# Laboratory Procedure Manual

| | |
|---|---|
| *Analyte:* | **Cadmium and Lead** |
| *Matrix:* | **Blood** |
| *Method:* | **Atomic Absorption Spectroscopy** |
| *Method No.:* | **1090A/02-OD** |
| *Revised:* | **August 22, 2001** |
| *as performed by:* | *Nutritional Biochemistry Branch*<br>*Division of Laboratory Sciences*<br>*National Center for Environmental Health, CDC* |
| *Contact:* | *Dr. Robert L. Jones*<br>*Division of Laboratory Sciences* |

**Important Information for Users**

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

## Public Release Data Set Information

This document details the Lab Protocol for NHANES 1999–2000 data.

A tabular list of the released analytes follows:

| Lab Number | Analyte | SAS Label |
|---|---|---|
| lab06 | LBXBCD | Cadmium (µg/L) |
| | LBDBCDSI | Cadmium (nmol/L) |
| | LBXBPB | Lead (µg/dL) |
| | LBDBPBSI | Lead (µmol/L) |

1. Clinical Relevance and Summary of Test Principle

Cadmium and lead analyses are performed to identify cases of either cadmium toxicity or lead toxicity. Occupational exposure is the most common cause of elevated cadmium levels. Occupational and residential exposures to lead are the most common causes of elevated blood lead levels (1).

Cadmium and lead can be measured simultaneously in blood by adapting the methods recommended by Miller et al. (2), Darsons et al. (3), and Stoeppler et al. (4).

Cadmium and lead quantification is based on the measurement of light absorbed at 228.8 nm and 283.3 nm, respectively, by ground-state atoms of cadmium and lead from either an electrodeless discharge lamp (EDL) or by a hollow cathode lamp (HCL) source. Human (patient or study) blood samples, bovine blood quality-control (QC) pools, and aqueous standards are diluted with a matrix modifier (nitric acid, Triton X-100, and ammonium phosphate). The cadmium and lead contents are determined on a PerkinElmer Model SIMAA 6000 simultaneous multi-element atomic absorption spectrometer with Zeeman background correction.

Cadmium and lead contamination must be carefully avoided throughout all procedures. All materials used to collect, store, and process specimens need to be screened for possible cadmium and lead contamination. All processing work must be performed under clean conditions, which includes using laminar-flow hoods.

2. Safety Precautions

Use Universal Precautions when handling blood products. These precautions include wearing gloves, a lab coat, and safety glasses. The hepatitis B vaccination series is recommended for all analysts working with blood or serum samples. Place disposable plastic, glass, and paper that contact blood (e.g., pipette tips, autosampler cups, gloves, etc.) in a biohazard autoclave bag. Keep these bags in appropriate containers until they are sealed and autoclaved. Wipe down all work surfaces with 10% sodium hypochlorite solution when work is finished. Use of the foot pedal on the Micromedic Digiflex reduces analyst contact with work surfaces that have been exposed to blood. The foot petal also keeps the analyst's hands free to hold the specimen vials and autosampler cups and to wipe off the tip of Micromedic Digiflex.

*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.*

*Exercise caution when handling and dispensing concentrated nitric acid. Always remember to add acid to water. Nitric acid is a caustic chemical capable of severe eye and skin damage. Wear metal-free gloves, a lab coat, and safety glasses.*
*If nitric acid comes in contact with any part of the body, quickly wash the exposed area with copious quantities of water for at least 15 minutes.*

Material safety data sheets (MSDSs) for nitric acid, Triton X-100, ammonium phosphate, metal standards, and argon are available through the DLS computer network.

3. Computerization; Data System Management

A. Maintain integrity of specimen and analytical data by proofreading all transcribed data and storing data in multiple computer systems. Store data files containing the date, analytical run identification (ID), specimen analytical results by specimen ID, and method code in a personal computer (PC) database

environment. Archive data from the PerkinElmer Model SIMAA 6000 graphite furnace instrument on 3.5” high-density discs. Use Microsoft Access, with a custom-written file system to track specimens.

B. Routine backup procedures include weekly backup of hard discs, archival on 3.5" floppy discs, and archival on CD-R optical disc. Store off site those floppy (or optical) discs that contain sensitive data. Contact either the supervisor or local area network manager for emergency assistance.

C. Accomplish statistical evaluation and calculation of the run with a "linear" calibration curve used by the PerkinElmer Model SIMAA 6000 software. After the data is calculated and the reviewing supervisor approves the release of the final values, the data entry clerk should transcribe the results into the database, which is located in Microsoft Access on the NCEH/DLS PC network. The supervisor and clerk should then proof data entry.

D. Files stored on the local PC network will automatically be backed up to tape each night by DLS LAN support staff and CDC data center staff, respectively.

E. Documentation for system maintenance is available in hard copy as well as in system log files on the local hard drives that are used to archive data.

4. Procedures for Collecting, Storing, and Handling Specimens; Criteria for Specimen Rejection

A. No special instructions for fasting or special diets are required.

B. The specimen type is blood with anticoagulant, preferably K3EDTA at 1.5 mg/mL blood.

C. The optimal amount of specimen required is 2-3 mL in an unopened collection tube; however, the minimum is about 500 µL (0.5 mL).

D. Acceptable containers include 2- to 7-mL vacuum tubes (e.g., lavender-top Vacutainers) and plastic (metal-free) syringes with plastic caps. Recommended anticoagulants are either K3EDTA or Na2EDTA (1.5 mg/mL). EDTA is preferred over heparin because heparin tends to allow the formation of microclots. Use heparinized blood, if necessary. Use sterile collection systems for specimen acquisition.

E. Specimen stability has been demonstrated for 1 year at both 4°C and –20°C.

F. The criteria for unacceptable specimen are either a low volume (< 0.5 mL) or suspected contamination due to improper collection procedures or collection devices. Clotted specimens are not acceptable. In all cases, request a second blood specimen.

G. Specimen characteristics that may compromise test results are indicated above and include clotted blood.

H. The division protocol for blood collection and handling (copies available in the branch laboratory and special activities specimen-handling office) outlines specimen-handling conditions. Collection, transport, and special equipment required are discussed. In general, transport and store blood specimens at 4°C. Upon receipt, freeze the specimens at –20°C until time for analysis. Refreeze at –20°C portions of the sample that remain after analytical aliquots are withdrawn. Samples thawed and refrozen several times are not compromised unless inadvertent contamination occurs due to improper handling. If there is more than one analyte of interest in the specimen and it needs to be divided, transfer the appropriate amount of blood into a sterile polypropylene cryovial labeled with the participant's ID.

