Laboratory Procedure Manual

Analyte: **Iodine**

Matrix: **Salt**

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

Method No: 3047.1-01

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

Contact: Amir Makhmudov
Phone: 770-488-7086
Fax: 770-488-4097
Email: AMakhmudov@cdc.gov

James L. Pirkle, MD, PhD, Director
Division of Laboratory Sciences

Important Information for Users

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

Public Release Data Set Information

This document details the Lab Protocol for NHANES 2019–2020 data.

Dataset name	Variable name	Description
IODS_K_R	LBXSL1	Iodine – Salt, 1st collection (mg/kg)
	LBXSL2	Iodine – Salt, 2nd collection (mg/kg)

1. Clinical relevance & summary of test principle

a. Clinical relevance:

Iodine (I), an essential element for thyroid function, is necessary for normal growth, development and functioning of the brain and body. Iodine-deficiency disorders (IDDs) are well-documented global health problems affecting over one billion people worldwide. Consequences of IDD include goiter, cretinism, intellectual impairment, brain damage, mental retardation, stillbirth, spontaneous abortions, miscarriages, congenital deformities, and increased perinatal mortality(1). Progress toward eliminating IDDs has been substantial; an estimated 71% of the world's edible salt is currently iodized as many countries moved toward universal salt iodization, requiring all salt to contain a standardized amount of iodine (2). The United States has not adopted this particular food fortification approach and in fact only 50-60% of salt in the US contains iodine (3). Due to differences in storage conditions in stores and households, the actual amount of iodine may vary significantly from the value advertised on the labels. For those relying solely on iodized salt for their iodine intake, this difference may pose health consequences.

b. Test principle:

This method directly measures the iodine content of salt samples using inductively coupled mass spectrometry (ICP-MS) after a simple dilution sample preparation step.

The samples are prepared by weighing out 0.625 g of each sample and diluting with 250 mL of ≥18 Mohm·cm water. The salt/water solution is mixed using a shaker until all salt is completely dissolved. This mixing step is vital to provide a homogeneous sample from which to sub-sample.

Dilution of the salt mixture in the sample analysis preparation step prior to analysis is a simple dilution of 1 part dissolved salt sample + 49 parts diluent. The effects of the dilution are to reduce ionization suppression of the salt matrix, prevent clogging of the sample introduction system pathways from high dissolved solids, and allow introduction of internal standards which will be utilized in the analysis step. Tetramethylammonium hydroxide (TMAH, 0.4% v/v) in the sample diluent solubilizes organic components. The internal standard, rhenium, is at a constant concentration in all blanks, calibrators, QC, and samples. Monitoring the instrument signal ratio of a metal to its internal standard allows correction for instrument noise and drift and sample- to-sample matrix differences.

Liquid samples are introduced into the mass spectrometer through the inductively coupled plasma (ICP) ionization source. The liquid diluted salt sample is forced through a nebulizer which converts the bulk liquid into small droplets in an argon aerosol. The smaller droplets from the aerosol are selectively passed through the spray chamber by a flowing argon stream into the ICP. By coupling radio-frequency power with flowing argon, plasma is created in which the predominant species are positive argon ions and electrons at a temperature of 6000 - 8000 K. The small aerosol droplets pass through a region of the plasma where the

thermal energy vaporizes the liquid droplets, atomizes the molecules of the sample and then ionizes the atoms. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP (at atmospheric pressure, ~760 torr) from the mass spectrometer (operating at a pressure of 10^{-5} torr). The ions first pass through a focusing region, then the dynamic reaction cell (DRC), the quadrupole mass filter, and finally are selectively counted in rapid sequence at the detector allowing individual isotopes of an element to be determined.

Generally, the DRC operates in one of two modes. In 'vented' (or 'standard') mode the cell is not pressurized and ions pass through the cell to the quadrupole mass filter unaffected. In 'DRC' mode, the cell is pressurized with a gas for the purpose of causing collisions and/or reactions between the fill gas and the incoming ions. In general, collisions or reactions with the incoming ions selectively occur to either eliminate an interfering ion, change the ion of interest to a new mass, which is free from interference, or collisions between ions in the beam and the DRC gas can focus the ion beam to the middle of the cell and increase the ion signal. With this method, the instrument is operated in standard mode.

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

2. Limitations of Method; Interfering Substances and Conditions

c. Interferences addressed by this method
None applicable.

d. Limitations of method
None applicable.

3. Procedures for collecting, storing, and handling samples; criteria for sample rejection; sample accountability and tracking

a. Procedures for collecting, storing, and handling samples:

Sample handling conditions, special requirements, and procedures for collection and transport are discussed in the Division of Laboratory Science's (DLS) Policies and Procedures Manual [4].

i. 0.625 g is needed for one analytical measurement preparation. Optimal amount of sample is 2.0 g. Request a minimum amount of 2.5 g.

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

iii. Sample characteristics that compromise test results include humidity, light exposure, and high storage temperatures.

iv. Recommended storage conditions: Samples should be stored in an airtight container. The container should be either opaque or stored in the dark, at room temperature (20 – 25º C) with little to no humidity.

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

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

ii. Small quantity (≤1.0g).

iii. Nonhomogenous salt that cannot be homogenized without comprimising the integrity of the analysis.

In all cases, request a second salt sample.

c. Transfer or referral of samples; procedures for sample accountability and tracking: Location, status, and final disposition of the samples will be tracked electronically through the Laboratory Information Management System. Maintain records for a minimum of 2 years. Use only numerical identifiers for samples within the laboratory (i.e., case ID numbers) in order to safeguard confidentiality. Only the medical supervisor (MS) or project coordinator (PC) i.e. non CDC personnel will have access to the personal identifiers.

4. Safety precautions

a. General safety

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

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

iii. Take special care when handling and dispensing bases and concentrated acids. Use additional personal protective equipment which protects face, neck, and front of body.

Warning: If concentrated TMAH or concentrated hydrochloric or nitric acid comes in contact with any part of the body, quickly wash with copious quantities of water for at least 15 minutes.

iv. Use secondary containment for containers holding corrosive liquids.

v. The use of the foot pedal on a benchtop automatic pipette is recommended because it keeps the analysts' hands free to hold the sample cups and autosampler tubes and to wipe off the tip of the benchtop automatic pipette.

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

vii. Wipe down all work surfaces at the end of the day with a broad spectrum disinfectant, or equivalent. Spray the surface with the disinfectant, allow 15 minutes contact time and then wipe up with clean water.

b. Waste disposal:

i. Autoclaving: Although these samples are not biological in nature, dispose all items that come into contact with samples in an autoclave waste container. This will avoid confusion about the safety of lab waste in normal waste containers. Use sharps containers for broken glass/quartz or items which puncture autoclave bags (i.e. pipette tips).

5. Instrument & material sources

a. Sources for ICP-MS instrumentation

i. ICP-MS: Inductively Coupled Plasma Mass Spectrometer with Dynamic Reaction Cell Technology (NexION) (PerkinElmer Norwalk, CT, www.perkinelmer.com), or equivalent.
ii. Recirculating chiller/heat exchanger for ICP-MS: Refrigerated chiller (PolyScience 6105PE) or heat exchanger (PolyScience 3370) (PerkinElmer Norwalk, CT, www.perkinelmer.com), or equivalent.
iii. Autosampler: ESI SC4-DX autosampler (Elemental Scientific Inc., Omaha, NE) or equivalent.
iv. Computer: Computer controller provided or recommended by ICP-MS manufacturer is recommended to ensure proper communication between computer and ICP-MS. Recommend 1-2 gigabytes of RAM and secondary internal hard disk for nightly backups (if network backups are not possible).
v. FAST sample introduction system (optional): Standard peristaltic pump on ICP-MS replaced by DXi-FAST micro-peristaltic pump/FAST actuator and valve combination unit. Like part # DXI-54-P4-F6. If DXi-FAST upgrade on ICP-MS is not used, a separate FAST actuator (built-in option on ESI SC4-DX

autosampler or stand-alone FAST actuator) will be necessary to complete the FAST sample introduction system.

b. Sources for ICP-MS parts & consumables

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

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

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

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

iii. Carboy and cap assembly for waste collection: 10-15 L, polypropylene wide-mouth carboy (100 mm neck size) with handles and no spigot (Like part #7BE-25126, Lab Safety Supply, Janesville, WI, www.lss.com) with cap assembly like part # N0690271 (PerkinElmer, Norwalk, CT, www.perkinelmer.com) with tubing connections built into the cap for addition of liquid waste.

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

v. Cones: Platinum or Nickel cones have been used. Platinum cones are more expensive, but will last longer, can be refurbished, and will frequently yield higher sensitivity.

1. Sampler (nickel/platinum): PerkinElmer part # WE021140/WE027802 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or cross-referenced part number manufactured by Spectron Inc. (Ventura, CA, www.spectronus.com) or Glass Expansion (Pocasset, MA, www.geicp.com). # *Spares* = *4*.
2. Skimmer (nickel/platinum): PerkinElmer part # WE021137/WE027803 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or cross-referenced part number manufactured by Spectron Inc. (Ventura, CA, www.spectronus.com) or Glass Expansion (Pocasset, MA, www.geicp.com). # *Spares* = *4*.

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

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

viii. FAST accessories

1. Valve: CTFE High-flow valve head for SC-FAST (uses ¼-28 fittings). Like part # SC-0599-1010 (Elemental Scientific Inc., Omaha, NE., www.icpms.com).
2. Stator: CTFE Stator for 6 port SC-FAST high flow valve (¼-28 fittings). Like part # SC-0599-1010-01 (Elemental Scientific Inc., Omaha, NE., www.icpms.com).
3. Rotor: Composite rotor for 6 port SC-FAST high flow valve (¼-28 fittings). Like part # SC-0599-1010-05 (Elemental Scientific Inc., Omaha, NE., www.icpms.com).
4. Sample loop: 1 mL Teflon, white connector-nuts, 1.6 mm i.d. Like part # SC-0315-10 (Elemental Scientific Inc., Omaha, NE., www.icpms.com).
5. Probe, autosampler: Teflon, carbon fiber support, 0.8 mm i.d., blue marker, 1/4-28 fittings. Like part number SC-5037-3751 (Elemental Scientific Inc., Omaha, NE., www.icpms.com). # *Spares = 2.*
6. Probe, carrier solution: Teflon, carbon fiber support, 0.5 mm i.d., orange marker, 1/4-28 fittings. Like part number SC-5037-3501 (Elemental Scientific Inc., Omaha, NE., www.icpms.com). # *Spares = 2.*
7. Tubing, carrier solution: 0.5 mm i.d. Teflon tubing (orange marker) with red ¼-28 male nut. Connects to high flow FAST valve head, port #2. Like part # SC-0316-0500 (Elemental Scientific Inc., Omaha, NE., www.icpms.com).
8. Tubing, nebulizer: See “Nebulizer, PolyPro-ST micro flow”
9. Tubing, rinse station: Teflon tubing and adapters (to attach to back of SC autosampler for filling rinse stations and to attach to rinse containers). Like part # SC-0302-0500, Elemental Scientific Inc., Omaha, NE., www.icpms.com).
10. Tubing, vacuum: Vacuum line for SC-FAST high flow valve, connects to port #6, black nut for connection to valve head, natural brown color nut on other end for connection to SC autosampler vacuum port. Like part # SC-0321 (Elemental Scientific Inc., Omaha, NE., www.icpms.com).

ix. Hose, for connection to chiller: Push on hose. I.D. = ½”, O.D. = ¾”. Use part # PB-8 (per inch, Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. Do not normally need spare hose (unless moving instrument into a new location).

x. Hose, for exhaust of ICP-MS: Available as part of ICP-MS installation kit from Perkin Elmer (PerkinElmer Norwalk, CT, www.perkinelmer.com), or equivalent. Available direct from manufacturer as part # S LP-10 air connector (Thermaflex, Abbeville, SC, www.thermaflex.net). # *Spares = 10 feet of 4” diameter and 10 feet of 6” diameter hose.*

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

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

1. Gas connection:
    a. Teflon tubing: 4mm O.D., 2.4 mm i.d. Teflon tubing (like part # ES-2502, Elemental Scientific Inc., Omaha, NE., www.icpms.com). # *Spares = 1*.
    b. Adapter kit: Plastic adapters to conn*ect Teflon t*ubing (2.4 mm i.d.) to ¼" male Swagelok (compression) port on ICP-MS. Parts can be obtained as components in a "gas fittings kit for microflow nebulizer", kit part # ES-2501-1000, Elemental Scientific Inc., Omaha, NE., www.icpms.com). # *Spares = 1*.
2. Liquid connection: Connects nebulizer to port #3 of high flow FAST valve head with green, 1/4-28 fitting. Like part # SC-0317-0250 (Elemental Scientific Inc., Omaha, NE., www.icpms.com). # *Spares = 2*.

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

xiv. 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.*

xv. 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.*

xvi. Oil for roughing pumps:

1. Welch Directors Gold: For Varian roughing pumps. Available direct from manufacturer as part # 8995G-15 (1 gallon, Welch Rietschle Thomas, Skokie, IL, www.welchvacuum.com), or equivalent. # *Spares = 4.*
2. Fomblin Y14/5 fluid: For Fomblin-based interface roughing pump PerkinElmer part # N8122265 (1 kg bottle, PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares =1 per instrument.*

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

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

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

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

1. Internal o-rings: ID = ¼", OD = 3/8", thickness = 1/16". Need 2 o-rings per injector support setup. PerkinElmer part # N8122008 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent (such as part # V75-010, O-rings West, Seattle, WA, www.oringswest.com). # *Spares = 20.*
2. External o-rings: ID = 3/8", OD = 1/2", thickness = 1/16". Need 2 o-rings for each injector support setup. PerkinElmer part # N8122009 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent (such as part # V75-012, O-rings West, Seattle, WA, www.oringswest.com). # *Spares = 20.*

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

xxii. Plugs, quick change for roughing pump oil: These plugs will only work on the Varian roughing pumps which come standard on ELAN DRC II ICP-MS 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.

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

xxiv. Spray chamber, quartz concentric:

1. Standard cyclonic: Like PerkinElmer part # WE025221 (PerkinElmer, Shelton, CT, www.perkinelmer.com), or equivalent. # *Spares = 2.*
2. Mini-cyclonic: Like ESI part #ES-3450-1010-20 (Elemental Scientific Inc., Omaha, NE., www.icpms.com), or equivalent. Use with socket adapter like quartz ESI part # ES-5510 (Elemental Scientific Inc., Omaha, NE., www.icpms.com), or equivalent. # Spares = 2.

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

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

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

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

xxviii. Tubing, peristaltic, 0.125” i.d. (spray chamber drain): Standard Santoprene, 3-stop (grey/ grey/ grey) peristaltic pump tubing, i.d. 1.30 mm. Spectron part # SC0311 (Spectron, Ventura, CA, www.spectronus.com) or equivalent. #Spares = 6 packs of 12 tubes. *For ESI DXi micro-peristaltic pump.*

xxix. Tubing, PVC, i.d. = 1/8”, O.D. = 3/16”. Used to transfer liquid

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

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

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

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

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

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

c. Sources for ICP-MS maintenance equipment & supplies

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

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

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

iv. Cutter (for 1/8" O.D. metal tubing): Terry tool with 3 replacement wheels. Like part # TT-1008 (ChromTech, Inc., Saint Paul, MN, www.chromtech.com) or equivalent.

v. 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).

