



Laboratory Procedure Manual

Analyte: **Total and Inorganic Mercury**

Matrix: **Blood**

Method: **Blood mercury FIMS CVAA (formerly: Flow Injection Cold Vapor Atomic Absorption (CVAA))**

Method No: **ITB003A (formerly: 1190B/06-OD)**

Revised: **September 13, 2004**

as performed by:

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Important Information for Users

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

Public Release Data Set Information

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

Data File Name	SAS Label
THG_F	Mercury, total ($\mu\text{g/L}$)
	Mercury, total ($\mu\text{mol/L}$)
IHG_F	Mercury, inorganic ($\mu\text{g/L}$)
	Mercury, inorganic ($\mu\text{mol/L}$)

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1. Clinical Relevance and Summary of Test Principle

Total mercury in blood is measured by flow injection cold vapor atomic absorption analysis with online microwave digestion, which is based on the method that Guo and Bassner developed. Decomposition of organic mercury is the result of the blood sample mixed with a bromate-bromide reagent, flowing through a microwave and reacting with sodium borohydride (the reductant). The online addition of potassium permanganate promotes further decomposition of organic mercury. The mercury vapor is measured by the spectrometer at 253.7 nm. Inorganic mercury in blood is measured using stannous chloride as a reductant, also utilizing microwave digestion. Mercury vapor (reduced from inorganic mercury compounds) is measured via the same quartz cell at 253.7 nm.

The difference between total and inorganic mercury represents organic mercury in blood. Mercury analysis identifies cases of mercury toxicity. The main organs affected by mercury are the brain and kidneys. Psychic and emotional disturbances are the initial signs of chronic intoxication by elemental mercury vapor or salts. Paresthesia and neuralgia may develop. Renal disease, digestive disturbances, and ocular lesions can also develop. Kidney toxicity is an important consequence of exposure to mercury salts.

2. Safety Precautions

Use universal precautions when handling blood products. Wear gloves, a lab coat, and safety glasses. The hepatitis B vaccination series is recommended for all analysts who work with blood or serum samples. Place in a biohazard autoclave bag disposable plastic, glass, and paper (e.g.; pipette tips, autosampler cups, gloves, etc.) that contact blood. Keep these bags in appropriate containers until they can be sealed and autoclaved. Wipe down all work surfaces with a 10% sodium-hypochlorite solution when work is finished.

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 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 metal-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.

Material safety data sheets for hydrochloric acid, nitric acid, sodium hydroxide, Triton X-100™, potassium permanganate, sodium borohydride, potassium bromide, potassium bromate, stannous chloride, and argon are available through the DLS computer network.

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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 multiple computer systems. Store data files containing the date, analytical run identification (ID), specimen analytical results by specimen ID, and method code on a local hard drive. The computer used in this FIMS system is not connected to the DLS computer network.
- B. Routine backup procedures include weekly backup of data files, archival on 3.5" floppy diskettes, and archival on a WORM™ optical disk or compact disk. Store sensitive data, on floppy disks (compact or optical) disks that are stored off site. Contact either the supervisor or LAN manager for emergency assistance.
- C. Accomplish statistical evaluation and calculation of the run with the calibration curve used by the AA WinLab™ software. Reformat data by using the AA WinLab™ Reformat program and save to 3.5" diskette. Import these data into the Inorganic Toxicology and Nutrition Branch lab data management system for evaluation of quality control parameters and for reporting and archiving.
- D. DLS LAN support staff and CDC data center mainframe make sure that files stored on the network or CDC mainframe are automatically backed up to tape each night.
- E. Both the laboratory research notebook and record book contain documentation for system maintenance and daily laboratory activities.

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

- A. No special instructions for fasting, or special diets are required.
- B. Specimen type is blood with anticoagulant, preferably K₃EDTA 1.5 mg/mL of blood.
- C. Optimal amount of specimen is 2-3mL in an unopened collection tube; minimum amount is about 500 µL (0.5mL).
- 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 (1.5mg/mL). EDTA is preferred over heparin because heparin allows 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 specimens are either a low volume (< 0.5mL) 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 listed above and include blood clotting.
- H. The division's protocol outlines specimen-handling conditions for blood collection and handling. (Copies are available in the branch laboratory and the division office). The protocol discusses collection, transport, and special equipment requirements. 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 the portions of the sample that remain after analytical aliquots are withdrawn. Thawing and refreezing do not compromise the samples unless inadvertent contamination occurs due to improper handling. If there is more than one analyte of interest in the specimen and it needs to

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be divided, transfer the appropriate amount of blood into a sterile Nalgene® cryovial labeled with the participant's ID.

