Laboratory Procedure Manual

Analytes: **Cadmium, Lead, Manganese, Mercury, and Selenium**

Matrix: **Whole Blood**

Method: triple quadrupole inductively coupled plasma mass spectrometer (ICP-QQQ-MS)

Method No: DLS 3040.1-05

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As performed by: Inorganic and Radiation Analytical Toxicology Branch
Division of Laboratory Sciences
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Important Information for Users

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

Images are included in this document as visual aids for certain topics. They are intended to be representative images only and should not be construed as absolute references. Discrepancies between the images in this document and the actual application design are not a cause for revisions to this documen

Public Release Data Set Information

This document details the Lab Protocol for testing items in the following table:

Data File Name	Variable Name	SAS Label
PBCD_L	LBXBCD	Cadmium (µg/L)
	LBDBCDSI	Cadmium (µmol/L)
	LBXBPB	Lead (µg/dL)
	LBDBPBSI	Lead (µmol/L)
	LBXTHG	Mercury, total (µg/L)
	LBDTHGSI	Mercury, total (µmol/L)
	LBXBMN	Manganese (µg/L)
	LBDBMNSI	Manganese (µmol/L)
	LBXBSE	Selenium (ug/L)
	LBDBSESI	Selenium (µmol/L)

1. Clinical relevance and summary of test principle

A. Clinical relevance

Metals ions affect human health in various ways. Some metals (i.e., lead, cadmium, and mercury) show only deleterious effects on human health. Some (i.e., selenium and manganese) play an essential role in the human biological system if within certain concentration ranges, while negative health implications are observed when concentrations in biological systems are in deficit or excess. Determination of a person's level of environmental exposure to chemicals through direct measurement of the substances or their metabolites in human samples such as blood is called biomonitoring. Biomonitoring reduces the uncertainty of determining levels of exposure over making these determinations through calculations of estimated dose based on analysis of environmental samples and assumptions about exposure pathways [2]. Biomonitoring measurements are the most health-relevant assessments of exposure because they indicate the amount of the chemical that actually gets into people from all environmental sources (e.g., air, soil, water, dust, or food) combined. The laboratory method described here is a multi-element technique for monitoring the total elemental concentrations of cadmium (Cd), lead (Pb), manganese (Mn), mercury (Hg), and selenium (Se) in whole human blood for the purpose of biomonitoring.

There is no known biological role of mercury in the human body. The main sources of mercury intake in humans are fish, dental amalgams, and occupational exposures [3]. The main organs affected by mercury are the brain and the kidneys. Exposure of childbearing-aged women is of particular concern because of the potential adverse neurologic effects of Hg in fetuses. The health effects of mercury are diverse and depend on the form of mercury encountered and the severity and length of exposure. The general population is exposed to three forms of mercury: elemental, inorganic, and organic (predominantly methyl). However, this method tests only for the total amount of mercury in the blood without regard to chemical form. In the general population, total blood mercury is due mostly to the dietary intake of organic forms which are formed through microbial action from inorganic mercury that has deposited in aquatic environments and bioaccumulated through the food chain (especially into large predatory fish) [4]. Exposure to inorganic or elemental mercury (e.g., dental amalgams or occupational exposures) is particularly reflected in urine excretion rather than blood. Psychic and emotional disturbances are the initial signs of chronic intoxication by elemental mercury vapors or salts. Those exposed are at increased risk for parasthesia, neuralgias, renal disease, digestive disturbances, and ocular lesions [5]. Massive exposure over a longer period of time results in violent muscular spasms, hallucinations, delirium, and death [6]. Except for methylmercury exposures, blood is considered useful if samples are taken within a few days of exposure. This is because most forms of mercury in the blood decrease by one-half every three days if exposure has been stopped. Thus, mercury levels in the blood provide more useful information after recent exposures than after long-term exposures. Several months after an exposure, mercury levels in the blood and urine are much lower. Recent blood mercury reference ranges for the U.S. population are listed in Table 12 of **Appendix C**.

There is no known biological role of lead in the human body. Lead, a naturally occurring metal, has had many different commercial uses from which a person can be exposed either in the

occupational / manufacturing process or by the manufactured products such as paint (paint chips, or dust and soil contaminated from deteriorating paint), solder or pipes (only now in older homes), gasoline (now outlawed for all but specialized applications), glazes on pottery, hobby uses (e.g., stained glass), commercial products (e.g., batteries, lead-containing jewelry), home remedy medicines containing lead compounds and non-Western cosmetics. Soil contains lead naturally, or from man-made uses of lead such as paint (near older homes), gasoline (near roadways), mining, manufacturing, and disposal. Lead exposure has been determined to affect nearly every system in the body. The developing biological systems of children are most sensitive to the effects of Pb, where effects are being recognized at low blood lead levels [7-13]. Acute, elevated lead exposure is associated with anorexia, dyspepsia, and constipation followed by diffuse paroxysmal abdominal pain. When lead exposure is high, particularly in children, the person is at increased risk for encephalopathy [14]. The alkyl lead species are highly toxic to the central nervous system [15]. The primary screening method for lead exposure is blood lead, which primarily reflects recent exposures (excretory half-life in blood is approximately 30 days) [16]. Lead in blood is primarily (approximately 99%) in the red blood cells. Recent blood lead reference ranges for the U.S. population are listed in Table 12 of **Appendix C**. The CDC now uses a blood lead reference level to identify children with blood lead levels that are higher than most children's levels[12]. This level is based on the U.S. population of children ages 1-5 years who are in the highest 2.5% of children when tested for lead in their blood. The blood lead reference value is calculated as the 97.5th percentile of blood lead concentrations for children ages 1-5 from four years of the National Health and Nutrition Examination Survey (NHANES). The blood lead reference value is currently 3.5 µg/dL based on NHANES cycles 2015-2018 [12, 13].

There is no known biological role of cadmium in the human body. The predominant commercial use of cadmium is in battery manufacturing. Other uses include pigment production, coatings and plating, plastic stabilizers, and nonferrous alloys. Since 2001, U.S. cadmium use has declined in response to environmental concerns. In the United States, for nonsmokers the primary source of cadmium exposure is from the food supply. People who regularly consume shellfish and organ meats will have higher exposures. In general, leafy vegetables such as lettuce and spinach, potatoes and grains, peanuts, soybeans, and sunflower seeds contain high levels of cadmium due to bioaccumulation from the soil. Tobacco leaves accumulate high levels of cadmium from the soil, and smoking is the primary non-occupational source of cadmium exposure for smokers. Generally, the critical organ for Cd is the kidney. Kidney dysfunction is one of the most characteristic signs of exposure to Cd. Workers in an environment with high exposure levels have developed proteinuria, renal glucosuria, aminoaciduria, hypercalciuria, phosphaturia, and polyuria. Chronic obstructive lung disease of varying degrees of severities is frequently seen in Cd workers. Concentration of cadmium in blood of healthy unexposed adults internationally is reported in the range 0.1 – 4 µg/L [17]. Newborn babies were reported to have low or non-detectable concentrations of Cd in blood[18]. Exposure to high concentration of fumes appearing from heated cadmium metal or compounds has led to acute poisoning and in some cases to the death of workers; the principal symptoms reported were respiratory distress due to chemical pneumonitis and edema. It has been estimated that 8 hours exposure to 5 g Cd/m^3 will be lethal [19]. Ingestion of high amounts of Cd puts a person at increased risk to a rapid onset with severe nausea, vomiting, and abdominal pain. Cadmium levels in blood, urine, feces, liver, kidney, hair, and other tissues have been used as biological indicators of exposure to cadmium. Blood

cadmium levels are principally indicative of recent exposure(s) to cadmium rather than whole-body burdens [20-23]. Urine cadmium levels primarily reflect total body burden of cadmium, although urine levels do respond somewhat to recent exposure [24]. Recent blood cadmium reference ranges for the U.S. population are listed in Table 12 of **Appendix C**.

Manganese is a trace element essential to humans and is associated with the formation of connective and bony tissue, growth and reproductive functions and with carbohydrate and lipid metabolism [25]. Manganese is also a known neurotoxin but little information exists about levels of manganese that cause toxicity. Symptoms of manganese toxicity are similar to Parkinson's Disease and can also include disorientation, memory impairment, anxiety and compulsive behavior [26]. There is much concern for the levels of manganese in humans whom are occupationally exposed to it [27-33]. Recently, there are growing concerns over exposure due to contamination of drinking water with manganese [34-36] and as a result of methylcyclopentadienyl mangangese tricarbonyl (MMT) used as an anti-knocking additive in gasoline [37-43]. Populations suffering from iron deficiencies are at an increased risk to manganese toxicity because iron deficiency can result in an accumulation of manganese in the central nervous system[40]. To fully understand the essentiality and toxicity of manganese, further investigations are needed regarding the levels of manganese in biological matrices. Group average levels in blood appear to be related to manganese body burden, while average urinary excretion levels appear to be most indicative of recent exposures [44]. On an individual basis the correlation between the level of workplace exposure and the levels in blood or urine has not always been found to be a reliable predictor of exposure [28, 44-46]. Measurements of manganese levels in blood are useful in detecting groups with above-average current exposure, although levels are sometimes related to exposures that have already ceased. In addition to individual variability, another factor that limits the usefulness of measuring manganese in blood, urine, or feces as a measure of excess manganese exposure is the relatively rapid rate of manganese clearance from the body. Excess manganese in blood is rapidly removed by the liver and excreted into the bile, with very little excretion in urine [47, 48]. Thus, levels of manganese in blood or urine are not expected to be the most sensitive indicators of exposure [49]. Recent, blood manganese reference ranges for the U.S. population are listed in Table 12 in **Appendix C**.

Selenium is an essential element that is required to maintain good health but both selenium deficiency and excessive levels of selenium are associated with several disorders [50, 51]. Selenium is a naturally occurring mineral element that is distributed widely in nature in most rocks and soils. Most processed selenium is used in the electronics industry, but it is also used: as a nutritional supplement; in the glass industry; as a component of pigments in plastics, paints, enamels, inks, and rubber; in the preparation of pharmaceuticals; as a nutritional feed additive for poultry and livestock; in pesticide formulations; in rubber production; as an ingredient in antidandruff shampoos; and as a constituent of fungicides. Radioactive selenium is used in diagnostic medicine. In the body, selenium is incorporated into proteins to make selenoproteins, which are important antioxidant enzymes. The antioxidant properties of selenoproteins help prevent cellular damage from free radicals. Free radicals are natural by-products of oxygen metabolism that increase risk of chronic diseases such as cancer and heart disease [51, 52]. Other selenoproteins help regulate thyroid function and play a role in the immune system [53-56]. Human selenium deficiency is rare in the U.S. but is seen in other countries where soil

concentration of selenium is low[57]. There is evidence that selenium deficiency increases the risk of a form of heart disease, hypothyroidism, and a weakened immune system [58, 59]. There is also evidence that selenium deficiency does not usually cause illness by itself. Rather, it can make the body more susceptible to illnesses caused by other nutritional, biochemical or infectious stresses [60]. Symptoms of very high exposure to selenium, a condition called selenosis, include gastrointestinal upsets, hair loss, white blotchy nails, garlic breath odor, fatigue, irritability, and mild nerve damage [50]. Selenium can be detected in the blood, feces, urine, hair, and nails of exposed individuals, however, field studies have used primarily blood or urine levels to indicate the degree of selenium exposure [50]. Recent blood selenium reference ranges for the U.S. population are listed in Table 12 of **Appendix C**.

The laboratory method presented here can be used to achieve rapid and accurate quantification of five elements of toxicological and nutritional interest in whole human blood: cadmium, lead, mercury, manganese, and selenium. Use this method to screen blood when people are suspected to have been acutely exposed; to evaluate chronic environmental exposure; or other non-occupational exposure to these elements.

B. Test principle

This method directly measures the cadmium, lead, mercury, manganese, and selenium content of whole blood samples using mass spectrometry after a simple dilution sample preparation step.

During the sample dilution step, a small volume of whole blood is extracted from a larger whole blood patient sample is mixed (vortexed) to create a uniform distribution of cellular components. The mixing step is important because some metals (e.g., Pb) are known to be associated mostly within the red blood cells in the sample and a uniform distribution of this cellular material must be produced before a small volume, or sub-sample, is extracted from the larger sample. Coagulation is the process in which the cellular components of blood form solid clots. If steps are not taken to prevent coagulation, i.e., the addition of anti-coagulant reagents such as EDTA (ethylenediaminetetraacetic acid) in the blood collection tube prior to blood collection, blood will immediately begin to form clots once leaving the body and entering the blood collection tube. These clots prevent uniform distribution, or homogeneity, of cellular material throughout the blood sample even after rigorous mixing, making a representative sub-sample of the larger sample unattainable. It is important that before or during sample preparation, the analyst identify any sample having clots or micro-clots (small clots). Clotted samples are not analyzed by this method due to the inhomogeneity concerns (i.e., all results from the sample are designated as “not reportable”).

Dilution of the blood in the sample preparation step prior to analysis is a simple dilution of 1 part sample + 1 part water + 18 parts diluent. The effect of the chemicals in the diluent are to release the metals bound to red blood cells making them available for ionization in the plasma of the inductively coupled plasma mass spectrometer (ICP-MS); reduce suppression of that ionization by the biological matrix; prevent undissolved biological solids from clogging the sample introduction system pathways of the ICP-MS; and allow the introduction of internal standards used in the analysis step.

Tetramethylammonium hydroxide (TMAH, 1.0% v/v) and Triton™ X-100 (0.05%) in the sample diluent solubilizes blood components. Triton™ X-100 also helps prevent formation of biological

deposits on the internal surfaces of the instrument's sample introduction system and reduce collection of air bubbles in the sample transport tubing. Ammonium pyrrolidine dithiocarbamate (APDC, 0.01%) in the sample diluent aids in solubilizing metals released from the biological matrix. Ethanol (1% v/v) in the sample diluent aids solubility of blood components and aerosol generation at the nebulizer by reducing the surface tension of the solution. The internal standards, rhodium, iridium, and tellurium, are at a constant concentration in all dilutions of blanks, calibrators, QC, and samples. Monitoring the instrument signal ratio of a metal to its internal standard allows correction for sample-to-sample matrix differences, instrument noise, and signal drift across time during the analytical run.

Liquid samples are introduced into the mass spectrometer through the inductively coupled plasma (ICP) ionization source. The liquid diluted blood sample is forced through a nebulizer with argon gas, which converts the bulk liquid into an aerosol of small droplets. The smaller droplets in the aerosol are selectively passed through the spray chamber by a flowing argon stream into the ICP. A plasma is created by coupling radio-frequency power with flowing argon. The plasma is characterized by a temperature of 4500-6500 K and predominant species of positive argon ions and electrons. 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 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). After the interface, the ions pass through a focusing region, then the first quadrupole mass filter (Q1), the collision-reaction cell (or octopole reaction system, ORS), a second quadrupole mass filter (Q2), and finally are selectively counted in rapid sequence at the electron multiplier detector allowing the individual isotopes of an element to be determined.

Generally, the ORS can operate in one of two modes. In "vented" (or "no gas") mode the cell is not pressurized and ions pass through the cell to the quadrupole mass filter unaffected. In collision or reaction mode, the cell is pressurized with a gas to cause collisions and/or reactions between the cell gas and the incoming ions. In general, collisions or reactions with the incoming ions selectively occur to either eliminate an interfering ion or change the ion of interest to a different mass-to-charge ratio (m/z), which is free from interferences. Collisions in the cell between ions in the beam and the cell gas can also focus the ion beam to the middle of the cell and increase the ion signal.

For selenium, the pressurized ORS cell contains oxygen and hydrogen gases (O_2 and H_2) to react with $^{80}Se^+$ to form $^{80}Se^{16}O^+$. The instrument operates in MS/MS mode to selectively filter for m/z 80 on Q1 before the ORS cell, then selectively filters m/z 96 on Q2 which avoids the argon dimer ($^{40}Ar_2^+$) interference at m/z 80.

For manganese, the oxygen and hydrogen gases reduce the ion signal from several interfering ions ($^{40}Ar^{15}N^+$, $^{38}Ar^{16}O^1H^+$, $^{54}Fe^1H^+$, $^{39}K^{16}O^+$) while allowing the $^{55}Mn^+$ ion stream to pass relatively unaffected through the ORS cell on toward the analytical quadrupole and detector. MS/MS mode is needed to avoid the creation of $^{54}Fe^1H^+$ in the ORS by selectively filter for m/z 55 on Q1 before the ORS cell (Q2 is also set to m/z 55). This avoids ^{54}Fe from entering the ORS and reacting with hydrogen gas.

In this method, the instrument is operated in ORS reaction MS/MS mode for all analytes: Cd, Hg, Pb, Mn and Se. They are analyzed at the same flow rate of oxygen and hydrogen gas into the ORS cell, and all in MS/MS mode, to avoid lengthening analysis time due to pause delays that would be necessary if different gas flows were used for each analyte, or if some analytes were tested in vented mode.

Once ions pass through the ORS cell and are electrically selected for passage through the analytical quadrupole, electrical signals resulting from the ions striking the discrete dynode detector are processed into digital information that indicates the intensity of the ions. The intensity of the ions detected while aspirating an unknown sample is correlated to an elemental concentration through comparison of the analyte / internal standard signal ratio of the unknown sample with the signal ratio obtained when aspirating calibrators. This method was based on the method by Jones et. al [61].

2. Safety precautions

A. General safety

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

ii. Observe Universal Precautions when working with blood.

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

iv. Exercise special care when handling and dispensing concentrated bases and acids. Use additional personal protective equipment which protects face, neck, and front of body. Add acid to water. **If a concentrated base (TMAH or Lysol I.C.) or concentrated acid (hydrochloric acid or nitric acid) comes in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.**

v. Use secondary containment when storing containers holding hazardous liquids.

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

vii. Operation of an ICP-MS instrument has many potential hazards including ultraviolet radiation, high voltages, radio-frequency radiation, and high temperatures. The specific hazards are detailed in the ICP-MS manufacturer's manuals.

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

ix. Wipe down all work surfaces at the end of the day with disinfectant. Disinfectant may be either a solution of diluted bleach prepared daily (1 part household bleach containing 5.25% sodium hypochlorite + 9 parts water) or an equivalent disinfectant. Allow 15 minutes contact time and then wipe up with clean water.

B. Waste disposal

i. Autoclaving: Autoclave all diluted biological samples, original biological samples being disposed, and non-metallic consumables which come into contact with biological samples (even diluted or aerosolized). Use sharps containers or labeledautoclave pans for broken glass / quartz or items which puncture autoclave bags (e.g., pipette tips). Consult the hazardous waste disposal program for specific instructions on items that require inceneration after autoclaving.

ii. Other liquid waste

1) Waste discarded down sink: Do not discard solutions at the sink having a pH lower than 6.0 or higher than 11.5 (limits defined by Dekalb County, GA). Inactivate biological compounds and cellular constituents in whole blood or mixed chemical and biological waste, such as the waste carboy of the ICP-MS, by adding an approved disinfectant (e.g., Lysol I.C. or equivalent) prior to drain disposal. Flush the sink with copious amounts of water.

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

Additional information on hazard identification, risk evaluation, and risk mitigation for this method can be found in the method risk assessment form.

3. Computerization; Data System Management

A. Sample identification and tracking

DLS uses a unique barcoded specimen ID number affixed to each sample to identify and track individual samples, and which links the laboratory information to demographic data recorded by those who collected the sample. To safeguard confidentiality, only the person outside of DLS requesting the laboratory analysis will maintain information which links the specimen ID number to the patient's name. Personal identifiers (e.g., names) are not included on samples. Barcoded sample IDs are indexed in STARLIMS to both a CDC specimen identifier (CSID) and a CDC unique identifier (CUID).

During sample preparation and analysis, samples are identified by their sample ID. Sample IDs are entered into the ICP-MS instrument software using a barcode scanner, whenever possible. If hand entry of a sample ID is necessary, proofread the transcribed data after entry.

Location, status, and final disposition of the specimens will be tracked using a specimen tracking sheet or electronic tracking in the laboratory information system. Records will be maintained for a minimum of two years.

Retention of samples is determined by specifications in each study protocol. A copy of each study protocol is kept in DLS STARLIMS.

B. Data file types, what they include, how they are used, and where they are stored and backed up

Data files are created by the Sample Logistics Laboratory during sample accession which are used to log the samples into the laboratory information system (STARLIMS). The software files used to control the ICP-MS system and raw data files created during analysis by the ICP-MS control software are stored on the ICP-MS control computer. The data files exported from the instrument computer and uploaded to STARLIMS for review are described in **Appendix E**. The file types reported to the customer are described in the Division Policies and Procedures Manaul and copies of those files are stored in STARLIMS. STARLIMS data is backed up by the Division of Laboratory Sciences regularly to network drives. Instrument computer files are backed up per the procedure and schedule described in **Appendix F**.

4. Limitations of Method; Interfering Substances and Conditions

A. Interferences addressed by this method

i. Reaction of selenium ($^{80}Se^{+}$) with oxygen gas to create selenium oxide ($^{80}Se^{16}O^{+}$) and avoid argon dimer ($^{40}Ar_2^{+}$) interference on selenium: We add oxygen gas to the ORS to react with $^{80}Se^{+}$ and create $^{80}Se^{16}O^{+}$. This reaction avoids the $^{40}Ar_2^{+}$ polyatomic ion as an interference on $^{80}Se^{+}$. $^{40}Ar_2^{+}$ is formed in the plasma as a result of a reaction between argon ions in the plasma gas. We also use MS/MS mode with Q1 at 80 m/z and Q2 at 96 m/z. In MS/MS mode there is no interference for $^{80}Se^{16}O^{+}$ at m/z 96.

ii. Reaction of selenium ($^{80}Se^{+}$) with oxygen gas to create selenium oxide ($^{80}Se^{16}O^{+}$) and avoid gadolinium double charged ($^{160}Gd^{++}$) interference on selenium: We add oxygen gas to the ORS to react with $^{80}Se^{+}$ and create $^{80}Se^{16}O^{+}$. This reaction avoids the $^{160}Gd^{++}$ ion as an interference on $^{80}Se^{+}$. $^{160}Gd^{++}$ is formed in the plasma if present in whole blood. We also use MS/MS mode with Q1 at 80 m/z and Q2 at 96 m/z. In MS/MS mode there is no interference for $^{80}Se^{16}O^{+}$ at m/z 96.

iii. Reaction of selenium ($^{80}Se^{+}$) with oxygen gas to create selenium oxide ($^{80}Se^{16}O^{+}$) and avoid calcium containing ion (e.g., $^{40}Ca^{40}Ar^{+}$, $^{44}Ca^{36}Ar^{+}$, $^{48}Ca^{32}S^{+}$) interferences on selenium: We add oxygen gas to the ORS to react with $^{80}Se^{+}$ and create $^{80}Se^{16}O^{+}$. This reaction avoids the calcium polyatomic ions as interferences on $^{80}Se^{+}$. The calcium polyatomic ions are formed in the plasma as a result of reactions between Ca and Ar or S. We also use MS/MS mode with Q1 at 80 m/z and Q2 at 96 m/z. In MS/MS mode there is no interference for $^{80}Se^{16}O^{+}$ at m/z 96.

iv. Reduction of argon nitride ($^{40}Ar^{15}N^{+}$), argon hydroxide ($^{38}Ar^{16}O^{1}H^{+}$) on manganese ($^{55}Mn^{+}$): We add oxygen and hydrogen gasses to the ORS cell to reduce $^{40}Ar^{15}N^{+}$ and $^{38}Ar^{16}O^{1}H^{+}$ polyatomic ions. These ions are formed in the plasma as a result of reactions between the plasma gas (Ar) and atmospheric gases (N_2, O_2) or the solvent (H_2O). The ORS cell is filled with oxygen (O_2) gas and hydrogen (H_2) gas which react with $^{40}Ar^{15}N^{+}$ and $^{38}Ar^{16}O^{1}H^{+}$ ions through either charge transfer reactions or transfer reactions. The products of the reactions are either neutral molecules and are not detected (charge transfer), or a new ion with higher mass (oxygen or hydrogen transfer). In either case, attenuation of the background ion signal at m/z 55 occurs.

v. Reduction of $^{39}K^{16}O^{+}$ and $^{54}Fe^{1}H^{+}$ on manganese ($^{55}Mn^{+}$): We add oxygen and hydrogen gasses to the ORS cell to reduce $^{39}K^{16}O^{+}$ and $^{54}Fe^{1}H^{+}$ polyatomic ions. These ions are formed in the plasma as a result of reactions between elements present in the blood matrix (K, and Fe) and the solvent (H_2O). The reaction cell is filled with oxygen (O_2) gas and hydrogen (H_2) gas which react with $^{39}K^{16}O^{+}$, $^{54}Fe^{1}H^{+}$ ions through either charge transfer reactions or transfer reactions. The products of the reactions are either neutral molecules and are not detected (charge transfer), or a new ions with higher mass (oxygen or hydrogen transfer). In either case, attenuation of the background ion signal at m/z 55 occurs.

vi. Reduction of $^{130}Xe^{+}$ on tellurium ($^{130}Te^{+}$): Oxygen gas will undergo a charge transfer reaction with $^{130}Xe^{+}$ to create neutral ^{130}Xe and O_2^{+}. Xenon is not expected to be in blood samples, but we have observed it in the argon gas used in the plasma. If not removed, the presence of $^{130}Xe^{+}$, could increase the signal at m/z 130 and cause erroneously low net intensities for Se and Hg (net intensity = measured intensity for analyte isotope / measured intensity for internal standard isotope).

vii. Use of MS/MS mode to avoid $^{98}Mo^{16}O_2^{+}$ interference on tellurium (^{130}Te): Previous research determined that molybdenum and molybdenum oxide (e.g., $^{98}Mo^{+}$ and $^{98}Mo^{16}O^{+}$) will combine with oxygen to form the polyatomic ion, $^{98}Mo^{16}O_2^{+}$ and will interfere with the measurement of the internal standard for Se and Hg, $^{130}Te^{+}$. In MS/MS mode instrument Q1 and Q2 are set to 130 m/z for Te measurements, so neither $^{98}Mo^{+}$ and $^{98}Mo^{16}O^{+}$ are in the ORS at the same as $^{130}Te^{+}$, and therefore the reaction to form $^{98}Mo^{16}O_2^{+}$ does not occur.

viii. Interference testing: Interference testing was performed on this method as part of its method validation and is documented in **Appendix A**.

ix. Ruggedness testing: This method has also undergone a series of in-house ruggedness testing experiments designed to assess how much method accuracy changes when certain experimental parameters are varied while holding all other experimental variables constant. The ruggedness testing findings for this method are presented in **Appendix B**.

B. Limitations of method:

i. Contamination control: Accuracy and precision of this method can be critically impacted by the influence of elemental contamination. See Section 3.A regarding contamination control in the pre-analytical processes. Use high purity water and chemicals (See Section 5.E) and pre-rinsed or pre-screened containers in reagent and sample preparation (See Section 6). Occasionally we observe contamination sample preparations due to the Hamilton dilutor components (e.g. valve and syringes), especially if the components are new, or infrequently used. Manganese, especially, can be problematic from the valve of the Hamilton. Monitor instrument and diluter cleanliness before and during analysis (See 8.B.ii) and rinse or replace components identified as causing elevated background levels in the reagent and blank checks.

ii. Control for loss of Se signal in intermediate working calibrators: During working calibration standard preparation, take great care to not allow contact between the sample diluent (see Section 6.C) and the intermediate working calibration standard (see Section 6.J). We have discovered that repeated contact at the Hamilton pipette tip of these two liquids will cause the selenium to be removed from the intermediate working calibration standard solutions. The mechanism of this removal is not well understood. We do know that adding the intermediate working calibration standard with a handheld pipette (e.g. Sartorius Picus NxT

electronic pipette, 10-300 uL) to the sample dilution tube will eliminate this problem. Dilution of acidic standards (e.g., intermediate working calibrators, S0-S7) should follow this guidance. Pipette tips should be rinsed with 3% (v/v) HCl (i.e. intermediate working calibration standard 0) at least three times before use for both preventing contamination and pre-wetting the tip for best pipetting accuracy.

5. 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[1] and have been published [62, 63]. In general:

i. Sample types acceptable is whole blood, drawn either by venipuncture or capillary (fingerstick).

ii. No fasting or special diets are required before collection of blood.

iii. Use sterile, lot screened collectors for sample acquisition. Lot screening of materials is highly advised. If lot screening is not possible, use collection devices that are manufactured as "metal free," "for trace elements," (i.e., royal blue top) or "for lead testing." (i.e., tan top). The designation of "sterile" does not indicate that the device is free of metals contamination. If collectors are not lot screened or manufactured for trace element analysis, Include one or more field blanks (empty collection tubes) so potential metal contamination can be evaluated in the analytical phase.

iv. Avoid heparin anticoagulant because clots may form in the blood after collection (e.g., >24 hours); EDTA anticoagulant is preferable.

v. If blood is drawn by venipuncture, draw the blood through a stainless steel needle into a pre-screened collection materials.

vi. If the focus of the study is metals, collect venous blood tubes for metals analysis first.

vii. If blood is drawn by capillary (fingerstick) collection, follow recommended sample collection steps to minimize contamination [64, 65].

viii. Acceptable containers for analytical aliquots for venous blood include lot screened polypropylene (PP) cryo-vials or tubes (e.g., 2 to 5 mL). Acceptable containers for capillary blood include lot screened micro-collection containers (e.g., 0.2 mL). Avoid colored plastics and containers containing o-rings, when possible, because of the increased risk of trace element contamination from coloring agents or o-ring materials.

ix. Venous specimens collected in the field are stored at refrigerated temperatures (+2°C to +8°C) while in blood tubes (i.e., evacuated tubes) or transferred to plastic pre-screened cryo-vials before freezing (-10 °C to -90 °C) prior to shipment to the lab. Venous blood is not frozen in blood collection tubes because it could cause them to crack. Capillary (fingerstick) blood is typically stored frozen (-10 °C to -90 °C) prior to shipment to the lab. Samples are typically kept at the collection site for 1-4 weeks and then shipped by overnight carrier in blood tubes on cold packs (+2°C to +8°C) in pre-screened cryo-vials on cold packs or dry ice.

x. Once received, samples in blood tubes are stored at refrigerated temperatures (+2°C to +8°C) and samples in cryo-vials are stored frozen (-10 °C to -90 °C) through 'in-processing', which is typically completed in less than 4 hours, until they are transferred to the testing laboratory, typically within 15 business days, and then before and after analysis.

xi. Sample stability in sealed polypropylene vials has been demonstrated for over 4 years at ≤ -70 °C by storage of quality control materials[66]. Storage temperatures of −20 °C and 4 °C are equivalent to −70 °C for stability of Cd, Mn, Pb, Se, and Hg in human whole blood for at least 36 months when blood is stored in sealed polypropylene vials. The best analytical results are obtained when storage time at higher temperature conditions (23 °C and 37 °C) is minimized because recovery of Se and Hg is reduced. Blood samples stored in polypropylene cryovials also lose volume over time and develop clots at higher temperature conditions (23 °C and 37 °C), making them unacceptable for elemental testing after 10 months and 2 months, respectively.

xii. Thawing and refreezing samples has not been found to compromise sample results.

xiii. Criteria for sample rejection (in all cases request a second blood sample)

1) Contamination: Improper collection procedures, collection devices, or sample handling can contaminate the blood through contact with dust, dirt, etc. Manganese is present in the general environment, found often in combination with iron, and is present in many alloys (especially stainless steel).

2) Clotted samples: It is important that prior to or during sample preparation the analyst identify any sample having clots or micro-clots (small clots). Do not analyze clotted samples by this method due to the inhomogeneity issues.

3) Low Volume: Minimum volume is 0.20 mL whole blood. Volume used for one analytical measurement is 0.05 mL. The additional volume is for tube wetting and to allow for the potential of re-testing if needed for quality control or confirmation practices.

B. Transfer or referral of samples; procedures for sample accountability and tracking

This protocol does not involve referral of specimens for testing the analytes of this method at another laboratory. See Section 3.A for additional details about sample identification and tracking.

6. Instrument & material sources

In the case of simple laboratory instrumentation (e.g., pipettes, vortex mixer, analytical balance, etc.), a product listed herein may be substituted with equivalent product from a different manufacturer provided that it meets or exceeds the specifications of the product listed. In the case of analysis instrumentation (e.g., ICP-MS) equivalent performance must be demonstrated experimentally in accordance with DLS Policies and Procedures Manual if a product substitution is made. Equivalent performance must also be demonstrated in accordance with DLS policies and procedures when multiple analysis systems are used in parallel, even if they are of the exact same type.

With some exceptions, a material listed herein may be substituted with an equivalent product from a different manufacturer provided that it meets or exceeds the specifications of the product listed.

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

A. Sources for ICP-MS instrumentation

i. Inductively Coupled Plasma Triple Quadrupole Mass Spectrometer (ICP-QQQ-MS): such as Agilent 8900 (part# G3665A) or equivalent, (Agilent, Santa Clara, CA, www.agilent.com)

ii. Recirculating chiller: such as PolyScience Model 6106T (part# G3292A) or equivalent, (Agilent, Santa Clara, CA, www.agilent.com)

iii. Autosampler: such as ESI SC4-DX autosampler or equivalent (part# 8150391), (Elemental Scientific Inc., Omaha, NE, www.elementalscientific.com)

iv. Computer: Computer controller provided or recommended by ICP-MS manufacturer is recommended to ensure proper communication between computer and ICP-MS (Agilent, Santa Clara, CA, www.agilent.com)

v. FAST sample introduction system (optional): such as FAST actuator and valve (part # VM-FR), (Elemental Scientific Inc., Omaha, NE, www.elementalscientific.com) Standard peristaltic pump on ICP-MS replaced by DXi-FAST micro-peristaltic pump/FAST actuator and valve combination unit. (Like ESI part # DXI-54-P4-F6, Elemental Scientific Inc, Omaha, NE, www.icpms.com). 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

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

ii. Female nut: for 1.6mm O.D. (1/16") tubing. Like part P-420 (IDEX Health & Science, LLC , Oak Harbor, WA,www.idex-hs.com)

iii. PEEK ferrule: Like part P-260 or P-260x (10pk SuperFlangeless ferrule, IDEX Health & Science, LLC , Oak Harbor, WA, www.idex-hs.com).