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

d. Sources for general laboratory equipment and consumables

i. Bar code scanner: Like Xenon 1902 cordless area-imaging scanner (Honeywell International Inc., Morristown, NJ, www.honeywellaidc.com). For scanning sample IDs during analysis setup. Any bar code scanner capable of reading Code 128 encoding at a 3 mil label density and 2D bar codes can be substituted.

ii. Bottles, Plastic: Like USP 250mL Item #069030 (United States Plastic Corp, Lima, OH, www.usplactic.com

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

iv. Containers for diluent and rinse solution: Two liter Teflon™ containers (like catalog# 02-923-30E, Fisher Scientific, Pittsburgh, PA., www.fishersci.com or equivalent) and 4L polypropylene jugs (like catalog# 02-960-10A, Fisher Scientific, Pittsburgh, PA, www.fishersci.com or equivalent).

v. Flasks, volumetric:

1. 50mL volumetric flask (like plastic flask catalog # 40000050, Thermo Scientific, Fisher Scientific, Pittsburgh, PA., www.fishersci.com).
2. 100mL volumetric flask (like glass flask catalog # 92812G50, DWK Life Sciences (Kimble), Fisher Scientific, Pittsburgh, PA., www.fishersci.com).
3. 250mL volumetric flask (like glass flask catalog # 92812G50, DWK Life Sciences (Kimble), Fisher Scientific, Pittsburgh, PA., www.fishersci.com).
4. 1L volumetric flask (like plastic flask catalog # 40001000, Thermo Scientific, Fisher Scientific, Pittsburgh, PA., www.fishersci.com).
5. 2L volumetric flask (like glass flask catalog # 92812G2000, DWK Life Sciences (Kimble), Fisher Scientific, Pittsburgh, PA., www.fishersci.com).

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

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

viii. Pipette, benchtop automatic (for preparation of salt dilutions to be analyzed): Like the Microlab 625 advanced dual syringe diluter (Hamilton, Reno, NV, http://www.hamilton.com/) equipped with a 2.5 mL left and right syringe, a 12 gauge Concorde CT probe dispense tip, the Microlab cable management system and a foot pedal. PEEK valves like part # 60676-01 (left) and part # 60675-01 (right) may reduce metal background in prepared samples.

ix. Pipettes (for preparation of intermediate stock working standards & other reagents): Either,

1. Like Picus® NxT electronic, single-channel pipettes (Sartorius AG, Göttingen, Germany, www.sartorius.com). 5-120 µL (catalog # LH-745041), 10-300 µL (catalog #LH-745061), 50-1000 µL (catalog #LH-745081), 100-5000 µL (catalog #LH-745101).
2. Hamilton Microlab 600 Diluter Dispenser Dual Dispense Kit equipped with a 50.0 mL and a 1.0 mL syringe, 12 gauge fill and dispense tubing, and foot pedal or equivalent (Hamilton, Reno, NV http://www.hamiltoncompany.com/products/laboratory-products/laboratory-instruments/microlab-600-diluter-dispenser) or equivalent programmable automatic diluter and dispenser.

x. 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), or equivalent. Clear plastics tend to have lowest trace metal contamination. Blue colored caps have also been used successfully for this method.

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

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

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

xiv. Shaker: Like Stuart, gentle rocking and rolling system. (Fisher Scientific, Hanover Park, Illinois, fishersci.com), or equivalent.

e. Sources of chemicals, gases, and regulators

i. Acid, nitric acid: Environmental grade, 68-70% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com), or equivalent. For use in cleaning any bottles, vials, tubes, and flasks. This is referred to as “concentrated” nitric acid in this method write-up.

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

1. Regulator for argon (at dewar): Stainless steel, single stage, specially cleaned regulator with 3000 psig max inlet, 0-200 outlet pressure range, CGA 580 cylinder connector, and needle valve shutoff on delivery side terminating in a ¼" Swagelok connector. Part number "KPRCGRF415A2/AG10-AR1" (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com), or equivalent. # *Spares = 1*.
2. Regulator for argon (between bulk tank and PerkinElmer filter regulator): Single Stage 316SS Regulator, with 0-300 psi Inlet Gauge, 0-200 psi Outlet Gauge, Outlet Spring Range, 0-250 psi, ¼" Swagelok Inlet Connection, ¼ turn Shut off Valve on Outlet with ¼" Swagelok Connection and Teflon Seals. Part number KPR1GRF412A20000-AR1 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com), or equivalent. # *Spares = 1*.
3. Regulator for argon (filter regulator on back of ICP-MS): Argon regulator filter kit. Catalog number N812-0508 (PerkinElmer, Shelton, CT, www.perkinelmer.com).

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

iv. Standard, rhenium: 1,000 µg/mL, item #CGRE1-1 (Inorganic Ventures, Christiansburg, VA http://www.inorganicventures.com), or equivalent. Used as an internal standard in diluent. Standard must be traceable to the National Institute for Standards and Technology.

v. Standard, iodide: 1,000 µg/mL, item #IC-II-M (High Purity Standards, Charleston, SC, http://www.hps.net/), or equivalent. Standard must be traceable to the National Institute for Standards and Technology.

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

6. Preparation of reagents and materials

a. Internal standard intermediate:

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

ii. Preparation: To prepare 50 mL of 100 µg/mL Re in 3% v/v HNO_3:

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

b. Sample diluent and carrier

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

ii. Preparation: To prepare 2 L of 5 μg/L Re, 0.4% v/v TMAH:

1. If not previously dedicated to this purpose, acid wash a 2 L container (PP, PMP, or Teflon™) with 1% v/v HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Dedicate to purpose.
2. Partially fill the 2 L container with ≥18 Mohm·cm water.
3. Add 8 mL of 25% v/v TMAH.
4. Fill to 2 L using ≥18 Mohm·cm water.
5. Spike 100 μL of 100 mg/L Re (Internal Standard Intermediate) to the final diluent.
6. Invert bottle a few times to insure thorough mixing.
7. Store at ambient temperature and label appropriately. Expiration is 1 year from date of preparation.

c. ICP-MS rinse solution

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

ii. Preparation: To Prepare 4 L of 0.4% v/v TMAH:

1. If not previously dedicated to this purpose, acid wash a 4 L container (PP, PMP, or Teflon™) with 1% v/v HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Dedicate to purpose.
2. Partially fill the 4 L container with ≥18 Mohm·cm water.
3. Add 16 mL of TMAH.
4. Fill to 4 L using ≥18 Mohm·cm water.
5. Invert container a few times to ensure thorough mixing.
6. Store at ambient temperature and label appropriately. Expiration is 1 year from date of preparation.

d. Standards, calibrators, base salt and QC

Use of volumetric flasks with handheld pipettes or volumetric preparation using a benchtop automatic pipette are both acceptable. Use of a benchtop automatic pipette for this process is faster and reduces chance for error.

i. Stock calibration standards

1. Purpose: The single-element standards used to prepare the single-element intermediate stock calibration standards.
2. Contents: An aqueous solution containing iodide.
3. Purchase & Storage:
    a. Purchasing: Standards must be NIST-traceable.
    b. Storage: Store at ambient temperature and label appropriately. Expiration is determined by manufacturer or is 1 year after the container is opened (whichever comes first).

ii. Iodide (I^-) intermediate stock calibration standard

1. Purpose: Used to prepare the S1–S5 intermediate working calibration standards
2. Preparation & storage: To prepare 100 mL of an aqueous solution containing I at concentrations listed in Table 3:
    a. If not previously dedicated to this purpose acid-rinse a 100 mL volumetric flask. For example, with 3% v/v HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Mark flask according to intended use. Dedicate to purpose.
    b. Partially fill (50–75% full) the 100 mL glass flask with ≥18 Mohm·cm water. Add 0.25g NaCl (Noniodized) to the 100 mL flask. Rinse weighing vessel with 18 Mohm·cm water to ensure complete transfer of NaCl.
    c. Pipette the appropriate volume (see Table 3) of the iodine stock standard solution into the volumetric flask. Dilute to the volumetric mark with ≥18 Mohm·cm water. Mix solution thoroughly. Final concentration is listed in Table 3.
    d. Once mixed, transfer to a labeled, lot tested or cleaned PP tube (i.e. 50 mL). Store solutions at room temperature (~20–25 ºC). Expiration is 6 months from the date of preparation.

iii. Preparation of NaCl Matrix for Calibration Standards:

1. Purpose: Used to prepare the S0-S5 working calibration standards.
2. Preparation & Storage:
    a. If not previously dedicated to this purpose acid-rinse a 500 mL volumetric flask. For example, with 3% v/v HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Mark flask according to intended use. Dedicate to purpose.
    b. Partially fill 500 mL volumetric flask with ≥18 Mohm·cm water.
    c. Add 1.25g NaCl to flask.
    d. Fill to the mark with ≥18 Mohm·cm water.
    e. Expiration is 6 months from the date of preparation.

iv. Intermediate working calibration standards

1. Purpose: Used each day of analysis to prepare the final working calibrators that will be placed on the autosampler.
2. Preparation and storage: To prepare 50 mL of each in a 0.25% NaCl matrix at concentrations listed in Table 4.
    a. Preparation by volumetric flasks
        i. If not previously dedicated to this purpose, acid-rinse six 50 mL volumetric flasks with 3% v/v HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Mark each flask according to intended use (S1–S6). Dedicate to purpose.
        ii. Fill each 50 mL flask 15–75% with the 0.25 % NaCl solution (see Table 4 Preparation of intermediate working standards).
        iii. Pipette the appropriate volume (see Table 4) of the intermediate stock calibration standard solution into each of the volumetric flasks. Dilute each to the volumetric mark with the 0.25% NaCl solution using a pipette for the final drops. Mix each solution thoroughly. Final concentrations are listed in Table 4.
        iv. Once mixed, transfer to labeled, lot tested or cleaned PP tubes/containers for storage (e.g. 15mL for daily use). Store at room temperatures (~20–25 ºC). Expiration for iodine is 3 months from the date of preparation. Iodine only calibrator sets can be stored in plastic containers.

v. Working calibrators

1. Purpose: The working calibrators will be analyzed in each run to provide a signal-to-concentration response curve for each analyte in the method. The concentration of the analyte of interest in a salt sample dilution is determined by comparing the observed signal ratio (element/internal standard) from the dilution of the salt sample to the signal ratio response curve from the working calibrators.
2. Preparation & use: To make dilutions of the corresponding six intermediate working calibration standards, use a benchtop automatic pipette and follow the volumetric directions in Table 7 along with directions in Section 8: “The run: quality, execution, evaluation, and reporting”. Analyze the calibrators within 48 hours after preparation.

vi. Internal quality control materials (“bench” QC)

1. Purpose: Internal (or “bench”) quality control (QC) materials are used to evaluate the accuracy and precision of the analysis process, and to determine if the analytical system is “in control” (is producing results that are acceptably accurate and precise). They are included in the beginning and at the end of each analytical run.
2. Content: The internal (or “bench”) quality control (QC) materials used in this method are Salt solutions created by Morton Iodized salt and a mixture of Morton Iodized and Noniodized salt. The analyte concentrations are in the low-normal (“low QC”), and high-normal (“high QC”) concentration ranges.
3. Preparation & storage: Quality control materials can be either prepared by and purchased from an external laboratory or prepared within the CDC laboratories. Quality control must always be traceable to the National Institute for Standards and Technology (NIST). The CDC laboratory currently prepares its own bench QC materials using the following procedures:

    a. Dispensing and storage of salt

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

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

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

1. Allow solution to reach ambient 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 the benchtop automatic pipette) with a length of clean Teflon™ tubing long enough to reach into the bottom of the 10 L carboy while it is sitting on the stir plate.
3. Check cleanliness of the benchtop automatic pipette before use by analyzing 1–2% v/v HNO_3 which has been flushed through the benchtop automatic pipette with a portion of the same solution which has not been through the benchtop automatic pipette.
4. Approximately one hour before dispensing begins:
    a. With the large stir plate close to the left side of the benchtop automatic pipette, begin stirring the salt pool to be dispensed.
    b. Also during this time, flush the benchtop automatic pipette with salt solution from the pool to be dispensed. Place the ends of the tubing attached to both the sample and dispensing syringes into the carboy of salt solution so that the solution 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 salt solution into the vials, cap the vials and label them. Placing labels on vials after capping minimizes the chance for contamination during the process.

iv. Homogeneity test: Check homogeneity of analyte concentrations in pool aliquots. Keep samples pulled for homogeneity analysis in the sequence that they were dispensed. Label vials in the order they were pulled. Analyze the samples in a random order for the purpose of looking for trends in concentrations without interference of possible instrument related trends. Once dispensed and homogeneity has been shown to be good throughout the tubes of a pool, store tubes at ≤ -20°C and pull tubes out as needed for analysis.

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

e. Optimization solutions

i. Dual detector calibration:

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

7. Analytical instrumentation setup

(See Section 5: Instrument and Material sources for details on hardware used, including sources)

a. Instrumentation and equipment setup:

i. Configuration for liquid handling

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

2. Carrier solution uptake: Use peristaltic pump to control uptake flow rate of carrier solution to the SC-FAST valve. Use of a 'peristaltic to Teflon tubing adapter' for prevents damage to small i.d. tubing when making connections (see consumables descriptions in Section 5.b: FAST/ESI SC4-DX autosampler accessories.

3. Spray chamber waste removal

   Use of a 'peristaltic to Teflon tubing adapter' for prevents damage to small i.e. tubing when making connections (see consumables descriptions in Section 5.b FAST/ESI SC4-DX autosampler accessories).
   a. Between spray chamber and peristaltic tubing:
      i. Spray chambers with threaded connection: Use vendor-supplied threaded connector on base of chamber, connecting tubing directly to peristaltic pump tubing through a PEEK adapter or directly.
      ii. Spray chambers without threaded connection: Use of specialized push-on connectors available from various vendors (like UFT-075 from Glass Expansion, Pocasset, MA) are preferred for safety reasons to direct connection of PVC tubing (i.e. 1/8" i.d. x ¼" O.D.).

b. Between peristaltic pump tubing and waste container: Connect 1/8" i.d. x ¼" O.D. PVC tubing to the grey/grey Santoprene peristaltic pump tubing using a tubing connector (PerkinElmer item # B3140715). Place the free end of the PVC tubing through the lid of the waste jug (be sure it is secure). In case of overflow, place the waste jug in a secondary containment tray (>110% the volume of the waste jug).

4. Rinse solution for autosampler:

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

ii. Gas delivery and regulation

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

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

   a. Step down regulator (if source of argon is a bulk tank): Place this single stage regulator in the lab so that incoming argon pressure can be monitored and adjusted. Set delivery pressure to at least 10 psig above the delivery pressure of the filter regulator on the ICP-MS.
   b. Filter regulator at ICP-MS: Single stage "argon regulator filter kit" supplied with the ICP-DRC-MS. Set the delivery pressure depending on the instrument setup:
      i. NexION: Argon regulator filter kit. Catalog number N8145023 (PerkinElmer, Shelton, CT, www.perkinelmer.com).

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

b. Instrument and method parameters: See Table 1 for a complete listing of the instrument and method parameters and software screen shots.

