



Laboratory Procedure Manual

Analyte: **Lead Cadmium Mercury**

Matrix: **Whole Blood**

Method: **Blood Lead Cadmium Mercury
ICPDRCMS**

Method No.: **ITB001A**

Revised: **September 9, 2004**

as performed by: *Inorganic Toxicology and Nutrition Branch
Division of Laboratory Sciences
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Important Information for Users

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

Public Release Data Set Information

This document details the Lab Protocol for NHANES 2003–2004 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
L06_c	LBXBCD	Cadmium ($\mu\text{g/L}$)
	LBDBCDSI	Cadmium (nmol/L)
	LBXBPB	Lead ($\mu\text{g/dL}$)
	LBDBPBS	Lead ($\mu\text{mol/L}$)
	LBXTHG	Mercury, total ($\mu\text{g/L}$)
	LBPTHGSI	Mercury, total ($\mu\text{mol/L}$)

1. Summary of Test Principle and Clinical Relevance

A. Clinical relevance

Mercury (Hg), 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 (1). The main organs affected 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 (1). Massive exposure over a longer period of time results in violent muscular spasms, hallucinations, delirium, and death (2). For nonoccupationally burdened population (3): normal whole blood Hg levels < 3 µg/L, the value is inconspicuous; at 3–10 µg/L, the value is increased, danger to health not recognizable; at >10 µg/L, the value is distinctly increased, on a 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 (4). 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 (5, 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 (1). The alkyl lead species are highly toxic to the central nervous system (7).

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 (1). Principal symptoms reported were respiratory distress due to chemical pneumonitis and edema. It has been estimated that 8 hrs exposure to 5 gm of Cd/m³ will be lethal (1). Ingestion of high amounts of Cd may lead to a rapid onset of 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 environments at high exposure levels, workers have developed proteinuria, renal glucosuria, aminoaciduria, hypercalcuria, phosphaturia, and polyuria. Chronic obstructive lung disease of varying degrees of severity is frequently seen in Cd workers. Concentration of cadmium in blood of healthy unexposed adults is in the range 0.1–4 µg/L (9).

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 ICP-MS. This multi-element analytical technique is based on quadrupole ICP-MS technology (10). 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 the mass spectrometer, the ions pass first through the ion optics, then the mass analyzing quadrupole before being detected as they strike the surface of the detector. The ion optics focus 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 mega-ohm water and with diluent, containing 1% v/v tetramethylammonium hydroxide (TMAH), 0.5% disodium ethylenediamine tetraacetate (EDTA), 10% ethyl alcohol, 0.05% Triton X-100, 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

PerkinElmer provides safety information that should be read before operating the instrument. This information can be found in the PerkinElmer 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 6 M 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 regularly 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 Toxicology and Nutrition (ITN) 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 Norton Utilities 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

(1) Schedule of backups

Weekly full data backups on one recordable compact disc (active “elandata” directory and all subdirectories).

Daily differential backups on a second hard disk (saves all data files created or altered since the last full backup). Only one daily backup is necessary. It will be overwritten each night when the backup is performed.

(2) Procedures of backups

Whenever making a backup (daily or weekly or CD-R) the active “elandata” directory, including all subdirectories, should be included. Before making weekly backups, saving a copy of the Windows event log in the active “elandata” directory will ensure archiving of all recent software system events (including communications between ICP-DRC-MS and ELAN software, as well as times of hard drive defragmentation, and other Windows system events).

(3) Compact Disc Backups:

- Use CD-R discs only (recordable compact discs), not CD-RW discs (rewritable compact discs).
- The CD-R should be recorded in such a way that after creation, the recordable compact disc cannot be written to again (to prevent any accidental over-writing of stored data).
- Compact disc backups can be made using Adaptec “Easy CD Creator” or similar software. (For Easy CD Creator v.4 software, use the following settings: Create Data CD, ‘Create CD’ under Create Options, ‘Track-At-Once’ and ‘Close CD’ under Write Method)

(4) Disk Backups:

- Disk backups can be made using Microsoft “Backup Exec” or similar software.
- Set up the differential backup to run automatically at a time when instrument will not be in use (i.e. 2 am). This will reduce the possibility of the backup software and the ICP-DRC-MS software interfering with one another.

Removing Data from the ICP-DRC-MS computer hard drive:

When the active “elandata” directory on the ICP-DRC-MS computer hard drive becomes too large to fit onto a single recordable compact disc, the oldest data on the hard drive should be removed so that a regular backup can be done onto a single CD-R. Usually this procedure can be done annually.

- The oldest data on the hard drive should be backed up in duplicate onto two CD-R discs. Each dataset folder (subdirectories under “elandata/dataset”) along with all other relevant files (i.e. - optimization, tuning, and sample files), to be included on these backups must be selected manually in the CD-R software. The entire elandata directory (and all subdirectories) can be backed up on disks since its capacity is much larger than CD-R.
- Correct operation of backup CD-R discs must be verified after they have been created before any data is deleted from the hard drive.
- To verify the operation of a CD-R disc, open any file on the disc using the appropriate computer software (ICP-DRC-MS software or otherwise).
- Once all backups are verified for operation, the original data can be deleted from the hard drive.
- One copy of the CD-R disc should be kept in a building other than the lab building (in case of fire in one structure). The other should be kept near the ICP-MS lab.

(5) Backup of sensitive Data

In case of sensitive data, duplicate recordable compact disc backups can be made, and the two CD-R discs stored in two different buildings.

D. Documentation of system maintenance.

(1) 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.

(2) Instrument maintenance

Documentation for system maintenance is contained in hard copies of data records (i.e. daily maintenance checklist, PerkinElmer 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. No special instructions such as fasting, special diets are required.

