Laboratory Procedure Manual

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

Matrix: **Whole Blood**

Method: blood multi-element analysis by a triple quadrupole inductively coupled plasma mass spectrometer (ICP-QQQ-MS)

Method No: DLS 3040.1-02

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As performed by: Inorganic and Radiation Analytical Toxicology Branch
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Important Information for Users

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

Public Release Data Set Information

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

Data File Name	Variable Name	SAS Label
PBCD_K PBY_K_R	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 even at blood lead levels <5 µg/dL [7-12]. 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 [13]. The alkyl lead species are highly toxic to the central nervous system [14]. The primary screening method for lead exposure is blood lead, which primarily reflects recent exposures (excretory half-life in blood is approximately 30 days) [15]. 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 5 µg/dL based on NHANES cycles 2007-2008 and 2009-2010 [16].

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. 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.

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.

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

A. Procedures for collecting, storing, and handling samples

Sample handling conditions, special requirements, and procedures for collection and transport are discussed in the Division of Laboratory Science's (DLS) Policies and Procedures Manual[1] and have been published by CDC[62]. In general:

i. Sample type – whole blood

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 labeled as "metal free," "for trace elements," or "for lead testing." The designation of "sterile" does not indicate that the device is free of metals contamination.

iv. Include one or more field blanks (empty collection tubes) so potential metal contamination can be evaluated in the analytical phase.

v. Avoid heparin anticoagulant; EDTA anticoagulant is preferable.

vi. Draw the blood through a stainless steel needle into a pre-screened vacutainer

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

viii. Do not freeze blood in blood collection tubes because it could cause them to crack. Transfer blood/blood products to plastic pre-screened cryo-vials before freezing. Store samples in blood tubes (, evacuated tubes) in refrigerator at approximately 4˚C and ship on cold packs. Store blood samples in vials (i.e., cryo-vials) preferably at ≤20˚C and ship frozen (i.e., on cold packs or dry ice). Sample stability in sealed polypropylene vials has been demonstrated for over 4 years at ≤ -70 °C by storage of quality control materials[63]. 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 (e.g., 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 (e.g., 23 °C and 37 °C), making them unacceptable for elemental testing after 10 months and 2 months, respectively.

ix. Acceptable containers for analytical aliquots include lot screened polypropylene (PP) cryo-vials or tubes (e.g., 2 to 5 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.

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

xi. Criteria for sample rejection

The criteria for an unacceptable sample include

xii. 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).

xiii. 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.

xiv. Low Volume: Optimal amount of sample is 1+ mL. Request a minimum volume of 0.25 mL. Volume for one analytical measurement is 0.05 mL.

In all cases, request a second blood sample.

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

Location, status, and final disposition of the samples will be tracked at least by paper document in the "Study Folder" (created before analysts receive the samples). Apart from this sample tracking form, this folder will also contain the paper print outs of results from analysis of the samples. Maintain records for a minimum of 3 years. Use only numerical identifiers for samples within the laboratory (e.g., case ID numbers) in order to safeguard confidentiality. Access to personal identifiers for samples will be limited to the medical supervisor or project coordinator (e.g., non-CDC personnel).

4. Safety precautions

A. General safety

i. Observe all safety regulations as detailed in the Laboratory Safety Manual and the Chemical Hygiene Plan. Participate in training regarding 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. Take special care when handling and dispensing concentrated bases and acids. Use additional personal protective equipment which protects face, neck, and front of body. If 25% w/w TMAH or concentrated hydrochloric acid comes in contact with any part of the body, quickly wash with copious quantities of water for at least 15 minutes.

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

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

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

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

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

B. Waste disposal

i. Autoclaving: Autoclave all diluted biological samples, original biological samples being disposed, or consumables which come into contact with biological samples (even diluted or aerosolized). Use sharps containers or special autoclave pans for broken glass / quartz or items which puncture autoclave bags (e.g., pipette tips).

ii. Other liquid waste

1) Waste discarded down sink: Do not discard solutions at the sink having a pH lower than 5.0 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 liquid waste generated in the CDC laboratory for this method.

5. Instrument & material sources

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.icpms.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.icpms.com)

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.

1) Female nut: for 1.6mm O.D. (1/16") tubing. Like part P-420 (Upchurch Scientific, Oak Harbor, WA, www.upchurch.com)

2) PEEK ferrule: Like part P-260x (10pk SuperFlangeless ferrule, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com).

3) Conical Adapter Body: Like part P-692 (Upchurch Scientific, Oak Harbor, WA, www.upchurch.com)

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

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

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

v. 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

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

vii. 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

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

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

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

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

xii. 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).

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

xiv. 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 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). # Spares = 2.

xv. 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 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xvi. 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).

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

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

xix. 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).

xx. 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.

xxi. 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

xxii. 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

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

xxiv. Torch: Agilent part #G3280-80053 (Agilent, Santa Clara, CA, www.agilent.com) # spares = 4

xxv. 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

xxvi. 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

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

C. Sources for ICP-MS maintenance equipment & 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. 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).

viii. 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.

ix. 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.

x. 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.

xi. 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) 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, 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.

xvii. 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).

xviii. 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.

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

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

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

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

6. Preparation of reagents and materials

A. Internal standard intermediate mixture:

i. Purpose: Preparation of 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) If not previously dedicated to this purpose, acid wash a 50 mL volumetric flask (PP, PMP, or Teflon™). For example, with 1% v/v HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.

2) Partially fill the 50 mL volumetric flask with ≥18 Mohm·cm water (approximately 25-40 mL).

3) Add 0.5 mL of high purity concentrated nitric acid and mix thoroughly.

4) 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.

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

6) Store at ambient temperature and label appropriately. Expiration is 1 year from date of preparation.

B. Intermediate Triton™ X-100 solution:

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

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

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

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 and label appropriately. Expiration is 1 year from date of preparation.

C. 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. Make all samples, standards, blanks, and QC 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 in 1.0% v/v TMAH, 1% ethanol, and 0.05% Triton™ X-100:

1) If not previously dedicated to this purpose, acid wash a 2L container (PP, PMP, or Teflon™). For example, with 1% v/v HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.

2) Partially fill the 2 L container with ≥18 Mohm·cm water.

3) Add 0.2 g of APDC, 20 mL of concentrated (25% w/w) TMAH, 20 mL of ethanol, and 5 mL of 20% Triton™ X-100.

4) Dilute to volume (2L) with ≥18 Mohm·cm water.

5) Spike 500 µL of 20 mg/L Rh, Ir, Te to the final diluent.

6) 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.

7) Store at ambient temperature and label appropriately. Expiration is 1 year from date of preparation.

D. 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) If not previously dedicated to this purpose, acid wash a 4L container (PP, PMP, or Teflon™). For example, with 1% v/v HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.

2) Partially fill the 4 L bottle with ≥18 Mohm·cm water (approximately 2-3 L). Use of volumetric flask is not required.

3) Add 0.4 g of APDC

4) Add 40 mL of 25% v/v TMAH

5) Add 40 mL of ethanol,

6) Add 10mL of 20% Triton™ X-100, (See Section 6.b for details on preparation)

7) Fill to 4 L using ≥18 Mohm·cm water.

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 and label appropriately. Expiration is 1 year from date of preparation.

E. 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 as smaller portions for daily use (e.g., 2 mL cryovials) according to the same storing and handling criteria described in Section 3.

F. 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 and label appropriately. Expiration is as defined by the manufacturer or 1 year from date of opening, whichever comes first.

G. 3% v/v HCl diluent:

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) If not previously dedicated to this purpose, acid wash a 2L volumetric flask (PP, PMP, or Teflon™). For example, with 3% v/v HCl and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.

2) In the 2 L flask, add 1-1.5L ≥18 Mohm·cm water.

3) Add 60 mL high purity concentrated HCl.

4) Fill to the mark and mix thoroughly.

5) Store at ambient temperature and label appropriately. Expiration is 1 year from date of preparation.

H. 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) If not previously dedicated to this purpose, acid-rinse one 100 mL PP (or PMP) volumetric flask. For example, with 3% v/v HCl and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Mark flask according to intended use. Dedicate to purpose.

2) Partially fill (50-75% full) the 100 mL flask with the 3% v/v HCl diluent prepared in Section 6.G. 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. 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.

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

4) Store at ambient temperature and label appropriately. Expiration is 1 year from date of preparation.

I. 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) If not previously dedicated to this purpose, acid-rinse one 100 mL PP (or PMP) volumetric flask. For example, with 3% v/v HCl and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Mark flask according to intended use. Dedicate to purpose.

2) Partially fill (50-75% full) the 100 mL flask with the 3% v/v HCl diluent prepared in Section 6.G. 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. 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.

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

4) Store at ambient temperature and label appropriately. Expiration is 1 year from date of preparation.

J. 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) If not previously dedicated to this purpose, acid-rinse seven 100 mL, PP (or PMP) volumetric flasks. For example, with 3% v/v HCl and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Mark each flask according to intended use. Dedicate to purpose.

2) Fill each 100 mL flask 50-75% with the 3% v/v HCl diluent prepared in Section 6.G.

3) 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. 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-cleaned, labeled containers (PP, PMP, or Teflon™) for storage.

5) Store at ambient temperature and label appropriately. Expiration is 1 year from date of preparation.

6) Pour aliquots of each standard into smaller containers (PP, PMP, or Teflon™) and label for daily use.

K. 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.

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 and label appropriately before analysis, within 24 hours of preparation.

L. 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 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[64]).

d) Mix the blood in the carboys using stirrers and large stir plates. Keep blood refrigerated whenever possible.

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).

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

e) 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.

f) 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.

g) Approximately one hour before dispensing begins,

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

i) 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.

j) 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.

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

l) 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 3.

M. 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) Store at ambient temperature and prepare as needed.

6) Label appropriately. Expiration date is one year from preparation date.

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) Store at ambient temperature and prepare as needed.

6) Label appropriately. Expiration date is one year from preparation date.

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) Store at ambient temperature and prepare as needed.

6) Label appropriately. Expiration date is one year from preparation date.

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) Store at ambient temperature and prepare as needed.

6) Label appropriately. Expiration date is one year from preparation date.

N. P/A Factor tune solution

i. Purpose: Use as necessary to perform the P/A factor tune. 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. To prepare a total of 50 mL of the multi-element solution:

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

2) Add 0.1 mL of multi-element stock standard.

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

4) Label appropriately. Expiration date is one year from preparation date.

7. 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 73 – 102 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.

8. 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. Before QC materials can be used in the QC process, they must be characterized by at least twenty (20) analytical runs to determine appropriate QC parameters. Bench QC pool analyte concentrations in this method span the analyte concentration range of the calibrators including "low-normal" ('Low QC'), "high-normal" ('High QC'), and "above-normal" ('Elevated QC') concentrations.

In each analytical run the analyst will test each of the three bench QC samples two times, subjecting them to the complete analytical process. Bench QC pool samples are analyzed first in the run after the 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. 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 seven NIST-traceable calibrators. These calibrators, along with the QCs and blanks, are used to verify that the test system is performing properly. Because the system's calibration procedure includes three or more levels of calibration material, and includes low, mid, and high value, and is performed at least once every six months, the requirement for calibration verification is met.