1) Conical Adapter Body: Like part P-692 (IDEX Health & Science, LLC , Oak Harbor, WA,www.idex-hs.com)

iv. 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.elementalscientific.com)

v. 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) or(Like part # 2235-0020, ThermoFisher Scientific, Waltham, MA, www.thermofisher.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.

vi. Coolant, for polyscience chiller: such as polyclean Mix 30 part# 004300062 (www.fishersci.com) # spares = 2

vii. Cones:

1) Sampler (platinum): Agilent part# G3280-67036 (Agilent, Santa Clara, CA, www.agilent.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 (platinum): Agilent part# G8400-67201 (Agilent, Santa Clara, CA, www.agilent.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

3) Skimmer base (brass): For use with Pt skimmer cones. Agilent part# G8400-60625 (Agilent, Santa Clara, CA, www.agilent.com). # spares = 1

4) Graphite gasket: Agilent part# G3280-67009 (Agilent, Santa Clara, CA, www.agilent.com). # spares = 2

5) Retaining ring, sampling cone: Agilent part# G3280-20504 (Agilent, Santa Clara, CA, www.agilent.com). # spares = 1

viii. Connector tube, straight, quartz: Agilent part# G3270-80025 (Agilent, Santa Clara, CA, www.agilent.com). # spares = 4

ix. Clamp, torch ball joint connector: to connect spray chamber to connector tube and connector tube to torch. Agilent part# G8400-60327 (Agilent, Santa Clara, CA, www.agilent.com). # spares = 2

x. Detector, electron multiplier: like part# 5190-0154 (Agilent, Santa Clara, CA, www.agilent.com). # spares = 1

xi. FAST accessories, Valve: CTFE High-flow valve head for SC-FAST (uses ¼-28 fittings). Like part # SC-0599-1010 or PF-P6 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xii. FAST accessories, Stator: CTFE Stator for 6 port SC-FAST high flow valve (¼-28 fittings). Like part # SC-0599-1010-01 or PF-P6S (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xiii. FAST accessories, Rotor: Composite rotor for 6 port SC-FAST high flow valve (¼-28 fittings). Like part # SC-0599-1010-05 or PF-P6R (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xiv. FAST accessories, Sample Loop: 0.50 mL Teflon, white connector-nuts for high flow valve head(¼-28 fittings). Like part # SC-0315-05 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xv. FAST accessories, Probe, Autosampler: Teflon, carbon fiber support, 0.8mm i.d., blue marker, 1/4-28 fittings. Like part number SC-5037-3751 or SC-5037-3755-150 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). # Spares = 2.

xvi. FAST accessories, Probe, Carrier Solution: Teflon, carbon fiber support, 0.5mm i.d., orange marker, 1/4-28 fittings. Like part number SC-5037-3501 or ES-5037-3500-100 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). # Spares = 2.

xvii. FAST accessories, Tubing, FAST vacuum: Vacuum line for SC-FAST high flow valve, connects to port #6, black nut for connection to valve head, natural brown color nut on other end for connection to SC autosampler vacuum port. Like part # SC-0321-32 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xviii. FAST accessories, Tubing and nut for carrier solution: 0.5 mm i.d. Teflon tubing (orange marker) with red ¼-28 male nut. Connects to high flow FAST valve head, port #2. Like part # SC-0316-0500 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xix. FAST p-port valve module: Agilent part # VMR-FR (Agilent, Santa Clara, CA, www.agilent.com)

xx. FAST valve mounting bracket: Agilent part # ES-2999-7779 (Agilent, Santa Clara, CA, www.agilent.com)

xxi. Hose, 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).

xxii. Hose, exhaust of ICP-MS: Flexible exhaust hose like part # S-LP-10 air connector (Thermaflex, Abbeville, SC, www.thermaflex.net), or equivalent. # spares = 10 feet of 6" diameter hose.

xxiii. Lenses

1) Extraction lens 1: For 8900 ICP-QQQ-MS with x-lens Agilent part# G3666-60302 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 4

2) Extraction lens 2: For for 8900 ICP-QQQ-MS with x-lens Agilent part# G3666-60303 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 4

3) Omega lens: Agilent part# G8400-60217 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 2

4) Omega bias lens: Agilent part# G8400-00240 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 2

5) Screw and spacer kit: Agilent part# G3280-67037 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 1

xxiv. Nebulizer: MicroMist nebulizer w/ PEEK gas connector, for 8900, 0.4mL/min with ratchet gas fitting Agilent part# G3266-80005 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 4

xxv. Spray chamber: Double pass w/ UHMI type, Agilent part#G8400-67150 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 4

1) End cap: For spray chamber, Agilent part# G3280-60008 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 2

2) Retainer Ring: For spray chamber Agilent part # G8400-40200 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 2

3) Nut: to hold nebulizer in place, Agilent part# 0535-1082 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 2

4) Connector for gas line at end cap: Agilent part# 5042-4775 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 2

5) Drain tubing assembly: from spray chamber to peristaltic pump tubing. Agilent part# G3280-60555 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 2

xxvi. Torch: Agilent part #G3280-80053 (Agilent, Santa Clara, CA, www.agilent.com) # spares =

xxvii. Tubing, peristaltic sample: Orange Green Tubing 2 stop PVC Flared MP2 Peripump Tubing 0.38mm id, 12 pack part # MPP-038-F-PVC # spares = 6. This tubing is used for analysis.

xxviii. Tubing, peristaltic sample: White-White Tubing 2 stop PVC Flared MP2 Peripump Tubing 1.02mm id, 12 pack part # MPP-102-F-PVC # spares = 6. This tubing can be used during troubleshooting to compare against Agilent performance specifications.

xxix. Tubing, peristaltic waste: Ismaprene, 3-stop, yellow-blue, 1.52 mm i.d. 12/pk, Agilent part# G1833-65570 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 6

xxx. Tubing, stainless steel: 1/8 in od, 6 m. Agilent part# G3270-65035 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 1

xxxi. Shield plate for torch: Agilent part #G1833-65419 (Agilent, Santa Clara CA, www.agilent.com) # spares = 3

xxxii. Bonnet for torch: Agilent part # G1833-65421 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 3

xxxiii. RF Coil for 8900: Agilent part # G8400-60434 (Agilent, Santa, CA, www.agilent.com) # spares = 3

xxxiv. Alignment plate for RF Coil: Agilent part # G1833-66011 (Agilent, Santa Clara, CA, www,agilent.com) # spares = 3

xxxv. Interface Wrench for sampling cone: Agilent part # G3280-01507 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 3

C. Sources for ICP-MS maintenance equipment and supplies

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

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

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

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

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

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

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

D. Sources for general laboratory 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. Carboy (for preparation of blood quality control pool and waste jug for ICPMS sample introduction system): Polypropylene 10-L carboy (like catalog # 02-960-20C, Fisher Scientific,

Pittsburgh, PA, www.fischersci.com) or equivalent. Carboys with spouts are not advised due to potential for leaking.

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

iv. Flask, volumetric:

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

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

3) 1L volumetric flask (like catalog # 40001000, Thermo Scientific, Fisher Scientific, Pittsburgh, PA., www.fishersci.com). Plastic or glass is acceptable.

4) 2L volumetric flask (like glass flask catalog # 92812G2000, DWK Life Sciences (Kimble), Fisher Scientific, Pittsburgh, PA., www.fishersci.com). Plastic or glass is acceptable for the making of the S_0 intermediate working calibration standard.

v. Dropper bottle: volumetric 50mL PFA dropper bottle, part # 700-550, (Savillex, Eden Prairie, MN, www.savillex.com).

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

vii. KVM switch: A recommended, but not required accessory is a small controller connected to the instrument computer that provides keyboard, video, and mouse (hence KVM) control of a computer from a remote location. Rather than connect the computer itself to the network, the KVM controller is connected to the network and then to the computer. Raritan Dominion KX II 101 V2, or product with equivalent capabilities. Check with the Division of Laboratory Sciences Informatics Support Team through a LIST ticket in STARLIMS for latest purchasing information.

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

ix. Pipette, benchtop automatic (for preparation of blood dilutions to be analyzed): Like the Microlab 625 advanced dual syringe diluter (Hamilton, Reno, NV, http://www.hamilton.com/) equipped with a 1.0 mL left syringe, a 100 µL 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 (e.g., manganese) background in prepared samples. Alternatives are acceptable, including the Micromedic Digiflex™ Model 33010 (Titertek, Huntsville, AL, http://www.titertek.com/) equipped with 10.0-mL dispensing syringe, 200 µL sampling syringe, 0.75-mm tip, and foot pedal.

x. Pipettes (for preparation of intermediate working calibration standards and other reagents): Like Picus® NxT electronic, single-channel pipettes (Sartorius AG, Göttingen, Germany, www.sartorius.com). 5-120 µL (catalog # LH-745041), 10-300 µL (catalog #LH-745061), 50-

1000 µL (catalog #LH-745081), 100-5000 µL (catalog #LH-745101). Equivalent pipettes and tips can be substituted.

xi. Tubes for sample analysis (for autosampler): Like polypropylene 5-mL conical tubes, Eppendorf model #0030122305 (Eppendorf North America, Hauppauge, NY, www.eppendorf.com) or equivalent. Clear plastics tend to have lowest trace metal contamination. Blue colored caps have also been used successfully for this method.

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

E. Sources of chemicals, gases, and regulators

i. Acid, hydrochloric acid: Veritas™ double-distilled grade, 30–35% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com) or equivalent. This is referred to as "concentrated" hydrochloric acid in this method write-up. For use in preparation of intermediate stock and intermediate working calibration standards.

ii. Acid, nitric acid: Veritas™ double-distilled grade, 68-70% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com). For use in cleaning any bottles, vials, tubes, and flasks. This is referred to as "concentrated" nitric acid in this method write-up.

iii. Blood, whole (human or bovine): Bags of human blood can be purchased from various sources such as American Red Cross (http://www.redcross.org) or ZenBio (RTP, NC, www.zen-bio.com/). Request that human blood be screened for infectious diseases such as Hepatitis B and HIV. Source for bovine blood includes the Wisconsin State Laboratory of Hygiene (WSLH, Madison, WI, http://www.slh.wisc.edu).

iv. Ethanol (EtOH): USP dehydrated 200 proof part #111000200CSPP (Pharmco Products, Inc.) or equivalent.

v. Ammonium pyrrolidine dithiocarbamate (APDC): Laboratory grade, like part number A18210 (Fisher Scientific, Fairlawn, NJ, www.fischersci.com) or equivalent.

vi. 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 (180-250 L). Bulk tank (e.g., 1,500 gallon^{+} is preferred).

vii. Regulator for argon (between bulk tank and instrument): 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. If source of argon is a smaller liquid dewar or a cylinder where large differences in argon pressure may occur from the argon source, a dual stage regulator is recommended.

viii. Regulator for hydrogen gas: Part # 0101-1535 (Agilent, Santa Clara, CA, www.agilent.com), or equivalent.

ix. Regulator for oxygen gas: Part # 0101-1537 (Agilent, Santa Clara, CA, www.agilent.com), or equivalent.

x. Gas clean carrier filter kit: Part# CP17976 (Agilent, Santa Clara, CA, www.agilent.com), or equivalent.

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

xii. Hydrogen gas (research grade, 99.999%): Part # HYR35, or equivalent (Airgas, www.airgas.com)

xiii. Oxygen gas (research grade , 99.999%): Part # OXR33A, or equivalent (Airgas, www.airgas.com)

xiv. Standard, iridium: Like 1,000 µg/mL, item #CGIR1-1 (Inorganic Ventures, Christiansburg, VA http://www.inorganicventures.com). Used as an internal standard in diluent. Standard must be traceable to the National Institute for Standards and Technology.

xv. Standard, multi-element stock calibration standard: Item number SM-2107-057 (High Purity Standards, Charleston, SC, http://www.hps.net/). Standard must be traceable to the National Institute for Standards and Technology.

xvi. Standard, ICP-MS Stock Tuning Solution. 10 µg/mL Li, CLi, Y, Ce, Tl and Co: Agilent Catalog No. 5188-6564 (Agilent, Santa Clara, CA, www.agilent.com) or equivalent. Used to make performance check solution.

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

xviii. Standard, single element stock standards for preparation of calibrators and blood quality control pools: National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs): 3108 (Cd), 3132 (Mn), 3128 (Pb), 3133 (Hg), 3149 (Se). (Gaithersburg, MD, www.nist.gov).

xix. Standard, tellurium: Like 1,000 mg/L, item #CGTE1-1 (Inorganic Ventures, Christiansburg, VA http://www.inorganicventures.com).Used as an internal standard in diluent. Standard must be traceable to the National Institute for Standards and Technology.

xx. Tetramethylammonium hydroxide: Like part number 20932, 25% w/w, or equivalent (AlfaAesar, 30 Bond St., Ward Hill, MA 01835).

xxi. Triton™ X-100 surfactant: Like “Baker Analyzed” Triton™ X-100, product number X198-05 (J.T. Baker Chemical Co., www.jtbaker.com).

xxii. Cone cleaning detergent: for cleaning sampler and skimmer cones, like part # 5188-5359 (Agilent, Santa Clara, CA, www.agilent.com)

xxiii. Alumina powder: For cleaning stainless steel lenses, like part # 8660-0791, or equivalent (Agilent, Santa Clara, CA, www.agilent.com)

7. Preparation of reagents and materials

For all containers (including PMP, PP, Teflon™, glass, or quartz) used for preparation or storage of solutions, the containers should be cleaned by acid-washing prior to first use. Containers can be acid washed at least three times using first a dilute (≤5% v/v) nitric acid solution and second ≥18 MOhm·cm water. If the prepared reagent solution has an HCl matrix, containers may instead be acid-washed at least three times using first a dilute (i.e. ≤3% v/v) hydrochloric acid solution and

second ≥18 MOhm·cm water. Verify the cleanliness of the container is fit for purpose by ICP-MS analysis of rinsate before and after rinsing the container. After this cleaning and verification, the containers may be dedicated to purpose and labeled appropriately for the intended solution preparation. Throughout this section, these cleaned containers are referred to as acid-washed.

To facilitate tracking of solvents and other chemicals transferred from the original manufacturer containment into a secondary container (e.g., solvent bottle), include the expiration date provided by the manufacturer or the lot number on the secondary container.'

Though each reagent preparation specifies a total volume of reagent prepared, these directions may be scaled up or down to prepare larger or smaller quantities if desired by adjusting all volumes proportionally.

The following reagent preparation instructions are summarized in **Appendix D** help sheets for use at the bench in the laboratory.

A. Water

i. Purpose: All solutions used in this method are prepared in water.

ii. Storage conditions: Store at ambient temp. (+15°C to + 30°C).

iii. Expiration:

1) Water used to prepare reagents:

One month after refilling from the water purification unit. Document refills on a date strip on the container.

2) Water used to flush the probe of the ICP-MS autosampler:

Replace each day to avoid contamination from repeated use. Document refills on a date strip on the container or on the container itself.

3) Water used in squirt bottle for Hamilton diluter tip rinse:

One month after refilling from the water purification unit. Document refills on a date strip on the container.

4) Water used to flush the Hamilton diluter:

One month after refilling from the water purification unit. Document refills on a date strip on the container.

5) Water used to prepare final dilutions for analysis:

Replace each day to avoid contamination from repeated use. Document refills on a date strip on the container or on the container itself.

iv. Preparation: Dispense ≥ 18 MOhm·cm water from a water purification unit.

B. Intermediate Internal standard solution:

i. Purpose: Preparation of a single intermediate solution containing all internal standards simplifies the addition of the internal standard(s) into the final diluent solution. This solution can be purchased rather than prepared.

ii. Preparation and storage: To prepare 50 mL of 20 mg/L Rh, Ir, Te in 1% v/v HNO_3:

1) Partially fill a acid-washed 50 mL volumetric flask (PP, PMP, or Teflon™).
with ≥18 MOhm·cm water (approximately 25-40 mL).

2) Add 0.5 mL of high purity concentrated nitric acid and mix solution thoroughly. See section 4.A.iv for safety precautions. Take special care when handling and dispensing concentrated acids. Use additional personal protective equipment which protects face, neck, and front of body.

3) Add 1 mL of 1,000 µg/mL Rh standard, 1 mL of 1,000 µg/mL Ir standard, and 1 mL of 1,000 µg/mL Te standard. If initial Rh, Ir, or Te standard concentration is different, adjust volume proportionally.

4) Fill to mark (50 mL) with ≥18 MOhm·cm water and mix thoroughly.

5) Store at ambient temperature (+15°C to + 30°C) and label appropriately. Expiration is 1 year from date of preparation.

C. Intermediate Triton™ X-100 solution:

i. Purpose: For use in preparation of the diluent, carrier, and rinse solutions as a more dilute solution of (20% v/v) of Triton® X-100.

ii. Preparation and storage: To prepare 1 L of 20% Triton™ x-100:

1) Partially fill an acid-washed 1 L container with ≥18 MOhm·cm water.

2) Add 200 mL of Triton™ X-100 to the 1L container that is partially filled with ≥18 MOhm·cm water.

3) Fill to 1 L with ≥18 MOhm·cm water and mix until the Triton™ X-100 has completely dissolved into solution (overnight). A magnetic stirring plate can be used to assist mixing by adding an acid-washed Teflon™ coated stirring bar to the bottle.

4) Store at ambient temperature (+15°C to + 30°C) and label appropriately. Expiration is 1 year from date of preparation.

D. Sample diluent and carrier

i. Purpose: This solution will be used in the preparation of all samples and calibrators during the dilution process prior to analysis. It is important to make all samples, standards, blanks, and QC, etc., in a run from the same diluent solution so that the concentration of the internal standards will be the same among all calibrators and samples in the run. When using a flow-injection component in the sample introduction system (i.e., the Elemental Scientific SC4-FAST autosampler), use the same solution for the the 'carrier' and sample diluent. The diluent is an aqueous solution of 5 µg/L internal standard mixture (Rh, Ir, Te), in 1.0% v/v tetramethyl ammonia hydroxide (TMAH), 1% ethanol, 0.01% APDC, and 0.05% Triton™ X-100. Larger volumes of these solutions can be prepared by adjusting component volumes proportionally.

ii. Preparation and storage: To prepare 2L of 5 µg/L Rh, Ir and Te, 0.01% APDC, 1.0% v/v TMAH, 1% ethanol, and 0.05% Triton™ X-100:

1) Partially fill an acid-washed 2 L container with ≥18 MOhm·cm water.

2) Add 0.2 g of APDC.

3) Add 20 mL of concentrated (25% w/w) TMAH. Take special care when handling and dispensing concentrated bases. Use additional personal protective equipment which protects face, neck, and front of body. See section 4.A.iv.

4) Add 20 mL of ethanol.

5) Add 5 mL of 20% Triton™ X-100(see Intermediate Triton X-100)

6) Fill to volume (2L) with ≥18 MOhm·cm water.

7) Spike 500 µL of 20 mg/L Rh, Ir, Te (Internal Standard Intermediate) to the final diluent.

8) Invert bottle a few times to ensure thorough mixing. Wait several hours before using, until the Triton X-100 is mixed evenly throughout the solution.

9) Store at ambient temperature (+15°C to + 30°C) and label appropriately. Expiration is 1 year from date of preparation. When refilling the carrier solution bottle at the instrument, document each refill on the date strip and update the expiration date, as needed, to match the expiration date of the source container.

E. ICP-MS rinse solution

i. Purpose: The rinse solution used in this method is an aqueous solution of 0.01% APDC in 1.0% v/v TMAH, 1% ethanol, and 0.05% Triton™ X-100. This solution will be pumped through the autosampler rinse station, autosampler probe, and sample loop between sample analyses to prevent carry-over of analytes from one sample measurement to the next.

ii. Preparation and storage: To prepare 4 L of 0.01% APDC in 1.0% v/v TMAH, 1% ethanol, and 0.05% Triton™ X-100:

1) Partially fill an acid-washed 4 L container with ≥18 MOhm·cm water (approximately 2-3 L). Use of volumetric flask is not required.

2) Add 0.4 g of APDC.

3) Add 40 mL of 25% v/v TMAH. Take special care when handling and dispensing concentrated bases. Use additional personal protective equipment which protects face, neck, and front of body. See section 4.A.iv.

4) Add 40 mL of ethanol.

5) Add 10mL of 20% Triton™ X-100, (See Section Intermediate Triton X-100 for details on preparation)

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

7) Invert bottle a few times to ensure thorough mixing. Wait several hours before using, until the Triton X-100 is mixed evenly throughout the solution.

8) Store at ambient temperature (+15°C to + 30°C) and label appropriately. Expiration is 1 year from date of preparation. If refilling a larger rinse solution reservoir at the instrument from a container in which the rinse solution was made, document each refill of the reservoir on the date strip and update the expiration date, as needed, to match the expiration date of the source container.

F. Base blood

i. Purpose: This blood pool material will be mixed with the intermediate working calibration standards just prior to analysis to matrix-match the diluted working calibrators to the blood matrix of the unknown samples.

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

1) Purchase several bags of whole blood.

2) Screen each individual bag of blood for concentration of analytes of interest. See Table 3 of **Appendix C** for suggested concentrations.

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

4) Store long-term frozen (-10°C to -90°C) as smaller portions for daily use (e.g., 2 mL cryovials) according to the same storing and handling criteria described in Section 5.

5) Label appropriately and store frozen (-10°C to -90°C). Blood stored at ≤ -70°C has been stable for use as QC materials for up to 5 years.

G. Multi-element stock calibration standards

i. Purpose: This multi-element stock standard will be used to prepare the intermediate working calibration standards.

ii. Purchase and storage: Whether purchased as a special mix or prepared in-house, the starting materials must be NIST-traceable. Matrix and concentrations of Pb, Cd, Hg, Mn and Se are listed in Table 4 of **Appendix C**. Store at ambient temperature (+15°C to + 30°C) and label appropriately. Expiration is as defined by the manufacturer or 1 year from date of opening, whichever comes first.

H. Diluent (3% v/v HCl) for stock and intermediate stock calibration standards:

i. Purpose: This diluent is used to dilute stock and intermediate stock calibration standards, not to prepare working calibrators or blood samples for analysis.

ii. Preparation and storage: To prepare 2L of 3% v/v HCl:

1) Partially fill an acid-washed 2 L flask, with 1-1.5L ≥18 MOhm·cm water.

2) Add 60 mL high purity concentrated HCl. See section 4.A.iv for safety recommendations. Take special care when handling and dispensing concentrated acids. Use additional personal protective equipment which protects face, neck, and front of body.

3) Fill to the mark and mix thoroughly.

4) Store at ambient temperature (+15°C to + 30°C) and label appropriately. Expiration is 1 year from date of preparation.

I. Multi-element intermediate stock calibration standard A

i. Purpose: The multi-element intermediate stock standard A will be used to prepare the intermediate working calibration standards.

ii. Preparation and storage: To prepare intermediate stock calibration standard A containing 3% v/v HCl solutions with Cd, Pb, Hg, Se, and Mn concentrations listed in Table 5 of **Appendix C**:

1) Partially fill an acid-washed (50-75% full) 100 mL flask with the 3% v/v HCl diluent prepared in Section 7.H.

2) Using the volume listed in Table 5 of **Appendix C**, pipette the appropriate volume of the multi-element stock calibration standard solution into the volumetric flask.

3) Dilute to the volumetric mark with the 3% v/v HCl diluent using a pipette for the final drops. Mix the solution thoroughly. Final concentrations are listed in Table 5 of **Appendix C**.

4) Once mixed, transfer to acid washed labeled containers (PP, PMP, or Teflon™) for storage.

5) Store at ambient temperature (+15°C to + 30°C) and label appropriately. Expiration is 1 year from date of preparation.

J. Multi-element intermediate stock calibration standard B

i. Purpose: The multi-element intermediate stock standard will be used to prepare the intermediate working calibration standards.

ii. Preparation and storage: To prepare intermediate stock calibration standard B containing 3% v/v HCl with Cd, Pb, Hg, Se, and Mn concentrations listed in Table 5 of **Appendix C**:

1) Partially fill an acid-washed (50-75% full) 100 mL flask with the 3% v/v HCl diluent prepared in Section 7.H.

2) Using the volume listed in Table 5 of **Appendix C**, pipette the appropriate volume of the multi-element stock calibration standard solution into the volumetric flask.

3) Dilute to the volumetric mark with the 3% v/v HCl diluent using a pipette for the final drops. Mix the solution thoroughly. Final concentrations are listed in Table 5 of **Appendix C**.

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

5) Store at ambient temperature (+15°C to + 30°C) and label appropriately. Expiration is 1 year from date of preparation.

K. Intermediate working calibration standards

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

ii. Preparation and storage: To prepare intermediate working calibration standards containing 3% v/v HCl solutions with Cd, Pb, Hg, Se, and Mn concentrations listed in Table 6 of **Appendix C**:

1) Partially fill each acid-washed 100 mL flask 50-75% with the 3% v/v HCl diluent prepared in Section 7.H.

2) Using the volumes listed in Table 6 of **Appendix C**, pipette the appropriate volume of the multi-element intermediate stock calibration standard solutions into each of the volumetric flasks.

3) Dilute each to the volumetric mark with the 3% v/v HCl diluent using a pipette for the final drops. Mix each solution thoroughly. Final concentrations are listed in Table 6 of **Appendix C**.

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

5) Store at ambient temperature (+15°C to + 30°C) and label appropriately. Expiration is 1 year from date of preparation.

6) Pour aliquots of each standard into lot screened or acid-washed smaller containers (PP, PMP, or Teflon™) and label for daily use. Document the expiration date of the source solution on the secondary containers.

L. Working calibrators

i. Purpose: The working calibrators will be analyzed in each run to provide a signal-to-concentration response curve for each analyte in the method. The concentration of the analyte of interest in a patient blood sample dilution is determined by comparing the observed signal ratio (analyte/internal standard) from the dilution of the patient blood sample to the signal ratio response curve from the working calibrators. A bulk preparation of a working calibrator (e.g., S2 or S3) will be prepared to measure repeatedly prior to the run to condition the ICP-MS system (referred to as 'reaction gas stability time'), reaching a stable analyte-to-internal standard ratio over consecutive measurements.

ii. Preparation and storage: Dilutions (1:20) of the corresponding seven intermediate working calibration standards with base blood and sample diluent. Base blood and diluent (Section 6.c) can be added to tubes using a benchtop automatic pipette. Use a handheld pipette to add the intermediate working calibrators immediately prior to analysis (see Table 9 of **Appendix C**). Store at ambient temperature (+15°C to + 30°C) and label appropriately before analysis, within 24 hours of preparation. Expiration for use in reaction gas stability conditioning time is 24 hours at ambient temperature (+15°C to + 30°C) or 1 week if refrigerated (+2°C to +8°C). If refilling the DRC stability solution container at the instrument from a source container, document each refill of the container at the instrument on a date strip and update the expiration date, as needed, to match the expiration date of the source container.

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

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

ii. Preparation and storage: To prepare pooled animal or human blood at low-normal, high-normal, and elevated concentrations: Both purchased or in-house prepared quality control materials are suitable for this purpose if volumes and concentrations meet method requirements and any spikes of elemental levels are traceable to the National Institute for Standards and Technology (NIST).

1) Screening blood: Screen bags of blood for concentrations of analytes. Then select blood to use for the difference QC pools based on the starting concentrations.

2) Keep blood refrigerated (+2°C to +8°C) whenever possible to minimize microbial growth.

3) Select blood for the low bench QC pool which has analyte concentrations in the low-normal population range. Select blood for the high and elevated bench QC pools which has analyte concentrations less than some pre-selected target concentration values in the high normal population range. See Table 12 of **Appendix C** for normal population reference ranges.

4) Combining collected blood: The goal for combining samples is to approach an 'average' matrix for each pool.

a) Graduate and acid-wash one PP or PMP carboy for each QC pool being created (e.g., one acid-washed 10L carboy graduated in 0.5L increments). If the volume of the blood pool will

be determined by weighing, rather than estimation, weigh the empty carboy and any stir bar that will be used in the carboy.

b) Combine blood into separate acid-washed carboys according to their concentrations, for each QC pool being made.

c) Determine the volume of blood in the carboy either by estimation or, if possible, weighing (e.g., using the density formula and an estimated blood density of 1.057 g/mL[67]).

d) Mix the blood in the carboys using stirrers and large stir plates. Keep blood refrigerated whenever possible (+2°C to +8°C).

5) Spiking of blood

a) Analyze aliquots of each blood pool to determine the pre-spike pool concentrations. Record these results for future recovery calculations.

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

c) Calculate the volume of single element standards needed to spike each pool to the desired concentrations. See Table 12 of **Appendix C** for normal population reference ranges.

d) While stirring the pools on large stir plates, spike each pool with calculated volumes of single element standards (all spiking standards used must be traceable to NIST). Use small spike volumes that can be transferred accurately (e.g., >50 uL), but which do not greatly modify the blood matrix (e.g., <0.5%).

e) Continue to stir pools overnight after spiking, then reanalyze.

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

6) Dispensing and storage of blood

a) Container types: Dispense blood 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.

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

c) Dispensing: Dispensing can be accomplished most easily using a benchtop automatic pipette in continuous cycling dispense mode. Dispense the pools in a clean environment (i.e., a class 100 cleanroom area or hood).

(i) Allow blood to reach ambient temperature (+15°C to + 30°C) before dispensing (to prevent temperature gradients possibly causing concentration gradients across the large number of vials being dispensed and to prevent condensation problems during labeling of vials).

(ii) 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.

(iii) Check cleanliness of the benchtop automatic pipette before use by analyzing dilute acid (e.g., 1% 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.

(iv) Approximately one hour before dispensing begins,

(v) With the large stir plate close to the left side of the benchtop automatic pipette, begin stirring the blood pool to be dispensed.

(vi) Also during this time, flush the benchtop automatic pipette with blood from the pool to be dispensed. Place the ends of the tubing attached to both the sample and dispensing syringes into the carboy of blood so that blood won't be used up during this process. Be sure to secure both ends of tubing in the carboy (e.g., with Parafilm) so they will not come out during the flushing process.

(vii) After dispensing the blood into the vials, cap the vials and label them. Placing labels on vials after capping minimizes the chance for contamination during the process.

(viii) Homogeneity test: Check homogeneity of analyte concentrations in pool aliquots.

(ix) Storage: Store long-term as smaller portions for daily use (e.g., 2 mL cryovials) according to the same storing and handling criteria described in Section 5. QC materials stored at these conditions are valid for at least 15 years. Beyond that, monitor stability quarterly along with external reference materials (see Section 13.A).

N. ORS cell gas optimization solutions:

i. Purpose: For periodic testing of the ORS cell parameters. Procedure requires at a minimum a blank (i), an analyte solution (ii), a blank with interference (iii), and an analyte and interference containing solution (iv). Interferences are discussed in Sections 1.B. and 2.A. Interference concentrations can be prepared higher as needed by adjusting the volume of the spikes. Keep interference spike volume small (0.05 – 0.3 mL) using a high concentration stock solution (i.e., 1000 µg/mL). Analyte concentrations can be made higher if needed for sensitivity reasons by preparing a higher concentration calibrator. If elimination of the interference is difficult to verify, replace the use of blood in these preparations with ultrapure water to minimize trace amounts of the analyte in the preparation. Diluent in this section refers to sample diluent (5 µg/L internal standard mixture (Rh, Ir, Te), 1.0% v/v tetramethyl ammonia hydroxide (TMAH), 1% ethanol, 0.01% APDC, and 0.05% Triton™ X-100 as described in Section 6c.

ii. Preparation and storage: ($^{54}Fe^{1}H$ interference on ^{55}Mn):

1) Base blood in diluent (1 + 19): In a 15 mL lot screened or acid-washed polypropylene tube, prepare a 10 mL portion of working calibrator 0 as described in Table 9 of **Appendix C** (multiply volumes by 10).

2) Base blood in diluent (1 + 19) + 1.6 µg/L Mn: In a 15 mL lot screened or acid-washed polypropylene tube, prepare a 10 mL portion of working calibrator 3 as described in Table 9 of **Appendix C** (multiply volumes by 5).

3) Base blood in diluent (1 + 19) + 500 µg/L Fe: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 0 as described in Table 9 of **Appendix C** (multiply volumes by 50). Add 0.025 mL of 1000 µg/mL Fe.

4) Base blood in diluent (1 + 19) + 1.6 µg/L Mn + 500 µg/L Fe: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 3 as described in Table 9 of **Appendix C** (multiply volumes by 50). Add 0.025 mL of 1000 µg/mL Fe.

5) Label appropriately. Expiration date is 24 hours at ambient temperature (+15°C to + 30°C) and one week in the refrigerator (+2°C to + 8°C).

iii. Preparation and storage: ($^{39}K^{16}O^{+}$ interference on ^{55}Mn):

1) Base blood in diluent (1 + 19): In a 15 mL lot screened or acid-washed polypropylene tube, prepare a 10 mL portion of working calibrator 0 as described in Table 9 of **Appendix C** (multiply volumes by 10).

2) Base blood in diluent (1 + 19) + 1.6 µg/L Mn: In a 15 mL lot screened or acid-washed polypropylene tube, prepare a 10 mL portion of working calibrator 3 as described in Table 9 of **Appendix C** (multiply volumes by 5).

3) Base blood in diluent (1 + 19) + 200 µg/L K: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 0 as described in Table 9 of **Appendix C** (multiply volumes by 50). Add 0.010 mL of 1000 µg/mL K.

4) Base blood in diluent (1 + 19) + 1.6 µg/L Mn + 200 µg/L K: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 3 as described in Table 9 of **Appendix C** (multiply volumes by 50). Add 0.010 mL of 1000 µg/mL K.

5) Label appropriately. Expiration date is 24 hours at ambient temperature (+15°C to + 30°C) and one week in the refrigerator (+2°C to + 8°C).

iv. Preparation and storage: ($^{160}Gd^{++}$ interference on ^{80}Se):

1) Base blood in diluent (1 + 19): In a 15 mL lot screened or acid-washed polypropylene tube, prepare a 10 mL portion of working calibrator 0 as described in Table 9 of **Appendix C** (multiply volumes by 10).

2) Base blood in diluent (1 + 19) + 37.5 µg/L Se: In a 15 mL lot screened or acid-washed polypropylene tube, prepare a 10 mL portion of working calibrator 4 as described in Table 9 of **Appendix C** (multiply volumes by 10).

3) Base blood in diluent (1 + 19) + 10 µg/mL Gd: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 0 as described in Table 9 of **Appendix C** (multiply volumes by 50). Add 0.05 mL of 10,000 µg/mL Gd.

4) Base blood in diluent (1 + 19) + 37.5 µg/L Se + 10 µg/mL Gd: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 4 as described in Table 9 of **Appendix C** (multiply volumes by 50). Add 0.05 mL of 10,000 µg/mL Gd.

5) Label appropriately. Expiration date is 24 hours at ambient temperature (+15°C to + 30°C) and one week in the refrigerator (+2°C to + 8°C).

v. Preparation and storage: (Ca interferences on ^{80}Se):

1) Base blood in diluent (1 + 19): In a 15 mL lot screened or acid-washed polypropylene tube, prepare a 10 mL portion of working calibrator 0 as described in Table 9 of **Appendix C** (multiply volumes by 10).

2) Base blood in diluent (1 + 19) + 37.5 µg/L Se: In a 15 mL lot screened or acid-washed polypropylene tube, prepare a 10 mL portion of working calibrator 4 as described in Table 9 of **Appendix C** (multiply volumes by 10).

3) Base blood in diluent (1 + 19) + 100 µg/mL Ca: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 0 as described in Table 9 of **Appendix C** (multiply volumes by 50). Add 0.1 mL of 10,000 µg/mL Ca.

4) Base blood in diluent (1 + 19) + 37.5 µg/L Se + 10 µg/mL Ca: In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 4 as described in Table 9 of **Appendix C** (multiply volumes by 50). Add 0.10 mL of 10,000 µg/mL Ca.

5) Label appropriately. Expiration date is 24 hours at ambient temperature (+15°C to + 30°C) and one week in the refrigerator (+2°C to + 8°C).

O. Pulse / Analog (P/A) Factor tune solution

i. Purpose: Use to perform the P/A factor tune to ensure cross-calibration of the pulse and analog modes of the detector. Lead and manganese typically require both pulse and analog mode of the detector.

ii. Preparation & storage: Prepare different volumes, if needed, by adding proportionally larger or smaller volumes of solution constituents. A solution with higher analyte concentrations may be prepared as needed by using a larger volume of stock solution. The concentration of analytes needed to measure analog mode may differ between instruments due to unique instrument sensitivity. To prepare a total of 50 mL of the multi-element solution with a concentration including 100 µg/L Pb and 50 µg/L Mn among other analytes:

1) Partially fill a 50 mL lot screened or acid-washed polypropylene tube with 10-40 mL 2% v/v nitric acid.

2) Add 0.5 mL of each multi-element stock standard (Agilent part number # 5188-6524 has 2 solutions).

3) Dilute to the 50 mL mark with 2% v/v nitric acid.

4) Label appropriately. Expiration date is one year from preparation date. If transferred to a secondary container, document the expiration date of the source on the secondary container.

8. Analytical instrumentation setup

A. Configuration for liquid handling

i. FAST valve setup: See Figure 8. Configuration of tubing and devices for liquid handling using FAST sample introduction of **Appendix C** for diagram and Section 5.b “FAST / ESI SC4-DX autosampler accessories” for source information.

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

2) Port 2: 0.5 mm ID probe (red nut) for carrier solution.

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

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

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

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

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

iii. Spray chamber waste removal: Use the peristaltic pump to control the removal of liquid waste from the spray chamber. The spray chamber drain tubing connects directly to the Santoprene™ peristaltic pump tubing. Connect the other end of the peristaltic pump tubing to 0.5 mm i.d. PFA tubing. Place the free end of the PFA tubing through the lid of the waste jug (be sure it is secure). Between peristaltic pump tubing and waste container: Connect 1/8" i.d. x ¼" o.d. PVC tubing to the orange/green peristaltic pump tubing using a tubing connector. Place the free end of the PVC tubing through the lid of the waste jug (be sure it is secure). Place waste container in a deep secondary containment tray in case of overflow.

iv. Between spray chamber and peristaltic tubing: Use vendor-supplied drain kit on base of chamber, connecting tubing directly to peristaltic pump tubing through a PEEK adapter or directly.

v. Rinse solution for autosampler:

1) 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.

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

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

B. Gas delivery and regulation

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

1) Regulator for argon source (if a dewar): Set delivery pressure of this regulator at least 10 psi higher than the delivery pressure of the step-down regulator to allow for pressure drop across tubing that stretches to the instrument.

2) Step down regulator (if source of argon is a bulk tank): Place this single stage regulator in the lab so that incoming argon pressure can be monitored and adjusted. Set delivery pressure to 10-20 psi higher than the setting at the instrument's filter regulator.

3) Filter regulator at the instrument: 90 (±10) psi

ii. Hydrogen gas: Used in the ORS for interference removal

1) Connect to Hydrogen Cell Gas inlet

2) Set the delivery pressure of regulator to 13 - 17 psig when gas is flowing. See section 5.e for part numbers and details.

iii. Oxygen gas: Used in the ORS for interference removal

1) Connect to 4th Cell Gas inlet

2) Set the delivery pressure of regulator to 13 - 17 psig when gas is flowing. See Section 5.e for part numbers and details.

C. Chiller / heat exchanger:

If using refrigerated chiller, set temperature control to approximately 18 °C (+10°C to +26°C).