B. Specimen type: whole blood

C. Optimal amount of specimen required is 1–2 mL, minimum is 0.25 ml.

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 (< 0.25 ml) 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. Preparation of Reagents, Calibration (Calibrators), Controls, and All Other Materials; Equipment and Instrumentation

A. Reagent Preparation

(1) Diluent

The diluent used in this method is an aqueous solution of $5\ \mu\text{g/L}$ internal standards (rhodium and bismuth), $10\ \mu\text{g/L}$ gold (for reduced Hg memory effect), 1 g of EDTA in 1% v/v tetramethyl ammonia hydroxide (TMAH), 10% ethyl alcohol, and 0.05% v/v Triton X-100. 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. To prepare the solution, acid rinse a 2-L Teflon container, and partially fill with 18 mega-ohm water. Add 1g of EDTA, 20 ml of TMAH, 200 ml of ethyl alcohol, and 100 ml of 1% Triton X-100. For ease of daily preparation of the diluent, first prepare a 1% Triton X-100 by adding 20 ml of Triton X-100 to a pre-acid washed 2L Teflon container that is partially filled with 18 mega-ohm water. Fill to 2 L with 18 mega-ohm water, add an acid-washed, Teflon-coated stirring bar, and stir on a magnetic stirrer until the Triton X-100 has completely dissolved into solution. Spike 500 µl of 20 mg/L Rh and Bi and 1 ml of 20 mg/L Au to the final diluent. Dilute to volume (2 L) with 18 mega-ohm water. Store at room temperature and prepare as needed. Larger volumes of diluent can be prepared, if desired, by adding proportionally larger volumes of the solution constituents.

(2) ICP-DRC-MS Rinse Solution

The rinse solution used in this method is an aqueous solution of 1% v/v TMAH, 5% ethyl alcohol, and 0.05% Triton X-100. 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 by adding 20 ml of Triton X-100 to a pre-acid washed 2-L Teflon container that is partially filled with 18 M-ohm water. Fill to 2 L with 18 mega-ohm water, add an acid-washed, Teflon-coated stirring bar, and stir on a magnetic stirrer until the Triton X-100 has completely dissolved into solution. To prepare the final rinse solution, acid rinse a 2 L Teflon container, and partially fill with 18 mega-ohm water. Add 20 ml of TMAH, 100 ml of ethyl alcohol, and 100 ml of the 1% Triton X-100, dilute to 2 L with 18 mega-ohm water. Store at room temperature and prepare as needed.

B. Calibrators Preparation

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

Note: The concentrations of some stock calibrators (such as some NIST standard reference materials (SRMs) are certified relative to the mass of the solution, while others are certified relative to the volume of the solution. Refer to the certificate of analysis to determine this (specific for the lot number of the calibrator being used). If the calibrator was certified relative to mass, concentration calculations must be performed based on the weight measurements recorded during calibrator's preparation and the calibration information in the ELAN ICP-DRC-MS software must be updated to reflect the new calibrator concentrations each time the calibrators are remade. This is necessary because the same mass of solution will not be pipetted each time the dilutions are made, resulting in differing calibrator concentrations. Instructions here are for a stock solution that is certified relative to the mass of the solution.

(1) Mercury, lead, and cadmium intermediate stock calibrator solutions

Three intermediate stock calibrator solutions used in this method were prepared using double distilled 6 M hydrochloric acid diluted with 18 mega-ohm water to approximately 3%. The intermediate stock calibrator solution is the first dilution of the primary calibrator from which all intermediate working calibrators will be made. As the primary calibrator solutions 1000 mg/L of Hg, Cd, and Pb solutions in 2% nitric acid from Spex CertiPrep were used. To prepare the intermediate stock solution for each element, partially fill an acid washed 50 ml volumetric flask (polypropylene or poly-methyl pentane flask preferred) with 3% hydrochloric acid. For ease of preparation of calibrator's solution, first prepare 1 L of 3% hydrochloric acid by diluting 140 ml of double distilled 6M hydrochloric acid with 18 mega-ohm water till 1 L in 1L acid-rinse polyethylene or Teflon bottle. Determine the mass of a 50 µl aliquot of the Hg and Cd and 500 µl aliquot of Pb calibrator addition to the each of three flasks. Next, dilute the solution in the flask to approximately 49.5 ml volume using 3% hydrochloric acid. Mix the solution thoroughly, and carefully add the remaining little drops of 3% hydrochloric acid needed to dilute to exact volume.

The concentration (in µg/L) of the resulting intermediate stock calibrator solution can then be calculated using the following formulas:

$$\text{Hg, Cd, Pb Conc (mg/L)} = \text{mass Calibrator spike (g)} \times \text{conc. Calibrator (mg V / g)} / 0.050 \text{ L}$$

$$\text{Hg, Cd Conc (}\mu\text{g/L)} = \text{Hg, Cd Conc. (mg/L)} \times 1,000 \mu\text{g/mg}$$

$$\text{Pb Conc. (}\mu\text{g/dL)} = \text{Pb Conc. (10 mg/L)} \times 1,000 \mu\text{g/mg}$$

For Hg and Cd, the concentration of the intermediate stock calibrator solution was 1 mg/L; for Pb, it was 10 mg/L.

Store the solution in several smaller portions (i.e. 4- to 25-mL portions) in acid-washed containers at room temperature, and prepare as needed.

These intermediate stock calibrator solutions were used for the next calibrator's preparation.

(2) Hg, Pb, and Cd intermediate working calibrators

The intermediate working calibrator solutions used in this method are a series of four aqueous dilutions of the Hg, Pb, and Cd intermediate stock calibrator solutions in 3% double-distilled hydrochloric acid. These solutions will be used each day of analysis as the final working calibrators that will be placed in the autosampler of the ICP-DRC-MS. To prepare, acid rinse four 50-mL volumetric flasks, and partially fill it with 3% hydrochloric acid. Spike each flask with the appropriate volume of Hg, Pb, and Cd intermediate stock calibrator solution, as is shown in Table 1.

