Laboratory Procedure Manual

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

Matrix: Whole Blood

Method: Blood Metals Panel 2 (BMP2) ICP-DRC-MS

Method No: DLS 3016.8

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

Contact: Jeffery M. Jarrett, MS
Phone: 770-488-7906
Fax: 770-488-4097
Email: JJarrett@cdc.gov

Dr. Jim Pirkle, MD, PhD, Director
Division of Laboratory Sciences

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

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

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<th>Data File Name</th>
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<tr>
<td>PBCD_G</td>
<td>LBXBCD</td>
<td>Cadmium (µg/L)</td>
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<tr>
<td></td>
<td>LBDBCDSI</td>
<td>Cadmium (µmol/L)</td>
</tr>
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<td>LBXBPB</td>
<td>Lead (µg/dL)</td>
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<td></td>
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1. Clinical Relevance & 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 specimens 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[1]. 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, rather than the amount that may get into them. The laboratory method described here is a multi-element technique for monitoring the 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[2]. 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 may be 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)[3]. 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. Parasthesia, neuralgias, renal disease, digestive disturbances, and ocular lesions may develop[4]. Massive exposure over a longer period of time results in violent muscular spasms, hallucinations, delirium, and death[5]. 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.

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 may contain lead naturally, or from man-made uses of lead such as paint (near older homes), gasoline (near roadways), mining, manufacturing, and disposal. The main target for lead toxicity is the nervous system, both in adults and children. The developing biological systems of children are most sensitive to the effects of Pb, where effects are being recognized even at blood lead levels <10 µg/dL[6]. In its initial phase, acute lead poisoning is associated with anorexia, dyspepsia, and constipation followed by diffuse paroxysmal abdominal pain. Lead exposure may cause encephalopathy, particularly in children[7]. The alkyl lead species are highly toxic to the central nervous system[8]. The primary screening method for lead exposure is blood lead, which primarily reflects recent exposures (excretory half-life in blood is approximately 30 days)[9]. Lead in blood is primarily (99%) in the red blood cells.

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, hypercalcuiuria, 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 are in the range 0.1 – 4 µg/L[10]. Newborn babies are practically free of Cd[11]. 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[7]. Principal symptoms reported were respiratory distress due to chemical pneumonitis and edema. It has been estimated that 8 hrs exposure to 5 gm Cd/m³ will be lethal[7]. Ingestion of high amounts of Cd may lead 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[12-15]. Urine cadmium levels primarily reflect total body burden of cadmium, although urine levels do respond somewhat to recent exposure[16].

Manganese (Mn) 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 [17]. 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 [18]. There is much concern for the levels of manganese in humans whom are occupationally exposed to it [19-25]. Recently, there are growing concerns over exposure due to contamination of drinking water with manganese [26-28] and as a result of methylcyclopentadienyl mangangese tricarbonyl (MMT) used as an anti-knocking additive in gasoline[29-35]. Populations suffering from iron deficiencies may be particularly susceptible to manganese toxicity because iron deficiency may lead to an accumulation of manganese in the central nervous system [32]. 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[36]. On an individual basis the correlation between the level of workplace exposure and the levels in blood or urine has always been found to be a reliable predictor of exposure[20, 36-38]. Manganese in blood or urine may be useful in detecting groups with above-average current exposure, but measurements of manganese in these body fluids in individuals may only be related to exposure dose after the exposure has 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[39, 40]. Thus, levels of manganese in blood or urine are not expected to be the most sensitive indicators of exposure[41].

Selenium is an essential element that is required to maintain good health but both selenium deficiency and excessive levels of selenium are associated with
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 may contribute to the development of chronic diseases such as cancer and heart disease. Other selenoproteins help regulate thyroid function and play a role in the immune system. Human selenium deficiency is rare in the U.S. but is seen in other countries where soil concentration of selenium is low. There is evidence that selenium deficiency may contribute to development of a form of heart disease, hypothyroidism, and a weakened immune system. 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. 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. 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.

The laboratory method presented here can be used to achieve rapid and accurate quantification of five elements of toxicological and nutritional interest including cadmium (Cd), lead (Pb), mercury, manganese (Mn) and selenium (Se) in whole human blood. The method may be used to screen blood when people are suspected to be acutely exposed to these elements or to evaluate chronic environmental or other non-occupational exposure.

b. **Test Principle:**
This method directly measures the Cd, Mn, Hg, Pb, and Se content of whole blood specimens 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 specimen after the entire specimen is mixed (vortexed) to create a uniform distribution of cellular components. This mixing step is important because some metals (e.g. Pb) are known to be associated mostly with the red blood cells in the specimen and a uniform distribution of this cellular material must be produced before a small volume extracted from the
larger specimen will accurately reflect the average metal concentration of all fractions of the larger specimen. Coagulation is the process in which blood forms solid clots from its cellular components. If steps are not taken to prevent this process from occurring, i.e. addition of anti-coagulant reagents such as EDTA in the blood collection tube prior to blood collection, blood will immediately begin to form clots once leaving the body and entering the tube. These clots prevent the uniform distribution of cellular material in the blood specimen even after rigorous mixing, making a representative sub-sample of the larger specimen unattainable. It is important that prior to or during sample preparation the analyst identify any sample having clots or micro-clots (small clots). Consequently, blood samples containing clots should not be analyzed by this method due to the inhomogeneity issues and expected results from the sample should be documented 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 + 48 parts diluent. The effects of the chemicals in the diluent are to release metals bound to red blood cells making them available for ionization, reduce ionization suppression by the biological matrix, prevent clogging of the sample introduction system pathways by undissolved biological solids, and allow introduction of internal standards to be utilized in the analysis step. Tetramethylammonium hydroxide (TMAH, 0.4% v/v) and Triton X-100® (0.05%) in the sample diluent solubilizes blood components. Triton X-100® also helps prevent biological deposits on internal surfaces of the instrument’s sample introduction system and reduce collection of air bubbles in sample transport tubing. Ammonium pyrrolidine dithiocarbamate (APDC) in the sample diluent (0.25%) aids in solubilizing metals released from the biological matrix. Ethyl alcohol in the sample diluent (1%) aids solubility of blood components and aids in aerosol generation by reduction of the surface tension of the solution. The internal standards, rhodium, iridium and tellurium, are at a constant concentration in all blanks, calibrators, QC, and samples. Monitoring the instrument signal ratio of a metal to its internal standard allows correction for instrument noise and drift, and sample-to-sample matrix differences.

Liquid samples are introduced into the mass spectrometer through the inductively coupled plasma (ICP) ionization source. The liquid diluted blood sample is forced through a nebulizer which converts the bulk liquid into small droplets in an argon aerosol. The smaller droplets from the aerosol are selectively passed through the spray chamber by a flowing argon stream into the ICP. By coupling radio-frequency power into flowing argon, plasma is created in which the predominant species are positive argon ions and electrons and has a temperature of 6000-8000 K. The small aerosol droplets pass through a region of the plasma and the thermal energy vaporizes the liquid droplets, atomizes the molecules of the sample and then ionizes the atoms. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP (at atmospheric pressure, ~760 torr) from the mass spectrometer (operating at a
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pressure of $10^{-5}$ torr). The ions first pass through a focusing region, then the dynamic reaction cell (DRC), the quadrupole mass filter, and finally are selectively counted in rapid sequence at the detector allowing individual isotopes of an element to be determined.

Generally, the DRC operates in one of two modes. In ‘vented’ (or ‘standard’) mode the cell is not pressurized and ions pass through the cell to the quadrupole mass filter unaffected. In ‘DRC’ mode, the cell is pressurized with a gas for the purpose of causing collisions and/or reactions between the fill gas and the incoming ions. In general, collisions or reactions with the incoming ions selectively occur to either eliminate an interfering ion, change the ion of interest to a new mass, which is free from interference, or collisions between ions in the beam and the DRC gas can focus the ion beam to the middle of the cell and increase the ion signal. In this method, the instrument is operated in DRC mode when analyzing for manganese, mercury and selenium. For selenium, the DRC is pressurized with methane gas (CH$_4$, 99.999%) which reduces the signal from $^{40}$Ar$_2^+$ while allowing the $^{80}$Se$^+$ ions to pass relatively unaffected through the DRC on toward the analytical quadrupole and detector. Manganese and mercury are both measured when the DRC is pressurized with oxygen gas (O$_2$, 99.999%). They are analyzed at the same flow rate of oxygen to the DRC cell to avoid lengthening analysis time due to pause delays that would be necessary if different gas flows were used for the two analytes. The oxygen reduces the ion signal from several interfering ions ($^{37}$Cl$^{18}$O$^+$, $^{40}$Ar$^{15}$N$^+$, $^{38}$Ar$^{16}$O$^+$, $^{54}$Fe$^+$) while allowing the Mn$^+$ ion stream to pass relatively unaffected through the DRC on toward the analytical quadrupole and detector. In the case of mercury, collisional focusing of the mercury ions occurs, increasing the observed mercury signal at the detector by approximately a factor of two (2x).

Once ions pass through the DRC cell and electrically selected for passage through the analytical quadrupole, electrical signals resulting from the ions striking the discrete dynode detector are processed into digital information that is used to indicate the intensity of the ions. The intensity of ions detected while aspirating an unknown sample is correlated to an elemental concentration through comparison of the analyte:internal standard signal ratio with that obtained when aspirating calibration standards. This method was originally based on the method by Lutz et al.[54] The DRC portions of the method are based on work published by Tanner et al. [55, 56].