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

a. Bench QC, reference materials and calibration verification:

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

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

In each analytical run the analyst will test each of the bench QC samples two times, subjecting them to the complete analytical process. Bench QC pool samples are analyzed first in the run after the calibrators but before any samples are analyzed. This permits making judgments on calibration linearity and blank levels prior to analysis of samples. The second analysis of the bench QC pools is done after analysis of all samples in the run (typically 30–40 samples total) to ensure analytical performance has not degraded across the time of the run. If more samples are analyzed on the same calibration curve after the second run of the bench QC, all bench QC must be reanalyzed before and after the additional samples. For example, the schemes shown in Table 5 are both acceptable ways to analyze multiple consecutive "runs".

ii. Use standard reference material (SRM, e.g. SRM 3530) from the National Institute of Standards and Technology (NIST) to verify method accuracy. Use previously characterized samples from proficiency testing program or commercially-produced reference materials when NIST SRMs are unavailable.

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

b. Perform, evaluate and report a run

i. Starting the equipment for a run

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

5. Start the plasma
6. Start the peristaltic pump: Start the pump running slowly, making sure that the rotational direction is correct for the way the tubing is set up.
7. Place probe in adequate volume of liquid (i.e. carrier, rinse solution): If using an ESI FAST, manually place carrier probe into liquid which will be aspirated during the warm-up time prior to the daily performance test. If not using an ESI FAST, send the autosampler probe to that solution.
8. Warm-up time: Allow warm-up time suggested by the manufacturer for the ICP-MS (i.e. RF generator) after igniting the plasma.
9. Daily performance check: Perform and document a daily performance check and any optimizations necessary.

   Save new parameters to the “default.tun” and “default.dac” files.
10. Place probe in adequate volume of carrier or rinse solution: If using an ESI FAST, manually place carrier probe into carrier solution. If not, send the autosampler probe to a rinse solution (i.e. autosampler rinse station).

11. Readying the instrument for quick-start analysis: Leave the plasma running to eliminate the need for an initial instrument warm-up period and/or a DRC stabilization period as long as appropriate planning is made for sufficient solution supply and waste collection. Analysis of conditioning samples (diluted salt matrix) can also be scheduled to occur at roughly a predetermined time. Accomplish this by setting up multiple sample analyses with extended rinse times (i.e. one analysis with a 1500s rinse time will take approximately 30 minutes to complete). Initial samples would be non-matrix, while final samples would be diluted matrix for conditioning.
12. Software setup for analysis:
    a. Workspace (files & folders): Verify & set up the correct files and data directories for your analysis (See Table 1 for defaults).
    b. Samples/batch window: Update the software to reflect the current sample set. Use a bar code scanner to input data whenever possible. See Table 1 for times and speeds.

        1. Salt vs. aqueous method files:
            a. The difference: There are two method files for this one method (see Table 1). 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 salt blank and salt-based calibrators (the “Sblk” method file) and the other lists the autosampler position of the aqueous blank (the “aqblk” method file).
            b. Use: The ONLY TIME when it matters which of these files is used is when the measurement action *includes* “Run

blank" or "Run standards". When the measurement action is only 'run sample', it does not matter whether the "Sblk" or "aqblk" method file is used. Analysts typically follow the pattern below, however, for the sake of consistency and as a reminder of which blank must be used for which type of sample see Table 6.

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

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

ii. Preparation of samples for analysis (See Table 7)

1. Sample Preparation:
   a. Allow sample container to shake and roll on the Shaker for at least 25 minutes.
   b. Weigh 0.625g of the salt sample and add to a 250 mL volumetric flask.
   c. Rinse weigh boat with ≥18 Mohm·cm water into the volumetric flask.
   d. Fill to the 250 mL mark with ≥18 Mohm·cm water.
2. Prepare the following solutions into pre-labeled containers using a benchtop automatic pipette. See Table 7 for a summary.
   a. *Aqueous Blank*: Prepare a minimum of two aqueous blanks. One will be the actual aqueous blank and the other will be a backup ("Aqueous Blank Check") in case the original aqueous blank is unusable.
   b. *Calibrators*: Prepare the working calibrators (S0–S6). Three preparations of the S0 calibrator will be needed. One of these S0 preparations will be the zero standard (Matrix blank) for the calibrators; two will be analyzed after the last calibrator to perform washout after the calibrators.
   c. *Salt samples & QC Samples*: Before taking an aliquot for analysis, homogenize the sample thoroughly (e.g. vortex for 3-5 seconds, or invert 5-10 times).

   After preparation, cover and mix the diluted samples then uncover and place them on the ICP-MS autosampler in the order corresponding to the sequence setup in the ICP-MS software.

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

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

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

Monitor the analysis for the following:

1. Verify proper operation of the instrument (e.g. loop filling, sample introduction and measurement timing, etc…).
2. Verify that background signal from instrument and reagents are low. Helpful checks when diagnosing high background problems include:
   a. Water to be used in Aq Blank Checks and dilutions.
   b. Diluent before and after being flushed through the benchtop automatic pipette.

      If contamination is observed from the benchtop automatic pipette, flush the benchtop automatic pipette with nitric acid solution (e.g. ≥500 mL) no greater than 5% v/v HNO_3 and retest.

   c. Comparison with other instruments.
3. Verify analyte/internal standard ratio stability (esp. DRC measurements)

   The net intensity (analyte / internal standard ratio) of the measurements made while stabilizing the DRC gives indication of the readiness of the system to begin analysis. Continual trending in this ratio indicates that unwanted instrument drift will occur within the run.
4. Verify calibration curves meet correlation coefficient (R^2) requirements of a minimum of 0.98 (R^2 is typically 0.99 to 1.00).
5. Verify bench QC results are within acceptable limits.

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

   a. Evaluate the blank results.
   b. Evaluate the reproducibility of the 3 replicates within the measurements.
   c. Evaluate the consistency of the internal standard across the measurements (esp. the calibrators).
   d. Evaluate calibration curves. If a particular calibrator is obviously in error, it can be re-analyzed as a sample (old or new dilution) and incorporated into the curve through data reprocessing as a calibrator. As a last resort, a single calibration point per analyte between or including S2 and S5 can be removed from the curve (Do not drop S0, S1 or S6). If repeated problems are observed with calibrators, follow up with appropriate corrective actions (i.e. re-preparation of intermediate working standards or troubleshooting instrument parameters).
   e. Prepare a fresh dilution of the failing QC material (same vial) and reanalyze it to see if the QC dilution was not properly made.

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

g. Prepare and analyze new working calibrators.

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

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

6. Verify that the precision among replicates of each measurement is acceptable in terms of rep delta requirements.

7. *Verify consistent measured intensities of the internal standards*.

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

8. Verify elevated sample results.

Refer to Figure 17 in Appendix C for flowchart.

a. Confirming an elevated concentration: Repeat for confirmation any sample having a concentration greater than the 1UB threshold. See Table 9.

b. Dilution of a sample to within the calibration range: Repeat in duplicate with extra dilution any sample having a concentration greater than the highest calibrator to bring the observed result within the concentration range of the calibrators. See Table 8 for high calibrator concentrations and validated extra dilutions.

c. Confirming proper washout after an elevated sample: When monitoring the analysis in real-time, if sample concentrations following an elevated sample are greater than the highest concentration validated for washout for either analyte (see Table 9), do the following to verify that the run is still in control for low concentration samples before proceeding with analysis.

i. Stop run following elevated sample

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

Example:
3047 SBlkChk Wash1
3047 SBlkChk Wash2
LUXXXXX Wash

iii. If the run is not verified in-control for low concentration samples before the next samples are analyzed, see Section 8: “Analytical results” for directions.

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

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

1. Electronic file transfer to laboratory information system (LIMS): Transfer data electronically to the LIMS. When keyboard entry must be used, proofread transcribed data after entry.

a. Export data from the ICP-MS software using “original conditions” or files and folders used during the analysis. Use descriptive report filenames (e.g. 2019-0118_group08.txt). In the NexION software under “Report Format” (METHOD window, REPORT tab) choose the “Use Separator” option, and under the “File Write” Section choose “Append.”

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

c. Import the instrument file into the laboratory information system with appropriate documentation.

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

i. Analyst initials

ii. Instrument ID

iii. Date of analysis and run # for the day

vii. Analyst evaluation of run results:

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

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

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

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

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

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

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

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

Abbreviations:

S_i = Standard deviation of individual results.

S_m = Standard deviation of the run means.

S_w = Within-run standard deviation.

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

2. Analytical results:

a. Concentrations outside of the normal range: (refer to Figure 17 for flowchart for elevated concentration samples)

i. Boundaries requiring confirmatory measurement:

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

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

   a. Results greater than the first upper boundary (1UB): Confirm by repeat analysis of a new sample preparation any concentration observed greater than the 1UB. Report the first analytically valid result, as long as the confirmation is within ±10% of the initial result or ±3SD of the nearest QC, whichever is greater. Continue repeat analysis until a concentration can be confirmed.

b. Analyst reporting of results outside of the normal range: Report any sample results confirmed to be greater than the second upper boundary (2UB) as an “elevated result”.

2. Results greater than highest calibrator: Samples that exceed the high calibrator must be prepared with extra dilution in duplicate to bring the observed result within the calibration range. Report the first analytically valid result (i.e. the first one within the calibration range), as long as the confirmation is within 10% of the initial result or ±3SD of the nearest QC, whichever is greater. Continue repeat analysis until a concentration can be confirmed. Use of the lowest possible dilution level is preferred to minimize differences between the calibrators and the samples (i.e. 2x dilution is preferred over 10x if 2x is sufficient to dilute analyte into the documented linearity range).

ii. Concentrations requiring verification of washout: after a result is observed that is greater than the highest concentration validated for washout, do the following:

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

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

iii. Unacceptable reproducibility: If the range of the three replicate readings (maximum replicate concentration value - minimum replicate concentration value) for a single sample analysis is greater than the range maximum criteria listed in Table 9 ***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.

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

9. Routine equipment maintenance and data backups

Maintenance activities will be documented in the instrument logbook.

a. Equipment maintenance: Analysts are expected to regularly evaluate the need for, and when necessary perform, cleaning, replacement, or re-positioning of components in ICP-MS the sample introduction system, interface, ion optics region, and equipment required resources (i.e. autosampler, exhaust,

compressed gases, and coolant). Frequency of equipment maintenance will be dependent on instrument throughput.

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

   i. Dual detector calibration: Perform dual detector calibration regularly as instrument stability requires (e.g. weekly or monthly) for any element exceeding 1,000,000 cps for calibrator 6. This is typically I. Dual detector calibration solution is described in Section 6: “Preparation of reagents and materials”.

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

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

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

10. Reporting Thresholds

a. Reportable range: Salt element concentrations are reportable in the range between the method limit of detection (LOD) and the high calibrator times the maximum permitted extra dilution (see Table 8). Above the high calibrator, extra dilutions are made of the salt sample solution to bring the observed concentration within the calibration range.

b. Reference ranges (normal values): None have been established for this method yet.

c. Action levels: There are no routine notification for levels of iodine in salt determined with this method.

11. Method Calculations

d. Method limit of detection (LODs): The method detection limits for elements in salt samples are defined as 3 times s_0, where s_0 is the estimate of the standard deviation at zero analyte concentration. S_0 is taken as the y-intercept of a linear or 2nd order polynomial regression of standard deviation versus concentration (3 concentration levels of the analytes in salt each measured 20 times). Method LODs are re-evaluated periodically.

e. Method limit of quantitation (LOQ): The Division of Laboratory Sciences does not currently utilize limits of quantitation in regards to reporting limits [3].

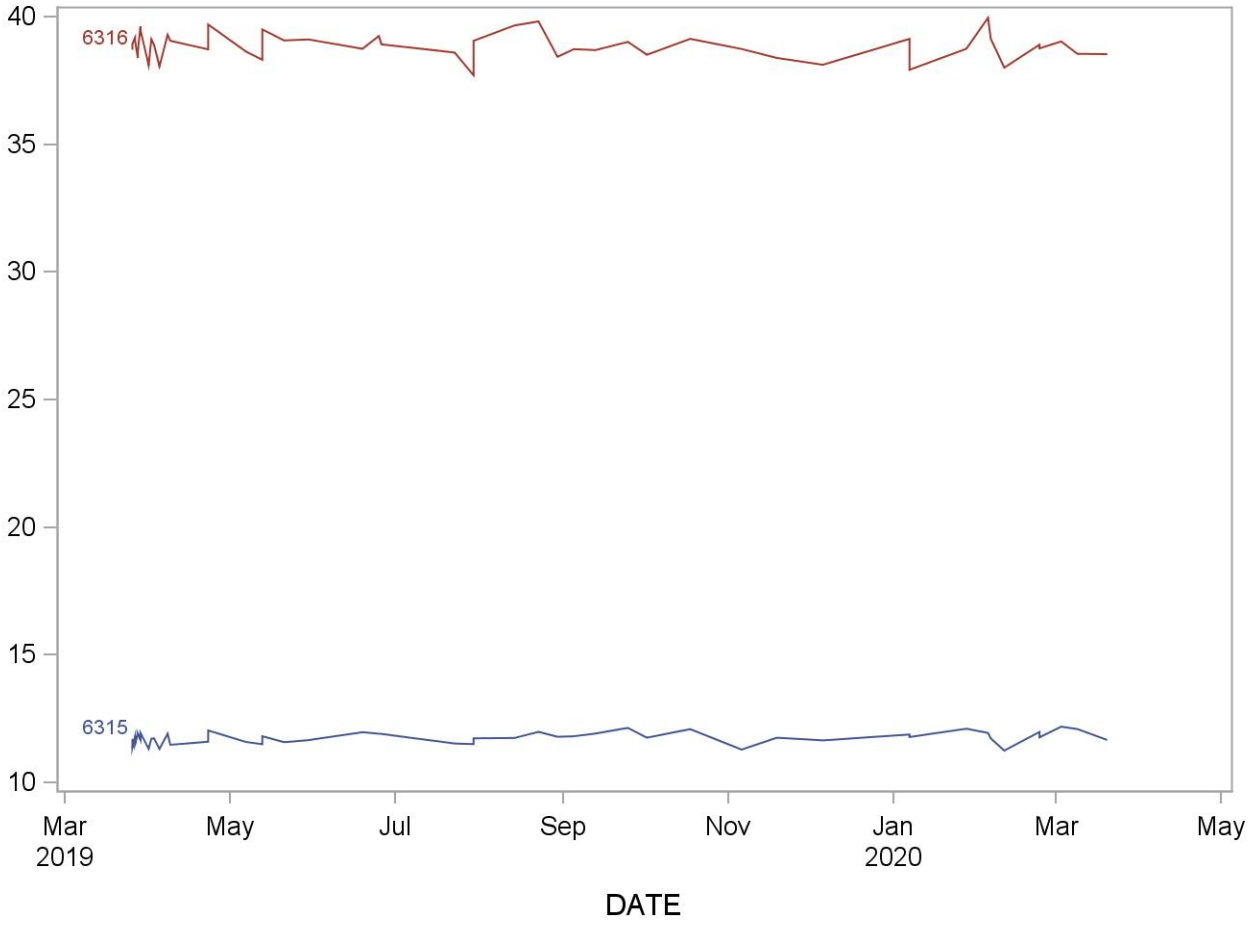
f. QC Limits: Quality control limits are calculated based on concentration results obtained in at least 20 separate runs. It is preferable to perform separate analyses on separate days and using multiple calibrator lot numbers, instruments, and analysts to best mimic real life variability. The statistical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

12. Alternate methods for performing test and storing samples if test system fails:
If the analytical system fails, setup analysis on other ICP-MS instrument, if available. If no other instrument is available, store the samples at room temperature (20-25 ºC) in the dark in an area with little to no humidity until the analytical system can be restored to functionality.