- I. Screen each lot of collection tubes for mercury contamination; do the same for all shipping and storage containers.

5. Procedures for Microscopic Examinations; Criteria for Rejecting of 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

1. Sodium Borohydride Solution. (0.05% sodium borohydride (NaBH₄) in 0.05% sodium-hydroxide (NaOH). Weigh out 1.0 g sodium borohydride and 1.0 g NaOH, and dissolve in 2 L of ultrapure water. Add 3.0 mL Dow Corning™ DB-110A antifoam agent to 1 L of reduction solution and shake it. Prepare this solution daily.
2. Potassium Permanganate Solutions. For 0.05% (w/v) dissolve 0.5 g of potassium permanganate in 1L of ultrapure water. For 0.2% (w/v) dissolve 2.0 g of potassium permanganate in 1 L of ultrapure water. For 5.0% (w/v) dissolve 2.5 g potassium permanganate in 50 mL of ultrapure water. Dissolve the potassium permanganate crystals completely. Store all the solutions in dark bottles and prepare weekly.
3. Oxidation Reagent. Dissolve 4.71 g potassium bromide and 1.32 g potassium bromate in 500 mL of ultrapure water. Add 0.6 mL of 100% Triton X-100™ and mix well.
4. Hydrochloric-Acid Solution. (10.0% [v/v] add 100 mL of concentrated HCl to several hundred mL of ultrapure water, then make up to 1 L with more water.
5. Stannous Chloride Solution. (2.5% [w/v]) dissolve 25.0 g stannous chloride in 1 L of 5% HCl solution. Add 3.0 mL of Dow Corning™ DB-110A antifoam agent and mix it. Prepare this solution daily.
6. Triton Solution. (0.12% [v/v]) dissolves 0.6 mL of 100% Triton X-100™ in 500 mL of ultrapure water, and mix well.
7. Carrier Solution. Use ultrapure water.

B. Standards Preparation

1. 1,000-mg/L Stock Mercury Standard. Using an Eppendorf® pipette, dilute 1.00 mL of National Institute of Standards and Technology (NIST SRM 3133), to 10-mL with ultrapure water in an acid-cleaned volumetric flask. Store in refrigerator and prepare every 6 months in a flask dedicated to this solution.
2. 10-mg/L Intermediate Mercury Standard. Using an Eppendorf® pipette, dilute 1.00 mL of the 1,000-mg/L stock mercury standard to 100 mL with ultrapure water in an

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acid-cleaned volumetric flask. Store in refrigerator and prepare monthly in a flask dedicated to this solution.

3. Working Mercury Standards. Using the Micromedic Digiflex™, transfer the following volumes of 10-mg/L intermediate standard to 50-mL volumetric flasks and dilute to volume with 1 M of HCl.

Table 1. Working Mercury Standards

Intermediate Stock (µL)	Working Standard Concentration (µg/L)
500	100
125	25

4. Calibration Standards

- a. Using a calibrated Eppendorf® pipette, dispense 0.2 mL of bovine base blood into each of a series of five 15-mL conical tubes.
- b. Using a Digital Dispensette®, dispense 2.0 mL of oxidation reagent for total mercury measurement or 0.12% Triton solution for inorganic mercury measurement to each of the five tubes.
- c. Using a series of calibrated micropipettes, dispense 0 (Base), 10, 20, and 40 µL of 25- µg/L mercury working standard to the first four tubes, respectively. Dispense 40 µL of 100-µg/L-mercury working standard to the last tube. Mix well. Place the standards into autosampler position 9-13.
- d. Perform a series of measurements on these five tubes. These measurements will correspond to mercury concentration at the levels of 1.25 µg/L, 5.0 µg/L, 10.0 µg/L and 20.0 µg/L.
- e. AA WinLab™ software automatically constructs the calibration curve (absorbance versus µg/L mercury).

C. Preparation of Quality Control (QC) Materials

Four pools of bovine blood were evaluated for total mercury. All of the pools were preserved with the anticoagulant EDTA. One pool was unspiked and, therefore, had a very low concentration. The low pool is suitable for use as the base blood from which the calibration standards are prepared. The other three pools were spiked either with inorganic (mercuric) mercury chloride (COXEL and INOHG) or a mixture of inorganic (mercuric chloride) and organic (methyl mercury iodide) mercury (NIST SRM 966-2); therefore their blood mercury levels were elevated. The QC pools were dispensed into prescreened vials and stored frozen at -20°C.