2. Safety Precautions

a. General Safety

i. Observe all safety regulations as detailed in the Division (DLS) Safety Manual. Additional information can be found in your lab’s
chemical hygiene plan. 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. Special care should be taken when handling and dispensing bases and concentrated acids. Wear powder free gloves, a lab coat, safety glasses, and face / neck protection. **If 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 containers holding biological or corrosive liquids.

vi. Dispose of all biological samples and diluted specimens in a biohazard autoclave bag at the end of the analysis according to CDC/DLS guidelines for disposal of hazardous waste.

vii. The use of the foot pedal on the Digiflex™ 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 specimen cups and autosampler tubes and to wipe off the tip of Digiflex™.

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

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

x. Wipe down all work surfaces at the end of the day with bleach-rite spray or freshly prepared 10% (v/v) sodium-hypochlorite solution.

b. **Waste Disposal:** Operators of this method should take the CDC-OHS Hazardous Chemical Waste Management Course (initial and yearly refreshers).
i. Waste to be Placed Into Biohazard Autoclave Bags & Pans:

1. All biological samples and diluted specimens (after analysis run).

2. All disposable plastic and paper which contact blood (autosampler tubes, gloves, etc.).

3. Used non-glass/quartz ICP-MS consumables (i.e. probes, tubing, cones, ion lenses).

ii. Waste to be Placed Into Sharps Containers: Pipette Tips, broken glass or quartz instrument consumables (broken spray chambers, torches, nebulizers, etc.). Large broken glass which will not fit in the sharps container should be placed in a separate autoclave pan from other waste and labeled as “broken glass” (see the “Autoclaving” section of the CDC safety policies and practices manual located in the laboratory).

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

1. Electronic Records:

   a. Transfer of Results to the Laboratory Information System / Database: Transfer data electronically between computers or software to reduce errors. When keyboard entry must be used, proofread transcribed data after entry.

   b. Long-Term Storage of ELAN software files: Files used and produced by the ELAN software in analyzing samples will be backed up long term on compact disk and kept a minimum of three years.

2. Paper Records: The paper copy of the results from the run should be put into the study folder(s) and should include

   a. A summary of the calibration curve statistics.

   b. A printout of analysis of each measurement made during the run.

   c. On the front sheet of the printed records, write the following

      i. Analyst initials

      ii. Instrument ID

      iii. Date of Analysis

      iv. Run # for the day on this instrument

      v. Study ID and Group Number
vi. Database batch ID (Not known until the run is imported into the database)

3. Transfer of Results to the Laboratory Database: Every analytical run performed for the analysis of patient samples should be entered into the laboratory results database unless the run is not useable for obvious reasons (e.g. the run is stopped for some reason before ending QC is analyzed, no internal standard spiked into the diluent, etc. . . ).

a. Data Export Process (from ELAN® software to .TXT file): If the data file was not created during the initial analysis, reprocess the data of interest either with “original conditions” option, or by loading the method file used during the analysis. Use report options file “CDC_Database Output.rop” and type in a descriptive report filename using a format such as “2005-0714a_DRC2F_group55.txt” to designate data from analysis of group 55 from July 14, 2005, run #1 of instrument “DRC2F”. Under “Report Format”, choose the “Use Separator” option, and under the “File Write” section, choose “Append.”

b. Data Import Process (from .TXT file to Laboratory Information System):
   a. Move the .TXT file created in the data export process to the appropriate subdirectory on the network drive where exported data are stored. Directories for data storage are named according to instrument \ year \ month.
   b. Import the instrument file into the LIMS.
   c. Enter the appropriate information to identify the instrument, assay, analysis date & time, run number, analyst, calibrator lot number and prep date used (use the “IS Lot Number” field) and study. If other than default values for Method LOD, High Calibrator, Rep Delta Limit, and units were used in the run, document accordingly.
   d. In the “Import Instrument Results” table, correct sample IDs and document dilution factors if dilution factor notations were added to the ID in the ELAN software prior to analysis.
   e. Once transferred into the database, the data should be evaluated for QC pass / fail, then appropriate settings entered for QC accept / reject, final value status, and comments.

4. Procedures for Collecting, Storing, and Handling Specimens; Criteria for Specimen Rejection; Specimen Accountability and Tracking
   a. Procedures for Collecting, Storing, and Handling Specimens: Specimen handling conditions, special requirements, and procedures for collection and transport are discussed in the division (DLS) Policies and Procedures Manual
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a. No fasting or special diets are required before collection of blood

b. Specimen type – whole blood

c. Optimal amount of specimen is 1-2 ml. Request a minimum volume of 0.4 ml. Volume for one analytical measurement is 0.1 ml.

d. Sample collection devices and containers should be verified to be free of significant contamination (“pre-screened”) before use.

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

f. Blood specimens should be transported and stored at ≤ 4°C. Once received, they can be frozen at ≤ -20°C until time for analysis. Specimen stability has been demonstrated for several months at ≤ -20°C.

c. Criteria for Specimen Rejection: The criteria for an unacceptable specimen include:

a. 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).

b. Low Volume: Request a minimum volume of 0.4 ml. Volume for one analytical measurement is 0.1 ml.

In all cases, a second blood specimen should be requested.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.
6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

A. Instrument & Material Sources

1. Sources for ICP-MS Instrumentation


   DXi-FAST upgrade: Standard peristaltic pump replaced by DXi-FAST micro-peristaltic pump / FAST actuator and valve combination unit. For ELAN DRC2, part # DXI-54-P4-F6.

   Recirculating chiller / heat exchanger for ICP-MS: Refrigerated chiller (PolyScience 6105PE for ELAN® 6100 DRCPlus instruments) if unit is to be placed remotely from ICP-MS or heat exchanger (PolyScience 3370 for ELAN® DRC II instruments) if unit is to be placed alongside ICP-MS (PerkinElmer Norwalk, CT, www.perkinelmer.com).

   Autosampler:

   1. ESI SC4 autosampler: Dual rinse station supplied by two independent pumps built internal to the autosampler (Elemental Scientific Inc., Omaha, NE).

   2. FAST: Purchase as an option onto the ESI SC4 autosampler (Elemental Scientific Inc., Omaha, NE).

2. Sources for ICP-MS Parts & Consumables

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

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

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


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Carboy and cap assembly for waste collection: 10-15L, 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).

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


Cone, skimmer (nickel): PerkinElmer part # WE021137 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Part # SC2012-Ni (Testing has also found Spectron, Ventura, CA, www.spectronus.com cones to be comparable) # Spares = 4.


FAST accessories


Sample Loop: 1 mL Teflon, white connector-nuts for high flow valve head. Like part # SC-0315-10 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

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.
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**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.

**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).

**Tubing, connects nebulizer to valve:** See “Nebulizer, PolyPro-ST micro flow”

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

**Hose, for exhaust of ELAN:** Available as part of ELAN installation kit from PerkinElmer (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available direct from manufacturer as part # S-LP-10 air connector (Thermaflex, Abbeville, SC, www.thermaflex.net). Equivalent part may be substituted. # Spares = 10 feet of 4” diameter and 10 feet of 6” diameter hose.

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

**Injector support (for pass-through injector):** PerkinElmer part # WE023951 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available direct from manufacturer as part # 400-37 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com) or equivalent from various distributors. # Spares = 2.

**Ion Lens:** PerkinElmer part # WE018034 (PerkinElmer Norwalk, CT, www.perkinelmer.com). # Spares = 3.


**Gas connection:**

Adapter kit: Plastic adapters to connect Teflon tubing (2.4mm i.d) to ¼” male Swagelok (compression) port on ICP-DRC-MS. Parts can be obtained as components in a “gas fittings kit for microflow nebulizer”, kit part # ES-2501-1000, Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). # Spares = 1.


Nut and Ferrule set, 1/8” Swagelok: Such as part # SS-200-NFSET (stainless steel) or part # B-200-NFSET (brass) (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. For part numbers listed here a quantity of 1 means 1 nut, 1 front ferrule, and 1 back ferrule. Spares = 20.

Nut and Ferrule set, 1/4” Swagelok: Such as part # SS-400-NFSET (stainless steel) or part # B-400-NFSET (brass) (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. For part numbers listed here a quantity of 1 means 1 nut, 1 front ferrule, and 1 back ferrule. Spares = 20.

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

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

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

O-ring: (for ELAN DRC II standard injector support).

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

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

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


Plugs, Quick Change for Roughing Pump Oil: These plugs will only work on the Varian roughing pumps which come standard on ELAN DRC II ICPMS instruments.  These plugs will not fit the Leybold pumps which come standard on the ELAN DRC Plus instruments.  Part # W1011013 (PerkinElmer, Shelton, CT, www.perkinelmer.com).  No spares typically needed.

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

Spray chamber, quartz concentric:  PerkinElmer part # WE025221 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent.  Available direct from manufacturer as part # 400-20 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com) or from various distributors.  # Spares = 2.

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

Torch, quartz:  PerkinElmer part # N812-2006 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent.  Available direct from manufacturer as part # 400-10 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com) or various distributors.  Damaged torches can often be repaired for substantially lower cost than purchasing a new one by companies such as Wilmad LabGlass (Buena, NJ, www.wilmad-labglass.com) or Precision Glass Blowing (Centennial, CO, www.precisionglassblowing.com).  # New Spares = 2.

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

Tubing, drains waste liquid from spray chamber:  PVC 1/8" i.d., 3/16" o.d tubing used to transfer waste liquid between spray chamber waste port and peristaltic pump waste tubing and between peristaltic pump waste tubing and liquid waste carboy.  Like part # 14-169-7A (pkg. of 50ft, Fisher Scientific International, Hampton, NH, www.fishersci.com) or equivalent.  # Spares = 20ft.
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Connector: Use to connect 1/8" I.D. PVC tubing to 0.125" I.D peristaltic pump tubing. Use part # 3140715 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or equivalent. # Spares = 4.