I. It is important to screen each lot of collection tubes and shipping and storage containers for lead contamination prior to use.

5. Procedures for Microscopic Examinations; Criteria for Rejecting Inadequately Prepared Slides

Not applicable for this procedure.

6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation

A. Reagent Preparation

Matrix Modifier (0.2% [v/v] nitric acid, 0.5% [v/v] Triton X-100, and 0.2% [w/v] ammonium phosphate). Using micropipettes, dilute 2 mL redistilled concentrated nitric acid, and 5,000 µL (5.0 mL) of Triton X-100 in approximately 750 mL18 MΩ ultra-pure water in an acid-cleaned 1,000-mL volumetric flask. Weigh out 2.0 g of dibasic ammonium phosphate and add it to the flask by washing down the weighing boat with ultra-pure water delivered from a wash bottle. Add a magnetic stirring bar and stir the solution on a stirring plate until the Triton X-100 has dissolved. Remove the stirring bar and bring the solution to volume with ultra-pure water. After preparation, this solution should be checked for contamination at the beginning of each analytical run and discarded if absorbance greater than 0.005 Abs/sec is observed. Store at room temperature and prepare as needed in a flask dedicated to this solution.

B. Standards Preparation

(1) 1,000-mg/L stock cadmium standard

Dilute 1 mL National Institute of Standards Technology (NIST) Standard Reference Manual (SRM) 3108 (or 2121-1) delivered by a class-A volumetric pipette (or the Micromedic Digiflex) to 10 mL with ultra-pure water in an acid-cleaned volumetric flask. Store at room temperature and prepare every 6 months in a flask dedicated to this solution.

(2) 10-mg/L stock cadmium standard

Using either a micropipette or the Micromedic Digiflex, dilute 1 mL of the 1,000- mg/L stock cadmium standard to 100 mL with 18 MΩ ultra-pure water in an acid-cleaned volumetric flask. Store at room temperature and prepare monthly in a flask dedicated to this solution.

(3) 1-mg/L intermediate cadmium standard

Using either a micropipette or the Micromedic Digiflex, dilute 1 mL of the 10-mg/L stock cadmium standard to 10 mL with 18 MΩ ultra-pure water in an acid-cleaned volumetric flask. Store at room temperature and prepare daily in a flask dedicated to this solution.

(4) 1,000-mg/L stock lead standard

If using NIST SRM 3128 (or 2121-2), dilute 1 mL (delivered by either a class-A volumetric pipette or the Micromedic Digiflex) to 10 mL with 18 MΩ ultra-pure water in an acid-cleaned volumetric flask. Store at room temperature and prepare every 6 months in a flask dedicated to this solution.

(5) 10-mg/L intermediate lead standard

Using either a class-A volumetric pipette or the Micromedic Digiflex, dilute 1 mL of the 1,000-mg/L stock lead standard to 100 mL with 18 MΩ ultra-pure water in an acid-cleaned volumetric flask. Store at room temperature and prepare monthly in a flask dedicated to this solution.

(6) Working Cadmium and Lead standards

Using the Micromedic Digiflex, transfer the following volumes of the 1-mg/L cadmium intermediate standard and the 10-mg/L lead intermediate standard to 10-mL acid-cleaned volumetric flasks and dilute to volume with ultra-pure water:

| Intermediate Stock (µL) | | Standard Concentration (µg/L) | | Sample Concentration (µg/L) | |
|---|---|---|---|---|---|
| 1 ppm Cd | 10 ppm Pb | Cd | Pb | Cd | Pb |
| 20 | 200 | 20 | 200 | 2 | 20 |
| 60 | 500 | 60 | 500 | 6 | 50 |

Store at room temperature and prepare daily in flasks dedicated to these solutions.

(7) Calibration Standards:

(a) Using the Micromedic Digiflex, dispense 100 µL of ultra pure water followed by 900 µL of matrix modifier into an autosampler cup for use as a process blank. Place this cup in position 1 and 73 on the AS-72 autosampler tray for the PerkinElmer SIMAA 6000.

(b) Using the Micromedic Digiflex, dispense 100 µL of each of the standards and 900 µL of the matrix modifier into the autosampler cups. Standards should be in positions 74 (2/20 µg/L) and 75 (6/50 µg/L).

(c) Pipette 100 µL of blood and aqueous QC samples into autosampler cups, followed by 900-µL matrix modifier.

(d) Place the QC samples in positions 2, 3, and 4. If a fourth QC sample is used, place the QC sample in position 5.

C. Preparation of Quality-Control (QC) Materials

Evaluate levels of lead and cadmium in bovine blood QC materials. The blood was drawn from two animals: one had not been lead-dosed (oral lead nitrate or acetate), one had been recently dosed with lead nitrate to obtain a final blood lead level of 20-30 µg/dL. One liter of blood was drawn from each animal into evacuated bottles containing EDTA as coagulant. The elevated blood lead pool (ca 20 µg/dl) was spiked with aqueous cadmium nitrate standard (SRM 3108) to a final concentration of 5 µg/L. The two pools were mixed using a magnetic stirrer for at least one hour in an acid-cleaned flask.

Using a sterile technique under a laminar-flow hood, dispense the blood by using an automatic dispenser into 3-mL cryovials previously screened for lead and cadmium. Dispense the pools in 2-mL aliquots, screw cap, and label. Freeze the pools at –20°C. Twenty vials of each level were randomly selected for characterization of the QC limits and for testing of homogeneity.

D. Other Materials

(1) Stock solution of lead: NIST SRM 2121-2 or 3128, 10,000 mg/L (National Institute of Standards and Technology, Gaithersburg, MD).

(2) Stock solution of cadmium: NIST SRM 2121-1 or SRM 3108, 10,000 mg/L (National Institute of Standards and Technology, Gaithersburg, MD).

(3) Redistilled concentrated nitric acid (G. Frederick Smith Chemical Co., Columbus, OH).

(4) Triton X-100 (Fisher Scientific, Fairlawn, NJ)

(5) Ammonium phosphate, dibasic ("Baker Analyzed," J.T. Baker Chemical Co., or any source whose product is low in lead and cadmium contamination).

(6) Ultra-pure 18 MΩ water (from the Milli-Q Water Purification System).