B. Execution: perform, evaluate and report a run

i. Starting the equipment for a run:

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

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

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

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

5) Place probe in adequate volume of carrier or rinse solution: lf 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).

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

7) Warm-up time: Allow warm-up time suggested by the manufacturer for the ICP-MS (e.g., RF generator) after igniting the plasma.

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

9) Reaction gas stability time: Best analyte-to-internal standard ratio stability is typically observed after one hours 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) 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.

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

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

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

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

1) Thaw blood samples; allow them to reach ambient temperature.

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.

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

b) Calibrators: Prepare the working calibrators (S0-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.

c) 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.

3) 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.

4) Ambient temperature storage is acceptable for the original samples during the work day.

5) 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).

vii. 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).

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 following steps are recommended:

a) Evaluate the blank results.

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

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

d) Evaluate calibration curves. If a particular calibration standard is obviously in error, it can be re-analyzed as a sample (old or new dilution) and incorporated into the curve through data reprocessing as a calibrator. As a last resort, a single calibration point per analyte can be removed from the curve. 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).

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

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

g) Prepare and analyze new working calibrators.

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

i) 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.

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.

(i) 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.

1. Stop run following elevated sample.

2. 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

(ii) lf the run is not verified in-control for low concentration samples before the next samples are analyzed, see Section 8.C.ii.1)b) 6or directions.

viii. 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.

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

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

a) Export data from the ICP-MS software

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

c) lmport the instrument file into the laboratory information system.

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

a) Analyst initials

b) lnstrument lD

c) Date of analysis and run # for the day

C. Evaluation: Analyst evaluation of run results:

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

1) Rules for bench quality control evaluation: The following are the CDC DLS QC rules for three QC pools per run with two or more QC results per pool. (Note: Si = Standard deviation of individual results; Sm = Standard deviation of the run means; Sw = Within-run standard deviation)

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: lf 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.

1. 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.

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

(ii) Results greater than highest calibrator: Samples that exceed the high calibrator must be prepared with minimum extra dilution in duplicate to bring the observed result within the calibration range (< S7). Report the first analytically valid result (i.e., the first one within the calibration range), 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.

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

(ii) lf 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

2) Unacceptable reproducibility: lf the range of the three replicate readings (maximum replicate concentration value - minimum replicate concentration value) for a single sample analysis is greater than the range maximum criteria listed in Table 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. After appropriately documenting the run in the laboratory information system (e.g., sample and run QC, and run and sample comments), inform the first level reviewer of the completed work and submit any printed documentation.

9. Routine equipment maintenance and data backups

Maintenance activities will be documented in the instrument logbook.

A. Equipment maintenance:

Analysts are expected to regularly evaluate and maintain the equipment and document that maintenance. When necessary clean, reposition, or replace components in the ICP-MS (e.g., components of the sample introduction system, interface, and ion optics), maintain a clean and functioning autosampler and chiller, maintain the computer, and ensure proper supply of exhaust and compressed gases. Frequency of equipment maintenance will be dependent on instrument throughput.

B. Parameter optimizations:

i. Analysts are expected to optimize instrument parameters, as needed, each day of analysis to maintain the ICP-MS performance comparable to specifications described by the manufacturer and those observed during method validation. See the manufacturer user manual for recommended daily optimizations. Typical parameters include torch axis alignment, detector EM voltages, plasma correction (i.e., nebulizer gas flow), standard lenses tune (i.e., ion optics), resolution axis (i.e., quadrupole resolution).

ii. P/A factor tune: Perform the P/A factor tune regularly. Preparation of P/A factor tune solution is described in Section 6.N. Frequency of tune is typically every six months when throughput requires multiple analytical runs per week, or as needed for optimized linearity.

iii. 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:

Files used and produced by the ICP-MS during sample analysis will be backed up via two backup routines and kept a minimum of two years after analysis to prevent loss of data from failure of primary hard drive.

i. Daily backups to a secondary hard drive: Setup automatic backups of the relevant computer files to occur daily either to a network location or onto a secondary hard drive within the instrument control computer.

ii. Backups to removable media: Backup relevant computer files regularly (e.g., every 5-10 analytical runs) to removable media such as CD or DVD. Store them outside of the laboratory.

10. 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: Experts now use a reference level of 5 μg/dL 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 2007-2008 and 2009-2010. 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[65]

ii. Cadmium: Levels of concern for cadmium in blood is >5 μg/L[66, 67]

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)[67].

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[68, 69]

11. 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:

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

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

If the analytical system fails, 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 3 until the analytical system can be restored to functionality.

13. Method performance documentation

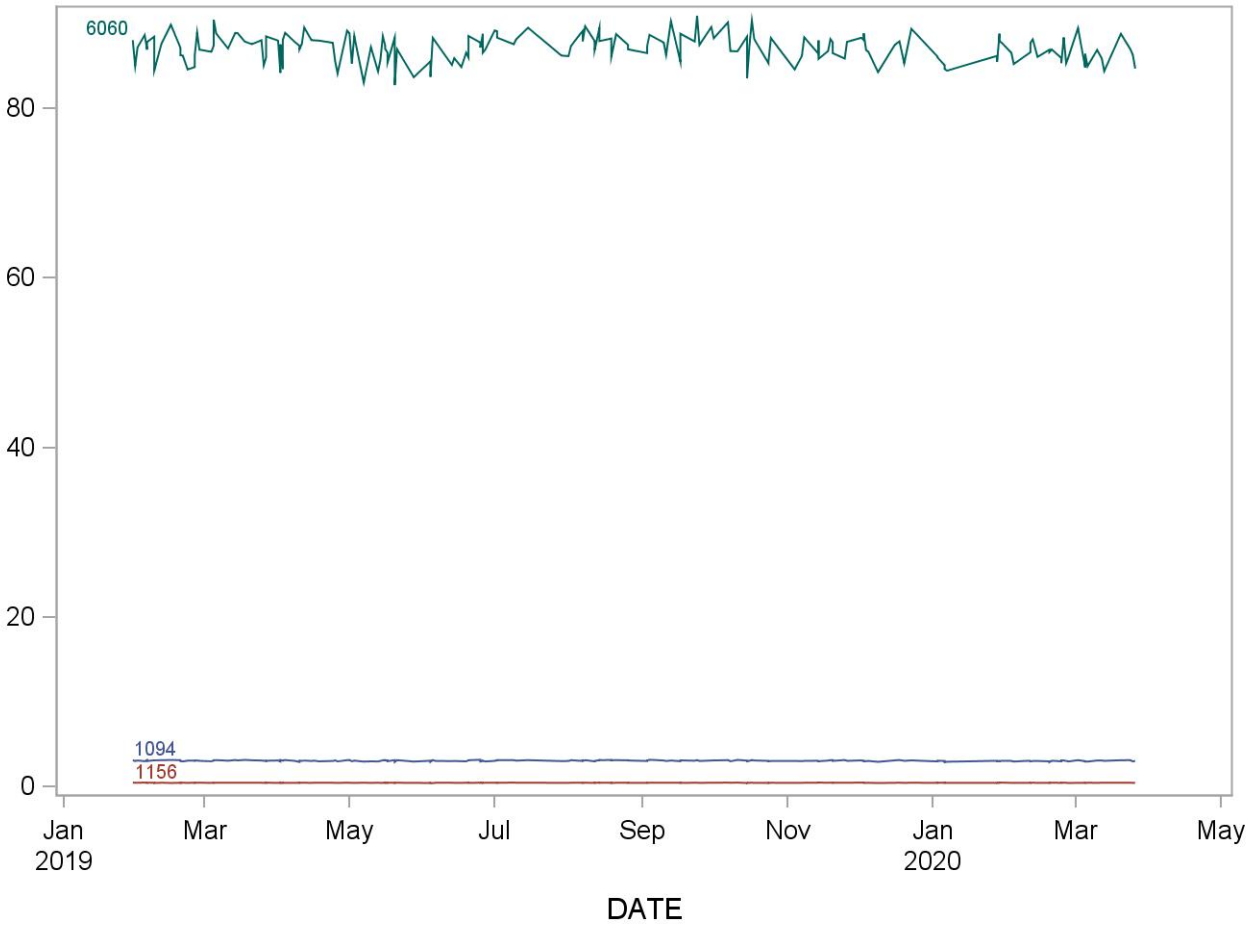
Method performance documentation for this method including accuracy, precision, sensitivity, specificity, and stability is provided in Appendix A of this method documentation. **The signatures of the branch chief and director of the Division of Laboratory Sciences on the first page of this procedure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

14. Summary Statistics and QC Charts

Please see following pages.

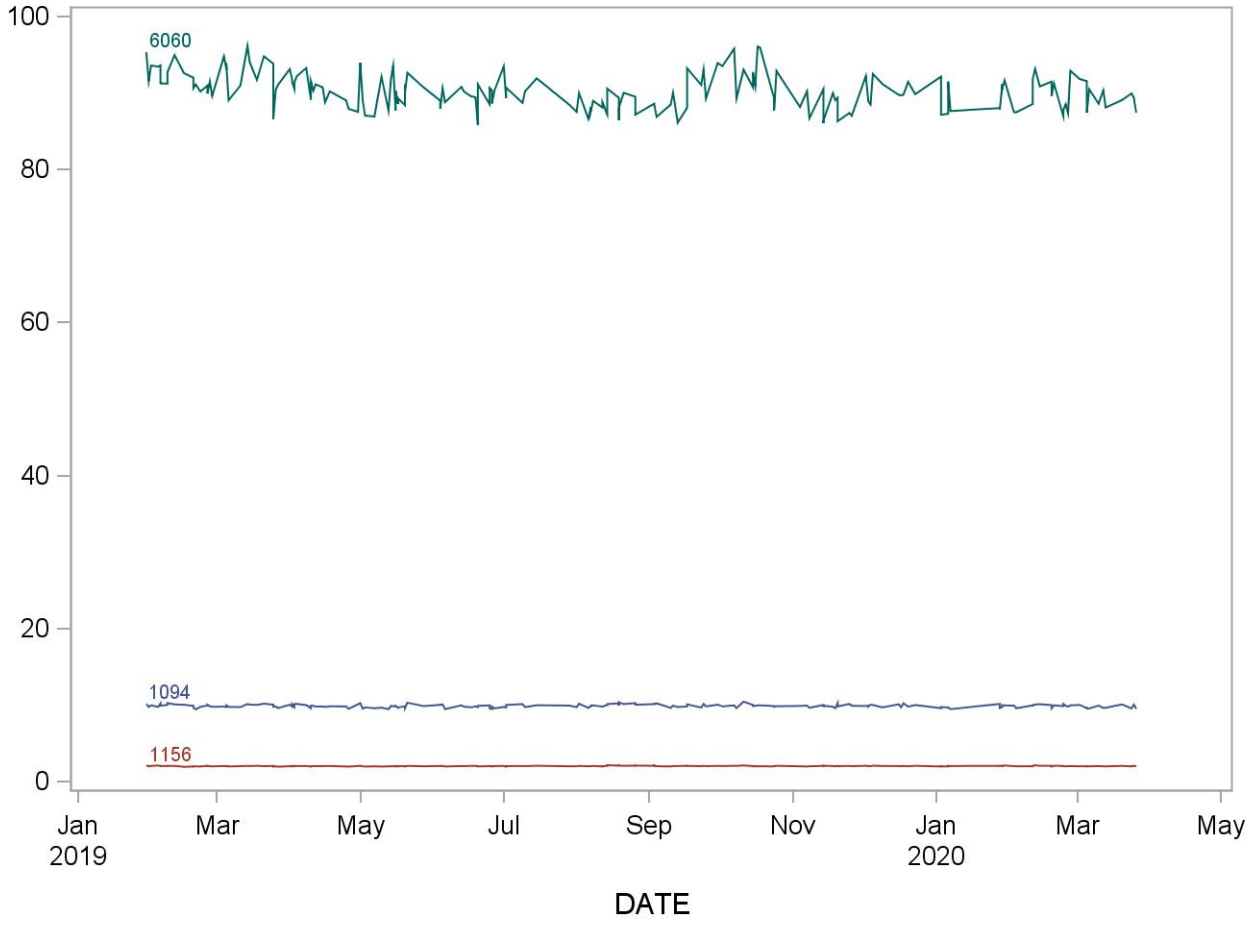
2019-2020 Summary Statistics and QC Chart
LBXBCD (Blood cadmium (µg/L))