Tubing, peristaltic, 0.03” i.d. (sampling/carrier solution):

Standard PVC, 2-stop (black / black) peristaltic pump tubing, i.d. = 0.03". PerkinElmer part # 09908587 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # Spares = 6 packs of 12 tubes. Use this type tubing with standard ELAN peristaltic pump.

Standard PVC, 3-stop. (blank / black) peristaltic pump tubing, i.d. 0.76 mm. Spectron part # SC0056 (Spectron, Ventura, CA, www.spectronus.com) or equivalent. # Spares = 6 packs of 12 tubes. Use this type tubing with ESI DXi micro-peristaltic pump.

Tubing, peristaltic, 0.045” i.d. (spray chamber drain):

Standard PVC, 2-stop (red / red) peristaltic pump tubing, i.d. = 0.045”. PerkinElmer part # N0680375, (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # Spares = 6 packs of 12 tubes.

Standard Santoprene, 3-stop (grey / grey / grey) peristaltic pump tubing, i.d. 1.30 mm. Spectron part # SC0311 (Spectron, Ventura CA, www.spectronus.com) or equivalent. # Spares = 6 packs of 12 tubes. Use this type tubing with ESI DXi micro-peristaltic pump.

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


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

Union Tee, PTFE, ¼” Swagelok: Connects argon tubing to torch plasma gas sidearm and holds igniter inside torch sidearm. Like part # T-400-3 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. Spares = 2.

3. Sources for ICP-MS Maintenance Equipment & Supplies

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

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

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

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

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

- Getter Regeneration Kit: Part # WE023257 (PerkinElmer, Shelton, CT, [www.perkinelmer.com](http://www.perkinelmer.com)). Use this as needed (at least annually) to clean the getter in the pathway of channel A DRC gas.

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

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

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

4. Sources for General Laboratory Consumable Supplies

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

- Carboy (for preparation of 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](http://www.fischersci.com)) or equivalent. Carboys with spouts are not advised due to potential for leaking.

- Containers for diluent and Rinse Solution: Two liter Teflon™ containers (like catalog# 02-923-30E, Fisher Scientific, Pittsburgh, PA., [www.fischersci.com](http://www.fischersci.com)) and 4L polypropylene jugs (like catalog# 02-960-10A, Fisher Scientific, Pittsburgh, PA, [www.fischersci.com](http://www.fischersci.com)) have both been used. Acid rinse before use. Equivalent containers may be substituted.
Gloves: Powder-free, low particulate nitrile (like Best CleaN-DEX™ 100% nitrile gloves, any vendor). Equivalent nitrile or latex gloves may be substituted.

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

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

Pipettes (for preparation of intermediate stock working standards & other reagents): Like Brinkmann Research Pro Electronic pipettes (Brinkmann Instruments, Inc., Westbury, NY, http://www.brinkmann.com/home/). 5-100 µL (catalog #4860 000.070), 20-300 µL (catalog #4860 000.089), 50-1000 µL (catalog #4860 000.097), 100-5000 µL (catalog #4860 000.100). Note: pipette catalog numbers are without individual chargers. Can purchase individual chargers (pipette catalog numbers will differ) or a charging stand that will hold four pipettes (catalog #4860 000.860). When purchasing pipette tips (epTips), purchase one or more boxes, then “reloads” for those boxes after that: 5-100 µL (box catalog # 22 49 133-4, reload catalog # 22 49 153-9), 20-300 µL (box catalog # 22 49 134-2, reload catalog # 22 49 154-7), 50-1000 µL (box catalog # 22 49 135-1, reload catalog # 22 49 155-5), 100-5000 µL (box catalog # 22 49 138-5, reload catalog # 22 49 198-9, bulk bag catalog # 22 49 208-0). Equivalent pipettes and tips can be substituted.

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

Tubes for storage of intermediate working stock standards: Like polypropylene 50-mL conical tubes, BD Falcon model #352098 (Becton Dickinson Labware, Franklin Lakes, NJ, www.bd.com). For use in storage of intermediate working stock standards. Equivalent tubes may be substituted which are shown by lot screening to be free of trace metal contamination.
of trace metal contamination. Clear plastics tend to have lowest trace metal contamination. Blue colored caps have also been used successfully for this method.

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

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

### 6. Sources of Chemicals, Gases, and Regulators

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

b. **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. An equivalent nitric acid product may be substituted, but it must meet or exceed the purity specifications of this product for trace metals content.

c. **Alcohol, Ethyl:** USP dehydrated 200 proof (Pharmco Products, Inc.) or equivalent.

d. **Ammonium pyrrolidine dithiocarbamate:** laboratory grade (Fisher Scientific, Fairlawn, NJ) or equivalent.

e. **Argon Gas (for plasma & nebulizer) and Regulator:** High purity argon (>99.999% purity, Specialty Gases Southeast, Atlanta, GA, www.sgsqgas.com) for torch and nebulizer. Minimum tank source is a dewar of liquid argon (180-250L). Bulk tank (1500*L is preferred).

1. **Regulator for argon (at dewar):** Stainless steel, single stage, specially cleaned regulator with 3000 psig max inlet, 0-100 outlet pressure range, CGA 580 cylinder connector, and needle valve shutoff on delivery side terminating in a ¼” Swagelok connector. Part number KPRAFPF415A2AG10 (Georgia Valve and Fitting, Atlanta,
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GA, www.swagelok.com). An equivalent regulator from an alternate vendor may be substituted. # Spares = 1.

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


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

g. Methane: Methane (Research Grade 5.0, 99.99% purity), for DRC channel A. Typically purchased in cylinder size 200 (part # ME R200, Airgas South, Atlanta, GA, www.airgas.com).


2. Flash Arrestor: Like part # 6103 (Matheson Tri Gas, Montgomeryville, PA, www.mathesontrigas.com) or equivalent.

h. Oxygen: Oxygen ("Research Grade Research Grade 5.0", 99.9999% purity) for DRC channel B. Typically purchased in cylinder size 300 (9.5” x 54”) (Airgas South, Atlanta, GA, www.airgas.com).

1. Regulator for oxygen: High purity brass body with monel trim, two stage regulator. Stainless steel is not used for this application due to safety concerns of working with oxygen at high pressure. For one regulator, order the following parts, and ask that they be tested and assembled (Engineered Specialty Products, Kennesaw, GA, www.espgauges.com).

   a. Tescom part # 44-3410S24-555
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*Regulator body:* Brass bar stock, two stage, Monel trim, TFE seats, Elgiloy diaphragms, Cv=0.05, 3000 psig max inlet, 1-25 psig outlet range, 1/4 FNPT inlet / outlet / gauge ports, O₂ cleaned to ASTM G93 and CGA4.1.

b.  **Tescom part # 60500-3000N**  
*Inlet pressure gauge:* 2" diameter, 0-3000 psig range, O₂ cleaned, ¼" MNPT bottom, brass.

c.  **Tescom part # 60500-0015N**  
*Delivery pressure gauge:* 2" diameter, 0-15 psig range, O₂ cleaned, ¼" MNPT bottom, brass.

d.  **Tescom part # 63842-540-B**  
*NPT to CGA Adaptor:* ¼" NPT to CGA 540 adapter, brass.

e.  **Swagelok part # B-200-1-4**  
*Adapter:* Brass male connector, ¼" MNPT to 1/8" Swagelok (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com).

An equivalent regulator from an alternate vendor may be substituted.  
# Spares = 1.

2.  **Flash Arrestor (brass):** Like part # 6103 (Matheson Tri Gas, Montgomeryville, PA, www.mathesontrigas.com) or equivalent.

i.  **Standard, Iridium:** Like 1,000 mg/L, item #CGIR1-1 (Inorganic Ventures, Christiansburg, VA http://www.inorganicventures.com). Used as an internal standard in diluent. Any vendor whose standards are traceable to the National Institute for Standards and Technology may be substituted. The standard must have low trace metal contamination.

j.  **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. Any vendor whose standards are traceable to the National Institute for Standards and Technology may be substituted. The standard must have low trace metal contamination.

k.  **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.
diluent. Any vendor whose standards are traceable to the National Institute for Standards and Technology may be substituted. The standard must have low trace metal contamination.

l. 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). Other sources of standards can be used if they are NIST traceable.

m. Tetramethylammonium hydroxide, 25% w/w, or equivalent (AlfaAesar, 30 Bond St., Ward Hill, MA 01835)

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

7. Preparation of Reagents and Materials

a. Internal Standard Intermediate Mixture (20 µg/mL Rh, Ir and Te):

   a. 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.

   b. Preparation: To prepare 50 mL of the intermediate internal standard solution:

      1. Partially fill a 50 mL acid-washed volumetric flask (PP, PMP, or Teflon™) with 1% v/v HNO₃ (approximately 25-40 mL).

      2. 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.

      3. Fill to mark (50 mL) with 1% v/v HNO₃ and mix thoroughly.

      4. Label appropriately (e.g. “Internal Standard Intermediate Mixture. 20 µg/mL Rh, Ir and Te, 1% v/v HNO₃”, preparation date, expiration date 1 year from preparation date, and preparer’s initials).

b. 20% Triton X-100® intermediate solution:

   a. Purpose: Addition to diluent and rinse solutions where Triton X-100® acts as a surfactant. For ease of daily preparation of the
diluent and rinse solutions, first prepare a 20% Triton X-100 \textsuperscript{®} solution.

b. Preparation: To prepare 1 L of 20% Triton x-100 \textsuperscript{®}

1. Add 200 ml of Triton X-100 \textsuperscript{®} to a pre-acid washed 1L Teflon \textsuperscript{®} container that is partially filled with 18 M-ohm water.

