



## **Laboratory Procedure Manual**

*Analyte:*           **Lead Cadmium Mercury**

*Matrix:*           **Whole Blood**

*Method:*           **Blood Lead Cadmium Mercury ICPDRCMS**

*Method No:*       **3001.1**

*Adopted:*          **January 22, 2003**

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*as performed by:*

Inorganic Toxicology and Nutrition  
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### **Important Information for Users**

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.

**Blood Cadmium and Lead using ICPMS  
NHANES 2009-2010**

**Public Release Data Set Information**

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

<b>Data File Name</b>	<b>Variable Name</b>	<b>SAS Label</b>
PBCD_F	LBXBCD	Cadmium ( $\mu\text{g/L}$ )
	LBDBCDSI	Cadmium ( $\mu\text{mol/L}$ )
	LBXBPB	Lead ( $\mu\text{g/dL}$ )
	LBDBPBSI	Lead ( $\mu\text{mol/L}$ )
	LBXTHG	Mercury, total ( $\mu\text{g/L}$ )
	LBDTHGSI	Mercury, total ( $\mu\text{mol/L}$ )

## **1) Summary of Test Principle and Clinical Relevance**

### **a. Clinical relevance**

Lead (Pb), and cadmium (Cd) are considered to be toxic at certain levels.

The main sources of mercury intake in humans are fish, dental amalgams, and occupational exposure. Occupational exposure also is the most common cause of elevated cadmium levels while environmental, occupational, or residential exposure is the most common cause of elevated lead levels. The main organs effected by mercury are the brain and the kidneys. 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.

Massive exposure over a longer period of time results in violent muscular spasms, hallucinations, delirium, and death]. For nonoccupationally burdened population: normal whole blood Hg levels < 3 µg/L – value inconspicuous, 3-10 µg/L – value increased, danger to health not recognizable, > 10 µg/L – value distinctly increased, on long-term basis danger to health cannot be excluded. Lead is not an essential element for humans. Nearly all lead in the body reflects exposure sources associated with human activities. In general, lead in whole blood ranges from 0.15 to 1.5 µmol/L depending on several factors. Children are most sensitive to the effect of Pb, and it has been suggested that even Pb blood levels below 1 µmol/L probably account for a tiny 2-3% reduction of cognitive performance, or around 4-5 IQ points. 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, particular in children. The alkyl lead species are highly toxic to the central nervous system. Newborn babies are practically free of Cd [8]. 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. Principal symptoms reported were respiratory distress due to chemical pneumonitis and edema.

It has been estimated that 8 hrs exposure to 5 gm Cd/m<sup>3</sup> will be lethal. Ingestion of high amounts of Cd may lead to a rapid onset with severe nausea, vomiting, and abdominal pain. Generally, the critical organ for Cd is the kidney. Kidney dysfunction is one of the most characteristic signs of exposure to Cd. In working environment at high exposure levels workers have developed proteinuria, renal glucosuria, aminoaciduria, hypercalciuria, phosphaturia, and polyuria. Chronic obstructive lung disease of varying degrees of severities is frequently seen in Cd workers. Concentration of cadmium in blood of healthy unexposed adults are in the range 0.1 – 4 µg/L. There are several methods for Hg, Pb, and Cd analyses. Hg may be analyzed by cold vapor atomic absorption spectrometry (CV-AAS).

Pb and Cd are commonly analyzed by graphite furnace atomic absorption spectrometry (GF-AAS). These methods are precise and dependable, but are generally single element determinations. Inductively coupled plasma mass spectrometry (ICP-MS) often enhances productivity because of its multi-element analysis capability.

## **b. Test principle**

Whole blood Hg, Pb, and Cd concentrations are determined using inductively coupled plasma mass spectrometry. This multi-element analytical technique is based on quadrupole ICP-MS technology. Coupling radio frequency power into a flowing argon stream seeded with electrons creates the plasma. Predominate species in the plasma are positive argon ions and electrons. Diluted whole blood samples are converted into an aerosol using a nebulizer inserted within a spray chamber. A portion of the aerosol is transported through the spray chamber and then through the central channel of the plasma, where it experiences temperatures of 6000-8000 K. This thermal energy atomizes, and ionizes the sample. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP, operating at atmospheric pressure (approximately 760 torr), from the mass spectrometer, operating at approximately  $10^{-5}$  torr. The mass spectrometer permits detection of ions at each mass-to-charge ratio in rapid sequence, allowing individual isotopes of an element to be determined. Once inside of the mass spectrometer, the ions pass through the ion optics, then the mass analyzing quadrupole before being detected as they strike the surface of the detector. The ion optics focuses the ion beam using an electrical field.

Electrical signals resulting from the detection of the ions are processed into digital information that is used to indicate the intensity of the ions and subsequently the concentration of the element. In this method blood samples are diluted with 18 M-ohm water and with diluent, containing 1% v/v tetramethylammonium hydroxide (TMAH), 0.05% disodium ethylenediamine tetraacetate (EDTA), 5% ethyl alcohol, 0.05% Triton X-100<sup>®</sup>, Au is added to reduce intrinsic Hg memory effects, Rh for internal standardization of Cd, and Bi for internal standardization of Hg and Pb [11-13]. The samples were prepared with the following ratio Sample: Water: Diluent = 1:1:48 correspondingly.

## **2) Safety Precautions**

Perkin Elmer provides safety information that should be read before operating the instrument. This information can be found in the Perkin Elmer ELAN 6100 ICP-DRC-MS Plus System Safety Manual. Possible hazards include ultraviolet radiation, high voltages, radio frequently radiation, and high temperatures.

Wear gloves, lab coat, and safety glasses while handling human blood. Disposable plastic, glass, and paper (pipette tips, autosampler tubes, gloves, etc.) that contacts blood is to be placed in a biohazard autoclave bag. These bags should be kept in appropriate containers until sealed and autoclaved. Wipe down all work surfaces where blood was handled with 10% v/v sodium hypochlorite solution when work is finished. The use of the foot pedal on the Micromedic Digiflex is recommended because it reduces analyst contact

with work surfaces that have been in contact blood and keeps the hands free to hold the specimen cups and autosampler tubes.

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.

Special care should be taken when handling and dispensing bases and concentrated acids. Wear powder free gloves, a lab coat, and safety glasses.  
**If TMAH or hydrochloric acid comes in contact with any part of the body, quickly wash with copious quantities of water for at least 15 minutes.**

### 3) Computerization; Data System Management

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

#### a. Data entry and transfer.

Bar code scanners should be used whenever possible to enter sample identifiers into the ICP-DRC-MS computer software to avoid errors associated with the keyboard-entry process and to speed up sample processing. When bar code scanners cannot be used, transcribed data must be proofread after entry. Data should be handled / transferred electronically when reporting or moving to other computerized data handling software. In the Inorganic Radiation and Analytical Toxicology (IRAT) Branch, sample analysis results generated by this method are stored long-term in Microsoft Access or a sequel server database software (including at least the analysis date, analytical run number, quality control results for the run, specimen analytical results by specimen ID, and method identifier).

#### b. Routine computer hard drive maintenance.

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

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

1. Daily Backups to External Hard Drive: Automatic backups of the "elandata" directory and all subdirectories should be programmed to occur each night onto an external hard disk.
2. Weekly Backup to CD: Backup all files in the active "elandata" directory and all subdirectories onto one recordable compact disc during the weekly maintenance SOP. When the active "elandata" directory on the ICP-DRC-MS computer hard

drive becomes too large to fit onto a single recordable compact 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.

**d. Documentation of system maintenance.**

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

Instrument maintenance: Documentation for system maintenance is contained in hard copies of data records (i.e. daily maintenance checklist, Perkin-Elmer service records, instrument log book) as well as electronic records relating to instrument optimization (default.dac), tuning (default.tun).

**4) Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection**

- a. Specimen type – whole blood
- b. No special instructions (i.e. fasting or special diet) are required prior to blood collection.
- c. Analytical volume is 0.05 mL; minimum recommended sample size **0.2 mL(0.25mL?)**; ideal >1mL.
- d. Acceptable containers include pre-screened polyethylene vials and pre-screened vacutainers should be used for specimen acquisition.
- e. Specimen stability has been demonstrated for several months at -20°C or at -70°C for several years.
- f. The criteria for an unacceptable specimen are either a low volume or suspected contamination due to improper collection procedures or collection devices. In all cases, a second blood specimen should be requested.
- g. Specimen characteristics that may compromise test results are as indicated above including contamination of blood by contact with dust, dirt, etc. from improper handling.