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
1094	188	30JAN19	26MAR20	3.0391	0.0614	2.0
1156	188	30JAN19	26MAR20	0.4368	0.0090	2.1
6060	188	30JAN19	26MAR20	87.1043	1.5989	1.8



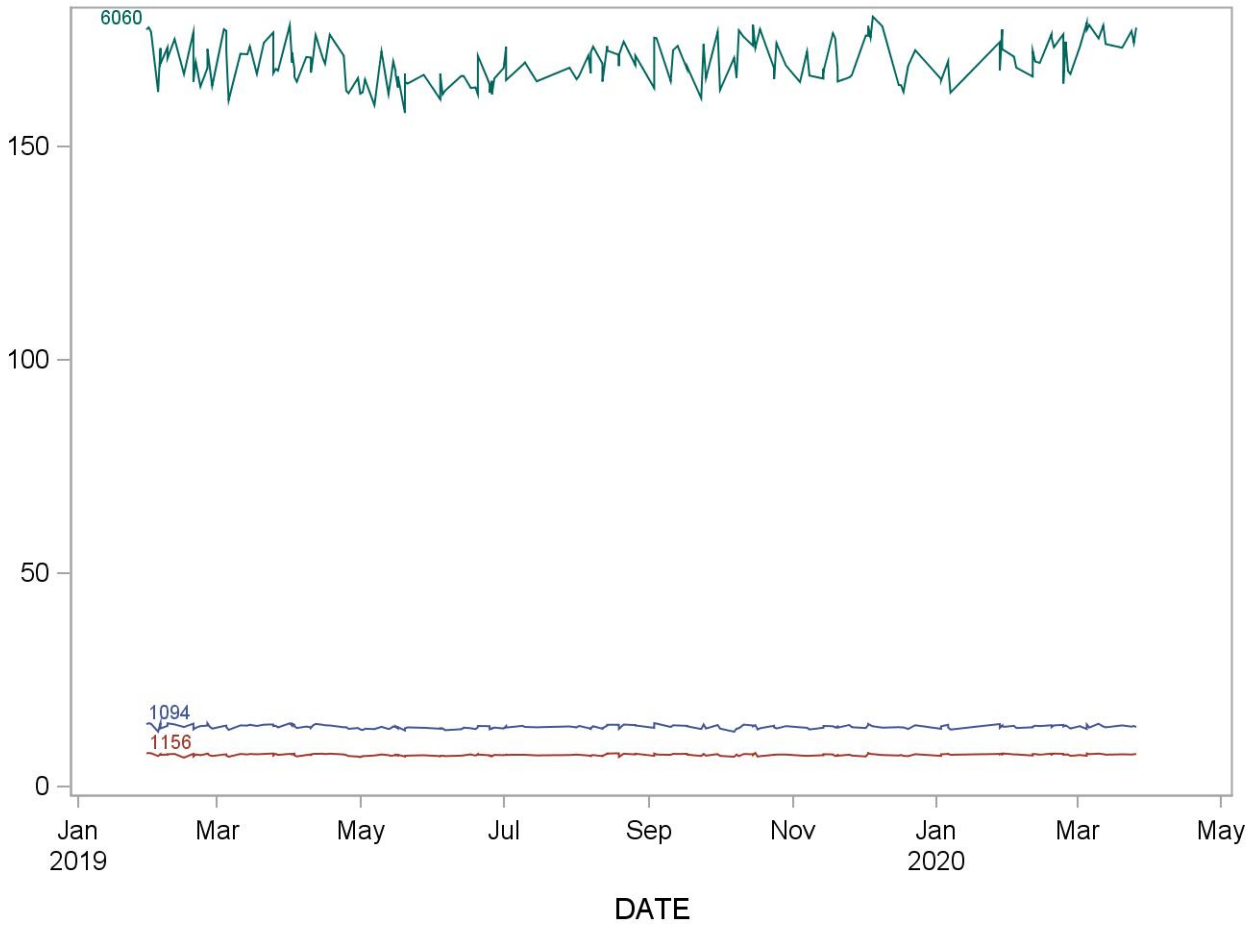
2019-2020 Summary Statistics and QC Chart
LBXBPB (Blood lead (µg/dL))

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
1094	187	30JAN19	26MAR20	9.8909	0.2053	2.1
1156	187	30JAN19	26MAR20	2.0348	0.0370	1.8
6060	187	30JAN19	26MAR20	90.2177	2.2802	2.5



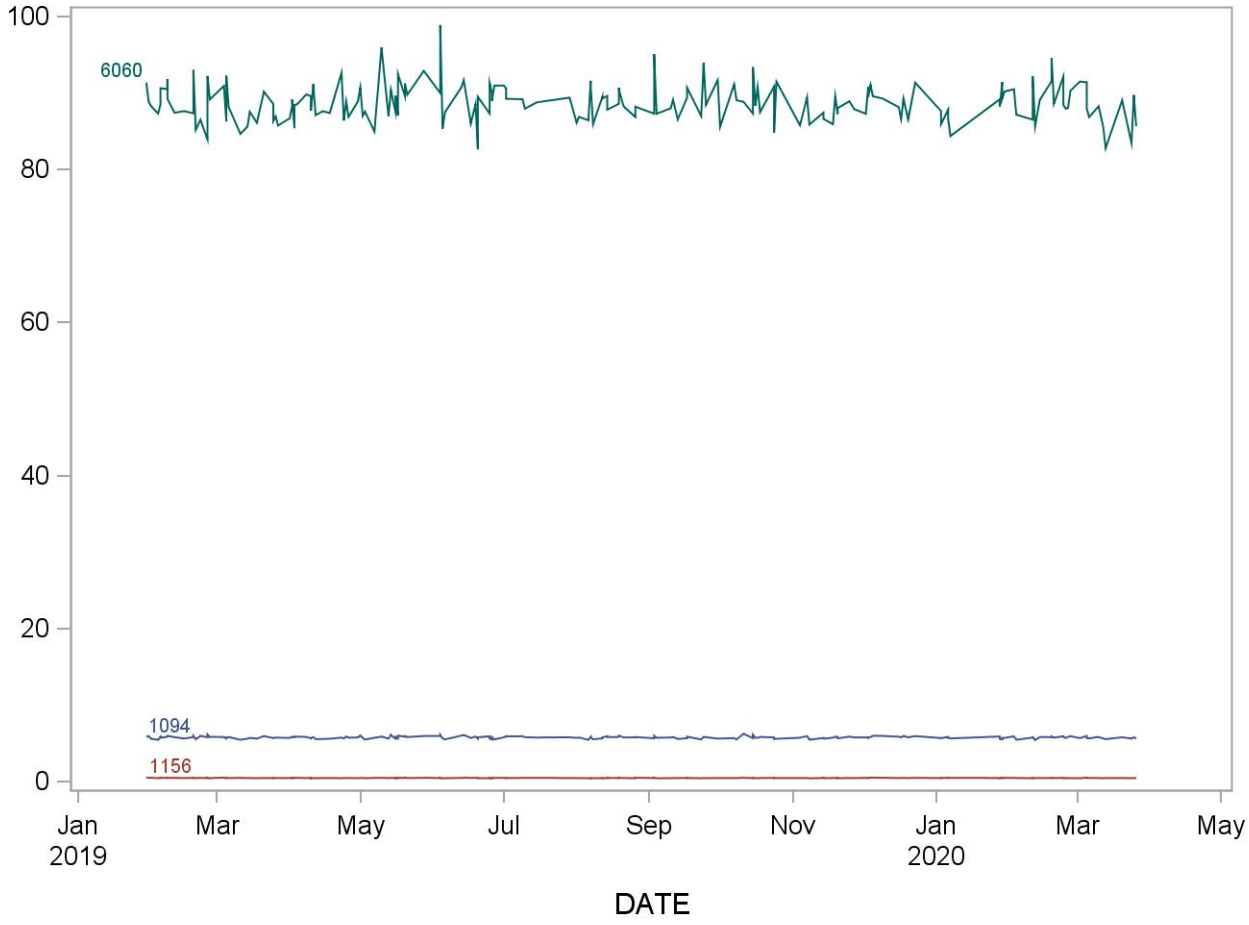
2019-2020 Summary Statistics and QC Chart
LBXBMN (Blood manganese (µg/L))

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
1094	190	30JAN19	26MAR20	14.039	0.409	2.9
1156	190	30JAN19	26MAR20	7.457	0.221	3.0
6060	190	30JAN19	26MAR20	169.884	4.919	2.9