2. Fill to 1 L with 18 M-ohm water and mix until the Triton X-100 \textsuperscript{®} has completely dissolved into solution (overnight). A magnetic stirring plate can be used to assist mixing by adding an acid-washed Teflon \textsuperscript{®} coated stirring bar to the bottle.

c. Sample Diluent

a. Purpose: The diluent used in this method is an aqueous solution of 5 \( \mu \text{g/L} \) internal standard mixture (Rh, Ir, Te), in 0.4\% v/v tetramethyl ammonia hydroxide (TMAH), 1\% ethyl alcohol, 0.01\% APDC, and 0.05\% v/v Triton X-100 \textsuperscript{®}. This solution will be used in the preparation of all calibrators and samples during the dilution process just prior to analysis. It is important that all samples in a run should be made 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), the ‘carrier’ solution should be the same as the diluent used for the method. Larger volumes of these solutions can be prepared by adjusting component volumes proportionally.

b. Preparation:

1. Acid rinse a 2 L Teflon \textsuperscript{®} container, and partially fill with 18 M-ohm water.

2. Add 0.2 g of APDC, 8 ml of 25\% v/v TMAH, 20 ml of ethyl alcohol, and 5 ml of 20\% Triton X-100 \textsuperscript{®}.

3. Dilute to volume (2L) with 18 M-ohm water

4. Spike 500 \( \mu \text{l} \) of 20 mg/L Rh, Ir, Te to the final diluent.

5. Invert bottle a few times to insure thorough mixing. Allow to sit for several hours or overnight before using.

6. Label appropriately (e.g. “5 \( \mu \text{g/L} \) Rh, Ir and Te”, “0.01\% APDC in 0.4\% v/v tetramethyl ammonia hydroxide (TMAH), 1\% ethanol, and 0.05\% v/v Triton X-100”, preparation date, expiration date (1 year from prep), and preparer’s initials).
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7. Store at room temperature and prepare as needed.

d. **DRC Stability Test Solution**
   a. **Purpose:** The DRC Stability Test Solution is a “dummy” blood matrix sample analyzed for 1-1.5 hr before the beginning of the analytical run.
   b. **Preparation:**
      1. Fill an acid rinsed 1 L Teflon® container with 960 mL of Sample Diluent.
      2. Add 20 mL of rejected screened human or bovine blood
      3. Add 1.5 mL of Intermediate Stock Calibration Standard. (As an alternate, add 20 mL of Intermediate Working Calibration Standard 1 or 2.)
      4. Store at 4°C and prepare as needed.

e. **Base Blood**
   a. **Purpose:** This blood pool material will be mixed with the intermediate working calibrators just prior to analysis to matrix-match the calibration curve to the blood matrix of the unknown samples.
   b. **Contents:** A mixture of multiple blood sources collected from anonymous donors are used to approximate an average blood matrix.
   c. **Preparation & Storage:**
      1. Purchase several bags of whole blood. Bovine blood or human blood can be used. Human blood should be screened for infectious diseases such as Hepatitis B and HIV.
      2. Screen each individual bag of blood for concentration of analytes of interest.
      3. Once screened, mix the acceptable 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. For short term storage, store at 2-4°C. For long-term storage, dispense into smaller-volume tubes (i.e., 2 mL cryovials) and store at ≤ -20°C.
      5. Labels on 2 mL cryovials should be labeled appropriately (e.g. “Base Blood for Blood metals panel 2, Cd, Hg, Mn, Pb, Se”, dispensed date and vial number).

f. **ICP-DRC-MS Rinse Solution**
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a. **Purpose:** The rinse solution used in this method is an aqueous solution of 0.4 % v/v TMAH, 1% ethyl alcohol, 0.01% APDC, and 0.05 % Triton X-100®. This solution will be pumped through the autosampler rinse station, probe, and sample loop between sample analyses to prevent carry-over of analytes from one sample measurement to the next.

b. **Preparation:** To Prepare 4 L of the Rinse Solution:

1. Partially fill a 4 L acid-washed bottle (PP, PMP, or Teflon™) with ≥18 Mega-ohm·cm water (approximately 2-3 L). Use of volumetric flask is not required.
2. Add 0.4 g of APDC
3. Add 16 ml of TMAH
4. Add 40 ml of ethyl alcohol,
5. Add 10ml of 20% Triton X-100®, (See Section 6.b for details on preparation)
6. Fill to 4 L using ≥18 Megaohm·cm water.
7. Store at room temperature and prepare as needed. To prepare volumes other than specified here, add proportionally larger or smaller volumes of the solution constituents.
8. Invert bottle a few times to ensure thorough mixing. Allow to sit for several hours or overnight before using.
9. Label appropriately (e.g. “0.4 % v/v TMAH, 1% ethyl alcohol, and 0.05 % Triton X-100®, 0.01% (w/v) APDC”, preparation date, expiration date one year from preparation date, and preparer’s initials).

g. **Single-Element Stock Standards For Preparation of Intermediate Stock Calibration Standard**

a. **Purpose:** These single-element standards will be used to prepare the intermediate stock calibration standard.

b. **Contents:** Separate, aqueous single-element standards of Cd, Pb, Hg, Se, and Mn. Concentrations should be 1,000 mg/L or 10,000 mg/L.

c. **Purchase & Storage:**

1. **Purchasing from vendors:** If the intermediate stock calibration standard is purchased as a special-mix standard, these single-element stock standards are not required. Purchase only NIST-traceable single-element standards at the highest purity (don’t contain other metal impurities).
2. Storage: Store at room temperature.

h. 3% (v/v) HCl Diluent:
   a. Purpose: 3% HCl is used to dilute single element stock standards into a single intermediate stock calibration solution and finally to the intermediate working calibration standards.
   b. Preparation:
      1. In a cleaned 2 L flask, add 1-1.5L > 18 Megaohm·cm water.
      2. Add 60 mL high purity concentrated HCl.
      3. Fill to the mark and mix thoroughly.
      4. Label appropriately (e.g. “3 % v/v HCl”, preparation date, expiration date one year from preparation date, and preparer’s initials).

i. Intermediate Stock Calibration Standard
   a. Purpose: This multi-element solution will be used to prepare the five working calibration standards.
   b. Preparation & Storage: This solution may be purchased as a special-mix standard or prepared in-house from separate single-element stock standards.
      1. Purchasing from vendors: The intermediate stock calibration standard may be purchased as custom mixture from any vendor which prepares multi-element solutions that are traceable to the National Institute for Standards and Technology (NIST) for their accuracy.
      2. In-house Preparation from NIST single element standards: Different volumes may be prepared by adding proportionally larger or smaller volumes of solution constituents.
         a. Acid-rinse one 100 mL, PP (or PMP) volumetric flask. Mark the flask according to intended use. Dedicate to purpose.
         b. Partially fill the 100 mL flask with the 3% (v/v) HCl diluent (50-75% full).
         c. Add necessary volumes of single-element stock standards to achieve final concentrations.
         d. Dilute to the volumetric mark with the 3% (v/v) HCl diluent using a pipette for the final drops. Mix the flask solution thoroughly. Once mixed, transfer to an
acid-cleaned, labeled, 50-mL container (PP, PMP, or Teflon™) for storage. Label appropriately (e.g. “Whole Blood Metals Panel 2 Intermediate Stock Calibration Standard”, “3% (v/v) HCl”, date of preparation, expiration date (1 year from date of preparation), initials of preparer, and concentrations for each element).

3. **In-house Preparation from other single element standards**: Different volumes may be prepared by adding proportionally larger or smaller volumes of solution constituents.

   a. Acid-rinse one 100 mL, PP (or PMP) volumetric flask. Mark the flask according to intended use. Dedicate to purpose.
   
   b. Partially fill the 100 mL flask with the 3% (v/v) HCl diluent (50-75% full).
   
   c. Add necessary volumes of single-element stock standards to achieve final concentrations.
   
   d. Dilute to the volumetric mark with the 3% (v/v) HCl diluent using a pipette for the final drops. Mix the flask solution thoroughly.
   
   e. Once mixed, transfer to an acid-cleaned, labeled, 50-mL container (PP, PMP, or Teflon™) for storage. Label appropriately (e.g. “Whole Blood Metals Panel 2 Intermediate Stock Calibration Standard”, “3% (v/v) HCl”, date of preparation, expiration date (1 year from date of preparation), initials of preparer, and concentrations for each element).

4. **Storage**: Store at room temperature. If purchased, label bottle with additional information such as “store at room temperature”, date received, date opened, and initials of person to first open.

j. **Intermediate Working Calibration Standards**

   a. **Purpose**: Used each day of analysis to prepare the final five working calibrators that will be placed on the autosampler.
   
   b. **Content**: Five aqueous dilutions of the intermediate stock calibration standard solution with a 3% (v/v) hydrochloric acid (HCl) matrix.
   
   c. **Preparation & Storage**: Different volumes may be prepared by adding proportionally larger or smaller volumes of solution constituents.
1. **Cleaning flasks:** Acid-rinse five 100 mL, PP (or PMP) volumetric flasks. Mark each flask according to intended use. Dedicate to purpose.

2. **3% (v/v) HCl Diluent Preparation:** use the same 3% (v/v) HCl prepared in Section 6.g.

