



# Laboratory Procedure Manual

*Analyte:*           **Total Mercury**

*Matrix:*           **Whole Blood**

*Method:*           **Blood mercury ICPDRCMS**

*Method No:*       **ITB001A**

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

*Revised:*          **September 9, 2004**

*as performed by:*

Inorganic Toxicology and Nutrition  
Division of Laboratory Sciences  
National Center for Environmental Health

*contact:*

Dr. Robert L. Jones  
Phone: 770-488-7991  
Fax: 770-488-4097  
Email: [RLJones@cdc.gov](mailto:RLJones@cdc.gov)

Dr. Eric J. Sampson, Director  
Division of Laboratory Sciences

## **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.

## Procedure Change Log

Procedure: Blood lead cadmium mercury ICPMS

DLS Method Code: ITB001A

Date	Changes Made	By	Rev'd By (Initials)	Date Rev'd
9 sep 04	Name change Method code added	klc		
8/7/06	Added details for use of SC2-FAST autosampler	JJ		
8/7/06	Clarified backup procedures to CD-R	JJ		
8/7/06	Clarified preparation of blood quality control pools	JJ		
8/7/06	Corrected definition of concentrated hydrochloric acid from 6M to "approximately 12M or 37% w/v"	JJ		
8/7/06	Clarified acid washing steps for containers	JJ		
8/7/06	Updated calibration verification procedures	JJ		
8/7/06	Updated section on "Instrument & Software setup for the ICP-MS"	JJ		
8/7/06	Modified computer method file names	JJ		
8/7/06	Updated LOD section	JJ		
8/7/06	Updated number of samples in run	JJ		
8/7/06	Clarified procedures of evaluating calibration curves	JJ		
8/7/06	Updated page numbering	JJ		
2/8/2007	Updated sections 13 and 16 in regards to when to confirm sample results and the reporting of elevated results	JJ		
27/08/2007	Changes to Table 7	DJK		

## 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>
THGIHG_E	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

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  $< 3 \mu\text{g/L}$  – value inconspicuous,  $3\text{-}10 \mu\text{g/L}$  – value increased, danger to health not recognizable,  $> 10 \mu\text{g/L}$  – 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  $1.5 \mu\text{mol/L}$  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  $1 \mu\text{mol/L}$  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, particular 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 exposures to  $5 \text{ gm Cd/m}^3$  will be lethal [1]. 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  $0.1 - 4 \mu\text{g/L}$  [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 inductively coupled plasma mass spectrometry. 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 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), 0.5% disodium ethylenediamine tetraacetate (EDTA), 10% 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
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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 Toxicology and Nutrition (ITN) Branch, sample analysis results generated by this method are stored long-term in Microsoft Access or 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).

#### 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)

#### Disk Backups:

- Disk backups can be made using Microsoft “Backup Exec” or similar software.
- Setup 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.
- It is best to not store duplicate copies in the same location. If possible store CD-R discs in a fire-proof environment.

#### 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.

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. 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 / Carrier Solution**

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<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. To prepare the solution, acid rinse a 2 L Teflon<sup>®</sup> container, and partially fill with 18 M-ohm water. Add 1g of EDTA, 20 ml of TMAH, 200 ml of ethyl alcohol, and 100 ml of 1% Triton X-100<sup>®</sup>. 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. The internal standards can be added by spiking 500 µl of 20 mg/L Rh and Bi to the solution. Gold can be spiked into the solution by adding 200µL of 1000mg/L SPEX CertiPrep Au standard to the final diluent. Dilute to volume (2L) with 18 M-ohm water. Store at room temperature and prepare as needed. Larger volumes of diluent and 1% Triton X-100 can be prepared, if desired, by adding proportionally larger volumes of the solution constituents.

When using a flow-injection component in the sample introduction system (i.e. the Elemental Scientific SC2-FAST autosampler), the 'carrier' solution should be the same as the diluent used for the method.

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

a. For single-rinse station autosamplers (i.e. PerkinElmer AS93 or CETAC ASX500 series autosamplers):

When using only a single rinse solution with a single-rinse station autosampler, the rinse solution should be an aqueous solution of 1 % v/v TMAH, 5% ethyl alcohol, and 0.05 % Triton X-100<sup>®</sup> and 10 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 20 ml of TMAH, 100 ml of ethyl alcohol, 100 ml of the 1% Triton X-

100<sup>®</sup> solution and 200 $\mu$ L 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.

