



Laboratory Procedure Manual

Analytes: **Zinc, Copper and Selenium**

Matrix: **Serum**

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

Method No: ICPDRCMS-3006.7

Revised:

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

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Important Information for Users

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

**Multi-Element in serum
NHANES 2011-2012**

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_G	LBXSCU	Copper($\mu\text{g/dL}$)
	LBXSSE	Selenium ($\mu\text{g/dL}$)
	LBXSZN	Zinc ($\mu\text{g/dL}$)

1. Clinical Relevance & 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 may be used 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 of these. Liquid samples are introduced into the ICP through a nebulizer and spray chamber carried by a flowing argon stream. By coupling radio-frequency power into flowing argon, plasma is created in which the predominant species are positive argon ions and electrons and has a temperature of 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. In this method, the instrument is operated in 'DRC' mode where 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 0.7amu. 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 include 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. Safety Precautions

a. General Safety

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- 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 Micromedic Digiflex™ is recommended because it reduces analyst contact with work surfaces that have been in contact serum and also keeps the analyst's hands free to hold the specimen cups and autosampler tubes and to wipe off the tip of Micromedic Digiflex™.
 - vii. 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. Ammonia gas cylinders (either in use or on storage) should be placed in a cabinet which is well ventilated to the house exhaust. Ammonia cylinders in use should not be placed on their side 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 bleach-rite spray or freshly prepared 10% (v/v) sodium-hypochlorite solution.
- b. Waste Disposal: Operators of this method should take the CDC-OHS Hazardous Chemical Waste Management Course (initial and yearly refreshers).
- i. Waste to be Placed in Biohazard Autoclave Bags & Pans:

1. All biological samples and diluted specimens (after analysis run).
 2. All disposable plastic and paper which contact serum (autosampler tubes, gloves, etc.). Pipette tips can be placed in either autoclave pans or sharps containers.
 3. Used non-glass/quartz ICP-MS consumables (i.e. probes, tubing, cones, ion lenses).
- ii. Waste to be Placed Into Sharps Containers: Broken glass or quartz instrument consumables (broken spray chambers, torches, nebulizers, etc. . .). Pipette tips can be placed in either autoclave pans or sharps containers. Large broken glass which will not fit in the sharps container should be placed in a separate autoclave pan from other waste and labeled as “broken glass” (see the “Autoclaving” section of the CDC safety policies and practices manual located in the laboratory).
- iii. Liquid Waste
1. Waste discarded down sink: Only liquid waste from the ICP-DRC-MS instrument can be discarded at the sink. Flush the sink with copious amounts of water.
 2. Waste to be Picked up by Hazardous Waste Program: Submit request for hazardous waste removal of all other liquid waste.

3. Computerization: Data System Management

Integrity of specimen and analytical data generated by this method is maintained by eliminating hand-entry of specimen identifiers or analytical results whenever possible, proofreading all transcribed data, and regular defragmenting and backing up the ICP-MS computer’s hard drive.

a. Data entry and transfer.

Bar code scanners should be used whenever possible to enter sample identifiers into the ICP-DRC-MS computer software to avoid errors associated with the keyboard-entry process and to speed up sample processing. When bar code scanners cannot be used, transcribed data must be proofread after entry. Data should be handled / transferred electronically when reporting or moving to other computerized data handling software. In the Inorganic Radiation and Analytical Toxicology (IRAT) Branch, sample analysis results

generated by this method are stored long-term in Microsoft Access or a sequel server database software (including at least the analysis date, analytical run number, quality control results for the run, specimen analytical results by specimen ID, and method identifier).

b. Routine computer hard drive maintenance.

The computer hard drive should be defragmented regularly using software such as MS Windows to maximize computer performance and maintain data integrity for files on the hard drive. An entry will automatically be made in the Windows system event log when this process is done, providing documentation of this step.

c. Data backup: Data on the ELAN computer will be backed up via two backup routines.

1. Daily Backups to External Hard Drive: Automatic backups of the “elandata” directory and all subdirectories should be programmed to occur each night onto an external hard disk.
2. Weekly Backup to CD: Backup all files in the active “elandata” directory and all subdirectories onto one recordable compact disc during the weekly maintenance SOP. When the active “elandata” directory on the ICP-DRC-MS computer hard drive becomes too large to fit onto a single recordable compact disc, the oldest data can be removed from the computer to make it easier to backup the entire directory weekly. This can usually be done annually.
 - a. Backup the oldest data on the hard drive to two duplicate compact disks and verify that the files on the CD are readable
 - b. Label them with the name of the instrument, the date range of the data, the current date, your name, and “Copy 1 of 2” or “Copy 2 of 2”
 - c. After verifying that the CDs are readable, the oldest, backed up data can be deleted from the ICP-MS computer hard drive.
 - d. It is best to not store duplicate copies in the same location.

d. Documentation of system maintenance.

Computer maintenance: Any maintenance of computer hardware or ICP-DRC-MS software is contained in the instrument logbook. Other electronic records relating to integrity of the data and hard drive are located in the Windows event log. The event log should be backed up on a regular basis by saving a copy of it in the active elandata directory. It will then be backed up along with the ELAN data when backup CD-R discs are made.

Instrument maintenance: Documentation for system maintenance is contained in hard copies of data records (i.e. daily maintenance checklist, Perkin-Elmer

service records, instrument log book) as well as electronic records relating to instrument optimization (default.dac), tuning (default.tun).

4. 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 [9]. Copies are available in branch, laboratory, and special activities specimen-handling offices. An electronic copy is available at: http://inside.nceh.cdc.gov/dls/pdf/policiesprocedures/Policies_and_Procedures_Manual.DLS.2006mod.pdf In general, if more than one vacutainer of blood is to be drawn from an individual, the trace metals tube should be drawn 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. Serum specimens should be transported and stored at $\leq 4^{\circ}\text{C}$. Once received, they can be frozen at $\leq -20^{\circ}\text{C}$ until time for analysis. Portions of the sample that remain after analytical aliquots are withdrawn should be refrozen at $\leq -20^{\circ}\text{C}$. Samples thawed and refrozen several times are not compromised.
- i. No fasting or special diets are required.
 - ii. Specimen type - serum
 - iii. Acceptable containers include pre-screened polyethylene vials and pre-screened 7 mL vacutainers should be used for specimen acquisition.
 - iv. Specimen stability has been demonstrated for several months at approximately -20°C or at approximately -70°C for several years.
- b. Criteria for Specimen Rejection: Specimen characteristics that may compromise test results are indicated above. Reasons for rejection of a sample for analysis include
- i. Low volume: Optimal amount of serum is 1-2 mL, minimum is about 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 at least by paper document in the “Study Folder” (created before analysts receive the samples). Apart from this specimen tracking form, this folder will also contain the paper print outs of results from analysis of the specimens. Maintain records for a minimum of 3 years. 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 should have access to the personal identifiers.

5. Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides

Not applicable for this procedure

6. Preparation of Reagents, Calibrators(Standards and Controls) and all other Materials, Equipment, and Instrumentation

A. Instrument & 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 & 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 (Thermafex, Abbeville, SC, www.thermafex.net). Equivalent part may be substituted. # 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: Initial work using this method has used the standard Type A, 3mL/min nebulizer. Alternatively, the Type C, 1mL/min nebulizer may be used to improve sensitivity and precision. The ELAN supplies 30psi argon to the nebulizer. Variations of these nebulizers may be substituted with or without quick connects for the gas and liquid ports. Quartz nebulizers are used to avoid potential contamination from borosilicate glass (i.e. barium, uranium). # Spares = 2.
 - 1. Type A, Standard ELAN 3mL/min nebulizer: PerkinElmer part # WE024371 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available directly from manufacturer as part # TQ-30-A3 (Meinhard Glass Products, Golden, CO, www.meinhard.com) or from various distributors. The flangeless nut and ferrule assembly has been used for liquid sample back-end connection to this nebulizer.
 - 2. Type C, 1 mL/min nebulizer with quick disconnects for liquid and gas ports: One example is 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 1/4" 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", 1/4-28, 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, Welch Director Gold: For roughing pumps. Available direct from manufacturer as part # 8995G-15 (1 gallon, Welch Rietschle Thomas, Skokie, IL, www.welchvacuum.com) or from various distributors. Equivalent oil may be substituted. # Spares = 4.
- 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 = 1/4", 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. = 1/4". 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, 0.03" i.d. (sampling): Standard PVC, 2-stop (black / black) peristaltic pump tubing, i.d. = 0.03". PerkinElmer part # 09908587 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares* = 6 packs of 12 tubes.

xxxviii. Tubing, peristaltic, 0.125" i.d. (spray chamber drain): Standard PVC, 2-stop (black / white) peristaltic pump tubing, i.d. = 0.125". PerkinElmer part # N812-2012 (PerkinElmer, Shelton, CT, www.perkinelmer.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, 1/4" 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 may be substituted. # Spares = 10ft.
 - xlvi. Union Elbow, PTFE 1/4" 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.
 - xlvii. Union Tee, PTFE, 1/4" 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 & Supplies
- 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).
- Pan, for changing roughing pump oil: Like part # 53216 (United States Plastics Corporation, Lima, OH, www.usplastic.com) or equivalent. # On hand = 1.
- Container, to hold acid baths for glassware: Polypropylene or polyethylene containers with lids (must be large enough for torch, injector, or spray chamber submersion). May be purchased from laboratory or home kitchen supply companies. # On hand = 4.
- Cotton swabs: Any vendor. For cleaning of cones and glassware.
- 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.
- 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.
- 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).