3. Partially fill each 100 mL flask with the 3% (v/v) HCl diluent (50-75% full).

4. Add the correct volume of the Intermediate Stock Standard Calibration Standard (according to Table 5)
   a. If a separate Pb Intermediate Stock Calibrator is used, add the appropriate volume of this solutions according to Table 5 to the same flask.

5. Dilute to the volumetric mark with the 3% (v/v) HCl diluent using a pipette for the final drops. Mix the flask solution thoroughly.

6. Once mixed, transfer to acid-cleaned, labeled, 50 mL containers (PP, PMP, or Teflon™) for storage. Label appropriately (e.g. “Whole Blood Metals Panel 2 Intermediate Working Calibrators”, “3% (v/v) HCl”, date of preparation, expiration date (1 year from date of preparation), initials of preparer, concentration of each element, and Lot # of the stock solution).

7. Pour 10-15 mL of each solution into 15 mL tubes for daily use.

**k. Working Calibration Standards**

a. **Purpose:** The working calibration standards 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 (element/internal standard) from the dilution of the patient blood sample to the signal ratio response curve from the working calibrators.

b. **Content:** Dilutions (1:50) of the corresponding five intermediate working calibration standards.

c. **Preparation & Use:** Make immediately prior to analysis when the intermediate working calibration standards are mixed with base blood (Section 6.d) and diluent (Section 6.c) using a Digiflex automatic pipette.

**l. Single-Element Stock Standards For Preparation of Intermediate Stock Calibration Verification Standard**
a. **Purpose:** These single-element standards will be used to prepare the intermediate stock calibration verification standard.

b. **Contents:** Separate, aqueous single-element standards of Cd, Pb, Hg, Se, and Mn. Concentrations should be 1,000 mg/L or 10,000 mg/L.

c. **Purchase & Storage:**
   1. **Purchasing from vendors:** If the intermediate stock calibration verification standard is purchased as a special-mix standard, these single-element stock standards are not required. Purchase only NIST-traceable single-element standards at the highest purity (don’t contain other metal impurities).
   2. **Storage:** Store at room temperature.

m. **Intermediate Stock Calibration Verification Standard**

a. **Purpose:** This multi-element solution will be used to prepare the three working calibration verification standards.

b. **Preparation & Storage:** This solution may be purchased as a special-mix standard or prepared in-house from separate single-element stock standards.
   1. **Purchasing from vendors:** The intermediate stock calibration verification standard may be purchased as custom mixture from any vendor which prepares multi-element solutions that are traceable to the National Institute for Standards and Technology (NIST) for their accuracy.
   2. **In-house Preparation:** Different volumes may be prepared by adding proportionally larger or smaller volumes of solution constituents.
      a. Acid-rinse two 50 mL, PP (or PMP) volumetric flasks. Mark the flasks according to intended use. Dedicate to purpose.
      b. Partially fill the 50 mL flasks with the 3% (v/v) HCl diluent (50-75% full).
      c. Add necessary volumes of single-element stock standards to achieve final concentrations.
      d. Dilute to the volumetric mark with the 3% (v/v) HCl diluent using a pipette for the final drops. Mix the flask solution thoroughly.
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e. Once mixed, transfer to an acid-cleaned, labeled, 50-mL containers (PP, PMP, or Teflon™) for storage. Label appropriately (e.g. “Whole Blood Metals Panel 2 Intermediate Stock Calibration Verification Standard (Cd, Mn, Hg)”, and “Whole Blood Metals Panel 2 Intermediate Stock Calibration Verification Standard (Pb)”, “3% (v/v) HCl”, date of preparation, expiration date (1 year from date of preparation), initials of preparer, and concentrations for each element).

3. Storage: Store at room temperature. If purchased, label bottle with additional information such as “store at room temperature”, date received, date opened, and initials of person to first open.

n. Intermediate Working Calibration Verification Standards:

a. Purpose: Used as needed to on the day of analysis to prepare the necessary working calibration verification standard(s) that will be placed on the autosampler.

b. Content: Three aqueous dilutions of the intermediate stock calibration verification standard with a 3% (v/v) hydrochloric acid (HCl) matrix

c. Preparation & Storage: Different volumes may be prepared by adding proportionally larger or smaller volumes of solution constituents.

1. Cleaning flasks: Acid-rinse three 100 mL, PP (or PMP) volumetric flasks. Mark each flask according to intended use. Dedicate to purpose.

2. 3% (v/v) HCl Diluent Preparation: use the same 3% (v/v) HCl prepared in previous section.

3. Partially fill each 100 mL flask with the 3% (v/v) HCl diluent (50-75% full).


5. Dilute to the volumetric mark with the 3% (v/v) HCl diluent using a pipette for the final drops. Mix the flask solution thoroughly.

6. Once mixed, transfer to acid-cleaned, labeled, 50 mL containers (PP, PMP, or Teflon™) for storage. Label appropriately (e.g. “Whole Blood Metals Panel 2 Intermediate Working Calibration Verification Standard #”, “3% (v/v) HCl”, date of preparation, expiration date (1 year from date of preparation), initials of preparer, and concentrations for each element).
from date of preparation), initials of preparer, concentration of each element and Lot # of the stock solution).

7. Pour 10-15 mL of each solution into 15 mL tubes for as-needed use.

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

a. **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.

b. **Content:** Pooled animal or human blood, and may have been spiked with NIST-traceable elemental standards to reach desired low-normal and high-normal concentrations.

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

1. **Purchase of whole blood:** Bags of human blood can be purchased from various sources such as American Red Cross of Tennessee Blood services (http://tennesseebloodservices.com/). Animal blood may be available from the Wisconsin State Laboratory of Hygiene (WSLH).

2. **Screening blood:** Screen bags of blood for analyte of interest concentration before mixing together to make 2 separate base blood pools (for preparing the low and high bench QC materials). Samples can be screened individually

   a. Keep blood refrigerated whenever possible to minimize microbial growth.

   b. Because this is only a quick screen of the analyte of interest concentration, the number of replicates in the blood method can be reduced to one in order to reduce analysis time.

   c. Analyte concentrations in the final blood pool to be spiked for the low bench QC pool should be in the low-normal population range. Analyte concentrations in the final blood pool to be spiked for the high bench QC pool should be less than some pre-selected target
3. **Combining Collected blood**: The goal is for combining samples is to approach an ‘average’ matrix for each pool.
   a. Graduate four acid-washed 10 L carboys (PP or PMP) in 0.5 L increments (two will be used for decanting into).
   b. Combine collected blood samples into two separate acid-washed 10 L carboys (PP or PMP), according to their concentrations, for the low bench and high bench QC pools.
   c. Mix each blood pool using carboy stirrers and large stir plates. Keep blood refrigerated whenever possible.

4. **Spiking of blood**
   a. Analyze three samples of each blood pool. 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. 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).
   d. Continue to stir pools overnight after spiking, then reanalyze.
   e. Repeat steps 4 and 5 until all analytes reach target concentrations keeping track of the total volume of spiking solution added to each blood pool.

5. **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.
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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.

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

1. Allow blood to reach room temperature before dispensing (to prevent temperature gradients possibly causing concentration gradients across the large number of vials being dispensed and to prevent condensation problems during labeling of vials). This may require leaving the carboy of blood at room temperature overnight before dispensing.

2. Replace the tubing attached to the dispensing syringe (left when looking at front of Digiflex) with a length of clean Teflon™ tubing long enough to reach into the bottom of the 10 L carboy while it is sitting on the stir plate.

3. Check cleanliness of Digiflex before use by analyzing 1-2% (v/v) HNO3 which has been flushed through the Digiflex with a portion of the same solution which has not been through the Digiflex.

4. Approximately one hour before dispensing begins,
   a. With the large stir plate close to the left side of the Digiflex, begin stirring the blood pool to be dispensed.
   b. Also during this time, flush the Digiflex 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 with Parafilm so they will not come out during the flushing process.

5. 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.

Homogeneity Testing: After dispensing, check homogeneity of analyte concentrations in pool aliquots. Keep samples pulled for homogeneity analysis in the sequence that they were dispensed for the purpose of looking for trends in concentrations. Once dispensed and homogeneity has been
shown to be good throughout the tubes of a pool, store tubes at ≤ -20°C and pull tubes out as needed for analysis.

Storage: Blood pools should be stored long term at ≤ -20°C. Short term storage (several days) at refrigerator temperature (~ 2-4°C).

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Calibration Verification:

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

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

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

For frequent verification needs (i.e. when certain studies have many elevated results) or when a concentration higher than those shown in Table 8 p.66 needs to
be verified, use NIST-traceable single element stock standards to prepare single element verification standards. This will limit the exposure of the instrument to elevated concentrations of only the elements needing verification.

Always verify that normal background levels have been re-achieved through adequate rinse time following analysis of elevated standards for calibration verification. An external reference material (i.e. historical proficiency testing sample) can be substituted in place of the Calibration Verification Standard sample in these situations IF:

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

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

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

iv. A note to file is made that this was done.

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

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

Analytical Instrumentation & Parameters

a. Instrumentation & Equipment Setup:

1. ICP-DRC-MS: Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectrometer ELAN® DRC II.

   Modifications made to ICP-DRC-MS

   Stainless steel tubing is preferred between the reaction gas cylinder / regulator and the back of the ICP-DRC-MS instrument. A second mass flow controller will be needed (channel B) that does not send the DRC gas through a ‘getter’. Standard built-in peristaltic pump replaced by DXi-FAST micro-peripump / FAST actuator unit.

b. Sample Introduction Setup Notes and Tips
1. SC-FAST valve setup: Valve connections must match this description.
   a. Port 1: 1mL sample loop (white nut).
   b. Port 2: 0.5 mm ID probe (red nut) for carrier solution.
   c. Port 3: nebulizer line (green nut) for transfer of liquid to nebulizer.
   d. Port 4: sample loop (white nut).
   e. Port 5: 0.8 mm ID probe (blue nut) for diluted samples.
   f. Port 6: 1/8” i.d. vacuum line (black nut).