- b. For dual-rinse station autosamplers (i.e. with the Elemental Scientific SC2-FAST autosampler).

Rinse Solutions: When using a dual-rinse station setup, use the 1 % v/v TMAH, 5% ethyl alcohol, and 0.05 % Triton X-100<sup>®</sup> and 10  $\mu$ g/L gold solution (described above for single-rinse station setups) in rinse station #1 and 0.5 % v/v nitric acid in rinse station #2. To prepare 4L of the 0.5 % v/v nitric acid solution, acid rinse a 4 L Teflon<sup>®</sup> polypropylene container, and partially fill with 18 M-ohm water. 20 mL of concentrated nitric acid. Fill to 4 L with 18 M-ohm water and mix well. Store at room temperature and prepare as needed. Other volumes of 0.5 % nitric can be prepared, if desired, by combining proportionally adjusted volumes of nitric acid and water.

Carrier Solution: When using a flow-injection component in the sample introduction system (i.e. the Elemental Scientific SC2-FAST autosampler), the 'carrier' solution should be the same as the diluent used for the method. See section 6.A.1 for details in preparing the diluent.

## 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 80 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  $\mu$ L of the 1000 mg/L

mercury stock standard. For Pb, add 500 uL 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 -25 mL 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.

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.

**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

### 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  $\mu\text{L}$  of the appropriate aqueous intermediate working standard, 50  $\mu\text{L}$  of base blood, and 2400  $\mu\text{L}$  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  $\mu\text{g/L}$  for Hg, 2.0  $\mu\text{g/dL}$  for Pb, and 0.5  $\mu\text{g/L}$  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  $\mu\text{g/L}$  for Hg, 13.0  $\mu\text{g/dL}$  for Pb, and 2.0  $\mu\text{g/L}$  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  $-20^{\circ}\text{C}$ .

#### D. 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	1.45 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

2. Ohaus AP310 analytical balance, or equivalent (Ohaus, Pine Brook, NJ).
3. Micromedic Digiflex Automatic pipette (or equivalent) equipped with 10.0 ml dispensing syringe, 2000  $\mu$ 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 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. 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 ([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 15 mL 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
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- a. Prepare an aqueous blank consisting of 100  $\mu\text{l}$  of 18 M-ohm water and 2400  $\mu\text{l}$  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  $\mu\text{l}$  of base blood (same material used for preparation of the blood calibration calibrators), 50  $\mu\text{l}$  of 3% v/v hydrochloric acid that was used for preparation of the calibrators (Std.0), and 2400  $\mu\text{l}$  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  $\mu\text{l}$  diluent, 50  $\mu\text{l}$  18 M-ohm water, and 50  $\mu\text{l}$  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 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

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

1. Power on the computer, printer, peristaltic pump, and autosampler, and log into the operating system.
2. ELAN software: Start the ELAN® ICPMS software from Windows™.
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. 10 - 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. 10 - 24 rpm) in the DEVICES window.
4. Daily Pre-Ignition Maintenance Checks: Perform daily maintenance checks as described in the ITN 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 24 Mg, 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 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 SC2 (non FAST model):
    - 1) Send the probe into the rinse solution using the software (METHOD/SAMPLING window, press the “Probe” button, and then press “Go to Rinse” button).
    - 2) Start the peristaltic pump at a low to mid-range speed (i.e. 10 - 24 rpm).
    - 3) 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.
    - 4) Optimizations and Daily Performance Check: After this warm-up time, perform a daily performance check and any optimizations necessary (as described in the ITN 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.

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

**(b)Saving the Files:** Save 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 (i.e. – default\_2006-0731.dac).

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

<b>A/S Location</b>	<b>Sample ID</b>	<b>Measurements Action</b>	<b>Method File*</b>
XX	Blood blank check (S0)	<b>Run Blk, Stds, &amp; Sample</b>	Blood metals panel_methITB001A_bloodblk.mth
XX	Aqueous Blank Low Pool bench QC	<b>Run Blk &amp; Sample</b>	Blood metals panel_methITB001A_aqblk.mth
XX	High Pool bench QC	Run Sample	Blood metals panel_methITB001A_aqblk.mth
XX	Sample 1	Run Sample	Blood metals panel_methITB001A_aqblk.mth
XX	Sample 1	Run Sample	Blood metals panel_methITB001A_aqblk.mth
XX	Sample 2	Run Sample	Blood metals panel_methITB001A_aqblk.mth
Etc . . .			Blood metals panel_methITB001A_aqblk.mth
XX	Sample 20	Run Sample	Blood metals panel_methITB001A_aqblk.mth
XX	Low Pool bench QC	Run Sample	Blood metals panel_methITB001A_aqblk.mth
XX	High Pool bench QC	Run Sample	Blood metals panel_methITB001A_aqblk.mth
XX	Aqueous Blank	Run Sample	Blood metals panel_methITB001A_aqblk.mth