(7) Argon, 99.996% purity (supplied as a compressed gas by Holox or other supplier) equipped with approved gas regulator (Matheson Gas Products, Secaucus, NJ).

(8) NIST SRM 966 (two levels) trace elements in bovine blood (National Institute of Standards and Technology). Run these periodically to verify accuracy.

(9) Bovine and human blood QC pools with low and high levels of lead, that has reference values established by external laboratory or isotope dilution mass spectrometry (IDMS).

(10) Pyrolytic graphite tubes, solid pyrolytic graphite L'vov platforms, insertion and alignment tools, and graphite contact rings (PerkinElmer, Norwalk, CT).

(11) Small plastic weighing boats (Scientific Products, McGaw Park, IL).

(12) Metal-free pipette tips: 1-100 µL and 1-1,000 µL sizes each (Rainin Instrument Co., Inc., Woburn, MA).

(13) Acid-cleaned volumetric flasks (1,000-, 100-, and 10-mL volumes). Rinse the glassware in 1% nitric acid or soak for 24 hours in 1% nitric acid, then rinse with 18 MΩ ultra-pure water and dry under clean conditions.

(14) Conical-bottom 2-mL polystyrene autosampler cups (Lancer, St. Louis, MO).

(15) Kay-Dry paper towels and Kim-Wipe tissues (Kimberly-Clark Corp., Roswell, GA).

(16) Cotton swabs (Hardwood Products Co., Guilford, ME).

(17) Dehydrated alcohol, USP (Midwest Grain Products of Illinois, Pekin, IL).

(18) Cadmium and lead free N-DEX nitrile examination gloves (Best Manufacturing Co., Menlo, GA) or equivalent.

(19) Biohazard autoclave bags (Curtin-Matheson Scientific, Inc., Atlanta, GA).

(20) Bleach (10% sodium hypochlorite solution), any vendor.

(21) Dental mirror (PerkinElmer Norwalk, CT).

E. Instrumentation

(1) PerkinElmer (Norwalk, CT) model SIMAA 6000 multielement atomic absorption spectrometer, including THGA 600/700 furnace, cadmium-source lamp, lead- source lamp, EDL power supply (if EDL sources are to be used), AS-72 autosampler, and Hewlett-Packard LaserJet printer, and ultraviolet starter source.

| **Parameter** | **Setting** | |
|---|---|---|
| | **Cd** | **Pb** |
| Wavelength | 228.8 nm | 283.3 nm |
| HCL current* | 6 mA | 10 mA |
| EDL power supply* | 6 W | 10 W |
| EDL mode | Continuous | Continuous |
| Slit | 0.7 nm (low) | 0.7 nm (low) |
| Signal mode | Peak area | Peak area |

*Only one source (HCL or EDL) is used.

| Furnace Temperature Program - Furnace | | | | |
|---|---|---|---|---|
| **Step** | **Temp °C** | **Ramp (sec)** | **Hold (sec)** | **Gas Flow mL/min** |
| Dry* | 130 | 5 | 20 | 250 |
| Dry* | 200 | 5 | 30 | 250 |
| Char | 400 | 1 | 35 | 250 |
| Atomize | 1700 | 0 | 5 | 0 |
| Burnout | 2450 | 1 | 3 | 250 |
| Baseline: -1 sec (BOC set automatically) | | | | |
| Read: 0 sec | | | | |

** Dry temperature and hold time may vary with different graphite tube and platform combinations.*

(2) Micromedic Digiflex Automatic pipette equipped with 2,000-µL dispensing syringe, 2,000-µL and 200-µL sampling syringes, 0.75-mm tip, and the foot pedal (Micromedic Division, ICN Biomedical, Horsham, PA).

(3) Mettler PL 200 top-loading balance (Mettler Instrument Corp., Hightstown, NJ).

(4) Milli-Q Water Purification System (Millipore Corporation, Bedford, MA).

(5) Vortex-Genie vortex mixer (Fisher Scientific, Atlanta, GA).

(6) Eppendorf fixed-volume micropipettes: 1,000-, 250-, 100-, and 50-µL volumes (Brinkmann Instruments, Inc., Westbury, NY) or equivalent.

(7) Magnetic stirrer (Corning Glass Works, Corning, NY) and stirring bars (Fisher Scientific).

7. Calibration and Calibration Verification Procedures

A. Construct a calibration curve for cadmium and lead by using the measured mean values of integrated absorbance (absorbance-seconds, or Abs-sec, or A.s) of standards at 0, 0.5, 1.0, 2.0, 3.0, and 6.0 µg/dL and 0, 5, 10, 20, 25, and 50 µg/dL for cadmium and lead, respectively, plotted versus concentration. The "linear" or “nonlinear” calibration curve option can be chosen in the CALIBRATION window of the AA WinLab software.

B. After performing calibration, calculate the characteristic mass by using the "Edit Calibration" function. Acceptable characteristic masses are between 24.0 and 36.0 pg/0.0044 A.s for the ZL instrument.

C. Click on the “Calib” icon on the toolbar. Calibration curves should be displayed in the "CALIBRATION" window, to verify the mathematical fit and to evaluate the slope and intercept. Slopes should be > 0.0050 A.s/µg/dL for cadmium and > 0.002 A.s/µg/dL for lead. (See Section 8.f.)

D. Verify calibration by using NIST SRMs (SRM 966 Levels 1 and 2 for blood cadmium and lead and SRM 955b for lead calibration curve verification). Run these materials once per month.

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 (Section 8.f.)

(2) Allow frozen blood specimens, QC specimens, and blood calibration material to reach ambient temperature and mix on a vortex mixer for 10 seconds.

(3) While the specimens are thawing, rinse enough autosampler cups for an analytical run with 18 MΩ ultra-pure water delivered from a wash bottle. Drain the cups upside down on Kay-Dry paper towels.

(4) Prime the Micromedic Digiflex pumps with the matrix modifier solution.

(5) Wipe the tip of the Micromedic Digiflex with cadmium- and lead-free laboratory tissue after inserting it into any blood specimen.

B. Sample Preparation

(1) Use the Micromedic Digiflex to dilute the specimens and controls tenfold (1:10) with the matrix modifier solution into clean autosampler cups. Use 100 µL of specimen and 900 µL of matrix modifier in the following procedure: pull up 100 µL of blood into the tip, wipe the tip with a Kim-Wipe tissue, and dispense the blood and 900 µL of matrix modifier into a cup. Examine the tip and release any trapped air bubbles before dispensing into the autosampler cups. To do this, remove the tip from its clamp and hold the tip end up to release air. If air becomes trapped in a blood sample in the tip, dispense it into the waste beaker and take another sample.