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).

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

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

d. Sources for General Laboratory Consumable Supplies

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.

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.fishersci.com) or equivalent. Carboys with spouts are not advised due to potential for leaking.

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

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

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

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

Pipettes (for preparation of intermediate stock working standards & other reagents): Like Brinkmann Research Pro Electronic pipettes (Brinkmann Instruments, Inc., Westbury, NY, <http://www.brinkmann.com/home/>). 5-

100 μL (catalog #4860 000.070), 20-300 μL (catalog #4860 000.089), 50-1,000 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.

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

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 may be substituted which are shown by lot screening to be free of trace metal contamination. Clear plastics tend to have lowest trace metal contamination. Orange colored caps have also been used successfully for this method.

Votexer: 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.

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

e. Sources of Chemicals, Gases, and Regulators

Acid, Hydrochloric acid: Veritas™ double-distilled grade, 30-35% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com). This is referred to as “concentrated” hydrochloric acid in this method write-up. It is approximately 12 molar in concentration. For use in preparation of intermediate working stock standards. An equivalent hydrochloric acid product may be substituted, but it must meet or exceed the purity specifications of this product for trace metals content.

Acid, Nitric acid: Veritas™ double-distilled 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. An equivalent nitric acid product may be substituted, but it must meet or exceed the purity specifications of this product for trace metals content.

Ethyl Alcohol (C₂H₅OH), USP dehydrated 200 proof (Pharmco Products, Inc.) or equivalent.

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

Argon Gas (for plasma & nebulizer) and Regulator: High purity argon (>99.999% purity, Specialty Gases Southeast, Atlanta, GA, www.sgsgas.com) for torch and nebulizer. Minimum tank source is a dewar of liquid argon (180-250L) 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). An equivalent regulator from an alternate vendor may be substituted. # *Spare*s = 1.
2. Regulator for argon (between bulk tank and PerkinElmer filter regulator): Single Stage 316SS Regulator, with 0-300 psi Inlet Gauge, 0-200 psi Outlet Gauge, Outlet Spring Range, 0-250 psi, ¼” Swagelok Inlet Connection, ¼ turn Shut off Valve on Outlet with ¼” Swagelok Connection and Teflon Seals. Part number KPR1GRF412A20000-AR1 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com). An equivalent regulator from an alternate vendor may be substituted. # *Spare*s = 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).

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).

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 (for lecture bottle cylinder) 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,

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PA, www.matheson-trigas.com). An equivalent regulator from an alternate vendor may be substituted. # Spares = 1.

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

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

Standard, multi-element intermediate 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 p.49 for concentrations). This solution is diluted to prepare the intermediate stock working standards, which are in turn diluted to prepare the working calibrators. This solution can be prepared in-house from NIST traceable single element stock solutions if necessary.

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

B. Preparation of Reagent and Materials.

Diluent

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.

Contents: An aqueous solution of 10 µg/L Ga, 2% v/v double-distilled nitric acid, 5% Ethyl Alcohol, 0.01% Triton X-100™.

Preparation (4L) & storage: This solution does not have to be made up in a volumetric flask. The important thing about the concentration of the internal standards is that they be consistent within all samples in one run. To prepare different volumes of diluent, add proportionally larger or smaller volumes of the solution constituents.

Acid-rinse a 4 L container (material may be polypropylene (PP), polymethylpentene (PMP), or Teflon™).

Partially fill (i.e. 70-80% full) the 4 L container with ≥ 18 Mega-ohm-cm water.

Carefully add 80 mL double-distilled, concentrated nitric acid and mix.

Carefully add 200 mL Ethyl Alcohol and mix.

Add 4 mL each of 10 mg/L Ga.

Add 20 mL 2% Triton X-100™ stock solution and mix.

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Make up to volume (approximately 4 L) with ≥ 18 Mega-ohm·cm water.

Store at room temperature and prepare as needed.

Label should include “10 $\mu\text{g/L}$ Ga, 2% (v/v) HNO_3 , 5% Ethyl alcohol, 0.01% Triton X-100TM”, “Store at room temperature”, preparation date, expiration date (1 year from prep), and preparer’s initials.

Base Serum

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.

Contents: 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.

Screening serum: Screen all sources of serum for metal content before mixing together to make the base serum pool. Keep serum at $\leq -20\text{C}$ whenever possible to minimize microbial growth. Analyte concentrations in the final base serum pool should be in the low-normal population range (see Table 10, p. 62).

Preparation & Storage:

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

For short term storage, store at 2-4°C. For long-term storage, dispense into smaller-volume tubes (i.e., 10 mL acid-washed or lot screened polypropylene tubes) and store at $\leq -20^\circ\text{C}$.

Labels on 10 mL tubes should include “Base Serum for Multi-Element Method”, “Store Long Term at $\leq 20^\circ\text{C}$ ”, “Store Short Term at 2-4°C”, preparation date, expiration date 3 years from prep date, and preparer’s initials.

ICP-DRC-MS Rinse Solution

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. For this method, we also need to pump ≥ 18 Mega-ohm·cm water into the sample introduction system for 30 minutes after each run to prevent the clog of the probe and tubing.

Contents: An aqueous solution of 0.01% Triton X-100TM and 2% (v/v) double-distilled nitric acid solution, 5% Ethyl Alcohol, 0.5% v/v Hydrochloric acid.

Preparation & Storage:

Intermediate Triton X-100 Solution: To avoid the process of dissolving pure Triton X-100 on a daily basis, prepare an intermediate 2% Triton X-100™ / 5% (v/v) double-distilled, nitric-acid solution for daily use.

To prepare 2 L of Intermediate Triton X-100 Solution:

Partially fill a 2 L acid-washed bottle (PP, PMP, or Teflon™) with ≥ 18 Mega-ohm·cm water (approximately 1-1.5 L). Use of volumetric flask is not required.

Add 20 mL of Triton X-100™ and stir until completely dissolved. Use a Teflon™ stir bar and stir plate if necessary (acid wash stir bar before use).

Carefully add 100 mL of double-distilled, concentrated nitric acid.

Fill to 2 L and stir thoroughly.

Label should include “2% Triton X-100™ / 5% (v/v) HNO₃”, “Store at room temperature”, preparation date, expiration date 1 year from preparation date, and preparer’s initials.

Final Rinse Solution:

To Prepare 4 L of the Final Rinse Solution:

Partially fill a 4 L acid-washed bottle (PP, PMP, or Teflon™) with ≥ 18 Mega-ohm·cm water (approximately 2-3 L). Use of volumetric flask is not required.

Carefully add 80 mL of double distilled concentrated nitric acid and mix well.

Carefully add 200 mL ethyl alcohol and mix.

Carefully add 20 mL double distilled concentrated hydrochloric acid.

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

Fill to 4 L using ≥ 18 Mega-ohm·cm water and mix well.

Store at room temperature and prepare as needed. To prepare volumes other than specified here, add proportionally larger or smaller volumes of the solution constituents.

Label should include “2% v/v HNO₃, 5% Ethyl alcohol, 0.01% Triton X-100™, 0.5% v/v Hydrochloric acid”, “Store at room temperature”, preparation date, expiration date (1 year from prep), and preparer’s initials.

C. Standards and Calibrators

Multi-Element Intermediate Stock Standard (calibrators and calibration verification)

Purpose: This is the master solution from which all working calibrators will be prepared. It will be diluted to prepare intermediate working calibrators which are in turn diluted and included in each analytical run on the ICP-DRC-MS. This same stock standard will be diluted to prepare the intermediate working calibration verification solution which will be in turn diluted and analyzed at least every 6 months for calibration verification purposes (and as needed by supervisor request).

Contents: An aqueous solution containing all 3 elements of interest for this method (does not include the internal standards). The matrix is 2% v/v HNO₃ in ≥ 18 Mega-ohm·cm water.