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D. Other Materials

1. Stock solution of mercury: NIST SRM 3133, 10,000 mg/L (National Institute of Standards and Technology, Gaithersburg, MD) or equivalent.
2. Ultrapure, concentrated hydrochloric acid (Ultrex™ J.T. Baker Chemical Co., Phillipsburg, NJ) or equivalent.
3. Triton X-100™ ("Baker Analyzed", J.T. Baker Chemical Co., Phillipsburg, NJ or any source whose product is low in mercury contamination).
4. Ultrapure water (from the Milli-Q™ water-purification system).
5. Stannous chloride, dihydrate, American Chemical Society (ACS) ("suitable for mercury determination" J.T. Baker Chemical Co. Phillipsburg, NJ or any source whose product is low in mercury contamination).
6. Potassium permanganate, ACS (GFS Chemicals, Inc., Powell, OH) or equivalent.
7. Sodium borohydride, 98% (Alfa Products, Danvers, MA) or equivalent.
8. Potassium bromide, ACS (GFS Chemicals, Inc., Powell, OH) or equivalent.
9. Potassium bromate, ACS (GFS Chemicals, Inc., Powell, OH) or equivalent.
10. Argon, 99.996% purity (supplied as a compressed gas by Holox or other contract agency) equipped with approved gas regulator (Matheson Gas Products, Secaucus, NJ) or equivalent.
11. NIST SRM 966-2, mercury spiked in bovine blood. Run this periodically to verify accuracy.
12. Bovine blood QC pools with different levels of mercury that has reference values established by Cold Vapor Atomic Absorption Spectrometry (CVAAS).
13. Pipette tips: 1- to 200- μ L and 200- to 1,000- μ L sizes (Rainin Instrument Co., Inc., Woburn, MA) or equivalent.
14. Acid-cleaned volumetric flasks (1000-, 100-, 50-, and 10-mL volumes).
15. Kay-Dry® paper towels and Kim-Wipe® tissues (Kimberly-Clark Corp., Roswell, GA) or equivalent.
16. Nitrile, powder free medical-examination gloves (Best Manufacturing Co., Menlo, GA) or equivalent.
17. Biohazard autoclave bags (Curtin-Matheson Scientific, Inc., Atlanta, GA).
18. Bleach (10% sodium-hypochlorite solution) any past vendor.
19. Cotton swabs (Hardwood Products Co., Guilford, ME) or equivalent.
20. Small plastic weighing boats (Scientific Products, McGaw Park, IL).
21. Falcon 15-mL and 50-mL conical tubes with caps (Becton, Dickinson Labware, Franklin Lakes, NJ) or equivalent.

E. Instrumentation

1. PerkinElmer Flow Injection Mercury System-400. The FIMS analysis system is a compact, software-driven analysis system for mercury determinations. The system consists of the FIMS spectrometer, an autosampler (AS-91), a microwave digester (Maxidigest MX 350™), Flow Injection Analysis System 400 flow injection system, a computer, and a printer.
 - a. The FIMS spectrometer is a single-beam atomic absorption spectrometer specifically designed to measure the absorption of mercury (photo cell with maximum sensitivity at 254 nm, low-pressure mercury lamp).

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- b. The AS-91 autosampler is a computer-controlled, multipurpose sampling system with 152 locations for 15-mL sample vessels.
- c. The FIAS software incorporated in the spectrometer controls the microwave digester. The microwave power level for this method is 20%.
- d. The FIAS-400 is a flow injection system used to transport various liquids. For total mercury measurement, pump 1 feeds the sample solution through the sample loop. Pump 2 feeds the carrier solution (water) through the sample loop to carry the sample into the first manifold block, where the sample mixes with hydrochloric acid (HCl) solution. The mixture flows into the microwave digester loop, and then the digested solution passes through a water cooler and flows into the second manifold block, where it mixes with potassium-permanganate solution. The reductant solution is introduced to the system at the third manifold block, where the reduction takes place. The reaction mixture meets with carrier gas at the fourth manifold block and is fed to the gas/liquid separator, where the mercury vapor separates from the liquid. The carrier gas carries the mercury vapor to the FIMS cell. Recommended tubing configuration for FIMS-400 is in the book *FIMS Installation, Maintenance, and System Description*.

Table 2. Analytical Parameters of FIMS 400

Parameter	Setting	
	Total Mercury	Inorganic Mercury
Wavelength	253.7 nm	253.7 nm
Slit width	0.7 nm	0.7 nm
Signal type	AA	AA
Signal measurement	Peak height	Peak height
Smoothing point	9	9
Microwave power level	20%	20%
Read time	35 (sec)	35 (sec)
Read delay	0	0
BOC Time	5 (sec)	5 (sec)

Table 3. Flow Injection Program: Total and inorganic Mercury Measurement*

Step	Time (sec)**	Pump 1 Speed	Pump 2 Speed	Valve Position	Read Step
Prefill	5	120	90	Fill	

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1	7	120	90	Fill	
2	40	0	90	Inject	
3	35	0	90	Inject	X
4	1	0	90	Fill	

Instrument model: FIAS 400. Sample volume: 500 μ L (amalgam)

** Adjust time for each step to allow enough volume of sample to be filled (prefill and step 1) and to allow appropriate time for reactions to take place and to signal measurement (step 2).