(2) Place the autosampler cups containing the specimens in positions 6-70 with an additional blank (100 µL of water and 900 µL of matrix modifier) in position 1.

(3) Place an autosampler cup on the tray as a sample in position 76 and fill to the mark with 18 MΩ ultra-pure water as used in the preparation of the run to maintain the humidity in the AS-72 tray.

C. Instrument Setup for the SIMAA 6000 and the AS-72

(1) Turn on the argon gas tank and verify that the tank pressure is ≈100 psi; the outlet pressure should be 40-50psi.

(2) Turn on the following in the order presented: PerkinElmer Model SIMAA 6000, computer, monitor, and printer.

(3) Verify that the water level in the cooling system is at maximum.

(4) The PerkinElmer Model SIMAA 6000 AA WinLab software controls the AA automatically. On boot-up, the software loads the operating system (Windows 95) under which the AA control program runs and opens to the PerkinElmer Model SIMAA 6000 window. Using the mouse, double click on the “AA WinLab Analyst” icon in the PerkinElmer Model SIMAA 6000 window. The system will check for the furnace, initiate the optical system, and rinse the autosampler. When finished, the MULTI-ELEMENT AA WinLab window will be open and the dialogue box of the PerkinElmer AA WinLab software will be displayed at the bottom of the screen. This box has choices of “Select Workspace,” ”Technique,” “Tutorials and References,” and “Menus and Toolbars.” The options in this dialogue box are available through the Multielement AA WinLab toolbar. For more information, see Chapter 5, “Performing Analyses” in the PerkinElmer Model SIMAA 6000 Setting Up and Performing Analyses guidebook (P/N 0993-5219).

(5) To check the condition of the furnace tube, go to the Multielement AA WinLab window, click on “Tools,” then “Diagnostics,” and select the “Furnace” tab at the bottom-right portion of the screen. The “Furnace Diagnostics” dialogue box appears, the “Tube Cycle” and “Cylinder Cycle” tabs appear at the bottom of the screen, and the “Reset Button” can be used to set the counts to 0 if a new furnace tube will be installed.

(6) Note the number of firings performed with the current tube. If the number of firings is > 400, change the tube-and-platform combination (see pp. 1-10 to 1-12 in Setting Up and Performing Analyses P/N 0993-5219).

(a) Unscrew the autosampler support door lock in the front of the SIMAA and swing away from the furnace. Click the “Furnace” icon on the menu bar. Click the “Open/Close” button to unlock the furnace in the FURNACE CONTROL window. Swing the furnace lock to the left. Pull the forward tube support down.

(b) Remove the graphite tube-and-platform and inspect the condition. Discard if it is visibly pitted or if is contains noticeable deposits.

(c) Clean the furnace before inserting the new tube. Wipe the graphite contact cylinders with methanol and a Kim-Wipe. Use a cotton-tipped stick to clean the hole in the rear graphite cylinder. Stubborn deposits may be removed with 1% (v/v) HNO3. Follow instructions in the User's Manual to replace cylinders, if they are worn or pitted. Worn contact cylinders will result in skewed peaks and poor precision.

(d) Note: Do not touch the graphite tube with your fingers. Handle with plastic tweezers, wood grips, or a Kim-Wipe and orient the graphite tube so that the injection port is on top. The side of the tube where the platform is attached is on the left towards the rear of the tube. Place the graphite tube in the rear graphite cylinder.

(e) Swing the forward tube support up. Swing the furnace lock to the right under the forward tube support. Click the "Open/Close" button in the FURNACE CONTROL window to close the furnace.

(f) Swing the autosampler support door back into position and screw the lock closed.

(g) Click on "Condition Tube" in the FURNACE window to condition the tube. When the conditioning program concludes, close the furnace window.

(7) Double click on the "Lamps" icon on the menu bar to activate the MULTIELEMENT AUTOMATED ANALYSIS window.

(8) If the cadmium and lead lamps are installed in the turret, the instrument will automatically recognize the lamps and their respective positions.

(9) Check the reservoir of the autosampler's and the waste container. If necessary, empty the latter and fill the former with approximately 20 mL of matrix modifier dissolved in 2 L of 18 MΩ ultra-pure water. Fill the blank container at position 71 with fresh 18 MΩ ultra-pure. Install the carousel with dustcover in the autosampler.

(10) Follow the procedures specified in the "Autosampler Alignment" (See Section pp. 1-14 to 1-21 of the Setting Up and Performing Analyses guidebook P/N 0993-5219).

NOTE: Do not attempt to move the AS-72 arm manually without first disabling the software control via the "AUTOMATED ANALYSIS" or "FURNACE CONTROL" windows.

(a) Click on the "AUTOMATED ANALYSIS" window to make it active. Click on the button marked "Align Tip." The "Autosampler Control Dialogue" box will open and will be displayed on the screen. Click on "Unlocked" and the autosampler arm will rise to permit manual rotation.

(b) Examine the tip of the capillary tubing. If it is bent or discolored, prepare a fresh one. Use a pair plier to pull more tubing through the sleeve. Measure 6-7 mm beyond the plastic sleeve and cut with a razor blade held vertically to produce a 45° angle. Note: If the tubing is cut too short, the sleeve will hit the graphite tube.

(c) Use the knob on the left-hand side of the autosampler control box to manually swing the arm through its normal cycle. With a flashlight or other local light source illuminating the furnace opening, check to ensure that the tip of the capillary tube enters the graphite tube without touching the wall. If the capillary tube does not enter, adjust the in/out (forward/backward) or left/right control knobs.

(d) Now that the tip enters the tube, adjust the depth. Swing down the mirror and backstop. Rotate the arm to its deepest penetration. Turn the depth-adjustment knob (the one farther from the furnace) until the angled point of the tip is just above the platform. Recheck the alignment and click on "Unlocked" again to lock the autosampler into place. The autosampler will reset and rinse the autosampler.

(e) Click on “Align in Tube” and the autosampler will cycle through and place the tip into the tube. Recheck the alignment and click on “Align in Tube” again to remove the autosampler from the tube and return the autosampler to the rinse location. The autosampler will reset and rinse the autosampler tip. Swing the mirror and backstop up.

