Laboratory of Inorganic and Nuclear Chemistry
Division of Environmental Health Sciences
Wadsworth Center
Department of Health
State of New York

NYS CLEP Laboratory ID 1067
CLIA Laboratory ID 33D0654341

Standard Operating Procedure

Determination of Lead, Cadmium, and Mercury in whole blood by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Approved: _____
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Distribution

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Revision Record

Rev	Date	**Responsible Person**	**Description of Change**
1	01/18/2005	C. Palmer	Initial Release of biomonitoring method. Modification of the NYS 'Multielement' method, reduced for just three analytes (Cd, Hg, Pb). Modification of calibration ranges for biomonitoring purposes. Implemented for NYC HANES biomonitoring, Spring 2005.
2	02/14/2005	C. Palmer	Mercury calibration table modified
3	05/06/2005	C. Palmer	Original values in Table 3 were for 1+19 dilution, not 1+49 dilution. Corrected.
4	08/01/2006	C. Palmer	Addendum to Cd correction equation in the Appendix.
5	11/9/2006	C. Palmer	Replacement of the cross-flow nebulizer (and Scott-type double pass spray chamber) with a Burgener nebulizer and Cinnabar spray chamber. 20 independent runs performed to recalculate new detection limits, and to validate the method for CDC LRN-C purposes.
6	01/28/2009	C. Palmer	Single element standards of Cd, Hg, Pb replaced with a the CDC's LRN-C multielement stock solution SM-2107-007. Minor SOPM edits. Reformatted for LINC controlled document format.

The following laboratory staff have read this Manual.
A copy of this page will be distributed to the employee training record file.

Name	Title	Date
Christopher D. Palmer, Ph.D.	Research Scientist III	C.D. Palmer 4/17/09
Michelle Morrissette	Assistant Research Scientist	Michelle Morrissette 4-17-09

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

a. Clinical relevance

This biomonitoring method is used to achieve rapid and accurate quantification of cadmium, lead and mercury in whole blood. The method is sensitive enough to be used to rapidly screen blood specimens from subjects suspected to be exposed to these toxic elements, or to evaluate environmental or other non-occupational exposure to these same elements.

b. Test principle

Inductively coupled plasma-mass spectrometry is a multi-element analytical technique. Liquid samples are introduced into the ICP through a nebulizer and spray chamber carried by a flowing argon stream. By coupling radio frequency power into flowing argon, a plasma is created in which the predominant species are positive argon ions and electrons. The sample passes through a region of the plasma having a temperature of 6000- 8000°K. The thermal energy atomizes the sample, then ionizes the atoms. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP, which is operating at atmospheric pressure, from the mass spectrometer, which is operating at a pressure of 10^{-6} torr. The mass spectrometer permits detection of ions at each mass in rapid sequence, allowing individual isotopes of an element to be determined. 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. Blood samples are diluted 1+ 49 with 0.5% v/v GFS double-distilled concentrated nitric acid containing iridium, yttrium and rhodium for multi-internal standardization.

2. SAFETY PRECAUTIONS

Use standard precautions when handling any bodily fluid. Wear gloves, a lab coat, and safety glasses. Prepare all blood samples in the SterilGARD® Bio-safety hoods (Baker Company, Sanford, ME). Place in a biohazard autoclave bag disposable plastic, glass, and paper (e.g. pipette tips, autosampler cups, gloves, etc.) that are contaminated with blood. Keep these bags in appropriate containers until they can be sealed and autoclaved. Wipe down all work surfaces with a 10% v/v sodium hypochlorite (Bleach) solution when preparation work is finished. The hepatitis B vaccination series is recommended and is available via NYS Employee Health Services, for all analysts who work with human specimens.

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

Exercise caution when handling and dispensing concentrated acids and bases. Always remember to add acid or base to water. Acids and bases are caustic chemicals that are capable of causing severe eye and skin damage. Wear powder-free gloves, a lab coat, and safety glasses. If the acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.

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

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

a. Maintain the integrity of specimen and analytical data generated by this method by proofreading all transcribed data and storing data in several computer systems. Store data files containing the date, analytical run identification (ID), specimen analytical results by specimen ID, and method code on the local hard drive of the ICP-MS PC. When a run is completed, the data file, is copied to the respective study folder on the TREL server.

b. Routine backup procedures on the networked drive include daily backup of data files. Contact either the supervisor or lab director for emergency assistance.

c. Accomplish statistical evaluation and calculation of the run with the calibration curve used by the ELAN 3.0 Hotfix 3 software.

d. Computer Science support staff make sure that files stored on TREL are automatically backed up to tape each night, and mirrored to a backup server at DAI.

e. The ELAN DRC Plus Daily Performance binder, located in D 146B contains documentation for system maintenance and daily laboratory activities.