- h. Specimen handling conditions are outlined in the Division protocol for blood collection and handling (copies available in Branch, laboratory and Special Activities specimen handling offices). Collection, transport, and special requirements are discussed. In general, if more than one evacuated tube of blood is to be drawn from an individual, the trace metals tube should be drawn second or later. Draw the blood through a stainless steel needle into a pre-screened vacutainer. Blood specimens should be transported and stored at  $\leq 4^{\circ}\text{C}$ . Once received, they can be frozen at  $\leq -20^{\circ}\text{C}$  until time for analysis. Portions of the sample that remain after analytical aliquots are withdrawn should be refrozen at  $\leq -20^{\circ}\text{C}$ . Samples thawed and refrozen several times are not compromised.

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

Not applicable for this procedure

**6) Equipment and Instrumentation, Material, Reagent Preparation, Calibrators (Standards), and Controls**

**a. Reagent Preparation**

**1) Diluent / Carrier Solution**

The diluent used in this method is an aqueous solution of 5  $\mu\text{g/L}$  (ppb) internal standards rhodium and bismuth, 100  $\mu\text{g/L}$  gold (for reduced Hg memory effect), 0.05% EDTA in 1% v/v tetramethyl ammonia hydroxide (TMAH), 5% ethyl alcohol, and 0.05% v/v Triton X-100<sup>®</sup>. This solution will be added 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. For ease of preparation of the final diluent / carrier solutions, first prepare the following intermediate solutions. Larger volumes of these solutions can be prepared by adjusting component volumes proportionally.

**i. 1% Triton X-100<sup>®</sup> intermediate solution**

For ease of daily preparation of the diluent, first prepare a 1% Triton X-100<sup>®</sup> solution by adding 20 ml of Triton X-100<sup>®</sup> to a pre-acid washed 2L Teflon<sup>®</sup> container that is partially filled with 18 M-ohm water. Fill to 2 L with 18 M-ohm water and mix until the Triton X-100<sup>®</sup> has completely dissolved into solution. A magnetic stirring plate can be used to assist mixing by adding an acid-washed Teflon<sup>®</sup> coated stirring bar to the bottle.

## **ii. 5% v/v Nitric Acid Solution**

For ease of preparation of the Internal Standard Intermediate Solution, first prepare a 5% v/v HNO<sub>3</sub> solution. To prepare 1L of 5% v/v HNO<sub>3</sub>, partially fill an acid washed 1L flask (glass or plastic) with 500-800 mL of 18 M-ohm water. Add 50 mL of concentrated HNO<sub>3</sub>, then fill to the mark with 18 M-ohm water and mix.

## **iii. Internal Standard Intermediate Solution**

For ease of preparation of the final diluent, prepare two intermediate internal standard solutions. One containing 20 ug/mL (ppm) Rh and one containing 20 ug/mL (ppm) Bi. Partially fill two 50 mL acid-washed volumetric flasks with 5% v/v HNO<sub>3</sub> (approximately 30 mL). Add 1 mL of 1,000 ug/mL Rh standard to the first flask. To the second flask, add 1 mL of 1,000 ug/mL Bi standard. Fill to mark (50mL) and mix thoroughly. If initial Bi or Rh standard concentrations are different, adjust spike volume proportionally. If desired, one intermediate internal standard solution with combined Rh/Bi may be prepared in a single 50mL flask.

Finally, to prepare the diluent / carrier solution, acid rinse a 2 L Teflon<sup>®</sup> container, and partially fill with 18 M-ohm water (approximately 1.5 L). Add 1g of EDTA, 20 mL of 25% w/v TMAH, 100 mL of ethyl alcohol, 100 mL of 1% Triton X-100<sup>®</sup>, 0.2 mL of 1000mg/L gold, and 0.5mL of each 20 mg/L Rh/Bi internal standard intermediate solution. Dilute to volume (2L) with 18 M-ohm water. Store at room temperature and prepare as needed.

## **2) ICP-DRC-MS Rinse Solution**

The rinse solution should be an aqueous solution of 1 % v/v TMAH, 5% ethyl alcohol, 0.05% EDTA and 0.05 % Triton X-100<sup>®</sup> and 100 µg/L gold (for reduced Hg memory effect). This solution will be pumped through the sample introduction system between samples to prevent carry-over of Hg, Pb, and Cd and the internal standards from one sample measurement to the next. For ease of daily preparation of the rinse solution, first prepare a 1% Triton X-100<sup>®</sup> by adding 20 ml of Triton X-100<sup>®</sup> to a pre-acid washed 2L Teflon<sup>®</sup> container that is partially filled with 18 M-ohm water. Fill to 2 L with 18 M-ohm water, and mix until the Triton X-100<sup>®</sup> has completely dissolved into solution. A magnetic stirring plate can be used to assist mixing by adding an acid-washed Teflon<sup>®</sup> coated stirring bar to the bottle. To prepare the final rinse solution, acid rinse a 2 L Teflon<sup>®</sup> container, and partially fill with 18 M-ohm water. Add 1 gram of EDTA, 20 ml of TMAH, 100 ml of ethyl alcohol, 100 ml of the 1% Triton X-100<sup>®</sup> solution and 200uL of a 1000mg/L SPEX CertiPrep Au standard, dilute to 2 L with 18 M-ohm water. Store at room temperature and prepare as needed. Larger volumes of rinse solution and 1% Triton X-100 can be prepared, if desired, by adding proportionally larger volumes of the solution constituents.

If the autosampler has two rinse stations, like the Elemental Scientific SC4-FAST autosampler, only one needs to be used.

**b. Calibrators Preparation**

(Intermediate stock standard and intermediate working standard solutions may be prepared by and purchased from an external laboratory, which then provides target concentration values to be used in the analysis.)

**1) Mercury, lead, and cadmium intermediate stock standard solutions**

Three intermediate stock standard solutions are prepared in a 3% v/v hydrochloric acid (HCl) matrix. The intermediate stock standard solutions are the first dilutions of the primary standards (1000 mg/L standards) from which all intermediate working standards will be made. For ease of preparation of standards, first prepare a 3% v/v HCl solution. To prepare 1L of 3% v/v HCl, add 30 mL of concentrated HCl into a 1L flask (acid washed glass or plastic) partially filled with 18 M-ohm water, then fill to the mark with 18 M-ohm water and mix. Partially fill each of three 50 mL volumetric flasks with 3% HCl solution. Label one for each element (for example: one "Hg Stock Std", one "Pb Stock Std" and one "Cd Stock Std"). For Hg, add 50 uL of the 1000 mg/L mercury stock standard. For Pb, add 500uL of the 1000 mg/L lead stock standard. For Cd, add 50 uL of the 1000 mg/L cadmium stock standard. Add enough 3% HCL to bring to the 50 mL mark for each flask. Mix well by gently inverting several times. The resulting intermediate stock standard solutions will be 1 mg/L for Cd and Hg, and 10 mg/L for Pb.

The intermediate stock standard solutions may be stored in smaller portions (i.e. 2 - 25mL portions) in acid-washed containers at room temperature. Prepare as needed. These intermediate stock standard solutions are used for preparation of the intermediate working standards.

**Table 1. Preparation of intermediate working standards.**

	Hg intermed. working standard Conc. (µg/L)	Hg Spike volume of intermediate stock standard solution (µl)	Pb intermed. working standard Conc. (µg/dL)	Pb Spike volume of intermed. stock standard solution (µl)	Cd intermed. working standard Conc. (µg/L)	Cd Spike volume of intermed. stock standard solution (µl)
Std.0	0	0	0	0	0	0
Std.1	1	50	1	50	1	50
Std.2	5	250	10	500	2	100
Std.3	10	500	20	1000	5	250
Std.4	20	1000	50	2500	10	500

## **2) Hg, Pb, and Cd intermediate working standards**

The intermediate working standard solutions used in this method are a series of 4 aqueous dilutions of the Hg, Pb, and Cd intermediate stock standard solutions in 3% HCl. These solutions will be used each day of analysis in preparing the final working standards that will be placed in the autosampler of the ICP-DRC-MS. To prepare, acid rinse four 50-mL volumetric flasks, and partially fill with 3% HCl. Spike each flask with the appropriate volume of Hg, Pb, and Cd intermediate stock standard solution, as is shown in Table 1. Next, dilute the solution in the flask to approximately 99% volume using the 3% HCl. Mix the solution thoroughly, and carefully fill to the mark by adding the solution in a drop-wise fashion. Final concentrations of these solutions are listed in Table 1. The final solutions may be dispensed into smaller volumes for storage in acid washed tubes (i.e. 15 mL polypropylene centrifuge tubes) for daily use. Store at room temperature and prepare as needed.