(f) Check the proper alignment of the autosampler: In the bottom of the AUTOMATED ANALYSIS window, click on “Select Location,” click in the dialogue box that appears, enter “37,” and click “OK” to run a blank. Wait a few seconds for the lamp to peak, and then swing the mirror down. Carefully observe the sample deposition. This is critical for unattended operation. The tip must deposit the entire sample onto the platform. If the tip is too high in the tube, the sample will creep up the tip and crawl onto the wall. Realign and recheck. Do not continue until the sample is deposited onto the platform and only onto the platform.

(11) When the alignment is correct, check the sample for proper drying. The sample must dry evenly, without boiling or sputtering. This is especially important when a new tube is installed. Check the drying cycle first with a blank (matrix modifier) and then with a diluted blood sample. If sample is not dry before the start of step 3 (Char), increase the drying temperature by 10°C and re-check. If the sample sputters during step 1 or 2 (Dry), decrease the drying temperature by 10°C and recheck. The optimum drying parameters are critical for accurate and precise operation. Do not continue until the sample dries evenly. If you cannot get the sample to dry in a controlled manner, call the supervisor.

D. Instrument Operation

(1) In the AUTOMATED ANALYSIS window, click on the box marked “Calibrate.” The software will run the blank and each standard in duplicate. If the blank's absorbance is > 0.005 for cadmium and > 0.003 for lead, stop the run and call the supervisor.

(2) When standards finish running, the calibration results will print. Notify the supervisor if the correlation coefficient is < 0.995, or if the slope is outside the range 0.005-0.0062 (*0.0039-0.0048) for cadmium or 0.002–0.003 for lead using the Electrodeless Discharge Lamp (EDL) lamp source *For Hollow Cathode Lamp (HCL).

(3) After the calibration curve is generated, prepare the sample information file. First, call up the “Sample Information” file by clicking on “File,” “Open,” and “Sample Info File.” The OPEN window will be displayed; scroll down to select a sample information file from the available *.SIF list. Double click on the file to open. The SAMPLE INFORMATION EDITOR window will open and changes can be made to the file. Enter the sample IDs for the corresponding samples in the same number position in the autosampler tray. Finally, select “Save As” from the “File” menu to name the “Sample Information” file (.SIF) file and use the following standard convention: Enter the date as mm/dd/yy plus a letter (A, B, C, etc.) if two or more carrousels are set up for that day, e.g., 020398A for tray #1 of February 3, 2002. Make a separate “Sample Information” file for each carousel. Each carousel holds up to 65 samples in autosampler positions 6 through 70.

(4) Proceed with the analysis by clicking on “ Analyze Samples.” Monitor the first run of reference samples to ensure that the results are in the acceptable range. If they are not, notify the supervisor.

(5) While the PerkinElmer SIMAA 6000 is operating, check each run at least every 30 minutes for problems, such as the following:

(a) Error messages on the screen, e.g., "Timeout Error," "Printer not on," "Gas Pressure Is Low."

(b) Premature completion, if recalibration does not correct an out-of-range reference.

(c) Jammed printer.

(d) Crooked or melted tip.

Stop the run manually at any time during a run by clicking on the highlighted button in the AUTOMATED ANALYSIS window. A dialogue box will appear, giving you the option to stop immediately (default), stop after current sample replicate, or stop after replicates of the current sample. Press, “Return to stop immediately or click to select the appropriate choice.

(6) When a run finishes, all the terms of the buttons in the Automated Analysis window will change from gray to blue, and the “Analyze Samples” button will be no longer highlighted in green. If a second carousel is to be run, open the appropriate “Sample Information” file, place the carousel in the autosampler, and execute “Analyze Samples” again.

(7) When all analyses are complete, click on the “Lamps” icon and turn off each lamp by clicking the green highlighted button. Next, click on “File” and select “Exit”, then turn off the gas and instrument. Close all windows and exit from the Windows™ software before turning off the computer and printer.

(8) Enter results for each reference material in the PC database files for plotting. For each level, enter the duplicate concentration values. Notify the supervisor of any unusual trends or biases. Record the number of cycles for the current tube on the PerkinElmer Model SIMAA 6000 Routine Maintenance sheet. Make a record of the slope of the standard line and the lamp energy (available from the ALIGN LAMPS window). Enter any other maintenance items or problems in the instrument log.

E. Replacement and Periodic Maintenance of Key Components

(1) Source lamps: A spare source lamp of each analyte should be available. Order another lamp if the spare is used for replacement.

(2) Printer cartridges: A supply of Desk-Jet printer cartridges should be on hand. Order more when the last one is installed.

(3) Graphite contact rings: Replace the graphite contact rings of the furnace housing approximately every 6 months. An apparent loss of temperature control or sounding of the alarm by the instrument indicates a problem with the tube and the need for replacement. Maintain a logbook to keep track of when replacements occur. Always keep at least one spare set of graphite contact rings on hand.

(4) Reposition and trim the sampling tip of the autosampler every few weeks, depending on how the dispensing is proceeding.

F. Calculations

(1) The method described here has upper calibration limits of 50 µg/dL for lead (1) and 6 µg/L for cadmium. Use the "linear" or “nonlinear” zero-intercept calibration curve from the AA WinLab software to calculate the specimen concentrations. The program generates slopes, intercepts, correlation coefficients, and plotted and fitted curves. The correlation coefficient, r2, for each curve should be 0.995 or greater. For optimum sensitivity, slopes should be more than 0.0050 for cadmium and more than 0.002 for lead.

(2) Repeat a specimen analysis when duplicate integrations differ by more than 1.0 µg/dL for lead and 1.0 µg/L for Cadmium. Reanalyze specimens containing more than 10–40 µg/dL lead for confirmation based on the requirements of the study. When reanalyzing any specimen with a concentration greater than 50 µg/dL of lead or 6 µg/L of cadmium, prepare a new specimen by

diluting it twenty fold (1+19) rather than tenfold (1+9). The results from the 1:20 dilution must then be multiplied by 2 to account for this higher dilution. (The software normally calculates a 1:10 dilution.)

(3) The detection limit, based on 3 times the standard deviation (SD) of 10 repeat measurements of a sample with low-lead concentration, is 0.2 µg/L for cadmium and is 0.03 µmol/L (or 0.6 µg/dL) for lead (1,2). Results below the detection limit are reported as nondetectable ("ND"; refer to Section 8.e., “Recording of Data,” in this document).