2. Micromedic Digiflex Automatic™ pipette equipped with 2,000- μ L dispensing syringe, 2,000- μ L and 200- μ L 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. Vortex-Genie™ vortex mixer (Fisher Scientific, Atlanta, GA).
5. Eppendorf™ fixed-volume micropipettes: 1,000-, 500-, 250-, 200-, 100-, 50-, 40-, and 10- μ L volumes (Brinkmann Instruments, Inc., Westbury, NY).
6. Magnetic stirrer (Corning Glass Works, Corning, NY) and stirring bars (Fisher Scientific).
7. Digital Dispensette™ (Brinkmann Instruments, Inc., Westbury, NY).
8. Flowmeter (Cole-Parmer Instrument Co., Vernon Hills, IL).

7. Calibration and Calibration-Verification Procedures

- A. Use the matrix-matched calibration method. Prepare calibration solutions at different mercury levels by spiking blood samples with appropriate amounts of mercury standard solution. The AA Winlab™ software constructs a calibration curve by using the measured mean values of absorbance of standards at 0.1, 5.1, 10.1, 20.1, and 50.1 μ g mercury plotted versus concentration.
- B. Once calibration has been performed, the slope and intercept will be generated. Calibration curves should be displayed in the CALCULATION DISPLAY window, both to verify the mathematical fit and to evaluate the slope and intercept. Acceptable slopes are between 0.0005 to 0.0009 for total mercury and 0.0003 to 0.00065 for inorganic mercury measurement.
- C. Verify calibration by using control material certified for mercury concentration. One suitable material is NIST SRM 966. This control material is run once per week. Agreement with certified or accepted values should be \pm 10%.

8. Operating Procedures; Calculations; Interpretation of Results

- A. Preliminaries and Sample Preparation

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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.e.).
2. Allow frozen blood specimens, QC control specimens, and blood calibration material to reach ambient temperature, and then mix on a vortex mixer for 10 seconds.
3. While the specimens are thawing, turn on the FIMS computer, printer, and allow them to warm up a minimum of 30 minutes.
4. Prepare the following solutions: a) for total mercury, prepare 0.05% sodium borohydride in 0.05% sodium hydroxide (NaOH) and 0.05% potassium permanganate and; b) for inorganic mercury, prepare 2.5% stannous chloride and 0.2% potassium-permanganate solution.
5. Set up a series of 15-mL conical tubes corresponding to the number of standards, QCs, and unknown samples to be run.

B. Instrument Setup

1. Turn on the argon gas tank and verify that the tank pressure is 100 psi; the outlet pressure should be 40-50 psi.
2. Set the flowmeter to 20.
3. Change the filter paper in the separator block.
4. The FIMS PC controls the FIMS/FIAS automatically. Using the mouse, double click on the icon named "AA WinLab Analyst" to run the FIMS/FIAS control program. The program will prompt you to select "Workplace"; select "Custom-designed Workplace." Open the file named "Automtd.fms." Note that five windows will appear on the screen. They are AUTOMATED ANALYSIS CONTROL, EXAMINE PEAK, RESULTS, EXAMINE CALIBRATION, and FIAS CONTROL.
5. Click on the FIAS CONTROL window to make it active. Start pumps 1 and 2 to pump ultrapure water through the FIAS system. Make sure the liquid flows through the system in the appropriate direction at the appropriate flow rate. (Place the inlets of the carrier and reagent tubes in a water container; while in the AUTOMATED ANALYSIS CONTROL window, click on "Move Probe Up/Down" to lower the sample probe into the wash beaker that is filled with water.)
6. Select "Open Method" from the "File" menu. Select the appropriate method for either the total mercury or inorganic mercury measurement.
7. Select "New Sample Info File" from the "File" menu and input the sample information as the order of sample loaded on the autosampler starting at location #14. Save the file.
8. In the AUTOMATED ANALYSIS CONTROL window, click on the "Setup" tab at the bottom of the window. Select the appropriate method and sample info file, fill them into the table. Take off the check marker for pumps when responding to the option; off after analysis. Type in the data file name.
9. Fill the blank container/wash beaker at autosampler position "0" with ultrapure water. Check the waste container; empty it, if necessary.