Preparation (Purchase) & Storage:

- a. Purchasing from vendors: The intermediate stock standard solution may be purchased as a custom mixture from any vendor which prepares multi-element solutions that are traceable to the National Institute for Standards and Technology (NIST) for their accuracy
- b. Current vendor & preparation process: Currently it is purchased from High Purity Standards (Charleston, SC, part number SM-2107-013). Details of the HPS preparation of the multi-element stock standard is as follows (per statement on their literature):

“Sub-boiled high purity acids were used to put the high purity metal, salts, or oxides into solution and to stabilize the standard. The solution matrix is 2% (v/v) nitric acid in ≥ 18 Mega-ohm·cm water. The standard was made gravimetrically by weighing the reference material to 5 significant figures. Volumetric glassware was calibrated gravimetrically to 5 significant figures.”

- c. In-house Preparation: If outside laboratories were not available to prepare the intermediate stock standard solution, it is also possible to make it in the laboratory from single element standards which are NIST traceable.
- d. Storage: Store the solution at room temperature. Label these bottles from HPS with additional information such as “store at room temperature”, date received, date opened, and initials of person to first open.

Multi-Element Intermediate Working Calibration Standards

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.

Content: The intermediate working standard solutions used in this method are aqueous dilutions of the multi-element intermediate stock standard solution in 2% (v/v) double-distilled nitric acid.

Preparation & Storage: To prepare different volumes, add proportionally larger or smaller volumes of the solution constituents.

Cleaning flasks: Acid-rinse five 100-mL and one 2L volumetric flasks. Check their cleanliness by comparing the counts observed on the ICP-DRC-MS for 2% (v/v) HNO₃ before and after contact with the flasks. Mark each of the flasks according to how they will be used. These flasks should be dedicated to this use in this method, and not used for other purposes.

HNO₃ Diluent Preparation: In the cleaned 2L volumetric flask, add 1-1.5L of ≥ 18 Mega-ohm·cm water, 40 mL high purity concentrated HNO₃. Fill to the mark and mix thoroughly. Use this diluent to fill the remaining flasks during preparation of the intermediate working standards.

Dilutions & Storage:

Partially fill the 100 mL flasks with the HNO₃ diluent (50-75% full).

Using the volumes listed (Table 4 p.49) pipette the appropriate volume of the multi-element intermediate stock standard solution into each of the five volumetric flasks. Dilute each solution to the mark with the HNO₃ diluent using a pipette for the final drops. Mix each solution thoroughly.

Once mixed, transfer to acid-cleaned, labeled, 50-mL containers (PP, PMP, or Teflon™) for storage. Labels should include information such as “Multi-Element Serum Working Calibrators”, “2% (v/v) HNO₃”, date of preparation, expiration date (1 year from date of preparation), “store at room temperature”, initials of preparer, and concentrations for each element.

Store at room temperature.

D. Internal Quality Control Materials (“Bench” QC)

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. Preparation of new pools should be made far enough in advance so that both old and new pools can be analyzed together for a period time (preferably at least 20 runs) before switching to the new quality control materials.

Content: The internal (or “bench”) quality control (QC) materials used in this method are pooled human serum and may have been spiked to reach a desired concentration. 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.

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

- a. Collection of 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).

Keep serum at $\leq -20\text{C}$ whenever possible to minimize microbial growth.

Analyte concentrations in the final serum pool to be spiked for the low bench QC pool should be in the low-normal population range (see Table 10, p. 62). Analyte concentrations in the final serum pool to be spiked for the high bench QC pool should be less than some pre-selected target concentration values in the high normal population range.

- c. Spiking of serum

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

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

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

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).

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

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

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.

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.

Dispensing: Dispensing can be accomplished most easily using a Digiflex automatic pipette in continuous cycling dispense mode. This process should be done in a clean environment (i.e. a class 100 cleanroom area or hood).

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. Replace the tubing attached to the dispensing syringe (left when looking at front of Digiflex) with a length of clean Teflon™ tubing long enough to reach into the bottom of the carboy while it is sitting on the stir plate.
3. Check cleanliness of Digiflex before use by analyzing 1-2% (v/v) HNO₃ which has been flushed through the Digiflex with a portion of the same solution which has not been through the Digiflex.
4. Approximately one hour before dispensing begins,
 - a. With the large stir plate close to the left side of the Digiflex, begin stirring the serum pool to be dispensed.
 - b. Also during this time, flush the Digiflex 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. Be sure to secure both ends of tubing in the carboy with Parafilm so they will not come out during the flushing process.
5. After dispensing the serum into the vials, cap the vials and label them. Placing labels on vials after capping minimizes the chance for contamination during the process.

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 $\leq -20^{\circ}\text{C}$ and pull tubes out as needed for analysis.

Storage: Serum pools should be stored long term at $\leq -20^{\circ}\text{C}$. Short term storage (several days) at refrigerator temperature ($\sim 2-4^{\circ}\text{C}$).

7. Calibration and Calibration Procedures

Working Multi-Element Calibrators

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.

Content: The working multi-element calibrators are 1:30 dilutions of the corresponding five intermediate working standards.

Preparation & Use: The working multi-element calibrators are made immediately prior to analysis when the intermediate working standards are mixed with base serum and diluent using a Digiflex automatic pipetter.

Multi-Element Intermediate Working Calibration Verification Standards

Purpose: Use the intermediate working calibration verification standard to satisfy calibration verification requirements for the method.

Content: The intermediate working standard calibration verification solution used in this method is an aqueous dilution of the multi-element intermediate stock standard solution (same as that used to prepare the intermediate stock calibration standards) in 2% (v/v) double-distilled nitric acid.

Preparation & Storage: To prepare different volumes, add proportionally larger or smaller volumes of the solution constituents.

Cleaning flasks: Acid-rinse one 100-mL and one 2L volumetric flask (the same 2L flask as was used in preparing the intermediate working calibration standards can be used here). Check their cleanliness by comparing the counts observed on the ICP-DRC-MS for 2% (v/v) HNO_3 before and after contact with the flasks. Mark the flasks according to how they will be used. This flask should be dedicated to this use in this method, and not used for other purposes.

HNO_3 Diluent Preparation: In the cleaned 2L volumetric flask, add 1-1.5L of ≥ 18 Mega-ohm-cm water, 40 mL high purity concentrated HNO_3 . Fill to the mark and mix thoroughly. Use this diluent to fill the remaining flasks during preparation of the intermediate working standards.

Dilutions & Storage:

Partially fill the 100 mL flask with the 2% v/v HNO₃ diluent (50-75% full).

Using the volumes listed (Table 4 p.49) pipette the appropriate volume of the multi-element intermediate stock standard solution into the 100mL volumetric flask. Dilute the solution to the mark with the 2% v/v HNO₃ diluent using a pipette for the final drops. Mix thoroughly.

Once mixed, transfer to acid-cleaned, labeled, 50-mL containers (PP, PMP, or Teflon™) for storage. Labels should include information such as “Multi-Element Serum Working Calibration Verification Standard”, “2% (v/v) HNO₃”, date of preparation, expiration date (1 year from date of preparation), “store at room temperature”, initials of preparer, and concentrations for each element.

Working Multi-Element Calibration Verification Standards

Purpose: The working multi-element calibration verification standard is a dilution of the intermediate working calibration verification standard. Analysis of this standard meets the calibration verification requirements detailed in section 8.a.ii.

Content: The working multi-element calibration verification standard is a 1:30 dilution of the corresponding intermediate working calibration verification standard.

Preparation & Use: The working multi-element calibration verification standards are made immediately prior to analysis when the intermediate working calibration verification standards are mixed with base serum and diluent using a Digiflex automatic pipetter.

1. *Linear calibration curves.*

a. Typical correlation coefficients will be 0.999 to 1.000.

b. The ELAN software generates a “simple linear” calibration curve (using a least squares calculation) for each of the 3 elements in this method. The curves are generated using the results from analysis of the serum blank and the 5 external serum calibrators whose concentrations are defined in the Calibration tab of the Method file. Specifically, the software plots the “net intensity” (y-axis) versus the analyte concentration (x-axis). The “net intensity” is the blank subtracted **ratio** of the measured intensity for the analyte to the measured intensity of the associated internal standard and is calculated as follows:

$$\text{net intensity} = \frac{\text{Analyte Meas Intensity}_{\text{sample}}}{\text{Internal Std Meas. Intensity}_{\text{sample}}} - \frac{\text{Analyte Meas Intensity}_{\text{Blank}}}{\text{Internal Std Meas Intensity}_{\text{Blank}}}$$

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

If an analyte result for the beginning QC material(s) falls outside of the 3SD (i.e. 99 percentile) limits, then the following steps are recommended:

- a. If a particular calibration standard is obviously in error, remake a new dilution at the Digiflex of that working calibrator, reanalyze it, and reprocess the sample analyses using this new result as part of the calibration curve.
- b. Prepare a fresh dilution of the failing QC material and reanalyze it.
- c. Prepare fresh dilutions at the Digiflex of all of the calibration standards (working serum multi-element standards) and reanalyze the entire calibration curve using the freshly prepared standards.