G. Special Procedure Notes – CDC Modifications

Use a Micromedic Digiflex automatic pipette for many of the dispensing needs in this method because it is less tedious for large numbers of samples and because it provides better precision and accuracy than micropipettes. The Micromedic Digiflex automatic pipette also helps prevent common sources of contamination in the sample preparation steps.

9. Reportable Range of Results

A. Cadmium

Blood cadmium results are reportable in a range from 0.01 µg/L to 6.0 µg/L without dilution. Dilute, reanalyze, and calculate samples with a cadmium level > 6.0 µg/L as described in the calculations section of this document. Samples with a cadmium level below 0.01 µg/L are below the detection limit and are reported as ≤0.01 µg/L (ND).

B. Lead

Blood lead values are reportable in the range lower detection limit (LDL) < PbB < 50 µg/dL without dilution, where LDL= the calculated (3 SD) lower detection limit. If a blood lead value is less than 0.6 µg/dL (the approximate LDL of this method), report it as ≤ 0.6 µg/dL; if greater than 50 µg/dL, dilute the specimen (1+19) and reanalyze.

10. Quality-Control (QC) Procedures

The method described in this protocol contains slight modifications to well established methods for BCd and BPb that have been used for several years in the Nutritional Biochemistry Branch for environmental and occupational health studies. This multielement method is as accurate, precise, and reliable as the previous single-analyte methods. The primary standard used is a NIST SRM, and NIST SRM blood materials are used for external calibration. Estimates of imprecision can be generated from long-term QC pool results.

This analytical method uses two types of QC systems. With one type of QC system, the analyst inserts bench specimens two times in each analytical run (a set of consecutive assays performed without interruption) so that judgments may be made on the day of analysis. With the other type of QC system, “blind" QC samples are placed in vials, labeled, and processed so that they are indistinguishable from the subject samples. The supervisor decodes and reviews the results of the blind specimens. With both systems, taking these samples through the complete analytical process assesses all levels of cadmium and lead concentration. The data from these materials are then used to estimate methodological imprecision and to assess the magnitude of any time-associated trends.

The bench QC pools comprise three to four levels of concentration spanning the "low-normal," "normal," and “high” ranges (at the CDC cutoff for cadmium and lead exposure in adults) for lead. Reference materials (blood products with certified values assigned by independent reference methods) are used periodically to check for accuracy. Analyze NIST SRM 966 (levels 1 and 2) once a week to once a month based on the requirements of the study for this purpose. If the stock of these materials becomes low, order another in time to analyze it concurrently with the QC materials currently in use so that a bridge may be

formed between the materials. If the material ordered from NIST is from the same lot, a full characterization is not necessary. However, there should be some overlap between the old and new stocks.

Establish QC limits for each pool. Perform an analysis of variance for each pool after performing 20 characterization runs in which previously characterized NIST SRM and bench QC pools are used for evaluation. In addition to providing QC limits, the characterization runs also serve to establish homogeneity of the pools. After the homogeneity of the bench and blind materials has been established, analyze them by another independent reference method, e.g., (IDMS).

A. Precision and Accuracy: QC Results Evaluation. After completing a run, consult the QC limits to determine if the run is "in control." **The QC rules apply to the average of the beginning and ending analyses of each of the bench QC pools.** The QC rules are as follows:

(1) If both the low and the high QC results are within the 2s limits, accept the run.

(2) If one of two QC results is outside the 2s limit, apply the rules below and reject the run if any condition is met.

(a) 13s – Average of both low QCs OR average of both high QCs is outside of a 3s limit).

(b) 22s – Average of both low QCs AND average of both high QCs is outside of 2s limit on the same side of the mean.

(c) R4s sequential – Average of both low QCs AND average of both high QCs is outside of 2s limit on opposite sides of the mean.

(d) 10x sequential – The previous nine average QC results (for the previous nine runs) were on the same side of the mean for either the low OR high QC.

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

B. Sample Results Precision Evaluation

If the range of the replicate readings (maximum replicate concentration value – minimum replicate concentration value) for a single sample analysis is greater than 30% of the mean of the replicates, then repeat the analysis of that sample.

11. Remedial Action If Calibration or QC Systems Fail to Meet Acceptable Criteria

If one or more QC samples fall outside 95% limits for mean or range of duplicate values, then perform the following steps in succession:

A. Prepare fresh dilutions and reanalyze all blood QC samples.

B. Prepare fresh calibration standards and run the entire calibration curve using freshly prepared standards.

C. Install and condition a new (unused) graphite furnace or pyrolytic graphite L'vov platform as described in Section 8.C.6, and reanalyze the entire sequence of calibrators, blanks, and QC samples.

If the steps outlined above do not result in correction of the "out of control" values for QC materials, consult the supervisor for other appropriate corrective actions. Do not report analytical results for runs not in statistical control.

12. Limitations of Method; Interfering Substances and Conditions

This cadmium, lead, and blood method was validated with blood specimens with target values obtained by IDMS, which is regarded as the definitive analytic method for metals (cadmium and lead) in blood. The

range of concentrations reportable was previously mentioned. No known chemical or physiochemical interferences have been documented for this analytical method. External contamination may limit the accuracy of blood lead values below 2 µg/dL as well as the accuracy of blood cadmium values below 0.5 µg/L.

13. Reference Ranges (Normal Values)

The data from National Health And Nutrition Evaluation Survey IV (NHANES) will be used to establish the national norm for the United States.

A. Cadmium

(1) World Health Organization recommendations

(2) The WHO recommends 10 µg of Cd/L as the critical level for cadmium in blood. A value of 5 µg of Cd/L is considered the health-based biological limit (4).

(3) Other References

(4) Ewers et al. proposed 2 µg of Cd/L as the “upper normal limit” of cadmium in blood (5, 6).

(5) CDC Data Univariate analysis on 3,189 control subjects from a 2001 study. National Report on Human Exposure to Environmental Chemicals.

B. Lead

(1) CDC recommendations: People with blood lead levels ≥ 10 µg/dL require intervention because some adverse health effects have been documented at this level (8,9).

(2) Other References

(a) The average blood lead value for 801 samples was listed as 138 ± 45 µg/L with a 95% range of 70-230 µg/L (10).

(b) A biological quality guide has been proposed for groups of the general population (11). The level of exposure is acceptable when the median group value is ≤ 200 µg/L and the distribution is 98% ≤ 350 µg/L, 90% ≤300 µg/L, and 50% ≤ 200 µg/L. For preschool children, the distribution should be 98% ≤ 300 µg/L, 90% ≤ 250 µg/L, and 50% ≤100 µg/L.