Table 1. Preparation of intermediate working calibrators.

Standards	Hg Calibration solution (µg/L)	Hg Spike volume of stock calibrator solution (µl)	Pb Calibration solution (µg/dL)	Pb Spike volume of stock calibrator solution (µl)	Cd Calibration solution (µg/L)	Cd Spike volume of stock calibrator 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

Next, dilute the solution in the flask to approximately 99% volume using a 3% hydrochloric acid. Mix the solution thoroughly, and carefully add the remaining little drops of 3% hydrochloric acid needed to dilute to exact volume. Dispense into smaller volume, acid washed tubes (i.e. 15 mL polypropylene centrifuge tubes) for daily use.

The final concentrations of Hg, Pb, and Cd in each of the intermediate working calibrators are dependent on the concentration of the intermediate stock calibrator used. Each calibrator concentration can be calculated using the formula

$$\text{Int. Work. Std. Conc. (}\mu\text{g/L)} = \text{Int. Stock Std. Conc. (}\mu\text{g/L)} \times \text{Int. Stock Std Spike (L)} / 0.050 \text{ L}$$

When a new set of intermediate calibration calibrators are prepared, the calibrator concentrations in the ELAN ICP-DRC-MS software must be updated. The values entered into the software should be the concentration of the intermediate working calibrators. Store at room temperature and prepare as needed.

(3) Working Calibrators

The working calibrator solutions are dilutions of the four intermediate working calibrators 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 Section 6.A.1). To prepare the working calibrators, transfer 50 μL of the appropriate aqueous intermediate working calibrator, 50 μL of base blood, and 2400 μL 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 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.

The screened blood is pooled in an acid washed bottle. The blood is divided into 2 parts for the preparation of 3 related pools: one part is for base and low level Hg, Pb, and Cd pool and another part is for high level pool, final concentration for low and base pool approximately 1 $\mu\text{g/L}$ for Hg, 2.0 $\mu\text{g/dL}$ for Pb, and 0.5 $\mu\text{g/L}$ for Cd. The high level Hg, Pb, and Cd pool is prepared by adding the appropriate volume of stock solutions to the pooled blood to get the desired final concentration that should be approximately 15 $\mu\text{g/L}$ for Hg, 25 $\mu\text{g/dL}$ for Pb, and 4 $\mu\text{g/L}$ for Cd. The dispensing must be done under a class 10-100 hood. 5 ml plastic vials are recommended for base pool, 2.5 ml vials are recommended for low and high pools. The vials are stored: long-term at -70°C or short-term at -20°C .

D. Other Materials

- (1) Stock solution of Hg: SPEX, 1,000 mg/L in 10% HNO_3 (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_3 (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_3 (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 6 M hydrochloric acid (GFS Chemicals Inc. 867 McKinley Ave. Columbus, Ohio 43223) or equivalent.
- (7) Ethyl Alcohol, USP dehydrated 200 proof (Pharmco Products, Inc.) or equivalent.
- (8) 18 mega-ohm water (from Barnsted or Elix 5 Reverse Osmosis water purification system or equivalent).
- (9) Liquid Argon (supplied by Specialty Gases or other contract agency) equipped with approved gas regulator (Matheson Gas Products, Secaucus, NJ – or equivalent).

- (10) Blood quality controls pools with low and high levels of Hg, Pb, and Cd.
- (11) Teflon-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% HNO₃ (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% HNO₃ (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 calibrator's preparation (polypropylene or polymethylpentene flasks preferred). To acid wash flasks, rinse with 10% v/v reagent-grade nitric acid, followed by rigorous rinsing with 18 mega-ohm water. This process may need to be repeated several times depending on prior use of the containers.
- (16) Acid-cleaned 2 L bottles (Teflon preferred). To acid wash containers, rinse with 10% v/v reagent-grade nitric acid, followed by rigorous rinsing with 18 mega-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, NJ 07417 or equivalent).
- (18) Triton X-100 ("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/w, or equivalent (AlfaAesar, 30 Bond St., Ward Hill, MA 01835)
- (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, 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

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.

Parameter	Setting
RF Power	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
Sweeps / Reading	20
Readings / Replicate	1
Replicates	3
Dwell Time	100 ms
RPQ	0.25

- (1) Ohaus AP310 analytical balance, or equivalent (Ohaus, Pine Brook, NJ).
- (2) Micromedic Digiflex Automatic pipette (or equivalent) equipped with 10.0 ml dispensing syringe, 2000 μ l 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

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 order to verify the concentrations of the calibrators being used in this method, low level and high level blood Hg, Pb, and Cd pools, and the Center of Toxicology for Quebec (blood Hg, Pb, and Cd materials prepared for their blood Hg, Pb, and Cd proficiency testing and ICP-MS laboratory comparison programs) are analyzed periodically (at least every 6 months) and the results compared to their target values. Agreement of the results for low and high level blood Hg, Pb, and Cd pools should be within the certified concentration range. Agreement of results for the Center of Toxicology for Quebec materials should be within 3 standard deviations of the median value for the participating laboratories. Calibration verification data is stored in the Laboratory Calibration Verification Log. Copies should be placed in the ICP-DRC-MS maintenance log in the laboratory with the instrument.

8. Procedure Operating Instructions; 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 (Section 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 15 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.