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

A. Quality: 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. See Section 12.C for description of QC calculations and monitoring of trends. In each analytical run bench QC samples are prepared from a minimum of two pools that represent "low-normal" ("Low QC"), "high-normal" (High QC"), and "above-normal" (Elevated QC") concentrations. This assay typically uses three urine pools that span the analyte concentration range of the calibrators including "low-normal" ('Low QC'), "high-normal" ('High QC'), and "above-normal" ('Elevated QC') concentrations. Samples from these pools are prepared in the same manner as patient samples and analyzed in duplicate as part of each run. Bench QC pool samples are analyzed first in the run after the calibrators but before any patient samples are analyzed. This permits making judgments on calibration linearity and blank levels prior to analysis of patient samples. The second analysis of the bench QC pools is done after analysis of all patient samples in the run (typically 20-50 patient samples total when analyzing for all elements in the method) to ensure analytical performance has not degraded across the time of the run. If more patient samples are analyzed on the same calibration curve after the second run of the bench QC, all bench QC must be reanalyzed before and after the additional samples. For example, the schemes shown in Table 7 of **Appendix C** are both acceptable ways to analyze multiple consecutive "runs".

ii. Reference materials: Use standard reference materials (SRM) from the National Institute of Standards and Technology (NIST) (i.e., SRM 955c Levels 1-4 and SRM 1401 Levels 1-2) to verify method accuracy quarterly. Use previously characterized samples from proficiency testing program or commercially-produced reference materials when NIST SRMs are unavailable (e.g., see materials used in accuracy tests summarized in Section 14.A of **Appendix A**.

iii. Calibration verification: The test system is calibrated as part of each analytical run with NIST-traceable calibrators. No additional calibration verification is required because multi-point calibration standards are included in every analytical run. However, for good laboratory practice, reference materials are measured quarterly (e.g., 4 times per calendar year).

B. Execution: perform, evaluate and report a run

i. Starting the equipment for a run:

1) Power is on: instrument computer controller and the autosampler.

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

3) Pre-start inspection: Perform the pre-start inspection as described in **Appendix F**.

4) Place probe in adequate volume of carrier or rinse solution: If using an ESI FAST, manually place carrier probe into carrier solution. If not, send the autosampler probe to a rinse solution (e.g., autosampler rinse station).

5) Start the plasma, verify peristaltic pump starts, that liquid flow is in the correct direction, and that the peristaltic pump tubing is under proper tension.

6) Post-start function checks: After igniting the plasma complete the steps described in **Appendix F** under "Function checks after igniting the plasma of the ICP-MS".

7) Reaction gas stability and conditioning time: Best analyte-to-internal standard ratio stability is typically observed after one hour of analysis of diluted blood samples (20 measurements of the 5 element panel can be made in 1 hour). Prepare 50 mL of a calibration standard (e.g., standard 2 or 3) to be analyzed repeatedly before the beginning of the run to achieve a stable analyte-to-internal standard ratio. Time to reach stability is instrument-specific and learned from performance of runs. See Table 8 of **Appendix C** for example of setup in the Samples / Batch window and Table 9 of **Appendix C** for details of making a working calibrator. The net intensity (analyte / internal standard ratio) of the measurements made while stabilizing the DRC can be evaluated to determine the readiness of the system to begin analysis. Continual trending in this ratio indicates that unwanted instrument drift will occur within the run. If the system has been recently cleaned, a longer stability time may be needed. After installing clean sample introduction components, the ICP-MS typically requires a period of system conditioning time or "pre-conditioning" where the interior components become lightly coated by the sample matrix.

ii. Verify that background signal from instrument and reagents are low. Prior to analysis, it is helpful to test the following solutions.

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

2) Diluent before and after being flushed through the benchtop automatic pipette. If contamination is observed from the pipette, flush the pipette with ≤ 500 mL of diluent and retest.

iii. Software setup for analysis:

1) Create a batch file (e.g., from an unused template). Verify the correct settings (acquisition method and data analysis method documented in Table 1 and 2 of **Appendix C**.

2) Update the software sample list / batch window to reflect the current sample set. Use a barcode scanner to input data whenever possible. See Table 1 of **Appendix C** for times and speeds. Verify the correct sample type for CalBlk and FQBlk samples.

a) Sample IDs for extra dilutions

For any sample prepared with an extra dilution, enter the sample ID into the ICP-MS software with the "^" symbol and the numerical extra dilution used at the end of the ID (e.g., sampleID^2). When the instrument run file is imported into STARLIMS, the suffix will be removed from the sample ID and the number will be set as the result dilution. The result prior to accounting for the result dilution will be the observed result and the Final and Reported Values will reflect the observed result times the result dilution. See Table 9 for description of how to prepare extra dilutions and Table 10 for maximum extra dilutions permitted.

iv. Prepare dilutions for analysis (See Table 9 of **Appendix C**)

1) Thaw blood samples; allow them to reach ambient temperature (+15°C to + 30°C).

2) Prepare the following solutions into pre-labeled containers using the benchtop automatic pipette or other volumetric sample transfer device. See Table 9 of **Appendix C** for a summary.

3) 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.

4) Calibrators: Prepare the working calibrators (S0-S7). Prepare S0 in triplicate to use for both the zero calibrator and blood blank checks after the calibrators. The base blood and diluent can be added with the automatic dilutor, but add the intermediate working calibrators to the dilution by handheld pipette using pipette tips that are pre-rinsed at least three times with 3% (v/v) HCl (i.e. S0) immediately prior to use. This is to avoid the intermediate working calibrator solutions from coming into contact with the diluent. Contact between the diluent and the intermediate working calibrators will result in loss of selenium from the intermediate working calibrator solution and will degrade method accuracy.

5) QC and Patient Samples: Before taking an aliquot for analysis, homogenize the blood sample thoroughly. To avoid wasting patient blood sample volume, wait, if possible, until after the beginning QC to verify the analytical system appears to be in control prior to preparing dilutions of patient samples.

6) After preparation, cap and mix. Place prepared dilutions on the autosampler of the ICP-MS in the order corresponding to the sequence setup in the ICP-MS software.

7) Ambient temperature (+15°C to + 30°C) storage is acceptable for the original samples during the work day.

8) Diluted samples have been validated for testing up to 24 hours after preparation (see Section 14.C of **Appendix A**).

v. Start the analysis using the ICP-MS software

vi. 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 Plasma Off At End (see below). Monitor the analysis for the following:

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

2) Verify calibration curves meet R^2 requirements (minimum of 0.98, typically 0.99 to 1.000). If a particular calibration standard is obviously in error, it can be re-analyzed as a sample (old or new dilution) within the run and incorporated into the curve through data reprocessing as a calibrator. As a last resort, a single calibration point per analyte can be removed from the curve to meet the coefficient requirements of the linear regression. Do not drop the lowest or highest calibrators because that would change the reportable range for the run. Follow up problems with calibrators with appropriate corrective actions (e.g., re-preparation of intermediate working calibration standards or troubleshooting instrument parameters).

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

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

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

f) Prepare and analyze new working calibrators.

g) Test a different preparation of intermediate working calibration standards.

4) Verify good precision among replicates of each measurement.

5) 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 preconditioning before the run or environmental conditions are changing too much around the instrument.

6) Verify elevated patient results.

a) Confirming an elevated concentration: Repeat for confirmation any sample having a concentration greater than the 1UB threshold (see Table 11 of **Appendix C**).

b) Dilution of a sample to within the calibration range: Repeat in duplicate with extra dilution any sample having a concentration greater than the highest calibrator to bring the observed result within the concentration range of the calibrators. See Table 10 of **Appendix C** for high calibrator concentrations and validated extra dilutions.

c) Confirming proper washout after an elevated sample: When monitoring the analysis in realtime, if a sample concentration is greater than the highest concentration validated for washout (see Table 11 of **Appendix C**), do the following to verify that the run is still in control for low concentration samples before proceeding with analysis.

(i) Stop run following elevated sample.

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

3040 BldBlkChk Wash1

3040 BldBlkChk Wash2

LBXXXXX Wash

(iii) If the run is not verified in-control for low concentration samples before the next samples are analyzed, see Section 8.C.ii.7) 6or directions.

vii. Overnight operation or using plasma off at end: The run may be left to complete itself unattended as long as appropriate planning is made (e.g., sufficient solution supply and waste collection). Turn on the “Plasma off at end” feature in the Queue of the ICP-MS software.

Delay the shutdown at least 10 min by analyzing several water samples at the end of the run to rinse the sample introduction system of blood matrix before turning off the plasma. lt will be necessary to replace the sample peristaltic pump tubing the next day since it will have been clamped shut overnight.

viii. Records of results: The data files created using the ICP-MS software containing the data from the analysis are stored on the CDC network and imported into the DLS STARLIMS database for review of the patient data, statistical evaluation of the QC data, and approval of the results. See **Appendix E** for a step-by-step description of how the analyst transfers data from the ICP-MS instrument computer to DLS STARLIMS, reviews and documents the results in DLS STARLIMS, and submits the results for review and approval. Request Helpdesk support for DLS STARLIMS issues using the link on the dashboard of DLS STARLIMS.

C. Evaluation: Analyst evaluation of run results:

i. Bench quality control: After completing a run, and importing the results into the laboratory information system, STARLIMS, (see **Appendix E**) a QC program written in SAS is available within STARLIMS and should be used to apply these rules to QC data and generate Shewhart QC charts.

1) Rules for bench quality control evaluation: The results from bench QC pools are checked after each run using a multi-rule quality control system [68] based their characterization data, namely: the pool mean; the pooled within-run standard deviation associated with individual QC results measured in the same run (Sw); the standard deviation associated with individual QC results (Si); and the standard deviation associated with run mean QC results (Sm). QC rules have been designed to accommodate the use of 1–3 different QC pools during a run, the use of 1–2 measurements of each pool per run, and as many instruments as needed. These QC rules are described in the DLS Policies and Procedures Manual and a relevant selection applicable to this assay is shown below. The following are the CDC DLS QC rules for three QC pools per run with two or more results per pool.

a) If all three QC run means are within 2Sm limits and individual results are within 2Si limits, then accept the run.

b) lf one of the three QC run means is outside a 2Sm limit - reject run if

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

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

(iii) 2S Rule - Two or more of the run means are outside the same 2Sm limit

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

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

(i) Extreme Outlier - One individual result is beyond the characterization mean + 4Sm

(ii) R 4S Rule - 2 or more of the within-run ranges in the same run exceed 4Sw (i.e., 95% range limit). Note: Since runs have multiple results per pool for 3 pools, the R 4S rule is applied within runs only.

2) Implications of QC failures: No results for a given analyte are to be reported from an analytical run that has been declared “out of control” for that analyte as assessed by internal

(bench) QC. If the DLS SAS QC program declares the run "out of control" for any analyte, use the following to determine the implications on usability of the data from the run.

a) 4-5 elements in the run

(i) 1 or 2 analytes "out of control": ONLY the analytes which were "out of control" are invalid for reporting from the run.

(ii) 3 or more analytes "out of control": All results, regardless of analyte, are invalid for reporting from the run.

b) 1-3 elements in the run

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

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

ii. Patient results:

1) Elevated concentrations:

a) Boundaries requiring confirmatory measurement:

(i) Results greater than the first (1UB) or second (2UB) upper boundaries. The concentrations assigned to 1UB and 2UB for an element is determined by study protocol but default concentrations are in Table 11 of **Appendix C**.

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

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

(iv) Results greater than highest calibrator: Samples with observed results exceeding the highest calibrator must be prepared with an extra dilution factor, to bring the observed result within the calibration range, and confirmed by a duplicate extra dilution before a result can be reported. Maximum allowable extra dilutions are described in Table 10 of **Appendix C**. Report the first analytically valid result (i.e. the first observed result within the calibration range), as long as the duplicate confirms that result within either ±10% of the first result or ±3SD of the nearest bench QC, whichever is greater. Continue to repeat analysis until a concentration is confirmed. The lowest possible extra dilution factor is preferred to minimize matrix differences between the calibrators and the sample (i.e. a 2x extra dilution is preferred over 10x if 2x dilutes the analyte into the calibration range of linearity)

b) Concentrations requiring verification of washout: Following a result greater than the highest concentrations validated for washout (see Table 11 of **Appendix C**) do the following:

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

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

c) Unacceptable reproducibility: If the range of the three replicate readings (maximum replicate concentration value - minimum replicate concentration value) for a single unknown sample analysis is greater than the range maximum criteria listed in Table 11 of **Appendix C** 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.

D. Reporting: Submitting final work for review:

All analyses must undergo quality control and quality assurance review. Follow instructions described in **Appendix E** before informing the first level reviewer of the completed work.

10. Routine equipment maintenance and data backups

A. Equipment maintenance:

i. ICP-MS system maintenance: Maintenance steps performed before igniting the plasma each run day, after igniting the plasma each run day, periodically (ever 10-15 run days), monthly, and biannually are described in **Appendix F**.

ii. Pipettes, single-channel: Pipette calibration verification is performed every 6 months for single-channel, handheld pipettes either onsite or by a certified company. Typically on-site calibration verification is performed using a dual-dye ratiometric photometry pipette calibration system.

iii. Pipette, benchtop automatic diluter: Pipette calibration verification is performed every 12 months for benchtop automatic diluters (e.g., Hamilton Microlab 625) onsite by a certified company.

iv. Balances and weight sets: Calibration verification of analytical balances are performed every 12 months on-site by a certified company. Calibration verifications of weight sets are performed every 12 months by shipping the weights to a certified company.

B. Parameter optimizations:

i. Regular parameter optimizations: ICP-MS optimizations performed after igniting the plasma each run day, periodically (ever 10-15 run days), monthly, and biannually are described in **Appendix F**.

ii. ORS cell gas flow verification: ORS cell gas flow rates can be verified by analyzing the ORS optimization solutions (see Section 6.M) as needed to ensure proper reduction of potential ICP-MS interferences (e.g., to troubleshoot performance).

C. Data backup:

The software files used to control the ICP-MS system and raw data files created during analysis by the ICP-MS control software are stored on the ICP-MS control computer. The instrument computer is backed up nightly to the laboratory (ISLE) network using SyncBack software on the instrument computer. The data from the ISLE is then backed up weekly to the CDC network

through a process setup by the Division's Laboratory Informatics Support team. The network backups are verified as part of planned function checks described in the periodic (10-15 run day) maintenance of Appedix F. Request Helpdesk support for data backup issues through the Laboratory Informatics Support Tool (LIST) in the Division of Laboratory Sciences (DLS) STARLIMS app. Occasionally electronic files need to be deleted from the hard drive (i.e., for computer replacement or upgrade, to free up hard drive space, etc.). Verify the files have been transferred to the ISLE before deleting. Record action in the instrument logbook.

11. Reporting thresholds

A. Reportable range:

Blood element concentrations are reportable in the range between the method LOD and the high calibrator times the maximum permitted extra dilution (see Table 10 of **Appendix C**). Above the high calibrator, extra dilutions are made of the blood sample to bring the observed concentration within the calibration range.

B. Reference ranges (normal values):

In this method the 95% reference ranges (see Table 12 of **Appendix C**) for these elements in blood fall within the range of the calibrators.

C. Action levels:

Report concentrations observed greater than the "second upper boundary" (defined in the laboratory database as the "2UB") to the QC reviewer as an "elevated result". The concentration assigned to the 2UB for an element is determined by study protocol but default concentrations are listed in Table 11 of **Appendix C**. The protocol for supervisors reporting elevated results to medical personnel is defined according to the study protocol. But typically,

i. Lead: CDC now uses a blood lead reference level of 3.5 µg/dL [13] to identify children with blood lead levels that are higher than most children's levels. This reference value is based on the 97.5th percentile of the National Health and Nutrition Examination Survey (NHANES)'s blood lead distribution in children. The current reference value is based on NHANES data from 2015-2016 and 2017-2018. Chelation treatment is recommended at blood lead levels ≥45 µg/dL [12]. The Occupational Safety and Health Administration regulations use a blood lead level of 40 µg/dL as cause for increased frequency of medical exams, and a blood lead level of 60 µg/dL as cause for medical removal from exposure[69]

ii. Cadmium: Levels of concern for cadmium in blood is >5 µg/L[70, 71]

iii. Mercury: The American Conference of Governmental Industrial Hygienists has a biological exposure index (BEI) of 15 µg/L for inorganic mercury in blood (end of shift at end of work week)[71].

iv. Manganese: Insufficient data to establish an action level.

v. Selenium: Greater than 500 µg/L selenium in whole blood may be associated with chronic toxicity[72, 73]

12. Method Calculations

A. Method limit of detection (LODs):

The method detection limits for elements in blood samples are defined as 3 times S0, where S0 is the estimate of the standard deviation at zero analyte concentration. S0 is taken as the y-intercept of a linear or 2nd order polynomial regression of standard deviation versus concentration (4 concentration levels of the analytes in blood each measured 60 times across at least a 2-month timeframe). Method LODs are re-evaluated periodically and are listed in Section 14.D of **Appendix A**).

B. Method limit of quantitation (LOQ):

The Division of Laboratory Sciences does not currently utilize limits of quantitation in regards to reporting limits[1].

C. QC Limits:

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 using the Division's SAS QC characterization program, available in STARLIMS. Records of QC limits calculations and resulting statistics are maintained in STARLIMS. Method performance is evaluated over time for potential shifts, trending, or changes in assay precision by evaluating the bench QC results. Quarterly statistics (mean, SD, CV) are calculated for each pool and compared to the characterization target values. As more QC data become available (covering multiple lots of reagents, multiple analysts, etc.), the initial QC limits can be reevaluated and updated. QC limits can also be reevaluated and updated as a result of a non-conforming event when the assay shows a higher than expected out of control rate and the root cause investigation does not reveal a correctable course of action to bring the assay back into control. This needs to be documented by a CAPA in STARLIMS.

13. Alternate methods for performing test and storing samples if test system fails

If the analytical system fails, the analysis may be setup on other ICP-MS instruments in the laboratory. If no other instrument is available, store the samples according to the storage requirements described in Section 5 until the analytical system can be restored to functionality.

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 approval of this procedure by the Branch Chief and CLIA Director denote that the method performance is fit for the intended use of the method.

15. Appendix A. Method performance documentation

A. Accuracy

i. Cadmium

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: Blood multi-element analysis by ICP-QQQ-MS
Method #: 3040
Matrix: Blood
Units: µg/L
Reference material: **NIST SRM 955c levels 2, 3, and 4**
Analyte: **cadmium**

Reference material	Replicate	Nominal value	Measured concentration					Mean	SD	CV (%)	Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5				
Material 1	1	2.14	2.14	2.18	2.14	2.07	2.08	2.13	0.05	2.29	-0.7
	2		2.19	2.16	2.15	2.10	2.05				
Material 2	1	5.20	5.23	5.14	5.20	5.08	5.00	5.16	0.09	1.73	-0.8
	2		5.32	5.17	5.18	5.17	5.09				
Material 3	1	9.85	10.3	10.3	10.3	10.1	10.1	10.2	0.20	1.90	4.0
	2		10.7	10.2	10.3	10.1	10.0				

ii. Lead

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: Blood multi-element analysis by ICP-QQQ-MS
Method #: 3040
Matrix: Blood
Units: µg/dL
Reference material: **NIST SRM 955c levels 2, 3, and 4**
Analyte: **lead**

Reference material	Replicate	Nominal value	Measured concentration					Mean	SD	CV (%)	Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5				
Material 1	1	14.0	13.5	13.7	13.4	13.3	13.0	13.4	0.26	1.92	-3.7
	2		13.7	13.7	13.5	13.5	13.0				
Material 2	1	27.8	27.1	27.2	26.6	26.8	25.8	26.8	0.50	1.85	-3.3
	2		27.3	27.4	26.6	27.1	26.3				
Material 3	1	45.5	44.9	48.1	46.6	46.5	45.2	46.3	1.07	2.31	1.7
	2		45.9	47.5	46.9	46.3	44.9				

iii. Manganese

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:	Blood multi-element analysis by ICP-QQQ-MS
Method #:	3040
Matrix:	Blood
Units:	µg/L
Reference material:	**NIST SRM 1401 L1, ClinChek I, Seronorm L3**
Analyte:	**manganese**

Reference material	Replicate	Nominal value	Measured concentration					Mean	SD	CV (%)	Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5				
Level 1	1	11.5	10.7	10.5	11.6	10.9	10.7	10.8	0.32	2.97	-6.3
	2		10.8	10.5	10.7	10.8	10.6				
Level 2	1	8.87	7.53	7.83	7.94	8.18	8.13	7.92	0.23	2.90	-10.7
	2		7.55	8.00	7.90	8.14	8.02				
Level 3	1	33.3	36.5	36.6	42.4	37.4	37.5	37.4	1.82	4.87	12.3
	2		36.5	36.7	36.0	37.4	37.0				

iv. Mercury

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:	Blood multi-element analysis by ICP-QQQ-MS
Method #:	3040
Matrix:	Blood
Units:	µg/L
Reference material:	**NIST SRM 955c levels 2, 3, and 4**
Analyte:	**mercury (total)**

Reference material	Replicate	Nominal value	Measured concentration					Mean	SD	CV (%)	Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5				
Level 1	1	4.95	5.38	5.30	5.25	5.33	5.26	5.32	0.05	0.95	7.6
	2		5.33	5.39	5.33	5.39	5.29				
Level 2	1	17.8	19.1	18.7	18.3	18.8	18.5	18.8	0.28	1.51	5.5
	2		19.1	18.9	18.6	19.1	18.8				
Level 3	1	33.9	35.5	35.2	34.6	35.0	34.8	35.0	0.42	1.20	3.3
	2		35.7	35.1	35.3	35.0	34.2				

v. Selenium

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:	Blood multi-element analysis by ICP-QQQ-MS
Method #:	3040
Matrix:	Blood
Units:	µg/L
Reference material:	**Clinchek L1, Clinchek L2, Seronorm L3**
Analyte:	**selenium**

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	75.3	77.0	77.7	77.6	76.5	72.2	76	2.20	2.89	0.8
	2		76.6	76.3	77.2	76.6	71.5				
Level 2	1	125	124	124	124	123	117	122.9	2.69	2.19	-1.7
	2		124	124	124	125	119				
Level 3	1	198	236	205	205	206	201	207	10.63	5.14	4.5
	2		203	203	205	206	197				

B. Precision

i. Cadmium

Precision

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

Method name:	Blood multi-element analysis by ICP-QQQ-MS
Method #:	3040
Matrix:	Blood
Units:	µg/L
Analyte:	**cadmium**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	2.96	2.91	2.93	0.00068	0.00068	17.21671
2	3.07	3.04	3.05	0.00013	0.00013	18.64772
3	3.09	2.97	3.03	0.00342	0.00342	18.36786
4	2.92	2.89	2.91	0.00036	0.00036	16.87805
5	3.03	3.14	3.08	0.00292	0.00292	19.02211
6	3.08	3.01	3.05	0.00116	0.00116	18.55623
7	3.00	2.87	2.93	0.00366	0.00366	17.22258
8	3.14	2.99	3.06	0.00563	0.00562	18.72720
9	3.02	2.93	2.97	0.00194	0.00194	17.66557
10	3.10	3.05	3.07	0.00058	0.00058	18.88666

Grand sum	60.185	**Grand mean**	3.00925

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.0409215	0.00409215	0.063969915	2.13
Between Run	0.07898625	0.00877625	0.048394731	1.61
Total	0.11990775		0.080213465	**2.67**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	86.4	86.2	86.3	0.0081	0.0081	14902.2848
2	90.1	88.7	89.4	0.511225	0.511225	15972.20645
3	86.4	84.3	85.3	1.113025	1.113025	14564.12445
4	85.5	83.7	84.6	0.801025	0.801025	14309.24445
5	89.7	89.6	89.7	0.0064	0.0064	16074.245
6	85.2	84.7	84.9	0.060025	0.060025	14417.71805
7	85.1	84.0	84.6	0.3364	0.3364	14300.7872
8	88.4	84.0	86.2	4.950625	4.950625	14852.26125
9	89.1	85.8	87.5	2.739025	2.739025	15303.75125
10	88.8	84.1	86.4	5.4289	5.4289	14936.8328

Grand sum	1729.58	**Grand mean**	86.479

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	31.9095	3.19095	1.786323039	2.07
Between Run	61.10688	6.789653333	1.341399145	1.55
Total	93.01638		2.233898312	**2.58**

ii. Lead

Precision

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

Method name:	Blood multi-element analysis by ICP-QQQ-MS
Method #:	3040
Matrix:	Blood
Units:	µg/dL
Analyte:	**lead**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	9.9	9.8	9.8	0.00090	0.00090	192.86480
2	9.4	9.3	9.3	0.00303	0.00302	173.91125
3	10.0	10.0	10.0	0.00002	0.00002	199.96000
4	10.0	10.0	10.0	0.00011	0.00011	198.58252
5	9.6	10.1	9.8	0.04709	0.04709	193.84805
6	9.9	9.9	9.9	0.00034	0.00034	195.80226
7	9.7	9.7	9.7	0.00011	0.00011	188.93736
8	10.2	10.2	10.2	0.00000	0.00000	209.18306
9	9.7	9.6	9.7	0.00397	0.00397	186.74714
10	10.1	10.4	10.3	0.01782	0.01782	210.59676

Grand sum 197.438 **Grand mean** 9.8719

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.14677	0.014677	0.121148669	1.23
Between Run	1.3450118	0.149445756	0.259585011	2.63
Total	1.4917818		0.286463571	**2.90**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	91.9	92.1	92.0	0.011025	0.011025	16922.48045
2	90.1	90.3	90.2	0.0064	0.0064	16279.2968
3	92.9	92.4	92.7	0.0576	0.0576	17171.7512
4	89.7	90.0	89.9	0.0169	0.0169	16153.2338
5	89.5	91.6	90.6	1.155625	1.155625	16400.41605
6	90.9	90.7	90.8	0.009025	0.009025	16501.99445
7	94.9	97.0	96.0	1.1025	1.1025	18420.4818
8	93.7	95.2	94.5	0.5329	0.5329	17845.3832
9	93.4	92.9	93.1	0.065025	0.065025	17340.80645
10	96.8	93.5	95.2	2.7556	2.7556	18107.045

Grand sum 1849.64 **Grand mean** 92.482

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	11.4252	1.14252	1.068887272	1.16
Between Run	84.48272	9.386968889	2.030326192	2.20
Total	95.90792		2.294503093	**2.48**

iii. Manganese

Precision

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

Method name:	Blood multi-element analysis by ICP-QQQ-MS
Method #:	3040
Matrix:	Blood
Units:	µg/L
Analyte:	**manganese**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	13.8	13.8	13.79	0.00003	0.00002	380.32820
2	14.5	13.5	13.99	0.23040	0.23040	391.32829
3	14.2	14.4	14.31	0.01277	0.01277	409.26605
4	14.5	14.3	14.35	0.01020	0.01020	411.95981
5	13.9	13.8	13.82	0.00270	0.00270	381.87425
6	14.1	14.0	14.05	0.00005	0.00005	394.74880
7	13.7	13.7	13.70	0.00014	0.00014	375.48961
8	14.2	14.0	14.08	0.01082	0.01082	396.38017
9	13.7	14.5	14.14	0.15920	0.15920	399.59645
10	14.1	13.9	14.02	0.00526	0.00526	393.20492

Grand sum 280.477 **Grand mean** 14.02385

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.8631305	0.08631305	0.293790827	2.09
Between Run	0.80917005	0.089907783	0.042395361	0.30
Total	1.67230055		0.296833988	**2.12**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	164	164	163.99	0.2116	0.2116	53785.4402
2	177	170	173.28	13.1044	13.1044	60051.9168
3	175	173	174.04	0.8464	0.8464	60579.8432
4	178	175	176.13	2.4964	2.4964	62043.5538
5	167	165	165.88	1.0816	1.0816	55032.3488
6	174	174	174.29	0.03258025	0.03258025	60750.87102
7	170	169	169.61	0.314721	0.314721	57533.0689
8	173	174	173.52	0.09891025	0.09891025	60215.25748
9	169	171	170.02	1.19793025	1.19793025	57813.94084
10	171	170	170.19	0.290521	0.290521	57927.91069

Grand sum 3421.873 **Grand mean** 171.09365

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	39.3501255	3.93501255	1.983686606	1.16
Between Run	273.4103211	30.37892456	3.636200765	2.13
Total	312.7604466		4.14209712	**2.42**

iv. Mercury

Precision

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

Method name:	Blood multi-element analysis by ICP-QQQ-MS
Method #:	3040
Matrix:	Blood
Units:	µg/L
Analyte:	**mercury (total)**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	5.35	5.52	5.43	0.00783	0.00783	59.04584
2	5.83	6.02	5.93	0.00865	0.00865	70.21125
3	6.14	6.52	6.33	0.03629	0.03629	80.02390
4	6.15	6.25	6.20	0.00250	0.00250	76.88000
5	6.13	6.36	6.24	0.01277	0.01277	77.97507
6	5.76	5.79	5.77	0.00011	0.00011	66.68970
7	5.78	5.82	5.80	0.00034	0.00034	67.29160
8	5.93	6.05	5.99	0.00391	0.00391	71.70031
9	5.67	6.09	5.88	0.04410	0.04410	69.14880
10	5.66	6.11	5.89	0.05018	0.05018	69.31354

Grand sum 118.915 **Grand mean** 5.94575

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.3333505	0.03333505	0.182578887	3.07
Between Run	1.24115725	0.137906361	0.228660568	3.85
Total	1.57450775		0.29261016	**4.92**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	80.7	81.9	81.3	0.4096	0.4096	13216.1282
2	88.5	91.6	90.0	2.4025	2.4025	16214.4032
3	97.2	101.6	99.4	4.84	4.84	19744.8192
4	89.7	92.7	91.2	2.265025	2.265025	16640.35245
5	91.9	97.0	94.5	6.375625	6.375625	17843.49405
6	92.2	92.4	92.3	0.009025	0.009025	17021.97005
7	90.9	89.9	90.4	0.275625	0.275625	16335.28125
8	89.8	92.2	91.0	1.44	1.44	16554.7208
9	89.3	93.5	91.4	4.3681	4.3681	16700.6088
10	89.8	92.0	90.9	1.155625	1.155625	16527.43805

Grand sum 1824.51 **Grand mean** 91.2255

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	47.08225	4.708225	2.169844464	2.38
Between Run	357.379045	39.70878278	4.183333466	4.59
Total	404.461295		4.712589934	**5.17**

v. Selenium

Precision

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

Method name:	Blood multi-element analysis by ICP-QQQ-MS
Method #:	3040
Matrix:	Blood
Units:	µg/L
Analyte:	**selenium**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	242	243	243	0.11022	0.11022	117838.61858
2	245	246	245	0.54538	0.54538	120473.24238
3	254	252	253	1.03836	1.03836	127540.78157
4	233	235	234	0.69639	0.69639	109413.27426
5	255	254	255	0.36180	0.36180	129680.51281
6	254	253	253	0.26214	0.26214	128107.07149
7	251	234	243	68.34329	68.34329	117753.19205
8	251	245	248	9.11436	9.11436	122717.51570
9	247	236	242	29.67526	29.67526	116695.22051
10	237	235	236	0.87236	0.87236	111658.36705

Grand sum 4900.998 **Grand mean** 245.0499

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	222.039132	22.2039132	4.712102843	1.92
Between Run	888.7265998	98.74739998	6.186416037	2.52
Total	1110.765732		7.776609582	**3.17**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1107	1096	1101	32.3761	32.3761	2426031.754
2	1098	1081	1090	70.7281	70.7281	2374935.768
3	1091	1078	1084	45.9684	45.9684	2351933.473
4	1042	1039	1041	1.221025	1.221025	2165592.661
5	1116	1109	1112	9.954025	9.954025	2474711.786
6	1063	1051	1057	35.5216	35.5216	2234117.496
7	1089	1054	1071	300.6756	300.6756	2295367.38
8	1078	1043	1060	309.4081	309.4081	2247666.424
9	1129	1085	1107	495.5076	495.5076	2450101.025
10	1013	996	1004	74.046025	74.046025	2017257.066

Grand sum 21455.77 **Grand mean** 1072.7885

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	2750.81315	275.081315	16.58557551	1.55
Between Run	20211.51911	2245.724345	31.38983139	2.93
Total	22962.33226		35.50215247	**3.31**

C. Stability

i. Cadmium

Stability

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

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Frozen three times at ≤ -70°C and then thawed (3 freeze-thaw cycles).
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Blood samples, not prepared for sample analysis, stored at room temperature for 1 day.
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: A set of processed samples, ready for analysis, including blanks, calibration, and QC materials, were stored at room temperature for 1 day
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: QM1 is a blood pool stored at ≤ -70°C for 10 years; QM2 is a blood pool stored at ≤ -70°C for 6 months

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

Method name:	Blood multi-element analysis by ICP-QQQ-MS
Method #:	3040
Matrix:	Blood
Units:	µg/L
Analyte:	**cadmium**

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	3.01	2.86	3.06	3.17	3.12	3.13	2.91	3.14
Replicate 2	2.97	2.88	3.04	3.12	3.12	3.16	2.81	3.02
Replicate 3	2.92	2.89	2.96	3.12	3.02	3.16	3.19	3.10
Mean	2.97	2.88	3.02	3.14	3.08	3.15	2.97	3.08
% difference from initial measurement	--	**-2.9**	--	**3.9**	--	**2.1**	--	**3.9**

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	85.8	84.0	85.2	89.6	87.6	87.6	89.9	88.4
Replicate 2	84.8	84.0	86.8	89.2	87.7	87.7	94.5	89.1
Replicate 3	85.5	83.3	85.9	89.6	86.6	87.3	88.5	88.8
Mean	85.3	83.8	86.0	89.5	87.3	87.5	91.0	88.8
% difference from initial measurement	--	**-1.8**	--	**4.1**	--	**0.3**	--	**-2.4**

ii. Lead

Stability

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

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Frozen three times at ≤ -70°C and then thawed (3 freeze-thaw cycles).
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Blood samples, not prepared for sample analysis, stored at room temperature for 1 day.
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: A set of processed samples, ready for analysis, including blanks, calibration, and QC materials, were stored at room temperature for 1 day
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: QM1 is a blood pool stored at ≤ -70°C for 10 years; QM2 is a blood pool stored at ≤ -70°C for 6 months

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

Method name:	Blood multi-element analysis by ICP-QQQ-MS
Method #:	3040
Matrix:	Blood
Units:	µg/dL
Analyte:	**lead**

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	10.1	9.9	10.1	10.0	10.1	10.4	10.2	10.2
Replicate 2	10.1	10.0	10.1	9.9	10.1	10.4	10.1	9.7
Replicate 3	10.0	10.0	10.0	9.9	10.0	10.4	10.0	10.1
Mean	10.0	10.0	10.0	9.9	10.0	10.4	10.1	10.0
% difference from initial measurement	--	**-0.9**	--	**-1.3**	--	**3.5**	--	**-0.8**

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	91.6	90.9	91.6	90.6	91.6	91.4	87.1	93.7
Replicate 2	92.1	92.3	92.1	90.6	92.1	92.1	86.5	93.4
Replicate 3	90.5	90.6	90.5	90.4	90.5	91.9	85.5	96.8
Mean	91.4	91.3	91.4	90.5	91.4	91.8	86.4	94.6
% difference from initial measurement	--	**-0.1**	--	**-0.9**	--	**0.5**	--	**9.5**

iii. Manganese

Stability

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

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Frozen three times at ≤ -70°C and then thawed (3 freeze-thaw cycles).
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Blood samples, not prepared for sample analysis, stored at room temperature for 1 day.
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: A set of processed samples, ready for analysis, including blanks, calibration, and QC materials, were stored at room temperature for 1 da
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: QM1 is a blood pool stored at ≤ -70°C for 10 years; QM2 is a blood pool stored at ≤ -70°C for 6 months

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

Method name:	Blood multi-element analysis by ICP-QQQ-MS
Method #:	3040
Matrix:	Blood
Units:	µg/L
Analyte:	**manganese**

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	14.5	14.1	15.0	14.3	14.3	14.5	14.4	13.8
Replicate 2	14.7	14.9	15.0	14.0	14.3	14.7	13.5	13.3
Replicate 3	14.3	14.3	14.3	14.1	14.0	14.5	14.6	13.7
Mean	14.5	14.4	14.8	14.1	14.2	14.6	14.2	13.6
% difference from initial measurement	--	**-0.4**	--	**-4.3**	--	**2.7**	--	**-3.9**

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	173	173	173	172	171	173	165	168
Replicate 2	173	174	175	172	171	174	167	164
Replicate 3	173	172	175	173	170	173	167	166
Mean	173	173	174	172	171	174	166	166
% difference from initial measurement	--	**0.2**	--	**-1.1**	--	**1.6**	--	**-0.2**

iv. Mercury

Stability

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

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Frozen three times at ≤ -70°C and then thawed (3 freeze-thaw cycles).
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Blood samples, not prepared for sample analysis, stored at room temperature for 1 day.
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: A set of processed samples, including blanks, calibration, and QC materials, were stored at room temperature for 1 day
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: QM1 is a blood pool stored at ≤ -70°C for 10 years; QM2 is a blood pool stored at ≤ -70°C for 6 months

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

Method name:	Blood multi-element analysis by ICP-QQQ-MS
Method #:	3040
Matrix:	Blood
Units:	µg/L
Analyte:	**mercury (total)**

Quality material 1								
	Initial measurement	**Four freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	6.0	5.8	6.0	5.8	6.0	5.9	5.8	5.9
Replicate 2	6.1	5.7	5.8	6.0	6.1	5.9	6.1	5.7
Replicate 3	6.0	5.8	6.0	5.9	6.0	5.8	5.5	5.7
Mean	6.0	5.8	5.9	5.9	6.0	5.9	5.8	5.8
% difference from initial measurement	--	**-4.4**	--	**-0.9**	--	**-3.0**	--	**-0.7**

Quality material 2								
	Initial measurement	**Four freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	92.3	91.5	91.7	88.6	92.3	89.6	85.9	89.8
Replicate 2	92.7	90.6	90.5	89.3	92.7	89.8	84.4	89.3
Replicate 3	90.9	90.2	91.4	89.2	90.9	89.2	81.5	89.8
Mean	92.0	90.8	91.2	89.0	92.0	89.5	83.9	89.6
% difference from initial measurement	--	**-1.3**	--	**-2.4**	--	**-2.6**	--	**6.8**

v. Selenium

Stability

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

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Frozen three times at ≤ -70°C and then thawed (3 freeze-thaw cycles).
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Blood samples, not prepared for sample analysis, stored at room temperature for 1 day.
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: A set of processed samples, ready for analysis, including blanks, calibration, and QC materials, were stored at room temperature for 1 day
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: QM1 is a blood pool stored at ≤ -70°C for 10 years; QM2 is a blood pool stored at ≤ -70°C for 6 months

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

Method name:	Blood multi-element analysis by ICP-QQQ-MS
Method #:	3040
Matrix:	Blood
Units:	µg/L
Analyte:	**selenium**

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	250	236	252	251	251	248	246	251
Replicate 2	242	233	252	246	256	249	250	247
Replicate 3	240	234	243	248	248	247	251	237
Mean	244	234	249	248	252	248	249	245
% difference from initial measurement	--	**-3.9**	--	**-0.2**	--	**-1.4**	--	**-1.7**

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	1065	1048	1066	1079	1101	1069	1061	1078
Replicate 2	1064	1048	1069	1077	1104	1065	1055	1129
Replicate 3	1086	1026	1073	1082	1088	1071	1075	1013
Mean	1072	1041	1069	1079	1098	1068	1064	1073
% difference from initial measurement	--	**-2.9**	--	**0.9**	--	**-2.7**	--	**0.9**

D. Analytical Sensitivity and Specificity

LOD, specificity and fit for intended use

Method name:	Blood multi-element analysis by ICP-QQQ-MS
Method #:	3040
Matrix:	Blood
Units:	µg/L (µg/dL for BPB)

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
cadmium (BCD)	0.065	yes	yes
lead (BPB)	0.049	yes	yes
manganese (BMN)	0.52	yes	yes
mercury (THG)	0.17	yes	yes
selenium (BSE)	9.9	yes	yes

16. Appendix B. Ruggedness testing results

A. Ruggedness Parameter #1: Oxygen gas flow rate

This test evaluated the significance of the ORS gas flow rate, oxygen, on method accuracy. The typical oxygen gas flow rate is 50%.

i. Test Details:

1) Prepared three sets of dilutions in three separate sets of tubes (calibrators, blanks, reference materials, dummy samples) for analysis.

