Zn (µg/dL)	Cu (µg/dL)	Se (µg/L)
LS-03601b	characterized mean 2SD range	50.7 41.9 - 59.5	64.9 61.9 – 67.9	75.0 66.7 – 83.3
	DRC RPq:0.56 (reduced by 20%)	52.5	63.1	70.8
	DRC RPq:0.70 (per method)	49.0	62.7	70.1
	DRC RPq:0.84 (increased by 20%)	54.1	63.9	75.9
HS-03601b	characterized mean 2SD range	175 142 – 209	203 191 – 215	144 130 – 157
	DRC RPq:0.56 (reduced by 20%)	178	197	129
	DRC RPq:0.70 (per method)	168	196	131
	DRC RPq:0.84 (increased by 20%)	178	199	145

Conclusion: Results are not compromised by changes in RPq within the range of 0.56 – 0.84.

Appendix A. Ruggedness testing results (continued).

Parameter test#4: Evaluate the impact on analysis results if the axial field voltage (AFV) is increased or decreased by 20% for the analytical run.

Test details:
1. Three different DRC AFV were tested in separately prepared, consecutive runs on the instrument without turning off the plasma. At least 15 minutes stabilization time was allowed between each run after the axial field voltage was changed. "Junk serum" samples (20) were analyzed between the beginning and ending QC of each run. All other method parameters were kept per method.
2. Run #1 (method default DRC AFV = 450); Run #2 (decreased DRC AFV to 360); Run #3 (increased DRC AFV to 500).

Ruggedness parameter #4 test results				
QC pool ID	axial field voltage tested	Zn (µg/dL)	Cu (µg/dL)	Se (µg/L)
LS-03601b	characterized mean 2SD range	50.7 41.9 - 59.5	64.9 61.9 – 67.9	74.9 66.7 – 83.3
	AFV-360 (reduced)	46.9	61.5	72.8
	AFV-360 (per method)	47.2	64.0	75.0
	AFV-500 (increased)	48.6	63.5	74.1
HS-03601b	characterized mean 2SD range	175 142 – 209	203 191 – 215	144 130 – 157
	AFV-360 (reduced)	163	195	142
	AFV-360 (per method)	170	205	147
	AFV-500 (increased)	168	200	146

Conclusion: Results are not compromised by changes in the axial field voltage within the range of 360 to 500V.

Appendix A. Ruggedness testing results. (continued)

Parameter test #5: Method descriptions and SOP assume preparation and analysis on same day. Evaluate the impact on analysis results if the analytical run is prepared to analyze but circumstances do not allow for analysis to occur until 24 or 48 hours later.

Test details (Part 1):
1. Three separate run sets (A, B, and C) were prepared at one sitting from the same starting materials. Set 'A' was analyzed immediately. Set's 'B' and 'C' were stored at room temperature for 24 and 48 hours, respectively before analysis. "Junk serum samples (20) were analyzed between the beginning and ending QC of each run, making each a normal length run. All other method parameters were kept per method. Results in table are average of beginning and ending QC.
2. On day two, a fresh run set ("D") was prepared and analyzed immediately for comparison to results from set "B" (Run 2 of the day. Results not shown).
3. On day three, another fresh run set ("E") was prepared and analyzed immediately for comparison to results from set "C" (Run 2 of the day. Results not shown).

Ruggedness parameter #5 test results (part 1)				
QC pool ID	**time from preparation**	**Zn (µg/dL)**	**Cu (µg/dL)**	**Se (µg/L)**
LS-03601b	*characterized mean* *±2SD range* *±3SD range*	50.7 41.9 – 59.5 37.5 – 63.9	64.9 61.9 – 67.9 60.4 – 69.4	74.9 66.7 – 83.3 52.5 – 87.4
	fresh preparation	47.2	65.7	74.7
	after 24 hours	51.9	67.1	***102 (150, 54.5)***
	after 48 hours	51.0	66.2	***57.1 (123, -8.8)***
HS-03602b	*characterized mean* *±2SD range* *±3SD range*	175 142 – 209 126 - 225	203 191 – 215 185 - 221	144 130 – 157 124 – 164
	fresh preparation	160	203	143
	after 24 hours	167	203	145
	after 48 hours	174	207	***130 (98.2, 161)***

Conclusion: The serum ICP-MS method is rugged for Zn and Cu to delays in analysis of samples after preparation for up to 48 hrs. and not rugged for Se to delay in analysis

Appendix A. Ruggedness testing results. (continued)

of samples after preparation for even 24 hrs. Suggested maximum amount of time from sample prep to end of the run is 450 min, which consists of 3 analytical runs.