If these three steps do not result in correction of the out-of-control values for QC materials, consult the supervisor for other appropriate corrective actions. Do not report analytical results for runs that are not in statistical control.

3. *Good precision among replicates.* If “air” was sampled into the system, the precipitated serum protein might be coated within the probe, tubing and the introduction system which might cause the bad precision among replicates and/or reduced sensitivity of the instrument. Use ≥ 18 Mega-ohm-cm water to rinse the system for recovering the instrument performance.

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

Some sample-to-sample variations are to be expected. However the intensities should be within a few percent of one another, and should fluctuate around an average value (not drift continuously in one direction).

5. Calibration Verification:

Bi-annual tests as defined in the DLS Policy and Procedures manual: CLIA requires the verification of accuracy of instrument response to analyte concentration be completed at least every 6 months. NIST traceable calibrators are analyzed in each run to define this response up to the concentration of the highest calibrator in the run. To verify accuracy of instrument response at concentrations higher than the highest calibrator in each run, analyze a NIST traceable standard with very high concentrations at least every 6 months. Prepare the Calibration Verification Standard for analysis just as a working calibrator is prepared. Use the “Serum Blank” as the blank when it is analyzed. If the observed concentrations for the Calibration Verification Standard are not within 10% of the target value the lab supervisor should be notified and the issue should be investigated. Do not substitute external reference materials (i.e. biological samples from a PT program) for the Calibration Verification Standard when performing this. Solutions needed for the Calibration Verification checks can be purchased from standards vendors (i.e. SPEX, High Purity Standards, etc . . .) or prepared in-house from NIST traceable single element standards. Always

verify that normal background levels have been re-achieved through adequate rinse time following analysis of elevated standards for calibration verification.

As-needed confirmations (per supervisor discretion): When a sample result is greater than the highest calibrator in the run by more than 10%, the supervisor may request that the result be confirmed in an analysis run which includes a standard or external reference material with equivalent (within 10%) or greater concentration than the sample. In order to avoid needless contamination of the instrument with high concentrations of analytes, the analyst should use the lowest appropriate calibration verification solution concentrations to meet the need.

For *infrequent* verification needs, the calibration verification stock solutions can be used to prepare verification standards to appropriate concentrations. This will, however, introduce elevated concentrations of all elements in the method to the sample introduction system. Frequent measurement of these very high concentrations can result in high background levels in the instrument which are difficult to rinse out and which may limit the ability to measure low concentrations.

For frequent verification needs (i.e. when certain studies have many elevated results on particular elements), use NIST-traceable single element stock standards to prepare single element verification standards. This will limit the exposure of the instrument to elevated concentrations of only the elements needing verification.

Always verify that normal background levels have been re-achieved through adequate rinse time following analysis of elevated standards for calibration verification.

8. Procedures Operating Instructions: Calculations, Interpretations of Results

A. Instrumentation & Equipment Setup:

- i. ICP-DRC-MS: Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectrometer ELAN[®] 6100 DRC^{Plus} or ELAN[®] DRC II.

Modifications made to ICP-DRC-MS

All plastic tubing for DRC reaction gases have been replaced with 1/8" O.D. stainless steel.

Sample introduction system setup:

(Adjustments of connections for the ESI SC4 autosampler are described below.

Concentric quartz nebulizer (quick connect arrangement for liquid and gas connections available from some vendors).

Quartz cyclonic spray chamber.

Quartz injector, 2 mm ID, ball joint end.

Configuration of tubing for liquid handling:

This is a recommended setup.

Tubing for liquid sample uptake:

i. Probe-to-peristaltic pump tubing: PFA tubing from ESI SC4 autosampler probe connects either directly to sample peristaltic tubing or through a connection adaptor.

ii. Nebulizer-to-peristaltic pump tubing:

1. 3mL/min nebulizer (TQ-30-A3): Hold square-cut end of 0.5mm x 1.59mm PFA tubing against the inside tapered nebulizer capillary using a flangeless nut and ferrule assembly. Angle-cut opposite end of tubing before inserting into end of black / black peristaltic pump tubing.
2. 1mL/min nebulizer (500-70QQDAC): Quick connect fitting fits inside back side of nebulizer. Use a PEEK adapter to securely connect the PFA tubing to the peristaltic tubing. Higher back-pressure from the 1mL/min nebulizer is likely to cause tubing become disconnected if the PFA tubing is merely inserted into the peristaltic pump tubing.

Tubing for autosampler rinse solution:

- iii. SC autosampler setup for non-FAST applications:
Differences for the ESI SC4 autosampler include
 1. Autosampler Probe: (SC4 probe has built-in PFA tubing extending from the Teflon-coated probe, so no nut and flanged tubing connection is necessary).
 2. Rinse station filling: ESI SC4 autosampler may have a built-in vacuum pump which pumps rinse solution from the rinse jug to the rinse station ports. If so, rinse solution will not need to be routed through the peristaltic pump.
 3. Rinse station waste: ESI SC4 autosampler liquid waste may be setup to drain by gravity (see comment below).
- iv. Tubing connection between autosampler rinse station and rinse solution reservoir: Tubing of different inner diameters can be obtained from Elemental Scientific, their distributors, or custom

built in the lab to optimize the rinse station fill rate between samples. Rinse station should not go empty at any point.

- v. Tubing for autosampler rinse station waste removal: Use minimum drain tubing to make this connection. If this tube is too long, the rinse station will not drain properly.
- vi. 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 and label appropriately (see solution preparation instructions).
- vii. Waste solution jug: Use secondary containment tray and label appropriately (see solution preparation instructions).
Configuration of tubing for spray chamber waste removal:
- viii. Chamber-to-peristaltic pump tubing: Connect 1/8" i.d. x 1/4 inch o.d. PVC tubing directly to the waste port on the spray chamber. Connect other end of PVC tubing to the white / black peristaltic pump tubing using a tubing connector (PerkinElmer item # B3140715).
- ix. Waste Jug-to-peristaltic pump tubing: Connect 1/8" i.d. x 1/4" o.d. PVC tubing to the white / black peristaltic pump tubing using a tubing connector (PerkinElmer item # B3140715). Place the free end of the PVC tubing through the lid of the waste jug (be sure it is secure). Waste jug should be sitting in a secondary containment tray in case of overflow.
- x. Cones used
Nickel or platinum cones from either PerkinElmer or Spectron have been used successfully. Platinum cones are preferred for durability.
- x. Gases & Regulators setup:
 - a. Argon: Argon stored as liquid in a dewar (180-250L) or bulk tank. Gaseous argon used for plasma and nebulizer.
 - i. Regulator for argon source (if a dewar): Keep the inlet pressure (headspace pressure of liquid argon dewar) above 100 psi. Set delivery pressure to 60-100psi to allow for pressure drop across tubing that stretches to the instrument. See Section 5.e. for part numbers and details.

- ii. 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 70-100 psig. See Section 5.e. for part numbers and details.
 - iii. Regulator at ICP-DRC-MS: Single stage “argon regulator filter kit” supplied with the ICP-DRC-MS. If the delivery pressure gauge range is 0-60psi, set the delivery pressure to 52±1 psig. If the delivery pressure gauge range is 0-100psi, set the delivery pressure to 60±1 psig. See Section 5.e. for part numbers.
- b. Ammonia gas for DRC channel A.
- i. Regulator for NH₃ gas: Set delivery pressure to 5-7 psig. See Section 5.e. for part numbers and details.
- .c. 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.
- d. Computer: Dell Optiplex GX150, GX270, or GX280 have all been used. Processors used have included Pentium III (1 GHz) through Pentium IV (2.8 GHz). Recommend 512Mb - 1Gb RAM. External hard disk drive for nightly backups of data connects via USB port. Software used includes Windows XP Professional, service pack 2 and ELAN v3.3.
- e. Autosampler: ESI SC4 autosampler without FAST sample introduction. Rack calibration, tubing ID for rinse supply, additional rinse time, probe movement speeds, and probe depth is optimized per autosampler.
- f. External Reference Standard

An external reference material (i.e. historical proficiency testing sample) can be used to verify the linearity of calibration within a run in these situations IF

The target value has been assigned by an external source (i.e. NIST, or the proficiency testing program).

The concentration of the external reference material is within 10% or is higher than the concentration of the material you need it to confirm.

There is confidence that there is no contamination of previously used external reference material.

A note to file is made that this was done.

- g. If the observed concentrations are not within 10% of the target value the lab supervisor should be notified and the issue should be investigated.

B. Daily Analysis of Samples

Preparation of the Analytical Equipment

For further details on any part of this description, see the ITN Daily Startup SOP for ELAN ICPMS instruments.