2) Analyzed each set of dilutions in separate runs on the same day, when possible, using the same instrument.

3) Changed the gas flow rate by 20% of the method default for each cell gas.

a) Run 1: method default O_2 = 50%
b) Run 2: decreased cell gas flow rates by 20% of method default O_2 = 40%
c) Run 3: increased cell gas flow rate by 20% of the method default O_2 = 60%

ii. Results: See Ruggedness Table 1

Conclusion: Accuracy of Pb, Cd, Hg, Mn and Se results are not compromised by changes in cell gas flow rate within the range tested (40% - 60%).

Ruggedness Table 1. Measured concentrations of Mn, Se, Cd, Hg, and Pb in NIST SRM or CRM materials at low, normal, and high oxygen flow rates

Analyte	Sample ID	Typical range*	Certificate 95% Conf. int.	Cell gas flow rate (% of maximum 100%)		
				Low (40%)	Normal (50%)	High (60%)
		Concentrations in µg/L, Pb in µg/dL (% difference relative to target value)				
Mn	NIST SRM 1401 L1	10.3 – 11.6	10.37 – 12.65	11.6 (+0.8%)	12.3 (+6.8%)	12 (+4.3%)
Se	Seronorm Level 3	197 – 236	158 – 238	224 (+13%)	215 (+8.6%)	219 (+11%)
Cd	NIST SRM 995C L2	2.05 – 2.19	1.9 – 2.38	2.10 (-1.9%)	2.16 (0.9%)	2.00 (-6.5%)
Hg	NIST SRM 995C L2	5.25 – 5.39	4.19 – 5.71	5.51 (+11.3%)	5.57 (+12.5%)	5.12 (+3.4%)
Pb	NIST SRM 995C L2	13.0 – 13.7	13.87 – 14.03	13.8 (-1.1%)	13.7 (-1.8%)	13.3 (-4.7%)
*typical range is the range of concentrations (N=10) measured in our lab during the PPM Accuracy experiments						

B. Ruggedness Parameter #2: Deflect lens voltage

This test evaluated the significance of the deflect lens voltage on method accuracy. The typical deflect lens voltage is 2V.

i. Test Details:

1) Prepared three sets of dilutions in three separate sets of tubes (calibrators, blanks, reference materials, dummy samples) for analysis.

2) Analyzed each set of dilutions in separate runs on the same day, if possible, using the same instrument.

3) Changed the deflect lens voltage by 20% of the method default for each run.

a) Run 1: method default deflect = 2V
b) Run 2: decreased deflect lens voltage by 20% of method default = 1.6V
c) Run 3: increased deflect lens voltage by 20% of the method default = 2.4V

ii. Results: See Ruggedness Table 2

Conclusion: Accuracy of Pb, Cd, Hg, Mn and Se results are not compromised by changes in deflect lens voltage within the range tested (1.6V – 2.4V).

Ruggedness Table 2. Measured concentrations of Mn, Se, Cd, Hg, and Pb in NIST SRM or CRM materials at low, normal, and high deflect lens voltages

Analyte	Sample ID	Typical range*	Certificate 95% Conf. int.	Deflect voltage		
				Low (1.8 V)	Normal (2.0 V)	High (2.2 V)
		Concentrations in µg/L, Pb in µg/dL				
Mn	NIST SRM 1401 L1	10.3 – 11.6	10.37 – 12.65	12.2	10.5	11.8
Se	Seronorm Level 3	197 – 236	158 – 238	201	213	206
Cd	NIST SRM 995c L2	2.05 – 2.19	1.9 – 2.38	2.34	2.04	2.26
Hg	NIST SRM 995c L2	5.25 – 5.39	4.19 – 5.71	5.37	5.4	5.29
Pb	NIST SRM 995c L2	13.0 – 13.7	13.87 – 14.03	14.9	13.3	14.4
*typical range is the range of concentrations (N=10) measured in our lab during the PPM Accuracy experiments						

C. Ruggedness parameter #3: APDC concentration

This test evaluated the significance of the ammonium pyrrolidinedithiocarbamate (APDC) concentration in diluent on method accuracy. The typical concentration is 0.01%.

i. Test Details:

1) Prepared three sets of dilutions in three separate sets of tubes (calibrators, blanks, reference materials, dummy samples) for analysis.

a) One set was prepared with diluent containing 0.008% APDC (low)

b) One set was prepared with diluent containing 0.012% APDC (high)

c) One set was prepared with diluent containing 0.01% APDC (normal)

2) Analyzed each set of dilutions in separate runs on the same day (if possible) using the same instrument.

3) Analyzed the three sets of dilutions

a) Run 1: normal APDC concentration

b) Run 2: low APDC concentration

c) Run 3: high APDC concentration

ii. Results: See Ruggedness Table 3

Conclusion: Accuracy of Pb, Cd, Hg, Mn and Se results are not compromised by changes in APDC concentration within the range tested (0.008% - 0.012%).

Ruggedness Table 3. Measured concentrations of Mn, Se, Cd, Hg, and Pb in CTQ PT sample QM-B-Q1721 low, normal, and high APDC concentrations;

Sample ID	Diluent APDC concentration		Mn (µg/L)	Se (µg/L)	Cd (µg/L)	Hg (µg/L)	Pb (µg/dL)
QM-B-Q1721	CTQ target value (range)		37.4 29.2 – 45.7	254 201 – 308	9.79 8.16 – 11.5	3.19 2.27 – 4.11	35.0 30.5 – 39.6
	CDC DLS 3040 results	Reduced	36.2*	281	10.1	3.12	36.2
		Normal (2018-1127)	-	280	10.2	3.24	36.4
		Normal (2018-1128)	35.7	262	10.1	3.23	36.5
		Increased	36.9	251	10.2	3.11	37.2

*BMN result for reduced APDC concentration diluent was taken from the run passing QC on 2018-1128

D. Ruggedness parameter test #4: TMAH concentration in the diluent

This test evaluated the significance of the tetramethylammonium hydroxide (TMAH) concentration in diluent on method accuracy. The typical concentration is 1.0%.

i. Test Details:

1) Prepared three sets of dilutions in three separate sets of tubes (calibrators, blanks, reference materials, dummy samples) for analysis.

a) One set was prepared with diluent containing 0.8% TMAH (low)

b) One set was prepared with diluent containing 1.2% TMAH (high)

c) One set was prepared with diluent containing 1.0% TMAH (normal)

2) Analyzed each set of dilutions in separate runs on the same day (if possible) using the same instrument.

3) Analyzed the three sets of dilutions

a) Run 1: normal TMAH concentration

b) Run 2: low TMAH concentration

c) Run 3: high TMAH concentration

ii. Results: See Ruggedness Table 4.

Conclusion: Accuracy of Pb, Cd, Hg, Mn and Se results are not compromised by changes in TMAH concentration within the range tested (0.8% - 1.2%).

Ruggedness Table 4. Measured concentrations of Mn, Se, Cd, Hg, and Pb in CTQ PT sample QM-B-Q1721 low, normal, and high TMAH concentrations

Sample ID	Cell gas flow rate		Mn (µg/L)	Se (µg/L)	Cd (µg/L)	Hg (µg/L)	Pb (µg/dL)
QM-B-Q1721	Target value Target range		37.4 29.2 – 45.7	254 201 - 308	9.79 8.16 – 11.5	3.19 2.27 – 4.11	35.0 30.5 – 39.6
	DLS 3040 results	Reduced (2018-1016)	35.5	233	10.1	2.77	35.7
		Normal (2018-1016)	37.8	301	9.84	3.06	37.4
		Normal (2018-1120)	36.1	233	10.2	3.14	36.9
		Increased (2018-1120)	36.1	242	10.2	3.12	36.2

E. Ruggedness parameter test #5: Extra sample dilutions

Evaluate the impact on observed concentration if an extra dilution is performed on the sample relative to the calibration standards.

i. Test details: Several blood samples (e.g., PTs, NIST SRMs) were prepared for analysis at various extra dilution levels and the observed results compared to results obtained with no extra dilution performed.

ii. Results: See Ruggedness Table 5.

iii. Conclusions: Results show that all analytes of the method (Pb, Cd, Hg, Mn, and Se) can be analyzed at up to a 2x extra dilution without significant effect (> ± 10% error) to the observed concentration.

Ruggedness Table 5. Average and 1SD of the normalized extra dilution results for Mn, Se, Cd, Hg, and Pb

	Mn	Se	Cd	Hg	Pb
No dilution	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
2x	0.98 ± 0.02	0.99 ± 0.01	1.01 ± 0.02	1.04 ± 0.02	0.97 ± 0.04
5x	0.98 ± 0.02	1.01 ± 0.04	1.03 ± 0.01	1.05 ± 0.05	0.96 ± 0.02
10x	0.97 ± 0.04	0.97 ± 0.02	1.04 ± 0.02	1.05 ± 0.06	0.96 ± 0.04
Highest calibrator concentration (S7)	400 µg/L	2500 µg/L	200 µg/L	200 µg/L	200 µg/dL

17. Appendix C. Tables and Figures

Table 1. Acquisition Method

Acquisition parameters: (see Figure 1 of Appendix C)			
Acquisition Mode	Spectrum		
Q2 Peak Pattern	1 point		
Replicates	3		
Sweeps/Replicated	50		
Tune Mode	O2 and H2 MS_MS mode		
Quick Scan	No (unselected)		
Stabilization Time [sec]	20		
Scan Type	MS/MS		
Element Name	Q1->Q2		Integ Time/Mass [sec]
Mn	55->55		1.0
Se	80->96		1.0
Rh	103->103		0.5
Cd	111->111		5.0
Te	130->130		0.5
Ir	193->193		0.5
Hg	200->200		5.0
Hg	202->202		5.0
Pb	206->206		1.0
Pb	207->207		1.0
Pb	208->208		1.0
Correction Equations (See Figure 2 of Appendix C)			
202Hg=(202Hg*1)+(200Hg*1)			
208Pb=(206Pb*1)+(207Pb*1)+(208Pb*1)			
PeriPump/ISIS: (see Figure 3 of Appendix C)			
	Time [sec]	Speed [rps] Nebulizer Pump	Vial#
Pre-run			
Sample Uptake	3	0.5	Sample
Stabilize	90	Tune Parameter	Sample
Acquisition			
Speed		Tune Parameter	Sample
Post-run			
Probe Rinse (Sample)	60	0.5	Rinse Port
Probe Rinse (Std)	60		Rinse Port
Intelligent Rinse = OFF		Preemptive Rinse = OFF	
Tune: (see Figure 4 of Appendix C)			
Scan Type	MS/MS		
RF power	1550 W		
Sample Depth	8.0 mm		
Option Gas	0.0 %		

Nebulizer Pump	0.25
S/C Temp	2 °C
Makeup/Dilution Gas	0.00 mL/min
Use Gas	checked
H2 flow	1.0 mL/min
4th Gas Flow (O_2)	50%
Axial Acceleration	1.5 V
Energy Discrimination	-7.0 V
Wait Time Offset	2
The remaining Tune parameters may optimized by Service Engineers and advanced users	

Table 2. Data Analysis Method

Basic Information: (see Figure 5 of Appendix C)						
FullQuant Analysis		checked				
Analysis Mode		Spectrum				
Bkg Subtraction if Exists		Ratio to ISTC Subtraction				
Interference Correction		Acq. Defined				
Sample Template		CDC_IRAT_quant report.analysis.acrt				
Analyte: (see Figure 6 of Appendix C)						
Tune Mode	ScanType	Transition	Q1	Name	Q2	Analyte/ISTD
1:O2 and H2 MS_MS mode	MS/MS	55 -> 55	55	Mn	55	Analyte
1: O2 and H2 MS_MS mode	MS/MS	80-> 96	80	Se	96	Analyte
1: O2 and H2 MS_MS mode	MS/MS	103 -> 103	103	Rh	103	ISTD
1: O2 and H2 MS_MS mode	MS/MS	111 -> 111	111	Cd	111	Analyte
1: O2 and H2 MS_MS mode	MS/MS	130 -> 130	130	Te	130	ISTD
1: O2 and H2 MS_MS mode	MS/MS	193 -> 193	193	Ir	193	ISTD
1: O2 and H2 MS_MS mode	MS/MS	200 -> 200	200	Hg	200	Analyte
1: O2 and H2 MS_MS mode	MS/MS	202 -> 202	202	Hg	202	Analyte
1: O2 and H2 MS_MS mode	MS/MS	206 -> 206	206	Pb	206	Analyte
1: O2 and H2 MS_MS mode	MS/MS	207 -> 207	207	Pb	207	Analyte
1: O2 and H2 MS_MS mode	MS/MS	208 -> 208	208	Pb	208	Analyte
Full Quant: (see Figure 7 of Appendix C)						
Calibration Method	External Calibration					
Weighting	checked					
Curve Fit	Linear					
Origin	Ignore					
Weight	1/x^2					
ISTD	Mn: 103 -> 103 Se: 130 -> 130 Cd: 193 -> 193 Hg: 130 -> 130 Pb: 193 -> 193					
Min Conc	<None>					

Units	Mn, Se, Cd, Hg: µg/L Pb: µg/dL						
Level	Level 1*	Level 2**	Level 3	Level 4	Level 5	Level 6	Level 7
Mn		0.48	1.6	6	60	200	400
Se			10.0	37.5	375	1250	2500
Cd	0.06	0.24	0.8	3	30	100	200
Hg		0.24	0.8	3	30	100	200
Pb	0.06	0.24	0.8	3	30	100	200
*no value is listed in level 1 for Mn, Se, or Hg **no value is listed in level 2 for Se							

Table 3. Suggested concentrations for base blood

Analyte	suggested concentration
Mn	< 8 µg/L
Se	<200 µg/L
Cd	<0.5 µg/L
Hg	<0.5 µg/L
Pb	<2 µg/dL

Table 4. Stock calibration standard concentrations.

Analyte	Stock calibration concentration (mg/L) High Purity Standards Item # SM-2107-057 10% v/v HCl
Mn	40
Se	250
Cd	20
Hg	20
Pb	200

Table 5. Preparation of intermediate stock calibration standards A and B.

	Int. stock std. A	Int. stock std.B
volume of flask (mL)	100	100
spike volume of stock standard solution (mL)	1	0.3
Analyte	**concentrations (**mg **/**L**)**	
Mn	0.4	0.12
Se	2.5	0.75
Cd	0.2	0.06
Hg	0.2	0.06
Pb	2	0.6

Table 6. Preparation of intermediate working calibration standards

Standard #	1	2	3	4	5	6	7
volume of flask (mL)	100	100	100	100	100	100	100
volume spike of stock std. (mL)					0.15	0.5	1.0
volume spike of int. stock std. A (mL)		0.12	0.4	1.5			
volume spike of int. stock Std. B (mL)	0.1						
Analyte	**Concentrations ***						
Mn (µg /L)	0.12**	0.48	1.6	6.0	60.0	200	400
Se (µg /L)	0.75**	3.0**	10	37.5	375	1250	2500
Cd (µg /L)	0.06	0.24	0.8	3.0	30.0	100	200
Hg (µg /L)	0.06**	0.24	0.8	3.0	30.0	100	200
Pb (µg /dL)	0.06	0.24	0.8	3.0	30.0	100	200

* These same concentrations, except for Mn, Se, and Hg in standard 1, and Se in standard 2 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 9 of Appendix C).

**Working calibrator 1 is not used for Mn, Se, and Hg, and working calibrator 2 is not used for Se, so no concentration is entered for these into the ICP-MS software.

Table 7. Acceptable ways to perform two consecutive analytical runs, bracketing with bench quality control samples.

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

Table 8. A typical SAMPLE/BATCH window.

Sample Type	Sample Name	Comment	Vial#*	File Name	Replicates	Level	Total Dil
Sample	Stability 1		1				
Sample	Stability 2		1				
Sample	Stability …		1				
Sample	Stability 19		1				
Sample	Stability 20		1				
Sample	Blood blank		1101				
CalBlk	3040 STD00		1102				
CalStd	3040 STD01		1103			Level 1	
CalStd	3040 STD02		1104			Level 2	
CalStd	3040 STD03		1105			Level 3	
CalStd	3040 STD04		1106			Level 4	
CalStd	3040 STD05		1107			Level 5	
CalStd	3040 STD06		1108			Level 6	
CalStd	3040 STD07		1108			Level 7	
Sample	3040 BldBlkChk Wash1		1109				
Sample	3040 BldBlkChk Wash2		1109				
Sample	3040 BldBlkChk1		1110				
Sample	3040 BldBlkChk2		1110				
Sample	Aq blank		1111				
FQBlk	3040 AQBLK		1111				
Sample	Aq blank		1112				
Sample	Low bench QC		1201				
Sample	High bench QC		1202				
Sample	Elevated bench QC		1203				
Sample	Unknown 1		1204				
Sample	Unknown 2		1205				
Sample	Unknown 3		1206				
Sample	Unknown 4		1207				
Sample	Unknown 5		1208				
Sample	Low bench QC		1209				
Sample	High bench QC		1210				
Sample	Elevated bench QC		1211				
Sample	H2O		8				
Sample	H2O		8				
Sample	H2O		8				
*Vial # refers to the autosampler tube position. The exact position does not need to be those shown above. QC samples do not have to be run in the order of low, then high, then elevated.							

Table 9. Preparation of samples, working calibrators, and QC materials for analysis.

These directions are written with the expectation of a 1,000 µL syringe on the left side and a 100 µL syringe on the right side of the benchtop automatic pipette. If a different total volume is prepared, adjust the volumes for each component proportionally.

Description	Water (µL)	Base Blood (µL)	AQ Intermediate Working Calibration Standard (µL)	Patient or QC blood sample (µL)	Diluent (µL)*	Total volume (µL)
Working Calibrators (S0-S7) and Bldblkchk (S0)	-	50 x 1	50 x 1 **	-	900 (450 x 2)	1,000
AQ Blank	100 x 1	-	-	-	900 (450 x 2)	1,000
Patient blood or Blood-based QC	50 x 1	-	-	50 x 1	900 (450 x 2)	1,000
Patient Blood *2x Extra Dilution* [H]	150 (50 x 3)	-	-	50 x 1	1,800 (450 x 4)	2,000
Patient Blood *3x Extra Dilution* [H]	250 (50 x 5)			50 x 1	2,700 (450 x 6)	3,000

* By splitting the dispense step of diluent into two or more portions, liquids pulled up into the right pipette tip are flushed out more completely. For example, when preparing a working calibrator, do the preparation in two steps: in step 1, dispense 450 µL diluent + 50 µL Int. working cal. standard; in step 2, dispense 450 µL diluent + 50 µL base blood to prepare a 1.0 mL total volume dilution.

** The base blood and diluent can be added with the automatic dilutor, but the intermediate working calibrators shall be added by handheld pipette, using pipette tips that are pre-rinsed at least three times with 3% (v/v) HCl (i.e. S0). The intermediate working calibrator solutions cannot come in contact with the diluent. Contact between the diluent and the intermediate working calibrators leads to loss of selenium.

[H] Extra dilution is performed on blood samples whose concentration is greater than the concentration of the highest calibrator listed in Table 10 of Appendix C. Any extra dilution within these limits can be prepared as long as the 4.5:5 ratio of diluent to total dilution volume is maintained. Use of the lowest possible dilution level is preferred to minimize differences between the calibrators and the samples.

Table 10. Limit of Detection, highest calibrator concentration, maximum allowable extra dilutions, and upper reportable range

Analyte	Limit of Detection (LOD)*	High Calibrator	Maximum Extra Dilution**	Reportable Range Upper Boundary
Mn (µg/L)	0.52	400	3x	1200
Se (µg/L)	9.9	2500	3x	7500
Cd (µg/L)	0.065	200	3x	600
Hg (µg/L)	0.17	200	3x	600
Pb (µg/dL)	0.049	200	3x	600

*Re-evaluated periodically (2+ years) or at significant method changes. LODs were calculated 2/21/2019.
**See ruggedness test 6 in Appendix B for supporting validation data.

Table 11. Boundary concentrations for whole blood concentrations.

Analyte (units)	1st upper boundary ("1UB") *	2nd upper boundary ("2UB") **	Range maximum ("Lim Rep Delta") †	Highest Concentration Validated for Washout
Mn (µg/L)	20	35	2.0 for values <20 10% of value at ≥20	400
Se (µg/L)	400	400	20 for values <200 10% of value at ≥200	2500
Cd (µg/L)	5.0	5.0	1.0 for values <10 10% of value at ≥10	200
Hg (µg/L)	10.0	10.0	1.0 for values < 10 10% of value at ≥10	200
Pb (µg/dL)	3.5	3.5	1.0 for values < 10 10% of value at ≥10	200

* Typically, the 1st upper boundary (1UB) is the 99th percentile of non-weighted concentration results from NHANES subset groups; a concentration significant to public health; or a concentration defined by study protocol. The default 1UB concentrations are listed in this table.
** The 2nd upper boundary (2UB) may be 2x the 1UB; a concentration significant to public health; or defined by study protocol.
† Range maximum is the range of the three replicate readings for a single sample analysis also called the "Lim Rep Delta" or "Rep Delta" in the division LIMS.

Table 12. Reference ranges for blood concentrations [74].

analyte (units)	survey years	geometric mean	50th	75th	90th	95th	N
Mn (µg/L)	11-12	9.35	9.22	11.5	14.4	16.7	7920
	13-14	9.52	9.41	11.8	14.6	16.7	5215
	15-16	9.59	9.52	12.0	14.8	16.4	4987
Se (µg/L)	11-12	190	190	206	223	236	7920
	13-14	193	193	208	223	235	5215
	15-16	191	191	206	223	233	4987
Cd (µg/L)	07-08	0.315	0.270	0.500	1.00	1.52	8266
	09-10	0.302	0.260	0.480	0.960	1.40	8793
	11-12	0.279	0.250	0.460	0.960	1.50	7920
	13-14	0.235	0.210	0.410	0.840	1.22	5215
	15-16	0.238	0.220	0.400	0.810	1.22	4988
Hg (µg/L)	07-08	0.769	0.740	1.48	2.95	4.64	8266
	09-10	0.863	0.790	1.68	3.43	5.13	8793
	11-12	0.703	0.640	1.38	2.87	4.40	7920
	13-14	0.683	0.620	1.29	2.65	4.36	5215
	15-16	0.678	0.600	1.26	2.55	4.25	4988
Pb (µg/dL)	07-08	1.27	1.22	1.90	2.80	3.70	8266
	09-10	1.12	1.07	1.70	2.58	3.34	8793
	11-12	0.973	0.930	1.52	2.38	3.16	7920
	13-14	0.858	0.830	1.32	2.10	2.81	5215
	15-16	0.820	0.780	1.32	2.14	2.75	4988

Figure 1. Batch Acquisition Method Acquisition Parameters (Stabilization time optimized per instrument. Elements to monitor are customizable.)

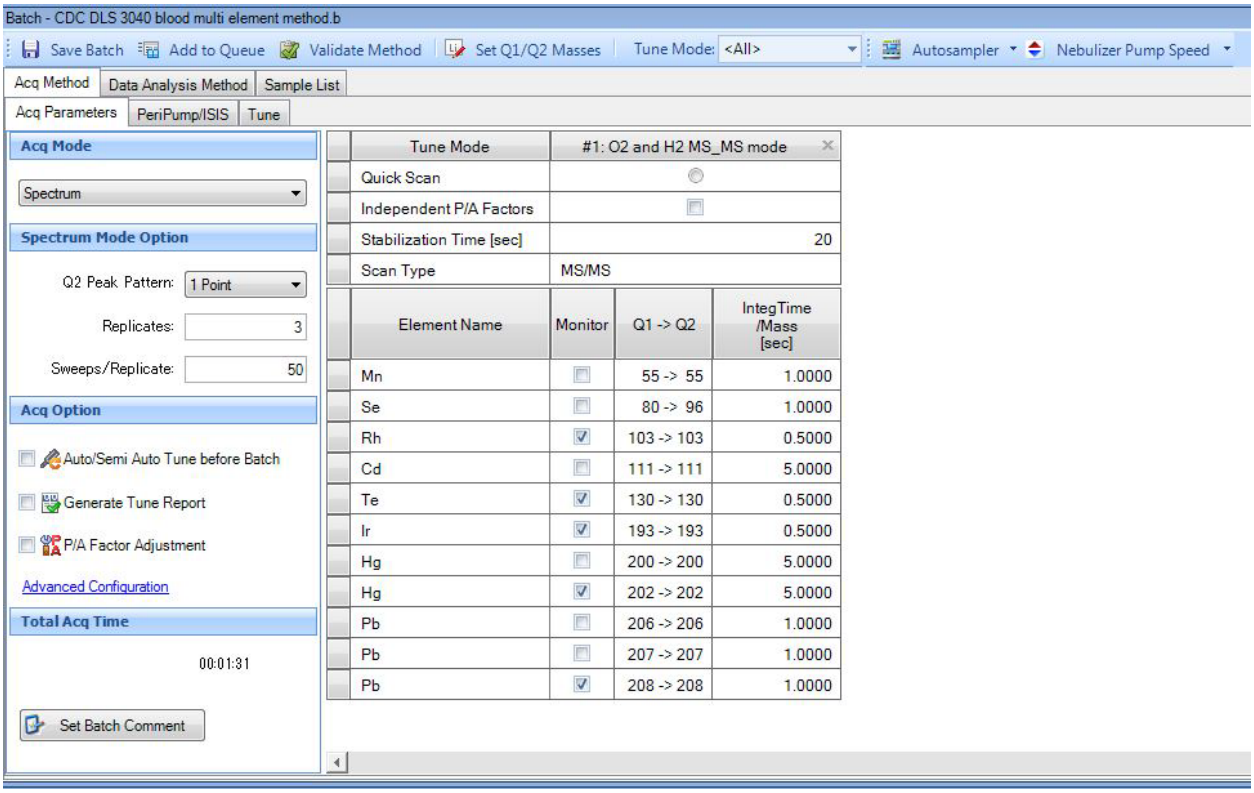


Figure 2. Batch Acquisition Method Set Q1/Q2 Masses and Correction Equations

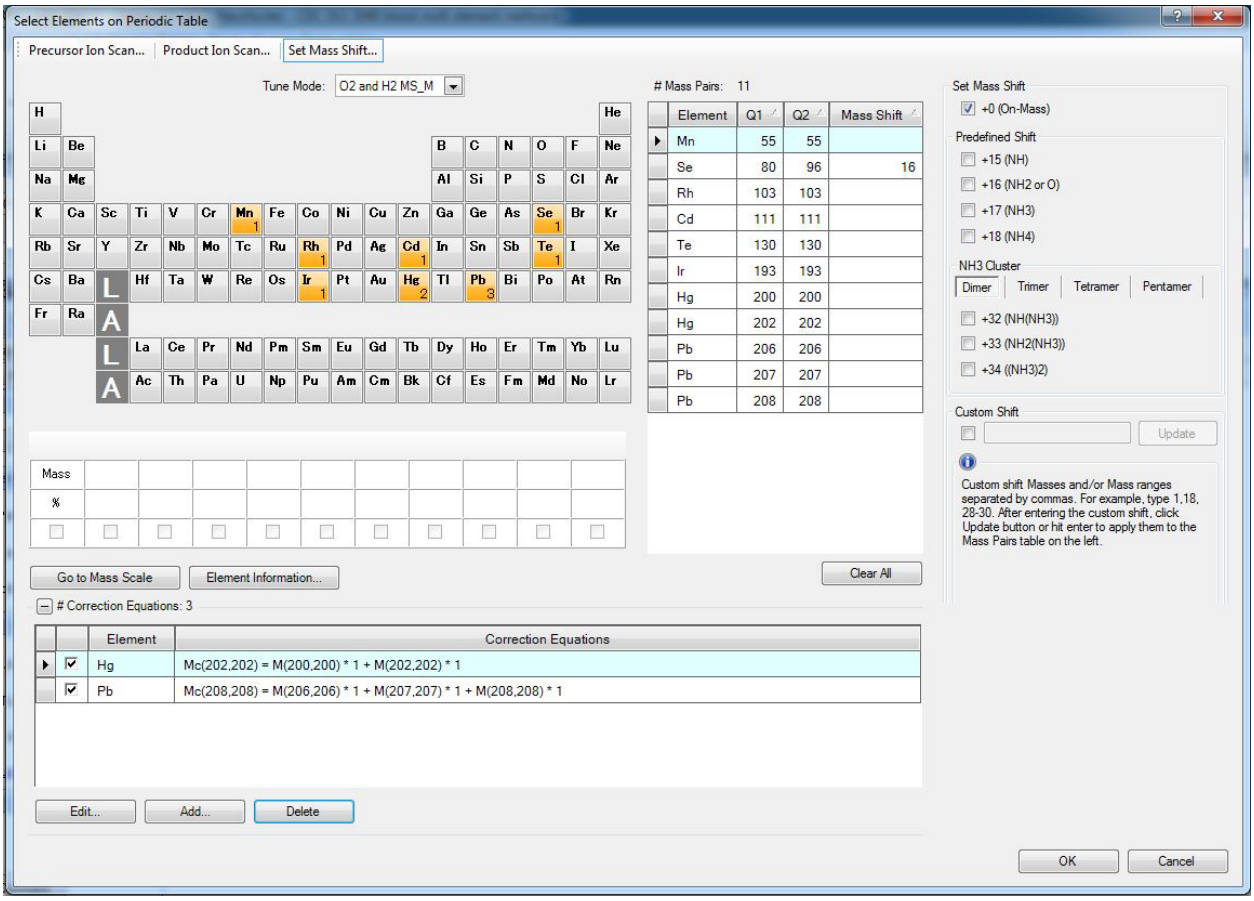


Figure 3. Batch Acquisition Method PeriPump settings

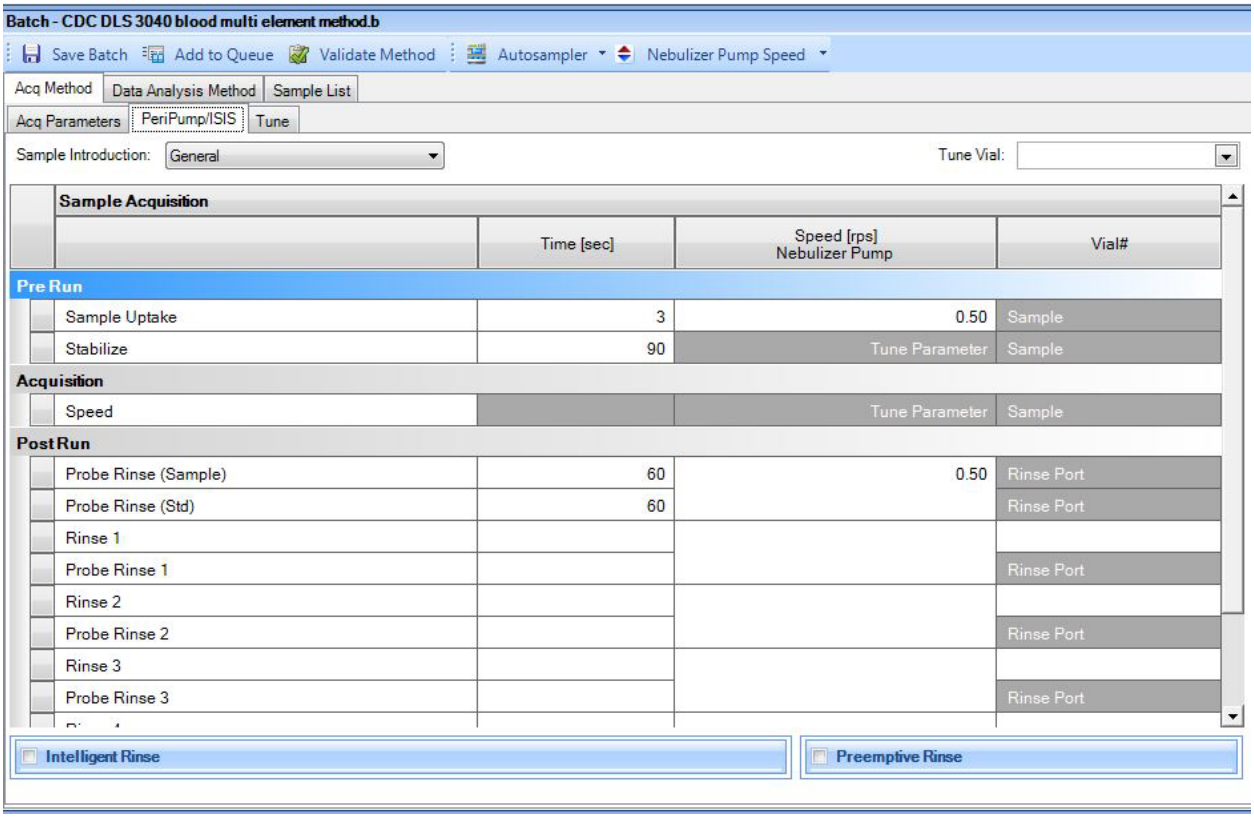


Figure 4. Batch Tune settings

The settings shown here under the “cell” section control the reaction gas flows into the octopole reaction cell and should not change. The “plasma” section controls sample introduction and plasma settings. The Nebulizer Gas is optimized daily, but typical values are 1.00 – 1.07 L/min. The remaining parameters under “plasma” may be optimized by advanced users.