Parameter test #5:

Test details (Part 2): Due to the observations in test one for selenium, a shorter time frame was examined in part two of this test.

1. Seven preparations of the low bench QC serum material were made at the beginning of the experiment. Each of these seven preparations were 4x the normal preparation volume (4 preparations into each vial).
2. Four consecutive runs of the serum method were then carried out. Each run included
   a. blanks, calibrators, and run judge QC (beginning and ending) which were prepared immediately prior to the beginning of each run.
   b. Seven preparations of the low bench QC which were prepared immediately prior to the beginning of each run.
   c. Measurements of the seven preparations of the low bench QC pool which were prepared before the first run (these were alternated with the freshly prepared low bench QC sequentially throughout the run).

Ruggedness parameter #5 test results (part 2)				
QC pool ID	axial field voltage tested	Zn (µg/dL)	Cu (µg/dL)	Se (µg/L)
LS-03601b	characterized mean 2SD range 3SD range	50.7 41.9 – 59.5 37.5 – 63.9	64.9 61.9 – 67.9 60.4 – 69.4	74.9 66.7 – 83.3 52.5 – 87.4
	Run 1 (up to 139 min elapsed)	41.8	58.3	72.5
	Run 2 (up to 303 min elapsed)	43.1	60.0	71.3
	Run 3 (up to 427 min elapsed)	51.4	65.9	71.0
	Run 4 *(up to 576 min elapsed)*	43.5	59.1	*54.7*

Conclusion: The serum ICP-MS method is rugged for Zn and Cu to delays in analysis of samples after preparation for up to 48 hrs (see part 1). The method is only rugged to delays in analysis for selenium for up to approximately 7 hours (one 90 patient sample run, or two 40 patient sample runs).

Appendix A. Ruggedness testing results. (continued)

Parameter test #6: Evaluate the impact on observed concentration if an extra dilution is performed on the sample relative to the calibration standards.

Test details: A large serum sample was spiked to elevated concentrations using single element standards, and mixed well. The spiked sample was then prepared for analysis on multiple days to dilution levels of 2x, 5x, 10x, and 20x using 18Mohm water as the makeup liquid.

Ruggedness parameter #6 test results

SCU (ug/dL)

Dilution level	4/20/12 b	5/8/12 b	5/9/12 b	6/6/12 b	SCU Average	SCU STDEV	SCU Normalized Average and Relative STDEV
No Extra	353	348	347	350	350	3	1.00 ± 0.01
2x dilution	363	355	396	354	367	20	1.05 ± 0.05
5x dilution	414	342	343	366	366	34	1.05 ± 0.09
10x dilution	378	345	342	347	353	17	1.01 ± 0.05
20x dilution	334	313	269	337	313	31	0.90 ± 0.10

SSE (ug/L)

Dilution level	4/20/12 b	5/8/12 b	5/9/12 b	6/6/12 b	SSE Average	SSE STDEV	SSE Normalized Average and Relative STDEV
No Extra	367	340	347	343	349	12	1.00 ± 0.03
2x dilution	361	343	387	331	355	24	1.02 ± 0.07
5x dilution	392	326	337	316	343	34	0.98 ± 0.10
10x dilution	347	316	340	262	316	38	0.91 ± 0.12
20x dilution	283	272	268	191	253	42	0.72 ± 0.17

Appendix A. Ruggedness testing results. (continued)

SZN (ug/dL)

Dilution level	4/20/12 b	5/8/12 b	5/9/12 b	6/6/12 b	SZN Average	SZN STDEV	SZN Normalized Average and Relative STDEV
No Extra	343	335	334	336	337	4	1.00 ± 0.01
2x dilution	348	340	382	338	352	20	1.05 ± 0.06
5x dilution	390	326	330	351	349	29	1.04 ± 0.08
10x dilution	344	320	329	340	333	11	0.99 ± 0.03
20x dilution	293	283	260	340	294	34	0.87 ± 0.12

Conclusion: SCU results are not unacceptably affected by the matrix difference in the dilution with water when performing a 2x, 5x or 10x dilution. However, the average observation of the 20x dilution is affected more than is acceptable (≥10% bias with noticeable impact on reproducibility).