13. Summary Statistics and QC Graphs
Please see follow page.

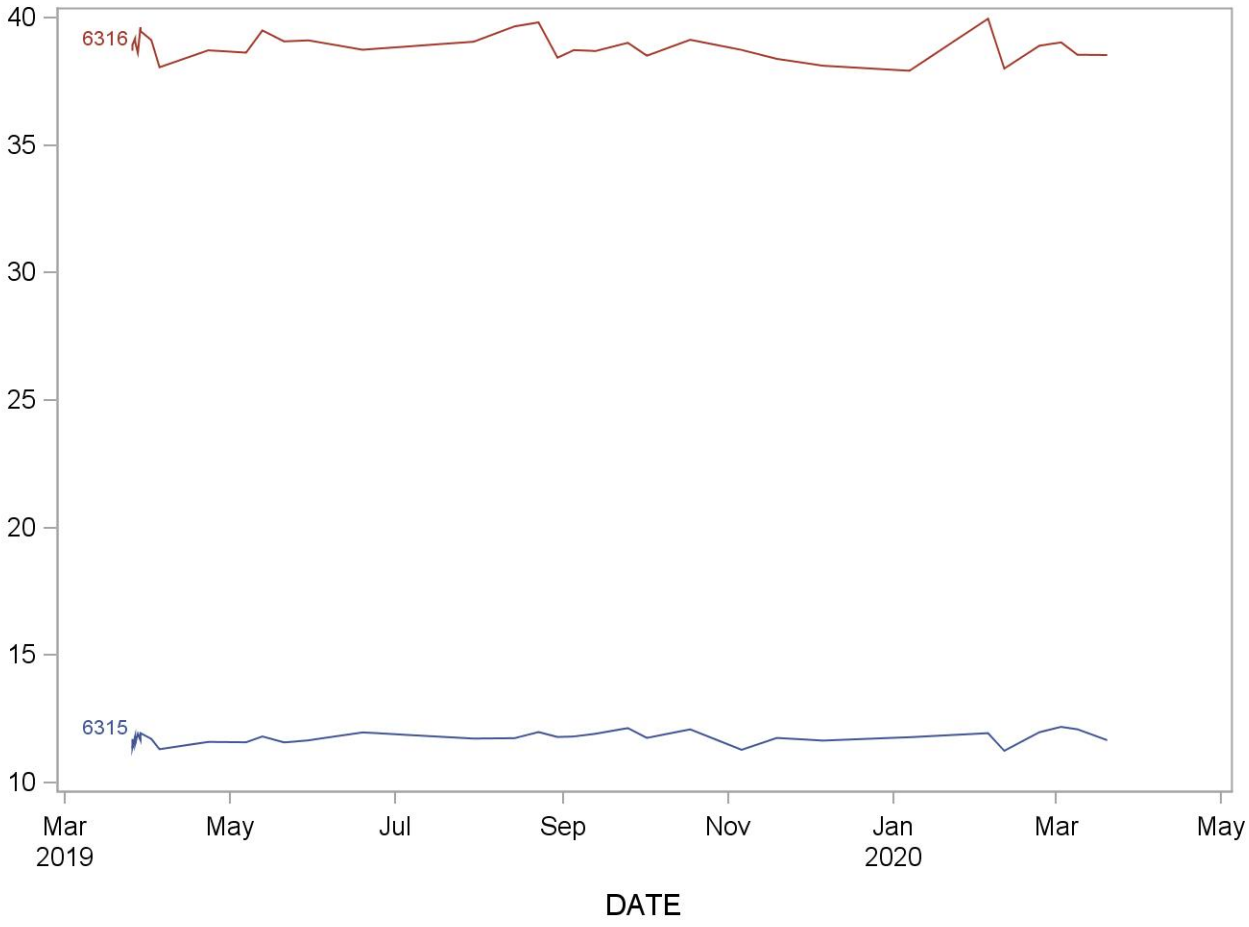
2019-2020 Summary Statistics and QC Chart
LBXSL1 (Iodine, salt - 1st collection (mg/kg))

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
6316	49	26MAR19	20MAR20	38.85	0.50	1.3
6315	49	26MAR19	20MAR20	11.75	0.23	2.0



2019-2020 Summary Statistics and QC Chart
LBXSL2 (Iodine, salt - 2nd collection (mg/kg))

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
6316	34	26MAR19	20MAR20	38.87	0.50	1.3
6315	34	26MAR19	20MAR20	11.76	0.24	2.0



14. Method performance documentation

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

Appendix A: Method performance documentation

a. Accuracy

Accuracy compared to Reference Material

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

Method name:	Iodine in Salt by ICP-MS
Method #:	3047
Matrix:	Salt
Units:	mg/kg
Reference material:	**NIST SRM 3530**
Analyte:	**Iodine**

Reference material	Replicate	Nominal value	Measured concentration								Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	CV (%)	
Level 1	1	34.0	35.5	36.1	36.5	35.8	35.3	35.89	0.44	1.22	5.41
	2		35.8	35.8	36.1	36.6	35.4				
Level 2	1	102.1	105.5	104.7	107.4	106.4	102.1	104.63	1.70	1.62	2.44
	2		105.4	103.8	103.6	105.1	102.3				
Level 3	1	153.2	155.5	155.4	156.2	155.2	151.5	154.08	2.04	1.33	0.57
	2		155.1	154.1	155.0	153.0	149.8				

b. Precision

Precision

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

Method name:	Iodine in Salt by ICP-MS
Method #:	3047
Matrix:	Salt
Units:	mg/kg
Analyte:	**Iodine**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	12.0	11.4	11.69	0.1	0.1	273.4
2	10.9	10.7	10.79	0.0	0.0	232.8
3	11.4	11.4	11.39	0.0	0.0	259.3
4	11.4	11.4	11.44	0.0	0.0	261.6
5	11.4	11.9	11.66	0.1	0.1	271.7
6	11.6	11.2	11.41	0.0	0.0	260.3
7	11.4	11.6	11.51	0.0	0.0	265.2
8	11.8	12.1	11.94	0.0	0.0	285.2
9	11.9	11.8	11.82	0.0	0.0	279.4
10	11.7	11.9	11.79	0.0	0.0	278.2

Grand sum 230.9 **Grand mean** 11.5

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.5	0.0	0.2	1.9
Between Run	1.9	0.2	0.3	2.5
Total	2.4		0.4	**3.1**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	38.2	37.3	37.73	0.2	0.2	2847.6
2	37.3	37.4	37.31	0.0	0.0	2784.0
3	37.5	36.7	37.10	0.2	0.2	2753.3
4	38.6	38.4	38.50	0.0	0.0	2964.6
5	37.2	37.7	37.43	0.1	0.1	2801.8
6	38.4	38.6	38.48	0.0	0.0	2961.8
7	38.6	38.2	38.37	0.0	0.0	2944.9
8	38.4	38.0	38.21	0.0	0.0	2919.9
9	38.7	39.0	38.81	0.0	0.0	3012.1
10	37.4	38.4	37.94	0.3	0.3	2879.2

Grand sum 759.8 **Grand mean** 38.0

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	1.5	0.2	0.4	1.0
Between Run	6.0	0.7	0.5	1.3
Total	7.5		0.6	**1.7**

c. Stability

Stability

The initial measurement can be from the same day for all stability experiments.
Freeze and thaw stability = Samples went through 3 freeze thaw cycles at ≤ -70°c prior to analysis.
Describe condition: QC pools LMS091819 and HMS091820 were used for the measurements.
Bench-top stability = Samples were left in the vials on the benchtop overnight prior to analysis.
Describe condition: QC pools LMS091819 and HMS091820 were used for the measurements.
Processed sample stability = Samples were processed(ready for instrument analysis) and stored capped at room temperature for 24 hours prior to analysis.
Describe condition: QC pools LMS091819 and HMS091820 were used for the measurements.
Long-term stability = Long term stability will be determined through a series of measurments over the span of 1 year starting at date of first sample collection and date of last sample analysis. This will be completed in January 2020.
Describe condition: QC pools LMS091819 and HMS091820 will be used for measurements.

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

Method name:	Iodine in Salt
Method #:	3047
Matrix:	Salt
Units:	mg/kg
Analyte:	**iodine**

Quality material 1								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	11.7	12.4	11.7	11.4	11.7	11.5	11.6	
Replicate 2	11.4	11.5	11.4	11.3	11.4	11.6	11.5	
Replicate 3	11.5	12.0	11.5	11.5	11.5	11.5	11.4	
Mean	11.5	12.0	11.5	11.4	11.5	11.5	11.5	#DIV/0!
% difference from initial measurement	--	**3.8**	--	**-1.2**	--	**0.0**	--	**#DIV/0!**

Quality material 2								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	38.8	38.7	38.8	38.4	38.8	38.7	38.2	
Replicate 2	38.8	39.0	38.8	38.0	38.8	38.2	38.4	
Replicate 3	38.5	39.1	38.5	38.3	38.5	38.6	38.5	
Mean	38.7	38.9	38.7	38.2	38.7	38.5	38.4	#DIV/0!
% difference from initial measurement	--	**0.6**	--	**-1.2**	--	**-0.5**	--	**#DIV/0!**

d. LOD, specificity and fit for intended use

LOD, specificity and fit for intended use

Method name:	Iodine in Salt
Method #:	3047
Matrix:	Salt
Units:	mg/kg

Analytes	Limit of Detection (LOD)	Interferences successfully checked in at least 50 human samples	Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use
Iodine	0.7	N/A	yes

Appendix B: Ruggedness parameter test results

a. Parameter test #1: stability of solution preparations

i. Test details: All analytical runs had approximately 30 samples between beginning and ending QC.

1. Run 1: Diluent solution was prepared with 0.2% v/v TMAH. All samples were prepared with the modified diluent. The carrier solution used was also poured from modified diluent.
2. Run 2: Diluent solution was prepared with 0.6% v/v TMAH. All samples were prepared with the modified diluent. The carrier solution used was also poured from modified diluent.
3. Run 3: Rinse solution was prepared with 0.2% v/v TMAH.
4. Run 4: Rinse solution was prepared with 0.6% v/v TMAH

ii. Results: See Ruggedness Table 1.

iii. Conclusion: Solution TMAH v/v concentrations are modifiable up to 50% of the optimal concentration of 0.4% v/v. All results are within expected ranges, however optimal analysis occurred at the specified concentration for regular analysis.

Ruggedness Table 1. Stability of solution preparations.*Values taken from NIST SRM 3530 certificate of analysis.

	Material ID	Target Value (mg/kg)	3SD range (mg/kg)	Average (mg/kg)
0.2%v/v TMAH Diluent	LMS091819	11.6	11.2 – 11.9	11.7
	HMS091820	38.1	36.2 – 41.0	38.6
	NIST SRM 3530	52.2*	48.0 – 56.4*	53.3
0.6%v/v TMAH Diluent	LMS091819	11.6	11.2 – 11.9	11.6
	HMS091820	38.1	36.2 – 41.0	38.8
	NIST SRM 3530	52.2*	48.0 – 56.4	50.7
0.2%v/v TMAH Rinse	LMS091819	11.6	11.2 – 11.9	11.4
	HMS091820	38.1	36.2 – 41.0	37.9
	NIST SRM 3530	52.2*	48.0 – 56.4*	51.6
0.6%v/v TMAH Rinse	LMS091819	11.6	11.2 – 11.9	11.8
	HMS091820	38.1	36.2 – 41.0	38.4
	NIST SRM 3530	52.2*	48.0 – 56.4*	54.1
Tests performed 2/13/2019, 2/14/2019, and 3/12/2019 by Katie Vance, instrument: NexION-J				

b. Parameter test #2: Evaluate the impact on observed concentration if an extra dilution is performed on the sample relative to the calibrators.

i. Test details:

1. Spike a volume of salt (10 to 100 mL) with I to concentrations approximating that of calibrator 6 and mix it well.
2. In at least 4 separate runs prepare the dilutions detailed below (2x, 5x, 10x, and 20x). Best precision is obtained when >10% of the benchtop automatic pipette's 2.0 mL syringe capacity is used. Analyze each as an unknown sample (i.e. subtract the aqueous blank).
   a. No extra dilution (5 mL total):
      100 µL salt sample + 4900 µL diluent
   b. 2x extra dilution (10 mL total):
      100 µL salt sample + 100 µL water + 9800 µL diluent
   c. 5x extra dilution (25 mL total):
      100 µL salt sample + 400 µL water + 24500 µL diluent
   d. 10x extra dilution (50 mL total):
      100 µL salt sample + 900 µL water + 49000 µL diluent
   e. 20x extra dilution (100 mL total):
      100 µL salt sample + 1900 µL water + 98000 µL diluent
   f. 100x extra dilution (500 mL total):

      100 µL: salt sample + 9900µL water + 490000 µL diluent
3. Keep the spiked sample frozen (≤-20 ºC) between experiments and mix it well before each sampling.

ii. Results: See Ruggedness Table 2.

iii. Conclusions: Results are acceptable at extra dilution levels of 2x up to 100x.