1. Power on the computer, printer, peristaltic pump, and autosampler, and log into the operating system.
2. Peristaltic pump: Set up the peristaltic pump tubing with proper tension for the sample rinse station.
 - a. *If using an external peristaltic pump*, after lighting the plasma go to the DEVICES window of the software and press the “Connect” button to establish communication between the computer and the autosampler. Next, start the peristaltic pump by pressing the appropriate arrow in the DEVICES window (make sure that the rotational direction is correct for the way the tubing is set up in the peristaltic pump). Set the pump speed to 10 rpm in the DEVICES window.
 - b. *If using the on-board ICP-MS peristaltic pump*, start the peristaltic pump by pressing the appropriate arrow in the DEVICES window (make sure that the rotational direction is correct for the way the tubing is set up in the peristaltic pump). Set the pump speed to a slow flow rate (6 to 10 rpm) in the DEVICES window.
3. Software: Starting the ESI software before starting the ELAN software may improve stability of software.
4. Daily Pre-Ignition Maintenance Checks: Perform daily maintenance checks as described in the ITN Daily Startup SOP for ELAN instruments (i.e., Ar supply pressure, interface components cleanliness and positioning, interface pump oil condition, vacuum pressure, etc.). Make appropriate notes in the Daily Maintenance Checklist and Instrument Log Book.
5. Start the Plasma: In the INSTRUMENT window of the software (or on the front of the ELAN), press the “Start” button to ignite the plasma.
6. Send Probe to Rinse Station: Through the METHOD/SAMPLING window in the software, press the “Probe” button, then the “Go to Rinse” button to lower the autosampler probe into the rinse solution.
7. Start the peristaltic pump:
8. Warm-up time: Allow at approximately 30 to 45 minutes warm-up time for the ICP-DRC-MS after igniting the plasma. This warm-up time is

for the RF generator. There will be another “Stability time” for the DRC later in this procedure.

9. Optimizations and Daily Performance Check: After this warm-up time, perform a daily performance check and any optimizations necessary (as described in the ITN Daily Startup SOP for ELANs). Include Be (m/z 9) in the daily performance check. Fill in the Daily Maintenance Checklist according to the optimization procedures performed.
 - a. Magnesium (²⁴Mg) may have high RSDs due to the use of Triton-X100 in the rinse solution. Avoid this problem by either temporarily using non-Triton-containing rinse solution during the daily check, or repeating the daily check multiple times in succession with no rinse time between.
 - i. Saving the Files: Save new tuning (mass calibration) parameters to the file “default.tun.” Save new optimization parameters (i.e., detector voltages, autolens values, nebulizer gas flow rate) to the file “default.dac.” monthly, or any time large changes are made in optimization parameters, save a separate copy of these optimization files under a different name (i.e. – default_070706.dac).

10. Software setup for Analysis:

- a. Workspace (files & folders): Click on “Open Workspace” from the “File” menu. Select the workspace file “CDC_Serum multi-element.wrk” (or one customized for user preferences). Select “Review Files” from the “File” menu. Verify & set up the correct files and data directories for your analysis .
- b. Samples / Batch Window: Update the window to reflect the current sample set. The only fields which need to be filled in include the autosampler location, sample identification (id), measurement action, method, sample flush time, sample flush speed, read delay time, read delay & analysis speed, wash time, wash speed. Use a bar code scanner to input data whenever possible. Save the Sample window file and re-use it on other days by simply replacing the sample IDs for the patient samples.
 1. DRC Stability Time: Best analyte-to-internal standard ratio stability is obtained after 1 hrs of analysis of serum samples using the DRC method. Analyze enough base serum sample dilutions prior to any DRC analysis run to fill at least one hours of analysis time. If analyzing the full set of method analytes, 10 samples will be sufficient.
 2. Serum vs. Aqueous Method Files:

- a. The difference: There are two method files for this one method . It is necessary to use both to accomplish each run because the current PerkinElmer software will not allow for more than one blank per method file. The ONLY DIFFERENCE between these two files is on the Sampling tab where one lists the autosampler positions of the 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.
 - i. The “sblk” method file: Use to analyze the initial serum blank (blank for the calibration curve), the serum calibrators, and the serum blank checks (sblkchk1 & sblkchk2) at the very beginning of the run. The serum blank method (set up for a ESI SC4 autosampler defines the serum blank in autosampler location 109 and the serum calibration standards 1-5 in autosampler locations 101-106, respectively.
 - ii. The “aqblk” method file must be used to analyze all QC materials and patient samples. The aqueous blank method (set up for a ESI SC4 autosampler) defines the aqueous blank in autosampler location 109.
3. Notation of Dilutions: To designate an extra dilution of a sample, edit the sample ID to reflect the level of dilution being performed (i.e., A 1:2 dilution of sample 1 would be reflected in the sample ID “sample 1 (2x dilution)”. This sample ID will be edited during the data-import process to the database so that it is recognized as the appropriate sample.
- ii. Preparation of Samples for Analysis

C. Preliminaries

1. Thaw the frozen serum specimens; allow them to reach ambient temperature.
2. Prepare diluted serum for analysis during the DRC stability period. A 40-minute DRC stability period will consume approximately 36mL of solution. Prepare the necessary volume according to the “patient sample constituent proportions. This can be prepared in a 50mL

polypropylene tube or a wide-mouth bottle (which can be put on the autosampler in place of one of the tube trays).

NOTE: Selenium is not stable in the diluted sample for more than 7 hours. Diluted serum must be analyzed within 7 hours of preparation.

3. Set up a series of 15-mL polypropylene tubes corresponding to the number of blanks, standards, QCs, and patient samples to be analyzed.
4. Prepare the following solutions in the 15-mL falcon tubes using the Micromedic Digiflex™ .
 - a. *Aqueous Blank:* Prepare two aqueous blanks consisting of 300 μL of ≥ 18 Mega-ohm-cm water and 4,200 μL of diluent (2 x 2100 μL). One will be the actual aqueous blank and the other will be a backup ("Aqueous Blank Check") in case the original aqueous blank gets contaminated....
 - b. *Serum Blank:* Prepare three serum blank dilutions consisting of 150 μL of base serum (same material used to prepare the serum calibration standards), 150 μL of ≥ 18 Mega-ohm-cm water, and 4,200 μL of diluent (2 x 2100 μL). One of these serum blanks will be the blank for the calibration standards; the others will be analyzed after standard 5 as sblkchk1 and sblkchk2, respectively. Results from sblkchk1 and sblkchk2 will be stored for periodic verification of the method limit of detection.
 - c. *Calibrators or Calibration Verification Standards:* Prepare the working calibration standards or the working calibration verification standards as 150 μL of the appropriate aqueous intermediate working solution, 150 μL of base serum, and 4,200 μL of diluent (2 x 2100 μL). To avoid carryover from working calibration standards and the working calibration verification standards to other samples, rinse tip of digiflex once with concentrated nitric acid.
 - d. *Patient & QC Samples:* Before taking an aliquot for analysis, mix the sample so that no particulates remain on the bottom of the tube. Prepare serum sample dilutions as 4,200 μL of diluent (2 x 2100 μL), 150 μL of the serum sample and 150 μL of ≥ 18 Mega-ohm-cm water.
 - e. Cap all of the blanks, standards, and samples and mix them well. Uncap them and place them in the autosampler of the ELAN® ICPMS in the order that was entered in the Samples / Batch window of the ELAN software.

Starting the Analysis: To begin analysis, highlight (click and drag with the mouse) the table rows of the samples that should be included in the run, and then click on "Analyze Batch."

Monitoring the Analysis: Initiate work in a timely manner so that the run may be monitored. Make every effort to complete analysis within the work day so that the entire run can be monitored. If it is not possible to complete the analysis by the end of the work day, the run may be left to complete itself unattended as long as appropriate planning is made for either overnight operation or Auto Stop (see below).

DRC stability (analyte / internal standard ratio stability)

After the analysis of the DRC stability base serum samples, these results can be reviewed to determine if sufficient stability of the analyte-to-internal standard ratio has been reached before beginning analysis. Importing data into an MS Excel template file is useful to simplify this procedure.

Proper operation of the instrument.

Contaminated blanks.

Elevated patient results.