## **3) Working Calibrators**

The working calibrator solutions are dilutions of the 4 intermediate working standards into a whole blood matrix for the purpose of external calibration of an analytical run. They are made up the day of the preparation and analysis of the patient samples. All calibrators, and patient samples in the same analytical run must be prepared using the same diluent (see sect. [6.a.1](#)). To prepare the working calibrators, transfer 50  $\mu$ L of the appropriate aqueous intermediate working standard, 50  $\mu$ L of base blood, and 2400  $\mu$ L of diluent to a 15 ml polypropylene centrifuge tube using the Micromedic Digiflex. Cap the tube and mix well before analysis by inverting several times or using a vortex mixer.

### **c. Preparation of Quality Control Materials**

#### **1) Preparation of Bench Quality Control Materials**

A low and high bench QC material is analyzed in each run to determine the validity of the concentration measurements being made. These pools will need to be prepared periodically, as supply dictates, by spiking base blood. Preparation of new pools should be made far enough in advance so that both old and new pools can be analyzed together for a period of time (preferably at least 20 runs) before switching to the new quality control materials.

All blood should be screened for Hg, Pb, and Cd before high and low pool preparation. The labware used to pool the blood must be acid washed. The storage vials must be screened for contamination.

Screened blood is pooled together into an acid washed bottle before separation into 2 smaller portions for making of 3 related pools. One portion is used for the preparation of the base and low QC pool. Low-normal concentrations are ideal for the blood used to

prepare the base and low QC pools ( i.e. approximately 0.5 µg/L for Hg, 2.0µg/dL for Pb, and 0.5 µg/L for Cd). The other portion of the screened blood is spiked with appropriate volumes of elemental stock standard solutions to concentrations in the high-normal range (i.e. approximately 6.0 µg/L for Hg, 13.0 µg/dL for Pb, and 2.0 µg/L for Cd). Large volumes of blood should be dispensed into smaller vials (i.e. screened 2 mL polypropylene cryovials) in a clean environment (such as a class 10-100) for daily use. Store the vials long-term at approximately  $-70^{\circ}\text{C}$  and short-term at approximately  $-20^{\circ}\text{C}$ .

### **Other Materials**

- 1) Stock solution of Hg: SPEX, 1,000 mg/L in 10% HNO<sub>3</sub> (SPEX Industries, Inc. 3880 Park Ave., Edison, NJ 08820), or equivalent NIST traceable stock solution.
- 2) Stock solution of Pb: SPEX, 1,000 mg/L in 2% HNO<sub>3</sub> (SPEX Industries, Inc. 3880 Park Ave., Edison, NJ 08820), or equivalent NIST traceable stock solution.
- 3) Stock solution of Cd: SPEX, 1,000 mg/L in 2% HNO<sub>3</sub> (SPEX Industries, Inc. 3880 Park Ave., Edison, NJ 08820), or equivalent NIST traceable stock solution.
- 4) Pipette tips: 1-200 µl (#RT-20, fits up to 100 µL pipettes ) and 200-1000 µl (#RT-200, fits between 100 µl and 1000 µl pipettes) sizes (Rainin Instrument Co., Inc., Woburn, MA – or equivalent vendor). Pipette tips should be acid rinsed with 1% v/v double distilled nitric acid immediately prior to use (equivalent tips may be used).
- 5) Eppendorf fixed-volume pipettes (or equivalent): 1000, 500,250, 50 µl volumes (Brinkmann Instruments, Inc., Westbury, NY)
- 6) Doubled distilled concentrated hydrochloric acid (GFS Chemicals Inc. 867 McKinley Ave. Columbus, Ohio 43223) or equivalent. Concentrated hydrochloric acid is approximately 12M or 37% w/v.
- 7) Ethyl Alcohol, USP dehydrated 200 proof (Pharmco Products, Inc.) or equivalent.
- 8) 18 M-ohm water (from Barnsted or Elix 5 Reverse Osmosis water purification system or equivalent)
- 9) Liquid Argon (supplied by Speciality Gases or other contract agency) equipped with approved gas regulator (Matheson Gas Products, Seraucus, NJ – or equivalent).
- 10) Blood quality controls pools with low and high levels of Hg, Pb, and Cd.
- 11) Teflon<sup>®</sup> coated magnetic stir bars (2). (Cat. Number 58948-974 or equivalent, VWR Scientific Products, West Chester, PA) or equivalent.

- 12) Rhodium: SPEX, 1,000 mg/L in 2% HNO<sub>3</sub> (SPEX Industries, Inc., Chemical Sales Dept. 3880 Park Ave, Edison, NJ, USA) or NIST traceable equivalent.
- 13) Bismuth: SPEX, 1,000 mg/L in 10% HNO<sub>3</sub> (SPEX Industries, Inc., Chemical Sales Dept. 3880 Park Ave, Edison, NJ, USA) or NIST traceable equivalent.
- 14) Gold: SPEX, 1,000 mg/L in 10% HCl (SPEX Industries, Inc., Chemical Sales Dept. 3880 Park Ave, Edison, NJ, USA) or NIST traceable equivalent.
- 15) Acid-cleaned volumetric flasks, seven 50-ml flask for standard preparation (polypropylene or polymethylpentene flasks preferred, glass may be used). To acid wash flasks, first rinse with dilute nitric acid (i.e. 1-5 % v/v) followed by rigorous rinsing with 18 M-ohm water. This process may need to be repeated several times depending on prior use of the containers.
- 16) Acid-cleaned 2L bottles (Teflon<sup>®</sup> preferred). To acid wash bottles, first rinse with dilute nitric acid (i.e. 1-5 % v/v) followed by rigorous rinsing with 18 M-ohm water. This process may need to be repeated several times depending on prior use of the containers.
- 17) 15 ml (# 352097) and 50 ml (#352098) polypropylene centrifuge tubes or equivalent: (Becton Dickinson Labware, 1 Becton Drive, Franklin Lakes, New Jersey 07417 or equivalent).
- 18) Triton X-100<sup>®</sup> ("Baker Analyzed", J.T. Baker Chemical Co., or any source found to be low in trace metal contamination or equivalent).
- 19) Tetramethylammonium hydroxide, 25% w/v, (AlfaAesar, 30 Bond St., Ward Hill, MA 01835, or equivalent).
- 20) Disodium Ethylenediamine Tetraacetate (Fisher scientific Comp., Chemical manufacture Division, Fair Lawn, NJ 07410 or equivalent).
- 21) Kay-Dry paper towels and Kim-Wipe tissues (Kimberly-Clark Corp., Roswell, GA – or equivalent).
- 22) Cotton swabs (Hardwood Products Co., Guilford, Maine – or equivalent.)
- 23) Nitrile or Latex, powder-free examination gloves (N-Dex, Best Manufacturing Co., Menlo, GA – or equivalent).
- 24) Biohazard autoclave bags (Curtin-Matheson Scientific, Inc., Atlanta, GA – or equivalent).
- 25) Bleach (10% sodium hypochlorite solution) – any vendor.

**e. Instrumentation**

- 1) Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectrometer ELAN series DRC (PerkinElmer Instruments, Headquarters Office, 710 Bridgeport Avenue, Shelton, CT 06484-4794). Parameters of x-y alignment, mass calibration, autolens voltages, and nebulizer gas flow rates are optimized periodically.

**Table 2. ELAN ICP-DRC-MS Method Parameters.**

<u>Parameter</u>	<u>setting</u>
RF Power	1.1 – 1.6 kW (Typically 1.45 KW)
Ar Nebulizer Gas Flow	Approx 0.75-1.2 LPM
Detector Mode	Pulse
Measurement Units	Cps
Autolens	On
Blank Subtraction	After Internal Calibrator
Curve Type	Simple Linear
Sample Units	ppb(Hg/Cd)/ug/dL(Pb)
Sweeps / Reading	20
Readings / Replicate	1
Replicates	3
Dwell Time	100 ms
RPQ	0.25

- 2) Navigator top loading balance, or equivalent (Ohaus, Pine Brook, NJ).
- 3) Micromedic Digiflex Automatic pipette (or equivalent) equipped with 10.0 ml dispensing syringe, 200 µl sampling syringe, 0.75 mm tip, and the foot pedal (Micromedic systems, Inc., Horsham, PA or equivalent).

**7) Calibration and Calibration Verification Procedures**

**a. Calibration curve**

- 1) A simple linear calibration curves for Hg, Pb, and Cd is generated using a series of 4 external calibrators whose concentrations are defined in the calibration page of the quantitative analysis method software. The calibration curve plots the ratio of the observed intensities for Hg, Pb, and Cd and the internal standards Bi and Rh versus the concentration of the calibrator. The ratio of the observed intensities for Hg, Pb, Cd and the internal standards in the patient sample are compared to those obtained from the calibrators to determine the concentration of Hg, Pb, and Cd in the sample.