SSE results are not unacceptably affected by the matrix difference in the dilution with water when performing a 2x or 5x dilution. However, the average observation of the 10x and 20x dilutions are affected more than is acceptable (≥10% bias with noticeable impact on reproducibility).

SZN results are not unacceptably affected by the matrix difference in the dilution with water when performing a 2x, 5x or 10x dilution. However, the average observation of the 20x dilution is affected more than is acceptable ≥10% bias with noticeable impact on reproducibility).

In summary, an extra dilution up to 10x with water was successful for SCU and SZN with less than a 10% impact on the observed concentration. However only up to a 5x extra dilution with water was acceptable for SSE.

Appendix B. Tables and figures.

Table 1. Instrument and method parameters	
Instrument: PerkinElmer ELAN DRC^{Plus} or DRC II ICP-MS ESI SC4 autosampler	
Optimization window parameters	
RF power	1.45 KW
plasma gas flow (Ar)	15 L/min
auxiliary gas flow (Ar)	1.2 L/min
nebulizer gas flow (Ar)	0.80 – 1.0 L/min (optimized as needed for sensitivity)
ion lens voltage(s)	AutoLens (optimized as needed for sensitivity)
QRO, CRO, CPV, AFV,Discriminator Threshold	Optimized per instrument by service engineer, or advanced user.
Parameters of x-y alignment, nebulizer gas flow, AutoLens voltages, mass calibration, and detector voltages are optimized regularly. Optimization file name = default.dac.	
Configurations window parameters	
cell gas changes pause times	pressurize delay (From Standard to DRC mode) = 30 exhaust delay (From DRC to Standard mode) = 30 flow delay (Gas changes while in DRC mode) = 25 channel delay (Gas channel change in DRC mode) = 25
File names and directories	
method file names	Serum multi panel_DLS3006.8_sblk.mth Serum multi panel_ DLS3006.8_aqblk.mth
dataset	Create a new dataset subfolder each day. Name as "2006-0718" for all work done on July 18, 2006
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: include date, instrument, and group being analyzed in file name (i.e. 20060724a_DRCC_HM-0364.txt)*
Method parameters	
Method parameters: timing page (see Figure 2a in the Appendix)	
sweeps/reading	90

readings/replicate	1
replicates	3
enable qc checking	on
isotopes monitored and internal standard associations (exact mass)	*use ^{71}Ga as an internal standard* ^{64}Zn (63.9291), ^{65}Cu (64.9278), ^{71}Ga (70.9247), ^{78}Se (77.9173)
dwell times	30 ms for ^{64}Zn, ^{65}Cu, ^{71}Ga (70.9247), ^{78}Se (77.9173)
scan mode	Peak Hopping for all isotopes (1 MCA channel)
drc channel a gas flow rate	Ammonia (5-7 psig delivery pressure) 0.5 L/min * (*Optimized per instrument)
RPa	0 for all isotopes
RPq	0.7 for all isotopes
Method Parameters: processing page (see Figure 2b in the Appendix)	
detector mode	pulse (see Section 6.f.ii)
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 3c in the Appendix)	
equations	On ^{64}Zn, use "-0.035297 * Ni60" On ^{78}Se, use "-0.030461 * Kr83"
Method Parameters: calibration page (see Figure 2d in the Appendix)	
calibration type	external std.
curve type	simple linear
sample units	μg/L
calibration standard concentrations	Zn (µg/dL): 3, 9, 30, 90, 300 Cu (µg/dL): 3, 9, 30, 90, 300 Se (µg/L): 3, 9, 30, 90, 300
Method Parameters: sampling page (see Figure 2e in the Appendix)	
"peristaltic pump under computer control"	On
sample flush	~40s at typically -10.8 rpm (optimize time so that solution reaches nebulizer before Read Delay begins)
read delay	45s at typically -8.1 rpm (optimize time so that signal is stable before analysis begins)
wash	60s at typically -10.8 rpm (optimize time as needed for effective washout of unusually elevated samples)

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."