- D. Records of Results: Run results will be documented daily in both electronic and paper form.
1. Electronic Records:
 - a. Transfer of Results to the Laboratory Information System / Database: Transfer data electronically between computers or software to reduce errors. When keyboard entry must be used, proofread transcribed data after entry.
 - b. Long-Term Storage of ELAN software files: Files used and produced by the ELAN software in analyzing samples will be backed up long term on compact disk and kept a minimum of three years.
 2. Paper Records: The paper copy of the results from the run should be put into the study folder(s) and should include
 - a. A summary of the calibration curve statistics.
 - b. A printout of analysis of each measurement made during the run.
 - c. Optional, but helpful, is a printout of the DRC stability check measurements in graphical form.
 - d. On the front sheet of the printed records, write the following
 - i. Analyst initials
 - ii. Instrument ID
 - iii. Date of Analysis

- iv. Run # for the day on this instrument
 - v. Study ID and Group Number
 - vi. Database batch ID (Not known until the run is imported into the database)
3. Transfer of Results to the Laboratory Database: Every analytical run performed for the analysis of patient samples should be entered into the laboratory results database unless the run is not useable for obvious reasons (i.e. the run is stopped for some reason before ending QC is analyzed, no internal standard spiked into the diluent, etc. . .).
 4. Data Export Process (from ELAN® software to .TXT file): If the data file was not created during the initial analysis, reprocess the data of interest either with “original conditions” option, or by loading the files and folders used during the analysis. In the ELAN® ICP-DRC-MS software, select “Review Files” from the “File” menu. From this window, you must open the files and directories that were used when collecting the data of the run that you wish to export. (If the analysis has just ended, all of these files and directories will still be open.) NOTE: A second copy of the ELAN® software can be run as an Edit/Reprocess copy without affecting an ongoing analysis by the first copy of the software running in Windows. After you open the relevant files, go to the “Report” page in the METHOD window. Deselect the box that prints a paper copy of data and select the box that sends data to a file. Select the “Report Options Template” named “CDC_Database Output.rop” and type in a report filename using a format such as “2006-0714a_group55.txt” to designate data from analysis of group 55 from July 14, 2006, run #1. Under “Report Format”, choose the “Use Separator” option, and under the “File Write” section choose “Append.” Finally, reprocess the data of interest. (See PerkinElmer ELAN® ICPMS Software Manual.) Make sure you apply the aqueous blank to all sample and quality control material analyses.
 5. Data Import Process (from .TXT file to Microsoft Access™ database):
 - a. Move the .TXT file to the appropriate subdirectory on the network drive where exported data are stored. Directories for data storage are named according to instrument \ year \ month\, such as I:\Instruments\ELANDRCC\2006\07\.
 - b. Using the ITN Database Frontends, import the instrument file into the database. On the GoTo window, click on “Add Sample Results to Database”, then “Import Instrument Data File”.
 - c. Enter the appropriate information to identify the instrument, assay, analysis date & time, run number, analyst, calibrator lot number and prep date used (use the “IS Lot Number” field) and study. If

other than default values for Method LOD, High Calibrator, Rep Delta Limit, and units were used in the run, document what was used by clicking on the “View/Set Batch Parameters” button, changing the appropriate values, and then clicking “Back”.

- d. Press the “Import” button and then browse to the correct network folder to select the file which contains the results from the run. Select the file and click “OK”.
- e. In the “Import Instrument Results” table, pressing the “Find X’s” button will show only those samples whose sample ID is not recognized as a valid QC pool ID or sample ID for this study. (Sample IDs are set up when the study is logged into the database.) Corrections to sample IDs and dilution factors can be made in this table (e.g., correction of transcription errors and adjustment for level of dilution). If samples were diluted for analysis, both the sample ID and the dilution factor need to be edited in this table before the values are transferred to the database (the Replace command under the Edit window is helpful in this case). When corrections to sample IDs are made, press the “Check IDs” button to re-evaluate the sample IDs. Any sample or analyte row marked “Not Recognized” will not be transferred to the database when the “Transfer” button is pressed. Once transferred into the database, the data should be evaluated for QC pass / fail, then set with the appropriate settings for QC accept / reject, final value status, and comment(s). See the database programmers for more detail on working in the database.

i. .

6. Patient Results:

- a. Results Outside the Normal Range: The normal range of concentrations observed for these elements in serum is listed in Table 10.
- b. Boundaries Requiring Confirmatory Measurement:
 1. Results Lower than the First Lower Boundary (1LB) or Higher than the First Upper Boundary (1UB):

Concentrations observed less than the “first lower boundary” (defined in the laboratory database as the “1LB”) or greater than the “first upper boundary (defined as the “1UB” in the laboratory database) should be confirmed by repeat analysis of a new sample preparation. The concentration assigned to the 1LB for an element is determined by study. Report the original result, as long as the confirmation is within 10% of the original. Continue repeat analysis until a concentration can be confirmed.

2. Results Greater than Highest Calibrator: When a sample result is greater than the highest calibrator in the run, the supervisor may request that the result be confirmed in an analysis run which includes a standard or external reference material with equivalent (within 10%) or greater concentration than the sample.
3. Results Greater than Range of Linearity Tested: Perform an extra dilution on any serum sample.
4. Analyst Reporting of Abnormally Low or Abnormally High Results: Concentrations observed lower than the “second lower boundary” (defined in the laboratory database as the “2LB”) or greater than the “second upper boundary” (defined in the laboratory database as the “2UB”) should be reported to the QC reviewer as an “abnormally low result” or an “elevated result”, respectively. The concentration assigned to the 2LB and 2UB for an element is determined by study protocol. There is no routine notification for elevated levels for the metals determined in this method. The protocol for supervisors reporting elevated results to medical personnel is defined according to the study protocol.
5. Inadequate Precision Within One Measurement: 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 criteria **and** the range of the three replicate readings is greater than 10% of the observed concentration, do not use the measurement for reporting. Repeat the analysis of the sample. This type of inadequate precision is noted in the database by an ‘X’ in the “>Lim Rep Delta” field.
6. Submitting final work for Review: Once results have been imported, reviewed, and set as final in the database by the analyst,

Submit an email to the QC reviewer informing them of the readiness of the data for final review. The email should include

Instrument ID, run Date, run number, study ID, group ID.

Any bench QC failures (include reasons if known).

Any patient sample result less than the 2LB or greater than the 2UB.

Anything out of the ordinary about this analytical work which could have a bearing on the availability (i.e.

insufficient sample to analyze), accuracy, or precision of the results.

Include all items called for by the study folder cover sheet in the study folder (i.e. printouts from the ICP-MS, bench QC evaluation) together in the study folder before submitting the folder for review when analysis is complete.

7. Overnight operation or Using Auto Stop: Make every effort to complete analysis within the work day so that the entire run can be monitored. If it is not possible to complete the analysis by the end of the work day, the run may be left to complete itself unattended as long as appropriate planning is made for either overnight operation or Auto Stop.

24 hrs / day operation in DRC mode:

To reduce startup time in the mornings, the analyst is encouraged to operate the ELAN in DRC mode 24hrs/day during the work week. This eliminates the need for daily 45 minute RF generator warm-up, and possibly the need for DRC stability time (if the DRC gas is not off for extended periods of time before analysis). To maintain the instrument in DRC mode when not analyzing patient samples, setup multiple sample rows in the Samples / Batch window with autosampler position n zero (rinse station of autosampler) and wash time of 1800s (30 minutes). Repeat this sample row enough times to keep the instrument in analysis mode overnight (1 sample with 15 minute wash will take ~ 20 minutes).

AutoStop: If 24 hrs / day ELAN operation is not desired, the instrument can shut the plasma off unattended after analysis. Setup this as follows:

On the "Auto Start / Stop" tab of the Instrument window, enable the Auto Stop feature.

Press the "Change" button within the Auto Stop box and set the Delayed shutdown time to 5 minutes. This will 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.

- E. Equipment Maintenance: Analysts are expected to follow a 4-day analysis / 1-day maintenance schedule in the laboratory.**
- i. ICPMS Maintenance: On the maintenance day, perform all maintenance per the Inorganic Toxicology and Nutrition Branch ELAN ICP-MS Weekly Maintenance SOP. All equipment maintenance should be documented in the instrument logbook. For this method we can not use straight ethanol to rinse the sample introduction system, otherwise the probe and tubing will be clogged because of the precipitation of the serum protein. Use the ≥ 18 Mega-ohm·cm water to rinse the whole system whenever it is necessary.
 - ii. Data Backup: Data on the ELAN computer will be backed up via two backup routines.
 1. Daily Backups to External Hard Drive: Automatic backups of the “elandata” directory and all subdirectories should be programmed to occur each night onto an external hard disk.
 2. Weekly Backup to CD: Backup all files in the active “elandata” directory and all subdirectories onto one recordable compact disc during the weekly maintenance SOP. When the active “elandata” directory on the ICP-DRC-MS computer hard drive becomes too large to fit onto a single recordable compact disc, the oldest data can be removed from the computer to make it easier to backup the entire directory weekly. This can usually be done annually.
 - a. Backup the oldest data on the hard drive to two duplicate compact disks and verify that the files on the CD are readable
 - b. Label them with the name of the instrument, the date range of the data, the current date, your name, and “Copy 1 of 2” or “Copy 2 of 2”
 - c. After verifying that the CDs are readable, the oldest, backed up data can be deleted from the ICP-MS computer hard drive.
 - d. It is best to not store duplicate copies in the same location.