4. BLOOD SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

a. Specimen type – whole blood, optimal amount of specimen required is 2-3 mL, minimum volume required for analysis is about 0.5 mL.

b. Acceptable containers include pre-screened vacutainers; 7-mL, Royal blue cap (unless Al is to be determined) or plastic 5-mL EDTA vacutainers (unless Zn is to be determined). Since no one specimen container can be used for all trace elements, consult contamination study data in the Appendix for details. All new lots of vacutainers and Falcon tubes should be screened for impurities of the target analytes prior to their use. The protocol for tube/vacutainer/vial prescreening is fully discussed in the 'Vial Screening' section of this SOPM.

c. The criteria for unacceptable specimen are either insufficient volume (< 0.5 mL), defined as QNS, presence of visible clotting, unable to verify collection date, or suspected contamination due to improper collection procedures or collection devices. Consult laboratory director for current lab policy on missing data. In some cases, a second specimen might be requested.

d. Specimen characteristics that may compromise test results are as indicated above including contamination of blood by contact with dust, dirt, etc. from improper handling.

e. In general, blood specimens should be transported and stored at 4 °C. Or in the case of biomonitoring projects they may be frozen at -20 °C or at -70 °C until time for analysis. Portions of the sample that remain after analytical aliquots are withdrawn and should be refrozen at ≤ -20 °C. Samples thawed and refrozen several times are not compromised. Blood specimens should be processed in a SterilGARD® Bio-safety hood (Baker Company, Sanford, ME). With the blower on, the cleanliness of the hood is class 100 or better. Furthermore, specimen aliquots shall be protected from dust contamination before and during analysis. For the DRC Plus Instrument in room D 146B, this is accomplished by housing the autosampler in a custom-made Terra Universal (Fullerton, CA) hood, and for the DRC II instrument by housing the autosampler in a class 100 cleanroom.

f. If venous blood specimens are collected for multiple analyses in other clinical sections, including trace element testing, a volume sufficient for the initial trace element test and any repeat testing should be transferred to a trace element-free tube under clean conditions (described above) before other processing or testing occurs to the specimen. Note: this should not happen for the Trace Elements Section at Wadsworth

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

Not applicable for this procedure.

6. PREPARATION OF REAGENTS, CALIBRATORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

Prepare all reagents, calibrators and controls in class 100 cleanroom conditions or better.

a. Reagent Preparation

1. Diluent.

The diluent used in this method is an aqueous solution of 25 µg/L yttrium, 25 µg/L rhodium, 25 µg/L iridium, and 1000 µg/L (1 mg/L) gold in 0.5% (v/v) double distilled nitric acid. This solution will be added in the preparation of all samples during the dilution process, just prior to analysis. To prepare, acid rinse a 2 L Teflon PFA container, and partially fill with 18.2 MΩ.cm, ultrapure water. Add 10 mL of GFS double distilled (or similar trace-metal free purity) concentrated nitric acid, and spike in 50 µL each of 1000 mg/L Rh, Y and Ir. Add 1 mL of 10% v/v Triton-X 100 to the diluent bottle (thus making the Triton-X 100 concentration 0.005% v/v). Add 2 mL of 1000 mg/L gold stock standard solution (or 200 µL of 10,000 mg/L gold stock standard). The gold will help reduce the mercury memory effect between samples. Make up to volume with 18.2 MΩ.cm ultrapure water. Store at room temperature and prepare as needed.

2. Base Blood preparation.

The base blood matrix used in this method is a pool of caprine (goat) blood. The base blood should be screened before its use as a base matrix, and should ideally have low concentrations of the elements of interest (i.e <5 µg/L Pb {0.5 µg/dL Pb}, <1 µg/L Hg, and <0.5 µg/L Cd). This base blood will be combined with intermediate working standards during the dilution process just prior to analysis. The base blood should be aliquoted to prescreened vials, and then stored at -80 °C. Individual vials can then removed from the freezer and stored at 4 °C whenever a vial of the base blood is needed to prepare standards and/or controls.

3. ICP-MS Rinse Solution.

The rinse solution used in this method is an aqueous solution of 0.005% v/v Triton X-100, 1 mg/L gold and 2% v/v GFS double-distilled (or similar trace metal free level) concentrated nitric acid. This solution will be pumped into the sample introduction system between samples to prevent carry over of the analytes of interest from one sample measurement to the next. To prepare, acid rinse a 2-L polypropylene or Teflon PFA container, and partially fill with 18.2 MΩ.cm, ultrapure water. Add 40 mL of GFS double distilled concentrated nitric acid, 1 mL of 10% v/v Triton X-100, and 2 mL of the 1000 mg/L gold stock standard (or 200 µL of 10,000 mg/L gold) solution to 18.2 MΩ.cm ultrapure water. Dilute to 2 L with 18.2 MΩ.cm, ultrapure water. Store at room temperature and prepare as needed.

b. Standards Preparation

1. Stock standards.

If the commercially available intermediate standard is unavailable, commercially available 1000 mg/L stock standard solutions should be used to prepare an intermediate standard. These are commercially available solutions may be purchased from reputable suppliers (e.g. Perkin-Elmer, SPEX or High Purity Standards). Standards used in the analysis should be National Institute of Standards and Technology (NIST) traceable. Stock standard calibration solutions typically have a 1000 mg/L trace element concentration.

2. Multi-element intermediate standards.

The standard solution used is SM-2107-007 (the "2200" series), from High Purity Standards (Charleston, SC). This is the stock standard used by Centers for Disease Control and Prevention's Emergency Response and Air Toxicants Branch, in the Hg, Pb and Cd ICP-MS method, that has been distributed to the State Labs as part of the Laboratory Response Network (LRN). Using modified calibration ranges, this stock solution can be used in this NYS biomonitoring method. The SM-2107-007 stock standard contains 1 µg/mL Cd, 20 µg/mL Pb, and 2 µg/mL Hg, in a solution of 5% HNO_3 and 1% HCl high purity acids.