C. Instrument Operation – Total and Inorganic Mercury

1. Total Mercury

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- a. For preparation of calibration standard solutions, see Section 6.b. (4)(a)-(c).
- b. Pipette 200 μ L of QCs and unknown specimen into 15-mL conical tubes. Load QCs and unknowns onto AS-91.
- c. Click on the FIAS window to activate it; start pump 1 at 100 and pump 2 at 90. Turn on the microwave at 20% power by clicking on "Remote 2." Make sure that the digester loop is inside the digester vessel and that the digester vessel is filled with about 20-30 mL of water. Then close the FIAS window.
- d. Place the carrier tubing inlet in a water container, the HCl tubing inlet in a 10% HCl solution, and the reductant-tubing inlet in a 0.05% sodium-borohydride solution. Finally, place the potassium-permanganate tubing inlet in 0.05% potassium-permanganate solution.
- e. In the AUTOMATED ANALYSIS CONTROL window, click on the "Analyze" tab on the bottom of this window. Click on the button marked "Calibrate." The software will run the calibration blank and each standard in duplicate. Blank's absorbance should be > 0.0003 . While the software is running, call up the DISPLAY CALIBRATION window. Correlation coefficient should be > 0.990 and the slope within the range (Section 7.b.). Proceed with the analysis by clicking the "Analyze Samples" button and typing in "QC" and the sample position. Monitor the first QC to ensure that the results are in.
- f. When a run is finished, the Analysis Progress information displayed on the AUTOMATED ANALYSIS window will be "Sample Complete . . . 100%, Current Status . . . Idle." If more than one set of samples are to be run, check QC between runs and recalibrate the system, if necessary. Load the samples on the autosampler, modify the "Sample Info" file, and execute "Analyze Samples" again.
- g. When all analyses are complete, let both pumps continue to run. Start the rinsing procedure in the following order: Take out the inlet of the potassium-permanganate tube from the brown bottle, place it in a wash container (deionized water), and pump until the tube is clear. Place the reductant reagent tube inlet in the same wash container, and pump for 30 seconds. Place the carrier and HCl tubes in the same container, and pump all the tubes for 5 minutes. Within this wash period, in the FIAS CONTROL window, click on "Valve Fill/Inject" several times while the pumps are running. This action ensures that the sample channel and the inside of the FIAS valve are rinsed effectively. Rinse all the tubes with a 10% HCl solution for 5 minutes. Rinse all the tubes with a solution of HCl:H₂O = 50:50 for 3-5 minutes. Place all the tubes back into the wash container and rinse them for 10 minutes.
- h. After rinsing with the last rinse solution (deionized water), remove all the tubes from the rinse-solution container. Click on "Move Probe Up/Down" to raise the sampling probe out of the wash beaker. Allow the pumps to run until all the tubes and the gas/liquid separator are empty. Click on the "Pump 1" and "Pump 2" buttons to stop the pumps, and then release the tension on the pump tubes. Make sure that the FIAS valve is in the "Fill" position.

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- i. Quit from the program (under "File"). Turn off the gas, instrument, and computer. Put the standards, QCs, and blood samples back into the refrigerator.
- j. Place the printed output of the runs in a data file folder. Make a record of the runs in the laboratory research notebook. Enter any other maintenance items or problems in the notebooks. Back up and archive the data files weekly.

2. Inorganic Mercury

- a. For preparation of calibration standard solutions, see Section 6.b. (4) (a)-(c).
- b. Pipette 200 μL of QCs and unknown specimens into 15-mL conical tubes. Load QCs and unknowns onto the AS-91 autosampler.
- c. Click on the FIAS window to activate it. Start pump 1 at 100 and pump 2 at 90. Then close the FIAS window. Turn on the microwave at 20% power by clicking on "Remote 2". Make sure that the digester loop is inside the digester vessel and that the digester vessel is filled with about 20-30 mL of water. Then close the FIAS window.
- d. Place the carrier tubing inlet in a water container, HCl tubing inlet in a 5% HCl solution, and the reductant-tubing inlet in a 2.5% stannous-chloride solution. Finally, place potassium-permanganate tubing inlet in a 0.2% potassium-permanganate solution.
- e. The remaining steps in the analysis of inorganic mercury correspond to the total mercury analysis listed in Section 8.d. (1) (f)-(l).