**Table 5. Sample File timing parameters.**

<b>For regular autosamplers such as PE AS93, CETAC ASX500 series, or ESI SC2 without 'FAST' module.</b>		
	<b>Pump Speed*</b>	<b>Duration</b>
Sample Flush	-24 rpm	50 s (or whatever time is necessary for sample to travel from probe to nebulizer tip)
Read Delay & Analysis	-18 rpm	40 s
Wash	-48 rpm	90 s

<b>For ESI SC2-FAST</b>		
	<b>Pump Speed*</b>	<b>Duration</b>
Sample Flush	-7 rpm	12 s (or whatever time is necessary for sample to travel from probe to nebulizer tip)
Read Delay & Analysis	-7 rpm	58 s
Wash	-7 rpm	0 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.

- 5). Software setup for Analysis:
  - (a) 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.

- (b) 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. The autosampler positions of QCs and patient samples do not have to be those shown in Table 5, but the order in which these are run should be as shown in the table.
- i. Blood vs. Aqueous Method Files: There are two method files for this one method (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.

- ii. 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.

iii. 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.

- (c) 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%.
- (d) 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 SC2 autosamplers not using the “FAST” flow injection option).
- (e) Verify cleanliness water, diluent, and sample introductions system by analyzing non-spiked samples (i.e. water, diluent through Digiflex).
- (f) 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.
- (g) Instrument Shutdown: Stop the peristaltic pump in the ELAN software and turn off the plasma. Release the tension on the pump tubing.

b. ESI SC2-FAST (flow injection) autosampler: Do the following when using this autosampler:

- 1) Manually move the carrier solution probe into a dilute nitric acid solution (i.e. 1% v/v HNO<sub>3</sub>).



- 2) Start the ESI software.
  - (a) If this is the first time to setup the ESI SC2 autosampler, change settings in the ESI to match that listed in Table 7 in the Appendix.
  - (b) Select "Fast" from the menu bar.
  - (c) Click the "Text file" button and open file "Blood metals panel\_methITB001A\_SC2 FAST parameters.txt"
  - (d) Uncheck "Enable Fast Control" (during all ICPMS optimizations) and close the "Fast" window.
  - (e) Click on the "Initialize autosampler" button if not already initialized.
  - (f) Flush the rinse stations. On the "Motion" 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 "Motion" window. Rinse #1 is a solution of 1% v/v TMAH, 0.05% Triton X-100, 5% Ethanol and 100ppb Au (gold). Rinse #2 is 0.5% v/v HNO<sub>3</sub>.
  - (g) Minimize the ESI software window (do not close the software).
- 3) Allow at least 45 minutes warm-up time for the ICP-MS (with all components operational).
- 4) 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. 1% v/v HNO<sub>3</sub>) and allow flushing for several minutes.
- 5) Setup ELAN software files:
  - (a) Workspace (files & folders): Select the appropriate workspace file (i.e. "CDC\_methITB001A\_SC2.wrk". Select "Review Files" from the "File" menu. Verify & set up the correct files and data directories for your analysis.
  - (b) 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. The autosampler positions of QCs and patient samples do not have to be those shown in

Table 5, but the order in which these are run should be as shown in the table.

- i. Method Files: There are two method files for this one method (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.