3. Working standards.

These solutions will be used, on each day of analysis, in the preparation of the final diluted working standards that will be placed on the autosampler of the ELAN DRC Plus. To prepare seven 100-mL polypropylene volumetric flasks and partially fill them with 18.2 MΩ.cm water. To the 100-mL volumetric flasks add 1-mL of doubly distilled concentrated nitric acid. To these flasks, spike in the appropriate aliquot of the intermediate SM-2107-007 standard (see Tables 1 for the appropriate volumes). Bring up the volume with 18.2 MΩ.cm water. The remaining seventh flask will not have an aliquot of intermediate stock standards, this flask will serve as a 1% v/v nitric acid Blank.

Table 1a. Preparation of intermediate working calibrators

Std.	Blank	Std. 1	Std. 2	Std. 3	Std. 4	Std. 5	Std. 6
Flask (mL)	100	100	100	100	100	100	100
mL HPS SM-2107-007 stock soln.	0.000	0.100	0.250	0.500	0.750	1.000	2.000

Table 1b. Concentration of analytes in intermediate standards, Hg (µg/L), Cd (µg/L), Pb (µg/dL).

Analyte	HPS SM-2107-007 Conc. (mg/L)	Std. 1	Std. 2	Std. 3	Std. 4	Std. 5	Std. 6
Mercury	2	2	5	10	15	20	40
Cadmium	1	1	2.5	5	7.5	10	20
Lead	20	2	5	10	15	20	40

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. Diluted working standards and diluted patient specimens.

The diluted working multielement standard solutions used in this method are a series of 1 + 49 dilutions of the corresponding multi-element intermediate working standards. In this step, the aqueous intermediate working standards are spiked into diluent and base blood to matrix match the working standards with the blood samples being analyzed. To prepare, transfer 200 µL (Finnipipette) of the appropriate aqueous intermediate working standard, 200 µL (Finnipipette) of base blood, and 9600 µL (Digiflex) of the diluent in to a 15 mL Falcon tube. NOTE: it is necessary to use a micropipette rather than the Digiflex to thoroughly mix the blood with the diluent mix. Mixing takes place by pumping the micropipette several times once the sample has been aliquoted. This mixes the solution and thoroughly cleans all of the blood sample from the inside of the pipette tip. Table 3 shows the concentrations of the analytes in the working calibration standards. Patient samples should be prepared in a similar manner by pipetting 200 µL (Finnipipette) of the patient whole blood specimen, 200 µL (Finnipipette) of 1% v/v nitric acid blank, and 9600 µL (Digiflex) of the diluent in to a 15 mL Falcon tube.

Table 2. Dilution Scheme – Preparation of samples for analysis. (All volumes in µL)

I.D.	D.I. Water	Std. Blank, (1% v/v HNO_3)	Std (in 1% v/v HNO_3)	Base Blood	Blood Sample or IQC	Diluent
Blood Blank	0	200	0	200	0	9600
Stds.	0	0	200	200	0	9600
Reagent Blank	200	200	0	0	0	9600
Blood Sample/IQC	0	200	0	0	200	9600

Table 3. Actual concentrations of analytes in the Falcon tubes following a 1+49 dilution with diluent.

	Std. 1	Std.2	Std. 3	Std. 4	Std. 5	Std. 6
Conc. of Hg in the Falcon tube/conc. introduced into the ICP-MS, µg/L	0.040	0.100	0.200	0.300	0.400	0.800
Conc. of Cd in the Falcon tube/conc. introduced into the ICP-MS, µg/L	0.020	0.050	0.100	0.150	0.200	0.400
Conc. of Pb in the Fan tube/conc. introduced into the ICP-MS, µg/dL	0.040	0.100	0.200	0.300	0.400	0.800

5. Preparation of Quality control materials

NY State trace element control materials are available, with target values assigned for Cd, Pb and Hg in blood. These materials are produced from caprine (goat) blood pools; e.g. TE03, TE04 and TE05 materials, or from bovine (cow) blood ; e.g. TE02 materials, that contain a mixture of endogenous and spiked levels of the target trace elements. The materials are also circulated as PT pools in the NYS PT program, so validation data are available to assess assay accuracy as well as monitor internal quality control (IQC). The median and standard deviation values for each analyte concentration in the PT materials is established by up to 25 labs enrolled in the NY State Trace Elements PT scheme. This "assigned target value" is used to assess on-

going accuracy. Additionally, the in-house running mean and standard deviation for each control level is established for IQC purposes. These PT/IQC materials are stored at – 80 °C until needed. Quality control materials are prepared in the same manner as patient samples (see Section 6.b.4)