2. Tubing connection between autosampler rinse station and rinse solution reservoir: Tubing of different inner diameters can be obtained from Elemental Scientific, their distributors, or custom built in the lab to optimize the rinse station fill rate between samples. Rinse station should not go empty at any point.

3. Tubing for autosampler rinse station waste removal: Use minimum drain tubing to make this connection. If this tube is too long, the rinse station will not drain properly.

4. Rinse solution jug: Leave one of the caps on the top of the rinse jug loose to allow air venting into the jug as liquid is removed. Otherwise the jug will collapse on itself as the liquid is removed and a vacuum is created inside. Use secondary containment tray and label appropriately (see solution preparation instructions).

5. Waste solution jug: Use secondary containment tray and label appropriately (see solution preparation instructions).

6. Configuration of tubing and probe for carrier solution: Can use a PEEK adapter to help with connecting peristaltic tubing to to Teflon tubing.

7. Nebulizer: Changing the nebulizer type will require re-optimization of the read delay time in the ELAN software. Polypropylene nebulizer type (ESI) tends to allow for shorter read delay times than quartz concentric nebulizers.

8. Configuration of tubing for spray chamber waste removal:
   a. Chamber-to-peristaltic pump tubing:
      i. Spray chambers with threaded connection: Use vendor-supplied threaded connector on base of chamber, connecting tubing directly to peristaltic pump tubing through a PEEK adapter or directly.
      ii. Spray chambers without threaded connection: Use push-on connectors with Teflon tubing available from various vendors or
connect 1/8” i.d. x ¼” o.d. PVC tubing directly to the waste port on the spray chamber. Connect other end of PVC tubing to the white / black peristaltic pump tubing using a tubing connector (PerkinElmer item # B3140715).

b. Waste Jug-to-peristaltic pump tubing: Connect 1/8” i.d. x ¼” o.d. PVC tubing to the white / black peristaltic pump tubing using a tubing connector (PerkinElmer item # B3140715). Place the free end of the PVC tubing through the lid of the waste jug (be sure it is secure). Waste jug should be sitting in a secondary containment tray in case of overflow.

c. Cones: Platinum or Nickel cones have been used and tested to be comparable in performance from either PerkinElmer or Spectron.

d. Gases & Regulators setup:

1. **Argon:** Argon stored as liquid in a dewar (180-250 L) or bulk tank. Gaseous argon used for plasma and nebulizer.

   a. **Regulator for argon source (if a dewar):** Keep the inlet pressure (headspace pressure of liquid argon dewar) above 100 psi. Set delivery pressure to 90-100psi to allow for pressure drop across tubing that stretches to the instrument. See Section 5.e for part numbers and details.

   b. **Step down regulator (if source of argon is a bulk tank):** Place this single stage regulator in the lab so that incoming argon pressure can be monitored and adjusted. Set delivery pressure to approximately 85 -100 psig. See Section 5.e for part numbers and details.

   c. **Regulator at ICP-DRC-MS:** Single stage “argon regulator filter kit” supplied with the ICP-DRC-MS. Set the delivery pressure to approximately 80 psi.

2. **Methane (99.99%) gas for DRC channel A**

   a. **Regulator for CH4 gas:** Set the delivery pressure = 5-7 psig. See section 5.e for part numbers and details.

3. **Oxygen (99.999%) gas for DRC channel B.**

   a. **Regulator for O2 gas:** Set the delivery pressure = 5-7 psig. See Section 5.e for part numbers and details.

   b. **Flash arrestor:** Brass flash arrestor is used on outlet side of regulator. See Section 5.e for part numbers and details.

**Chiller / Heat Exchanger:** Refrigerated chiller (for ELAN® 6100 DRC®Plus instruments) or heat exchanger (for ELAN® DRC II...
instruments). For refrigerated chiller, set temperature control to 18°C.

4. **Computer**: Dell Optiplex GX150, GX270, or GX280 have all been used. Processors used have included Pentium III (1 GHz) through Pentium IV (2.8 GHz). Recommend 512Mb - 1Gb RAM. External hard disk drive for nightly backups of data connects via USB port. Software used includes Windows XP Professional, service pack 2 and ELAN v3.3.

5. **Autosampler**: ESI SC-FAST series

**Daily Analysis of Samples**

a. **Preparation of the Analytical Equipment**

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

1. **Power on** the computer, printer, peristaltic pump, and autosampler, and log into the operating system.

2. **Peristaltic pump**: Set up the peristaltic pump tubing with proper tension for the sample rinse station.

3. **Software**: Start the ESI autosampler and ELAN® ICPMS software from Windows™.

4. **Daily Pre-Ignition Maintenance Checks**: Perform daily maintenance checks as described in the IRAT Daily Startup SOP for ELAN instruments (i.e., Ar supply pressure, interface component cleanliness and positioning, interface pump oil condition, vacuum pressure, etc.). Make appropriate notes in the Daily Maintenance Checklist and Instrument Log Book.

5. **Start the Plasma**: Press the “Start” button in the software or on the hardware to ignite the plasma.

   a. **Start the peristaltic pump**: Start the peristaltic pump in the software at 1.5rpm (~160uL/min, ESI DXi mini-peristaltic pump). Verify the rotational direction is correct.

6. **Aspirate liquid**: Place the carrier probe into dilute acid or water.

7. **Warm-up time**: Allow at least 45 minutes warm-up time for the ICP-DRC-MS after igniting the plasma. This warm-up time is for the RF generator. There will be another “Stability time” for the DRC later in this procedure.

8. **Daily Performance Check**: After this warm-up time, perform a daily performance check and any optimizations necessary (as described
in the IRAT Daily Startup SOP for ELANs). Fill in the Daily Maintenance Checklist according to the optimization procedures performed. Extra detail than can be documented in the checklist should go into the instrument logbook.

a. Magnesium ($^{24}\text{Mg}$) may have high RSDs due to the use of Triton-X100 in the rinse solution. Avoid this problem by either temporarily using non-Triton-containing rinse solution during the daily check, or repeating the daily check multiple times in succession with no rinse time between.

b. Saving the Files: Save updated tuning and optimization parameters to the “default.tun” and “default.dac” files, respectively.

9. Software setup for Analysis:
   a. Workspace (files & folders): Open the default (“CDC_WBMP2_methITB006A_.wrk”) or your own personalized workspace in the ELAN software. Verify & set up the correct files and data directories for your analysis (See Table 1 pp 60-62 “File Names & Directories”).
   b. Samples / Batch Window: Update software to reflect the current sample set. The only fields which need to be filled in include the autosampler location, sample identification (id), measurement action, method, sample flush time, sample flush speed, read delay time, read delay & analysis speed, wash time, wash speed. Use a bar code scanner to input data whenever possible. See Table 1 pp 60-62 for times and speeds. Save the Sample window file and re-use it on other days by simply replacing the sample IDs for the patient samples.

1. DRC Stability Time: Best analyte-to-internal standard ratio stability is typically obtained after 1-1.5 hrs of repeated analysis of blood samples using the DRC method. Analyze enough “dummy” blood sample dilutions prior to any DRC analysis run to fill 1-1.5 hours of analysis time.

2. Blood vs. Aqueous Method Files:
   a. The difference: There are two ELAN method files for this one method (see Table 1 pp 61-62). It is necessary to use both to accomplish each run because the current PerkinElmer software will not allow for more than one blank per method file. The ONLY DIFFERENCE between these two files is on the Sampling tab where one lists the autosampler positions of the blood blank and blood
calibrators (the “bldblk” method file) and the other lists the autosampler position of the aqueous blank (the “aqblk” method file).

b. Use: The ONLY TIME when it matters which of these files is used is when the measurement action includes “Run blank” or “Run standards”. When the measurement action is only ‘run sample’, it does not matter whether the “bldblk” or “aqblk” method file is used. Analysts typically follow the pattern below, however, for the sake of consistency and as a reminder of which blank must be used for which type of sample. See Table 10 p.68.

i. The “bldblk” method file: Use to analyze the initial blood blank (blank for the calibration curve), the blood calibrators, and the blood blank checks (WB Blank & WB Blank 2) at the very beginning of the run. The blood blank method defines the blood blank in autosampler location 105 and the blood calibration standards 1-5 in autosampler locations 106-110, respectively.

ii. The “aqblk” method file must be used to analyze all QC materials and patient samples. The aqueous blank method (set up for a ESI SC4 autosampler) defines the aqueous blank in autosampler location 113.

3. Notation of Dilutions: To designate an extra dilution of a sample, edit the sample ID to reflect the level of dilution being performed (e.g., A 1:2 dilution of sample 1 would be reflected in the sample ID “sample 1 (2x dilution)”. This sample ID will be edited during the data-import process to the database so that it is recognized as the appropriate sample. Do not use the ELAN® software to automatically correct for sample dilutions.

c. Method file modifications: This method can also be used to analyze whole human blood samples for a subset of the listed samples (i.e. Pb only). To do this, delete the unnecessary elements from the method windows (bldblk and aqblk) and save the file with a descriptive name such as “WBMP2_DLS3016_bldblk_Pb_only.mth” and “WBMP2_DLS3016_aqblk_Pb_only.mth”.