## **b. Calibration verification**

In this method, the ICPMS instrument is calibrated each run with NIST-traceable standards. Any samples with Pb, Cd, or Hg concentrations which are higher than the calibrated range are repeated with the appropriate dilution so that the observed concentration falls within the calibration range.

## **8) Operating Procedures; Calculations; Interpretation of Results**

### **a. Preliminaries**

- 1) For information regarding the reportable range of results and how to handle results outside this range, refer to the Reportable Range of Results section of this document ([sect 9](#)).
- 2) Allow frozen blood specimens, quality control specimens, and base blood calibration material to reach ambient temperature. Mix the sample, so that no particulates remain on the bottom of the tube, before taking an aliquot for analysis.

### **b. Sample preparation**

- 1) Thaw the frozen blood specimens, allowing them to reach ambient temperature (about 20 °C).
- 2) Set up a series of 15mL polypropylene centrifuge tubes corresponding to the number of blanks, calibrators, QCs, and patient samples to be analyzed.
- 3) Prepare the following solutions into the 15-mL polypropylene centrifuge tubes using the Micromedic Digiflex.
  - a) Prepare an aqueous blank consisting of 100  $\mu$ l of 18 M-ohm water and 2400  $\mu$ l diluent. The aqueous blank will be used as the blank for the quality control pools and patient samples.
  - b) Prepare 3 blood blanks consisting of 50  $\mu$ l of base blood (same material used for preparation of the blood calibration calibrators), 50  $\mu$ l of 3% v/v hydrochloric acid that was used for preparation of the calibrators (Std.0), and 2400  $\mu$ l of diluent. One of these blood blanks will be run as the blank for the calibration curves, the second as a blank check after calibrator 4, and the third at the beginning of the run (as whole blood blank check).
  - c) Prepare the working calibrators as described in [section 6.b.3](#).

- d) Prepare dilutions of the quality control and patient blood samples consisting of 2400  $\mu$ l diluent, 50  $\mu$ l 18 M-ohm water, and 50  $\mu$ l of the patient or quality control blood sample.
- e) Cap all of the blanks, calibrators, and samples and with a vortex mixer mix them for several seconds. Uncap them and place them in the autosampler of the ELAN ICP-DRC-MS.

**Table 3. Preparation of samples for analysis. (All volumes in  $\mu$ l)**

ID	Water	Intermediate Working Std.	Base Blood	Blood Sample or QC	Diluent
Blood Blank		50	50	-	2400
Calib. Stds	-	50	50	-	2400
Aqueous Blank	100	-	-	-	2400
Blood Sample or QC	50	-	-	50	2400
Blood Sample (2x dilution)	150	-	-	50	4800
Blood Sample (4x dilution)	175	-	-	25	4800
Blood Sample (5x dilution)	180	-	-	20	4800
Blood Sample (10x dilution)	190	-	-	10	4800

**c. Instrument & Software setup for the ICP-MS**

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) Software: Starting the ESI software before starting the ELAN software may improve stability of software.
- 3) Peristaltic pump: Set up the peristaltic pump tubing with proper tension for the sample rinse station then stop the pump.
  - a. If using an **external** peristaltic pump, go to the DEVICES window of the software and press the “Connect” button to establish communication between the

computer and the peristaltic pump. Next, start the peristaltic pump by pressing the appropriate arrow in the DEVICES window (make sure that the rotational direction is correct for the way the tubing is set up in the peristaltic pump). Set the pump speed to a low to mid-range speed (i.e. 7 - 24 rpm) in the DEVICES window.

- b. If using the **on-board** ICP-MS peristaltic pump, start the peristaltic pump by pressing the appropriate arrow in the DEVICES window (make sure that the rotational direction is correct for the way the tubing is set up in the peristaltic pump). Set the pump speed to a low to mid-range speed (i.e. 7 - 24 rpm) in the DEVICES window.
- 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 components cleanliness and positioning, interface pump oil condition, vacuum pressure, etc.). Make appropriate notes in the Daily Maintenance Checklist and Instrument Log Book. Better precision, especially on 24Mg, can be obtained in the daily performance check by using a rinse / carrier solution which contains no Triton X-100<sup>®</sup> (i.e. 0.5 % v/v nitric acid).
  - 5) Start the Plasma: In the INSTRUMENT window of the ELAN software (or on the front of the ELAN), press the "Start" button to ignite the plasma.
  - 6) Autosampler-specific instructions for operation:
    - a. Regular Autosampler: Do the following when using an autosampler such as a PE AS93, a CETAC ASX500, or an ESI SC4 (non FAST model):
      - i. Send the probe into the rinse solution using the software METHOD/SAMPLING window, press the "Probe" button, then press "Go to Rinse" button).
      - ii. Start the peristaltic pump at a low to mid-range speed (i.e. 7 - 24 rpm).
      - iii. Warm-up time: Allow at least 45 minutes warm-up time for the ICP-MS after igniting the plasma. This warm-up time is for the RF generator.
      - iv. Optimizations and 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). Include Be (m/z 9) in the daily performance check. Fill in the Daily Maintenance Checklist according to the optimization procedures performed.
        1. Magnesium (24Mg) may have high RSDs due to the use of Triton-X100 in the rinse solution. Avoid this problem by either temporarily using non-Triton-containing rinse solution during the daily check, or repeating the daily check multiple times in succession with no rinse time between.
        2. Saving the Files: Save new tuning (mass calibration) parameters to the file "default.tun." Save new optimization parameters (i.e., detector voltages, autolens values, nebulizer gas flow rate) to the

file "default.dac." Periodically, or any time large changes are made in optimization parameters, save a separate backup copy of these optimization files under a different name.

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\* Note: Negative values for pump speed indicate direction of pump rotation. Make sure that pump tubing is set up appropriately to match the direction of pump rotation.

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v. Software setup for Analysis:

1. Workspace (files & folders): Select the appropriate workspace file such as "CDC\_methITB001A.wrk" (or one customized for user preferences). Select "Review Files" from the "File" menu. Verify & set up the correct files and data directories for your analysis.
2. Samples / Batch Window: Update the window to reflect the current sample set. Fill in the autosampler location, sample identification (id), measurement action, method, sample flush time, sample flush speed, read delay time, read delay & analysis speed, wash time, and wash speed (see Tables 5 & 6). Use a bar code scanner to input data whenever possible. Save the Sample window file and re-use it on other days by simply replacing the sample IDs for the patient samples.
  - a. Blood vs. Aqueous Method Files: There are two method files for this one method (i.e. Blood metals panel\_methITB001A\_bloodblk.mth, and Blood metals panel\_methITB001A\_aqblk.mth). File names may vary to reflect autosampler parameters and analyte analyzed). 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 "bloodblk" method file) and the other lists the autosampler position of the aqueous blank (the "aqblk" method file).

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 "bloodblk" 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.

- b. The “bloodblk” method file: Use to analyze any sample into which base blood is spiked (i.e. blood blanks and blood-based calibrators). The blood blank method (set up for a CETAC ASX500 series autosampler, tray B) defines the blood blank in autosampler location 11 and the blood calibration standards 1-4 in autosampler locations 12-15, respectively.
      - c. The “aqblk” method file must be used to analyze all QC materials and patient samples. The aqueous blank method (set up for a CETAC ASX500 series autosampler, tray B) defines the aqueous blank in autosampler location 17.
  3. Notation of Dilutions: To designate an extra dilution of a sample, edit the sample ID to reflect the level of dilution being performed (i.e., A 1:2 dilution of sample 1 could 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. Extra dilution is performed on blood samples whose concentration is greater than the highest calibrator by more than 10%.
  4. Sample file timing parameters: The settings shown in Table 6 should be used for uptake and rinse times for all samples when using a standard autosampler with no flow injection components operating (i.e. PerkinElmer AS93 autosampler, CETAC ASX500 series autosamplers, or ESI SC4 autosamplers not using the “FAST” flow injection option).
  5. Verify cleanliness water, diluent, and sample introductions system by analyzing non-spiked samples (i.e. water, diluent through Digiflex).
  6. Sample Preparation and Analysis: Once diluent and instrument cleanliness is verified, prepare samples, and update SAMPLE / BATCH window with sample ID and autosampler positions. Highlight the table rows of the samples that are to be included in the run and then click on Analyze Batch.
  7. Instrument Shutdown: Stop the peristaltic pump in the ELAN software and turn off the plasma. Release the tension on the pump tubing.
- b. ESI SC4-FAST (flow injection) autosampler: Do the following when using this autosampler:

- i. Manually move the carrier solution probe into a dilute nitric acid solution (i.e. 0.5% v/v HNO<sub>3</sub>).
- ii. Setup the ESI software.
  1. If this is the first time to setup the ESI SC4 autosampler, change settings in the ESI to match that listed in Table 7 in the Appendix.
  2. Select "Fast" from the menu bar.
  3. Click the "Text file" button and open file "Blood metals panel\_methITB001A\_SC4 FAST\_parameters.txt"
  4. Uncheck "Enable Fast Control" (during all ICPMS optimizations) and close the "Fast" window.
  5. Click on the "Initialize autosampler" button if not already initialized.
  6. Flush the rinse stations. On the "Manual" menu, click on "Manually Move Probe". Enter 30-120s for "Manual Flush" at the "Flush 1" and "Flush 2" time boxes. Click on "Flush Rinse Stations." Wait for the rinse stations to flush, then close the "Manual" window. Rinse is a solution of 1% v/v TMAH, 0.05% Triton X-100, 5% Ethanol, 0.05% EDTA and 100ppb Au (gold).
  7. Minimize the ESI software window (do not close the software).
- iii. Allow at least 45 minutes warm-up time for the ICP-MS (with all components operational).
- iv. Move the carrier solution probe into the daily performance check solution. Complete the appropriate daily checking / optimization procedures. When an acceptable daily performance check has been obtained, move the carrier probe back into the dilute nitric acid solution (i.e. 0.5% v/v HNO<sub>3</sub>) and allow to flush for several minutes.
- v. Setup ELAN software files:
  1. Workspace (files & folders): Select the appropriate workspace file (i.e. "CDC\_methITB001A\_SC4.wrk"). Select "Review Files" from the "File" menu. Verify & set up the correct files and data directories for your analysis.
  2. Samples / Batch Window: Update the window to reflect the current sample set. Fill in the autosampler location, sample identification (id), measurement action, method, sample flush time, sample flush speed, read delay time, read delay & analysis speed, wash time, and wash speed. Use a bar code scanner to input data whenever possible. Save the Sample window file and re-use it on other days by simply replacing the sample IDs for the patient samples
    - a. Method Files: There are two method files for this one method (i.e. Blood metals panel\_methITB001A\_bloodblk.mth, and Blood metals panel\_methITB001A\_aqblk.mth). 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 "bloodblk" method file) and the other lists the

autosampler position of the aqueous blank (the “aqblk” method file).

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 “bloodblk” or “aqblk” method file is used. Analysts typically follow the pattern below, however, for the sake of consistency and as a reminder of which blank must be used for which type of sample.

- i. The “bloodblk” method file: Use to analyze any sample into which base blood is spiked (i.e. blood blanks and blood-based calibrators). The blood blank method (set up for an ESI SC4 autosampler) defines the blood blank in autosampler location 105 and the blood calibration standards 1-4 in autosampler locations 106-109, respectively.
  - ii. The “aqblk” method file must be used to analyze all QC materials and patient samples. The aqueous blank method (set up for an ESI SC4 autosampler) defines the aqueous blank in autosampler location 111.
  - iii. Method file name may be altered when analyzing a subset of the analytes.
3. Notation of Dilutions: To designate an extra dilution of a sample, edit the sample ID to reflect the level of dilution being performed (i.e., A 1:2 dilution of sample 1 could 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. Extra dilution is performed on blood samples whose concentration is greater than the highest calibrator by more than 10%.
- vi. Move the carrier solution probe from the dilute nitric acid solution to a bottle containing carrier (same as diluent used in the sample preparation). Allow to flush for several minutes.
  - vii. In the ESI software, check the “Enable Fast Control” box. Minimize the ESI software (do not close the software).
  - viii. Verify cleanliness water, diluent, and sample introductions system by analyzing non-spiked samples (i.e. water, diluent through Digiflex).
  - ix. Once diluent and instrument cleanliness is verified, prepare samples, and update SAMPLE / BATCH window with sample ID and autosampler

positions. Highlight the table rows of the samples that are to be included in the run and then click on Analyze Batch.

- x. When analysis is complete, maximize the ESI software window, and choose "Manual" on the tool bar. Click on the "Go Home" button to send the sample probe to the home position above the rinse station.
- xi. Move the peristaltic pump probe from the diluent bottle into dilute nitric acid (i.e. 0.5% HNO<sub>3</sub>) and flush for several minutes. Then, place the carrier probe into 18Mohm water and flush for several minutes. Lastly, place the carrier solution probe into an empty tube and allow the probe to run dry.
- xii. Stop the peristaltic pump in the ELAN software and turn off the plasma. Release the tension on the pump tubing.

#### **d. Recording of Data**

##### **1) Quality Control Data**

The results of the quality control samples analyzed in each run is stored in the Microsoft Access (or SQL server) database when all other data for the run is imported from the ELAN software. see [section 8.d](#) for a description of how to import data into the Microsoft Access database. The database allows for the printing of several types of QC reports.

##### **2) Analytical Results**

###### **a) Analysis Printouts and Analyst Run Report**

The analysis printouts should be bound together along with a printout of the calibration curve, and curve statistics and placed in the study folder(s). The results of the patient samples analyzed in each run is stored in the Microsoft Access (or SQL server) database when all other data for the run is imported from the ELAN software. See [section 8.d](#) for a description of how to import data into the Microsoft Access database. See [section 8.d](#) for description of how to import data into the database.

###### **b) Supervisor Review**

Using the Microsoft Access or SQL server database, it will be possible for the supervisor to review the QC and sample results directly in the database. After the supervisor reviews the data, the paper printouts from the analysis run should be filed in the study folder(s).

###### **c) Plotting QC Results**

Using the Microsoft Access or SQL server database, QC plots will be updated automatically when the data is imported into the database. These plots should be

monitored regularly to check for any trends in the bench QC results. If trends are observed, contact the lab supervisor.

**d) Use of the Microsoft Access Database**

After an analysis run, the results must be exported to a .TXT file, then imported into the Microsoft Access or SQL server database that handles data for the IRAT Branch. Once in the database, report summaries for QC and sample results should be printed out and kept with the hard copies of the data printout from the ICP-MS in the study folder.

**i) Data Export Process (from ELAN software to .TXT file)**

In the ELAN ICP-DRC-MS software, select 'Review Files' from the File menu. From this window you must open the files and directories that were used when collecting the data of the run that you wish to export (if the analysis has just ended, all of these files and directories will still be open). NOTE: A second copy of the ELAN software can be run as Edit / Reprocess" copy without affecting an ongoing analysis being done by the first copy of the software running in Windows. Once you have opened the relevant files, go to the Report page in the Method window. Deselect the box that print a paper copy of data, and select the box that sends data to a file. Select the Report Options Template named "database\_output.rep" and type in a report filename using a format such as '2006-0802\_study name.txt' to designate data from analysis of the study from August 2, 2006, run #1. The Report Format option 'Use separator' and the File Write Option 'Append' should be selected. Finally, reprocess the data of interest (see Perkin Elmer Elan Software manual), making sure to apply the correct blank to the correct samples & QCs. (The blood blank must be used for all of the calibrators. The aqueous blank must be used for all analyses of patient samples and QC samples).

**ii) Data Import Process (from .TXT file to Microsoft Access database)**

Transfer the .TXT file to the appropriate subdirectory on the network drive where exported data is stored (Note that directories are named according to instrument / year / month / and study name or ID, such as I:/Instruments/2F ELAN 6100 DRC/2001/08/Study 2001-01). From a computer that has access to Access or SQL server database used for tracking data, log in using your UserID. Once logged into the database, open the appropriate instrument data folder in the Go To window. Select "Import Instrument File". Enter the Appropriate information in the Instrument, Analyst, Assay, StudRefID, and Run Number fields and press the "Import" button. Select the location of the data file on the network drive, and press the "Open" button. In the "Imported Results" table, pressing the "Find X's" button will show only those samples whose Sample ID is not recognized as a valid QC pool ID, or sample ID for this study (sample IDs are setup when the study is logged into

the database). Corrections to sample IDs and dilution factors can be made in this table (i.e. – correction of transcription errors, adjustment for level of dilution). If samples were diluted for analysis (see [section 8.c.10](#)), both the sample ID and the dilution factor will need to be edited in this table before the values are transferred to the database. First change the dilution factor to reflect the way that the sample was analyzed, then edit the sample ID to remove any comments about the level of dilution the sample was analyzed at (The replace command is useful to do this.) Once any corrections to sample IDs have been made, pressing the “Check” button will again evaluate the sample IDs. Any sample or analyte row marked ‘Not Recognized’ will not be transferred to the database when the “Transfer” button is pressed.