C) Other Materials

1. Individual trace element stock standards 1000 mg/L Cd (10-03 Cd, Perkin-Elmer or equivalent), 1000 mg/L Pb (10-93 Pb, Perkin-Elmer or equivalent) and 1000 mg/L Hg (CL3-22HG, SPEX or equivalent).
2. Ultrex-II double-distilled Nitric Acid (J.T. Baker Chemical Co., Phillipsburg, NJ). Or, in-house made double-distilled high purity nitric acid.
3. Ultrapure water, 18.2 MΩ.cm from the Milli-Q water purification system (Millipore Systems Inc., Bedford, MA), or similar water purification system (e.g. Barnstead).
4. Liquid Argon equipped with approved gas regulator (Matheson Gas Products, Secaucus, NJ).
5. Base blood, pooled from healthy caprine (goat) or bovine (cow) subjects.
6. Iridium: SPEX PL1R3-2Y, 1000 mg/L (SPEX Industries Inc., Edison, NJ) or equivalent.
7. Rhodium: SPEX PLRH3-2Y, 1000 mg/L (SPEX Industries Inc., Edison, NJ) or equivalent.
8. Yttrium: SPEX PLY2-2Y, 1000 mg/L (SPEX Industries Inc., Edison, NJ) or equivalent.
9. Falcon 15-mL conical tubes (#2097) (Becton-Dickinson Labware, Franklin Lakes, NJ).
10. Triton-X-100 (t-octylphenoxypolyethoxyethanol) (Sigma Ultra purity, Sigma-Aldrich Co., St. Louis, MI, or any source found to be low in trace metal concentration).
11. N-DEX 100% Nitrile examination gloves.
12. Cotton swabs.
13. Dehydrated alcohol.
14. Biohazard autoclave bags.

15. Bleach (10% v/v sodium hypochlorite solution) – any vendor.

16. Reagent grade, concentrated nitric acid (J.T. Baker Chemical Co., or any source with comparable reagent) for use with acid washing. NOTE: This grade of acid is NOT to be substituted for the double distilled grade that is used for making standard solutions.

D) Instrumentation

(1) Inductively Coupled Plasma-Mass Spectrometer fitted with a Dynamic Reaction Cell, ELAN DRC Plus (Perkin-Elmer Corp., Shelton, CT). Burgener nebulizer gas flow rate and Autolens voltage are optimized daily. Other instrumental parameters are given below.

Table 4: PE-Sciex ELAN DRC Plus ICP-MS Operating Conditions

Parameter	Setting
RF power	1.1 kW
Ar nebulizer gas flow	Typically 0.7 – 1.0 LPM
Detector mode	Dual
Measurement Units	Cps
Autolens	On
Blank Subtraction	After internal standard
Curve Type	Simple Linear
Sample Units	µg/L (Cd and Hg), µg/dL (Pb)
Sweeps/Reading	90
Readings/Replicate	1
Replicates	3
Dwell Time	15 ms
Integration Time	1350 ms

(2) Milli-Q plus 18 MΩ.cm water purification system (Millipore Corporation, Bedford, MA), or Barnstead equivalent.

(3) Titertek DigiflexTP Totally Programmable pipette (Huntsville, AL) for automated dispensing of the 9600 µL of diluent.

(4) Finnipipette Digital micropipette for manual dispensing of 200 µL aliquots of blood (Finnipipette, Finland).

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a) Calibration

A simple linear calibration curve for each of the three elements in this method is generated using a series of external standards whose concentrations are defined in the calibration page of the quantitative analysis method. The ratios of the analyte versus the internal standards are calculated as the intensities of the analytes.

b) Verification

(1) In order to verify the calibration curve use NYS 'Low', 'Medium 1', 'Medium 2' and 'High' trace element control levels, or archived CTQ PT materials (if the NYS materials are unavailable). Calibration verification data is stored in the ICP-MS accession file that is kept in the same laboratory as the ELAN DRC Plus ICP-MS.

(2) Agreement with certified or accepted values should typically be within the ± 2 S.D. limits. Apply the modified Westgard Rules, consistent with section policy, and track QC on a daily basis.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a) Preliminaries

(1) For information regarding the range of linearity and how to handle results outside this range, refer to the Calculations section of this document (Sect. 10 - Remedial Action)

(2) Allow frozen blood specimens, quality control specimens, and base blood calibration material to reach ambient temperature. Mix each of the samples thoroughly on an automated rocker, before taking an aliquot for analysis.

b) Sample Preparation

(1) Thaw the frozen blood specimens; allow to reach ambient temperature (about 20 °C).

(2) Set up a series of pre-screened 15-mL polypropylene tubes (Falcon or similar) corresponding to the number of blanks, standards, QCs, and patient samples to be analyzed. Screening results for each tube lot number is kept in the Screening section of the SOPM binder.

(3) Prepare the following solutions into the 15-mL Falcon tubes using the Digiflex automatic pipettor (9600 µL volume) and Finipette micro-pipettes (200 µL volumes)

(a) Prepare five aqueous blanks consisting of 200 µL of 18.2 MΩ.cm ultra pure water, 200 µL of 1% v/v nitric acid, and 9600 µL of diluent solution.

(b) Prepare two blood blanks consisting of 200 µL of base blood, 200 µL of 1% v/v nitric acid, and 9600 µL of diluent solution.

(c) Prepare the patient blood samples consisting of 200 µL of the patient blood sample, 200 µL of 1% v/v nitric acid, and 9600 µL of diluent solution (i.e. a 1+49 dilution).

(d) Prepare working calibration standards and working QC standards as described in sections 6.B 4 and 5.

(4) Mix samples well for approximately sixty seconds, a vortexer may be used to ensure proper mixing.

(5) A base blood blank should be run as the blank for the calibration standards, in the ELAN software.

c) Instrument and Software set-up for the ICP-MS

(1) Turn on the computer (log in to the TREL server). Make sure the printer, peristaltic pump and autosampler are also turned on.