Table 3. Preparation of samples for analysis. (All volumes in μl)

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

- (a) Prepare an aqueous blank consisting of 100 μl of 18 mega-ohm water and 2400 μl 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 μl of base blood (same material used for preparation of the blood calibration calibrators), 50 μl of 3% hydrochloric acid that was used for calibration calibrator's preparation (Std. 0), and 2400 μl 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 end of the run (as blood blank).
- (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 μl diluent, 50 μl 18 mega-ohm water, and 50 μl 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 approximately 10 seconds. Uncap them and place them in the autosampler of the ELAN ICP-DRC-MS.

C. Instrument & Software setup for the ICP-MS

- (1) Turn on the computer, printer, peristaltic pump, and autosampler. Log in as Administrator (press the ENTER key rather typing in a password).
- (2) Start the ELAN ICP-DRC-MS software from Windows and note if all graphical indicators of instrument readiness are green. If not, take the appropriate actions described in the software and hardware manual for the instrument.
- (3) Perform necessary daily maintenance checks as described in Chapter 5 of the ELAN 6100 Hardware Guide (i.e. argon supply, interface components cleanliness and positioning, interface pump oil condition, etc...). Note the base vacuum pressure in the INSTRUMENT window of the software (before igniting the plasma, the vacuum is typically between 8×10^{-7} and 1.8×10^{-6} torr). Record any maintenance procedures, along with the base vacuum pressure in the Daily Maintenance Checklist Book (see example of Daily checklist in Table 4 of the APPENDIX).
- (4) Set up the peristaltic pump tubing for the autosampler, rinse station, and spray chamber waste line. Position the tubing and close the pump clamps. Adjusting the tension on the pump tubing will be done later.
- (5) In the INSTRUMENT window of the software, press the "start" button to ignite the plasma.
- (6) Once the plasma has ignited, press the "connect" button (in the DEVICES window of the software) to establish communication between the computer and the autosampler. 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). Fill the rinse station reservoir quickly by pressing the 'fast' button in the DEVICES window. Once the rinse

station is filled with the rinse solution, type in '12' in the rpm field of the DEVICES window to set the pump speed. If the spray chamber rinse line is not draining the spray chamber correctly, or the rinse solution is not flowing properly to the rinse station, adjust the tension screws on the back of the peristaltic pump.

- (7) Read this step entirely before proceeding. It is important to get the tension on the autosampler tubing correct, or it will adversely affect the precision of the ICP-DRC-MS measurements. Through the METHOD / SAMPLING window in the software, press the "Probe" button, then the "Goto Rinse" button to lower the autosampler probe into the rinse solution. Watch as solution is taken up through the autosampler probe tubing. When the leading edge of the solution is visible, press stop in the DEVICES window. The leading edge of solution in the autosampler tubing line should stop moving. If not, tighten the tension screw for this line on the back of the peristaltic pump. Loosen the peristaltic pump tubing screw for the autosampler tubing until the leading edge of solution in the autosampler tubing begins to move again then tighten the screw just enough to make the solution edge stop. Tighten the screw another 1/8 to 1/4 of a turn. 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).
 - (8) Allow at least 45 minutes warm-up time for the ICP-DRC-MS (with all components operational). After this warm-up time, complete the appropriate daily optimization procedures as described in Chapter 3 of the ELAN 6100 DRC Software Guide. Include beryllium (m/z 9) in the mass calibration, auto lens optimization, and daily performance check using a 1 µg/L multi-element solution. Fill in the Daily Maintenance Checklist Book according to the optimization procedures performed. Save new tuning (mass calibration) parameters to the file "default.tun", then in a separate file containing the analysis date "default_MMDDYY.tun" (where MM = month, DD = day, and YY = year). Save new optimization parameters (i.e.-detector voltages, auto lens values, neb gas flow rate) to the file "default.dac" then in a separate file containing the analysis date "default_MMDDYY.dac" (where MM = month, DD = day, and YY = year).
 - (9) To setup the run in the software, click on Open Workspace from the File menu. select workspace file "Element analysis.wrk". Select Review Files from the File menu. From this window, you will be able to set up the correct files and directories for data for your analysis. (The method, report template, tuning, and optimization files will be selected later. Also, there is no need to select a calibration or polyatomic file.)
- D. Dataset: If this is the first run of the day, create a new dataset using the date as the name (use the format 010101 for January 1, 2001). If a run has already been performed today, select the dataset for today's date.

Sample: If an analysis has been performed that is similar to the one you are going to do, select the sample file corresponding to it. You will edit it later for the present analysis.

- (1) In the SAMPLES / BATCH window, update the table to reflect the current sample set (i.e. autosampler locations, sample id, analysis methods, peristaltic pump speeds, etc...). There are two method files WBHgPbCd_std. mth and WBHgPbCd.aq. mth that will be used. These two methods differ only in the autosampler locations of the blank and calibration solutions. The 'WBHgPbCd_std' method file is used to run the base blood blank and the calibrators at the very beginning of the run. Because of the autosampler positions defined in the method file (these are editable), the blood blank must go in autosampler location 9 and the blood calibrators 1–4 must go in autosampler locations 10–13, respectively, the second blood blank must go in autosampler location 14. The 'WBHgPbCd_aq.' method file must be used to run the aqueous blank before the first sample. Because of the autosampler positions defined in the method file (these are editable), the aqueous blank must go in autosampler location 15. Apart from defining the blank and calibrators' autosampler locations, it does not matter which of these files is used when analyzing a sample since all other analysis parameters are identical in the method files. A typical SAMPLE / BATCH window for this method will look like Table 5. (Note: all other autosampler positions chosen besides those specified above are arbitrary):

Table 4. Typical Sample File setup for a total blood Hg, Pb, Cd analysis run.