F. Interpretation of the Results

- a. Reportable Range: Serum multi-element values are reportable in the range between the method LOD and the highest concentration verified accurate by bi-annual calibration verification tests. For example, if a serum Se value is less than the method LOD, report it as < "LOD" $\mu\text{g/L}$ where "LOD" is the numerical LOD. Above the highest concentration verified, extra dilutions are made of the serum sample to bring the concentration within the verified range.
- b. Reference Ranges (Normal Values): In this method the normal reference ranges 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.

G. Method Calculations

- a. Method Limit of Detection (LODs): The detection limits for elements in serum specimens are based on 3 times the concentration standard deviation of serum blanks (named sblkchk1 or sblkchk2) analyzed in at least 20 separate runs. 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 [9].
- 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).

10. **Quality Control (QC) Procedures**

Quality Control: Quality control procedures implemented in this method are defined by the Division Procedures and Practices Guidelines and include one type of QC system which is subjected to the complete analytical process. The data from this material is then used to estimate methodological imprecision and to assess the magnitude of any time-associated trends. The concentrations of this material should cover the expected concentration range of the analytes for the method. Before QC material can be used to judge patient analytical runs, acceptable QC concentration limits must be calculated from the concentration results observed in at least 20 characterization runs. During the 20 characterization runs, previously characterized QCs or pools with target values assigned by outside laboratories should be included to evaluate the analysis.

The process of limits calculation is performed using the laboratory database and the SAS division QC characterization program.

Types of Quality Control:

1. “Bench QC”: The bench QC pools used in this method comprise two levels of concentration spanning the “low-normal” and “high-normal” ranges of the analyte of interest. The intent of bench QC is for the analyst to evaluate the performance of the analytical system on the day of analysis. The analyst inserts both the “low” and the “high” bench QC specimens two times in each analytical run (a set of consecutive assays performed without interruption) so that judgments may be made on the day of analysis. The first analysis of the two bench QC pools is done after the calibration standards are analyzed but before any patient samples are analyzed (so that judgments on the calibration curves may be made before analysis of patient samples). The second analysis of the two bench QC pools is done at the end of the run (approximately 20 patient samples total). If more patient samples are analyzed on the same calibration curve after the second run of the bench QC, both the low-normal and high-normal bench QC must be reanalyzed before and after the additional samples. For example, the schemes shown in Table 5 p.50 are both acceptable ways to analyze multiple consecutive “runs”.
2. External Reference Materials: Materials produced by laboratories outside of the CDC which have assigned target concentrations can be helpful in verifying method performance. Some examples include Standard Reference Materials (SRM) from the National Institute of Standards and Technology (NIST) (i.e. SRM 1598a) and samples from previous challenges of proficiency testing programs (i.e. Centre de Toxicologie du Quebec (CTQ)). However, only the results for the bench QC materials are used to determine if the run results can be used.
3. Bench Quality Control: After completing a run, and importing the results into the database, export the QC results to the SAS program where the run will be judged to be in or out of control. 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 *officially* accepted or rejected until it is completed.
 - a. Quality Control Rules: The SAS program applies the division QC rules to the data as follows:
 - i. If both QC run means (low & high bench QC) are within $2S_m$ limits and individual results are within $2S_i$ limits, then accept the run.
 - ii. If 1 of the 2 QC run means is outside a $2S_m$ limit - reject run if:
 1. Extreme Outlier – Run mean is beyond the characterization mean $\pm 4S_m$

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

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

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).

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

If an analyte result for the beginning QC material(s) falls outside of the 3SD (i.e. 99 percentile) limits, then the following steps are recommended:

- a. If a particular calibration standard is obviously in error, remake a new dilution at the Digiflex of that working calibrator, reanalyze it, and reprocess the sample analyses using this new result as part of the calibration curve.
- b. Prepare a fresh dilution of the failing QC material and reanalyze it.
- c. Prepare fresh dilutions at the Digiflex of all of the calibration standards (working serum multi-element standards) and reanalyze the entire calibration curve using the freshly prepared standards.

If these three steps do not result in correction of the out-of-control values for QC materials, consult the supervisor for other appropriate corrective actions. Do not report analytical results for runs that are not in statistical control.

5. *Good precision among replicates.* If “air” was sampled into the system, the precipitated serum protein might be coated within the probe, tubing and the introduction system which might cause the bad precision among replicates and/or reduced sensitivity of the instrument. Use ≥ 18 Mega-ohm·cm water to rinse the system for recovering the instrument performance.

11. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

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.

If only one analyte of the three fails bench QC, then the other two which passed bench QC may be reported.

If two analytes of the three fail bench QC, then none of the results from the run should be used for reporting. The cause of the QC failures should be investigated and then the entire run should be repeated

12. Limitations of Method; Interfering Substances and Conditions

A. Interferences Addressed by This Method

- i. Correction & Elimination of Interferences (^{64}Ni , $^{36}\text{Ar}^{14}\text{N}_2$) on Zinc (^{64}Zn).

 1. Mathematical Correction for Nickel (^{64}Ni) Interference:
The correction equation ($-0.035297 * \text{Ni}60$) 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 $^{36}\text{Ar}^{14}\text{N}_2$ Interference Using DRC: The dynamic reaction cell of the ELAN ICP-DRC-MS is used in this method to eliminate interference from $^{36}\text{Ar}^{14}\text{N}_2$ onto zinc at m/z 64. See Section 1.b for an explanation of this process.

- ii. Elimination of Interferences ($^{40}\text{Ar}^{25}\text{Mg}$, $^{36}\text{Ar}^{14}\text{N}_2^1\text{H}$) 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 $^{40}\text{Ar}^{25}\text{Mg}$, $^{36}\text{Ar}^{14}\text{N}_2^1\text{H}$ on copper at m/z 65. See Section 1.b for an explanation of this process.
- iii. Correction & Elimination of Interferences (^{78}Kr , $^{38}\text{Ar}^{40}\text{Ar}$, $^{38}\text{Ar}^{40}\text{Ca}$) on Selenium (^{78}Se).

 1. Mathematical Correction for Krypton (^{78}Kr) Interference:
The correction equation ($-0.030461 * \text{Kr}83$) 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 $^{38}\text{Ar}^{40}\text{Ar}$, $^{38}\text{Ar}^{40}\text{Ca}$ Interference Using DRC:
The dynamic reaction cell of the ELAN ICP-DRC-MS is used in this method to eliminate interference from $^{38}\text{Ar}^{40}\text{Ar}$,

$^{38}\text{Ar}^{40}\text{Ca}$ onto selenium at m/z 78. See Section 1.b for an explanation of this process.

B. Limitations of Method (Interferences Remaining in Method)

i. $^{48}\text{Ca}^{16}\text{O}^{1}\text{H}$ 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

13. Reference Ranges (Normal Values)

Selenium: 6- 11 yrs 89.0 – 142 $\mu\text{g/dL}$
 12 150 yrs 104.0 - 187.0 $\mu\text{g/dL}$
Copper : 6 11yrs 90.0 – 190 $\mu\text{g/dL}$
 12 150 yrs Male 70.0 – 140 $\mu\text{g/dL}$
 12 150 Female 80.0 - 155.0 $\mu\text{g/dL}$
Zinc: 6-150 yrs > 75.0 $\mu\text{g/dL}$

14. Critical Call Results (“Panic Values”)

Critical results for copper is < 63.50 $\mu\text{g/dL}$ and > 250. $\mu\text{g/dL}$. Critical results for selenium is < 63.50 $\mu\text{g/dL}$ and > 250. $\mu\text{g/dL}$. Critical results for zinc is < 63.50 $\mu\text{g/dL}$.

15. Specimen Storage and Handling during Testing

Specimens may be left at room temperature during analysis in case confirmation analyses must be made. Take stringent precautions to avoid external contamination by the metals to be determined. Specimens may be stored short term at refrigerated temperatures, but should be stored long term (>4 weeks) at $\leq -20\text{ }^{\circ}\text{C}$.

16. Alternate Methods for Performing Test and Storing Specimens If Test System Fails

If the analytical system fails, the analysis may be setup on other ELAN DRC instruments in the laboratory. If no other instrument is available, store the specimens at $\leq 4^{\circ}\text{C}$ until the analytical system can be restored to functionality. If interruption longer than 4 weeks is anticipated, then store serum specimens at $\leq -20^{\circ}\text{C}$.

17. Test Results Reporting System: Protocol for Reporting Critical Calls (If Applicable)

Not applicable.