(2) Set up the peristaltic pump tubing on the pump. A daily visual inspection of the pump tubing is necessary to make sure that it is suitable for use and not overly worn. It may not be necessary to change the pump tubing everyday, in fact slightly worn pump tubing provides a smoother, less pulsed flow of sample to the nebulizer. If the pump tubing is changed, make a note in the *DRC Plus Daily Maintenance Log Book*.

(3) Perform necessary daily maintenance checks as described in chapter 5 of the ELAN 6100 DRC Hardware Guide (i.e. argon supply, interface components cleanliness and positioning, interface pump oil condition). Record any routine maintenance that is performed in the Daily Maintenance Log. Make a note of the base vacuum pressure displayed in the INSTRUMENT window of the software (before igniting the plasma, the vacuum is typically 8×10^{-7} to 1.8×10^{-6} torr. Record this pressure in the *DRC Plus Daily Maintenance Log Book*.

(4) Ensure that the 2-L rinse solution container is sufficiently full enough with rinse solution, so that there is enough solution to last through the duration of the analysis. Take the autosampler probe and make sure that it is in the ICP-MS rinse solution.

(5) Open up the INSTRUMENT window in the ELAN software. Press the "START" button to ignite the plasma.

(6) In the DEVICES window, select the autosampler tab to open up the autosampler window. Press the "CONNECT" button to establish communication between the computer and the autosampler, then start the peristaltic pump by pressing the appropriate arrow (make sure that the rotational direction is correct for the way that the tubing is set up in the

peristaltic pump). Type in '-18' into the 'rpm' field of the DEVICES-AUTOSAMPLER window to set the pump head speed at -18 revolutions per minute.

(7) Allow at least a 45 minute warm-up time for the mass spectrometer. Complete daily optimization procedures as required according to Chapter 3 'Tuning and Optimization' of the ELAN 6100 DRC Inductively Coupled Plasma-Mass Spectrometer Software Guide (Software Kit for Elan Software Version 3.0, P-E Part Number 1006920). Prepare a 1 µg/L multielement solution for instrument tuning purposes (dilute 1+9999 of CLARITAS PPT 10 ppm Tuning Solution 1, CL-TUNE1, typically 50 µL into a 500 mL PP volumetric flask, with 1% v/v nitric acid, 5 mL nitric acid per 500 mL total volume). Record the results for the daily optimization procedures in the *DRC Plus Daily Maintenance Log Book.*

(7) Click on **Open Workspace** from the **File** menu. Select workspace file "CHANESBlood". 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.

Method: "NYSHANESBlood"

Dataset: If this is the first run of the day, create a new dataset using the date as the name (use the format 031202 for March 12, 2002). 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 perform, select the sample file corresponding to it. This file will then need to be edited so that it contains sample information for the samples of the present analysis.

Report Template: Select "NYSHANESblood.rop"

Tuning: Save the present settings, using today's date as the file name (use the format 031202 for March 12, 2002).

Optimization: Save the present settings, using today's date as the file name (use the format 031202 for March 12, 2002).

Calibration: No need to select a file at the start of the analysis. However, once the calibration curves have been generated save them as the calibration file using today's date as the file name (use the format 031202 for March 12, 2002).

Polyatomic: elan.ply

(8) In the SAMPLES/BATCH window, update the table to reflect the current sample set (autosampler locations, sample i.d., analysis methods). Typically the matrix-matched calibration standards will go in autosampler positions 9-14, the blood blank in autosampler location 15, and the aqueous blank in autosampler location 17.

A typical sample file for this method will look like:

A/S	Sample ID	Measurement Action	Method.....
15	Base Blood Blank	Run Stds and Sample	NYSHANESblood.mth
16	Base Blood Check	Blank Run Sample	NYSHANESblood.mth
17	Reagent Blank 1	Run Sample	NYSHANESblood.mth
18	Reagent Blank 2	Run Sample	NYSHANESblood.mth
19	Reagent Blank 3	Run Sample	NYSHANESblood.mth
20	Reagent Blank 4	Run Sample	NYSHANESblood.mth
21	Reagent Blank 5	Run Sample	NYSHANESblood.mth
17	NYS Low Control	Run Sample	NYSHANESblood.mth
18	NYS Med 1 Control	Run Sample	NYSHANESblood.mth
19	NYS Med 2 Control	Run Sample	NYSHANESblood.mth
20	NYS High	Control Run Sample	NYSHANESblood.mth
21	*Sample 1*	Run Sample	NYSHANESblood.mth
22	*Sample 2*	Run Sample	NYSHANESblood.mth
23	*Sample 3*	Run Sample	NYSHANESblood.mth
	etc.....		

Close out samples with the NYS 'Low', 'Medium 1', 'Medium 2' and 'High' controls at the end of the sample run

The following settings should be used for uptake and rinse times for all samples (these values are already stored in the method files for blanks and standards)

	Pump speed (rpm)	Duration (sec)
Sample flush	-18	90
Read delay and analysis	-18	30
Wash	-24	120

The lengthy wash time is specifically intended to allow the tubing and spray chamber to wash out 'sticky' elements such as mercury.