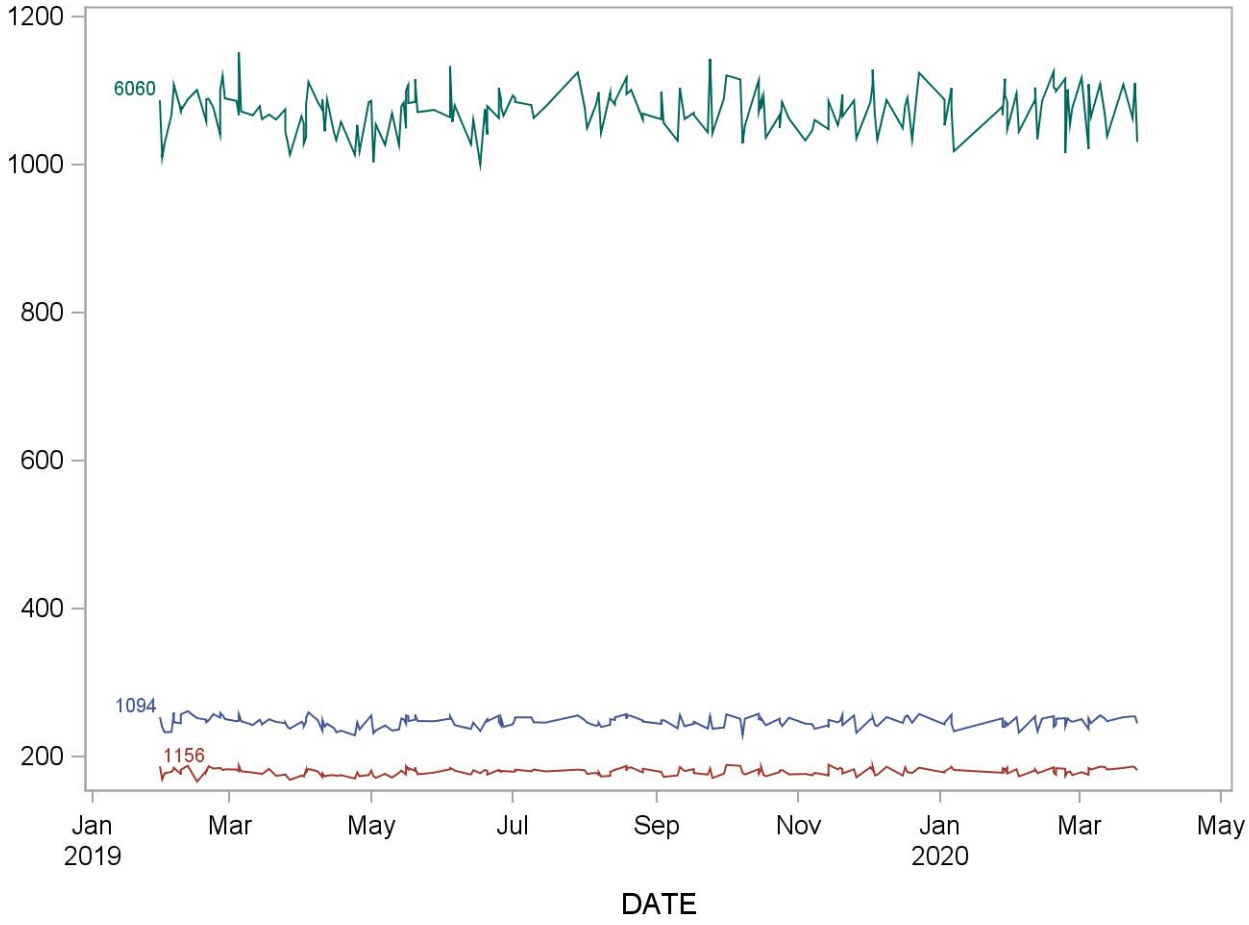
2019-2020 Summary Statistics and QC Chart
LBXTHG (Blood mercury,total (µg/L))

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
1094	188	30JAN19	26MAR20	5.779	0.151	2.6
1156	188	30JAN19	26MAR20	0.478	0.017	3.6
6060	188	30JAN19	26MAR20	88.749	2.413	2.7



2019-2020 Summary Statistics and QC Chart
LBXBSE (Blood selenium (µg/L))

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
1094	192	30JAN19	26MAR20	247.439	6.832	2.8
1156	192	30JAN19	26MAR20	180.063	4.548	2.5
6060	192	30JAN19	26MAR20	1073.732	29.035	2.7



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	10.6	10.4	10.7	0.37	3.46	-7.3
	2		10.8	10.5	10.7	10.5	10.3				
Level 2	1	8.87	7.53	7.83	7.94	7.95	7.90	7.83	0.16	2.09	-11.7
	2		7.55	8.00	7.90	7.91	7.79				
Level 3	1	33.3	36.5	36.6	42.4	36.4	34.3	36.8	2.08	5.65	10.5
	2		36.5	36.7	36.0	36.4	36.4				

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	13.7	13.6	13.65	0.00005	0.00005	372.53581
7	13.3	13.3	13.30	0.00013	0.00013	353.85980
8	13.8	13.6	13.68	0.01030	0.01030	374.47634
9	13.3	14.1	13.73	0.15016	0.15016	376.94342
10	13.7	13.5	13.62	0.00497	0.00497	370.87261

Grand sum 276.464 **Grand mean** 13.8232

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.843418	0.0843418	0.290416597	2.10
Between Run	1.8274232	0.203047022	0.243623913	1.76
Total	2.6708412		0.379070457	**2.74**

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	169	169	169.32	0.030625	0.030625	57335.13845
7	165	164	164.66	0.297025	0.297025	54222.53805
8	168	169	168.65	0.093025	0.093025	56882.27205
9	164	166	165.13	1.134225	1.134225	54532.53125
10	166	165	165.29	0.275625	0.275625	54638.26245

Grand sum 3372.69 **Grand mean** 168.6345

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	39.14185	3.914185	1.978429933	1.17
Between Run	351.953245	39.10591611	4.194742609	2.49
Total	391.095095		4.6378929	**2.75**

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	14.1	13.8	14.6	13.9	13.9	14.1	14.4	13.8
Replicate 2	14.3	14.5	14.6	13.6	13.9	14.3	13.5	13.3
Replicate 3	13.9	13.9	13.9	13.7	13.6	14.1	14.6	13.7
Mean	14.1	14.0	14.3	13.7	13.8	14.2	14.2	13.6
% difference from initial measurement	--	**-0.3**	--	**-4.4**	--	**2.8**	--	**-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	168	168	168	167	166	168	165	168
Replicate 2	168	169	170	167	166	170	167	164
Replicate 3	168	168	170	168	165	168	167	166
Mean	168	168	169	167	166	169	166	166
% difference from initial measurement	--	**0.3**	--	**-1.1**	--	**1.7**	--	**-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 12/21/2018.
**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)	5.0	5.0	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 [70].

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.)