Ruggedness Table 2. Impact of extra dilutions on observed concentrations of iodine

Dilution level	Concentration normalized to that observed with no additional dilution (± 1SD).
	I
No Extra Dilution	1.00 ± 0.00
2x dilution	1.02 ± 0.03
5x dilution	1.02 ± 0.03
10x dilution	1.02 ± 0.03
20x dilution	1.03 ± 0.03
100x dilution	1.07 ± 0.08
Test performed 2/8/2019, 2/11/2019, and 2/12/2019 by Katie Vance, instrument: NexION-J	

Appendix C

Table 1. Instrument and method parameters

Instrument: PerkinElmer NexION ICP-MS, ESI SC4 autosampler, ESI FAST sample introduction system and ESI DXi micro peristaltic pump	
Optimization window parameters	
RF power	1450 W
Plasma Gas Flow (Ar)	15 L/min
Auxiliary Gas Flow (Ar)	1.2 L/min
Nebulizer Gas Flow (Ar)	~0.90 – 1.0 L/min (optimized as needed for sensitivity)
Ion Lens Voltage(s)	AutoLens (optimized as needed for sensitivity)
AFV, QRO, CRO, CPV, Discriminator Threshold	Optimized per instrument by service engineer, or advanced user.
Parameters of x-y alignment, nebulizer gas flow, AutoLens voltages, mass calibration, dual detector calibration and detector voltages are optimized regularly. Optimization file name = default.dac.	
Configurations window parameters	
cell gas changes pause times	N/A
File names & directories	
method file names	*calibration curve (programmed for salt blank)* CDC_DLS3047_sblk.mth *For QC & sample analysis (programmed for aqueous blank)* CDC_DLS3047_aqblk.mth
dataset	Create a new dataset subfolder each day. Name as “2019-0808” for all work done on August 8, 2019
sample file	Create for each day’s work
report file name	*For sample results printouts* cdc_quant comprehensive.rop *For calibration curve information* CDC_Quant Comprehensive (6-cal curve info).rop
tuning	Default.tun
optimization	Default.dac
calibration	N/A
polyatomic	nexion.ply
report options template (transferring results to the database)	See Figure 8 of Appendix C. CDC_Database Output.rop *Report Format Options: select only “Use Separator”* *File Write Option: Append* *Report File name: make descriptive including date (e.g. 2019-0808_NexION-J_group1.txt)*
Method Parameters	
Method Parameters: Timing Page (see Figure 2 in Appendix C)	
sweeps/reading	30
readings/replicate	1

replicates	3
enable qc checking	On
isotopes monitored and internal standard associations (exact mass)	^{185}Re as internal standard for ^{127}I (126.9)
dwell times	30 ms for ^{185}Re, and ^{127}I
scan mode	Peak Hopping for all isotopes (1 MCA channel)
DRC channel A gas flow rate	None
DRC channel B gas flow rate	None
RPa	0 for all isotopes
RPq	Typically* 0.25 for ^{185}Re and ^{127}I (* Optimize per instrument, and periodically verified)
Method parameters: processing page (see Figures 3 in Appendix C)	
detector mode	Dual
process spectral peak	N/A
AutoLens	On
isotope ratio mode	Off
enable short settling time	Off
blank subtraction	After internal standard
measurement units	cps
process signal profile	N/A
Method parameters: equations page (see Figure 4 in Appendix C)	
equations	None
Method parameters: calibration page (see Figures 5 in Appendix C)	
calibration type	external std.
curve type	weighted linear
sample units	“μg/L” or “ppb”
calibrator concentrations (μg/L)	I: 1, 5, 25, 100, 250, 500
Method parameters: sampling page (see Figures 6 and 7 in Appendix C)	
“peristaltic pump under computer control”	On
autosampler tray port	***If using ESI autosampler*** Autosampler Type: AS-93plus Tray Name: esi.try

sampling device	Sampling Device: None If using other autosampler, refer to user guide.
sample flush	default is 3 s at 3 rpm (~320 μL/min, ESI DXi peristaltic pump, FAST sample introduction system) Time can be optimized as needed to adequately fill the FAST loop. Time and rpm can be optimized as needed to using a different style peristaltic pump (maintaining approximate liquid flow rate). As a matter of lab practice, set this time to equal the loop fill time in the ESI FAST program. As long as the combined time of sample flush + read delay is equal to the time required for signal to reach stability, analytical measurement will be good.
read delay	37 s at 3 rpm (~320 μL/min, ESI DXi peristaltic pump, FAST sample introduction system) Time can be optimized as needed to reach signal stability before beginning analysis. Time and rpm can be optimized as needed to using a different style peristaltic pump (maintaining approximate liquid flow rate). As a matter of lab practice, set this time equal to the total time required for the signal to reach stability minus the loop fill time. As long as the combined time of sample flush + read delay is equal to the time required for signal to reach stability, analytical measurement will be good.
wash	100 s at 10 rpm (~160 μL/min, ESI DXi peristaltic pump, FAST sample introduction system) Time can be optimized to allow for changes in FAST loop rinsing (must be greater than total time of steps in FAST program after the initial “on rinse” command). Time and rpm can be optimized as needed to using a different style peristaltic pump (maintaining approximate liquid flow rate).
extended wash (via ICP-MS software QC checking)	For sample concentrations greater than these, setup the ICP-MS software’s ‘QC checking’ feature to “Wash for X and continue.” See Figure 9 in Appendix C. **Analyte** — **Extended Rinse Trigger Conc.** — **Extended Rinse Time** I — >500 μg/L — 400 s
autosampler locations of blanks and standards	*For calibration curve (points to salt blank)* CDC_DLS3047_sblk.mth Calibration Stds 0 – 6 in autosampler positions 101 – 107 by default, but can be customized. *For QC & sample analysis (points to aqueous blank)*

	CDC_DLS3047_aqblk.mth Aqueous Blank in autosampler position 149 by default, but can be customized.
FAST parameters: See Figures 10 through 17 in Appendix C for details	
configuration file	default.sc (saved at C:\Program Files\ESI\ESI-SC\)
FAST program	cdc_1mL loop_SIO_dls3047.txt

Table 2. Stock calibration standard

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

Table 3. Preparation of intermediate stock calibration standards

Iodine	I Intermediate Stock Calibration Standard A (0.25%NaCl solution)
Flask Vol. (mL)	100
I Stock Calibration Standard Spike Vol. (mL)	0.05
I Stock Calibration Standard Concentration (µg/mL)	1000
Final solution concentration (µg/L)	500

Table 4. Preparation of intermediate working calibrators

Calibrator #	1	2	3	4	5
Total volume (mL)	50	50	50	50	50
I Int. Stock Std. (mL)	0.5	2.5	10	25	Direct transfer
I (µg/L)	5	25	100	250	500
* These same concentrations are entered in the ICP-MS software's calibration page to describe the concentrations of the working calibrators (preparations analyzed during a run). This eliminates the need to multiply ICP-MS observed results by a dilution factor except for the case of extra dilutions (see Table 8 in Appendix C).					

Table 5. Acceptable ways to perform two consecutive analytical runs bracketing with bench quality control samples

setup 1	setup 2
Run #1 **calibrators** low bench QC high bench QC samples low bench QC high bench QC *Run #2* **calibrators** low bench QC high bench QC samples low bench QC high bench QC	*Run #1* **calibrators** low bench QC high bench QC samples low bench QC high bench QC *Run #2* low bench QC high bench QC samples low bench QC high bench QC

Table 6. A typical SAMPLE/BATCH window

AS Location*	Sample ID	Measurements Action	Method
101	3047 S0 check	Run sample	…DLS3047_Sblk.mth
110	3047 SblkChk Wash 1	Run blank, standards, and sample **	…DLS3047_Sblk.mth
113	3047 SblkChk Wash 2	Run sample	…DLS3047_Sblk.mth
149	3047 AQBLK	Run blank and sample ¥	…DLS3047_aqblk.mth
301	L Bench QC	Run sample	…DLS3047_aqblk.mth
302	H Bench QC	Run sample	…DLS3047_aqblk.mth
305	Sample 1	Run sample	…DLS3047_aqblk.mth
306	Sample 2	Run sample	…DLS3047_aqblk.mth
307	L Bench QC	Run sample	…DLS3047_aqblk.mth
304	H Bench QC	Run sample	…DLS3047_aqblk.mth

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

** When executing this row, the NexION will first analyze the standard 0 (Salt blank) at AS position 101, then standards 1–6 at autosampler positions 102–107, then the “3047 SblkChk Wash 1” sample at A/S position 110. The sampling information about AS positions 101-107 are stored in the “Sblk” method file.

¥ When executing this row, the NexION will first analyze the aqueous blank at AS position 149, then the “Aq blank ” at AS position 150. The sampling information about AS position 149 is stored in the “aqblk” method file.

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

Description	Water (µL)	AQ Int Working Std (µL)	Sample or QC sample (µL)	Diluent * (µL)	Total Vol (µL)
working calibrators (S0-S8) and SBlkChk (S0)	-	100 x 1	-	4,900 (4,900 x 1)	5,000
AQ Blank	100 x 1	-	-	4,900 (4,900 x 1)	5,000
Salt Sample solution or Salt-Based QC	-	-	100 x 1	4,900 (4,900 x 1)	5,000
Salt Sample solution *2x Dilution* [H]	100 x 1	-	100 x 1	9,800 (4,900 x 2)	10,000
Salt Sample solution *5x Dilution* [H]	400 (100 x 4)	-	100 x 1	24,500 (4,900 x 5)	25,000
Salt Sample solution *10x Dilution* [H]	900 (100 x 9)	-	100 x 1	49,000 (4,900 x 10)	50,000
Salt Sample solution *20x Dilution* [H]	1,900 (100 x 19)	-	100 x 1	98,000 (4,900 x 20)	100,000
Salt Sample solution *100x Dilution* [H]	9,900 (100 x 99)	-	100 x 1	490,000 (4,900 x 100)	500,000

If a different total volume is prepared, adjust the volumes for each component proportionally. These directions are written with the expectation of a 10,000 µL syringe on the left and a 2000µL syringe on the right sides of the benchtop automatic pipette.

* By splitting the dispense step of diluent into two or more portions, liquids pulled up into the right pipette tip are flushed out more completely.

[H] Extra dilution is performed on salt samples whose concentration is greater than the concentration of the highest calibrator listed in Table 4 of Appendix B. Any extra dilution within these limits can be prepared as long as the 100:4900 (1:50) ratio of diluent to total dilution volume is maintained. Use of the lowest extra dilution level is preferred to minimize differences between the calibrators and the samples (i.e. 2x dilution is preferred over 5x if 2x is sufficient to dilute analyte into the documented linearity range).

Table 8. Reportable range concentrations (µg/L)

Analyte	Limit of Detection (LOD)*	High Calibrator	Maximum Extra Dilution **	Reportable Range Upper Boundary
I	0.7	500	100x	50,000
*Re-evaluated periodically (2+ years) or at significant method changes. LODs shown were calculated 03/07/2019.				
**See ruggedness test table 2 in Appendix B for supporting validation data.				

Table 9. Boundary concentrations for iodine in salt

analyte (units)	upper boundaries *		range maximum ("Lim Rep Delta") †	Highest Concentration Validated for Washout
	1UB	2UB		
I (mg/kg)	75	100	30 for values < 300 10% of value at ≥ 300	500

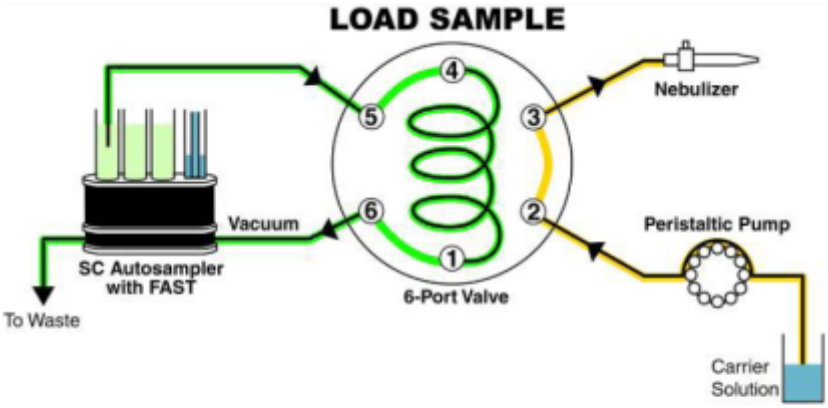
* These boundaries are set based on the FDA recommend level of iodine in salt (5). The concentrations assigned to these boundaries is determined by study protocol but default concentrations are listed in this table.

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

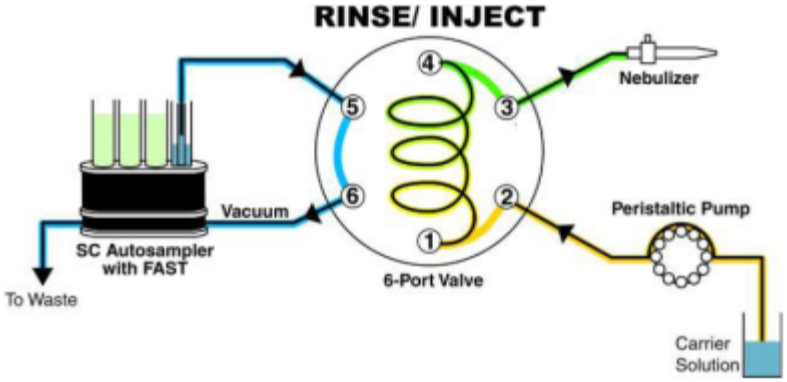
† Range maximum is the range of the three replicate readings for a single sample analysis. This value is also called the Rep Delta Limit in the LIMS

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

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



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



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

The connections to the valve are color-coded (see Figure 1).

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

Figure 2. NexION ICP-MS method screen shots (timing page)

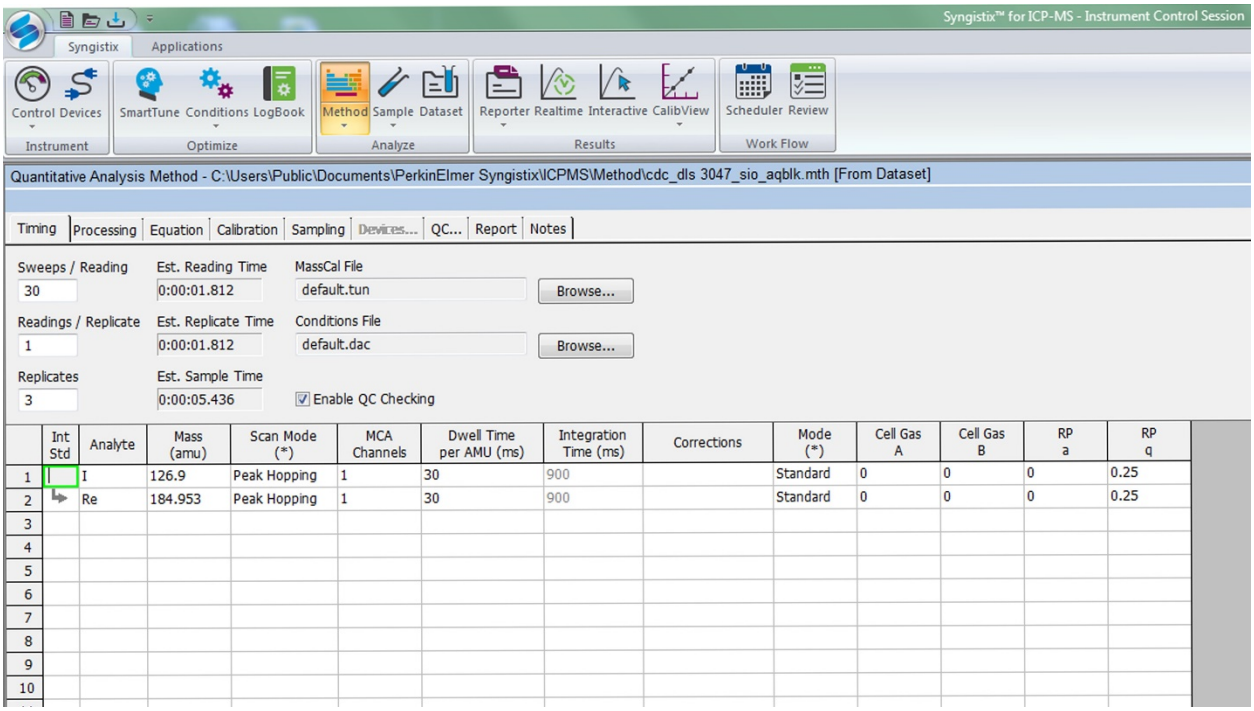


Figure 3. NexION ICP-MS method screen shots (processing page)