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
Cu	>330 µg/dL	120 s
Se	>330 µg/L	120 s
Zn	>330 µg/dL	120 s

autosampler locations of blanks and standards	*For calibration curve (points to serum blank)* Serum multi panel_DLS3006.8_sblk.mth Serum Blank and Calibration Stds 1 – 5 in autosampler positions 101 – 106. *For QC and patient sample analysis (points to aqueous blank)* Serum multi panel_DLS3006.8_aqblk.mth Aqueous Blank in autosampler position 109. See figures 3a through 3e in Appendix B for other default autosampler settings.

Table 2. Suggested maximum analyte concentrations for base serum.	
Analyte	Concentration (µg/L)
Zn	800 (80 ug/dL)
Cu	1100 (110 ug/dL)
Se	130

Table 3. Concentrations of analytes in the multi-element stock standard from High Purity Standards.

Analyte	stock standard concentrations (mg/L) High Purity Standards Item # SM-2107-013 (2% HNO_3)
Cu	300
Zn	300
Se	30

Table 4. Preparation of multi-element intermediate working standards (for calibrators).

Standard #	Units	1	2	3	4	5
Vol of flask (mL)		500	200	100	100	100
Vol spike of int. stock std. (mL)		0.050	0.060	0.10	0.30	1.0
		Concentrations				
Zn	ug/L ug/dL*	30 3	90 9	300 30	900 90	3,000 300
Cu	ug/L ug/dL*	30 3	90 9	300 30	900 90	3,000 300
Se	ug/L	3	9	30	900	300
* Use ug/dL units for Zn and Cu in the ELAN software and for reporting.						

Appendix B (continued).

Table 5. Acceptable ways to perform two consecutive analytical runs, bracketing with bench quality control samples.	
Setup 1*	**Setup 2** (typical)*

Run #1
     calibration standards
     low bench QC
     high bench QC
     patient samples
     low bench QC
     high bench QC
 	
 Run #2
     low bench QC
     high bench QC
     patient samples
     low bench QC
     high bench QC
 	
 * Use >18 Mohm·cm water
 to rinse the system
 for ~30 min.
 between the two runs.
	Run #1
	     calibration standards
	     low bench QC
	     high bench QC
	     patient samples
	     low bench QC
	     high bench QC
	
	 Run #2
	     calibration standards
	     low bench QC
	     high bench QC
	     patient samples
	     low bench QC
	     high bench QC
	
	 * Use >18 Mohm·cm water
	 to rinse the system
	 for ~30 min.
	 between the two runs.

Appendix B (continued).

Table 6. A typical SAMPLE/BATCH window.			
AS Location*	Sample ID	Measurements Action	Method
5	DRCstability1	Run sample	. . ._sblk.mth
5	DRCstability2	Run sample	. . ._sblk.mth
5	DRCstability3	Run sample	. . ._sblk.mth
5	DRCstability4	Run sample	. . ._sblk.mth
Continue DRC stability samples . . .			
5	DRCstability9	Run sample	. . ._sblk.mth
5	DRCstability10	Run sample	. . ._sblk.mth
100	Sblkchk1	Run blank, standards, and sample **	. . ._sblk.mth
101	Sblkchk2	Run sample	. . ._sblk.mth
127	Aq Blk Check	Run blank and sample ¥	. . ._aqblk.mth
138	L Bench QC	Run sample	. . ._aqblk.mth
134	H Bench QC	Run sample	. . ._aqblk.mth
146	Sample 1	Run sample	. . ._aqblk.mth
147	Sample 2	Run sample	. . ._aqblk.mth
148	Sample 3	Run sample	. . ._aqblk.mth
.	.	.	.
.	.	.	.
.	.	.	.
139	L Bench QC	Run sample	. . ._aqblk.mth
135	H Bench QC	Run sample	. . ._aqblk.mth
* The exact autosampler positions of QCs and patient samples do not have to be those shown above. ** When executing this row, the ELAN will first analyze the serum blank at AS position 101, then standards 1-5 at autosampler positions 102-106, then the "sblkchk1" sample at A/S position 100. The sampling information about AS positions 101-106 are stored in the "sblk" method file. ¥ When executing this row, the ELAN will first analyze the aqeous blank at AS position 109, then the "Aq Blk Check" at AS position 20. The sampling information about AS positions 109 is stored in the "aqblk" method file.			