Preparation of Samples for Analysis

1. Thaw the frozen blood specimens; allow them to reach ambient temperature.

2. Prepare enough DRC stability sample to be analyzed for 1-1.5 hr before the beginning of the run. This can be prepared using 50 mL
polypropylene tubes or a wide-mouth bottle (which can be put on the autosampler in place of one of the tube trays).

3. Set up a series of 15 mL polypropylene tubes corresponding to the number of blanks, standards, QCs, and patient samples to be analyzed.

4. Prepare the following solutions in the 15 mL falcon tubes using the Digiflex™

a. **Aqueous Blank**: Prepare a minimum of two aqueous blanks consisting of 200 µL of ≥18 Mega-ohm-cm water and 4800 µL of diluent. One will be the actual aqueous blank and the others will be backups (“Aqueous Blank Check”) in case the original aqueous blank gets contaminated.

b. **Blood Blank (Std 0)**: Prepare a minimum of three blood blank dilutions consisting of a 1:1:48 proportion of base blood:standard zero:diluent. Typical preparation will be 100 µL of base blood (same material used to prepare the blood calibration standards), 100 µL of > standard zero (3% (v/v) HCl), and 4800 µL of diluent. One of these blood blanks will be the blank for the calibration standards; the others will be analyzed after standard 5 as WB Blank and WB Blank2.

c. **Calibrators**: Prepare the working calibration standards as 100 µL of the appropriate aqueous intermediate working calibration standard, 100 µL of base blood, and 4800 µL of diluent.

d. **Patient & QC Samples**: Before taking an aliquot for analysis, mix the sample on the vortex for approximately 15 seconds. Prepare blood sample dilutions as 4,800 µL of diluent and 100 µL of the blood sample and 100 µL of ≥18 Mega-ohm-cm water.

e. Cap all of the blanks, standards, and samples and mix them on the Vortex for approximately 10 seconds. Uncap them and place them in the autosampler of the ELAN® ICPMS in the order that was entered in the Samples / Batch window of the ELAN software.

Patient Results:

a. **Elevated Results**:

**Boundaries Requiring Confirmatory Measurement**:

1. **Results Greater than the First Upper Boundary (1UB)**: Concentrations observed greater than the “first upper boundary” (defined in the laboratory database as the “1UB”) should be confirmed by repeat analysis of a new sample preparation. The concentration assigned to the 1UB for an element is determined by study 1. Continue repeat analysis until a concentration can be confirmed.

2. **Results Greater Than Highest Calibrator**: When a sample result is greater than the highest calibrator in the run, the supervisor may request that the result be confirmed in an analysis run which includes
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a standard or external reference material with equivalent (within 10%) or greater concentration than the sample.

3. Results Greater Than Calibration Verification Standard: Perform an extra dilution up to 20x on any blood sample whose concentration is greater than those listed.

Inadequate Precision in Confirmation of a Measurement: If a sample is reanalyzed to obtain a confirmation of an initially elevated result, the confirmation should be within 10% of the original result.

Analyst Reporting of Elevated Results: Concentrations observed greater than the “second upper boundary” (defined in the laboratory database as the “2UB”) should be reported to the QC reviewer as an “elevated result”. The analyst should report any patient results confirmed to be greater than the second upper boundary to the QC reviewer as an “elevated result”. There is no routine notification for elevated levels for the metals determined in this method. The protocol for supervisors reporting elevated results to medical personnel is defined according to the study protocol.

Inadequate Precision Within One Measurement: If the range of the three replicate readings (maximum replicate concentration value - minimum replicate concentration value) for a single sample analysis is greater than the criteria and the range of the three replicate readings is greater than 10% of the observed concentration, do not use the measurement for reporting. Repeat the analysis of the sample.

Submitting final work for Review: Once results have been imported, reviewed, and set as final in the database by the analyst,

Submit an email to the QC reviewer informing them of the readiness of the data for final review. The email should follow requirements specified by the QC reviewer and will include:

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

b. Any bench QC failures (include reasons if known).

c. Any patient sample result greater than the 2UB boundaries.

d. Anything out of the ordinary about this analytical work which could have a bearing on the availability (i.e. insufficient sample to analyze), accuracy, or precision of the results.

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

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

24 hrs / day operation in DRC mode:

To reduce startup time in the mornings, the analyst is encouraged to operate the ELAN in DRC mode 24hrs/day during the work week. This eliminates the need for daily 45 minute RF generator warm-up, and possibly the need for DRC stability time (if the DRC gas is not off for extended periods of time before analysis). To maintain the instrument in DRC mode when not analyzing patient samples, setup multiple sample rows in the Samples / Batch window with autosampler position in zero (rinse station of autosampler) and wash time of 1800s (30 minutes). Repeat this sample row enough times to keep the instrument in analysis mode overnight (1 sample with 15 minute wash will take ~ 25 minutes).

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

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

Press the “Change” button within the Auto Stop box and set the Delayed shutdown time to 5 minutes. This will rinse the sample introduction system of blood matrix before turning off the plasma.

It will be necessary to replace the sample peristaltic pump tubing the next day since it will have been clamped shut overnight.

Equipment Maintenance: Analysts are expected to follow a 4-day analysis / 1-day maintenance schedule in the laboratory.

ICPMS Maintenance: On the maintenance day, perform all maintenance per the IRAT ELAN ICP-MS Weekly Maintenance SOP. All equipment maintenance should be documented in the instrument checklist and logbook.

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

1. Daily Backups to External Hard Drive: Automatic backups of the “elandata” directory and all subdirectories should be programmed to occur each night onto an external hard disk using a three-file rotating backup scheme.

2. Weekly Backup to CD: Backup all files in the active “elandata” directory and all subdirectories onto one recordable compact disc during the weekly maintenance SOP. When the active “elandata” directory on the ICP-DRC-MS computer hard drive becomes too large to fit onto a single recordable compact disk, the oldest data can be removed from the computer to make it easier to backup the entire directory weekly. This can usually be done annually.
a. Backup the oldest data on the hard drive to two duplicate compact disks and verify that the files on the CD are readable.

b. Label them with the name of the instrument, the date range of the data, the current date, your name, and “Copy 1 of 2” or “Copy 2 of 2”.

c. After verifying that the CDs are readable, the oldest, backed up data can be deleted from the ICP-MS computer hard drive.

d. It is best to not store duplicate copies in the same location.

Interpretation of the Results

Reportable Range: Whole blood metals values are reportable in the range between the method LOD (see Section 10.a) and the highest concentration verified accurate by calibration verification tests. For example, if a blood metals concentration is less than the method LOD, report it as < LOD. Above the highest concentration verified, up to 20x extra dilutions are made of the blood sample to bring the measured (instrument) concentration within the verified range. Results greater than 20x the highest calibration verification concentration tested (“X” concentration) should be reported as “> X”.

Action Levels: Concentrations observed greater than the “second upper boundary” (defined in the laboratory database as the “2UB”) should be reported to the QC reviewer as an “elevated result”. The analyst should report any patient results confirmed to be greater than the second upper boundary to the QC reviewer as an “elevated result”. The protocol for supervisors reporting elevated results to medical personnel is defined according to the study protocol. Levels of concern for mercury in blood are >100 µg/L for children (6 yr and younger) and >200 µg/L for adults. Levels of concern for lead in blood are 25 µg/dL for children (6yr. and younger) and 40 µg/dL for adults. Levels of concern for cadmium in blood is >5 µg/L.

Method Calculations

Method Limit of Detection (LODs): The method detection limits for elements in blood specimens 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.

Method Limit of Quantitation (LOQ): The Division of Laboratory Sciences does not currently utilize limits of quantitation in regards to reporting limits.
QC Limits: Quality control limits are calculated based on concentration results obtained in at least 20 separate runs. It is preferable to perform separate analyses on separate days and using multiple calibrator lot numbers, instruments, and analysts to best mimic real-life variability. The statistical calculations are performed using the SAS program developed for the Division of Laboratory Sciences (DLS_QC_compute_char_stats.sas).

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

9. REPORTABLE RANGE OF RESULTS

Whole blood metals values are reportable in the range between the method LOD and the highest concentration verified accurate by calibration verification tests. For example, if a blood metals concentration is less than the method LOD, report it as < LOD. Above the highest concentration verified, up to 20x extra dilutions are made of the blood sample to bring the measured (instrument) concentration within the verified range. Results greater than 20x the highest calibration verification concentration tested (“X” concentration) should be reported as “> X”.

10. QUALITY CONTROL (QC) PROCEDURES

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

Types of Quality Control:

"Bench QC": The bench QC pools used in this method comprise two levels of concentration spanning the “low-normal” and “high-normal” ranges of the analyte of interest. The intent of bench QC is for the analyst to evaluate the performance of the analytical system on the day of analysis. The analyst inserts both the “low” and the “high” bench QC specimens two times in each analytical run (a set of consecutive assays performed without interruption) so that judgments may be made on the day of analysis. The first analysis of the two bench QC pools is done after the calibration standards are analyzed but before any patient samples are analyzed (so that judgments on the calibration curves may be made before analysis of patient samples). The second analysis of the two bench QC pools is done at the end of the run (approximately 20 patient samples total). If more patient samples are analyzed on the same calibration curve after the second run of the bench QC, both the low-normal and high-normal bench QC must be reanalyzed before and after the additional samples.