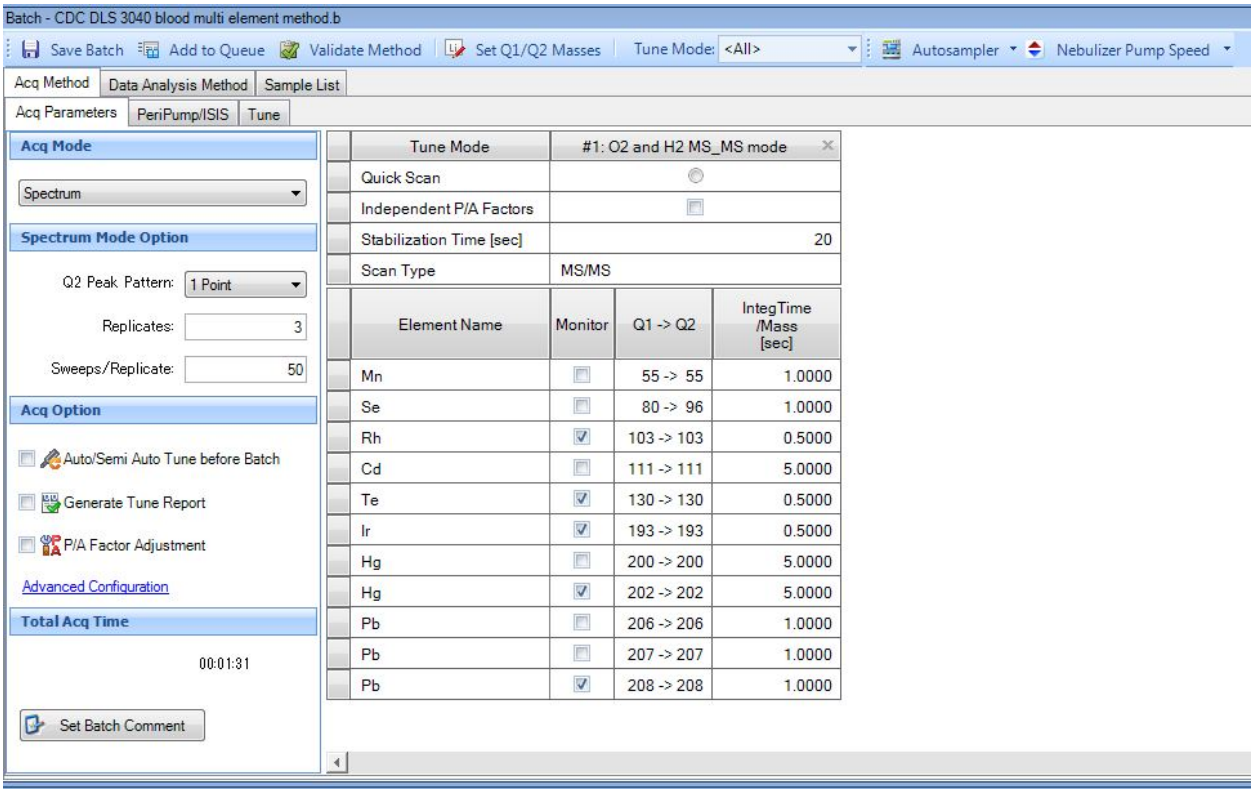


Figure 2. Batch Acquisition Method Set Q1/Q2 Masses and Correction Equations

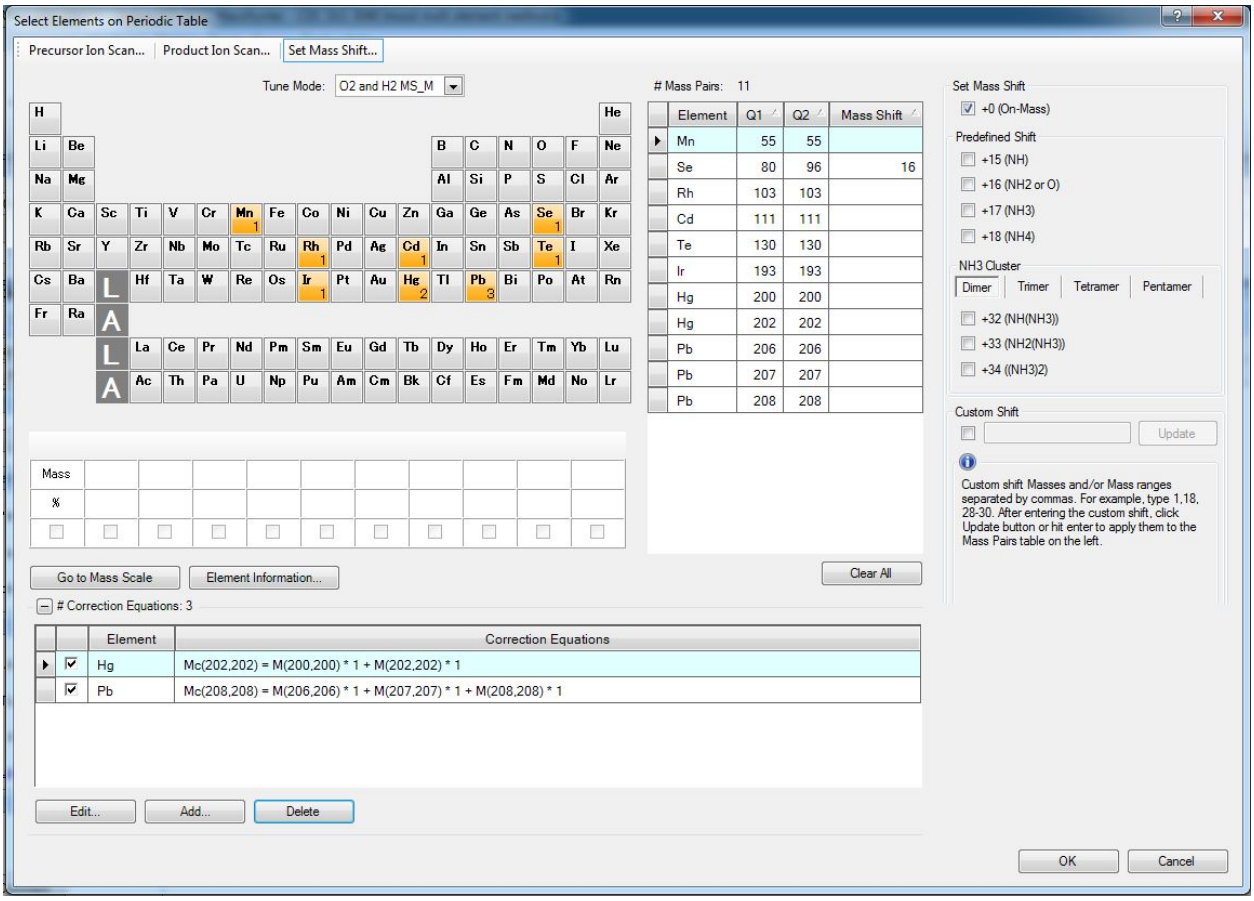


Figure 3. Batch Acquisition Method PeriPump settings

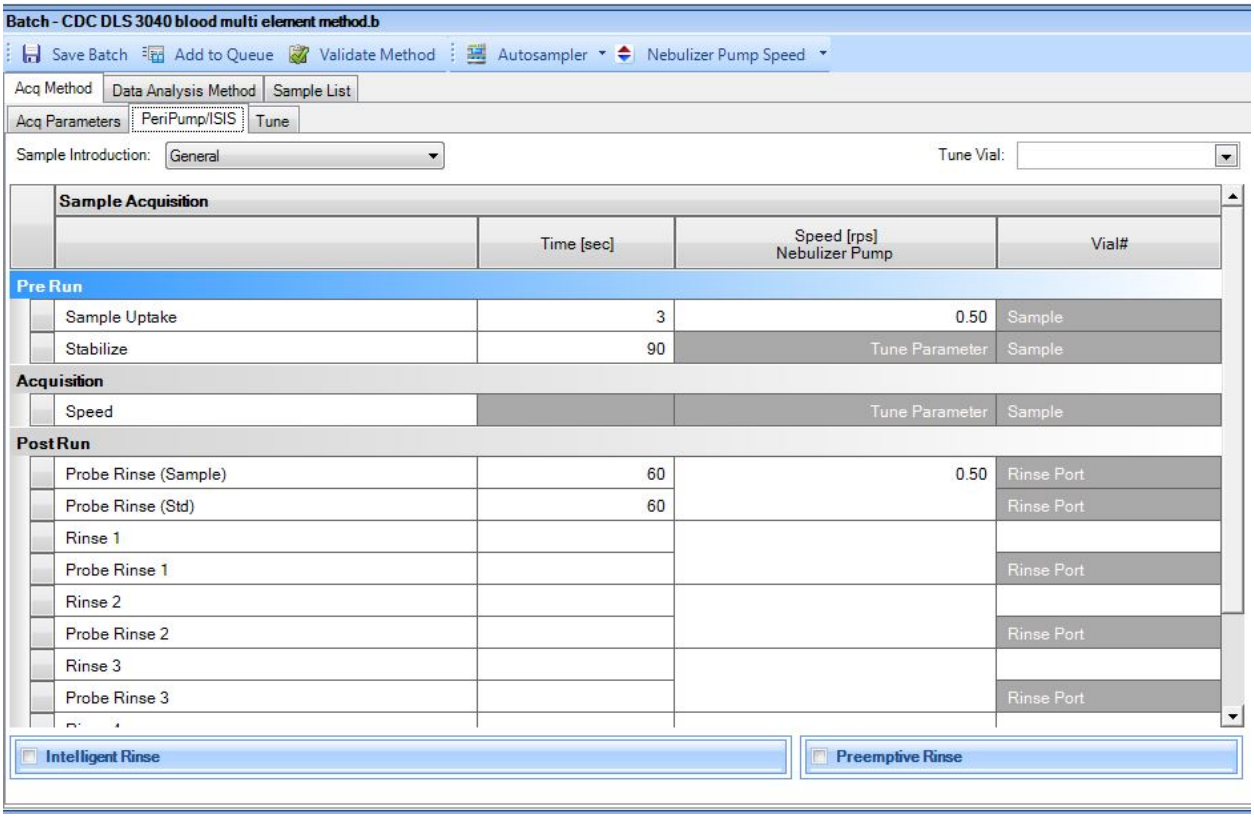


Figure 4. Batch Tune settings

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.

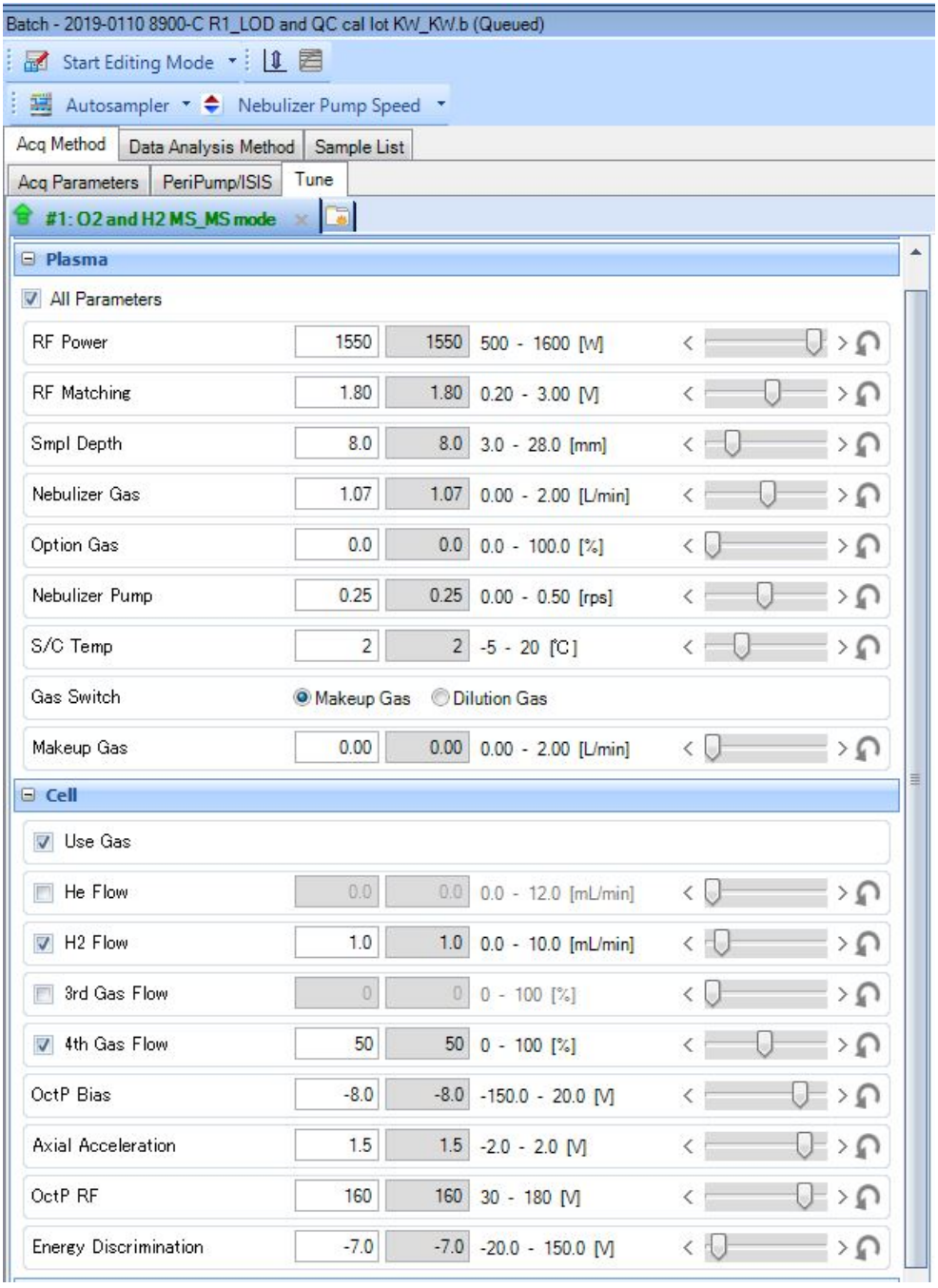


Figure 5. Batch Data Analysis Method Basic Information screen

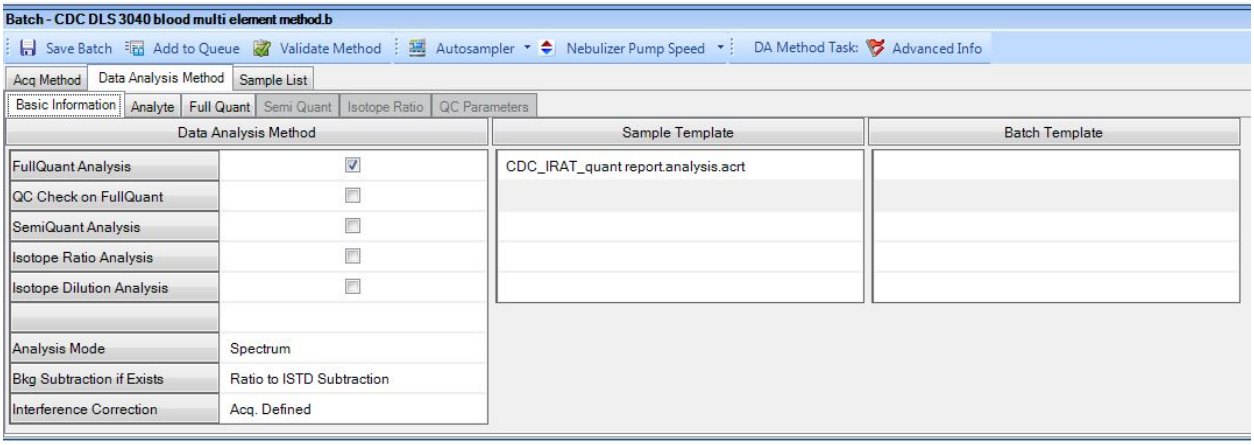


Figure 6. Batch Data Analysis Method Analyte list

Batch - CDC DLS 3040 blood multi element method.b

Save Batch | Add to Queue | Validate Method | Autosampler | Nebulizer Pump Speed

DA Method Task: | Advanced Info

Acq Method | Data Analysis Method | Sample List

Basic Information | Analyte | Full Quant | Semi Quant | Isotope Ratio | QC Parameters