Highlight the samples that you want to measure, then click on **Analyze Batch.**

d) Recording of data

Transfer data to the TREL server, record also onto a writeable CD-ROM.

e) Replacement and periodic maintenance of key components, (part nos. given are from Perkin Elmer Atomic Spectroscopy Supplies Catalog).

1) Nickel Skimmer (PE# WE02-1137) and sampler cones (PE# WE02-1140), or the Spectron equivalent: at least 2 of each on hand. Platinum cones may also be used.
2) Skimmer and sampler cone o-rings (# N812-0512 and N812-0511, respectively): at least 5 for each on hand.
3) Quartz torch: at least two spare torches should be on hand (PE# N812- 2006), or the Spectron equivalent.
4) RF coil (PE# WE02-1816): one spare should be on hand.

5) Injector Support / Torch Base (PE# N812-0116): one spare should be on hand.

6) Torch o-ring Kit (PE# N812-0100): one spare kit should be on hand.

7) Ryton Spray Chamber kit (PE# N812-0124): one spare kit should be on hand (PE# WE01-3060), retaining ring (PE# WE01-4081), and right angled drain connector (PE# WE01-3119) can be ordered individually. One spare of each should be on hand.

8) Burgener Teflon Mira Mist® nebulizer (Mississauga, ON, Canada).

9) Burgener polyethylene capillary tubing for Mira Mist® nebulizers (pack of 10) part # BRC 203X (Mississauga, ON, Canada).

10) Glass Expansion Cinnabar Spray Chamber (for ELAN instruments), Part # 20-809-0162 (West Melbourne, Vic, Australia).

11) Peristaltic pump tubing for sample (0.03-in i.d., #0990-8587), and for waste (0.125-in i.d., #N812-2012): Keep at least 2 packages of twelve on hand of the sample tubing, and 1 package of 12 on hand of the waste tubing. Other suppliers may offer the same size /type of peristaltic tubing.

12) Nebulizer Capillary tubing (used to connect the nebulizer and the peristaltic pump tubing, #0990-8265, or any source of polyethylene tubing, 0.6-mm i.d. x 0.97-mm o.d): one pack (10 ft) on hand.

13) Autosampler probe (# B300-0161): one spare should be kept on hand.

14) Varian General Purpose (Type GP) pump oil, #K7516-302 (Lexington, MA), or similar, for the interface and roughing pumps.

15) Neslab chiller coolant (PE Sciex Coolant, #016558A): two 1-L bottles should be on hand.

f. Calculations

1) Calibration

The ELAN has two on-board computers that work with the external system computer. The computers interface with other electronic components within the system to convert the detector signals to digital ion intensity values. As standard solutions are analyzed, the software plots the measured intensity versus the concentration of each element in the standard solution. These individual calibration curves are updated as each subsequent standard is analyzed. Internal standards are added to the diluent (Rh, Ir and Y), these internal standards

provide a means to correct for changes in instrument response. The software uses the ratio of analyte and the internal standard intensities to determine the actual intensities for the analyte. Because the responses to instrumental effects/drifts for all the elements in a standard group are assumed to be similar to the response for the internal standard, the ratio of each element's intensity to the internal standard's intensity is used for each element. Correlation coefficients (R^2) for the calibration curves should be ≥ 0.999, alert the supervisor if the calibration curves do not meet this criteria.

2) Limit of Detection

The method detections limits (MDLs) for Pb, Cd, and Hg in blood specimens using a Burgener Mira Mist® nebulizer, and cinnabar spray chamber, are presented in Table 5. These data are based on three times the standard deviation of a base blood analyzed against a calibration curve for 10 (n=10) and 20 (n=20) separate runs. The limit of quantitation (LOQ), or concentration at which quantitative results can be reported with a high degree of confidence (10 times the standard deviation of the base blood for n=10, n=20 separate runs) is also presented. For comparison, the MDLs, and LOQs presented by Centers for Disease Control and Prevention' National Health and Nutrition Examination Survey's Third National Report [3].

Table 5. NY State Pb, Cd, Hg in Blood by ICP-MS method detection limits (Burgener Mira Mist® nebulizer).

	CDC MDL's NHANES Third National report [3]	*CDC LOQ's NHANES Third National report [3]*	**NYS MDL's 10 separate runs (n=10)**	NYS LOQ's 10 separate runs (n=10)	**NYS MDL's 20 separate runs (n=20)**	NYS LOQ's 20 separate runs (n=20)
Pb, µg/dL	*0.3*	*1.0*	**0.41**	1.4	**0.34**	1.1
Cd, µg/L	*0.3*	*1.0*	**0.18**	0.6	**0.19**	0.63
Hg, µg/L	*0.14*	*0.47*	**0.13**	0.43	**0.24**	0.80

9. QUALITY CONTROL (QC) PROCEDURES

The bench quality control pools used in this method comprise four levels of concentration spanning the low to high ranges (normal population levels) for the three analytes of interest. These four pools are analyzed after the calibration standards, but before any patient samples are analyzed so that judgments on the analyte calibration curves may be made prior to analysis of patient samples. One of these controls is then analyzed again after approximately each 10 patient samples, and then the controls are run at the end of each day's run.

When using the Burgener nebulizer and cinnabar spray chamber configuration, the method has shown proven stability for 21 hours (see supporting documentation in the 'Stability' section of this SOPM). However, if a patient runs exceed an 8-hour run time, closely monitor the within run QC materials. If a found value of one of the within run QC material is outside of the 2 SD range of the QC target value, it will be necessary to perform a recalibration.