(c) Half of nonoccupationally exposed persons have blood lead values < 200 µg/L with a 95% value of < 350 µg/L (12,13).

14. Critical-Call Results (Panic Values)

A. Cadmium

According to National Institute of Occupational Safety and Health (NIOSH). Occupational exposure to cadmium. Blood cadmium levels > 5 µg/L may indicate toxicity. Fed. Regist., 57:42,102-142,462, Sept. 1992 (13).

Notify the supervising physician about laboratory results.

B. Lead

(1) Pediatric (age < 6 yr): If blood lead levels, > 25 µg/dL, medical intervention is indicated.

(2) Adult: If blood lead levels, > 40 µg/dL, OSHA regulations require removal from workplace.

(3) In either a. or b., notify the supervising physician about laboratory results.

15. Specimen Storage and Handling During Testing

    Specimens may reach and maintain ambient temperature during analysis. Exercise stringent precautions in order to avoid external contamination by cadmium and lead as previously described.

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

    Since the analysis of blood for lead is inherently complex and challenging, there are no acceptable alternative methods of analysis. If the analytical system fails, store blood at 4°C (refrigerated) until the analytical system is restored to functionality. If long-term interruption (greater that 4 weeks) is anticipated, store blood specimens at –20°C.

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

    *Notify the supervising physician or the principal investigator as soon as possible if blood lead results are > 25 µg/dL (pediatric) or > 40 µg/dL (adults). Utilize the most expeditious means of communication telephone, fax, etc. For NHANES, fax all values >10 µg/dL for physician transmittal and follow-up.*

    A. QC 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 binder(s).

    B. Analytical Results. Use a PC database file to record the specimen results. Record the results for blood cadmium and blood lead in µg/dL. If a result is below the detection limit of the method, write "ND" (for nondetectable) and enter a value of “0.1” in the blank. If a sample is missing from the rack, write "NOSAX" in the blank. If a sample is unsatisfactory, i.e., cannot be analyzed, write "UNSAX" in the blank. Print these subject data files and put a copy of the files in the study binder(s).

    C. Give all forms to the supervisor along with the hard copy of the data printout from the PerkinElmer SIMAA 6000. After the data are calculated and the final values are approved for release by the reviewing supervisor, the data-entry clerk should transcribed the results into the Study database, which is located in Microsoft Access on the NCEH/DLS PC network. Return the photocopies and the data printouts for filing in a notebook/binder. The supervisor should keep the original copies of the reporting sheets.

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

    The analyst who receives specimen/samples delivered to Nutritional Biochemistry Branch (NBB) will create a “Specimen” folder. Fill out a tracking form and place it in the folder for the analyst performing the analysis. The form tracks location, status, and final disposition of the specimens. When the sample analysis is completed, the tracking form will be placed in the Specimen Tracking Record Log Book located in the trace metals library.

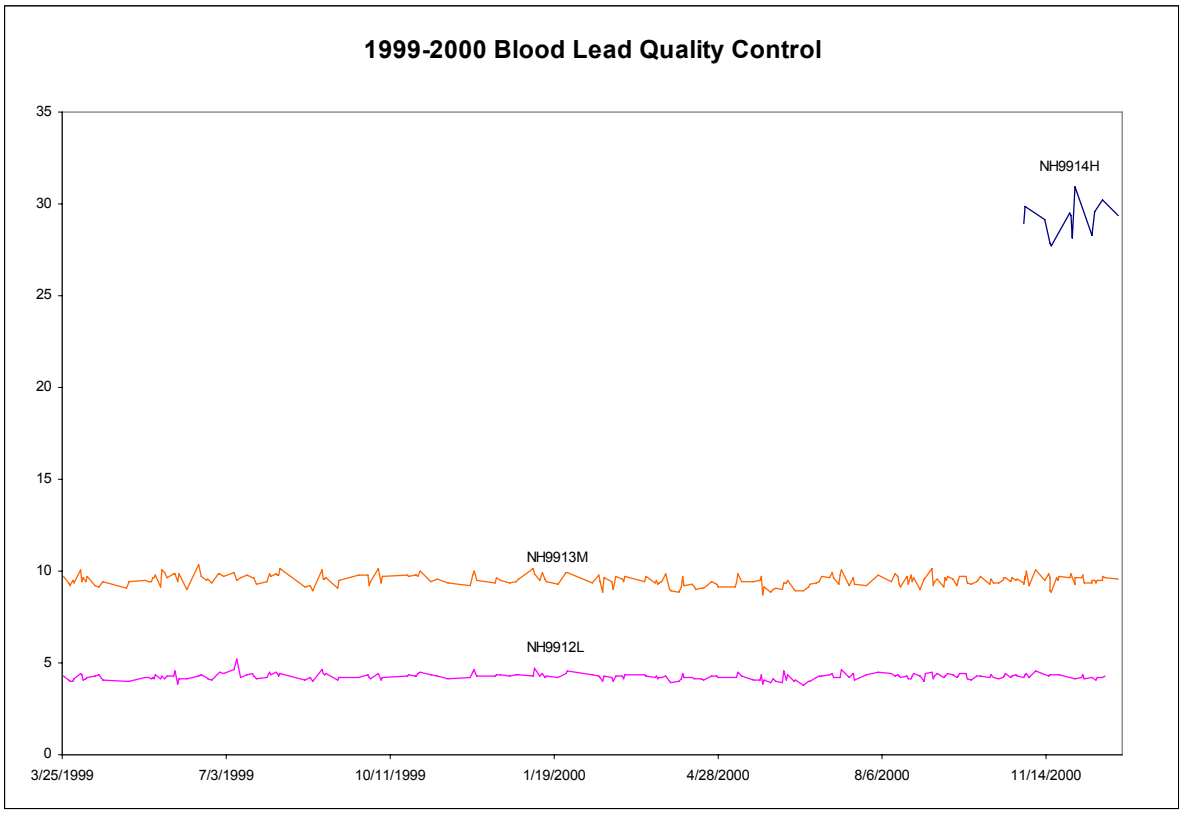
    Use standard record keeping means (e.g., electronic – Microsoft Access, optical disc, or tape backup) to track specimens. Records are maintained for at least 3 years. Records include related Quality Assurance/QC data. Duplicate records are kept in electronic or hardcopy format; if sensitive or critical, these duplicates are stored off site. Only numerical identifiers are used (e.g., case ID numbers); personal identifiers are available only to the medical supervisor or project coordinator to safeguard confidentiality.