D. Replacement and Periodic Maintenance of Key Components

1. A spare FIAS valve should be available. If there is any leakage, remove the valve from the pump unit and clean the individual parts of the valve with deionized water.
2. A spare manifold and gas/liquid separator should be available. Blood sample and reductant solutions can obstruct manifold blocks. Clean them with soapy water in an ultrasonic cleaner.
3. Change the separator filter daily.
4. Before the run, make sure that all the tubes are clean and free from kinks. Remove any tubes that may be damaged or blocked and install new tubes. To reduce wear on the pump tubes, place one drop of silicone oil on the part of the tube that comes in contact with the pump rollers. Release the tension on the pump tubes when you are not using FIMS. Replace pump tubes after several days of analysis (as needed).
5. A spare sample probe should be available. Clean the probe with a stainless steel cleaning wire for AS 91 Probe, if necessary.

E. Calculations

1. The method described here is linear up to at least 50 $\mu\text{g/L}$ (the highest concentration point in the calibration standards). The calibration curve (linear regression) and specimen concentration are generated by AA WinLab™ software.

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The software generates calibration-curve information such as correlation coefficients, slopes, and intercepts and also plots the fitted curves. The correlation coefficient, r^2 , for each curve should be 0.990 or greater. For optimum sensitivity, slopes should be more than 0.0007 for total mercury and 0.0004 for inorganic mercury; intercepts should be less than 0.0005 for total mercury and 0.0009 for inorganic mercury. The software also calculates the standard concentration and sample concentration (blank corrected) of each replicate and mean, standard deviation (SD) and % relative standard deviation (RSD) for each sample, and more.

2. In total mercury measurement, repeat a specimen analysis when duplicate integrated absorbencies below 0.0035 Abs/sec (mean) differ by more than about 0.0005 Abs/sec or when duplicate integrated absorbencies above 0.0035 Abs/sec (mean) differ by more than 0.001 Abs/sec. These absorbencies correspond to concentration differences of 0.8 µg/L and 1.425 µg/L, respectively.
3. In inorganic mercury measurement, repeat a specimen analysis when duplicate integrated absorbencies below 0.0022 Abs/sec (mean) differ by more than about 0.0003 Abs/sec or when duplicate integrated absorbencies above 0.0022 Abs/sec (mean) differ by more than 0.0005 Abs/sec. These absorbencies correspond to concentration differences of 1.27 µg/L and 1.65 µg/L, respectively.
4. Reanalyze specimens containing more than 30 µg/L of mercury for confirmation. For a specimen with a concentration greater than 50 µg/L, dilute the sample with base blood and reanalyze it. Multiply the result by the appropriate dilution factor.
5. The detection limit, based on 3 times the SD of 10 repeat measurements of a sample with low mercury concentration (2), is 0.137 µg/L in total mercury measurement and 0.446 µg/L in inorganic mercury measurement. Report results below the detection limits as nondetectable (ND; refer to Section 17.b., under "Test-Result Reporting System" in this document).

F. Special Procedure Notes – CDC Modifications

The sample preparation procedure in this method has been modified. Note that oxidation reagent (bromate-bromide) is now pre-mixed with the Triton X-100™ solution, then 2.05 mL of the mixture solution is dispensed into each 200-µL blood sample (standard or unknown), whereas in the original method (1) the Triton X-100™ solution was added to the tube first, followed by the blood sample, and finally the oxidation reagent. The modified procedure is much more efficient, and neither the accuracy nor the sensitivity of the method has been negatively affected. Also, the bromate-bromide solution no longer is added to the calibration blank. Ultrapure water is now used as the calibration blank.

9. Reportable Range of Results

Blood mercury values are reportable in the range $LDL < \text{blood mercury} < 50 \mu\text{g/L}$ without dilution, where $LDL =$ the calculated (3 SD) lower detection limit. If a blood total mercury value is less than 0.137 µg/L (the approximate LDL of this method), report it as $< 0.137 \mu\text{g/L}$. If a blood inorganic mercury value is less than 0.4 µg/L, report it as $< 0.4 \mu\text{g/L}$;

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if greater than 50 µg/dL, dilute the sample with bovine base blood, and reanalyze the specimen. Multiply the result by the appropriate dilution factor.

10. Quality Control (QC) Procedures

The Inorganic Toxicology and Nutrition Branch recently developed the method described in this protocol for the purposes of environmental and occupational health studies. The method is accurate, precise, and reliable. The primary standard used is a NIST SRM. Long-term QC control pool results can generate estimates of imprecision.

This analytical method utilizes two types of QC systems. With one type of QC system the analyst inserts "bench" QC 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 and are labeled and processed so that they are indistinguishable from the subject samples. The supervisor decodes and reviews results of the blind specimens. With both systems, taking these samples through the complete analytical process assesses all levels of mercury concentration. The data from these materials are then used to estimate methodological imprecision and to assess the magnitude of any time-associated trends.