Appendix B (continued).

| **Table 7. Preparation of samples, working standards, and QC materials for analysis** |

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

These directions are written with the expectation of a 5,000 μL syringe on the left side and a 250 μL syringe on the right side of the benchtop automatic pipettor.

Dilution ID	Water (μL)	Base Serum (μL)	AQ Intermediate Working Standard (μL)	Patient or QC Serum sample (μL)	Diluent * (μL)
Working Calibrators (S0-S5) And SBlkchk (S0)	-	150 x 1	150 x 1	-	4,200 (2,100 x 2)
AQ Blank	300 (150 x 2)	-	-	-	4,200 (2,100 x 2)
Patient Serum or Serum-Based QC	150 x 1	-	-	150 x 1	4,200 (2,100 x 2)
Patient Serum *2x Dilution* [H]	225 (225 x 1)	-	-	75 x 1	4,200 (1,400 x 3)
Patient Serum *5x Dilution* [H]	540 (150 x 3, 90 x 1)			60 x 1	8,400 (1,680 x 5)
Patient Serum *10x Dilution* [H]	570 (150 x 3, 120 x 1)	-	-	30 x 1	8,400 (1,680 x 5)

* By splitting the dispense step of diluent into two or more portions, liquids pulled up into the right pipette tip are flushed out more completely. For example, when preparing a working serum blank (S0) above, do the preparation in 2 steps: in step 1, dispense 150 μL intermediate working S0 + 2100 μL diluent; in step 2, dispense 150 μL base serum + 2100 μL diluent.

[H] Extra dilution is performed on serum samples whose concentration is greater than the concentration of the highest calibrator.

Maximum extra dilution (see ruggedness test results in Appendix A)
- 5x dilution for Se
- 10x dilution for Zn and Cu

Any extra dilution within these limits can be prepared as long as the 14:15 ratio of diluent to total dilution volume is maintained. Use of the lowest possible dilution level is preferred to minimize differences between the calibrators and the samples (i.e. 2x dilution is preferred over 10x if 2x is sufficient to dilute analyte into the documented linearity range).

Appendix B (continued).

Table 8. Boundary concentrations and replicate range maximums for serum.						
Analyte (units)	**Lower Boundaries**		**Upper Boundaries**		**Range Maximum ("Lim Rep Delta") †**	**Highest Concentration Validated for Washout**
	2LB*	**1LB***	**1UB***	**2UB***		
Zn (μg/dL)	35	35	120	240	17	330
Cu (μg/dL)	50	50	300	600	20	330
Se (μg/L)	45	45	165	330	20	330
* The concentrations assigned to these boundaries is determined by study protocol but default concentrations are listed in this table. † Range maximum is the range of the three replicate readings for a single sample analysis. This value is also called the "Lim Rep Delta" in the database which handles data for the Inorganic Toxicology and Nutrition Branch.						

Table 9. Reference ranges for serum concentrations; Se in μg/L; Zn and Cu in μg/dL.	
Analyte	**Reference Ranges**
Zn	70-120 [10]
Cu	20-302 [10]
Se	95-165 [11], [14]

Appendix C: Help Sheets

Reagent Preparation (page 1 of 2)

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

Rinse solution
(0.01% Triton X-100, 5% ethyl alcohol, 2% (v/v) HNO$_3$, 0.5% (v/v) HCl)

1. Partially fill the pre-cleaned 4 L bottle with >18 MΩ·cm water (approximately 2-3 L).
2. Add 80 mL of concentrated HNO$_3$ and mix well.
3. Add 200 mL ethyl alcohol and mix well.
4. Add 20 mL of concentrated HCl and mix well.
5. Add 20 mL of the 2% Triton X-100 and mix well.
6. Fill to 4 L using >18 MΩ·cm water and mix well.

Sample diluent
(10 ug/L Ga, 0.01% Triton X-100, 5% ethyl alcohol, 2% (v/v) HNO$_3$)

1. Partially fill (i.e. 70-80% full) the 2 L container with > MΩ·cm water.
2. Add 40 mL concentrated nitric acid and mix.
3. Add 100 mL ethyl alcohol and mix.
4. Add 1 mL of 20 μg/mL Ga internal standard intermediate solution.
5. Add 10 mL of the intermediate 2% Triton X-100 solution and mix.
6. Fill to 2 L with >18 MΩ·cm water and mix well.