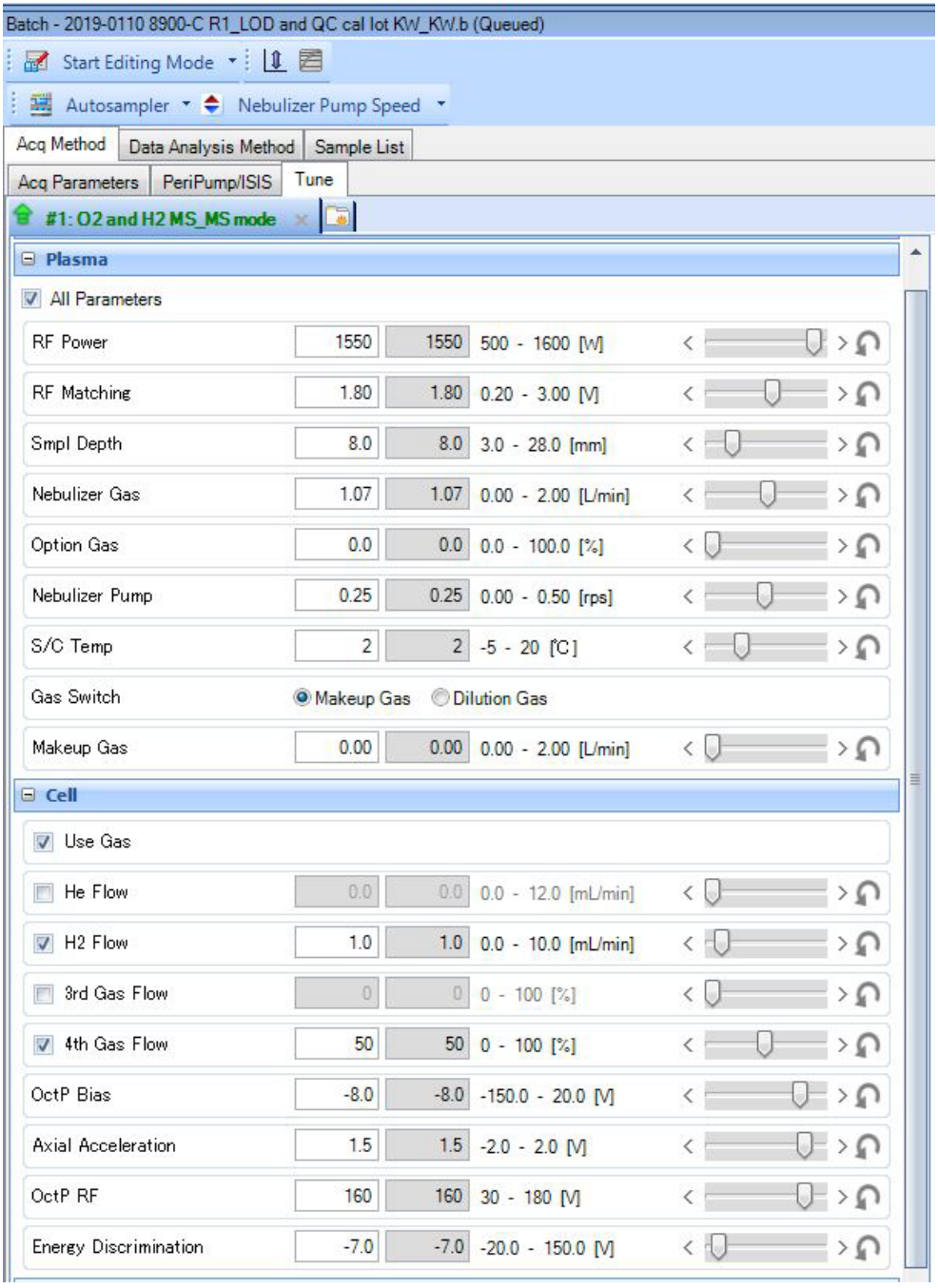


Figure 5. Batch Data Analysis Method Basic Information screen

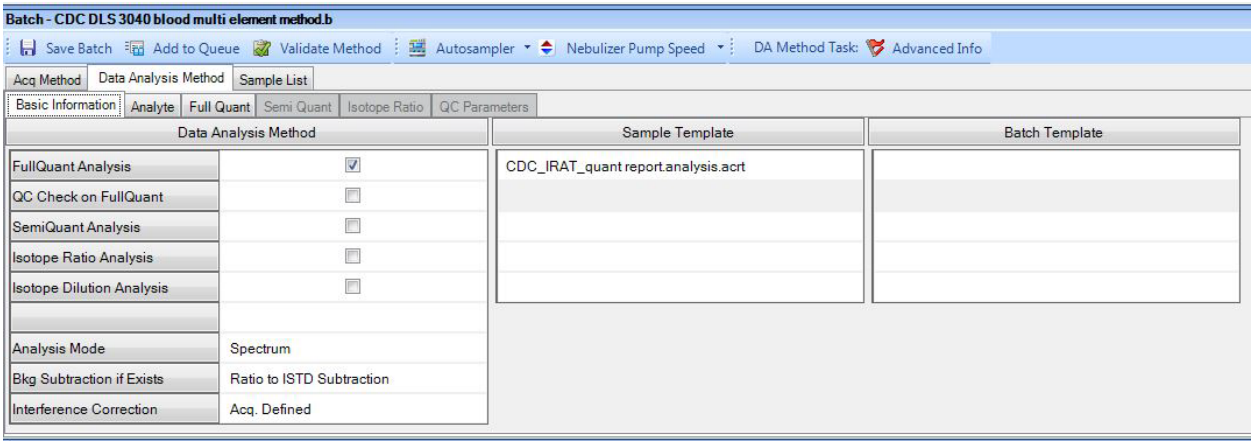
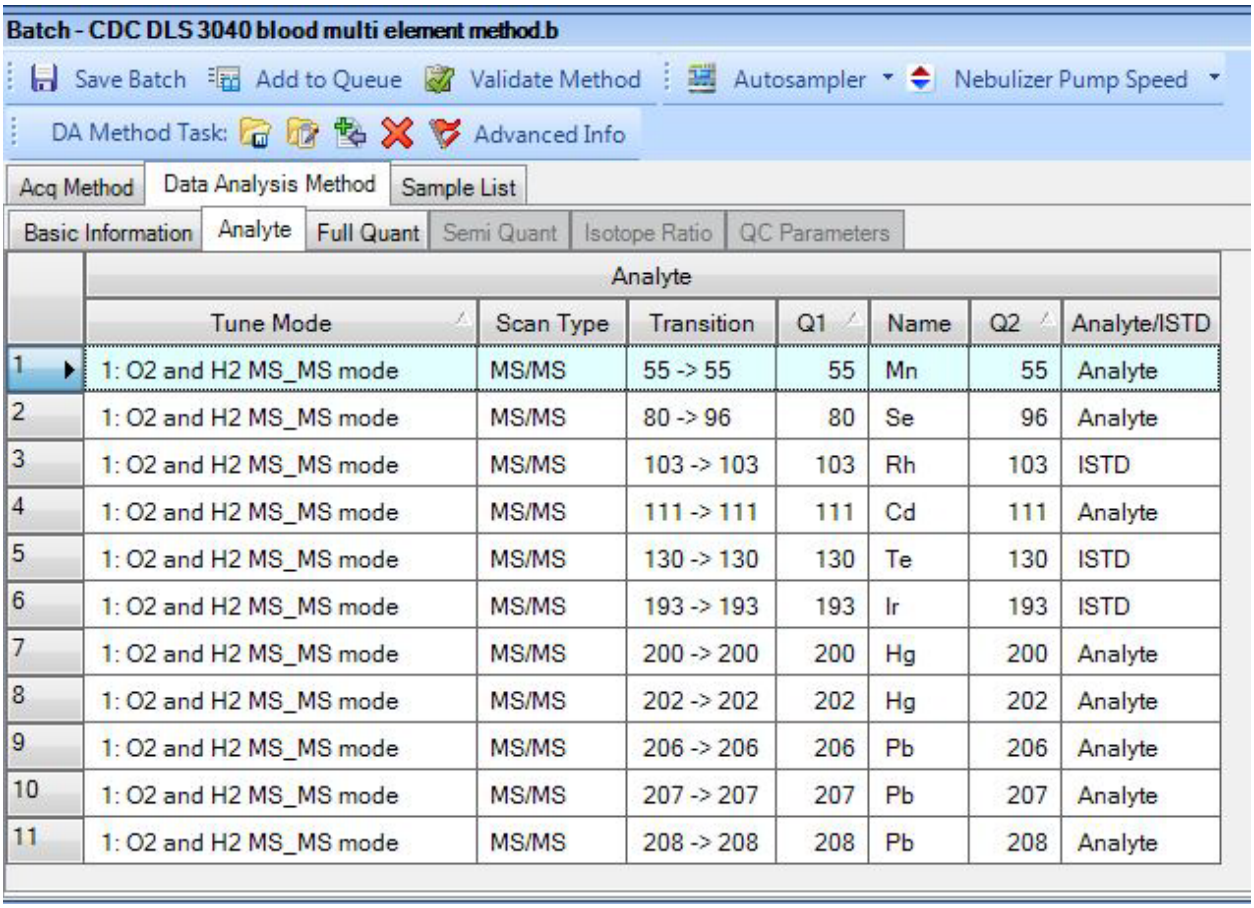


Figure 6. Batch Data Analysis Method Analyte list



	Analyte						
	Tune Mode	Scan Type	Transition	Q1	Name	Q2	Analyte/ISTD
1	1: O2 and H2 MS_MS mode	MS/MS	55 -> 55	55	Mn	55	Analyte
2	1: O2 and H2 MS_MS mode	MS/MS	80 -> 96	80	Se	96	Analyte
3	1: O2 and H2 MS_MS mode	MS/MS	103 -> 103	103	Rh	103	ISTD
4	1: O2 and H2 MS_MS mode	MS/MS	111 -> 111	111	Cd	111	Analyte
5	1: O2 and H2 MS_MS mode	MS/MS	130 -> 130	130	Te	130	ISTD
6	1: O2 and H2 MS_MS mode	MS/MS	193 -> 193	193	Ir	193	ISTD
7	1: O2 and H2 MS_MS mode	MS/MS	200 -> 200	200	Hg	200	Analyte
8	1: O2 and H2 MS_MS mode	MS/MS	202 -> 202	202	Hg	202	Analyte
9	1: O2 and H2 MS_MS mode	MS/MS	206 -> 206	206	Pb	206	Analyte
10	1: O2 and H2 MS_MS mode	MS/MS	207 -> 207	207	Pb	207	Analyte
11	1: O2 and H2 MS_MS mode	MS/MS	208 -> 208	208	Pb	208	Analyte

Figure 7. Batch Data Analysis Method Full Quant screen

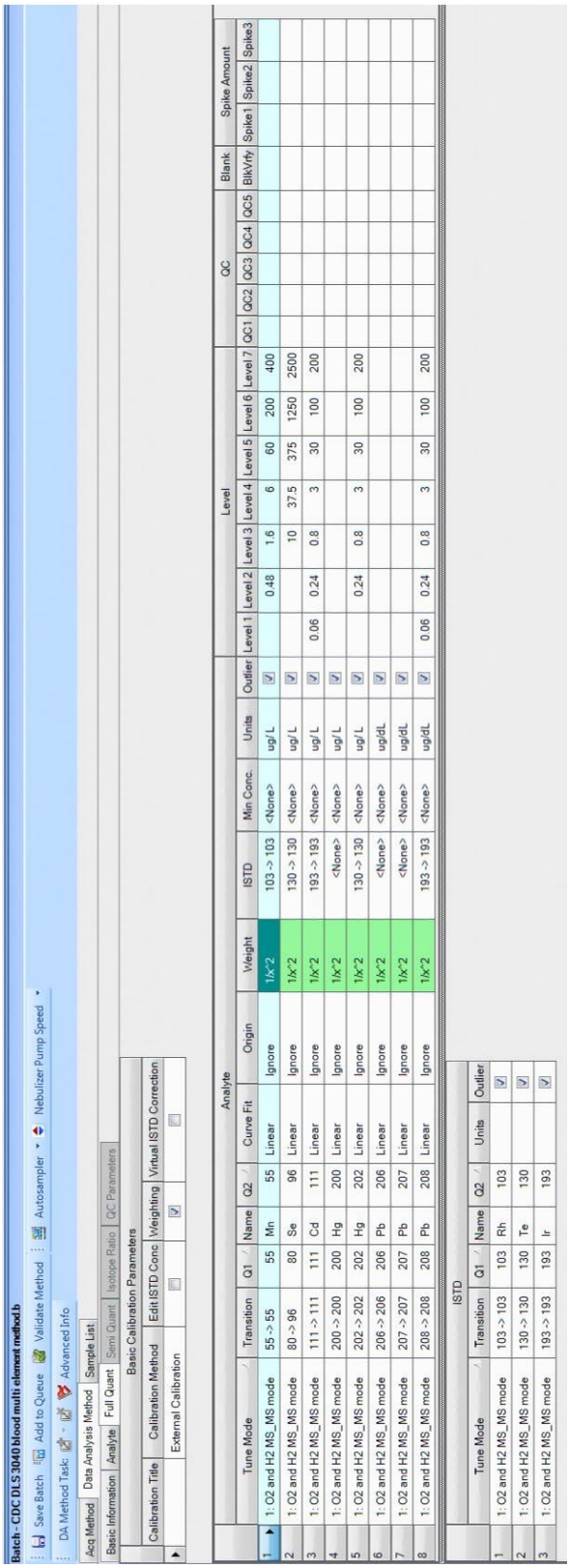
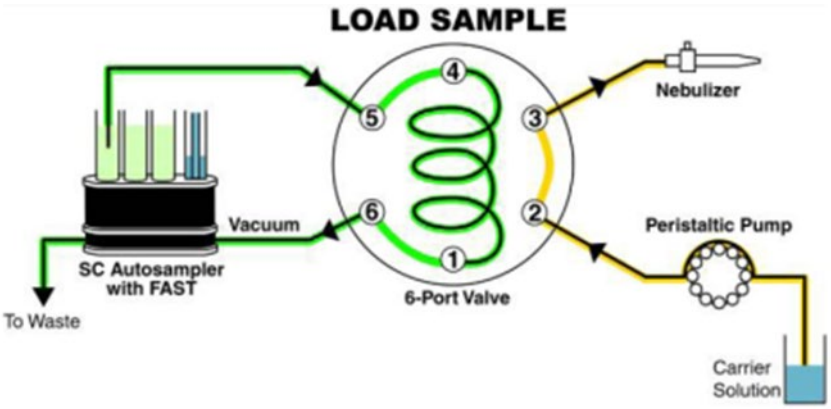


Figure 8. Configuration of tubing and devices for liquid handling using FAST sample introduction

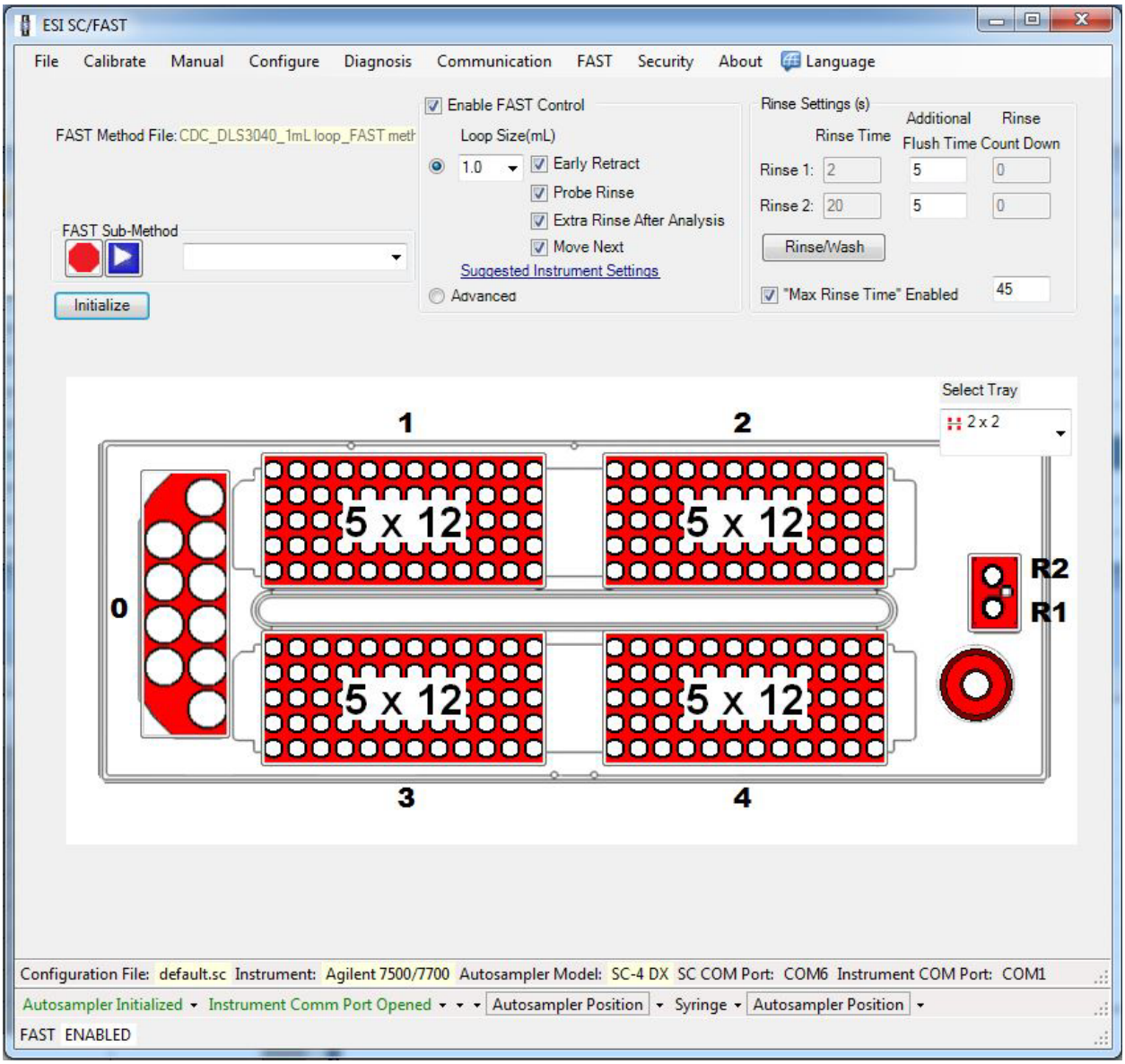
Below shows the correct connections to the 6-port FAST valve. The two diagrams show the differences in liquid flow directions when the valve changes from "Load" to "Inject" This change is internal to the valve. The shift of the valve cannot be seen, but it can be heard, and felt (with hand on the valve).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



The connections to the valve are color-coded (see Section 7.a.i).

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 9. ESI SC4 autosampler screen shots (main page)



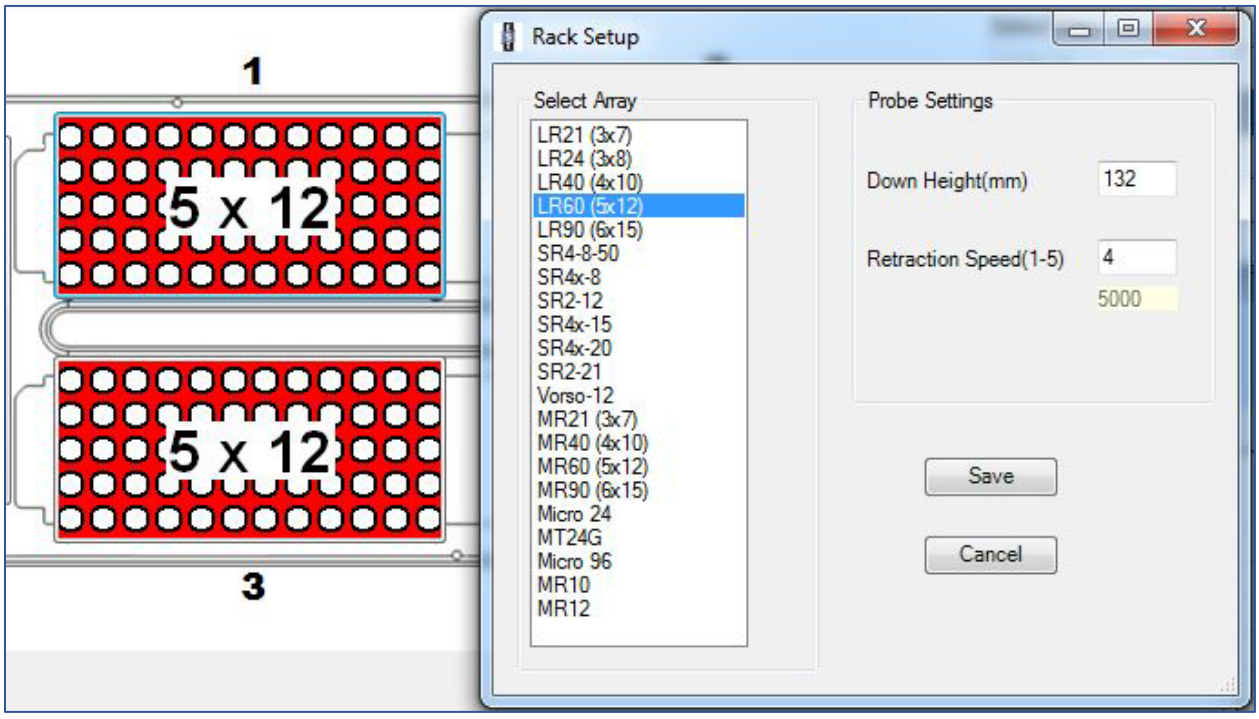
Additional flush times and “Max Rinse Time” are approximate. Optimize these for best reduction of elemental carry-over between samples. Tray types can be changed to allow for different volumes of diluted sample digests. “FAST control” must be enabled before start of method, but does not need to be used 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.

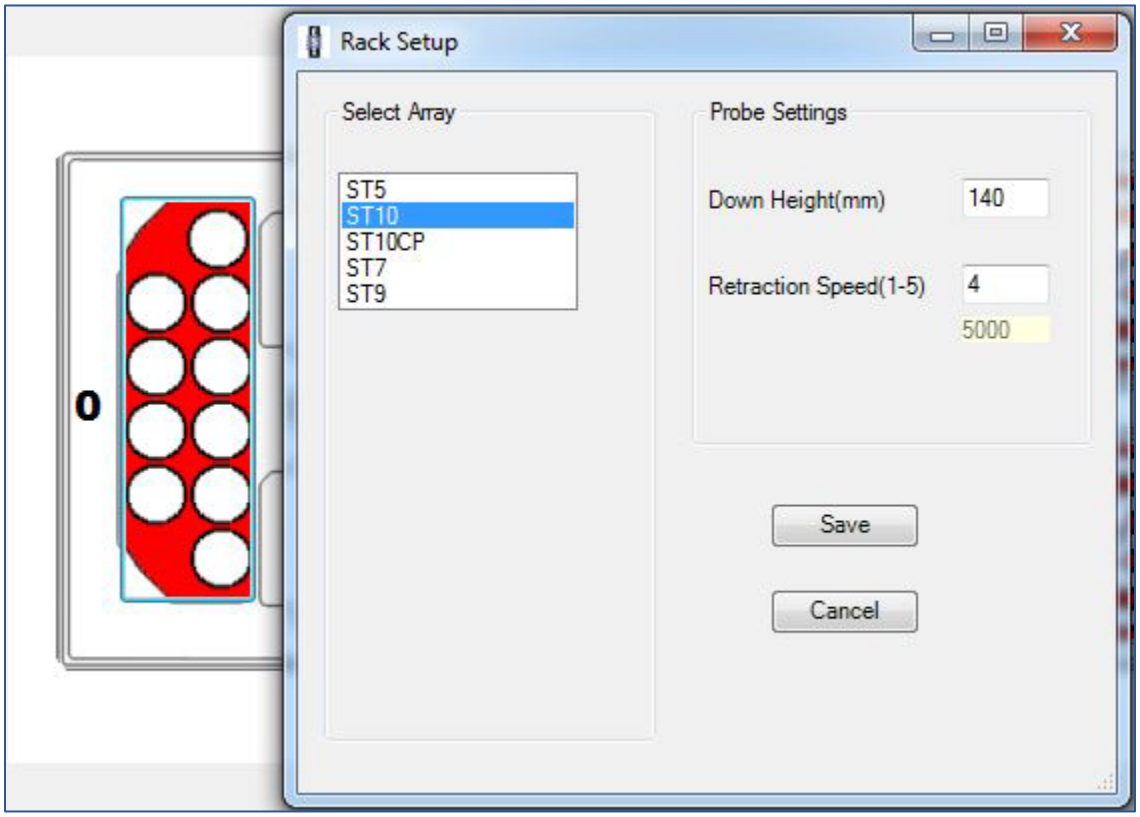
Additional flush times can be optimized to keep the rinse station full while not using too much rinse solution. The inner diameter size of the tubing providing the rinse solution to the rinse station determines how quickly the station will fill. Various sizes are available for purchase or can be made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

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



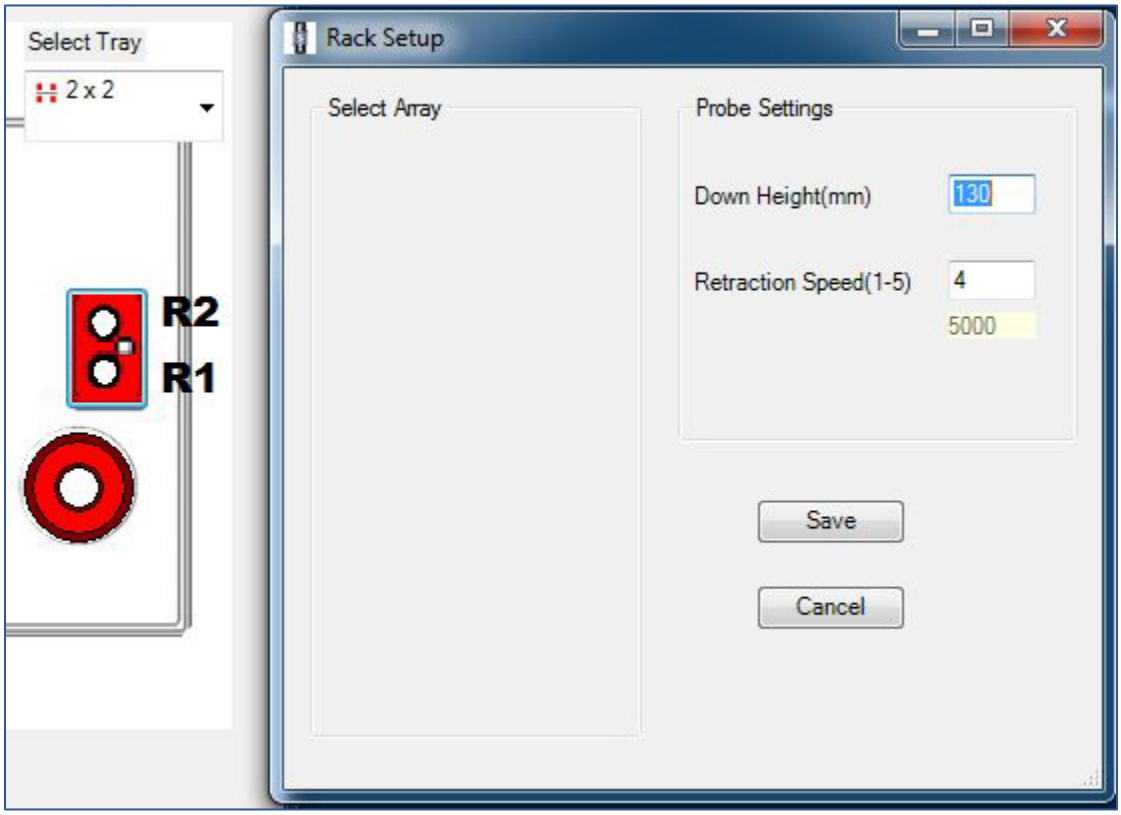
Settings are approximate. To be sure the loop is filled, set the probe to go close to the bottom of the cup, but not touch. Optimize retraction speed for least droplet splatter.

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



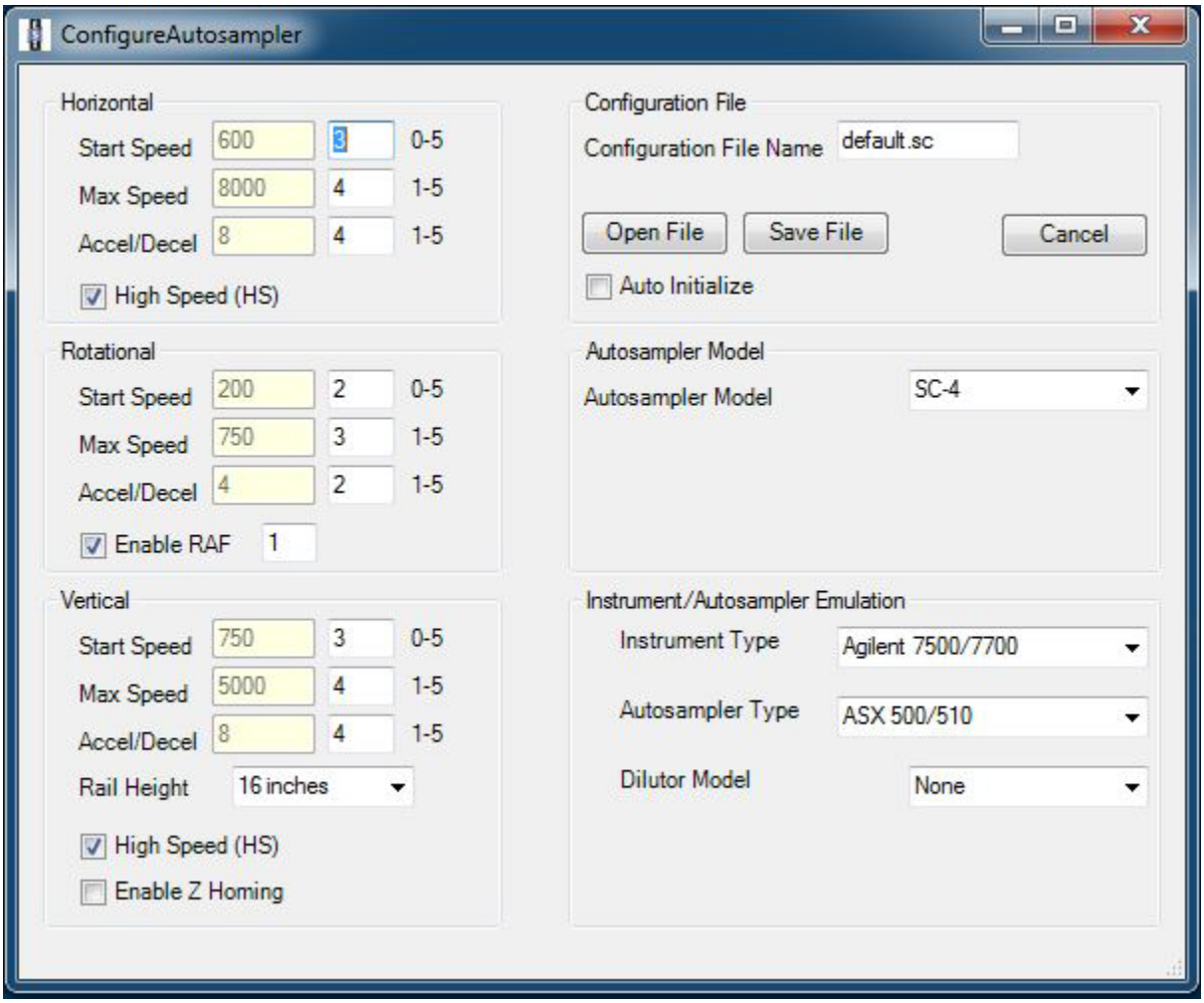
Settings are approximate. To be sure the loop is filled, set the probe to go close to the bottom of the cup, but not touch. Optimize retraction speed for least droplet splatter.

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



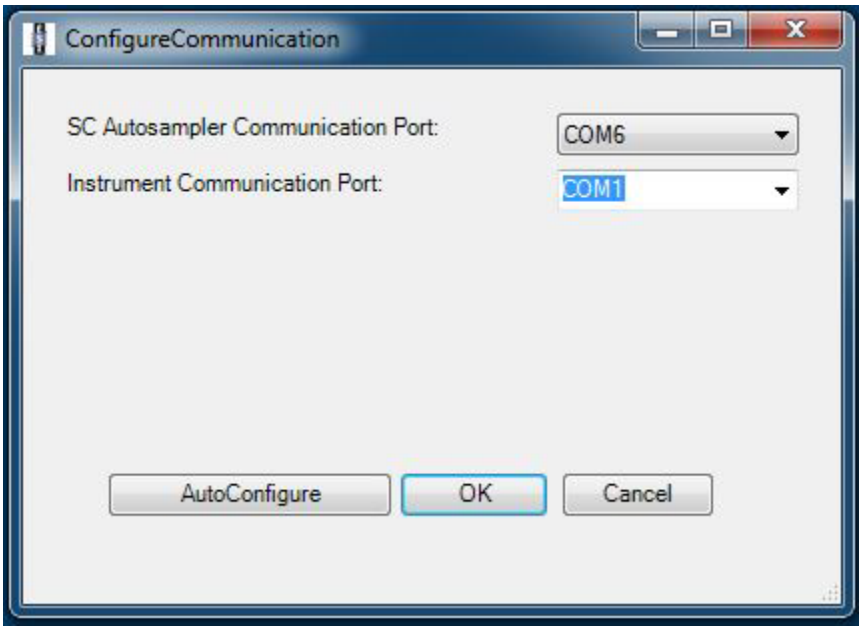
Settings are approximate. Optimize down height for best probe cleaning, and retraction speed for least droplet splatter.

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



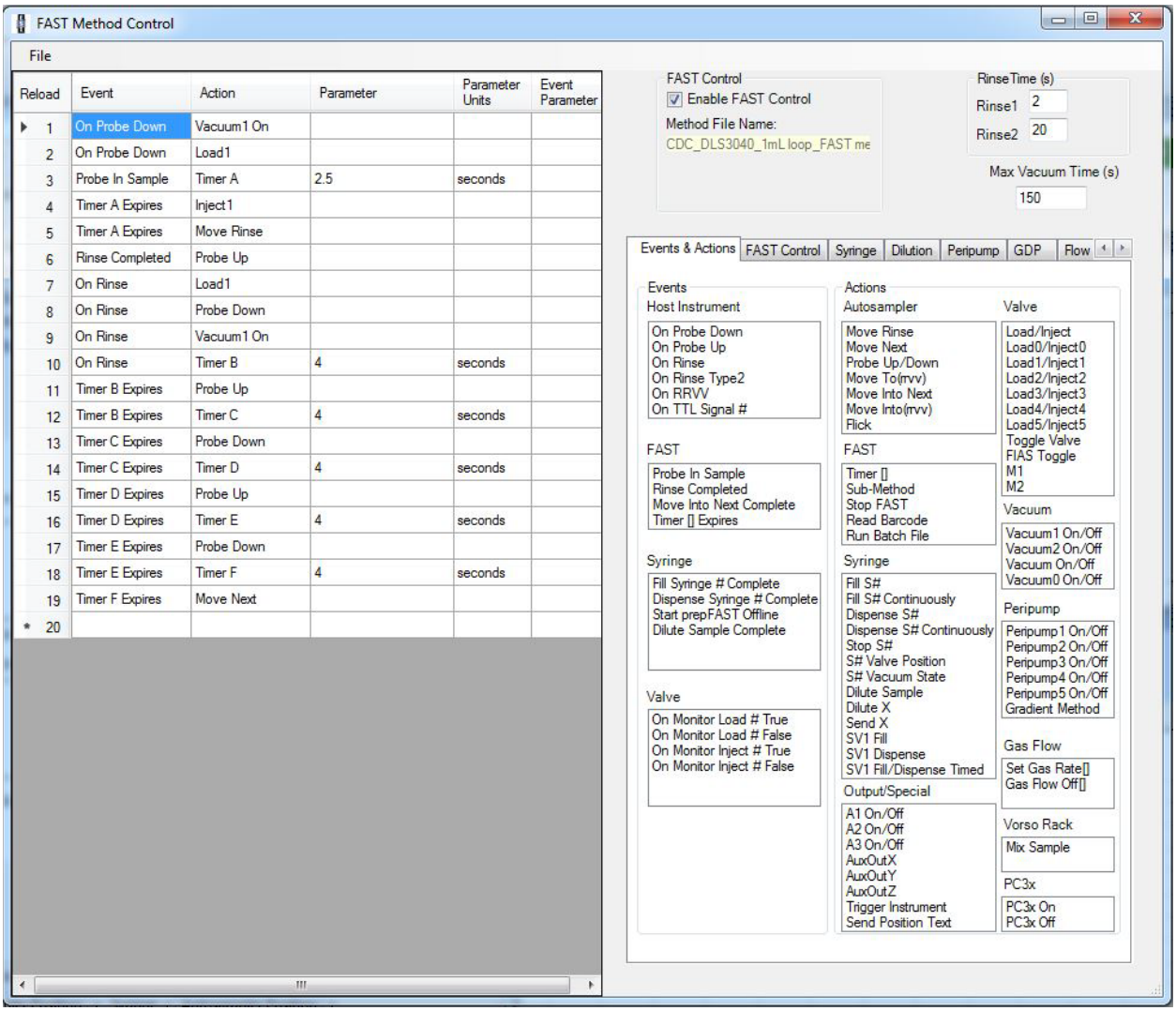
"High Speed" option is to only be used for "High Speed" models of the SC4 (look for "HS" in serial number). Speeds and accel / decel values can be optimized per analyst preference and to minimize droplet splatter off of probe.