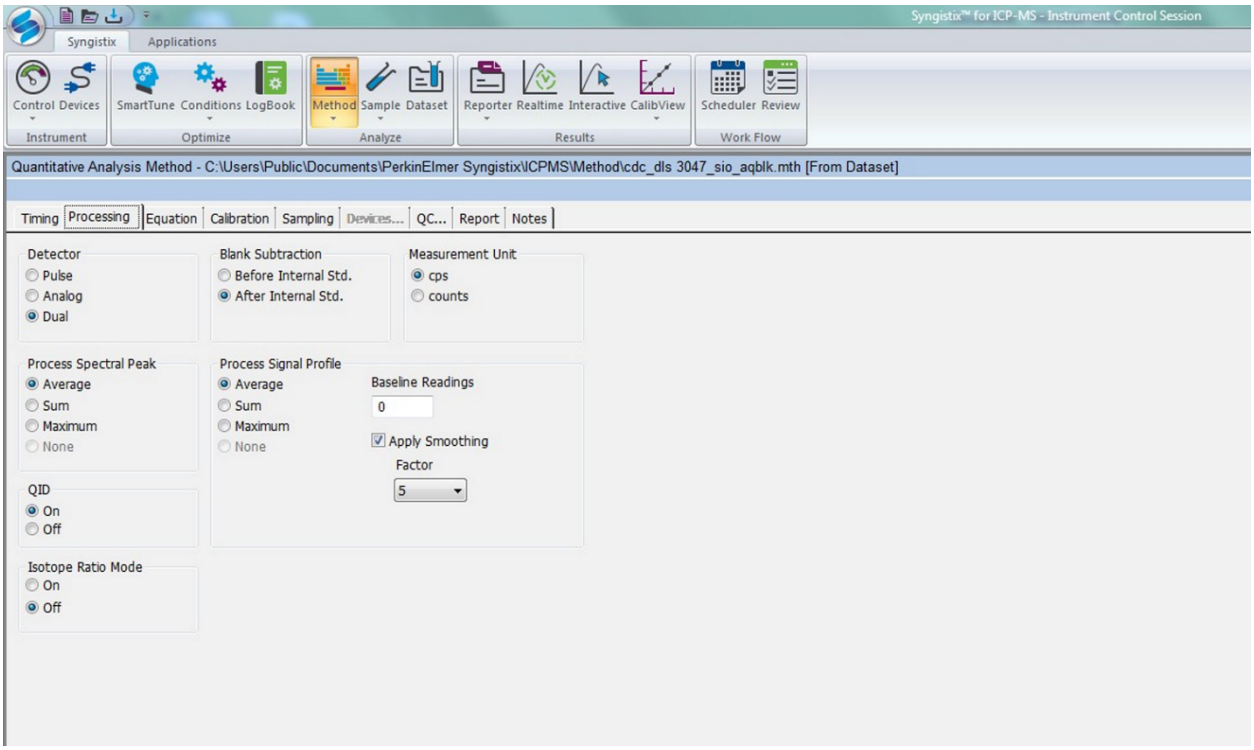


Figure 4. NexION ICP-MS method screen shots (equation page)

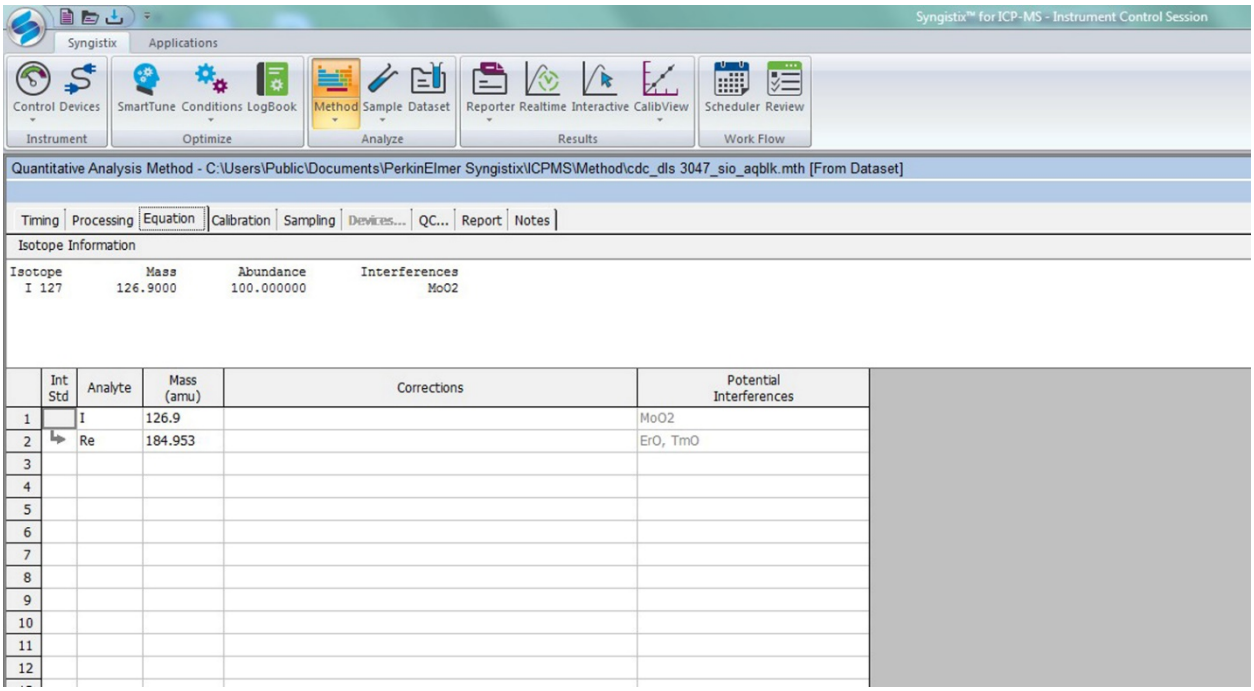


Figure 5. NexION ICP-MS method screen shots (calibration page)

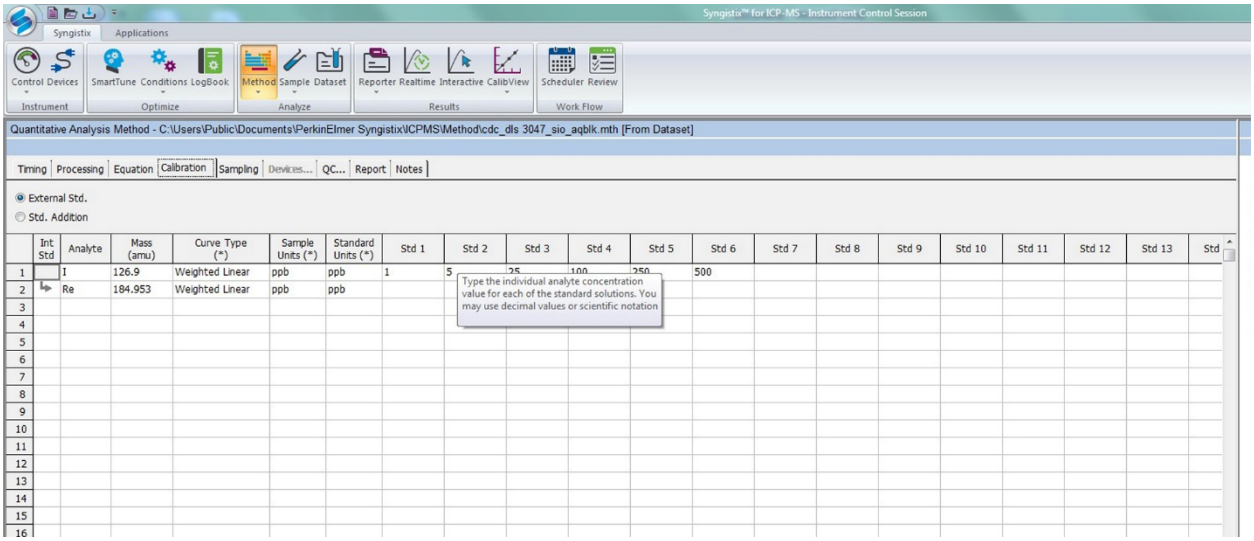


Figure 6. NexION ICP-MS method screen shots (sampling page, AqBlank method)

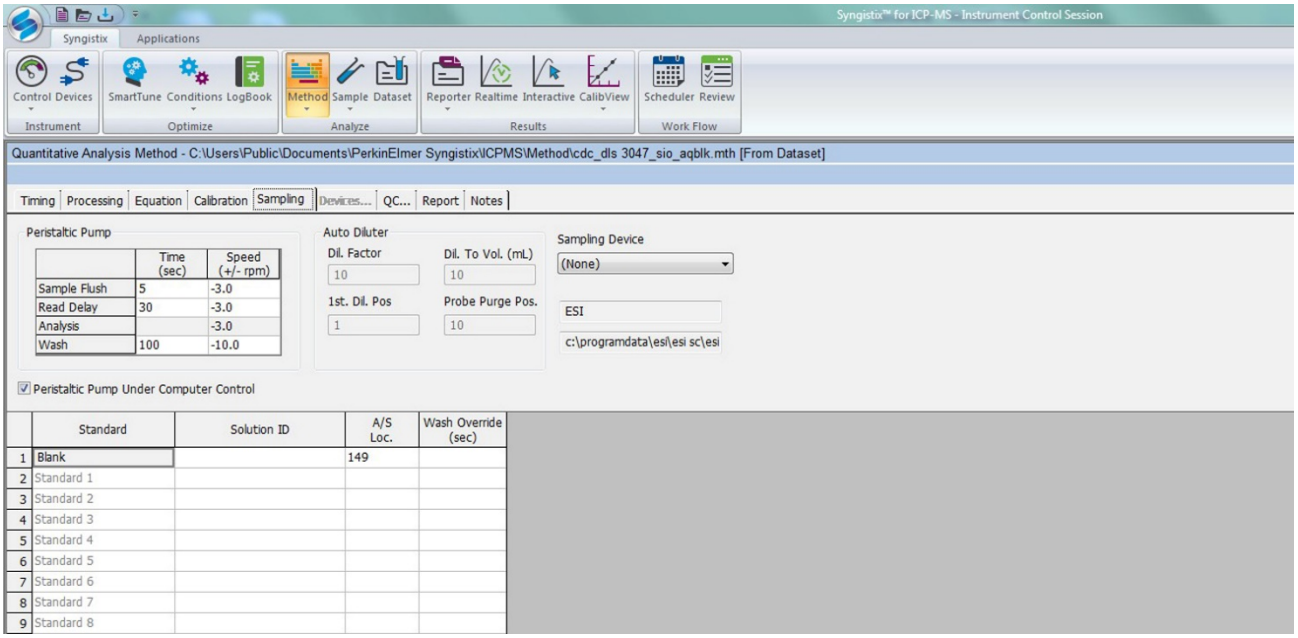


Figure 7. NexION ICP-MS method screen shots (sampling page, UrBlank method)

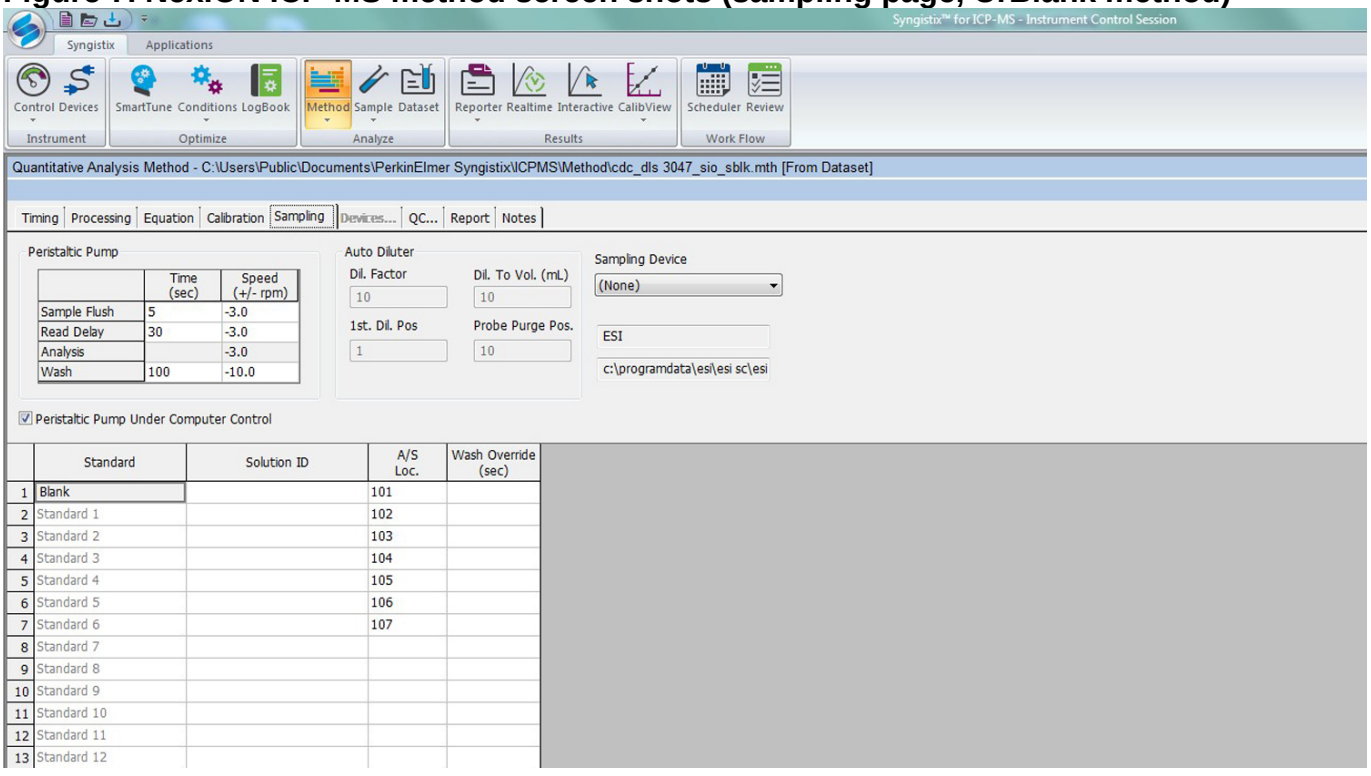


Figure 8. NexION ICP-MS method screen shots (report page)

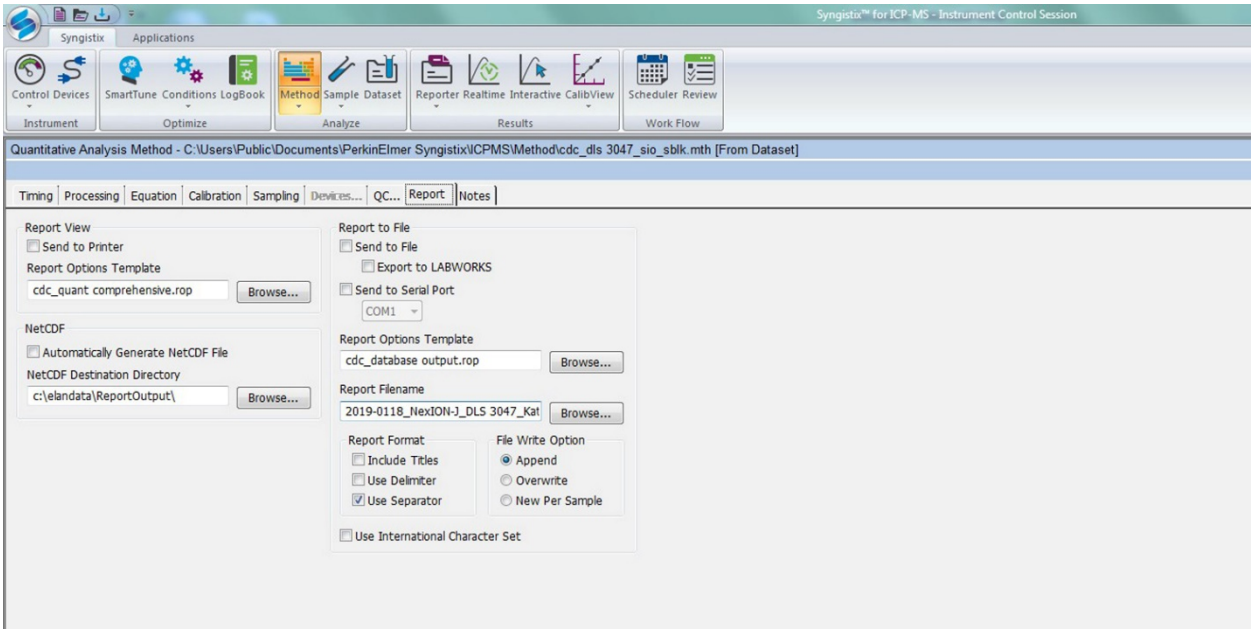


Figure 9. ESI SC4 autosampler screen shots (main page)

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

A rinse time of -1 causes the rinse station to be skipped.
A rinse time of 0 causes the probe to only dip into the station, but spends no time there.