A/S Location	Sample ID	Measurements Action	Method File*
14	Blood blank	Run Blk, Stds, & Sample	WBHgPbCd_std MMDDYY. mth
16	Low Pool bench QC	Run Blk & Sample	WBHgPbCd_aq MMDDYY. mth
17	High Pool bench QC	Run Sample	WBHgPbCd_aq MMDDYY. mth
18	Sample 1	Run Sample	WBHgPbCd_aq MMDDYY. mth
19	Sample 2	Run Sample	WBHgPbCd_aq MMDDYY. mth
Etc . . .			WBHgPbCd_aq MMDDYY. mth
57	Sample 40	Run Sample	WBHgPbCd_aq MMDDYY. mth
58	Low Pool bench QC	Run Sample	WBHgPbCd_aq MMDDYY. mth
59	High Pool bench QC	Run Sample	WBHgPbCd_aq MMDDYY. mth
60	Blood blank	Run Sample	WBHgPbCd_aq MMDDYY. mth

*(Where MMDDYY is the preparation date of the calibrators being used in the analysis. This date is written on the calibrator containers.)

The autosampler positions of QCs and patient samples do not have to be those shown above, but the order in which these are run (blood blank, calibrators 1–4, blood blank, low pool bench QC, high pool bench QC, 40 samples including 1 blind QC, low pool bench QC, high pool bench QC, blood blank at the end) should be as shown above.

The settings shown in Table 5 should be used for uptake and rinse times for all samples (these values are already stored in the method files for the blanks and calibrators).

Table 5. Sample File Timing Parameters for a Blood Hg, Pb, Cd Analysis Run.

	Pump Speed*	Duration
Sample Flush	–24 rpm	35 s
Read Delay & Analysis	–18 rpm	40 s
Wash	–24 rpm	180 s

* 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.

If using the Microsoft Access Database for long-term recording and handling of data (see Section 8.D), the Elan software should not be used to automatically correct for sample dilutions. When running dilutions of any sample, the sample ID should be edited to reflect the level of dilution

being performed (i.e. A two-fold dilution of 'Sample 1' could be recorded in the sample ID as 'Sample 1 (1–2 dilutions)'. The exact wording is not critical.). This sample ID will be edited during the data import process to the database so that it is recognized as the appropriate sample (see Section 8.D).

- (2) Once the parameters in the SAMPLE / BATCH window have been edited for the run, place the solutions in the autosampler tray according to the setup of the SAMPLE / BATCH window and method files. Highlight (click and drag with the mouse) the table rows of the samples that are to be included in the run, then click on Analyze Batch.

E. 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. One of these reports should be printed and kept in the analyst's laboratory analysis notebook for future reference. Another copy should be kept with the analysis printouts from the run.

(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. The database allows for the printing of a run summary report that indicates if any particular patient results are outside of the normal concentration reference range, or if any measurement failed precision limits. One of these reports should be printed and kept in the analyst's laboratory analysis notebook for future reference. Another copy should be kept with the analysis printouts from the run. See Section 8.D for description of how to import data into the database in order to print out a customized sample report.

(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 ITN 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 and 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 '08022001a_study_name.txt' to designate data from analysis of the study from August 2, 2001, run #1. The Report Format option 'Use separator' and the File Write Option 'Append' should be selected. Finally, reprocess the data of interest (see PerkinElmer Elan 6100 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, Blood Blk1, and Blood Blk2. 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 2F ELAN 6100 DRC directory 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.

F. Replacement and periodic maintenance of key components (part #'s given are PerkinElmer part #'s from the PerkinElmer 2000/2001 Consumables Catalog.)

- (1) Autosampler probe assembly (part # B3000161): One spare should be kept on hand.
- (2) 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.
- (3) Quartz Meinhard Type A3 Concentric Nebulizer for ELAN DRC (part # WE024371): at least one spare on hand.
- (4) Quartz Cyclonic Spray Chamber for ELAN DRC (part # WE025221): at least one spare on hand.
- (5) Liquid Connector for Concentric Nebulizer (part # WE024372) for use with Meinhard Nebulizer: at least one spare on hand.
- (6) 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. × 1.59 mm o.d.; one pack (60 cm length) on hand.
- (7) Injector Support for ELAN DRC (part # WE023951): one spare should be on hand.

- (8) Torch O-ring kit (pkg of 4, part# N8120100): 4 spare packages should be on hand.
- (9) Quartz torch: at least two spare torches should be on hand (part # N8122006).
- (10) Quartz injector, 2.0 mm i.d. sample injector (part # N8125029): at least two spare injectors should be on hand.
- (11) RF coil (part # WE021816): one spare should be on hand.
- (12) Nickel Skimmer (part # WE021137) and sampler cones (part # WE021140): at least 2 spares of each on hand.
- (13) Skimmer and sampler cone o-rings (part # N8120512 and N8120511, respectively): at least 10 spares of each on hand.
- (14) Series II Replacement Ion Lens (part# WE018034). Keep 2 spares on hand.
- (15) Pump oil for the roughing pump (part # N8122004): Should keep 4 bottles on hand.
- (16) NESLAB chiller coolant (NESLAB Coolant, part # WE016558): 2 1L bottles should be on hand.
- (17) If possible, having a backup A/S 93 Autosampler and NESLAB chiller are advised. See PerkinElmer sales representative for part numbers.

G. Calculations

(1) 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 ≥ 0.999 .

(2) 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 and Blood Blk2), 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 limit of detection calculation should be redone approximately twice a year to ensure that the LOD has not changed.

Table 6. Limit of detection for Hg, Pb, and Cd.

Analyte	LOD	Units
Hg	0.14	µg/L
Pb	0.25	µg/dL
Cd	0.075	µg/L

H. Special Procedure Notes – CDC Modifications

None 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 that the results falls 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 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 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.