18. Transfer of Referral of Specimens: Procedures for Specimen Accountability and Tracking

The analyst who receives specimen/samples delivered to IRAT sets up a "Specimen Folder." A tracking form is filled out and placed in the folder to be given to the analyst performing the analysis. The form tracks location, status, and final disposition of the specimens. When sample analysis is completed, the tracking form is placed in the Specimen Tracking Record Log book located in the trace metals library.

Calibrator record keeping means (e.g. electronic –Microsoft Access, optical disc, or tape backup) are to be used to track specimens. Records are maintained for ≥ 3 years, including related QA/QC data; duplicate records are kept (off-site, if sensitive or critical) in electronic or hardcopy format. Only numerical identifiers are used (e.g. Case ID numbers) – all personal identifiers are available only to the medical supervisor or project coordinator to safeguard confidentiality.

19. Summary Statistics and QC Graphs

See following pages.

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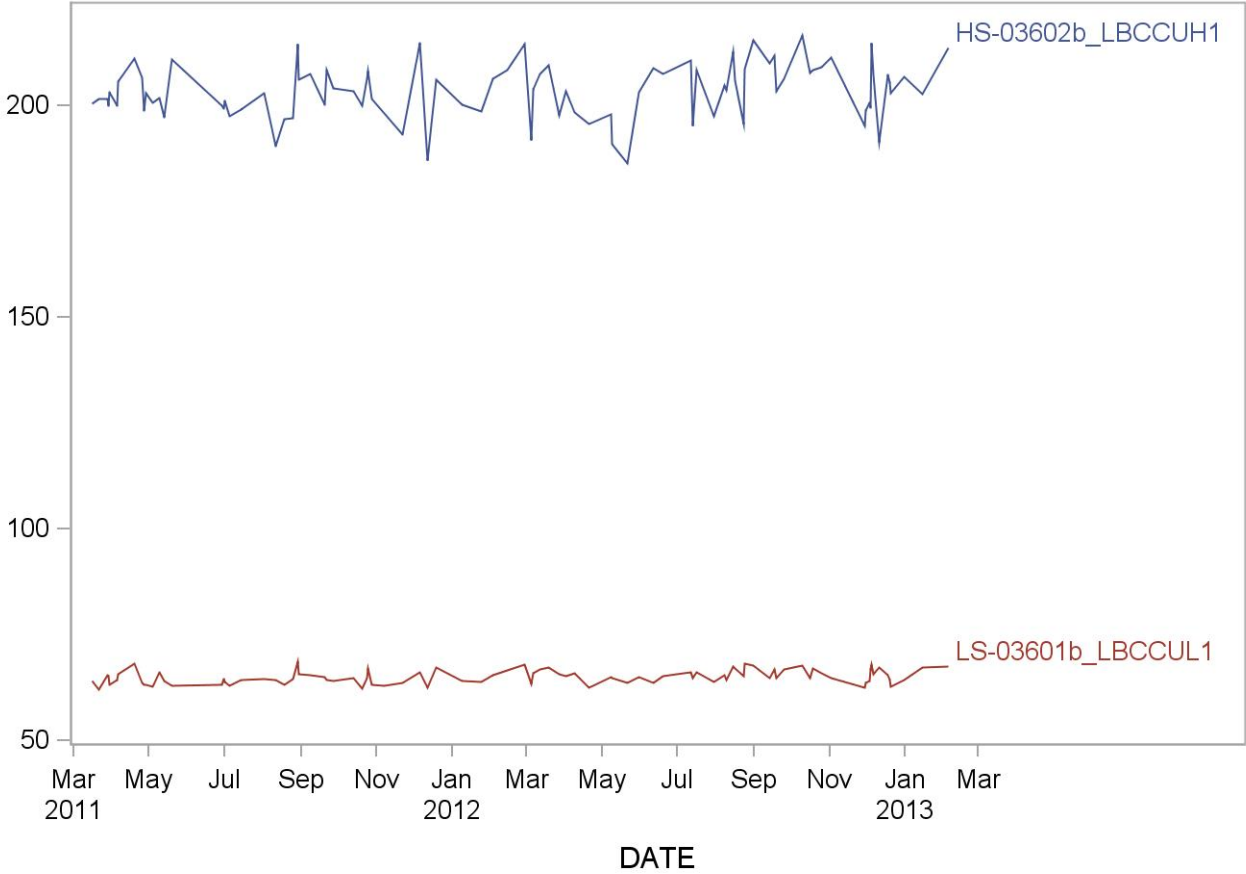
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Summary Statistics for Serum Copper (ug/dL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HS-03602b_LBCCUH1	124	16MAR11	05FEB13	202.84	6.66	3.3
LS-03601b_LBCCUL1	124	16MAR11	05FEB13	64.94	1.73	2.7

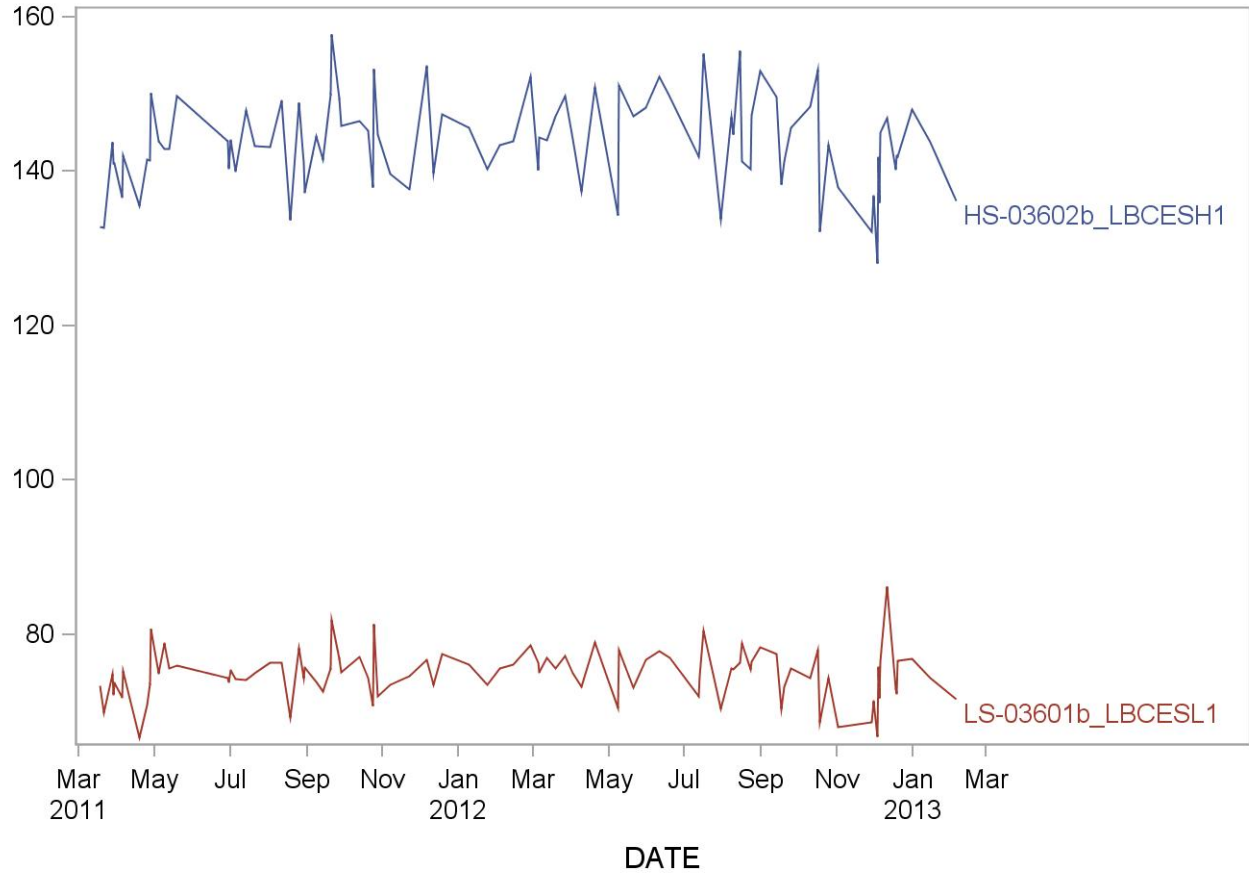
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Summary Statistics for Serum Selenium (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HS-03602b_LBCESH1	124	18MAR11	05FEB13	143.49	6.18	4.3
LS-03601b_LBCESL1	124	18MAR11	05FEB13	74.87	3.28	4.4

2011-2012 Serum Selenium (ug/L) Quality Control



Summary Statistics for Serum Zinc (ug/dL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HS-03602b_LBCZNH1	124	16MAR11	05FEB13	172.77	10.62	6.1
LS-03601b_LBCZNL1	124	16MAR11	05FEB13	49.60	2.80	5.7

2011-2012 Serum Zinc (ug/dL) Quality Control