	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

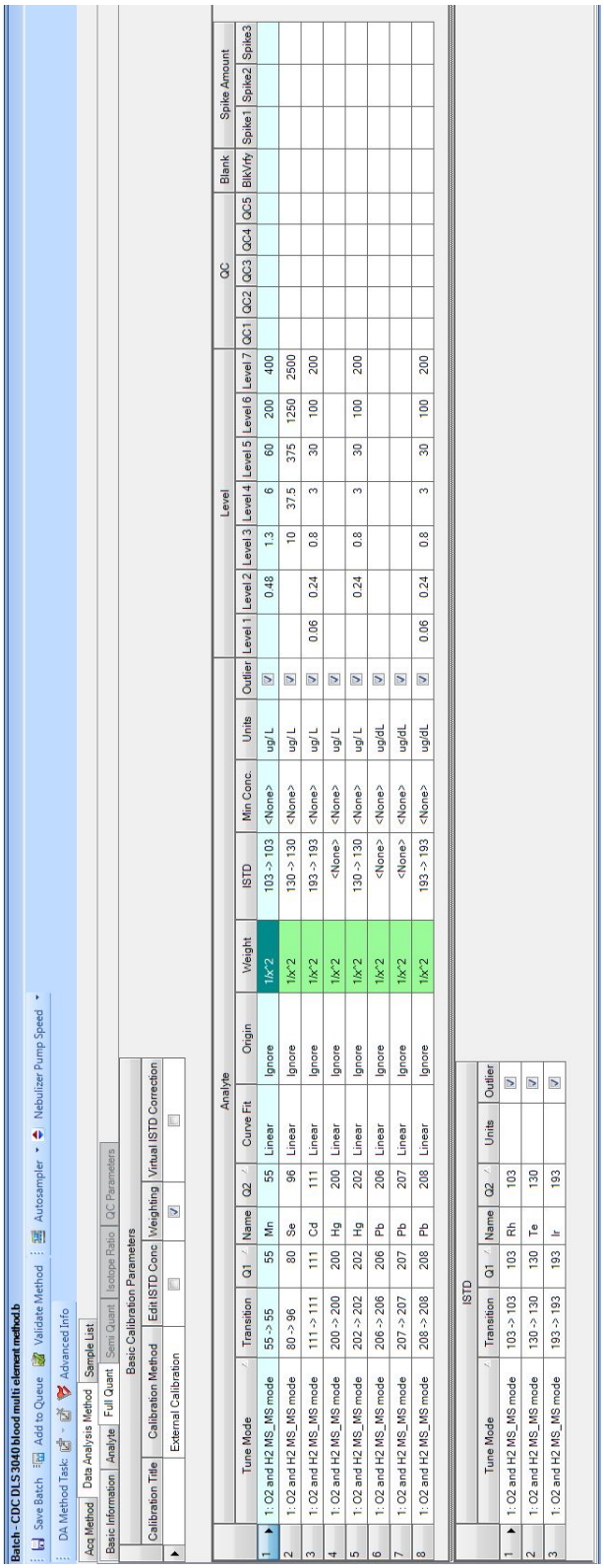
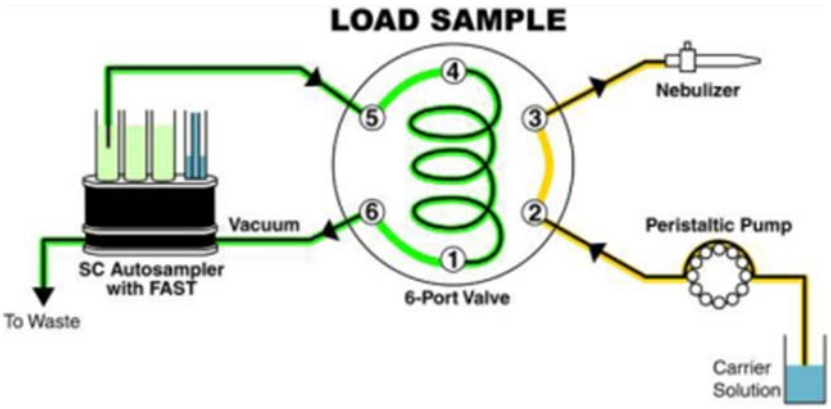


Figure 8. Configuration of tubing and devices for liquid handling using FAST sample introduction

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.

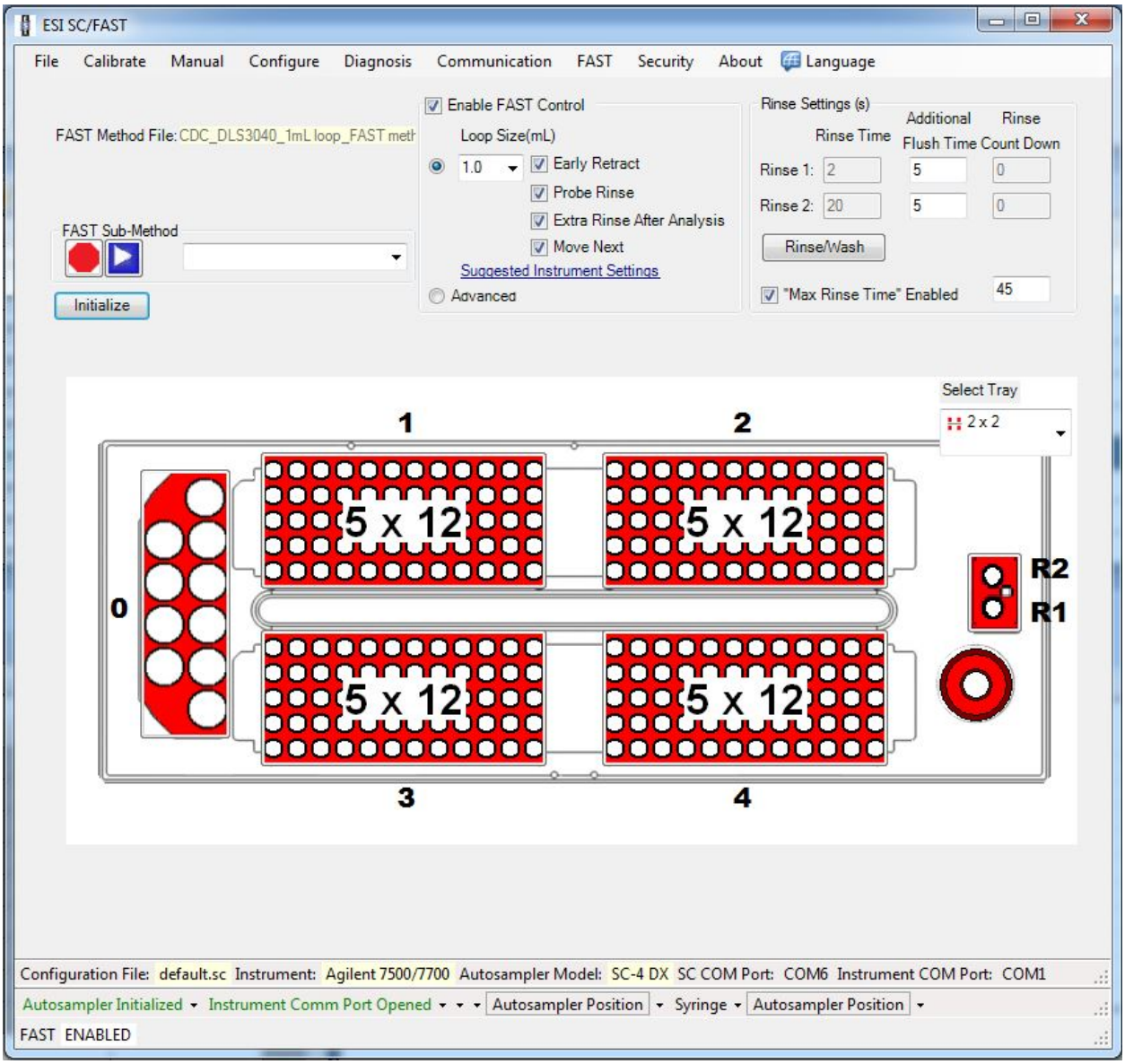


Teflon vacuum pump loads sample into loop while carrier solution is nebulized

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)



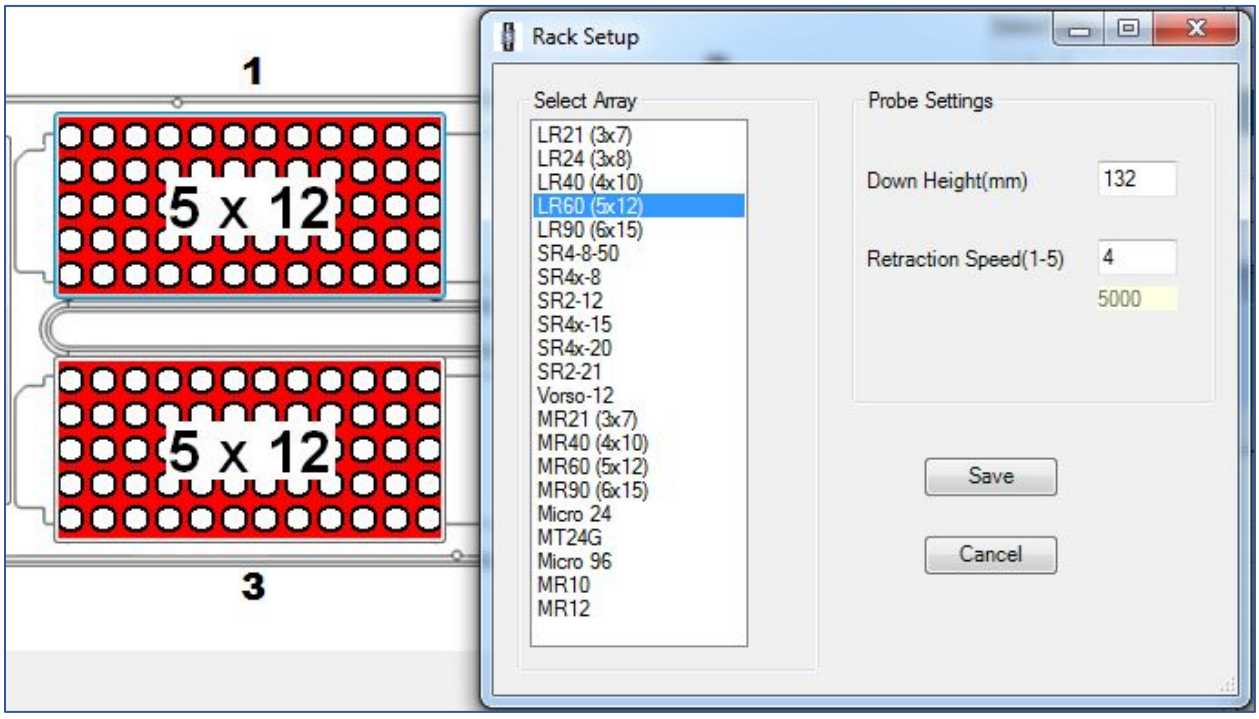
Additional flush times and “Max Rinse Time” are approximate. Optimize these for best reduction of elemental carry-over between samples. Tray types can be changed to allow for different volumes of diluted sample digests. “FAST control” must be enabled before start of method, but does not need to be used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