- e. Replacement and periodic maintenance of key components (part #'s given are Perkin Elmer part #'s from the Perkin Elmer 2000/2001 Consumables Catalog.)**
- a. Autosampler probe assembly (part # B3000161): One spare should be kept on hand.
  - b. Peristaltic pump tubing for sample (0.03 inch i.d., part # 09908587), rinse station (can use either same tube type as for sample, or 0.045 inch id, part# N0680375) and for waste (0.125 inch i.d., part # N812-2012): Keep at least 6 packages of twelve on hand of the sample tubing, 6 for rinse station, and 2 packages of 12 on hand of the waste tubing. Other suppliers may offer the same size / type of peristaltic tubing.
  - c. Quartz Meinhard Type A3 Concentric Nebulizer for ELAN DRC (part # WE024371) or equivalent: at least one spare on hand.
  - d. Quartz Cyclonic Spray Chamber for Elan DRC (part # WE025221) or equivalent: at least one spare on hand.
  - e. Liquid Connector for Concentric Nebulizer (part # WE024372) for use with Meinhard Nebulizer: at least one spare on hand.
  - f. Teflon Sample Capillary (used to connect the liquid connector for concentric nebulizer and the peristaltic pump tubing (part # WE0224375), or any source of teflon tubing, 0.5 mm i.d. x 1.59 mm o.d.; one pack (60 cm length) on hand.
  - g. Injector Support for ELAN DRC (part # WE023951) or equivalent: one spare should be on hand.
  - h. Torch O-ring Kit (pkg of 4, part# N8120100): 4 spare packages should be on hand.
  - i. Quartz torch: at least two spare torches should be on hand (part # N8122006).

- j. Quartz Injector, 2.0 mm i.d. sample injector (part # N8125029): at least two spare injectors should be on hand.
- k. RF coil (part # WE021816): one spare should be on hand.
- l. Nickel Skimmer (part # WE021137) and sampler cones (part # WE021140): at least 2 spares of each on hand.
- m. Skimmer and sampler cone o-rings (part # N8120512 and N8120511, respectively): at least 10 spares of each on hand.
- n. Series II Replacement Ion Lens (part# WE018034). Keep 2 spares on hand.
- o. Pump oil for the roughing pump (part # N8122004): Should keep 4 bottles on hand.
- p. NESLAB chiller coolant (NESLAB Coolant, part # WE016558): 2 1L bottles should be on hand.
- q. If possible, having a backup A/S 93 Autosampler and NESLAB chiller are advised. See Perkin Elmer sales representative for part numbers.

## **f. Calculations**

### **a. Calibration**

The ELAN has two on-board microcomputers that work with the external system computer. The computers interface with the other electronic components within the system to convert the detector signals to digital intensity values. As calibrators are analyzed, the software plots the ratio of the measured intensities of Hg, Pb, and Cd and the corresponding internal standards versus the concentration for Hg, Pb, and Cd in the calibrating solution. The resulting calibration curve is used as a reference point to determine the concentration of Hg, Pb, and Cd in each patient sample based on the ratio of the intensities of Hg, Pb, and Cd and the internal standards observed in the samples. The use of internal standards (Bi and Rh) allows for the correction of changes in instrument response during the run. The responses to instrumental effects for Hg, Pb, and Cd are assumed to be similar to the response for the internal calibrator, so basing the analysis on the ratio of the two should reduce effects of differing sample matrices and instrumental variations during the analysis run. The concentration for Hg, Pb, and Cd from the printout equals the concentration of Hg, Pb, and Cd detected in the blood samples. Typical correlation coefficients for the calibration curves will be  $\geq 0.999$ .

### **b. Limit of Detection**

The detection limit for Hg, Pb, and Cd in blood specimens are based on three times the standard deviation of blood blank run for a minimum of 20 runs. This represents the method detection limit. Since two blood blank checks are routinely analyzed in each run (Blood Blk1 or S0) and Blood Blk2 or S0), one of these blank checks should be used. Results below the detection limit are reported as <LOD (where LOD = the

calculated lower detection limit). The method limit of detection should be verified periodically to ensure that the LOD has not changed.

#### **g. Special Procedure Notes – CDC Modifications**

Not applicable for this operation.

#### **9) Reportable Range of Results**

Blood Hg, Pb, and Cd results are reportable in the range of greater than the LOD, where LOD is the calculated lower detection limit.

Results greater than the highest calibrator will be diluted appropriately and re-analyzed so the results fall within the concentration range covered by the calibrators.

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

The method described in this protocol is used in the Inorganic Toxicology and Nutrition Branch for environmental and occupational health screening studies.

Two types of quality control systems are used in this analytical method. These two systems are: (1) “bench” quality control specimens that are inserted by the analyst 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 and (2) “blind” quality control samples that are placed in vials, labeled, and processed so that they are indistinguishable if possible from the subject samples. The results of the blind specimens are decoded and reviewed by the supervisor. With both systems, taking these samples through the complete analytical process assesses all levels of the analyte concentrations. The data from these materials are then used in estimating methodological imprecision and in assessing the magnitude of any time-associated trends. The bench quality control pools used in this method comprise two levels of concentration spanning the “low-normal”, and “high-normal” ranges of Hg, Pb, and Cd. Both of these pools are analyzed after the calibrators, but before any patient samples are analyzed so that judgments on the Hg, Pb, and Cd calibration curves may be made prior to analysis of patient samples. These bench QCs should be analyzed again at the end of the run (approximately 40-80 patient samples total). If more patient samples are analyzed on the same calibration curve after the second run of the bench QC (after approximately 40-80 patient samples), both the “low-normal” and “high-normal” bench QC should be reanalyzed after the additional samples.

Quality control limits are established for each QC pool. An analysis of the mean and calibrator deviation (SD) is performed for each pool from the concentration results observed in at least 20 characterization runs. During the 20 characterization runs, previously characterized QC, or pools with target values assigned by outside laboratories are used for quality control evaluation of each run. In addition to providing quality control

limits, the characterization runs can also serve to establish homogeneity of the pools. Once the homogeneity of the bench materials has been established, it is useful to have them analyzed by another independent reference method, e.g. IDMS, if possible.

### **Precision and Accuracy:**

a. **Quality Control Results Evaluation.** After the completion of a run, the quality control limits are consulted to determine if the run is “in control”. **The quality control rules apply to the average of the beginning and ending analyses of each of the bench QC pools.** The quality control rules are as follows

1. If both the low and the high bench QC results are within the 2s limits, then accept the run.
2. If one of two QC results is outside the 2s limits, then apply the rules below and reject the run if any condition is met.
  - i. **13s** – Average of both low QC OR average of both high QC is outside of a 3s limit )
  - ii. **22s** – Average of both low QC AND average of both high QC is outside of 2s limit on the same side of the mean
  - iii. **R4s sequential** – Average of both low QC AND average of both high QC is outside of 2s limit on opposite sides of the mean
  - iv. **10x sequential** – The previous 9 average QC results (for the previous 9 runs) were on the same side of the mean for either the low OR high QC.

If the run is declared “out of control”, the analysis results for all patient samples analyzed during that run are invalid for reporting.

### **b. Sample Results Precision Evaluation**

If the range of the 3 replicate readings (maximum replicate concentration value – minimum replicate concentration value) for a single sample analysis is greater than 1 ug/L for Cd or Hg or greater than 1ug/dL for Pb, and the range is greater than 10% of the mean concentration of the three replicates, then the analysis of that sample should be repeated.

### **11) Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria**

If an analyte result for a quality control material falls outside of the 99% limits for mean or range, then the following steps should be taken if possible.

**If a particular calibrator is obviously in error, two approaches can be taken**

- a. If calibrator # 2 or #3 are in error on a particular run (not as a persistent day-to-day problem) a single calibration point may be removed from the calibration curve within the ELAN software, and samples analyzed using the resulting curve. If this problem persists with a particular calibrator, it should be remade.
- b. Remake a new dilution of that 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 re-analyze it.**

**Prepare fresh dilutions of the working calibrators, and re-analyze the entire calibration curve using the freshly prepared calibrators.**

If these three steps do not result in correction of the “out of control” values for QC materials, the supervisor should be consulted for other appropriate corrective actions. No analytical results should be reported for runs not in statistical control.

**12) LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS**

There are no significant interferences due to icterus, hemolysis, or lipemia.