- 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.
 - (a) 13s – Average of both low QC OR average of both high QC is outside of a 3s limit)
 - (b) 22s – Average of both low QC AND average of both high QC is outside of 2s limit on the same side of the mean
 - (c) R4s sequential – Average of both low QC AND average of both high QC is outside of 2s limit on opposite sides of the mean
 - (d) 10× 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 30% of the mean of the 3 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.

- A. If a particular calibrator is obviously in error, remake a new dilution of that 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 re-analyze it.
- C. 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. Reference Ranges

Table 7. References to normal blood Hg, Pb, Cd concentrations.

References	Concentration (µg/L)
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

13. Critical Call Results (Action Values)

The critical results for Hg in blood for children (6 yr and younger) $>100 \mu\text{g/L}$ (medical intervention is indicated), for adults $>200 \mu\text{g/L}$ (removal from workspace), for Pb in blood for adults, $40 \mu\text{g/dL}$; for children (6 yr. and younger), $25 \mu\text{g/dL}$; for Cd in blood, $5 \mu\text{g/L}$.

If a patient sample has concentration greater than $100 \mu\text{g/L}$ for Hg (in children), $200 \mu\text{g/L}$ for Hg (in adults), $5 \mu\text{g/L}$ for Cd, $40 \mu\text{g/dL}$ (in adults) for Pb, and $25 \mu\text{g/dL}$ (in children 6 yr or younger), the levels should be reported by FAX, phone, or email to the supervising physician.

14. 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.

15. 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.

16. Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

Test results reporting is carried out as outlined in the DLS Policies and Procedures Manual. As stated in section 14, the supervisor should notify the supervising physician as soon as possible. The most expeditious means should be utilized – telephone, FAX, etc.

17. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

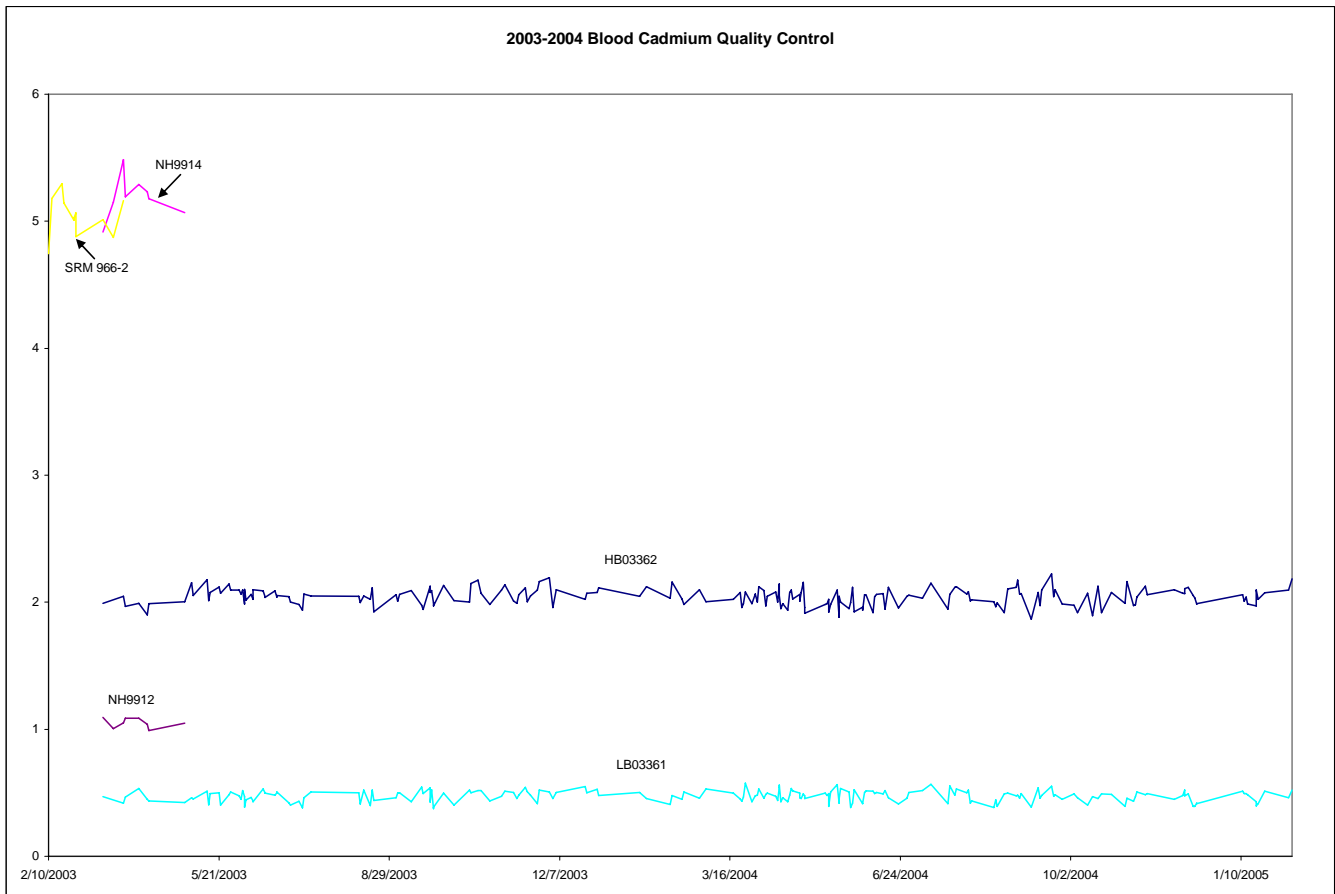
The analyst who receives specimen/samples delivered to ITN sets up a "Specimen Folder." A tracking form is filled out and placed in the folder to be given to the analyst performing the analysis. The form tracks location, status, and final disposition of the specimens. When sample analysis is completed, 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 ≥ 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.