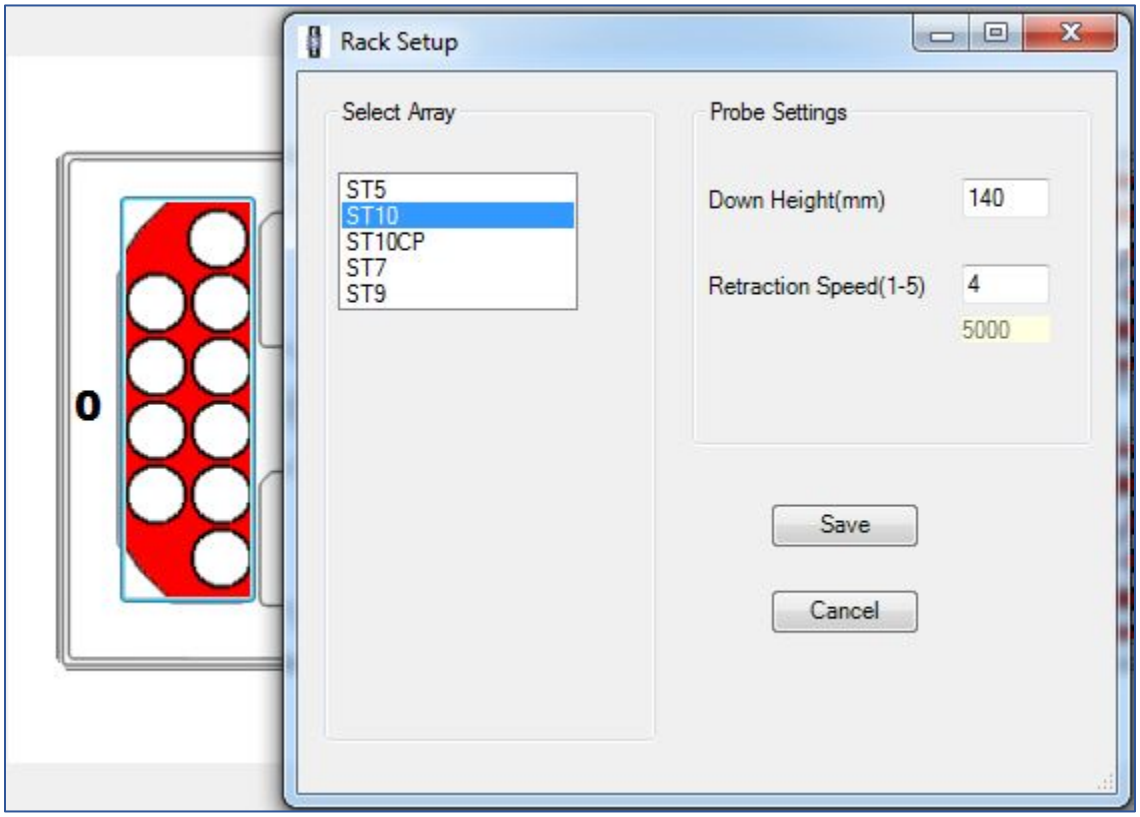
Additional flush times can be optimized to keep the rinse station full while not using too much rinse solution. The inner diameter size of the tubing providing the rinse solution to the rinse station determines how quickly the station will fill. Various sizes are available for purchase or can be made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

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



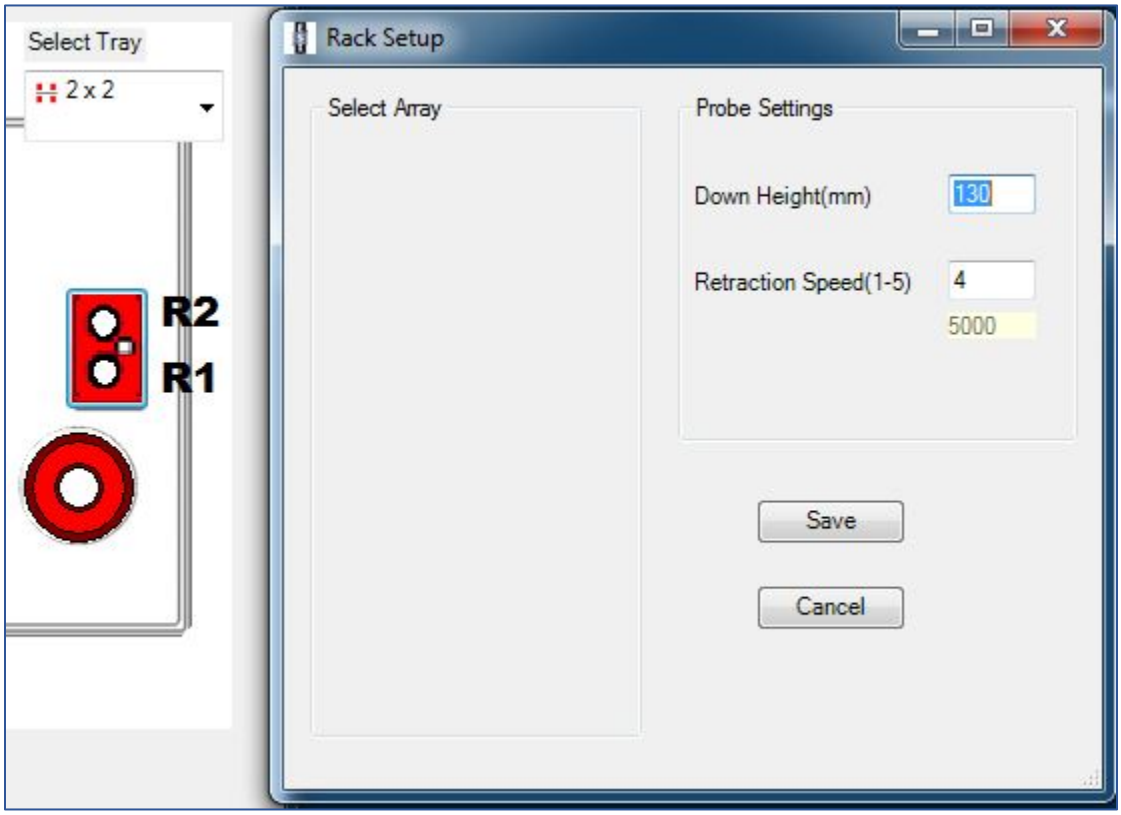
Settings are approximate. To be sure the loop is filled, set the probe to go close to the bottom of the cup, but not touch. Optimize retraction speed for least droplet splatter.

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



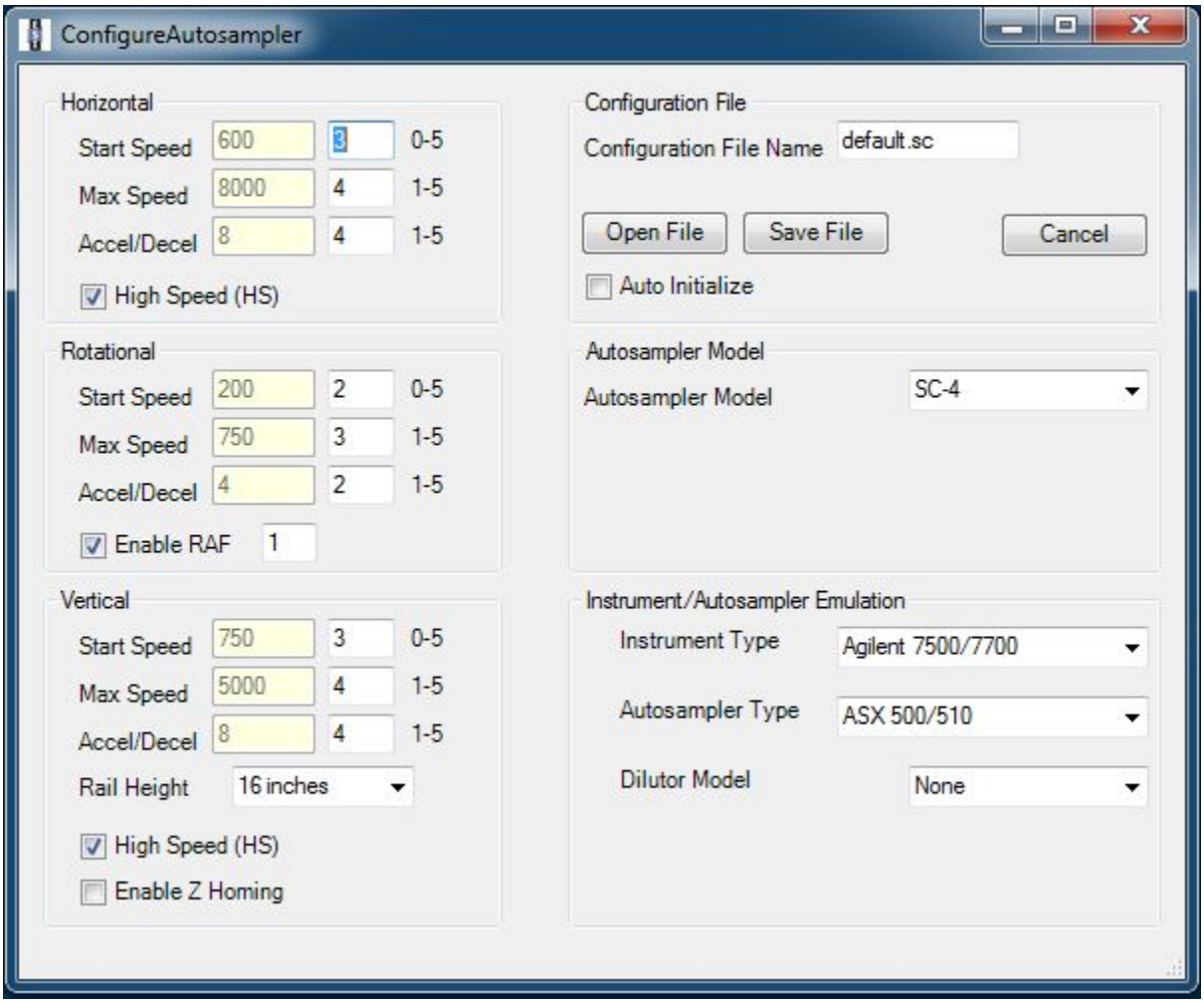
Settings are approximate. To be sure the loop is filled, set the probe to go close to the bottom of the cup, but not touch. Optimize retraction speed for least droplet splatter.