5% (v/v) HNO$_3$

1. Partially fill a 2 L bottle with 18 MΩ·cm water.
2. Add 100 mL of concentrated nitric acid.
3. Fill to 2 L using >18 MΩ·cm water

Appendix C: (continued)

Reagent Preparation (page 2 of 2)

2% Triton X-100 in 5% (v/v) HNO$_3$

1. Partially fill a 2 L bottle with 18 MΩ·cm water.
2. Add 40 mL of Triton X-100.
3. Add 100 mL of concentrated nitric acid.
4. Fill to 2 L using >18 MΩ·cm water.
5. Allow to dissolve overnight (or add a Teflon magnetic stirring bar and stir on stirrer until dissolved).
6. Mix well by gently inverting several times.

20 µg/ml Ga internal standard solution

1. Partially fill the 100-mL volumetric flask with >18 MΩ·cm water.
2. Carefully add 2 mL of concentrated nitric acid. Mix into solution.
3. Add 0.2 mL of 10 µg/mL Ga standard. If initial Ga concentration is different, adjust volume proportionally.
4. Fill to mark (100 mL) and mix thoroughly.

Daily solution (1 µg/L) in 2% (v/v) HNO$_3$

1. Partially fill a 1 L volumetric flask with 18 MΩ·cm water.
2. Add 1 mL of High Purity Standard SM-2107-018 (or current lot #)
3. Add 20 mL of concentrated nitric acid.
4. Fill to 1 L using >18 MΩ·cm water.
5. Mix well by gently inverting several times.

DRC Stability Solution (Junk Serum)

1. Add 33 mL of diluent into a plastic bottle
2. Add 1.2 mL of 18 MΩ·cm water
3. Add 1.2 mL of junk serum
4. Repeat until you've reached your desired volume

Appendix C: (continued)
Day-to-Day Operations (page 1 of 2)

Readying ICP-MS and materials

1. Remove "Junk Serum", QC materials and patient samples from the -70C freezer and place into a biological safety cabinet (BSC) to warm up to room temperature.
2. Check the peristaltic pump for proper tension on the tubing
3. Perform daily maintenance checks
    a. Ar supply pressure, interface components etc.
4. Start the plasma
5. Place autosampler probe into freshly poured >18 MΩ·cm water
    a. Allow for warm-up time (≈30 minutes)

Optimization of the ICP-MS

1. Perform daily performance checks
2. Record the daily into the Daily Logbook
3. Prepare materials for DRC stability time

Preparing and analyzing the curve

1. Prepare the calibrators while the DRC stability is running,
2. Evaluate the calibration curve
    a. The minimum acceptable R^2 value for each curve is 0.98.
    b. Check that the blank is not over-subtracting from the standards (i.e. each successive standard has a net intensity greater than the previous standard). Ensure that net intensity for all standards is positive.

Preparing and monitoring the run

1. Preparing the Run
    a. Thoroughly wash the benchtop automatic pipette probe in-between samples
    b. Ensure the prepared samples are homogenized before placing them in the autosampler.

Appendix C: (continued)
1. Day-to-Day Operations (page 2 of 2)

2. Monitoring the Run
   a. Ensure proper operation of the instrument (sample reaching nebulizer in correct timing, autosampler arm moving properly, etc…).
   b. Ensure DRC stability (analyte / internal standard ratio stability) before starting the run.
   c. Verify that bench QC results are within the acceptable limits.
      i. If an analyte result for the beginning QC material(s) falls outside of the ± 3SD limits, then follow the steps listed on page 37.
      ii. 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.
   d. Verify good precision among replicates
   e. Verify consistent measured intensities of the internal standards.
   f. Confirm elevated patient results.
      i. Repeat for confirmation any sample having a concentration greater than the 1UB.
      ii. Repeat with extra dilution (in duplicate) any sample having a concentration greater than the highest calibration standard.
3. After analysis,
   a. flush the ICP-MS sample introduction system with >18 MΩ·cm water
   b. turn off the plasma
   c. flush the benchtop automatic pipette
      i. 10% Ethanol
      ii. >18 MΩ·cm water
      iii. Leave the benchtop automatic pipette syringes dry and turn off the power