18. Summary Statistics and QC Graphs

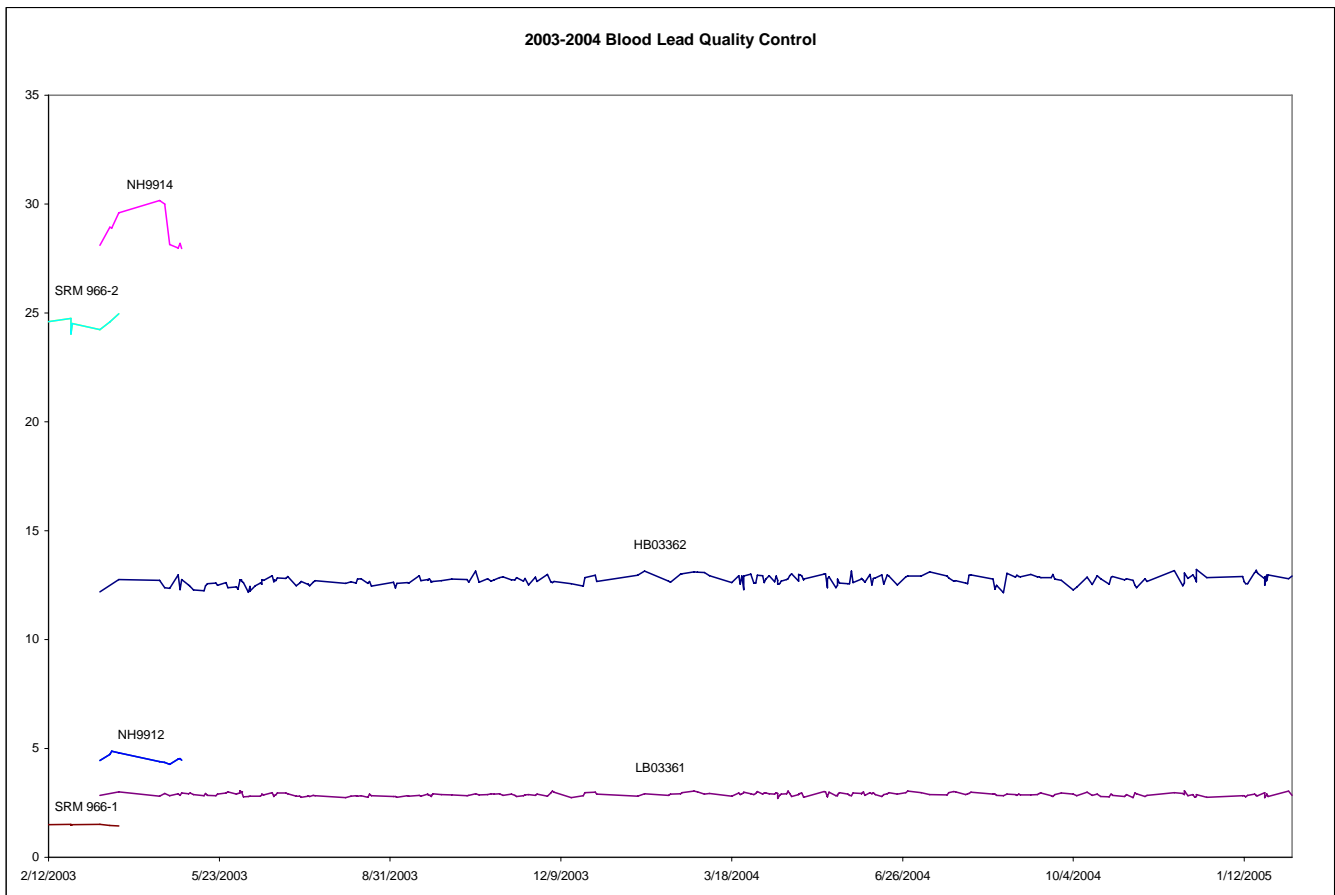
A. Blood Cadmium

Summary Statistics for Blood Cadmium by Lot						
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SRM 966-2	11	2/10/2003	3/26/2003	5.0360	0.1592	3.2
LB03361	200	3/14/2003	2/9/2005	0.4729	0.0444	9.4
NH9912	8	3/14/2003	5/1/2003	1.0498	0.0382	3.6
HB03362	200	3/14/2003	2/9/2005	2.0420	0.0679	3.3
NH9914	8	3/14/2003	5/1/2003	5.1892	0.1652	3.2