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



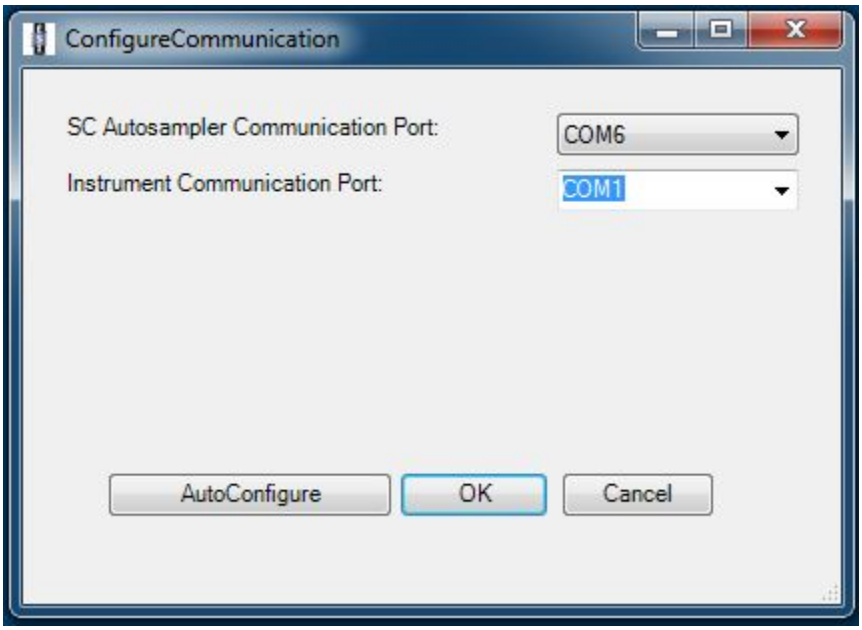
Settings are approximate. Optimize down height for best probe cleaning, and retraction speed for least droplet splatter.

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



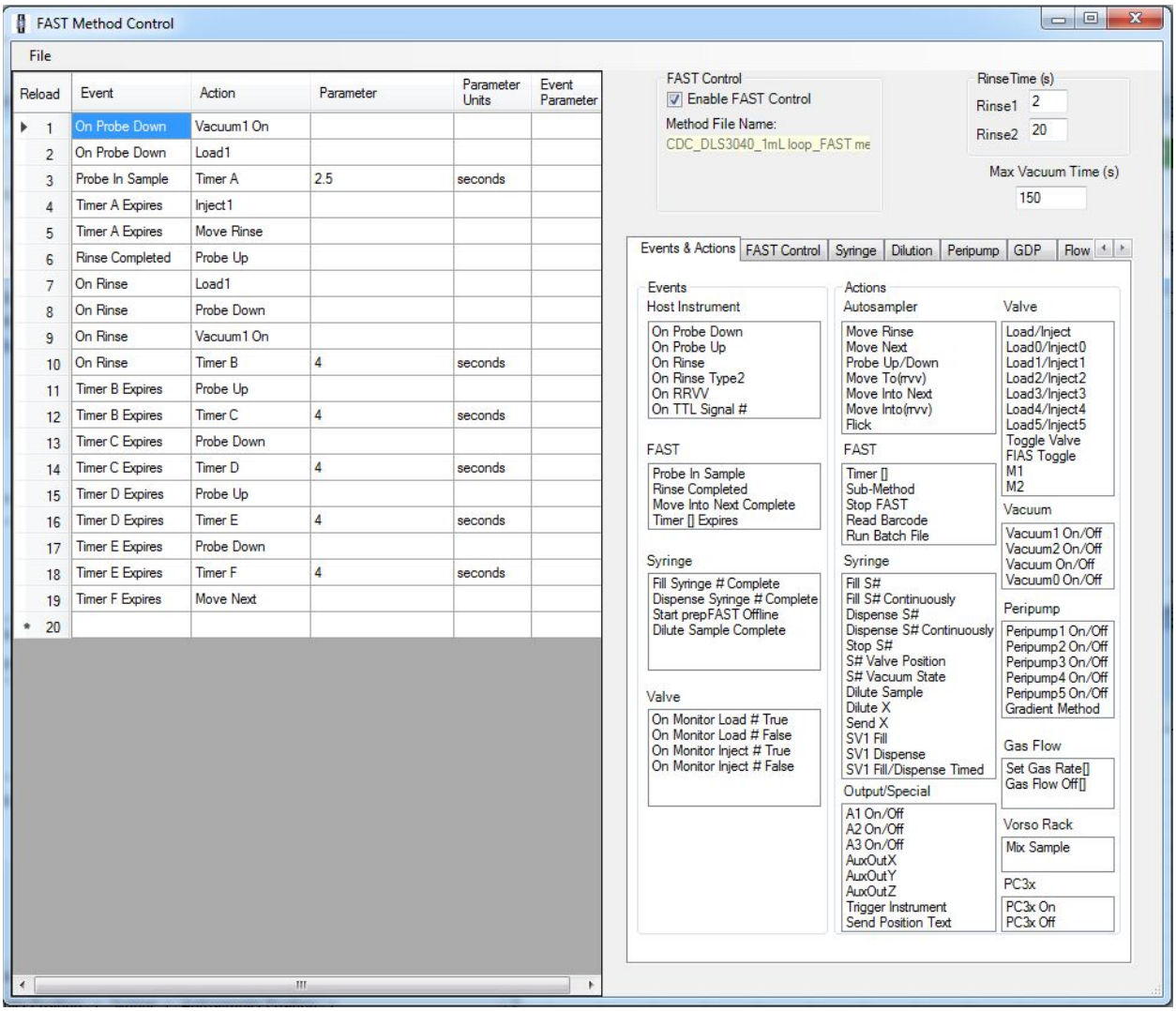
"High Speed" option is to only be used for "High Speed" models of the SC4 (look for "HS" in serial number). Speeds and accel / decel values can be optimized per analyst preference and to minimize droplet splatter off of probe.

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



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

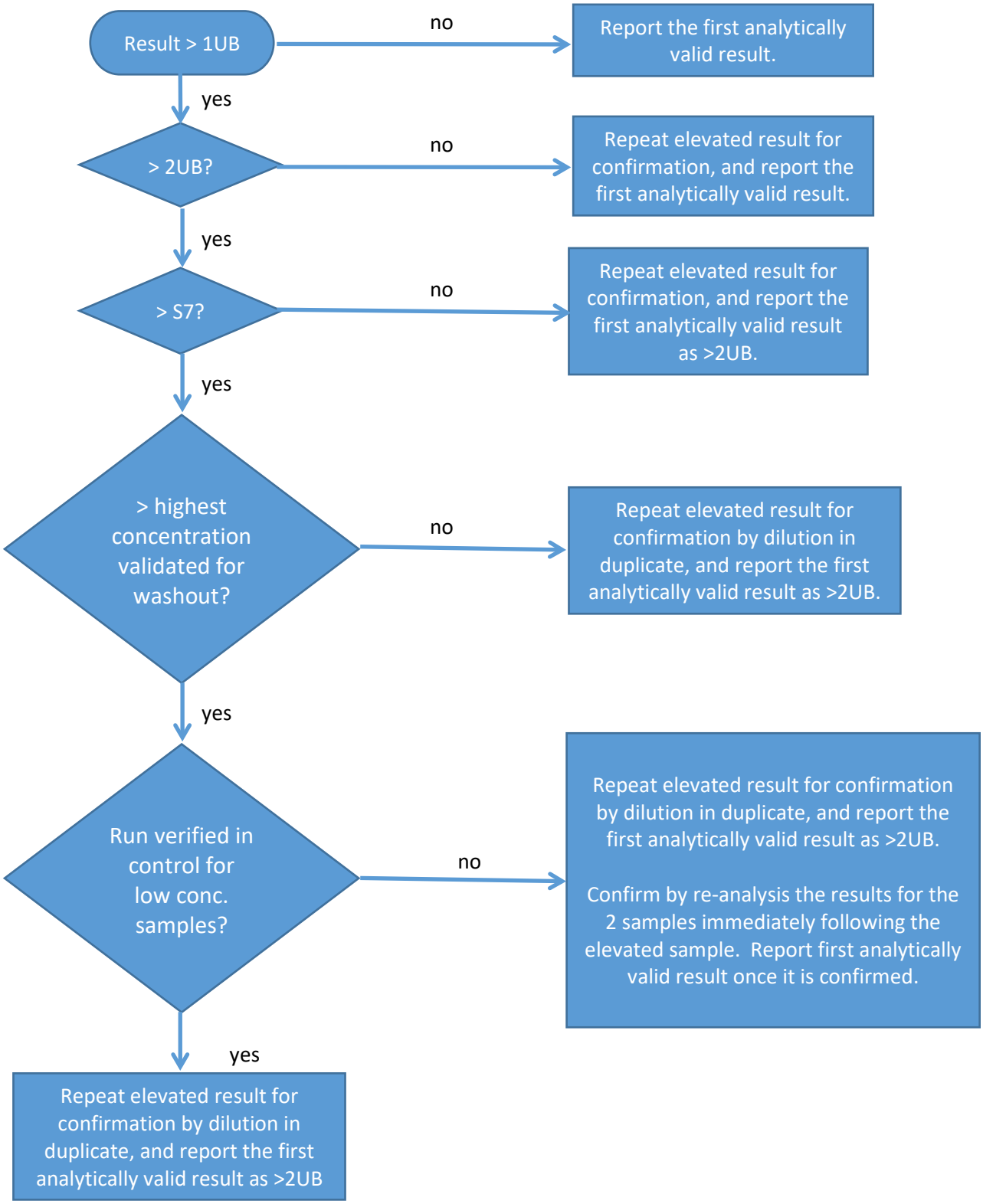
Figure 15. ESI SC4 autosampler screen shots ("FAST" page)



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

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

Sample diluent: 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

0.5% v/v HNO_3: Carrier solution for optimization

1. Partially fill a 2 liter bottle with ≥18 Mohm·cm water
2. Add 10 mL of concentrated HNO_3
3. Add enough ≥18 Mohm·cm water to bring to 2 liter mark
4. Mix well by gently inverting several times

1.0% v/v HNO_3: Carrier solution for optimization

1. Partially fill a 2 liter bottle with ≥18 Mohm·cm water
2. Add 20 mL of concentrated HNO_3
3. Add enough ≥18 Mohm·cm water to bring to 2 liter mark
4. Mix well by gently inverting several times

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

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

20 mg/L Rh, Te and Ir internal standard solution

1. Partially fill an acid rinsed, 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

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

1. Partially fill an acid rinsed, 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

Performance Report solution (1ppb) 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 (part# ICP-MS-TS-10-100)
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

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

Stability test solution (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

Prepare 3% v/v HCl solution:

1. Store in the refrigerator (when not using)
2. Partially fill a clean 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

Prepare 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

Prepare 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

Prepare 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)

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			

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