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



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

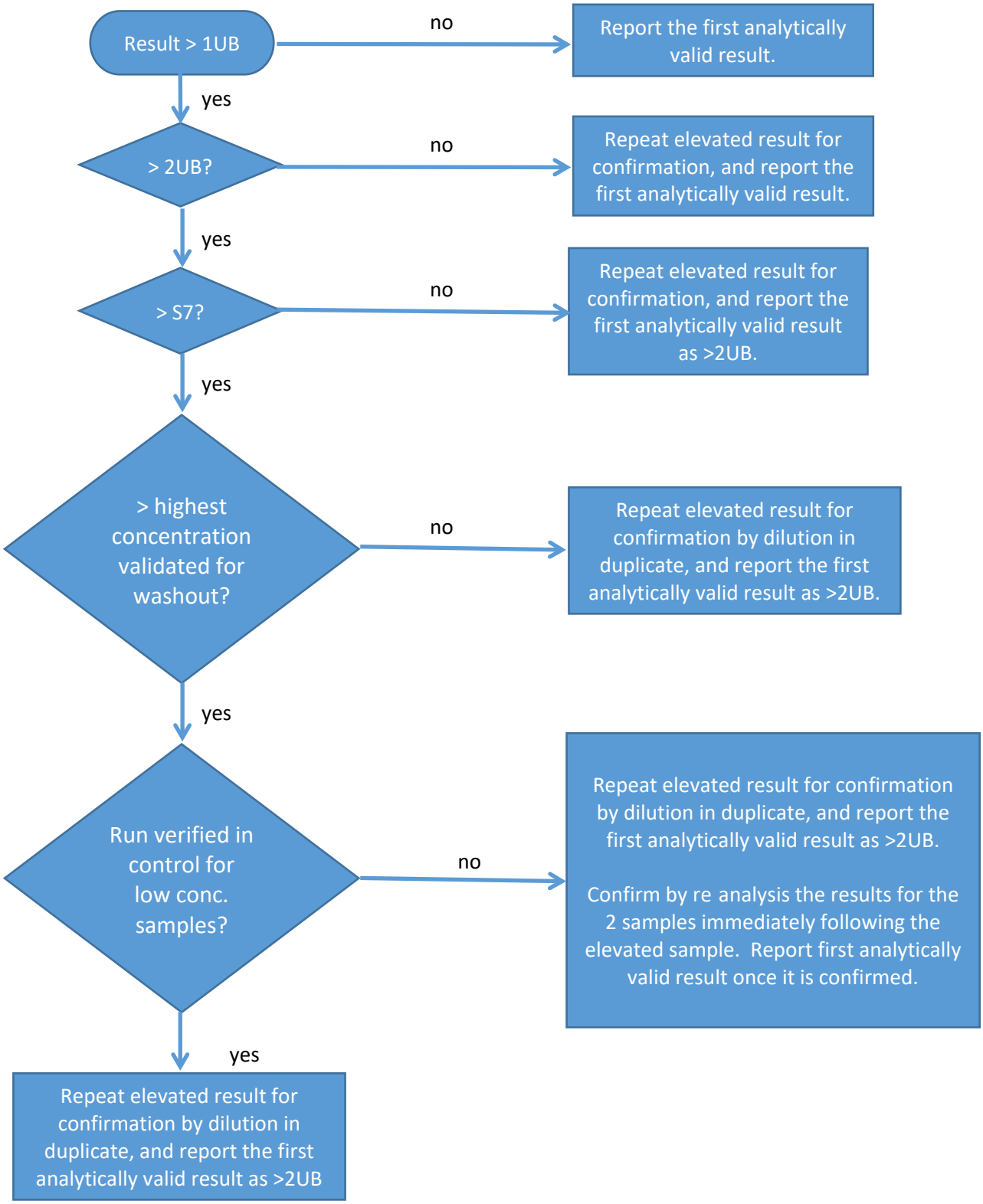
Figure 15. ESI SC4 autosampler screen shots ("FAST" page)



Timer A can be optimized to achieve proper filling of loop with diluted sample. 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_DLS 3040_1mL loop_FAST method.txt". The FAST file can be saved in either the directory C:\Program Files (x86)\ESI\ESI SC\Methods\FAST or C:\ProgramData\ESI\ESI SC\Methods\FAST.

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 (the vacuum pump may be slow to start for the first sample if this is not done, possibly resulting in loop filling inconsistencies).

Figure 16. Chart for handling an elevated result



18. Appendix D. Help sheets

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

When a solution is transferred to a secondary container, document the expiration date of the source on the secondary container or provide traceability to the expiration date on the source container (e.g., lot number).

Rinse solution: 1.0% (v/v) TMAH, 0.01% APDC, 0.05% Triton™ X-100, 1% ethanol

1. Partially fill a 4 liter bottle with ≥18 MOhm·cm water
2. Add 0.4 grams of APDC
3. Add 40 mL of 25% v/v TMAH (Tetramethylammonium hydroxide)
4. Add 40 mL of ethanol (200 proof)
5. Add 200 mL of 1% Triton™ X-100 (OR add 10 mL of 20% Triton™ X-100)
6. Add enough ≥18 MOhm·cm water to bring to 4 liter mark
7. Mix well by gently inverting several times
8. Label appropriately. Expires 1 year after prepared. Store at ambient temp. (+15°C to + 30°C).

Sample diluent / carrier: 1.0% (v/v) TMAH, 0.01% APDC, 0.05% Triton™ X-100, 1% ethanol, 5 ppb Te, Rh, Ir

1. Partially fill a 2 liter bottle with ≥18 MOhm·cm water
2. Add 0.2 grams of APDC
3. Add 20 mL of 25% v/v TMAH
4. Add 20 mL of ethanol
5. Add 500 µL of a 20 mg/L Te, Rh, and Ir stock solution (or 7 drops of 100 mg/L Te, Rh, and Ir stock solution from the Savillex volumetric dropper bottle, part # 700-550).
6. Add 100 mL of 1% Triton™ X-100 (OR, if using a 20% Triton™ X-100 solution, add 5mL)
7. Add enough ≥18 MOhm·cm water to bring to 2 liter mark
8. Mix well by gently inverting several times
9. Label appropriately. Expires 1 year after prepared. Store at ambient temp. (+15°C to + 30°C).

Intermediate Triton™ X-100 solution: 20% Triton™ X-100

1. Partially fill a 1 liter bottle with ≥18 MOhm·cm water
2. Add 200 mL of Triton™ X-100
3. Add enough ≥18 MOhm·cm water to bring to 1 liter mark
4. Allow to dissolve overnight (or add a Teflon magnetic stirring bar and stir until dissolved)
5. Mix well by gently inverting several times
6. Label appropriately. Expires 1 year after prepared. Store at ambient temp. (+15°C to + 30°C).

Intermediate Triton™ X-100 solution: 1% Triton™ X-100

1. Partially fill a 1 liter bottle with ≥18 MOhm·cm water
2. Add 10 mL of Triton™ X-100
3. Add enough ≥18 MOhm·cm water to bring to 1 liter mark
4. Allow to dissolve overnight (or add a Teflon magnetic stirring bar and stir until dissolved)
5. Mix well by gently inverting several times
6. Label appropriately. Expires 1 year after prepared. Store at ambient temp. (+15°C to + 30°C).

Intermediate internal standard solution: 20 mg/L Rh, Te and Ir in 1% (v/v) HNO_3

1. Partially fill an acid washed, 50 mL flask with 1% v/v HNO_3
2. Add 1 mL of Rh from 1000 ppm stock standard
3. Add 1 mL of Te from 1000 ppm stock standard
4. Add 1 mL of Ir from 1000 ppm stock standard
5. Add enough 1% v/v HNO_3 to fill to 50 mL mark
6. Mix well by gently inverting several times
7. Pour the standard solution over into an appropriately labeled 50 mL polypropylene tube
8. Expires 1 year after prepared. Store at ambient temp. (+15°C to + 30°C).

Intermediate internal standard solution: 100 mg/L Rh, Te and Ir in 0.5% (v/v) HNO_3

1. Partially fill an acid washed, 50 mL flask with 0.5% (v/v) HNO_3
2. Add 5 mL of Rh from 1000 ppm stock standard
3. Add 5 mL of Te from 1000 ppm stock standard
4. Add 5 mL of Ir from 1000 ppm stock standard
5. Add enough 0.5% v/v HNO_3 to fill to 50 mL mark
6. Mix well by gently inverting several times
7. Pour the standard solution over into an appropriately labeled 50 mL dropper bottle
8. Expires 1 year after prepared. Store at ambient temp. (+15°C to + 30°C).

Performance check solution: 1ppb multi-element standard in 2% (v/v) HNO_3

1. Partially fill a 0.5 liter volumetric flask with ≥18 MOhm·cm water
2. Add 0.5 mL of ICP-MS Stock Tuning Solution (catalog no. 5188-6564)
3. Add 10 mL of concentrated HNO_3
4. Add enough ≥18 MOhm·cm water to bring to 1 liter mark
5. Mix well by gently inverting several times
6. Label appropriately. Expires 1 year after prepared. Store at ambient temp. (+15°C to + 30°C).

Diluted blood solution for the reaction gas stability test solution (50 mL prep)

1. Use a 50 mL polypropylene tube
2. Add 45 mL of Sample Diluent
3. Add 2.5 mL of "junk" whole blood
4. Add 2.5 mL of ≥18 MOhm·cm water
5. Add 0.075 mL of intermediate stock solution A
6. Mix well by gently inverting several times
7. Label appropriately. is 24 hours at ambient temperature (+15°C to + 30°C) or 1 week if refrigerated (+2°C to +8°C).

Diluted blood solution for the reaction gas stability test (200 mL prep)

1. Use a 250 mL polypropylene bottle
2. Add 180 mL of Sample Diluent
3. Add 10 mL of "junk" whole blood
4. Add 10 mL of ≥18 MOhm·cm water
5. Add 0.3 mL of intermediate stock solution A
6. Mix well by gently inverting several times
7. Label appropriately. is 24 hours at ambient temperature (+15°C to + 30°C) or 1 week if refrigerated (+2°C to +8°C).

Diluent for intermediate stock and intermediate working cal.: 3% v/v HCl solution

1. Store in the refrigerator (+2°C to +8°C) (when not using)
2. Partially fill a acid washed 2 liter bottle with ≥18 MOhm·cm water
3. Using a clean 50 mL polypropylene tube to measure, add 60 mL of high purity concentrated HCl
4. Add enough ≥18 MOhm·cm water to bring to 2 liter mark
5. Gently invert to mix
6. Label appropriately. Expires 1 year after prepared. Store at ambient temp. (+15°C to + 30°C).

Intermediate stock standard A: see Table **5** of Appendix C

1. Partially fill a 100 mL volumetric flask with 3% v/v HCl solution
2. Label as: "DLS 3040 Intermediate Stock Std A"
3. Add 1 mL of DLS 3040 multi-element stock solution
4. Add enough 3% v/v HCl to bring to 100 mL mark
5. Mix well by gently inverting several times
6. Label appropriately. Expires 1 year after prepared. Store at ambient temp. (+15°C to + 30°C).

Intermediate stock standard B: see Table **5** of Appendix C

1. Partially fill a 100 mL volumetric flask with 3% v/v HCl solution
2. Label as: "DLS 3040 Intermediate Stock Std"
3. Add 0.3 mL of DLS 3040 multi-element stock solution
4. Add enough 3% v/v HCl to bring to 100 mL mark
5. Mix well by gently inverting several times
6. Label appropriately. Expires 1 year after prepared. Store at ambient temp. (+15°C to + 30°C).

Intermediate working calibration standards: see Table 6 of Appendix C

1. Partially fill seven, 100 mL volumetric flasks with 3% v/v HCl solution
2. Label as: Intermediate Working Std "S1", "S2", "S3" and "S4", "S5", "S6", and "S7"
3. For "S1 Intermediate Working Std": add 10 µL of the Intermediate Stock Std B
4. For "S2 Intermediate Working Std": add 120 µL of the Intermediate Stock Std A
5. For "S3 Intermediate Working Std": add 400 µL of the Intermediate Stock Std A
6. For "S4 Intermediate Working Std": add 1500 µL of the Intermediate Stock Std A
7. For "S5 Intermediate Working Std": add 150 µL of the Multi-Element Stock Std
8. For "S6 Intermediate Working Std": add 500 µL of the Multi-Element Stock Std
9. For "S7 Intermediate Working Std": add 1000 µL of the Multi-Element Stock Std
10. Add enough 3% v/v HCl solution to bring each to the 100 mL mark
11. Mix well by gently inverting several times
12. These intermediate working calibration standards may be poured over into clean 6 mL PFA vials for daily use (NOTE: "S0 Intermediate Working Std" is 3% v/v HCl only)
13. Expires 24 hours after prepared. Store at ambient temp. (+15°C to + 30°C).

P/A factor tune solution: 50mL multi-element standard in 2% (v/v) HNO_3

1. Partially fill a 50 mL lot screened or acid-washed polypropylene tube with 10-40 mL 2% v/v nitric acid.
2. Add 0.5 mL of each multi-element stock standard (Agilent part number # 5188-6524 has 2 solutions).
3. Dilute to the 50 mL mark with 2% v/v nitric acid.
4. Mix well by gently inverting several times.
5. Label appropriately. Expires 1 year after prepared. Store at ambient temp. (+15°C to + 30°C).

Barcodes for LIMS system

Table 13. These barcodes for calibrators, blanks, and QC pools can be used to enter these IDs into the instrument software instead of manually typing them.

Sample Name	Barcode	Sample Name	Barcode
3040 STD00		3040 BldBlkChk Wash1	
3040 STD01		3040 BldBlkChk Wash2	
3040 STD02		3040 BldBlkChk1	
3040 STD03		3040 AQBLK	
3040 STD04		LB08707	
3040 STD05		HB08708	
3040 STD06		EB18709	
3040 BldBlkChk2			

19. Appendix E. Exporting data from the instrument and uploading the run in STARLIMS

A. Creating the instrument performance report

Print the performance check completed prior to the run in .PDF format by choosing the time stamped report in the Performance Report software screen and clicking “generate”. The performance check must be completed within 24 hours of the start of the run (i.e., the matrix blank). In the MassHunter 4.5 Workstation Software for 8900 ICP-QQQ, generate the report based on the report template file “CDC PerformanceReport_ICP_QQQ.tune.tune.acrt” (example shown in Figure 17 and Figure18). Customize the .acrt file to include the name of the instrument in the sample information section title (see “How To…” in the Agilent Report Designer Help software for instructions).

i. During the performance check prior to the run (see Appendix F) save the PDF report using a descriptive filename (e.g., YYYY-MMDD_instrument_performance check_analyst.pdf).

ii. Save the PDF report in the appropriate batch folder in the “DATA” subfolder on the instrument computer.

iii. Save a copy of the PDF report in the instrument-specific subfolder of the CDC network IRATB_COMMON/Nutritional/Instrument.

B. Creating a batch tune report

Complete a batch tune prior to the run and save the batch tune report in .PDF format. The batch tune must be complete within 24 hours of the start of the run (i.e., the matrix blank). In the MassHunter 4.5 Workstation Software for 8900 ICP-QQQ, generate the report based on the report template file “CDC Batch TuneReport_ICPQQQ.tune.acrt.tune.acrt” to format the report (example shown in Figure 19 and Figure20). Customize the .acrt file to include the name of the instrument in the sample information section title (see ‘How To…’ in the Agilent Report Designer Help software for instructions).

i. During the batch tune prior to the run (see Appendix F) save the PDF report using a descriptive filename (e.g., YYYY-MMDD_instrument_tune report_analyst.pdf).

ii. Save the PDF report in the appropriate batch folder in the “DATA” sub-folder on the instrument computer.

iii. Save a copy of the PDF report in the instrument-specific subfolder of the CDC network IRATB_COMMON/Nutritional/Instrument.

C. Exporting calibration statistics to Excel

Export the calibration summary in .XLSX format that describes the curve statistics of each analyte.

i. In the ICP-QQQ Data Analysis software, accessible from the “Data Analysis” icon of the Masshunter instrument control software, expand the dropdown menu of the “Report” icon. Select “Export Calibration Summary” to export the curve statistics (example shown in Figure 21) into the appropriate batch subfolder in the “DATA” folder of the instrument computer.

ii. Save a copy of the XLSX file to the instrument-specific subfolder of the CDC network IRATB_COMMON/Nutritional/Instrument.

D. Creating the summary report

Print the run summary report in .PDF format that describes the measurements of the run. The report should include the following:

i. The first page should identify the analyst who performed the run, the instrument used, and the date of the analysis. Add the analyst identifier using PDF editing software.

ii. In the ICP-QQQ Data Analysis software, select all the samples in the run beginning with the calibration blank (matrix blank) through the ending bench QC material.

iii. Calibration information page. From dropdown arrow on the right of the "Report" icon, use "Print Calibration (Detail)" to generate the calibration information report (example shown in Figure 22). Print the generated report to pdfFactory Pro.

iv. Analysis summary page for each measurement that is part of the run. Use a report options template based on "CDC_IRAT_quant report.analysis.acrt" (example shown in Figure 23). Customize the .acrt file to include the name of the instrument in the sample information section title (see 'How To…' in the Agilent Report Designer Help software for instructions).

1) Click on the "Report" icon.

2) Select the radio button for "Selected Template" and load the "CDC_IRAT_quant report.analysis.acrt" report options template.

3) In the "Samples to be reported" Select the radio button for "Selected samples"

4) For the Output, select the radio button to print "To printer" PDF generating printer software "PDF factory Pro"

5) Click "Generate".

v. Save the PDF report in the appropriate batch folder in the "DATA" sub-folder on the instrument computer.

vi. Save a copy of the PDF report in the instrument-specific subfolder of the CDC network IRATB_COMMON/Nutritional/Instrument.

E. Creating the instrument run file in .CSV format that will be imported to STARLIMS

i. In the ICP-QQQ Data Analysis software, click the "Report" tab of the menu ribbon and select "Configure LIMS Settings…". Customize the reported data (example shown in Figure 24 and Figure 25) and match the expectations of the STARLIMS spec schema mapping sample schema action.

ii. In the Data Analysis software, select all the samples in the run beginning with the calibration blank (matrix blank) through the ending bench QC material.

iii. Expand the dropdown menu of the "Report" icon and select "LIMS – Export Selected Samples" to export the .CSV file of the reportable data

iv. Check the generated .CSV file to confirm that all samples and have been successfully exported (example shown in Figure 26b). Close the file.

v. Retrieve the run file (example shown in Figure 26c) from the Data (D:) drive "LIMS output" folder and save in the appropriate batch folder in the "DATA" sub-folder on the instrument computer.

vi. Save a copy of the PDF report in the instrument-specific subfolder of the CDC network IRATB_COMMON/Nutritional/Instrument.

F. Uploading and documenting the run in STARLIMS

i. Open the STARLIMS application and select “Pending Runs Assigned to My Labs”.

ii. Select the method from the Test drop down menu.

iii. Click “(+) Add.” Select the instrument system used with the run and click “OK.”

iv. Click the “All Results (S)” tab. Click “[0] Run Instrument Macro.” Once Excel opens, select “Enable Content” and follow the prompts on screen to select method, upload instrument data file (.csv), select solvent (aqueous or reagent) and matrix blanks. Save the file in the folder along with the instrument data file and then close it.

v. Click “[1] Upload Instrument File”. Use the browser to navigate to the Excel file that is to be imported (Excel file saved in step iv). Select your Excel Workbook file. Ensure the data in the sample data window matches the intended run and if it appears as expected click ‘Upload. If STARLIMS warns that there is a run date and time stamp conflict between this run and any previous run, ensure you selected the correct file for upload before proceeding. In the ‘add samples to run’ window ensure sample IDs for the run are recognized as eligible (one matrix blank, all calibrators, one solvent blank, QC, and patient samples) before clicking continue. On the ‘results not imported’ window ensure that only sample IDs not intended for upload are listed before closing the window.

vi. If your run included only a subset of the method analytes, a window will be presented listing sample results that have no value in the NUMRES field which are eligible to delete from the sample record (i.e., STARLIMS sample login records). Deleting an analyte record from a Retest > 0 only affects that particular sample ID | retest number combination (e.g., this import file). However, deleting an analyte from a retest 0 will permanently delete from the sample record (i.e., STARLIMS sample login records) ALL information associated with the targeted analyte(s) that are checked. Review the items checked for removal and adjust the selections as necessary before proceeding. Remove the check mark from any retest 0 analyte measurement if the sample needs to be tested later for that analyte (it is best practice that retest 0 for each sample should include all approved analytes to avoid the risk of accidentally deleting sample login information during this step). If you are uncertain about the use of this function, see additional guidance before proceeding.

vii. Document ‘not tested’ samples, if necessary (e.g., missing or empty vial, quantity not sufficient) using one of the options below that adds the sample ID into a run. Further sample information will be added in later steps as the entire run is processed. Use the ‘Set Not Tested’ function in the samples pending assignment section only to clear sample IDs that are not needed for CLIA reporting purposes (e.g., method development or training sample ID entries that were not needed).

1) Click on the Run Sheet Details tab. Click on ‘Click to Search’. Use the search criteria to populate the samples pending assignment list. Select the sample IDs to add to the run and click the “>” arrow button.

2) Click on the Run Sheet Details tab. Click on “Add Unknowns by Barcode” link. Enter the sample ID by typing or barcode scanning. Click ‘Ok’ to directly add the ID to the run list.

viii. Click the “All Results (S)” tab. Select “[2] Mark Null Results”. This will change any null values in NUMRES (i.e., observed result) to a pipe tab (|) (e.g., ‘not tested’ samples or analytes not tested for any samples).

ix. Select “[3] Evaluate Sample QC”. This will execute the STARLIMS spec schema for the method and assign values in the columns ‘Results Status’, ‘Status Message', ‘Sample QC Passed’, and (in some cases) the DLS comment code. Review these columns and take the follow up actions described in Table 14. Use the STARLIMS Modify SQC Assessments tools linked above the bottom data grid as needed to update the reported value, sample QC passed, DLS comment code, and analyst comment as needed (see Table 15) before submitting for review. ‘Results Status’ field entries of ‘check’ for blanks, calibrators, and QC measurement of the run do not invalidate the use of the measurement for the run but can indicate potential problem areas to troubleshoot. Notify the supervisor about ‘check’ events that occur frequently. If you are unsure, discuss with your supervisor.

x. Select “[4] Evaluate Run QC”. In the pop-up window, the QC pools from the run should appear with a date range automatically going back 3 months. Update the initial date if necessary to capture the data desired. In “For the following QC results” section, include at least the last 10 runs (i.e., select the radio button ‘the prior 20 results’). To include more historical runs, select the radio button for ‘The prior N* results within this date range,” and enter a larger number (e.g., N=40) or select the radio button “All results within this data range”. In “From the following instruments” section, select “All instruments” before proceeding to the next step and starting the SASQC Wizard.

xi. In the SASQC Wizard popup window, select “Save SAS Input File” and assign a filename. Including the run number in the filename can be helpful. The default location for this file is C:\Temp, change the network location to save the SAS file in the same /Nutritional/Instrument folder file that the data is stored in. Click “Save”. Select “OK” when export is complete. The next step is to “Send to SAS Server”. Check that your run number has passed and be sure to save a copy of this Report as a .pdf in the data network folder. Select “Finished”.

xii. Click “[5] Set Run QC Statuses”. Mark the Run QC statuses as was observed on the SASQC report. If more than one analyte fails QC, refer to the QC rules section of the APM to determine if the entire run needs to be rejected due to excessive run QC failures before proceeding.

xiii. Click “[6] Attach SAS QC File”. Select the PDF version of the SAS output previously saved.

xiv. Using the field filtering tool located in the top row of the data field, select “N/A” in the QC Type column.

xv. Click “Set Final Wizard” above the bottom results grid to change the ‘set final’ status to TRUE for all results recommended for reporting.

1) In the popup window, choose either to ‘process all samples displayed in the data grid” or “process only the samples selected in the data grid”. In this window also select “Run the Set Final Wizard” before proceeding.

2) The “batch processing criteria for marking results as set final’ window appears. In Step 1, check the boxes to “Require Sample QC passed” and “Require Run QC Passed” and to allow results statuses with “Pass” (and “Warn” only if samples have been previously tested and uploaded to STARLIMS) for setting final. In Step 2, verify the date range shown by default includes the dates necessary for capturing all relevant retests of the samples in the run being processed, and update the date range as necessary before proceeding.

3) The confirmation window will appear stating how many results were processed and how many result records have replicates (more than one retest). Verify the number is correct before clicking 'Yes' to process.

4) If the replicate wizard window appears, use it to review the different retests for each sample / analyte. Follow steps 1-4 listed on the right side of the window:

a) Step 1. Limit the algorithm to permit only 1 result to be set final.

b) Step 2. Choose which algorithm is appropriate (typically 'mark set final based on the first passing result that meets the parameters…" and then click the appropriate 'resolve' button to run the wizard.

c) Step 3. Select specimen ID/analytes to review in the upper left table and step through them using the 'Load previous record set' and 'Load Next Record set' buttons in the bottom table. Review each selection in the bottom data grid that was determined by the algorithm and manually adjust selections if necessary. Use the 'check selected as set final' and 'uncheck selected as set final' functions above the bottom data grid to set final the desired results. Use the STARLIMS Modify SQC Assessments tool linked above the bottom data grid as needed to update the reported value, sample QC passed, DLS comment code, and analyst comment as needed (see Table 15) before submitting for review. If you are unsure, discuss with your supervisor.

d) Step 4. Click the save button once all selections have been verified.

xvi. Before finishing results, verify "sample QC passed" and "run QC passed" are set correctly. Select "Manage Attachments" to attach the required files to the run per Table 16.

xvii. Fill in Run Comments and required User Fields associated with the run. See Table 17 for information.

xviii. Once you choose "Finish Results", you will not be able to make revisions. When you are ready to report the results, click "Finish Results". Select the names of the individuals that you want to send your run to.

Table 14. Possible outcomes of evaluating sample QC within STARLIMS.

QCType	Description	reported value	result status	sample QC	status message	DLS comment code	Analyst action
any	'not tested ' events or missing replicates	'|'	Incmp	FALSE	"not tested" or "no result reported"	60 or manual*	re-test, if needed
any	"S" (detector saturated)	'null'	FAIL	FALSE	"S value reported"	manual*	re-test with extra dil.
any	"[". Negative cps. Usually oversubtraction of a correction equation.	'null'	FAIL	FALSE	"[reported"	Manual*	re-test once to confirm
any	other non-numeric	'null'	FAIL	FALSE	"non-numeric reported"	manual*	re-test
blank	blank > lowest calibrator	no change	CHECK	N/A	"> lowCalibrator"	N/A	evaluate run QC, use different blank if possible
blank	measurement precision not as good as expected	no change	CHECK	N/A	"Rep Delta > 10% of mean" OR "Rep Delta > delta limit"	N/A	evaluate run QC, use different blank if possible
STD	calibrator result was below LOD	no change	CHECK	N/A	"< LOD"	N/A	evaluate calibration curve correlation
STD	measurement precision not as expected	no change	CHECK	N/A	"Rep Delta > 10% of mean" OR "Rep Delta > delta limit"	N/A	evaluate calibration curve correlation
QC	QC result was below LOD	no change	CHECK	N/A	"< LOD"	N/A	Evaluate QC results. Test new QC vial. Check for instrument sensititivity.
QC	measurement precision not as good as expected	no change	CHECK	N/A	"Rep Delta > 10% of mean" OR "Rep Delta > delta limit"	N/A	Evaluate QC results.
QC	QC result was > highest calibrator	no change	FAIL	N/A	"> high calibrator"	N/A	Treat as run QC fail.
Specimen	result < LOD (special approval permits >0, e.g., NHANES or PATH)	'null'	PASS	TRUE	"< LOD"	37	N/A
Specimen	Conc. greater than tested for washout.	no change	WARN	TRUE	"> highest validated washout"	N/A	See instructions to confirm washout after an elevated sample.

QCType	Description	reported value	result status	sample QC	status message	DLS comment code	Analyst action
Specimen	Conc. less than 2nd lower boundary threshold (unusually low result, notification threshold)	no change	WARN	TRUE	"<2 LB"	33 (manual after confirmed)	See instructions for patient results outside the normal range
Specimen	Conc. less than 1st lower boundary threshold (unusually low result, confirmation threshold)	no change	WARN	TRUE	"< 1LB"	33 (manual after confirmed)	See instructions for patient results outside the normal range
Specimen	Conc. greater than 1st upper boundary threshold (unusually high result, confirmation threshold)	no change	WARN	TRUE	"< 1UB"	33 (manual after confirmed)	See instructions for patient results outside the normal range
Specimen	Conc. greater than 2nd upper boundary threshold (unusually high result, notification threshold)	no change	WARN	TRUE	"<2 UB"	33 (manual after confirmed)	See instructions for patient results outside the normal range
Specimen	Prescreen failure	'null'	WARN	TRUE	"prescreen failure"	15	no retests needed
Specimen	measurement precision not as expected	no change	FAIL	FALSE	"Rep Delta > 10% of mean" OR "Rep Delta > delta limit"	N/A	Retest. If not possible document per Table 17.
Specimen	Conc. greater than highest calibrator.	no change	FAIL	FALSE	"> high calibrator"	N/A	Retest with extra dilution. If not possible, document per Table 17.
Specimen	Maximum permitted extra dilution exceeded.	no change	FAIL	FALSE	" > dilution max"	N/A	If observed result > high calibrator when using maximum extra dilution, document per Table 17.
Specimen	Conc. greater than upper reportable limit	no change	FAIL	FALSE	"> max rptbl conc"	103	Retest once with dilution. If observed > high cal. with max extra dilution, document per Table 17.

Table 15. Examples of non-reportable results. Analyst sets these manually using SQC Assessments.

Event	Retest to document	Reported value	Sample QC Passed	Set Final	DLS Comment code	Analyst comment
Blood clot	Add 'not tested', or document on any entered retest (if clot observed on sample re-prep)	Null	TRUE	TRUE	12	describe clot
Tube / vial broken	Add 'not tested'	Null	TRUE	TRUE	17	brief explanation
Missing or empty vial	Add 'not tested'	Null	TRUE	TRUE	18	brief explanation
Quantity not sufficient (QNS)	Add 'not tested'	Null	TRUE	TRUE	21	"QNS for initial testing"
QNS for repeat testing	Document on any retest	Null	TRUE	TRUE	22	"QNS for repeat testing"
Lab error	Add 'not tested'	Null	TRUE	TRUE	24	brief explanation
Test not performed	Add 'not tested'	Null	TRUE	TRUE	60	brief explanation
Concentration higher than upper reporting limit (i.e., observed > high calibrator)	Document on any retest	Null	TRUE	TRUE	103	none needed
Excessive run QC failure	Retests within the run	Null	TRUE	TRUE	200	none needed

Table 16. Files to upload in STARLIMS.

Files	File format	Comment
Instrument performance	PDF	Last performed prior to the run, at least within the last 24 hours
Run summary	PDF	Identifies instrument, analyst, date of analysis, calibration summary, and details each measurement performed in the run
Run results produced by STARLIMS instrument macro	Excel	Includes original instrument file
SAS QC plot	PDF	Created in the 'Evaluate Run QC' step within STARLIMS
Batch tune	PDF	Performed prior to the run, sets instrument parameters
Calibration summary	Excel	Includes the curve statistics for individual analytes

Table 17. Use of run user fields in STARLIMS.

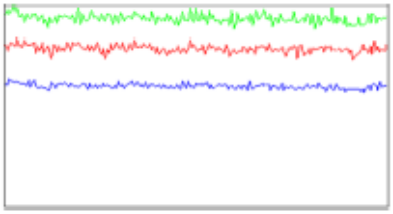
Field	Recommendations	Required	Examples
Run Comments	Brief info. related to the run outcome, e.g., ▪ QC failures ▪ Confirmations or Confirmation only (if no results set final) ▪ Elevated results (<2LB or >2UB) ▪ Dilutions performed ▪ Special purposes: LOD, instrument comparison, quarterly SRM, analyst evaluations. ▪ Rep Data>delta limits	Y	▪ UPB failed QC. ▪ Confirmations for BPB and BMN ▪ Confirmation run only, no results set final ▪ Elevated results (>2UB) ▪ Dilutions performed ▪ LOD run ▪ Elevated results, dilutions performed, confirmations for USR and UPB. ▪ BMN failed QC, confirmations for BPB. ▪ range of replicates > rep. delta limits
1	Other general comments about the run: ▪ If calibrator is dropped to correct curve linearity, specify calibrator and reason. ▪ If a different blank was used	N	▪ Dropped Std.2 for UPB calibration curve. ▪ Reprocessed with aq. blank ▪ Reprocessed with 2nd cal. Curve
2	Calibrator lot number or ID	Y	lot2035232-100_lot2213304-100_MN_2022-0817
3	DLS Study number of samples in the run: ▪ DLS study or PT name ▪ Note: R&D, LOD determination, Analyst comparison, if applicable	Y	▪ 2012-0036, NH99 ▪ CTQ QMEQAS, NH99 ▪ Analyst evaluation ▪ Quarterly SRM
4	Sample Group #	Y	HM-1837
5	Corrective action	Y, if performed	fresh QC vials
6	Column ID	if chrom. method	column serial number 308
7	Name of specific alternate reviewer	N	Name
8	Hamilton Identifier	Y	ML600FG9017
9	Alternate review by Lab Chief / Team Lead	N	TL / LC

Figure 17. Example of instrument performance report, page 1.

CDC Performance Report Agilent 8900-H

Acq. Date-Time	12/1/2022 8:13:41 AM
Instrument Name	G3665A SG19131160
Sample Introduction	PeriPump
Nebulizer Type	MicroMist
Ion Lens Model	x-Lens
Tune Parameters	Standard Tune

Sensitivity



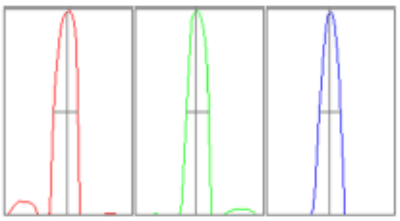
Mass	Range	Count	RSD%	Background
7	5000	3948	2.340	0.000
89	10000	9335	2.380	0.000
205	10000	6038	2.024	0.000

Sampling Period [sec]	0.311
Integration Time [sec]	0.1

Oxide/Doubly Charged Ratio

Oxide	156 / 140 0.537 %
Doubly Charged	70 / 140 1.651 %

Resolution/Axis



Mass	Peak Height	Axis	W-50%	W-10%
7	3932.54	7.00	0.58	0.68
89	9251.11	88.95	0.52	0.67
205	6038.69	205.00	0.51	0.69

Integration Time [sec]	0.1
Acquisition Time [sec]	20.37

Tune Parameters

Plasma Parameters

RF Power	1550 W	Option Gas	0.0 %	Makeup Gas	0.00 L/min
RF Matching	1.20 V	Nebulizer Pump	0.10 rps	Auxiliary Gas	0.90 L/min
Sample Depth	8.0 mm	S/C Temp	2 °C	Plasma Gas	15.0 L/min
Nebulizer Gas	1.07 L/min				

Lens Parameters

Extract 1	-16.0 V	Q1 Entrance	-50.0 V	Cell Exit	-50 V
Extract 2	-250.0 V	Q1 Exit	0.0 V	Deflect	13.2 V
Omega Bias	-150 V	Cell Focus	0.0 V	Plate Bias	-50 V
Omega Lens	9.8 V	Cell Entrance	-40 V		

Q1 Ion Guide Parameters

SLS Factor	0.60	SLG Factor	0.90

Q1 Parameters

Q1 Prefilter Bias	-5.5 V	Q1 Postfilter Bias	-5.0 V	Q1 Bias	-5.0 V

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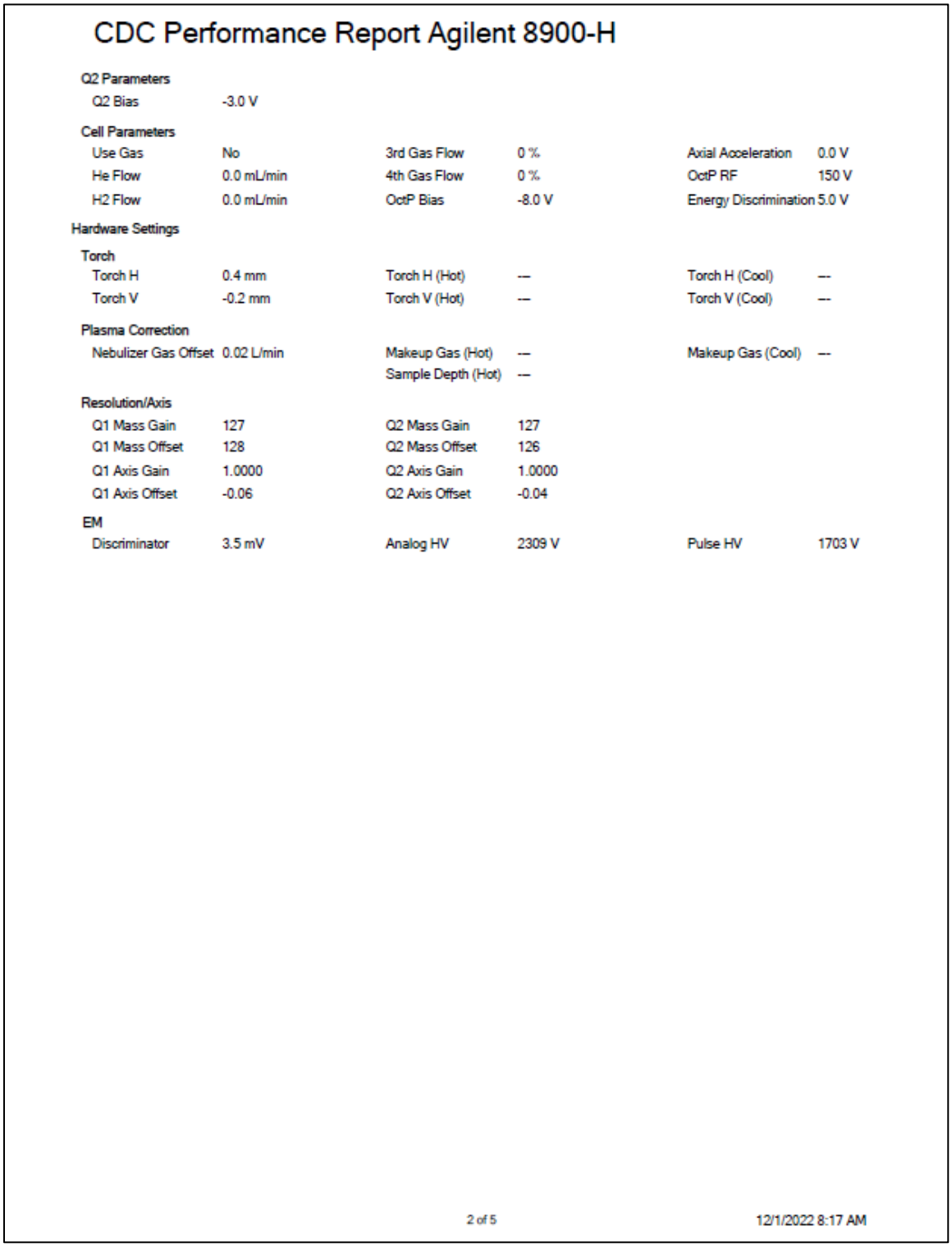
Figure 18. Example of instrument performance report, page 2.