**13) Reference Ranges (Normal Values)**

<b>References to normal blood Hg, Pb, Cd concentrations.</b>	
<u>References</u>	<u>Concentration (µg/L)</u>
Tietz Textbook of Clinical Chemistry, edited by C.A. Burtis and E.R.Ashwood, 1999 [14]	[Hg] <10 µg/L [Pb]<10 µg/dL for children [Pb]<30 µg/dL for adults [Cd]<5 µg/L
Carson B.L., Ellis III H.V., McCann J.L., Toxicology and Biological Monitoring of Metals in Humans, Lewis Publishers, 1986 1983 [15]	[Hg]<20 µg/L [Pb]=20-35 µg/dL for nonoccupationally exposed people [Pb]=60-70 µg/dL for male workers [Cd]<10 µg/L for nonoccupationally exposed people
Handbook on Metals in Clinical and Analytical Chemistry, edited by H.G. Seiler, A. Sigel, and H. Sigel, 1994 [1]	[Hg] <10 µg/L [Pb]=3.12-31.2 µg/dL [Cd]= 1-4 µg/L

**14) Critical Call Results**

**a. Results Requiring Confirmation analysis: 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 protocol but default concentrations are 5 ug/L for Cd, 10 ug/dL for Pb, and 10.0 ug/L for Hg. Report the**

**original result, as long as the confirmation is within 10% of the original. Continue repeat analysis until a concentration can be confirmed.**

b. **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 concentration assigned to the 2UB for an element is determined by study protocol but default concentrations are 5 ug/L for Cd, 10 ug/dL for Pb, and 5.8 ug/L for Hg (in this case the same as the 1UB boundaries). 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.

### **15) Specimen Storage and Handling During Testing**

Specimens may reach and maintain ambient temperature during analysis. Stringent precautions should be taken to avoid external contamination. Once the samples are analyzed, they should be returned to  $\leq -20^{\circ}\text{C}$  freezer storage as soon as possible.

### **16) Alternate Methods for Performing Test or Storing Specimens if Test System Fails**

If the analytical system fails, then freezer storage ( $\leq -20^{\circ}\text{C}$ ) is recommended until the analytical system is restored to functionally.

### **17) Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)**

Reporting of test results is carried out as outlined in the DLS Policies and Procedures Manual. As stated in section 13, the protocol for supervisors reporting elevated results to medical personnel is defined according to the study protocol.

### **18) Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking**

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

Calibrator record keeping means (e.g. electronic –Microsoft Access, optical disc, or tape backup) are to be used to track specimens. Records are maintained for  $\geq 3$  years, including related QA/QC data; duplicate records are kept (off-site, if sensitive or critical) in electronic or hardcopy format. Only numerical identifiers are used (e.g. Case ID numbers)

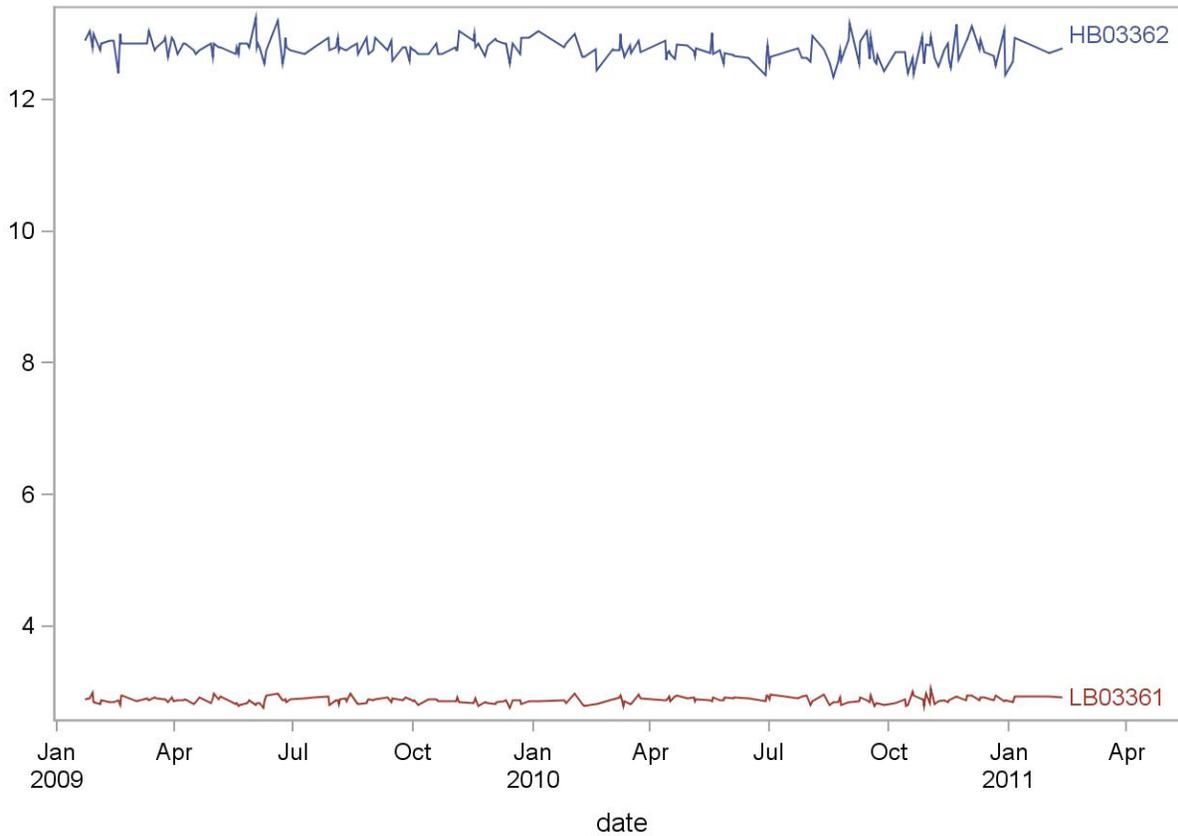
– all personal identifiers are available only to the medical supervisor or project coordinator to safeguard confidentiality.

**19) Summary Statistics and QC Graphs**

A. Lead (Blood)

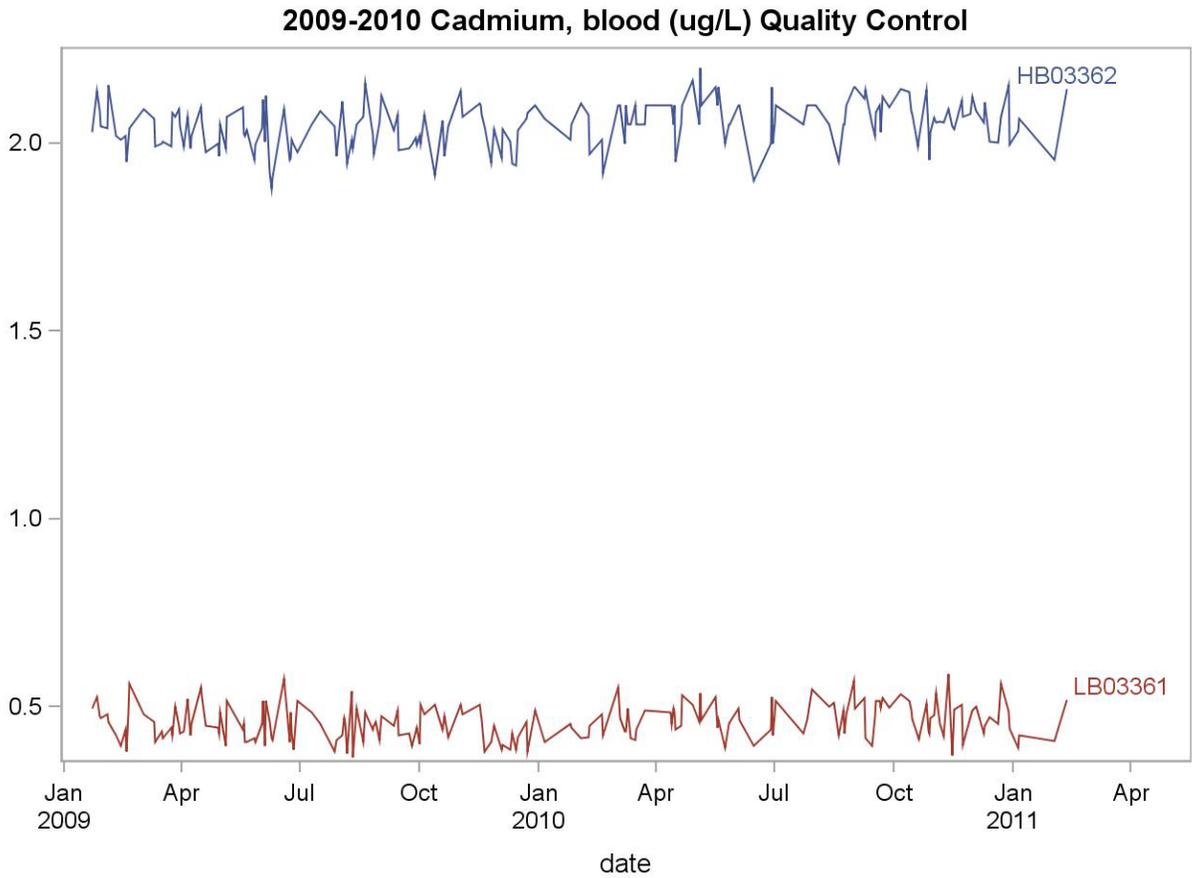
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HB03362	244	22JAN09	11FEB11	12.754	0.157	1.2
LB03361	250	22JAN09	11FEB11	2.876	0.050	1.7