Precision and Accuracy

Quality Control Results Evaluation: Consult the QC limits to determine if the run is in control. For each QC pool, the acceptable range is typically the mean ± 2 standard deviations of the results determined in an least 20 separate characterization runs ($\pm 2S_m$). The following modified Westgard QC rules apply to the average of the beginning and ending analyses of each of the QC pools:

a) If all QC results are within the $2S_m$ limits for a given QC pool, and the individual results are within $2S_i$ limits, then accept the run (S_w = within run standard deviation).

b) If one of the mean QC is outside the $2S_m$ limit, then reject the run if:
   1. Extreme Outlier – the run mean is beyond the characterization mean of $\pm 4S_m$.
   2. 1 3S Rule – Run mean is outside a $3S_m$ limit.
   3. 2 2S Rule – Run means are outside the same $2S_m$ limit.

c) If one of the 4 QC individual results is outside a $2S_i$ limit, then reject the run if: R 4S Rule – Within-run ranges for all pools in the same run exceed $4S_w$ (i.e., 95% of the range limit). The R 4S rule is applied to within run only.

Abbreviations: S_i = Standard deviation of individual results.
S_m = Standard deviation of the run means.
S_w = Within-run standard deviation.

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

If the QC rules are violated then the following steps should be taken.

1) Fresh working blood multi-element calibration standards should be prepared (see section 6.B.4), and the entire calibration curve be run using freshly prepared standards;

2) Fresh working blood multi-element QC samples should be prepared (see section 6.B.5), and re-analyzed.

If these two 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.

11. LIMITATIONS OF THE METHOD; INTERFERING SUBSTANCES AND CONDITIONS

A list of potential isobaric and polyatomic interferences for each target analyte are presented in Table 8. The correction equation used by the ELAN software to compensate for isobaric interferences on a given analyte are also listed in the table. Note that since the isotopic composition of lead varies in nature, the element must be measured as the sum of the four isotopes $^{204}Pb + {}^{206}Pb + {}^{207}Pb + {}^{208}Pb$.

12. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may reach and maintain ambient temperature during analysis – stringent precautions should be taken to avoid external contamination by the metals to be determined.

13. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

If the analytical system fails, then refrigerated storage (at 4 °C) is recommended until the analytical system is restored to functionality. If long-term interruption (greater than four weeks) is anticipated, then storage of blood specimens at ≤ - 20 °C is recommended. The DRC II ICP-MS instruments in D164 or D-134 may be used as back-up instruments to determine blood Cd, Hg and Pb by ICP-MS.

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

a. Quality Control Data

The reporting sheet has self-explanatory blanks for the means and ranges of duplicate determinations of QC pools. Put a copy of this form in the study folder(s).

b. Analytical Results

Reformat the data file by using the ELAN software, and then download the data file for calculation or reporting. Each of the sample concentrations calculated by the ELAN software needs to be corrected for a reagent blank value. Correction for the reagent blank, as well as arrangement of the data into a suitable comma separated variable (.csv) format, is performed by the BTE HANES Exporter software. A protocol for the operation of the BTE HANES Exporter software can be found in the Blood Metals SOPM binder located in D146 B.

c. Reference Ranges

Reference ranges for blood Pb, Hg, and Cd concentrations are calculated from (a) data published for New York City's Health and Nutrition Examination Survey (NYC HANES), 2004, (b) the Center for Disease Control and Prevention's Third National Report on Human Exposure to Environmental Chemicals, 2005, and (c) data regarded as generally acceptable from the literature.

Table 6a: NYC HANES data 2006

Reference	Concentration
A Biomonitoring Study of Lead, Cadmium and Mercury in the Blood of New York City Adults, McKelvey et al., 2006, [4].	Hg, 97.5th percentile = 15.4 µg/L Cd, 97.5th percentile = 2.5 µg/L Pb, 97.5th percentile = 6.3 µg/dL

Table 6b: CDC, NCEH – NHANES data 2005

Reference	Concentration
Third National Report on Human Exposure to Environmental Chemicals, 2005, [3].	95th percentile (95% confidence interval) Hg, = 4.60 µg/L (3.70 – 5.30), (*16-49 yr old females only)* Cd, = 1.30 µg/L (1.20 – 1.40) Pb, = 4.90 µg/dL (4.60 – 5.30)

Table 6c: Additional data from the literature

Reference	Concentration
Tietz Textbook of Clinical Chemistry, Edited by C.A. Burtis and E.R. Ashwood, 1999, [5].	Hg < 10 µg/L Pb < 10 µg/dL for children Pb < 30 µg/dL for adults Cd < 5 µg/L
Carson B.L., Ellis H.V., McCann J.L., Toxicology and Biological Monitoring of Metals in Humans, Lewis Publishers, 1986 [6].	Hg < 20 µg/L Pb = 20-35 µg/dL for a non-occupationally exposed population Pb = 60 – 70 µg/dL for male workers Cd < 10 µg/L for a non-occupationally exposed population.
Handbook on Metals in Clinical and Analytical Chemistry, edited by H.G. Seiler, A. Sigel, and H. Sigel, 1994 [7].	Hg < 10 µg/L Pb = 3.12 – 31.2 µg/dL Cd = 1-4 µg/L

d. Action Levels

The following table (Table 7) shows the Action Levels for blood metals analysis. Patient specimens that have a found trace element concentration above the Repeat and Critical Values, are highlighted in the BTE HANES Exporter software spreadsheets. For a repeat analysis the specimens are re-analyzed on a second day to confirm the initial result. Results that are still above the Critical Call value following a second days analysis should be reported immediately to the supervisor or study manager.