- ii. 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 SC2 autosampler) defines the blood blank in autosampler location 105 and the blood calibration standards 1-4 in autosampler locations 106-109, respectively.
  - iii. The “aqblk” method file must be used to analyze all QC materials and patient samples. The aqueous blank method (set up for an ESI SC2 autosampler) defines the aqueous blank in autosampler location 111.
- (c) 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%.
- 6) Move the carrier solution probe from the dilute nitric acid solution to a bottle containing diluent (same as what is used in the sample preparation). Allow to flush for several minutes.
  - 7) In the ESI software, select “Fast”, and then check the “Enable Fast Control” box. Under “Rinse Time”, enter 15 in the “Rinse 1” field, and 15 in the “Rinse 2” field then close the “Fast” window and minimize the ESI software (do not close the software).
  - 8) Verify cleanliness water, diluent, and sample introductions system by analyzing non-spiked samples (i.e. water, diluent through Digiflex).
  - 9) 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.
  - 10) When analysis is complete, maximize the ESI software window, and choose “Motion” on the tool bar. Click on the “Go Home” button to send the sample probe to the home position above the rinse station.
  - 11) Move the peristaltic pump probe from the diluent bottle into the dilute nitric acid bottle 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.
  - 12) 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. Analytica I 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 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.

1)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 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). The aqueous blank must be used for all analyses of patient samples and QC samples).

## 2)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.

- E Replacement and periodic maintenance of key components (part #'s given are Perkin Elmer part #'s from the Perkin Elmer 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. x 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 Perkin Elmer sales representative for part numbers.

## F. 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  $\geq 0.999$ .

### 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 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

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 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.

#### A. Precision and Accuracy:

1. **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
  2. If both the low and the high bench QC results are within the 2s limits, then accept the run.
  3. 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.  $1_{3s}$  – Average of both low QC OR average of both high QC is outside of a 3s limit )
    - b.  $2_{2s}$  – Average of both low QC AND average of both high QC is outside of 2s limit on the same side of the mean
    - c.  $R_{4s}$  sequential – Average of both low QC AND average of both high QC is outside of 2s limit on opposite sides of the mean
    - d.  $10_{\bar{x}}$  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.

- e. 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 1 ug/dL for Pb, 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, two approaches can be taken
  - 1. 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.
  - 2. 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. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS**

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

**13. REFERENCES RANGES (NORMAL VALUES)**

<b>Table 6. 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	[Hg] <10 µg/L [Pb] <10 µg/dL for children

E.R.Ashwood, 1999 [14]	[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 (“PANIC VALUES”)

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 5.8 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.

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 (6 yr. 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 ≤-20 °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**

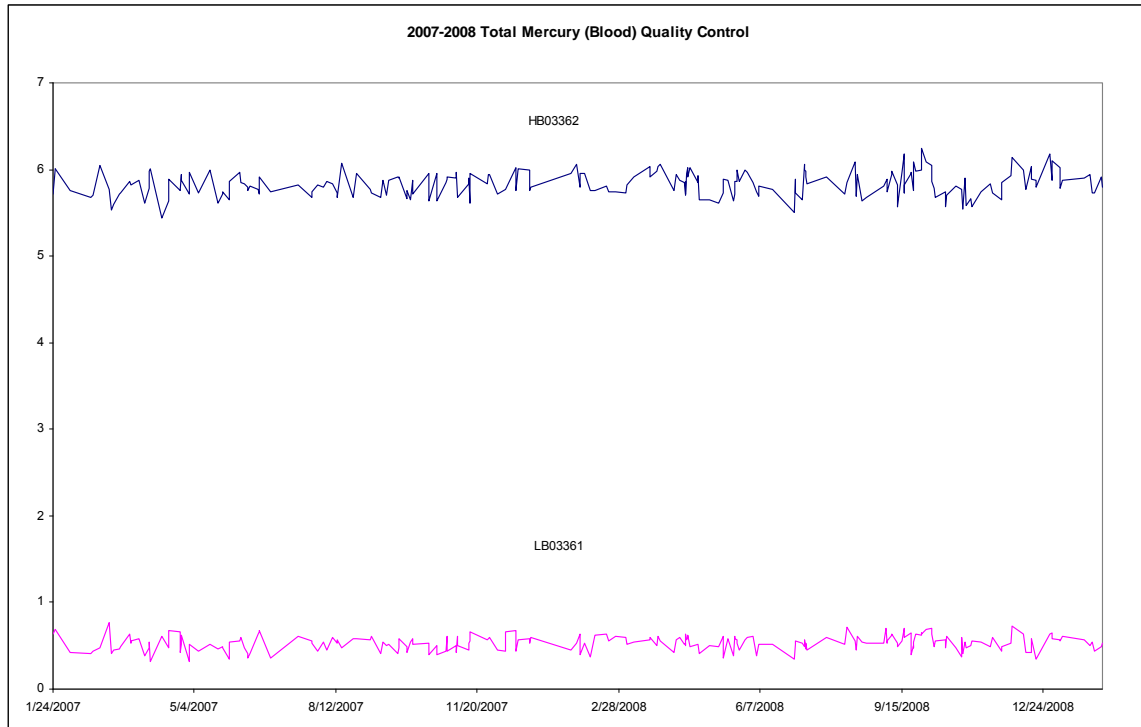
The analyst who receives specimen/samples delivered to ITN sets up a "Specimen Folder." A tracking form is filled out and placed in the folder to be given to the analyst performing the analysis. The form tracks location, status, and final disposition of the specimens. When sample analysis is completed, 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  $\geq 3$  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