**2009-2010 Lead, blood (ug/dL) Quality Control**



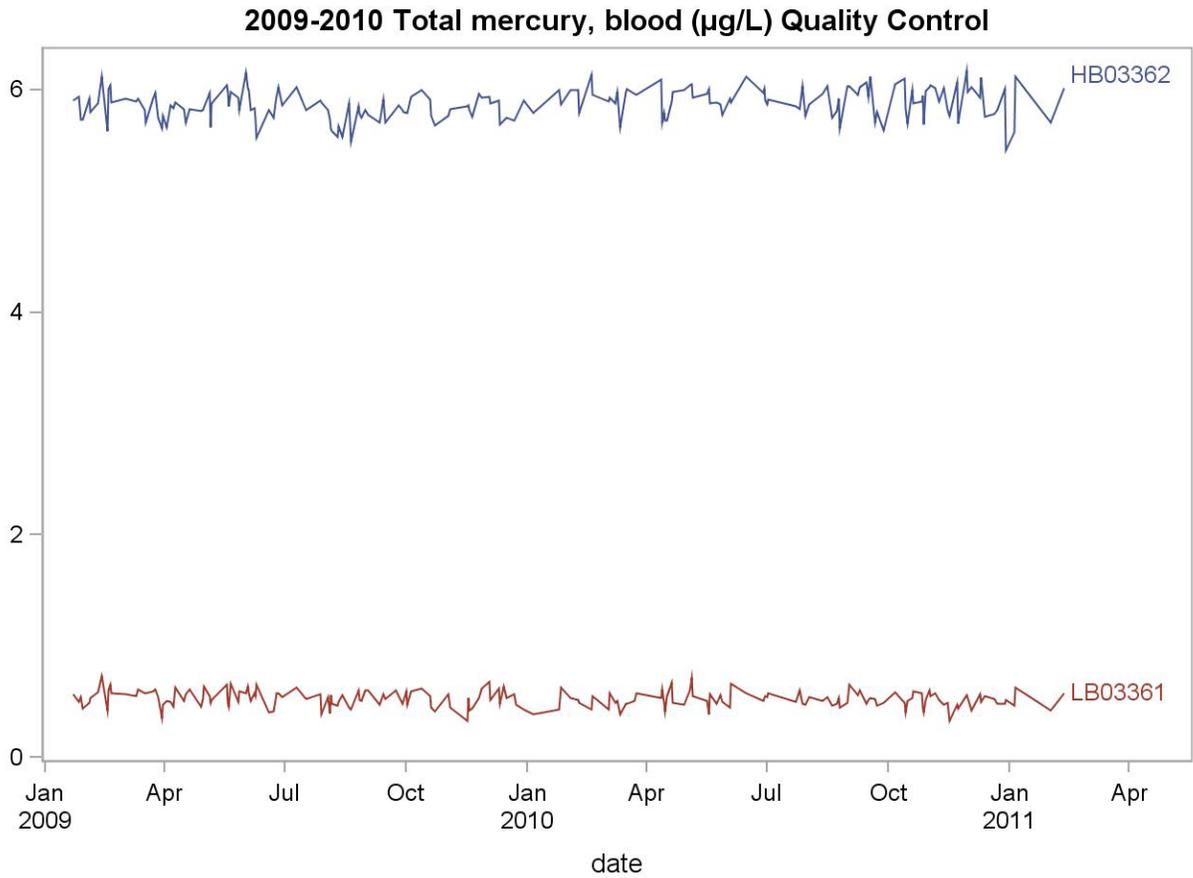
B. Cadmium (Blood)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HB03362	245	22JAN09	11FEB11	2.040	0.059	2.9
LB03361	248	22JAN09	11FEB11	0.453	0.047	10.3



C. Total Mercury (Blood)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HB03362	247	22JAN09	11FEB11	5.857	0.134	2.3
LB03361	248	22JAN09	11FEB11	0.516	0.078	15.1



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## **Appendix A. Ruggedness Testing**

**Ruggedness testing #1:** Evaluating the significance of time from preparation to analysis on sample stability.

This ruggedness testing documents the impact of waiting 24 or 48 hours after preparing a run set to analyze them. A “run set” is defined here as a set of blanks, calibrators, and beginning and ending bench QCs. Each run was made to be normal length by inserting matrix-matched ‘junk’ samples between beginning and ending bench QC.

Day 1: Prepared run sets #1, #2, and #3 in three separate sets of tubes. Analyzed run set #1 immediately using normal method practices. Capped run sets #2 and #3 and left at room temperature for later analysis. Temperature stability is not an issue, so refrigeration was not required.

Day 2: Prepared run set #4 and analyzed it sequentially with run set #2 using normal method practices.

Day 3: Prepared run set #5 and analyzed it sequentially with run set #3 using normal method practices.

**uggedness Testing # 2:** Evaluating the impact of different Plasma Gas flow rates on method ruggedness.

Prepared run sets #1, #2, and #3 in three separate sets of tubes from the same starting materials. Each run set was analyzed in a separate run on the same day, same instrument. Each run was made to be normal length by inserting matrix-matched ‘junk’ samples between beginning and ending bench QC. The plasma gas flow rate for run #1 was 15 L/min (per method). Plasma gas flow was reduced for run set #2 (13 L/min) and then increased for run set #3 (17 L/min).

### **Appendix A. Ruggedness Testing (continued)**

**Ruggedness Testing # 3:** Evaluating the impact of different concentrations of Ethanol (ETOH) in diluent on method stability.

Performed 3 separate preparations (Blanks, calibrators, QC, junk samples), where the ETOH concentrations of the diluents were adjusted. Each run was made to be normal length by inserting matrix-matched 'junk' samples between beginning and ending bench QC. Each run used a different concentration of ETOH.

Prepared run sets #1, #2, and #3 in three separate sets of tubes. Prepared run set 1 with diluent containing 5% ethanol and analyzed it with carrier and rinse solutions also having 5% ethanol content. Prepared run set 2 with diluent containing 10% ethanol and analyzed it with carrier and rinse solutions also having 10% ethanol content. Prepared run set 3 with diluent containing 1% ethanol and analyzed it with carrier and rinse solutions also having 1% ethanol content. Analyzed them in three separate runs. They were not all analyzed on the same day, but kept as many things constant between the three runs as possible (same Digiflex, same standards, same ICP-MS). Allowed the system to equilibrate to new ethanol content for at least 30 minutes before analyzing the next run set. Each run was made to be normal length by inserting matrix-matched 'junk' samples between beginning and ending bench QC.

### **Appendix A. Ruggedness Testing (continued)**

**Ruggedness Testing # 4:** Evaluating the impact of different concentrations of EDTA on method stability.

Prepared run sets #1, #2, and #3 in three separate sets of tubes. Prepared run set 1 with diluent containing 0.05% EDTA and analyzed it with carrier and rinse solutions also having a 0.05% EDTA content. Prepared run set 2 with diluent containing 0.01% EDTA and analyzed it with carrier and rinse solutions also having a 0.01% EDTA content. Prepared run set 3 with diluent containing 0.1% EDTA and analyzed it with carrier and rinse solutions also having a 0.1% EDTA content. Analyzed them in three separate runs. They were not all analyzed on the same day, but kept as many things constant between the three runs as possible (same Digiflex, same standards, same ICP-MS). Allowed the system to equilibrate to a new EDTA content for at least 30 minutes before analyzing the next run set. Each run was made to be normal length by inserting matrix-matched 'junk' samples between beginning and ending bench QC.

### **Appendix A. Ruggedness Testing (continued)**

**Ruggedness Testing # 5** Evaluate the impact on analysis results of the set RF Power on method stability.

#### Test Details:

1. Three different RF power settings were tested in separately prepared, consecutive runs on the instrument without turning off the plasma. At least 15 minutes stabilization time was allowed between each run after the RF power was changed. "Junk blood" samples were analyzed between the beginning and ending QC of each run. All other method parameters were kept per method.
2. Run #1 (method default, 1450W).
3. Run #2 (Decreased RF power by 20% to 1150W).
4. Run #3 (Increased RF power to instrument maximum, 1600W).