The method development utilizes three-bench QC pools. The mercury level of these QC pools ranges from 10 µg/L to 30 µg/L. Periodically use reference materials (blood products with certified values assigned by independent reference methods) to check accuracy. 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 is from the same lot, a full characterization is not necessary. However, there should be some overlap between the old and new stocks.

QC limits are established for each pool. An analysis of variance is performed for each pool after characterization runs have been performed in which previously characterized standard reference material (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 analyzing the standards and bench QC materials (at the beginning of an analytical run), consult the long-term QC charts for each control material to determine if the system is "in control." Two types of charts should be used. The first chart plots the means of the duplicate determinations and compares them to the 95% and 99% confidence limits as well as to the centerline (the overall mean of the characterization runs). The system is "out of control" if any of the following events occur for any one of the QC materials:

A. Precision and Accuracy

Quality Control Results Evaluation: After the completion of a run, consult the QC limits to determine if the run is in control. **The following QC rules apply to the average of the beginning and ending analyses of each of the bench QC pools:**

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1. If both the low and the high QC results are within the 2s limits, then accept the run.
2. If one of two QC results is outside the 2s limits, then apply the rules below and reject the run if any condition is met.
 - (a) **13s** – Average of both low QC results OR average of both high QC results is outside of a 3s limit).
 - (b) **22s** – Average of both low QC results AND average of both high QC results is outside of 2s limit on the same side of the mean.
 - (c) **R4s sequential** – Average of both low QC results AND average of both high QC results 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 results.

If the run is declared out of control, investigate the system (e.g., instrument, calibration standards) to determine the root of the problem before any analysis of specimens occurs.

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

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

1. Prepare fresh calibration standards and run the entire calibration curve using freshly prepared standards.
2. Prepare a fresh dilution of the failing QC material (working QC standard). Prepare fresh dilutions of all blood QC samples and reanalyze them.

If the two 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 any analytical results for runs not in statistical control.

12. Limitations of Method; Interfering Substances and Conditions

This method has been validated with blood specimens with target values obtained from reference (expert) laboratories. The reportable range of concentrations has been previously mentioned; no known chemical or physiochemical interferences have been documented for this analytical method. External contamination may limit the accuracy of blood mercury values below about 0.5 µg/L.

13. Reference Ranges (Normal Values)

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A. CDC Recommendations CDC recommendations have not been determined.

B. Other References:

1. The median blood mercury value for 812 samples was listed as < 5 µg/L with a 95% range of 0-30 µg/L (3).
2. Residents near a Superfund site with no known occupational exposure to mercury had a mean of 1.7-µg/L total mercury (SD = 1.5, N = 47 > LDL of 0.1 µg/L) (4).
3. In unexposed adults, the blood mercury level rarely exceeds 1.5 µg/dL; a blood concentration of 5 µg/dL or greater is considered the threshold for symptoms of toxicity (5).
4. 902 blood samples collected from a normal human population who had no extraordinary mercury exposure generated the following mean plus 2 SD skewed confidence-limit ranges of results: blood total mercury, 0-8.4 µg/L; blood inorganic mercury, 0-1.7 µg/L; and blood organic mercury, 0-7.5 µg/L (6).

14. Elevated Results including Critical Call Results or “Action Values”

- A. Results Requiring Confirmation analysis: Concentrations observed greater than the “first upper boundary” (defined in the laboratory database as the “1UB”) should be confirmed by repeat analysis of a new sample preparation. The concentration assigned to the 1UB for an element is determined by study protocol but the default concentration is 5.8 ug/L Hg. Report the original result, as long as the confirmation is within 10% of the original. Continue repeat analysis until a concentration can be confirmed.
- B. Analyst Reporting of Elevated Results: Concentrations observed greater than the “second upper boundary” (defined in the laboratory database as the “2UB”) should be reported to the QC reviewer as an “elevated result”. The concentration assigned to the 2UB for an element is determined by study protocol but default the concentration is 5.8 ug/L Hg (in this case the same as the 1UB boundaries). The analyst should report any patient results confirmed to be greater than the second upper boundary to the QC reviewer as an “elevated result”. The protocol for supervisors reporting elevated results to medical personnel is defined according to the study protocol. Levels of concern for mercury in blood are >100 ug/L for children (6 yr and younger) and >200 ug/L for adults.

15. Specimen Storage and Handling during Testing

Specimens may reach and maintain ambient temperature during analysis. Take stringent precautions to avoid external contamination by mercury.

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

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

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

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

1. 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).

2. Analytical Results

Reformat the data file by using Reformat AA WinLab™ program, and then download the data file for calculation or reporting. Record the results for blood mercury in µg/L. If a result is below the detection limit of the method, write "ND" (for nondetectable) or "< LDL" in the blank. If a sample is missing from the rack, write "NOSAX" in the blank. If a sample is not satisfactory, i.e., cannot be analyzed, write "UNSAX" in the blank. Print these subject data files and put a copy in the study folder(s).