Table 7. Analyte Action Levels.

Analyte	Level at which Repeat Analysis is Performed	Critical Call Level µg/L	Reporting Standards to NY Heavy Metals Registry
Cd	≥ 4.0 µg/L	≥ 10L	> 10 µg/L
Pb	≥ 10.0 µg/dL (100 µg/L)	≥ 45 µg/dL	All BPb results
Hg	≥ 10.0 µg/L	≥ 100 µg/L	> 5 µg/L

A third analysis may be necessary if the results from first two analyses are not consistent. Typically the difference between the first two analyses should be within 4 standard deviations of the long-term precision of QC material of closest concentration to the two samples (i.e. within ± 2 standard deviations of the average of the two repeats). The long-term precision of the QC material is the reference range (± 2SD) used for assessment of the QC data. If the first and repeated results agree within 4 standard deviations of the QC, report the average of the two results. If the results do not agree within 4 standard deviations of the QC, a third analysis must be performed. If the third analysis agrees with either of the first two results within 4 standard deviations of the closest QC, report the average of those values, and reject the other value. If the third result doesn't agree with either of the initial results, within 4 standard deviations of the closest QC, do not report a value for that sample. Notify the supervisor, and investigate the cause of the discrepancy.

This ICP-MS method has been shown to be linear well beyond the top calibration point (see the 'Validation' section of the SOPM binder located in D 146B). Further

dilution of a blood specimen that is above the top standard is not appropriate as it may produce erroneous data. If an abnormally high level of a target analyte is detected in a specimen, that would be far higher than the highest QC material ensure that an archived control material, or archived PT material is analyzed along with the repeat of the specimen. An example of such a material would be NYS RM 056, which has an assigned target value of 225.6 µg/L Hg.

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

The analyst who receives specimen/samples delivered to the Trace Elements Lab sets up a "Specimen Accessioning Sheet." Fill out an accessioning sheet and place it in the folder to be given to the analyst performing the analysis. The accession sheet tracks location, status, and final disposition of the specimens. When sample analysis is completed, place the accession sheet in the *NYC HANES Trace Elements in Blood (Cd, Pb, Hg) Accessioning Sheets Log Book* located in the Trace-Elements Lab.

All electronic records must be stored on the TREL server in the appropriate directory to ensure security and backup are maintained. Maintain all records indefinitely. Include related Quality Assurance (QA)/QC data keep duplicate records (off site, if sensitive or critical) in electronic or hardcopy format. Use only numerical identifiers (e.g. case ID numbers). Any personal identifiers and/or demographics are available only to the medical supervisor and/or project coordinator, lab director, bench supervisor and analyst to safeguard confidentiality.

References:

1. Palmer C.D., Lewis M.E. Jr., Geraghty C.M., Barbosa F.,Jr., Parsons P.J., *"Determination of lead, cadmium and mercury in blood for assessment of environmental exposure: A comparison between inductively coupled plasma-mass spectrometry and atomic absorption spectrometry"*, Spec. Acta B, **61**, 2006, 980 – 990.
2. Centers for Disease Control and Prevention, Emergency Response and Air Toxicants Branch, Division of Laboratory Services Laboratory Protocol Method No. CTL-TMS-3.01 "Mercury, Lead, Cadmium in Whole Blood, by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)", adopted May 30, 2006.
3. Centers for Disease Control and Prevention, National Center for Environmental Health *"Third National Report on Human Exposure to Environmental Chemicals"*, NCEH Pub. No. 05-0570, July 2005.
4. McKelvey W., Gwynn R.C., Jeffrey N., Kass D., Thorpe L.E., Garg R.K., Palmer C.D., Parsons P.J., "A Biomonitoring Study of Lead, Cadmium and Mercury in the Blood of New York City Adults", Environmental Health Perspectives, 115(10), 2007, 1435-1441.
5. Tietz Textbook of Clinical Chemistry, Third Edition, edited by Burtis C.A., Ashwood E.R., 1999.
6. Carson B.L. Ellis H.V., McCann J.L., Toxicology and Biological Monitoring of Metals in Humans, Lewis Publishers, 1986.
7. Handbook of Toxicity of Inorganic Compounds, edited by Sieler, H.G., Sigel, H., Sigel A., Marcel Dekker Inc., 1988.

Appendix

Table 8: Potential interferences on analytes of interest, and correction equation applied. The Internal Standard used for each analyte is also given.

Analyte	Mass (amu)	Internal Standard	Correction	Potential Interference
Cd	114	Rh	-0.026826 x Sn 118	Sn. MoO
Hg	202	Ir (Y)	None	WO
Pb	Sum of 204+206 +207+208	Ir	For Pb 204 only; -0.230074 x Hg 202	Pb 204 only; Hg, WO
*Rh**	*103*	-	*None*	*SrO*
*Y**	*89*	-	*None*	*None*
*Ir**	*193*	-	*None*	*HfO, LuO*

* = *internal standards*