Additional flush times can be optimized to keep the rinse station full while not using too much rinse solution. The inner diameter size of the tubing providing the rinse solution to the rinse station determines how quickly the station will fill. Various sizes are available for purchase or can be made in the laboratory.

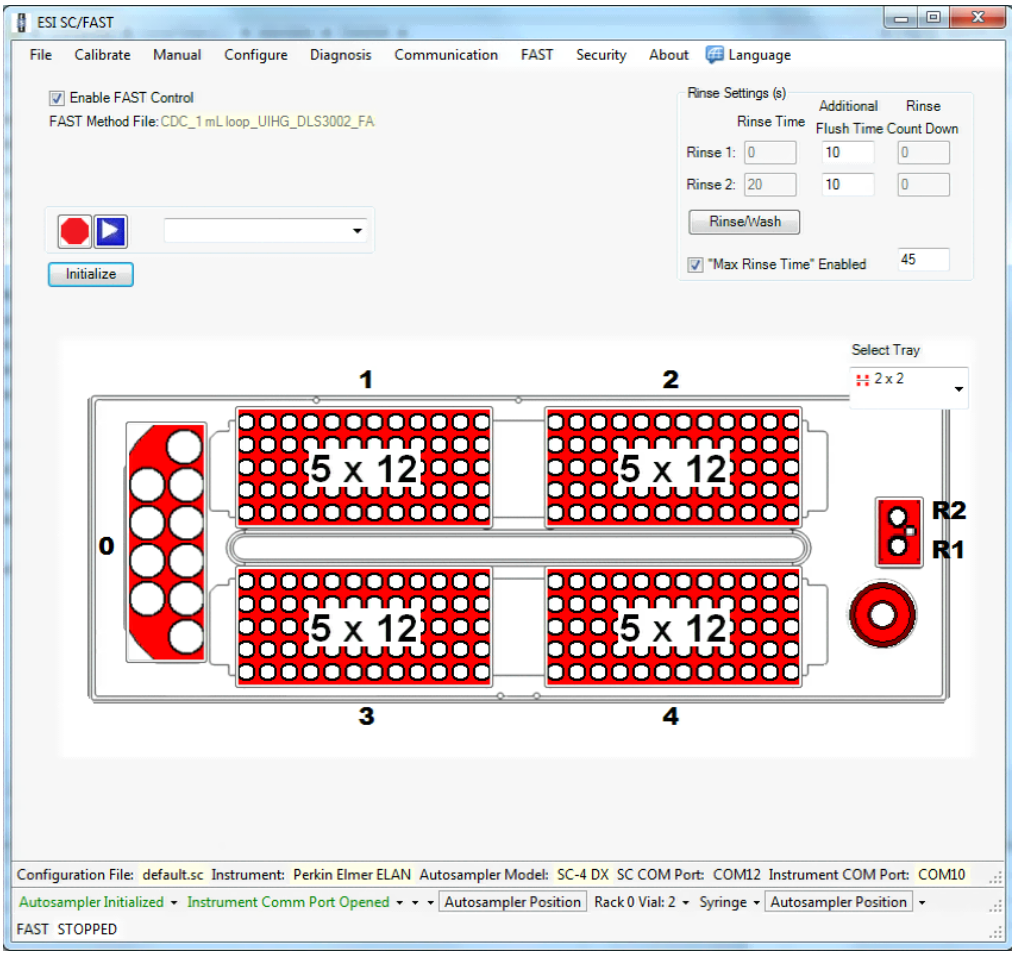


Figure 10. ESI SC4 autosampler screen shots (5x12 rack setup window)

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

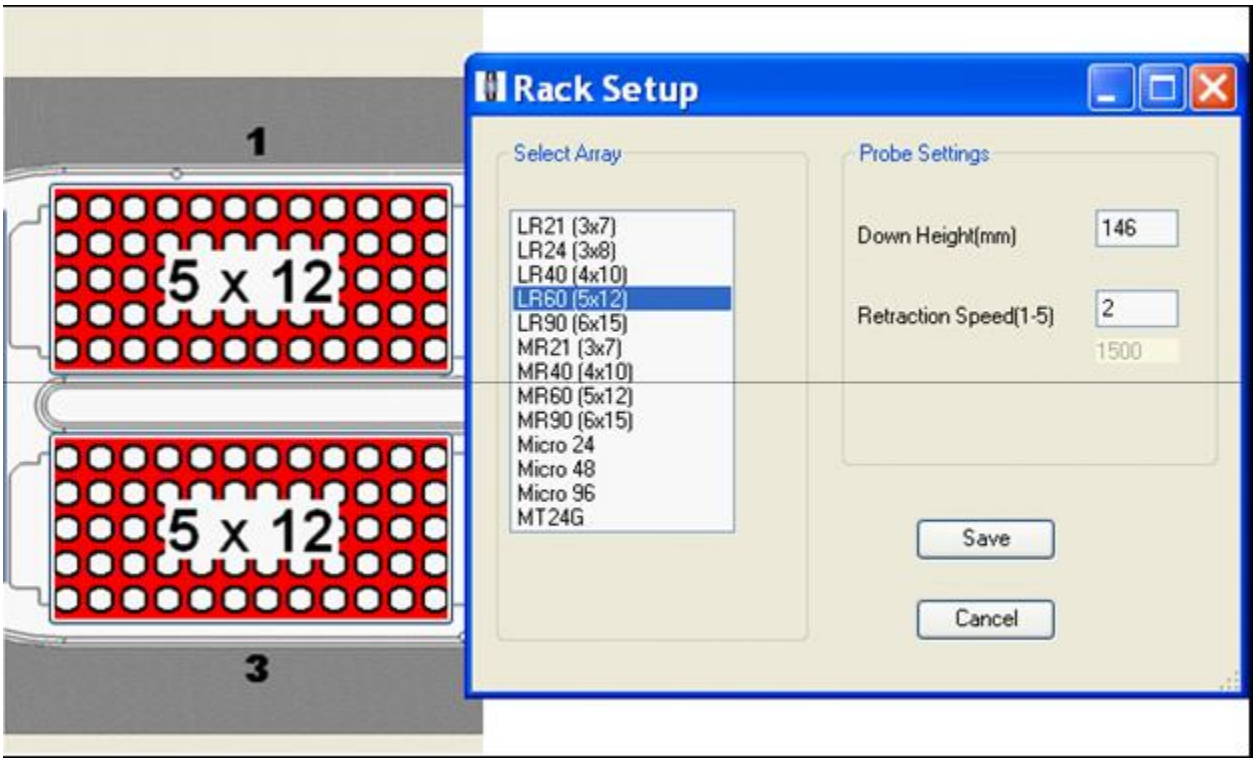


Figure 11. ESI SC4 autosampler screen shots (50 mL tube rack setup window)

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

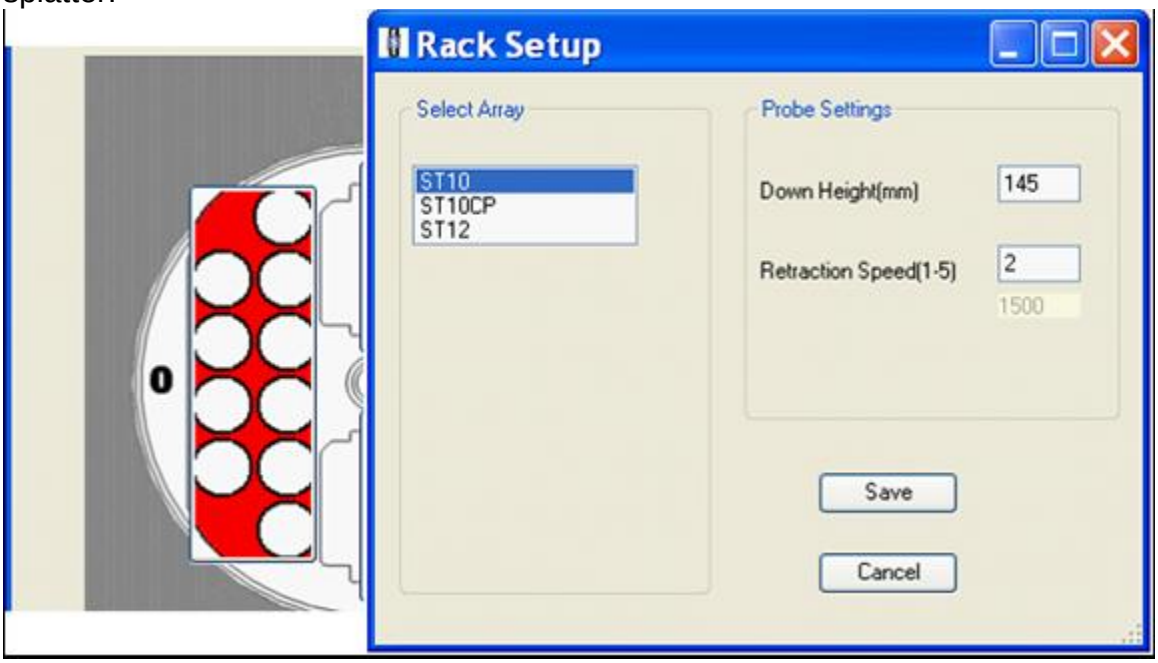


Figure 12. ESI SC4 autosampler screen shots (rinse station rack setup window)

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

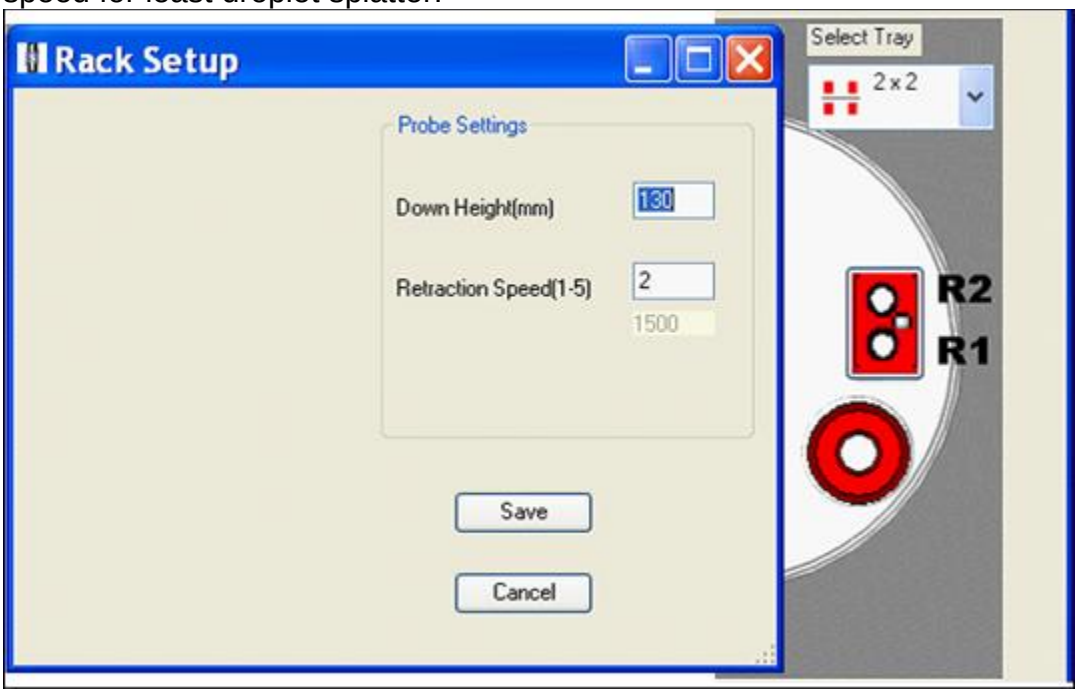


Figure 13. ESI SC4 autosampler screen shots (“Configure” page)

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

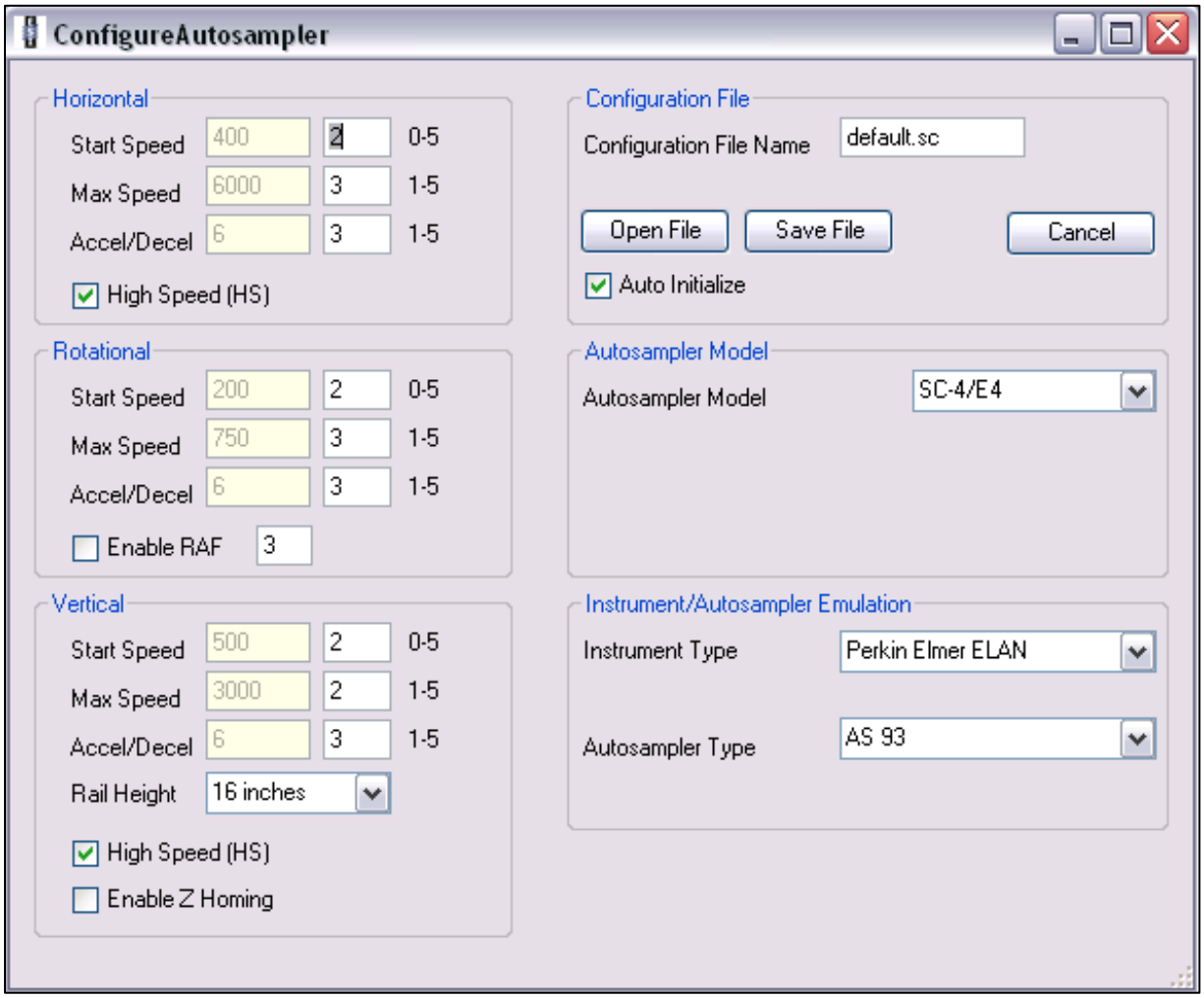


Figure 14. ESI SC4 autosampler screen shots ("Communication" page)

Communication ports will differ depending on available ports on instrument control computer.