Analyst Evaluation of Run Results:

Bench Quality Control: After completing a run, and importing the results into the LIMS, export the QC results to the SAS program where the analytes in the run will be judged to be in or out of control. The QC limits are based on the average and standard deviation of the beginning and ending analyses of each of the bench QC pools, so it will not be possible to know if the run is officially accepted or rejected until it is completed.

a. Quality Control Rules: The SAS program applies the division QC rules to the data as follows:

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

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

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

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

c. 2 2S Rule - Both run means are outside the same 2Sm limit
d. 10 X-bar Rule – Current and previous 9 run means are on same side of the characterization mean

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

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

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

Abbreviations:

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

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

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

Implications of QC Failures: If the division SAS program declares the run out of control", then all results from the run are invalid for reporting from the run. Set all run results as “QC Rejected” in the database.

External Reference Materials: Materials produced by laboratories outside of the CDC which have assigned target concentrations can be helpful in verifying method performance. Samples from previous challenges of proficiency testing programs (i.e. Centre de Toxicologie du Quebec (CTQ)) can be used. However, only the results for the bench and blind QC materials are used to determine if the run results can be used.

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

If an analyte result for the beginning QC material(s) falls outside of the 99% limits, then the following steps are recommended:

If a particular calibration standard is obviously in error, remake a new dilution at the Digiflex of that working calibrator, reanalyze it, and reprocess the sample analyses using this new result as part of the calibration curve.

Prepare a fresh dilution of the failing QC material and reanalyze it.

Prepare fresh dilutions at the Digiflex of all of the calibration standards (working blood multi-element standards) and reanalyze the entire calibration curve using the freshly prepared standards.
Blood Metals Panel in whole blood
NHANES 2011-2012

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

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

*Elevated patient results:* Confirmation by repeat measurement will be required for any result greater than the “1st upper boundary”. A calibration verification check of equal or greater concentration must be analyzed in the same run as the elevated study sample result if it is to be used for reporting.

11. **REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA**

If an analyte result for the beginning QC material(s) falls outside of the 99% limits, then the following steps are recommended:

a. If a particular calibration standard is obviously in error, remake a new dilution at the Digiflex of that working calibrator, reanalyze it, and reprocess the sample analyses using this new result as part of the calibration curve.

b. Prepare a fresh dilution of the failing QC material and reanalyze it.

c. Prepare fresh dilutions at the Digiflex of all of the calibration standards (working blood multi-element standards) and reanalyze the entire calibration curve using the freshly prepared standards.

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

12. **Limitations of Method; Interfering Substances and Conditions**

Reduction of argon dimer (40Ar2+) interference on selenium (80Se+) using ICP-DRC-MS: 40Ar2+ is a polyatomic ion formed in the plasma as a result of a reaction between the plasma gas (Ar) and itself. The dynamic reaction cell of the ELAN ICP-DRC-MS is used to reduce ion signals from polyatomic ions via ion-molecule reaction chemistry.

In the reaction cell, methane (CH4) molecules react with 40Ar2+ ions through a charge transfer reaction. The products of the reaction are 40Ar+ (ion at a different mass) and 40Ar (neutral). The background ion signal at m/z 80 is reduced by six orders of magnitude because of this reaction.
Reduction of argon nitride ($^{40}\text{Ar}^{15}\text{N}^+$), argon hydroxide ($^{38}\text{Ar}^{16}\text{O}^+\text{H}^+$) interference on manganese ($^{55}\text{Mn}$) using ICP-DRC-MS: $^{40}\text{Ar}^{15}\text{N}^+$ and $^{38}\text{Ar}^{16}\text{O}^+\text{H}^+$ are polyatomic ions 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$_2$O). The dynamic reaction cell of the ELAN ICP-DRC-MS is used to reduce ion signals from polyatomic ions via ion-molecule reaction chemistry. In the reaction cell, oxygen molecules react with $^{40}\text{Ar}^{15}\text{N}^+$ and $^{38}\text{Ar}^{16}\text{O}^+\text{H}^+$ ions through either charge transfer reactions or oxygen 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 transfer). In either case, attenuation of the background ion signal at m/z 55 occurs.

Reduction of $^{37}\text{Cl}^{18}\text{O}^+$, $^{39}\text{K}^{16}\text{O}^+$, $^{54}\text{Fe}^+\text{H}^+$ interferences on manganese ($^{55}\text{Mn}$) using ICP-DRC-MS: $^{37}\text{Cl}^{18}\text{O}^+$, $^{39}\text{K}^{16}\text{O}^+$, $^{54}\text{Fe}^+\text{H}^+$ are polyatomic ions created in the plasma as a result of reactions between elements present in the blood matrix (Cl, K, and Fe) and the solvent (H$_2$O). Due to the high concentrations of Cl, K, and Fe in the blood matrix the resulting ion signals of $^{37}\text{Cl}^{18}\text{O}^+$, $^{39}\text{K}^{16}\text{O}^+$, and $^{54}\text{Fe}^+\text{H}^+$ interfere with the measurement of $^{55}\text{Mn}^+$ at m/z 55. The dynamic reaction cell of the ELAN ICP-DRC-MS is used to reduce ion signals from polyatomic ions via ion-molecule reaction chemistry. In the reaction cell, oxygen molecules react with $^{37}\text{Cl}^{18}\text{O}^+$, $^{39}\text{K}^{16}\text{O}^+$, $^{54}\text{Fe}^+\text{H}^+$ ions through either charge transfer reactions or oxygen 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 transfer). In either case, attenuation of the background ion signal at m/z 55 occurs.

13. CRITICAL CALL RESULTS ("PANIC VALUES")

Concentrations observed greater than the “second upper boundary” (defined in the laboratory database as the “2UB”) should be reported to the QC reviewer as an “elevated result”. The analyst should report any patient results confirmed to be greater than the second upper boundary to the QC reviewer as an “elevated result”. The protocol for supervisors reporting elevated results to medical personnel is defined according to the study protocol. Levels of concern for mercury in blood are >100 µg/L for children (6 yr and younger) and >200 µg/L for adults. Levels of concern for lead in blood are 25 µg/dL for children (6yr. and younger) and 40 µg/dL for adults. Levels of concern for cadmium in blood is >5 µg/L.
14. SPECIMEN STORAGE AND HANDLING DURING TESTING

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

NOTE: Samples must be analyzed within 24 hours of preparation to obtain valid results for selenium. The method has been validated to produce valid results for other Pb, Cd, Hg, and Mn even 48 hrs after sample preparation.

i. Starting the Analysis: Begin the analysis using the ELAN software.

ii. Monitoring the Analysis: It is preferable to initiate work early enough in the day to permit the entire run to be monitored. If it is not possible to complete the analysis by the end of the work day, the run may be left to complete itself unattended as long as appropriate planning is made for either overnight operation or Auto Stop (see below).

Monitor the analysis for the following:

1. **DRC stability (analyte / internal standard ratio stability)**
   
   After the analysis of the DRC stability “dummy” samples, the stability of the analyte / internal standard ratios across these samples indicates the instrument stability going into the run.

2. **Proper operation of the instrument.**

3. **Contaminated blanks.**
   
   All QC and samples are treated with the same aqueous blank, but backup/new preparation of blanks may be substituted.

4. **Linear calibration curves.**
   
   a. Typical correlation coefficients will be 0.999 to 1.000.

   b. The ELAN software generates a “simple linear” calibration curve (using a least squares calculation) for manganese in this method. The curves are generated using the results from analysis of the blood blank and the 5 external blood calibrators whose concentrations are defined in the Calibration tab of the Method file. Specifically, the software plots the “net intensity” (y-axis) versus the analyte concentration (x-axis). The “net intensity” is the blank subtracted ratio of the measured intensity for the analyte to the measured intensity of the associated internal standard and is calculated as follows:
\[
\text{net intensity} = \frac{\text{Analyte Meas Intensity}_{\text{sample}}}{\text{Internal Std Meas Intensity}_{\text{sample}}} - \frac{\text{Analyte Meas Intensity}_{\text{Blank}}}{\text{Internal Std Meas Intensity}_{\text{Blank}}}
\]

c. One point, consisting of calibration standards 2, 3, or 4, may be removed from the calibration curve for each analyte, if necessary, to provide appropriate correlation coefficients. It is preferable, however, to re-analyze problematic calibration standards rather than dropping points. Recurring problems with calibration standards should be resolved expeditiously.

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

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

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

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

19. SUMMARY STATISTICS AND QC GRAPHS

See following pages.
### Summary Statistics for Cadmium (ug/L)

<table>
<thead>
<tr>
<th>Lot</th>
<th>N</th>
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<th>End Date</th>
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#### 2011-2012 Cadmium (ug/L) Quality Control

The graph shows the quality control of Cadmium levels over the period from March 2011 to March 2013, with data points for each lot as indicated by the legend.
## Summary Statistics for Lead (ug/dL)

<table>
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<th>Standard Deviation</th>
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## 2011-2012 Lead (ug/dL) Quality Control

![Graph showing lead levels over time for different lots](image-url)
Summary Statistics for Manganese (ug/L)

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<th>Lot</th>
<th>N</th>
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<th>End Date</th>
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### Summary Statistics for Selenium (ug/L)

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<th>Standard Deviation</th>
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### 2011-2012 Selenium (ug/L) Quality Control

![Graph showing Selenium levels from 2011 to 2012](image-url)
## Summary Statistics for Total mercury, blood (µg/L)

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<th>End Date</th>
<th>Mean</th>
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</tbody>
</table>

### 2011-2012 Total mercury, blood (µg/L) Quality Control
References

7. Sigel, H. and A. Sigel, Handbook of Toxicity of Inorganic Compounds, H.G. Sieler, Editor 1988, Marcel Dekker, INC.
<table>
<thead>
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<th>Reference</th>
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