CDC Performance Report Agilent 8900-H

Q2 Parameters					
Q2 Bias	-3.0 V				
Cell Parameters					
Use Gas	No	3rd Gas Flow	0 %	Axial Acceleration	0.0 V
He Flow	0.0 mL/min	4th Gas Flow	0 %	OctP RF	150 V
H2 Flow	0.0 mL/min	OctP Bias	-8.0 V	Energy Discrimination	5.0 V
Hardware Settings					
Torch					
Torch H	0.4 mm	Torch H (Hot)	---	Torch H (Cool)	---
Torch V	-0.2 mm	Torch V (Hot)	---	Torch V (Cool)	---
Plasma Correction					
Nebulizer Gas Offset	0.02 L/min	Makeup Gas (Hot)	---	Makeup Gas (Cool)	---
		Sample Depth (Hot)	---		
Resolution/Axis					
Q1 Mass Gain	127	Q2 Mass Gain	127		
Q1 Mass Offset	128	Q2 Mass Offset	126		
Q1 Axis Gain	1.0000	Q2 Axis Gain	1.0000		
Q1 Axis Offset	-0.06	Q2 Axis Offset	-0.04		
EM					
Discriminator	3.5 mV	Analog HV	2309 V	Pulse HV	1703 V

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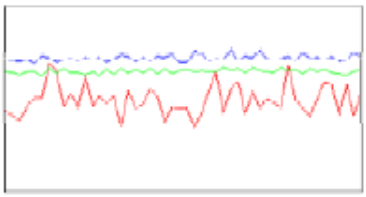
Figure 19. Example of instrument batch tune report, page 1.

Batch Tune Report - 8900-H

Operator Name	Admin
Acq/Data Batch	D:\Agilent\ICPMH\1\DATA\2022-1201_8900-H_NHANES_MF.b
Acq. Date-Time	12/1/2022 8:24:25 AM
Report Comment	—
Instrument Name	G3665A SG19131160

[O2 and H2 MS_MS mode]

Sensitivity



Mass	Range	Count	RSD%	Background
7 -> 7	100	50	16.233	0.000
89 -> 105	10000	6570	1.958	0.000
205 -> 205	10000	7306	2.214	0.000

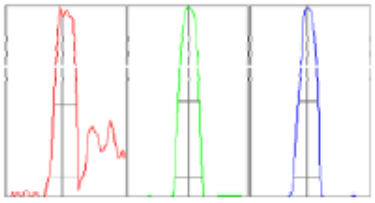
Sampling Period [sec] 0.31
Integration Time [sec] 0.1

Oxide/Doubly Charged Ratio

Oxide 156 -> 156 / 140 -> 140 1466.723 %
Doubly Charged 70 -> 70 / 140 -> 140 325.927 %

Resolution/Axis

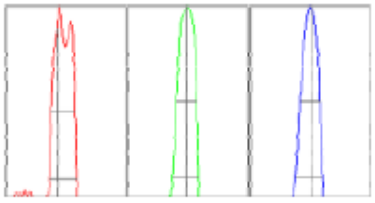
Q1



Mass	Peak Height	Axis	W-50%	W-10%
7	62,50	6,95	0,58	0,742
89	8277,22	89,05	0,54	0,691
205	8618,76	204,95	0,49	0,672

Integration Time [sec] 0.1
Acquisition Time [sec] 22.74
Y Axis Linear

Q2



Mass	Peak Height	Axis	W-50%	W-10%
7	56,00	6,80	0,60	0,683
105	9350,14	105,00	0,52	0,663
205	13691,65	205,05	0,51	0,684

Integration Time [sec] 0,1
Acquisition Time [sec] 22,64
Y Axis Linear

Tune Parameters

Plasma Parameters

Plasma Mode — Nebulizer Gas 1,07 L/min Makeup Gas 0,00 L/min

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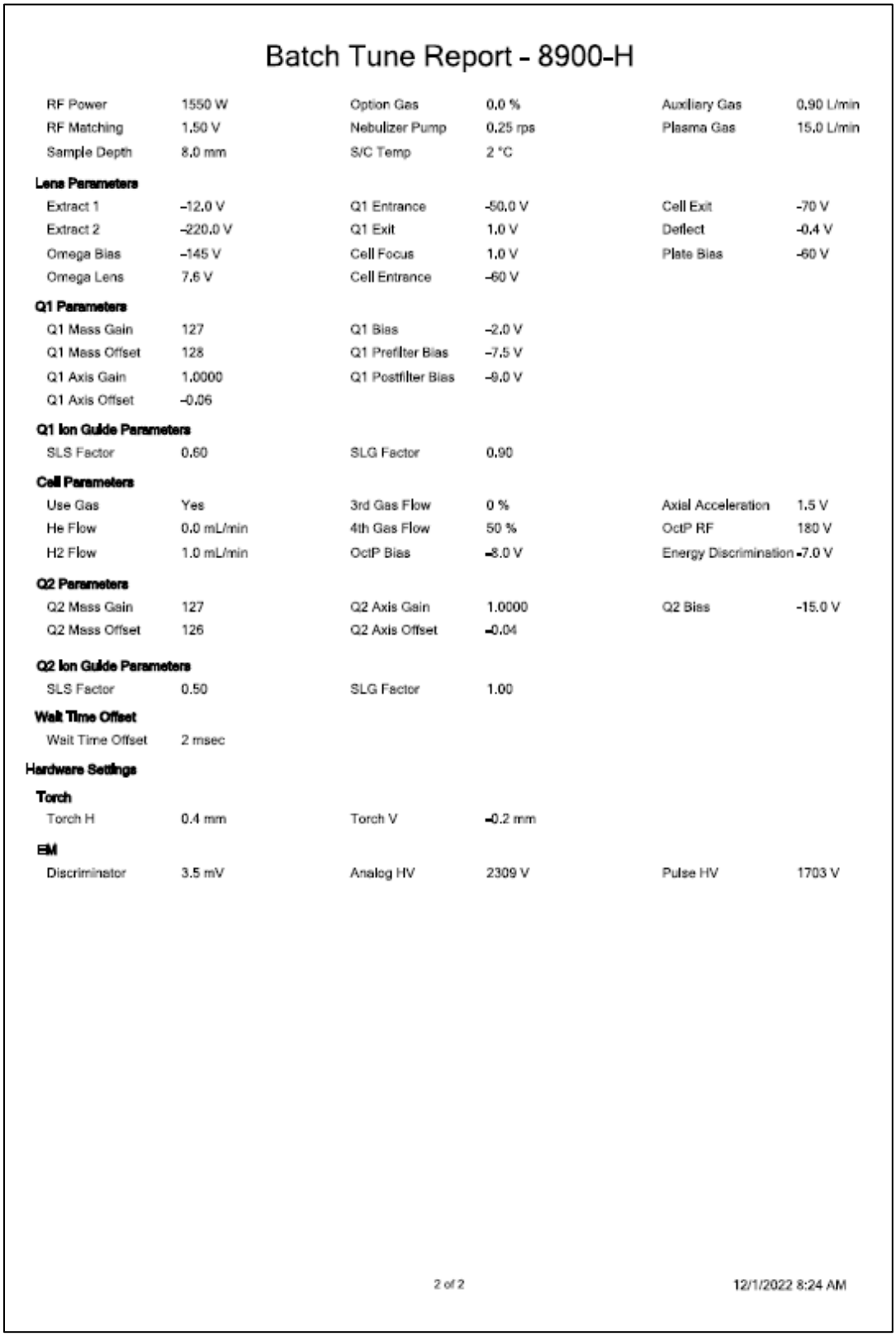
Figure 20. Example of instrument batch tune report, page 2.



Batch Tune Report - 8900-H

RF Power	1550 W	Option Gas	0.0 %	Auxiliary Gas	0.90 L/min
RF Matching	1.50 V	Nebulizer Pump	0.25 rps	Plasma Gas	15.0 L/min
Sample Depth	8.0 mm	S/C Temp	2 °C		
Lens Parameters					
Extract 1	–12.0 V	Q1 Entrance	-50.0 V	Cell Exit	-70 V
Extract 2	–220.0 V	Q1 Exit	1.0 V	Deflect	-0.4 V
Omega Bias	–145 V	Cell Focus	1.0 V	Plate Bias	-60 V
Omega Lens	7.6 V	Cell Entrance	-60 V		
Q1 Parameters					
Q1 Mass Gain	127	Q1 Bias	–2.0 V		
Q1 Mass Offset	128	Q1 Prefilter Bias	–7.5 V		
Q1 Axis Gain	1.0000	Q1 Postfilter Bias	–9.0 V		
Q1 Axis Offset	–0.06				
Q1 Ion Guide Parameters					
SLS Factor	0.60	SLG Factor	0.90		
Cell Parameters					
Use Gas	Yes	3rd Gas Flow	0 %	Axial Acceleration	1.5 V
He Flow	0.0 mL/min	4th Gas Flow	50 %	OctP RF	180 V
H2 Flow	1.0 mL/min	OctP Bias	-8.0 V	Energy Discrimination	-7.0 V
Q2 Parameters					
Q2 Mass Gain	127	Q2 Axis Gain	1.0000	Q2 Bias	-15.0 V
Q2 Mass Offset	126	Q2 Axis Offset	-0.04		
Q2 Ion Guide Parameters					
SLS Factor	0.50	SLG Factor	1.00		
Wait Time Offset					
Wait Time Offset	2 msec				
Hardware Settings					
Torch					
Torch H	0.4 mm	Torch V	-0.2 mm		
EM					
Discriminator	3.5 mV	Analog HV	2309 V	Pulse HV	1703 V

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Figure 21. Example exported calibration summary.

Tune Mode	Scan Type	Q1	Q2	Name	ISTD	R	a	b (blank)	DL	BEC	Units
O2 and H2 MS_MS mode	MS/MS	55	55	Mn	103 -> 103 Rh [O2 and H2 MS_MS mode]	0.99975823	0.01000874	0.07353419		7.34700076	ug/ L
O2 and H2 MS_MS mode	MS/MS	80	96	Se	130 -> 130 Te [O2 and H2 MS_MS mode]	0.9999945	0.00252241	0.3678226		145.82164	ug/ L
O2 and H2 MS_MS mode	MS/MS	111	111	Cd	193 -> 193 Ir [O2 and H2 MS_MS mode]	0.99998457	0.00245983	0.00133339		0.54206679	ug/ L
O2 and H2 MS_MS mode	MS/MS	200	200	Hg							ug/ L
O2 and H2 MS_MS mode	MS/MS	202	202	Hg	130 -> 130 Te [O2 and H2 MS_MS mode]	0.99999983	0.01657577	0.01161806		0.70090653	ug/ L
O2 and H2 MS_MS mode	MS/MS	206	206	Pb							ug/dL
O2 and H2 MS_MS mode	MS/MS	207	207	Pb							ug/dL
O2 and H2 MS_MS mode	MS/MS	208	208	Pb	193 -> 193 Ir [O2 and H2 MS_MS mode]	0.99980509	0.22210701	0.1796112		0.80866967	ug/dL
O2 and H2 MS_MS mode	MS/MS	103	103	Rh							
O2 and H2 MS_MS mode	MS/MS	130	130	Te							
O2 and H2 MS_MS mode	MS/MS	193	193	Ir							

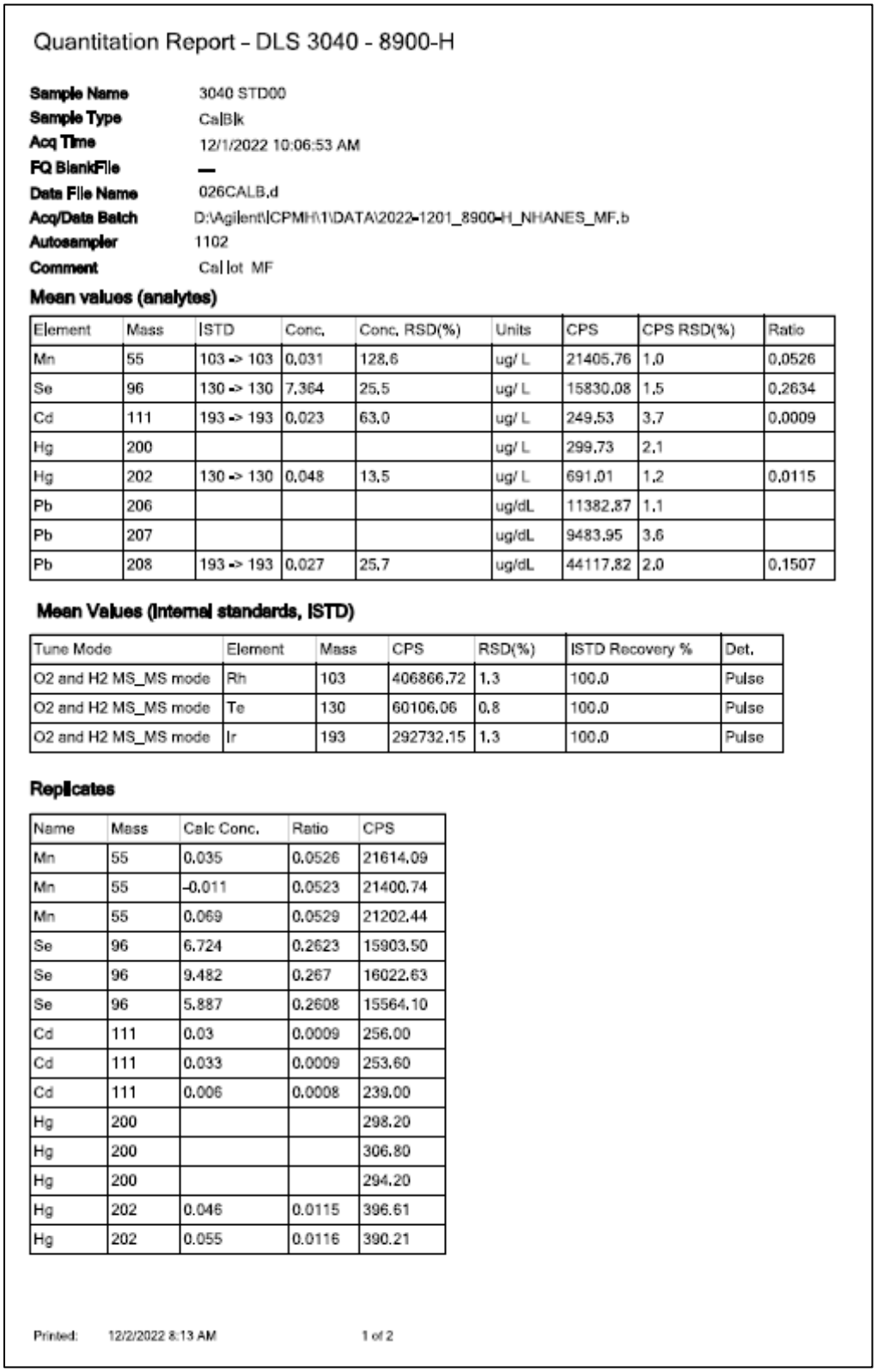
Figure 22. Example calibration information report.

Calibration for 099SMPL.d

Batch Folder: D:\Agilent\ICPMH\1\DATA\2022-1201_8900-H_NHANES_MF.b\
Analysis File: 2022-1201_8900-H_NHANES_MF.batch.bin
DA Date-Time: 12/2/2022 8:12:10 AM
Calibration Title:
Calibration Method: External Calibration
VIS Interpolation Fit:

Level	Standard Data File	Sample Name	Acq. Date-Time
1	027CALS.d	3040 STD01	12/1/2022 10:10:47 AM
2	028CALS.d	3040 STD02	12/1/2022 10:14:45 AM
3	029CALS.d	3040 STD03	12/1/2022 10:18:39 AM
4	030CALS.d	3040 STD04	12/1/2022 10:22:37 AM
5	031CALS.d	3040 STD05	12/1/2022 10:26:31 AM
6	032CALS.d	3040 STD06	12/1/2022 10:30:30 AM
7	033CALS.d	3040 STD07	12/1/2022 10:34:20 AM

Figure 23. Example analysis summary page.



Quantitation Report – DLS 3040 - 8900-H

Sample Name 3040 STD00
Sample Type Cal|Blk
Acq Time 12/1/2022 10:06:53 AM
FQ BlankFile —
Data File Name 026CALB.d
Acq/Data Batch D:\Agilent\ICPMH\1\DATA\2022-1201_8900-H_NHANES_MF.b
Autosampler 1102
Comment Cal|ot MF

Mean values (analytes)

Element	Mass	ISTD	Conc.	Conc. RSD(%)	Units	CPS	CPS RSD(%)	Ratio
Mn	55	103 -> 103	0.031	128.6	ug/ L	21405.76	1.0	0.0526
Se	96	130 -> 130	7.364	25.5	ug/ L	15830.08	1.5	0.2634
Cd	111	193 -> 193	0.023	63.0	ug/ L	249.53	3.7	0.0009
Hg	200				ug/ L	299.73	2.1	
Hg	202	130 -> 130	0.048	13.5	ug/ L	691.01	1.2	0.0115
Pb	206				ug/dL	11382.87	1.1	
Pb	207				ug/dL	9483.95	3.6	
Pb	208	193 -> 193	0.027	25.7	ug/dL	44117.82	2.0	0.1507

Mean Values (Internal standards, ISTD)

Tune Mode	Element	Mass	CPS	RSD(%)	ISTD Recovery %	Det.
O2 and H2 MS_MS mode	Rh	103	406866.72	1.3	100.0	Pulse
O2 and H2 MS_MS mode	Te	130	60106.06	0.8	100.0	Pulse
O2 and H2 MS_MS mode	Ir	193	292732.15	1.3	100.0	Pulse

Replicates

Name	Mass	Calc Conc.	Ratio	CPS
Mn	55	0.035	0.0526	21614.09
Mn	55	-0.011	0.0523	21400.74
Mn	55	0.069	0.0529	21202.44
Se	96	6.724	0.2623	15903.50
Se	96	9.482	0.267	16022.63
Se	96	5.887	0.2608	15564.10
Cd	111	0.03	0.0009	256.00
Cd	111	0.033	0.0009	253.60
Cd	111	0.006	0.0008	239.00
Hg	200			298.20
Hg	200			306.80
Hg	200			294.20
Hg	202	0.046	0.0115	396.61
Hg	202	0.055	0.0116	390.21

Printed: 12/2/2022 8:13 AM 1 of 2

Figure 24. Example LIMS output settings for data type (sample)

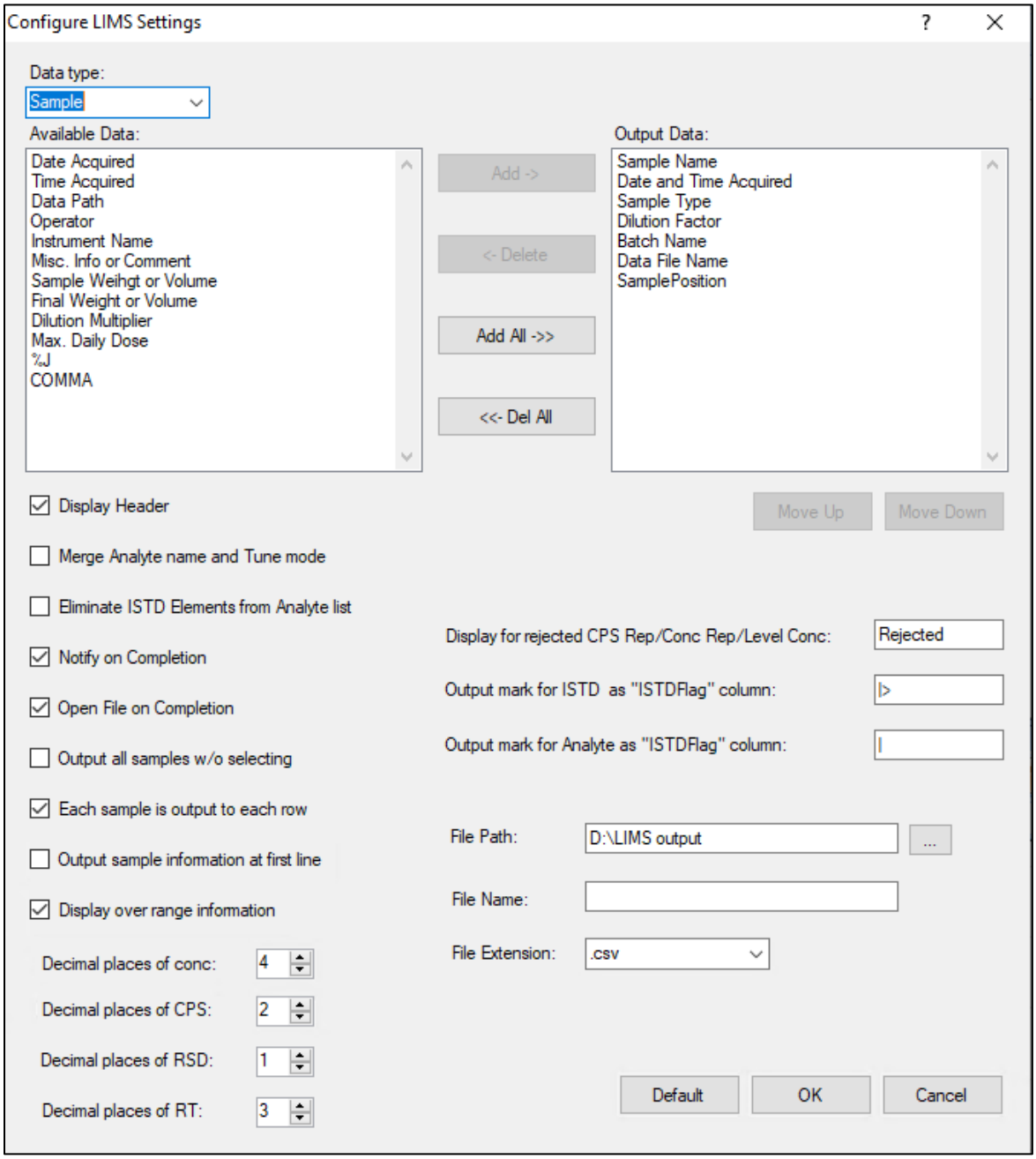


Figure 25. Example of LIMS output settings for data type (analyte).

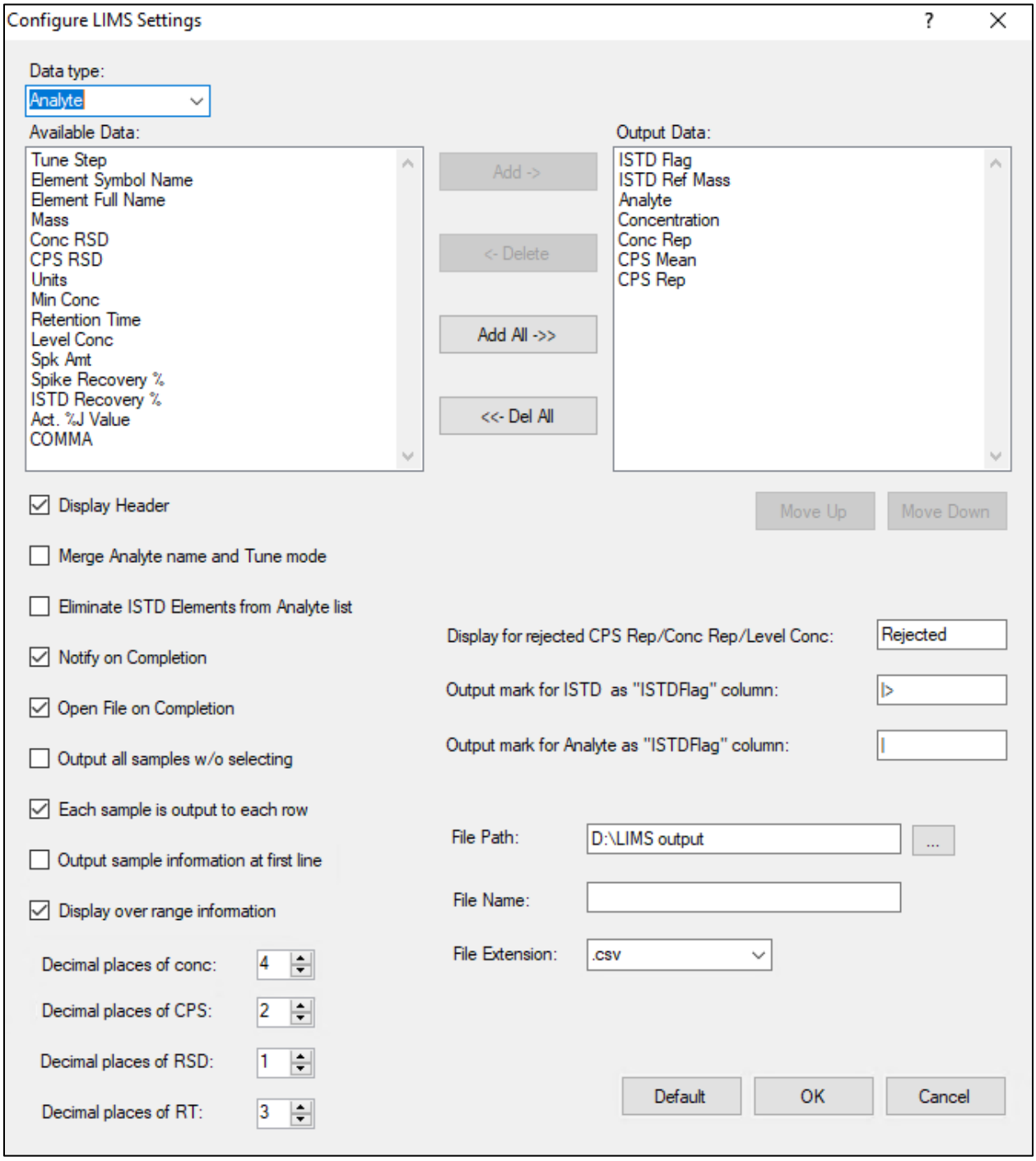


Figure 26. Example data format in instrument run file.

26a) For each measurement in the run the file must contain this information in this comma-delimited format. This format is required by the STARLIMS spec schema mapping sample schema action.

Sample Name, Date and Time Acquired, Sample Type, Dilution Factor, Batch Name, Data File Name,SamplePosition,ISTD Flag,ISTD Ref Mass,Analyte,Concentration,Conc Rep1,Conc Rep2,Conc Rep3,CPS Mean,CPS Rep1,CPS Rep2,CPS Rep3, …

(Repeat ISTD through CPS Rep3 fields for each analyte and internal standard)

26b) Notepad format of the an example file.

```
Sample Name,Date and Time Acquired,Sample Type,Dilution Factor,Batch Name,Data File Name,SamplePosition
Analyte,Concentration,Conc Rep1,Conc Rep2,Conc Rep3,CPS Mean,CPS Rep1,CPS Rep2,CPS Rep3,ISTD Flag,ISTD
3040 STD00,11/30/2022 4:36:42 PM,CalBlk,1,2022-1130_8900-C_InstrComp_KW.b,022CALB.d,1102,|,103,Mn,4.968
3040 STD01,11/30/2022 4:40:35 PM,CalStd,1,2022-1130_8900-C_InstrComp_KW.b,023CALS.d,1103,|,103,Mn,0.354
3040 STD02,11/30/2022 4:44:34 PM,CalStd,1,2022-1130_8900-C_InstrComp_KW.b,024CALS.d,1104,|,103,Mn,0.470
3040 STD03,11/30/2022 4:48:27 PM,CalStd,1,2022-1130_8900-C_InstrComp_KW.b,025CALS.d,1105,|,103,Mn,1.704
3040 STD04,11/30/2022 4:52:25 PM,CalStd,1,2022-1130_8900-C_InstrComp_KW.b,026CALS.d,1106,|,103,Mn,6.013
3040 STD05,11/30/2022 4:56:19 PM,CalStd,1,2022-1130_8900-C_InstrComp_KW.b,027CALS.d,1107,|,103,Mn,59.37
3040 STD06,11/30/2022 5:00:18 PM,CalStd,1,2022-1130_8900-C_InstrComp_KW.b,028CALS.d,1108,|,103,Mn,197.3
3040 STD07,11/30/2022 5:04:12 PM,CalStd,1,2022-1130_8900-C_InstrComp_KW.b,029CALS.d,1109,|,103,Mn,390.4
```

26c) Excel spreadsheet format of an example file.

Sample N	Date and T	Sample Ty	Dilution F	Batch Nan	Data File I	SamplePo	ISTD Flag	ISTD Ref N	Analyte	Concentra	Conc Rep1	Conc Rep2	Conc Rep3	CPS Mean	CPS Rep1	CPS Rep2	CPS Rep3
3040 STD0	########	CalBlk	1	2022-1130	022CALB.d	1102	|	103	Mn	4.9681	4.6263	5.1261	5.1519	29056.14	28809.32	29606.5	28752.59
3040 STD0	########	CalStd	1	2022-1130	023CALS.d	1103	|	103	Mn	0.3545	0.3844	0.3171	0.3618	19046.16	19533.49	19050.81	18554.17
3040 STD0	########	CalStd	1	2022-1130	024CALS.d	1104	|	103	Mn	0.4706	0.554	0.4322	0.4256	19593.25	20179.4	19529.48	19070.87
3040 STD0	########	CalStd	1	2022-1130	025CALS.d	1105	|	103	Mn	1.7041	1.654	1.7309	1.7273	21951.02	22445.79	22049.16	21358.12
3040 STD0	########	CalStd	1	2022-1130	026CALS.d	1106	|	103	Mn	6.0133	6.029	6.0565	5.9544	31323.64	32039.23	31433.86	30497.83
3040 STD0	########	CalStd	1	2022-1130	027CALS.d	1107	|	103	Mn	59.3751	58.8117	59.336	59.9777	144579.1	147947.4	144065.7	141724.2
3040 STD0	########	CalStd	1	2022-1130	028CALS.d	1108	|	103	Mn	197.3186	195.8324	198.4664	197.657	442012.3	448802.4	442302.2	434932.4
3040 STD0	########	CalStd	1	2022-1130	029CALS.d	1109	|	103	Mn	390.4426	389.8948	390.302	391.131	860034.3	880909.2	858030.5	841163.1

20. Appendix F. Routine function checks and maintenance

Analysts are expected to optimize instrument parameters, as needed, each operating day to maintain the ICP-MS performance that meets or exceeds specifications described by the manufacturer. The terms instrument function check and performance check are used interchangeably in this document.

Further information detailing maintenance instructions and troubleshooting can be found in the Agilent 8900 Triple Quadrupole ICP-MS Hardware Maintenance Manual [75], the ESI SC Installation and Software Guide [76], and the Agilent 8900 Triple Quadrupole ICP-MS MassHunter Workstation User Guide [77] manuals. These manufacturer guides are referenced in the instructions below as needed.

Document pre-ignition functions checks, inspections, performance checks, and maintenance information in the electronic instrument function check and maintenance log sheets (see Figures 27 and 28) which summarize the manufacturer and CDC recommendations. Document additional details (e.g,. results of troubleshooting) in the instrument notebook, and the instrument 3-ring binder. Upload instrument electronic function check and maintenance log sheets and service records at least bi-annually in STARLIMS.

To place a service call with Agilent either enter a request through the CDC CrossLab program service portal at https://reporting.chem.agilent.com/cdc/index.html, or call Agilent Service line at 1 (877)-467-6262.

A. Pre-start inspection before igniting the plasma of the ICP-MS

i. Record Date and Initials in the instrument logbook and on the pre-start table.

ii. Inspect the autosampler probe tubing for leaks and damaged tubing. Replace any damaged tubing or connections. [76]

iii. Inspect the Peristaltic pump tubing for flat spots or damage in both the carrier and waste tubing paying special attention to the carrier tubing. Replace if damaged, kinked, or worn flat. [75]

iv. Inspect the instrument waste collection carboy. If waste carboy is at ~3/4 full, follow waste decontamination and disposal procedures [78] to empty. Record in the pre-start table the volume and pH of the final waste solution prior to discard at the sink. Record adjustments made to the waste pH in the instrument logbook.

v. Argon gas delivery pressure to the instrument. Inspect the delivery regulator at the instrument is within acceptable range 475-725kPa [typically 90 (±10) psi]. Adjust the pressure at the regulator if needed to remain within target pressure range. If problems with low pressure, verify that the pressure at the in-line regulator between the instrument and the bulk tank source is 10-20 psi higher than the regulator at the instrument (e.g., 90-120 psi) and that the regulator at the bulk tank is at least 10-20 psi higher than the in-line regulator (typically at 130-170 psi). [75]

vi. Hydrogen and oxygen reaction cell gas delivery pressures to the instrument. Verify at the regulators that the pressures are in the within acceptable range 20-60kPa (7 – 9 psi). Adjust the pressure at the regulators if needed to remain within target pressure range.

vii. Check and record the Analyzer vacuum Pressure (kPa) before plasma ignition in the pre-start table. Typical pressure before the plasma is ignited is 1 x 10^{-5} Pa to 7 x 10^{-4} Pa. If pressure is >7 x 10^{-4} Pa or trending over days toward being >7 x 10^{-4} torr, then place a service call. [77]

viii. Install the peristaltic pump tubing and set the clamp tension as described in the Agilent hardware manual [75].

ix. With the instrument in Standby mode, from the middle screen, click the dropdown arrow next to the "Autosampler" button. Uncheck "Use Autosampler".

x. Click the Plasma gadget to see the startup pane. Click on "Select Custom Settings". Verify the "Vial #" says "Manual".

xi. Check the "On" box for these optimizations and the function check "Performance Report".

1) Torch Axis
2) EM
3) Plasma Correction
4) Standard Lenses Tune
5) Resolution/Axis
6) Performance Report (standard function check)

xii. Click the dropdown arrow next to the "Plasma" gadget and click on "configure ignition sequence".

1) Select the boxes to "Run Startup on Ignition" and "Wait for Warmup".
2) Select the radio button for the "Standard Tune" mode.

xiii. Set the carrier probe in the Agilent standard tuning solution (1 µg/L Li, Co, Ce, Y, Tl. See Appendix D).

xiv. Ignite the Plasma. Follow instructions from the software user guide [77] to start the plasma and run the "Startup" initiation sequence of optimizations including the Performance Report.

1) Torch Axis
2) EM
3) Plasma Correction
4) Standard Lenses Tune
5) Resolution/Axis
6) Performance Report (function check)

B. <u>Function checks after igniting the plasma of the ICP-MS</u>

i. After the plasma ignites, allow instrument to warm up for 20 minutes (referred to as the warm-up time in the instrument software). Typical pressure after the plasma is ignited is 1 x 10^{-4} to 2 x 10^{-3} Pa [77]. If the pressure is greater than that or is trending over days toward being greater than that, it may indicate that the orifice of the cones have enlarged and that the cones need to be replaced at the next scheduled periodic maintenence. If the function checks (e.g., sensitivity, precision, oxides, etc.) pass the expected criteria the cones can continue to be used. If the high operating pressure is not fixed by replacing the cones, place a service call.

ii. Check and record the analyzer vacuum pressure (Pa) in the function check table.

iii. Allow the “startup” initiation sequence to run the optimizations and the Performance Report [75].

1) Torch Axis

2) EM

3) Plasma Correction

4) Standard Lenses Tune

5) Resolution/Axis

6) Performance Report (function check)

iv. After the optimizations and Performance Report are complete, evaluate the results of the performance report against the Agilent instrument performance specifications to determine if the instrument is performing to manufacturer specifications. Use the method’s normal 0.38 mm id (orange-green) peripump tubing size for this function check. Though the Agilent instrument performance specifications are written with the expectation that a 1.02 mm id (white-white) peripump tubing will be used, which will deliver more liquid to the plasma and result in higher intensities, method validation history demonstrated that we typically meet the manufacturer specifications using the narrower tubing. The typical performance observed by within IRAT EAL during method validation is summarized along with the Agilent performance specifications in a sheet within the Excel function check and maintenance log file. The instrument should pass against the Agilent specifications before using it for analysis by this method. Comparison against the IRAT EAL specifications is for information only.