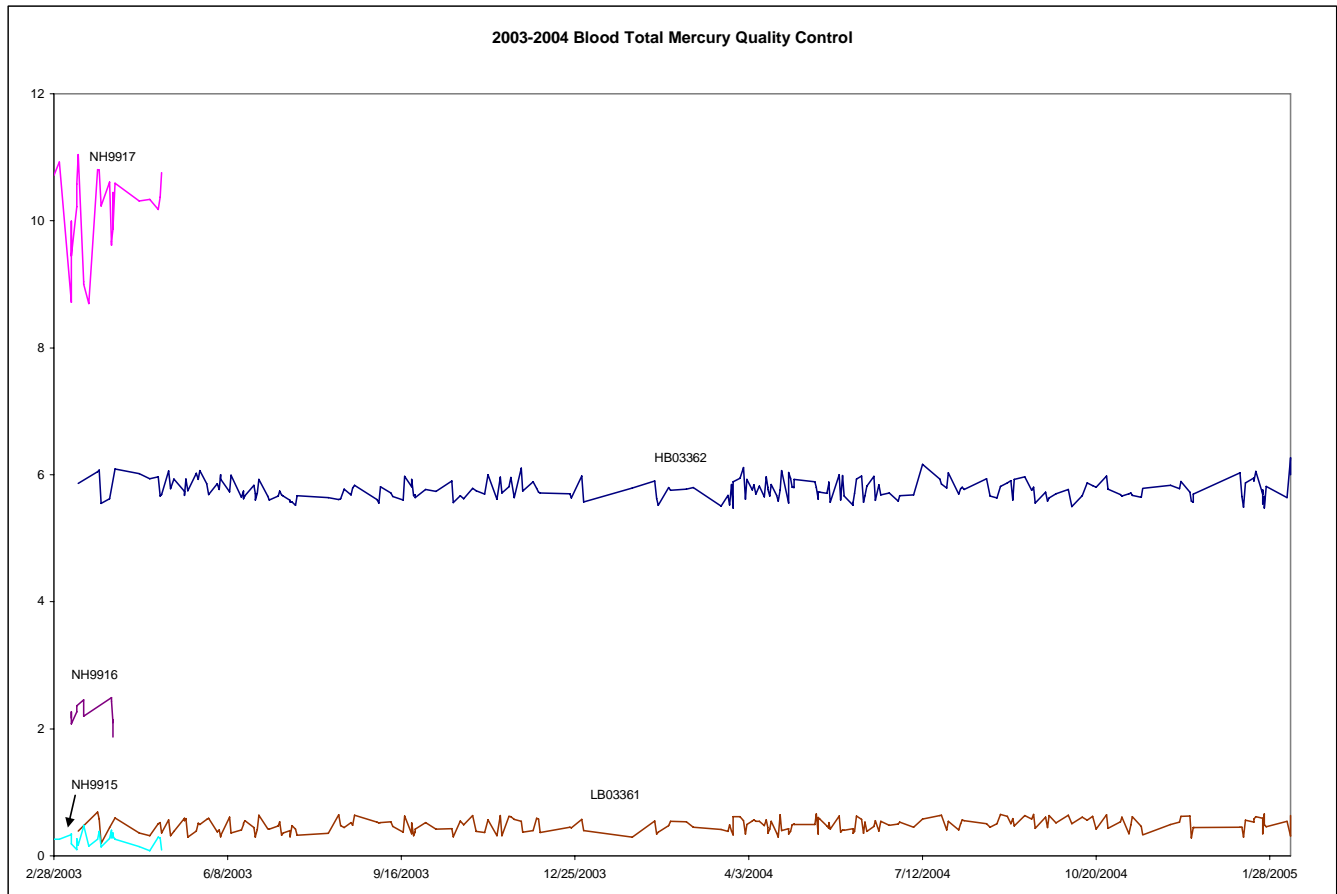
B. Blood Lead

Summary Statistics for Blood Lead by Lot						
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SRM 966-1	7	2/12/2003	3/25/2003	1.4846	0.0287	1.9
SRM 966-2	7	2/12/2003	3/25/2003	24.5310	0.3090	1.3
LB03361	235	3/14/2003	2/9/2005	2.8793	0.0738	2.6
NH9912	10	3/14/2003	5/1/2003	4.5321	0.2014	4.4
HB03362	235	3/14/2003	2/9/2005	12.7263	0.2153	1.7
NH9914	10	3/14/2003	5/1/2003	28.8016	0.8583	3.0



C. Blood Total Mercury

Summary Statistics for Blood Mercury by Lot						
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH9915	26	2/28/2003	5/1/2003	0.2645	0.1090	41.2
NH9917	26	2/28/2003	5/1/2003	10.1102	0.6816	6.7
NH9916	12	3/10/2003	4/3/2003	2.2373	0.1808	8.1
LB03361	224	3/14/2003	2/9/2005	0.4796	0.1051	21.9
HB03362	224	3/14/2003	2/9/2005	5.7695	0.1544	2.7



References

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Appendix

Table 9. Example of Daily Performance Checklist.

Perkin Elmer ELAN DRC (A) ICPMS Daily Maintenance / Performance Checklist		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Month: _____ Instrument: _____														
Day of the month >>>																
Hardware Maintenance Checklist																
SAMPLE INTRODUCTION SYSTEM																
Replace Peristaltic Pump Tubing - SAMPLE																
Replace Peristaltic Pump Tubing - RINSE																
Replace Peristaltic Pump Tubing - DRAIN																
Other Autosampler / Tubing Maintenance (file log for details)																
Nebulizer / Spray Chamber Parts Maintenance																
Empty Waste Container																
Injector Support / Injector / Torch Maintenance																
PLASMA INTERFACE																
Sampler & Skimmer Cone Clearing																
Other Interface Maint. (see log for details)																
Change Interface Roughing Pump Oil (monthly)																
HIGH VACUUM REGION COMPONENTS																
Ion Optic Lens Clearing																
Quadrupole Maintenance																
Detector Maintenance																
Other High Vacuum Region Components Maintenance																
Change High Vac. Pump Oil (every 6 months)																
Perkin Elmer Preventive Maint. Visit (twice yearly)																
Daily Performance Records																
Vacuum Pressure (Before lighting plasma)																
Vacuum Pressure (After lighting plasma)																
Nebulizer Gas Flow Rate																
X-Y Optimization Performed																
Autolens Optimization Performed																
Mass Calibration Tuning Performed (masses +/- 0.05 amu, res. 0.7 +/- 0.1 amu)																
Detector Voltage Optimization (Pulse - Analog - Pulse)																
Daily Performance Check Results																
Be																
Mg																
In																
U																
CeO %																
Bt++ %																
Background (220 amu)																
Be % RSD																
Mg % RSD																
In % RSD																
U % RSD																
Argon Tank Change																
Analyst Performing Maintenance																
Type of Analysis Work Run																

Via. Check = Visual Check of Components