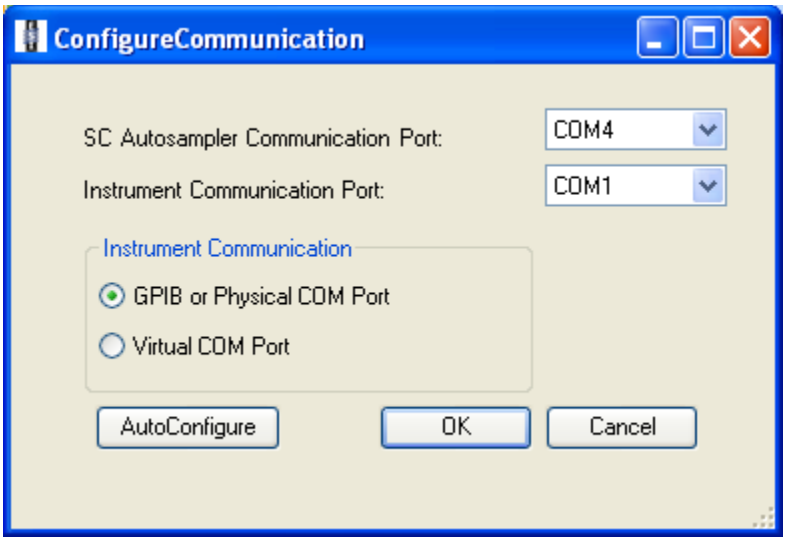


Figure 15. ESI SC4 autosampler screen shots (“FAST” page)

Timer A can be optimized to achieve proper filling of loop with diluted sample digestate. Timers B, C, D, E, and F control rinsing the loop after analysis and can be optimized for eliminating carry-over from one sample to the next while using the minimum amount of rinse solution. Save the file with the name “CDC_1 mL loop_SIO_DLS3047_FAST.txt”.

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

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

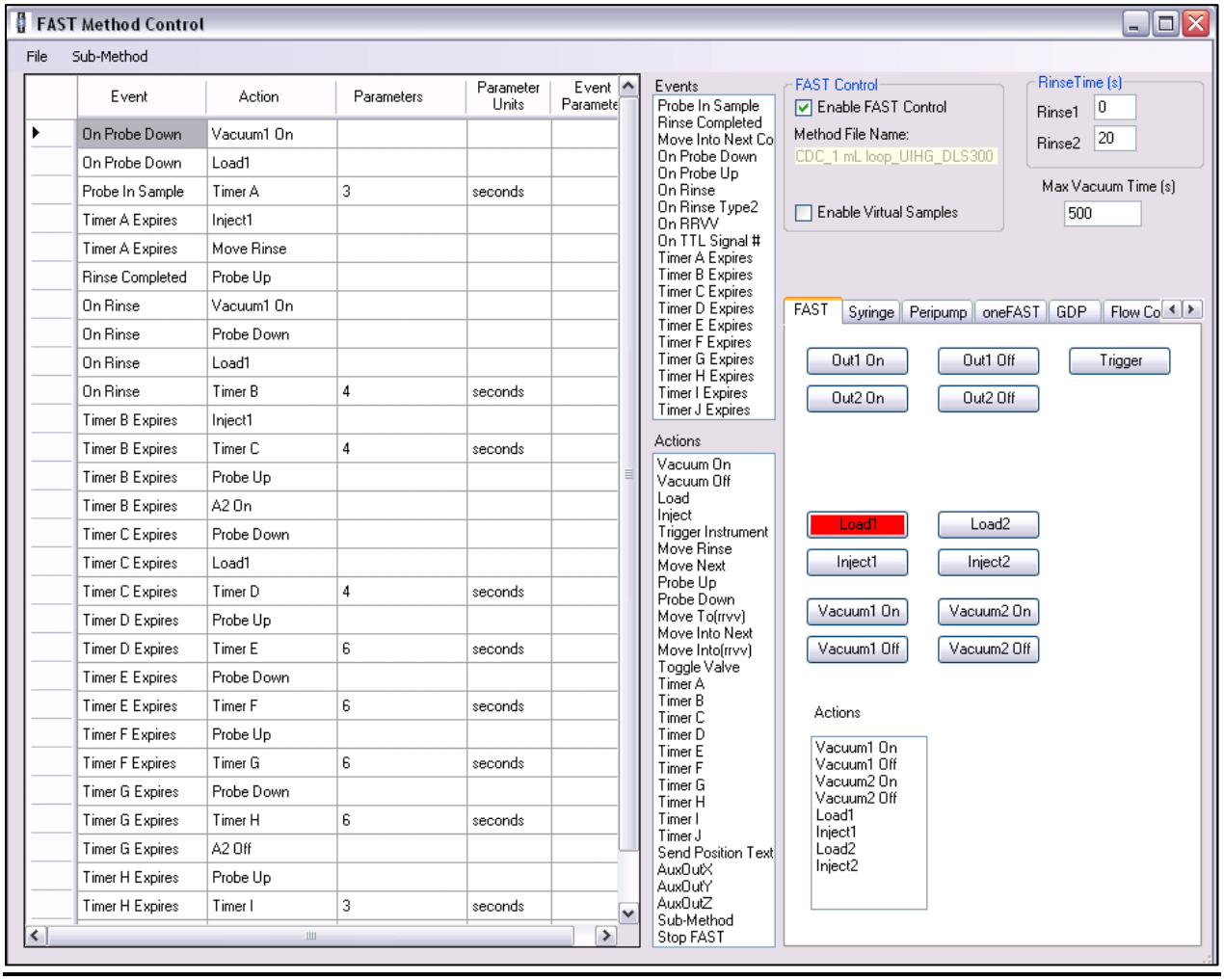
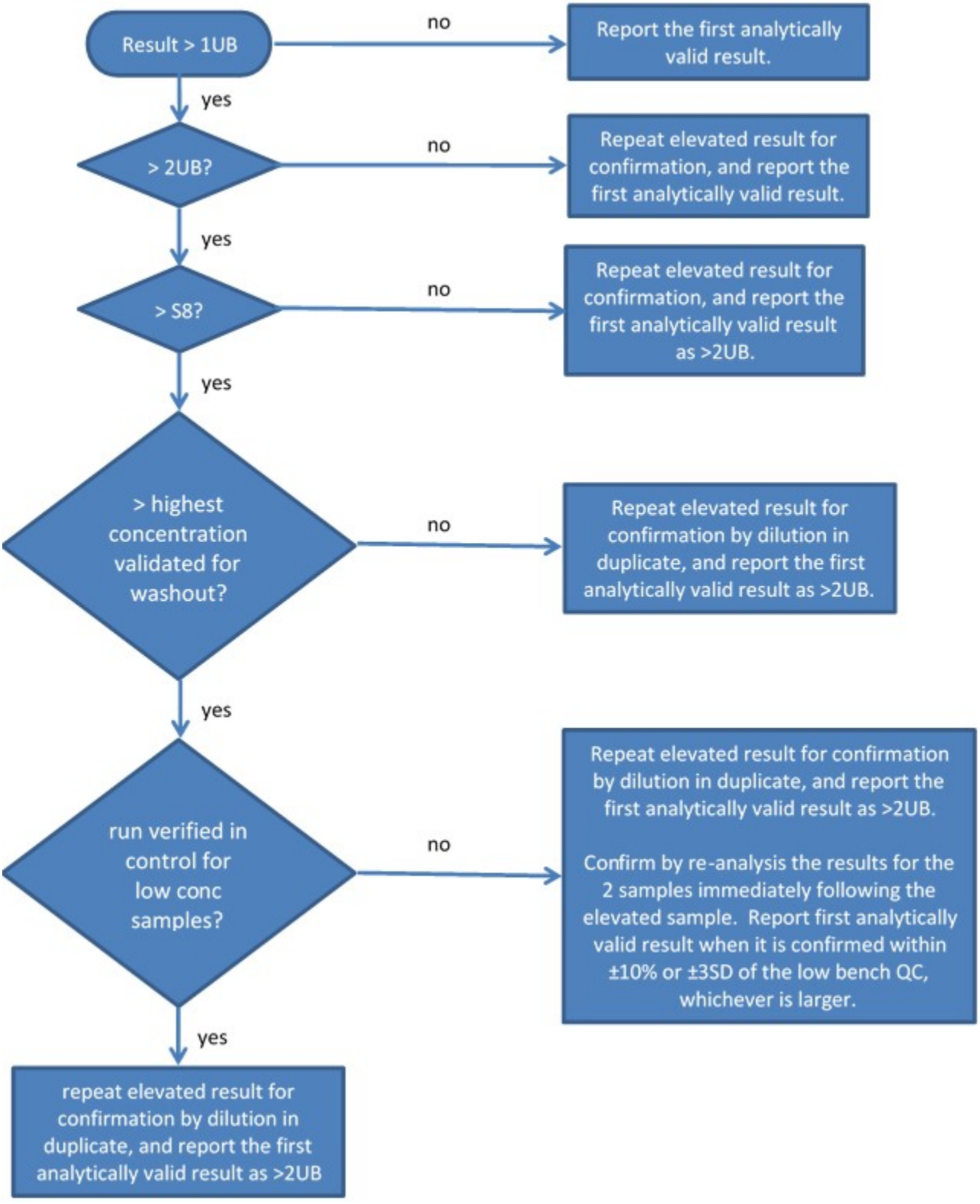


Figure 16. ESI SC4 autosampler screen shots (“FAST” program in full)

Event	Action	Parameters	Parameter Units
On Probe Down	Vacuum1 On		
On Probe Down	Load1		
Probe In Sample	Timer A	3	seconds
Timer A Expires	Inject1		
Timer A Expires	Move Rinse		
Rinse Completed	Probe Up		
On Rinse	Vacuum1 On		
On Rinse	Probe Down		
On Rinse	Load1		
On Rinse	Timer B	4	seconds
Timer B Expires	Inject1		
Timer B Expires	Timer C	4	seconds
Timer B Expires	Probe Up		
Timer B Expires	A2 On		
Timer C Expires	Probe Down		
Timer C Expires	Load1		
Timer C Expires	Timer D	4	seconds
Timer D Expires	Probe Up		
Timer D Expires	Timer E	6	seconds
Timer E Expires	Probe Down		
Timer E Expires	Timer F	6	seconds
Timer F Expires	Probe Up		
Timer F Expires	Timer G	6	seconds
Timer G Expires	Probe Down		
Timer G Expires	Timer H	6	seconds
Timer G Expires	A2 Off		
Timer H Expires	Probe Up		
Timer H Expires	Timer I	3	seconds
Timer I Expires	Move Next		

Figure 17. Flow chart for handling an elevated result



Appendix D: Help Sheets

Reagent Preparation (page 1 of 2)

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

Rinse solution
0.4% v/v TMAH

1) Partially fill a 4 L bottle with ≥18 Mohm·cm water.
2) Add 16 mL of TMAH (Tetramethylammonium hydroxide, 25% w/w $((CH_3)_4NOH)$.
3) Add enough ≥18 Mohm·cm water to bring to 4 L mark.
4) Mix well by gently inverting several times.
5) Label appropriately.

Sample diluent
(5 µg/L Re and 0.4% v/v TMAH

1) Partially fill a 2 L bottle with ≥18 Mohm·cm water.
2) Add 8 mL of TMAH.
3) Add 100 µL of a 100 mg/L stock solution of Re.
4) Add enough ≥18 Mohm·cm water to bring to 2 L mark.
5) Mix well by gently inverting several times.
6) Label appropriately.

1% v/v HNO_3 (for acid washing containers)

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

Reagent Preparation (page 2 of 2)

5% v/v HNO_3 (for soaking quartz and glass components)

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

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

1) Partially fill an acid rinsed, 50 mL flask with ≥18 Mohm·cm water.
2) Add 5 mL of Re from 1000 mg/L stock standard.
3) Add 1.5 mL of concentrated HNO_3.
4) Add enough water to fill to 50 mL mark.
5) Mix well by gently inverting several times.
6) Pour the standard solution over into a 50 mL tube.
7) Label appropriately.

1 µg/L (ppb) Daily Performance Test Solution in 2% v/v HNO_3

1) Partially fill a 1 L volumetric flask with ≥18 Mohm·cm water.
2) Add 1 mL of High Purity Standard: SM-2107-018
3) Add 20 mL of concentrated HNO_3
4) Add enough ≥18 Mohm·cm water to bring to 1 L mark.
5) Mix well by gently inverting several times.

References

1. Hollowell, J.G., et al., *Iodine nutrition in the United States. Trends and public health implications: Iodine excretion data from National Health and Nutrition Examination Surveys I and III (1971-1974 and 1988-1994).* Journal of Clinical Endocrinology & Metabolism, 1998. **83**(10): p. 3401-3408.
2. World Health Organization: Fortification of Food-grade salt with iodine for prevention and control of iodine deficiency disorders. 2014 World Health Organization, Geneva.
3. Caldwell, K.L., er al., *Iodine Status of the U.S. Population, National Health and Nutrition Examination Survey, 2005-2006 and 2007-2008*. Thyroid, 2011. **21**(4): p. 419-427.
4. Division of Laboratory Sciences, *Division of Laboratory Sciences Policies and Procedures Manual*. 2017, version 6.0, Centers for Disease Control and Prevention: Atlanta, GA.
5. National Institutes of Health, Office of Dietary Supplements, *Iodine: Fact Sheet for Consumers*. Updated September 26, 2018.