1) If the Performance Check results are acceptable, record the results in the function check table.

2) If the results do not meet the Agilent performance specifications, troubleshoot the instrument parameters and hardware and record troubleshooting actions in the instrument notebook. Refer to Appendix B in the Agilent hardware manual [75] for more detailed information or the Agilent Software Manual [77]. It is acceptable to troubleshoot instrument performance issues using the Agilent standard pump tubing (1.02 mm id wht-wht) to create a direct comparison against the Agilent specifications and historical performance of the instrument at the time it was installed. If troubleshooting and use of 1.02 mm id sample tubing fails to achieve performance that meets the Agilent performance specifications, place a service call.

v. After completing an acceptable Performance Report, create a new Batch folder from an existing DLS 3040 batch folder use the naming format “YYYY-MMDD_instrument_method_project detail_initials”.

vi. In the Batch Acquisition Method “Tune” tab, set the Nebulizer Gas flow to match the optimized nebulizer gas flow from the standard Performance Report.

vii. Save the batch.

viii. With the carrier probe still in the Agilent standard tune solution, click on “Autotune” to optimize the batch-specific lens and cell voltages and perform a Batch Tune check.

ix. After the Batch Tune check is complete, the software will autogenerate and save a .PDF file of the optimized batch tune results. Save the Batch file.

x. Remove the carrier probe from the tune solution and place it in the DLS 3040 carrier solution.

xi. Click the dropdown arrow next to the “Autosampler” button. Check “Use Autosampler”.

xii. Click save batch. Make any modifications necessary to the batch file “Sample” tab and the batch is ready to run. Click on “Add to Queue” to start the analysis of the batch beginning with sample row. [75]

C. Equipment maintenance

i. 5 run day instrument maintenance

1) Sampler cone. Inspect the outer surface of the sampler cone for debris and deformation at the orifice of the cone. If damaged, replace the cone. If large amounts of debris or dirt are present at the orifice, clean and re-use or replace the cone. [75]

2) Skimmer cone. If inspection of the sampler cone indicates cleaning or replacement, closely inspect the surface and orifice of the skimmer cone. [75]

ii. 10-15 run day instrument maintenance

1) Verify network backups of the data on the instrument computer

a) Nightly ISLE backup from instrument computer to the to the ‘Metals’ Z: drive. The Z:\Metals drive is a network attached storage (NAS) drive that is physically in the laboratory. Data from the instrument computer should occur nightly at the time scheduled in the SyncBack software running on the instrument computer (e.g. 3-4 am).

(i) Open the SyncBack software on the instrument computer.

1. Verify the last run date matches today’s date for the scheduled morning time (e.g., 3-4 am) and has a result of ‘success’ and that the next scheduled run is 24 hours later.
2. Verify that at least the following folders are in the ‘backup’ group.
    a. Desktop (e.g., Admin\Desktop\)
    b. Documents (e.g., Admin\Documents\)
    c. ESI_SC (e.g., C:\Program Files (x86)\ESI\ESI SC\)
    d. ICPMH (e.g., D:\Agilent\ICPMH\)
    e. LIMS output (e.g., D:\LIMS output\)

(ii) Open the ISLE-networked ‘Metals’ backup drive “Z:\” and navigate to the instrument subfolder under the folder Z:\InstrumentData. Verify the latest backup took place by comparing the ‘date modified’ for some of the latest created content (e.g., ELAN datasets) on the Z: drive matches the expected date from the instrument computer.If backups are not happening for expected folders each day at the expected time fix correct the settings in the SyncBack software. Submit a LIST ticket in STARLIMS to fix the backup, if necessary.

b) Weekly CDC-network backup of the ISLE network attached storage (NAS) drive.From a computer connected to the CDC network, open the instrument subfolder on the CDC network MUST SHARE drive folder ONDIEH_IRATBACKUP-RO\Instrumentdata. Verify the backups are taking place by comparing the ‘date modified’ for content created recently (e.g., ELAN datasets) on the ONDIEH_IRATBACKUP-RO folder with the expected date from

the instrument computer. If newest content is more than 1 week old, submit a LIST ticket in STARLIMS to fix the backup.

2) Nebulizer. Remove and inspect the nebulizer for debris, matrix build-up, chips, cracks, or breaks. Clean and re-use or replace the nebulizer if damaged. If the nebulizer was replaced, run the nebulizer test. [75]

3) Spray chamber and end cap. Remove and inspect the spray chamber and end cap for debris, matrix build-up, chips, cracks, or breaks. Clean and re-use or replace if damaged. [75]

4) Transfer pipe/connector tube. Remove and inspect the tube for chips, cracks, or breaks. Clean and re-use or replace if damaged. [75]

5) Torch/Injector and bonnet. Remove and inspect the torch/injector for matrix build-up on the interior channel, chips, cracks, breaks or deformation. Clean and re-use or replace the torch if damaged or deformed. If deformation is observed, carefully inspect the RF coil and coil alignment. Remove and inspect the torch bonnet for damage or deformation. Replace if damaged or deformed. If deformation is observed, carefully inspect the RF coil and coil alignment. [75]

6) Pt electrode / shield (platinum shield). Remove and inspect. Replace if deformed. [75]

7) Shield contact. Inspect the shield contact and torch area for contamination. If dirty, clean the area. [75]

8) RF coil. Inspect the surface of the RF coil for damage, severe discoloration, and water leaks. Inspect the alignment and spacing of the individual turnings of the coil. [75]

9) Sampler cone. Remove and inspect the outer surface of the sampler cone for debris and deformation at the cone orifice. Inspect the cone for an enlarged orifice. If damaged, replace the cone. If large amounts of debris or dirt are present at the orifice, clean and re-use or replace the cone. [75]

10) Skimmer cone. Remove and inspect the surface and orifice of the skimmer cone for debris, damage or an enlarged orifice. Clean and re-use or replace the cone if damaged. [75]

11) Graphite gasket (sampler cone). Inspect the graphite gasket in place. If the gasket is in good condition, leave in place and work carefully so as not to damage the gasket. Replace the gasket if it is damaged, creased, or has fissures. [75]

12) Ion lens stack (x-lens). Remove and inspect the ion lenses, spacers, and supports. Clean and replace the lenses and spacers if dirty or damaged. [75]

13) Octopole reaction cell gas cyinder internal pressure. Check and document the internal gas pressures on the cylinder regulators for hydrogen and oxygen. If the internal pressure is <200 psi, replace the cylinder.

iii. Monthly maintenance

1) Computer operating system updates. Run Windows updates manually on the instrument computer desktop through the “Windows10-Updater” shortcut. Log into the computer either in person or over the KVM switch. Follow the prompts to complete the update. Submit a LIST ticket in STARLIMS if problems are encountered with this process.

2) Autosampler FAST valve. Perform disassembly, cleaning, and reassembly of the P-port FAST valve as described in the ESI SC Installation and Software Guide section "Maintenance for P-Series FAST Valve" [75].

3) Optimization: Detector P/A Factor. Once per month, perform the P/A factor optimization using the P/A factor tune solution described in section 6.N. The P/A factor optimization may be performed more frequently as needed to maintain calibration linearity for analytes measured >1,000,000 cps in the calibration curve.

4) Optimization: RF (coil) matching. Once per month, perform the RF matching optimization using the Agilent standard tune solution. The RF optimization may be performed more frequently as needed or after the RF coil or RF generator is replaced. [75]

5) Detector: Check the EM Analog and Pulse stage voltages as recorded in the "Hardware Settings/Tune Parameters" section of the Performance Report. [75]

a) Record the Analog HV and Pulse HV stage voltages on the "Other Maint. Log" log sheet.

b) Place a service call to replace the EM detector before either the Analog HV reaches 3500V or the Pulse HV reaches 2000V.

6) Chiller: Inspect fluid level. Add fluid, if below minimum fluid level. Drain and replace fluid annually. (4) Cooling fluid is replaced as part of the Preventative Maintenance (PM) checklist performed by Agilent.

7) Foreline pump oil (MS40+). Inspect oil level and color. [75] Add fluid if oil level is below minimum fluid level. Replace pump oil every 6 months (±30 days). An oil replacement is included as part of an Agilent Preventative Maintenance (PM) performed by Agilent engineers.

iv. Biannual maintenance

1) Autosampler FAST vacuum check. Check the autosampler vacuum pump is achieving a flow rate of >60mL/min. [76]

a) Power on the autosampler and instrument computer.

b) Open the ESI software and initialize the autosampler.

c) Disconnect the tubing from FAST port #6 that connects the FAST valve to the FAST pump intake connection (port#6).

d) Fill a 50mL centrifuge tube with at least 40mL of ≥18.0 MΩ·cm water. Weigh the water-filled tube and record the initial mass in grams (accuracy to ± 1g is sufficient).

e) In the ESI software, open the manual control window. Under Dx FAST control, click on "Vacuum1 On".

f) Place the end of the port#6 tubing in the water and allow the vacuum to pull for 30 seconds. After 30 seconds, remove the probe from the tube of water.

g) In the ESI software manual control window click "Vacuum1 Off".

h) Weigh the tube+water and record the final mass in grams (accuracy to ± 1g is sufficient).

i) Subtract the final mass from the initial mass in grams to find the amount of water removed by the vacuum pump. Assume the density of water is 1 g/mL and convert the grams of water to mL.

j) The vacuum pump is acceptable if the water removed by the vacuum pump is >27 mL i.e., the water flow rate is >60mL/min ±10%.

k) If the water removed by the vacuum pump is <60mL/min ±10%, place a service call to replace the vacuum pump of the autosampler.

2) Foreline pump oil (MS40+). Replace pump oil every 6 months (±30 days).[75] An oil replacement may be included as part of an Agilent Preventative Maintenance (PM) performed by Agilent engineers.

v. Annual maintenance

1) Argon gas purifier and filter. Inspect and replace annually. Inspection and replacement are performed as part of the Agilent preventative maintenance (PM) checklist. [75]

2) Plasma gas and Auxillary gas tubing. Inspect annually for wear or damage. Place service call if replacement is needed.[75]

3) Chiller: cooling fluid and water strainer (also called the water mesh filter). Replace cooling fluid annually. Clean and inspect water filter annually, replace if damaged. Maintenance activities to drain and replace cooling fluid and clean the water strainer (filter) are performed as part of the PM checklist. [75]

4) Foreline pump oil mist filter (MS40+). Replace annually. Replacement of the pump oil mist filter is performed as part of the PM checklist. [75]

Figure 27. Instrument electronic function check and maintenance log sheet (checks at each use and every 10-15 days).

Instrument name: _____ **Year** _____

Information in the pre-start and performance tables below are only required on days the instrument was used.

Monthly supervisory review. Enter Date and user ID.	Jan	Feb	Mar	Apr	May	Jun
	Jul	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**

Pre-Start Inspection - inspect the following items each run day before powering on Instrument Plasma.
(Unless otherwise indicated, I=Inspect; R=Replaced)

Instrument Use Date										
Laboratorian user ID										
Autosampler: probe tubing - Inspect for leaks or damaged tubing. Replace if damaged										
Peri Tubing carrier (S), waste (W) - inspect tubing for flat spots or										
Waste carboy inspect, if emptied record the volume and pH										
Argon Gas pressure - record delivery pressure to instrument.										
DRC gas pressure to instrument, verified, changed (psi)										
Analyzer Vacuum Press. in Pa. (**Before** plasma ignition)										

Perform the following after the pre-start Inspection at the beginning of each run day or after hardware maintenance is performed.
Performance Records - Record results in the table below from Performance Report collected with 0.38 mm id [org-grn] peristaltic carrier/sample pump tubing installed

If no samples analyzed, mark with an 'X'										
Analyzer Vacuum Press. in Pa. (**After** plasma ignition)										
Run initiation sequence*, mark with an 'X'										
Nebulizer Gas (L/min)										
Extract 2 (V)										
Omega Lens (V)										
OctP RF (V)										
Li (counts)¥										
Y (counts)¥										
Tl (counts)¥										
Li % RSD¥										
Y % RSD¥										
Tl % RSD¥										
CeO %¥										
Ce^{++} %¥										
Max background at 7/89/205 amu (max cps)¥										
Other notes (optional) add longer text as comments on the cell										

*includes torch axis, EM, Plasma correction, standard lens tune, Resolution/Axis, and Performance Report

¥ IRAT EAL criteria for Performance specs are in "IRAT EAL 8900 Performance Report criteria" version 1

5-run day Instrument Maintenance - After every 5 run days, complete the following list. This may be done more frequently if components are damaged or dirty or to improve instrument performance. **(Unless otherwise indicated, I=Inspect; R=Replaced; C=Cleaned and re-used)**

	Date and user ID	
Sampler Cone - Every 5 run days, inspect sampler cone for debris/ deformation. Replace or clean and re-use if damaged.		
Skimmer Cone - Every 5 run days, if sampler cone was replaced, inspect skimmer cone for debris/ deformation. Replace or clean and re-use if damaged.		

Periodic Instrument Maintenance - After 10 run days (10-15) complete the following maintenance and optimizations. If the action has already been performed on run days 6-9 in the table immediately above, the maintenance does not need to be repeated - simply record the date it was performed in the table immediately below. **(Unless otherwise indicated, I=Inspect; R=Replaced; C=Cleaned and re-used)**

	Date and user ID
Computer backup: verify ISLE backup, verify CDC-network backup - Verify the backup is performed. If backup is not operating properly, manually run SyncBack software and place LIST ticket. Record details in Notebook.	
Nebulizer - Clean and replace if debris or matrix build-up is present or Neb test fails. If replaced, run Nebulizer Test. (NT=Neb Test)	
Spray Chamber and endcap - Replace or clean and re-use	
Transfer pipe/Connector tube - Replace or clean and re-use	
Torch/Injector & bonnet - Replace or clean and re-use	
Pt electrode/Shield - Inspect; replace if damaged or deformed.	
Shield contact - Inspect; clean if dirty.	
RF Coil - Inspect for damage or leaks. Replace RF coil if damaged.	
Graphite gasket (sampler cone) - Inspect; replace if damaged or bent.	
Lens stack - Inspect; replace or clean and re-use if dirty.	
DRC gas cylinder internal pressure - check and document psi, replace and document psi if low (<200 psi oxygen, <200 psi hydrogen)	

Figure 28. Instrument electronic function check and maintenance log sheet (1-12 month events).

Instrument name: _____ **Year** _____

After 1 year of use copy the 'Other Maint. Log TEMP' sheet as a new sheet and update the year

Bi-annually (6 months ± 1 month), complete the following.		
Task Details	**Date and User ID**	**Date and User ID**
Autosampler FAST vacuum - Every 6 months - check vacuum flow is pulling minimum 60 mL/min ≥18MΩ·cm water with 3.2mm tubing (port#6). Place service call to schedule pump replacement if vacuum is below minimum.		
Foreline pump oil (MS40+). Replace oil.		

Annually, verify the following tasks are completed. If any not included in the Preventative Maintenance Visit (PM) by Agilent, place a service call or complete by lab staff.	
Task Details	**Date and User ID**
Argon Gas filter - Annual (at PMV) replacement	
Plasma gas and Auxilary gas tubing - Annual (at PMV) inspection and replacement if worn/damaged.	
Chiller: water strainer - Annual (at PMV) inspection and cleaning.	
Foreline pump: oil mist filter (MS40+) - Annual (at PMV) replacement.	

Monthly, complete the following list of tasks, described more fully in Appenix F of the method procedure manual.
Document below including user ID / name and date performed. Document also in the instrument notebook, especially longer notes (e.g., troubleshooting).

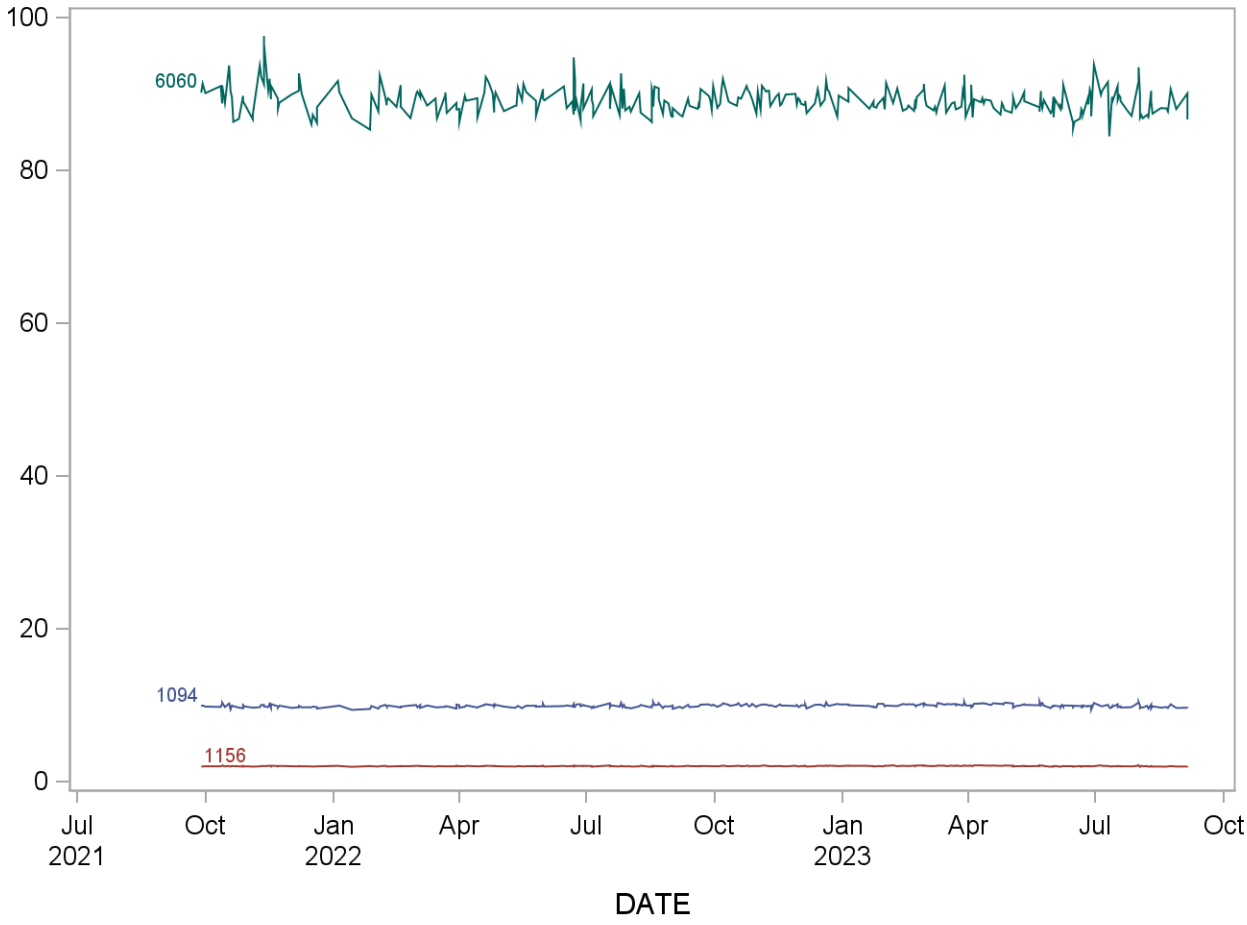
Month	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Computer operating system - Update the OS. Note when done along with date and user ID.												
Autosampler FAST valve - Dissassemble and clean interior face of rotor and stator. Note clean and re-used (C) or replaced (R), date and user ID / name.												
Optimization: Detector PA Factor - Note optimized (O), date and user ID / name.												
Optimization: RF (coil) matching - Note optimized (O), date and user ID / name.												
Electron Multiplier (EM) - Record pulse (P ####) and analog (A ####) voltages along with date and user ID / name. Place service call to replace EM if A voltage >3500 or P voltage >2000.												
Chiller: cooling fluid level and condition - Inspect (I), add fluid if below minimum fluid level (A), replace annually (R or R-PMV) along with date and user ID / name.												
Foreline pump: oil level and color - Inspect (I), add fluid if below minimum fluid level (A), Replace if dark, or every 6 months (R). Note date and user ID / name.												
Monthly supervisory review. Enter Date and user ID.												

21. Summary Statistics and QC Graph

Please see following pages.

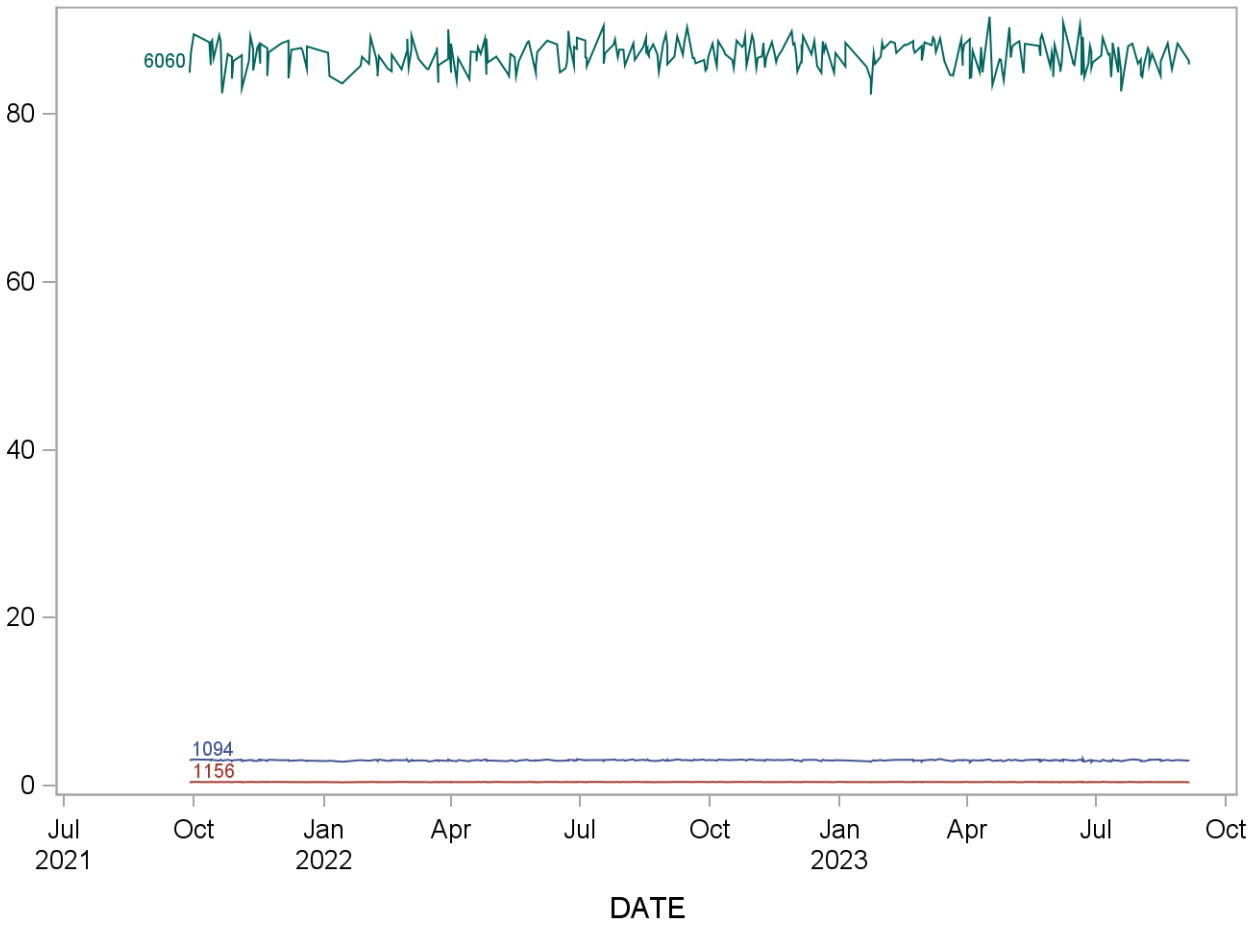
August 2021 – August 2023 Summary Statistics and QC Chart
LBXBPB (Blood lead (µg/dL))

Lot	N	Start Date	End Date	MEAN	Standard Deviation	Coefficient of Variation
1094	315	28SEP21	05SEP23	9.9312	0.1987	2.0
1156	315	28SEP21	05SEP23	2.0399	0.0423	2.1
6060	315	28SEP21	05SEP23	89.1415	1.7078	1.9



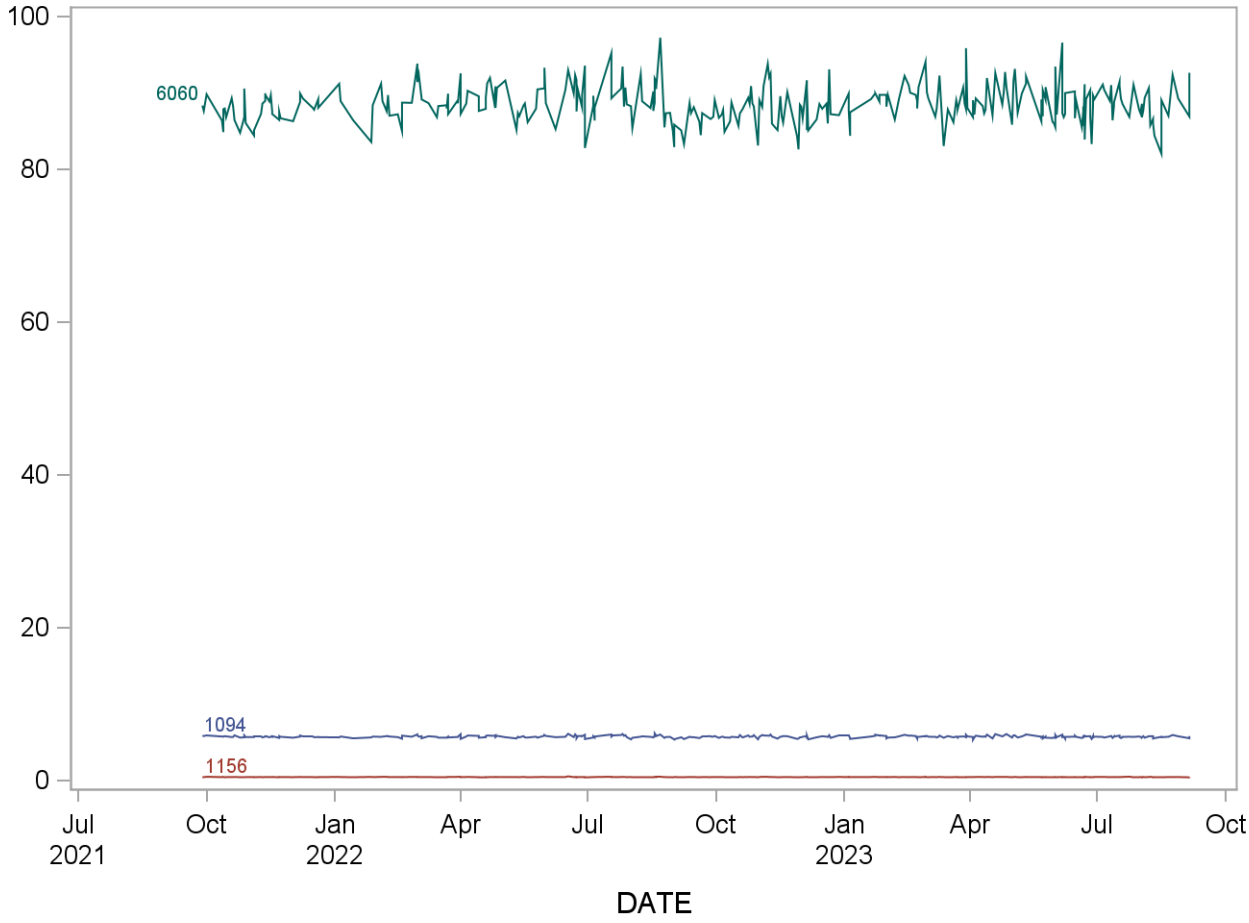
August 2021 – August 2023 Summary Statistics and QC Chart
LBXBCD (Blood cadmium (µg/L))

Lot	N	Start Date	End Date	MEAN	Standard Deviation	Coefficient of Variation
1094	315	28SEP21	05SEP23	3.0200	0.0661	2.2
1156	315	28SEP21	05SEP23	0.4343	0.0097	2.2
6060	315	28SEP21	05SEP23	86.9452	1.6316	1.9



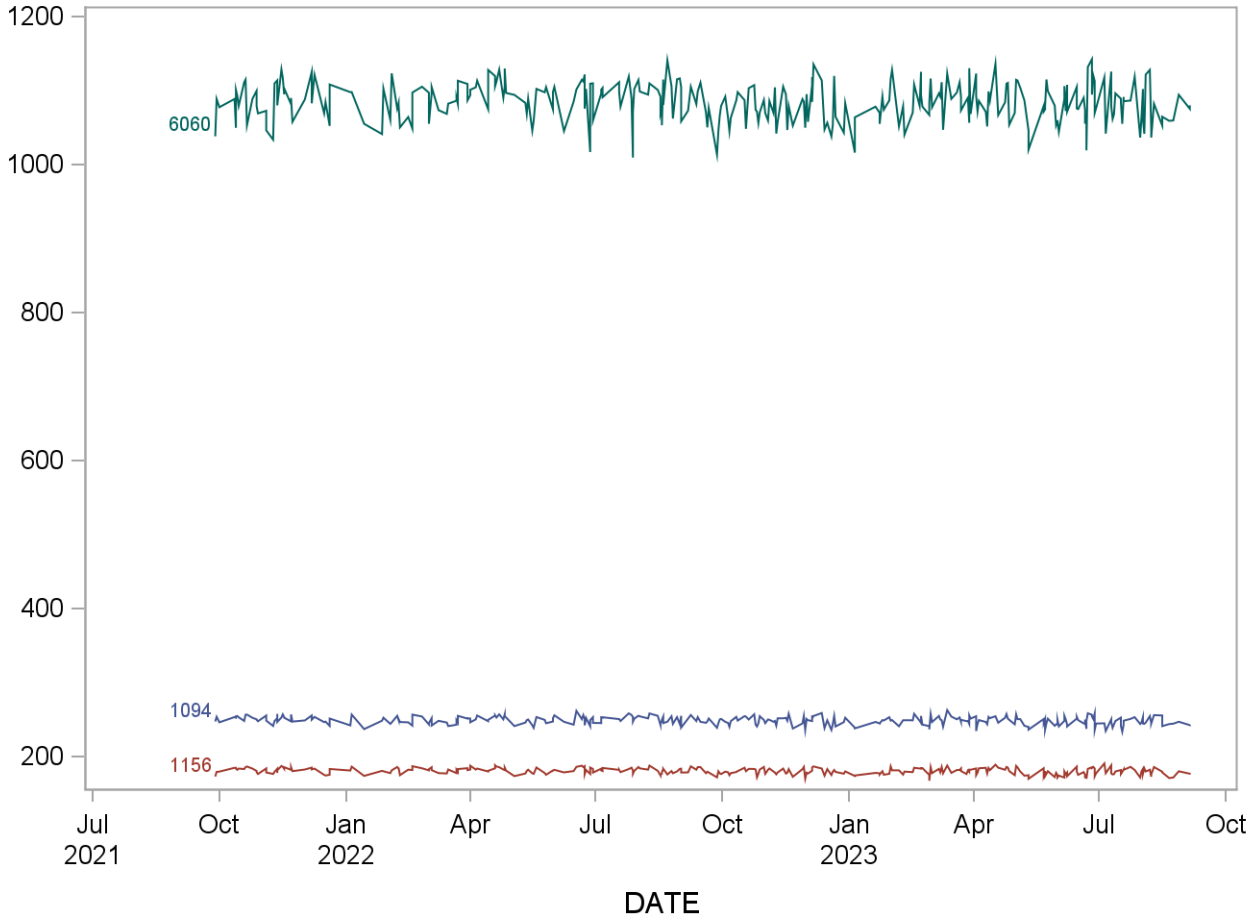
August 2021 – August 2023 Summary Statistics and QC Chart
LBXTHG (Blood mercury,total (µg/L))

Lot	N	Start Date	End Date	MEAN	Standard Deviation	Coefficient of Variation
1094	317	28SEP21	05SEP23	5.764	0.145	2.5
1156	317	28SEP21	05SEP23	0.474	0.016	3.4
6060	317	28SEP21	05SEP23	88.588	2.443	2.8



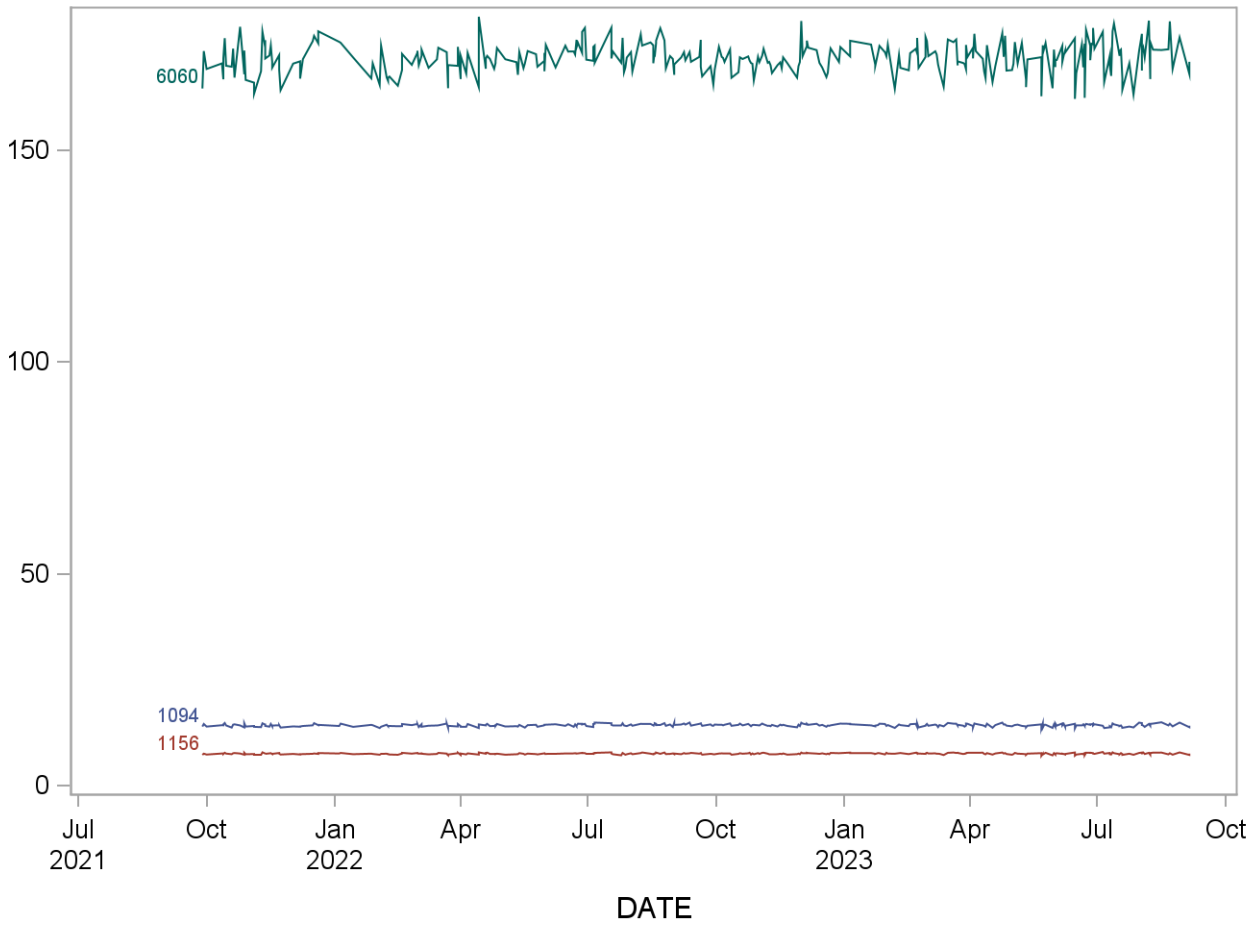
August 2021 – August 2023 Summary Statistics and QC Chart
LBXBSE (Blood selenium (µg/L))

Lot	N	Start Date	End Date	MEAN	Standard Deviation	Coefficient of Variation
1094	314	28SEP21	05SEP23	248.774	5.516	2.2
1156	314	28SEP21	05SEP23	180.434	4.277	2.4
6060	314	28SEP21	05SEP23	1084.077	25.647	2.4



August 2021 – August 2023 Summary Statistics and QC Chart
LBXBMN (Blood manganese (µg/L))

Lot	N	Start Date	End Date	MEAN	Standard Deviation	Coefficient of Variation
1094	317	28SEP21	05SEP23	14.283	0.306	2.1
1156	317	28SEP21	05SEP23	7.565	0.158	2.1
6060	317	28SEP21	05SEP23	171.805	3.536	2.1



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