19. Summary Statistics and QC Graphs

A. Lead

**Summary Statistics for Lead**

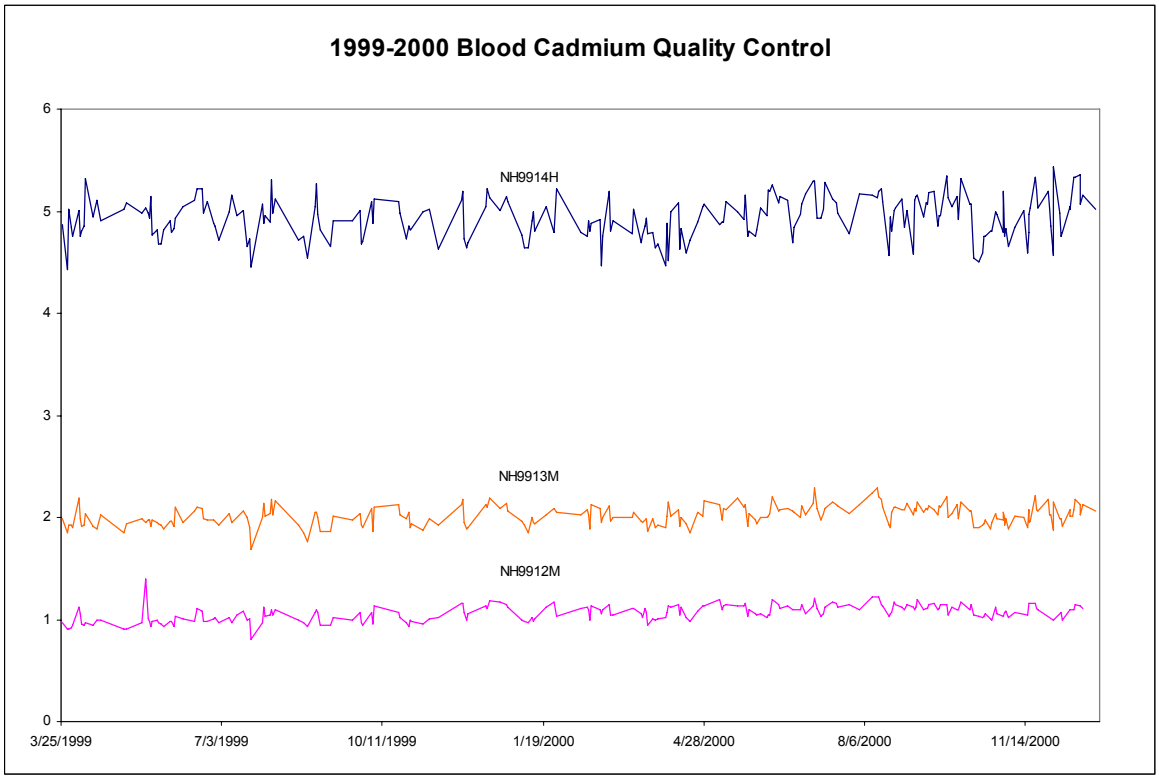
| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| NH9912L | 226 | 3/26/1999 | 12/20/2000 | 4.2649 | 0.169 | 4.0 |
| NH9913M | 239 | 3/26/1999 | 12/28/2000 | 9.5076 | 0.2976 | 3.1 |
| NH9914H | 13 | 10/31/2000 | 12/28/2000 | 29.1364 | 0.9442 | 3.2 |



B. Cadmium

**Summary Statistics for Cadmium**

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| NH9912L | 226 | 3/26/1999 | 12/20/2000 | 1.0614 | 0.0778 | 7.3 |
| NH9913M | 239 | 3/26/1999 | 12/28/2000 | 2.0172 | 0.0951 | 4.7 |
| NH9914H | 239 | 3/26/1999 | 12/28/2000 | 4.9395 | 0.2034 | 4.1 |



## References

1. Carson BL, Ellis HV III, McCann JL. Toxicology and biological monitoring of metals in humans. Chelsea MI: Lewis Publishers, Inc.; 1986. p. 130.

2. Miller DT, Paschal DC, Gunter EW, Stroud PE, D'Angelo J. Determination of blood lead with electrothermal atomic absorption using a L'vov platform and matrix modifier. Analyst 1987;112:1701–1704.

3. Parsons PJ, Slavin W. A rapid Zeeman graphite furnace atomic-absorption spectrometric method for the determination of lead in blood. Spectrochim Acta 1993;48B(6/7):925–939.

4. Stoeppler M, Brandt K. Determination of cadmium in blood and urine by electrothermal atomic-absorption spectrophotometry. Fresenius J Anal Chem 1980;300:372–380.

5. World Health Organization (WHO) Study Group. Recommended health-based limits in occupational exposure to heavy metals. Friberg L Vahter M. Assessment of exposure to lead and cadmium through biological monitoring: results of a UNEP/WHO global study 1983. Environ. Res.; 30:95–128.

6. Ewers U, Brockhaus A, Dolgner R, Freier I, Jermann E, Bernard A, et al. Environmental exposure to cadmium and renal function of elderly women living in cadmium-polluted areas of the Federal Republic of Germany. Int Arch Occup Environ Health 1985;55:217–239.

7. Verschoor M, Herber R, van Hemmen J, Wibowo A, Zielhuis R. Renal function of workers with low-level cadmium exposure. Scand J Work Environ Health 1987;13:232–238.

8. CDC published study 2001. National Report on Human Exposure to Environmental Chemicals.

9. Mueller PW, Smith SJ, Thun Mj, Steinberg KK. Chronic renal tubular effects in relation to urine cadmium levels. Nephron 1989; 52:45–54.

10. Preventing lead poisoning in young children: a statement by the Centers for Disease Control and Prevention. Final report. Atlanta (Ga): Dept. of Health and Human Services (US), Centers for Disease Control and Prevention; 2001.

11. Lentner C, editor. Geigy scientific tables. Vol. 3., 8th ed., Basle, Switzerland: Ciba-Geigy Co.; 1984. p. 87.

12. Long GL, Winefordner JD. Limit of detection: a closer look at the IUPAC definition. Anal Chem 1983; 55:712A–724A.

13. National Institute of Occupational Safety and Health (NIOSH) Fed. Regist., 57:42, 102–142, 462, September, 1992.