Summary Statistics for Total Mercury (Blood) by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
LB03361	231	1/24/2007	2/4/2009	0.531	0.086	16.3
HB03362	231	1/24/2007	2/4/2009	5.831	0.141	2.4



**20. Appendix.**

<b>Table 7 Parameters for the Elemental Scientific SC2-FAST autosampler.</b>	
Autosampler Model:	SC-2/E2
Configuration File:	cdc.sc
Text File:	Blood metals panel_methITB001A_SC2 FAST parameters.txt
Instrument Type:	Perkin Elmer ELAN
Autosampler Type:	AS 93
<b>Configure Communication</b>	
SC Autosampler Communication Port:	2
Instrument Communication Port:	1
<b>Configure Autosampler</b>	
	Optimize for speed while preventing dripping of solution off probe from one tube to another.
Horizontal:	
Start speed (May vary):	1564, 3 (0-5)
Max speed: (May vary)	3876, 3 (1-5)
Accel/decel (May vary):	6, 3 (1-5)
Rotational:	
Start speed (May vary):	50, 1 (0-5)
Max speed (May vary):	1050, 3 (1-5)
Accel/Decel (May vary):	6, 3 (1-5)
Vertical:	
Start speed (May vary):	850, 3 (0-5)
Max speed (May vary):	2500, 4 (1-5)
Accel/Decel (May vary):	6, 3 (1-5)
<b>Tray Type</b>	
Tray Configuration:	2 large racks 1x1
Array Type (rack 1):	SR2-80
Array Type (rack 3):	SR2-80
Standards Rack:	ST10
	<i>NOTE:</i> Number on the corner of the rack designates ST10 or an ST10CP ("CP" stands for "closely packed").
<b>NOTE:</b>	Rack 1: ID numbers start in left corner, closest to the Standard Rack, with 101, and end with 180  Rack 3: ID numbers start in left corner, closest to the Standard Rack, with 301, and end with 380

	These two large racks are numbered as if they were four smaller racks (first rack is one and two, second rack is three and four). This is why the second rack is designated as “Rack 3” and not a “Rack 2”. This also explains why Rack 3 starts with #301.
<b>Probe Settings (May vary)</b>	
Rinse Station:	
Probe Down Height :	Typically ~130. Range from 120 - 160mm.  Note: The probe should go down close to the bottom of each rinse tube.
Retraction Speed :	Typically ~ 2. Choose from 1-5 to optimize speed while preventing dripping of solution from probe into adjacent tubes.
Racks 1 and 3:	
Probe Down Height:	Typically 145. Range from 120 - 160mm.  Note: To be sure the loop is filled, the probe should go down close to the bottom of the cup, but not touch.
Retraction Speed:	Typically ~3. Choose from 1-5 to optimize speed while preventing dripping of solution from probe into adjacent tubes.
Standards Rack:	
Probe Down Height :	Typically ~ 140. Range from 120 - 160 mm.  <i>NOTE:</i> To be sure the loop is filled, the probe should go down close to the bottom of the cup, but not touch.
Retraction Speed:	Typically ~3. Choose from 1-5 to optimize speed while preventing dripping of solution from probe into adjacent tubes.
<b>Rinse Settings (sec)</b>	
Rinse 1:	
Rinse Time:	15s
Additional Flush Time:	60s
Rinse 2:	
Rinse Time:	15s
Additional Flush Time:	60s
<b>Fast Valve Control</b>	
	Event / Action / Time in sec
	On Probe Down / Vacuum On
	On Probe Down / Load
	Probe in Sample / Timer A / 12
	Timer A Expires / Inject
	Timer A Expires / Move Rinse
	Rinse Complete / Move Next
	On Rinse / Load

	On Rinse / Timer B / 1000
	Timer B Expires / Vacuum off



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