3. Reporting

Give both types of forms to the supervisor along with the hard copy of the data printout. After the data is calculated and the final values are approved for release by the reviewing supervisor. The supervisor keeps 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 Inorganic Toxicology and Nutrition Branch sets up a "Specimen Folder." Fill out a tracking form and place it in the folder to be given to the analyst performing the analysis. The form tracks location, status, and final disposition of the specimens. When sample analysis is completed, place the tracking form in the *Specimen Tracking Record Log Book* located in the trace-metals library.

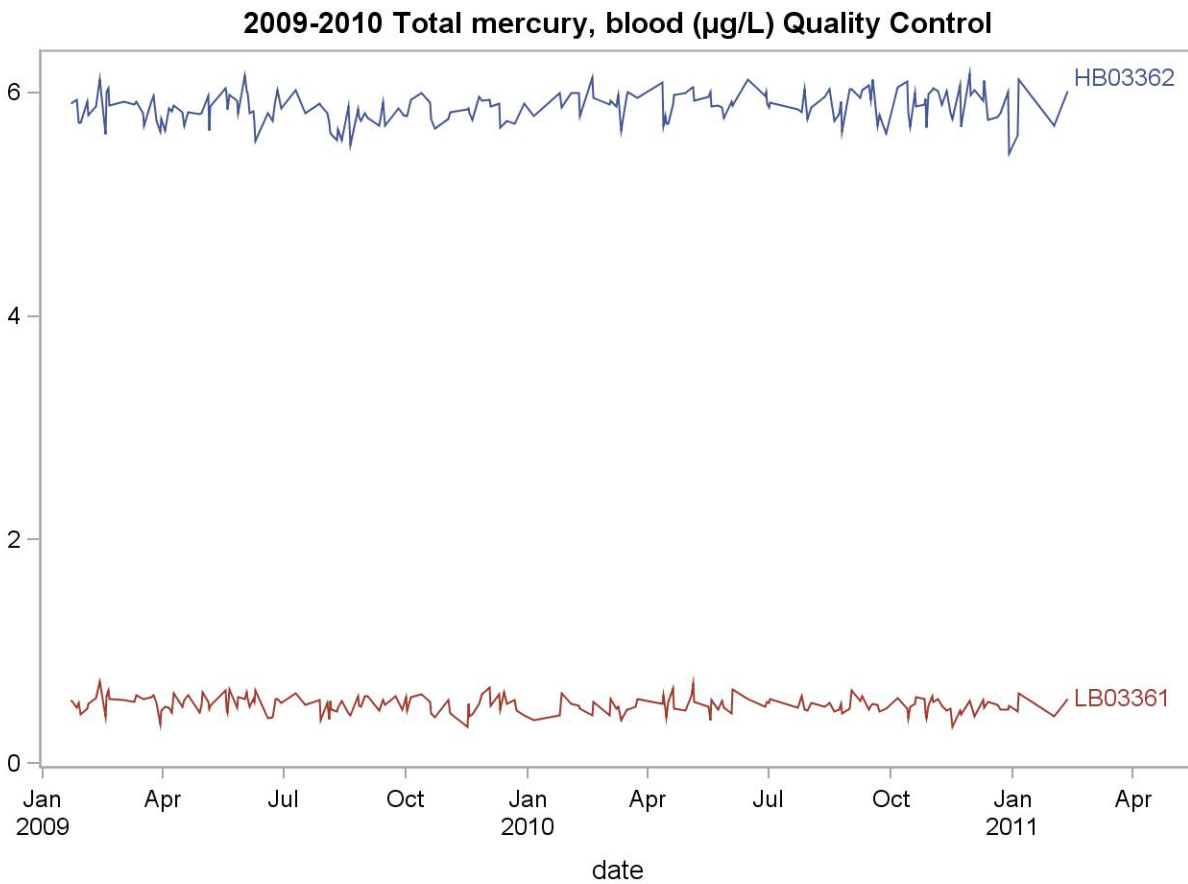
Use standard record keeping means (e.g., electronic – Microsoft Access™, optical disk, or tape backup) to track specimens. Maintain records at least 3 years. Include related Quality Assurance (QA)/QC data keep duplicate records (off site, if sensitive or critical) in electronic or hardcopy format. Use only numerical identifiers (e.g., case ID numbers). All personal identifiers are available only to the medical supervisor or project coordinator to safeguard confidentiality.

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19. Summary Statistics and QC Graphs

A. Total Mercury

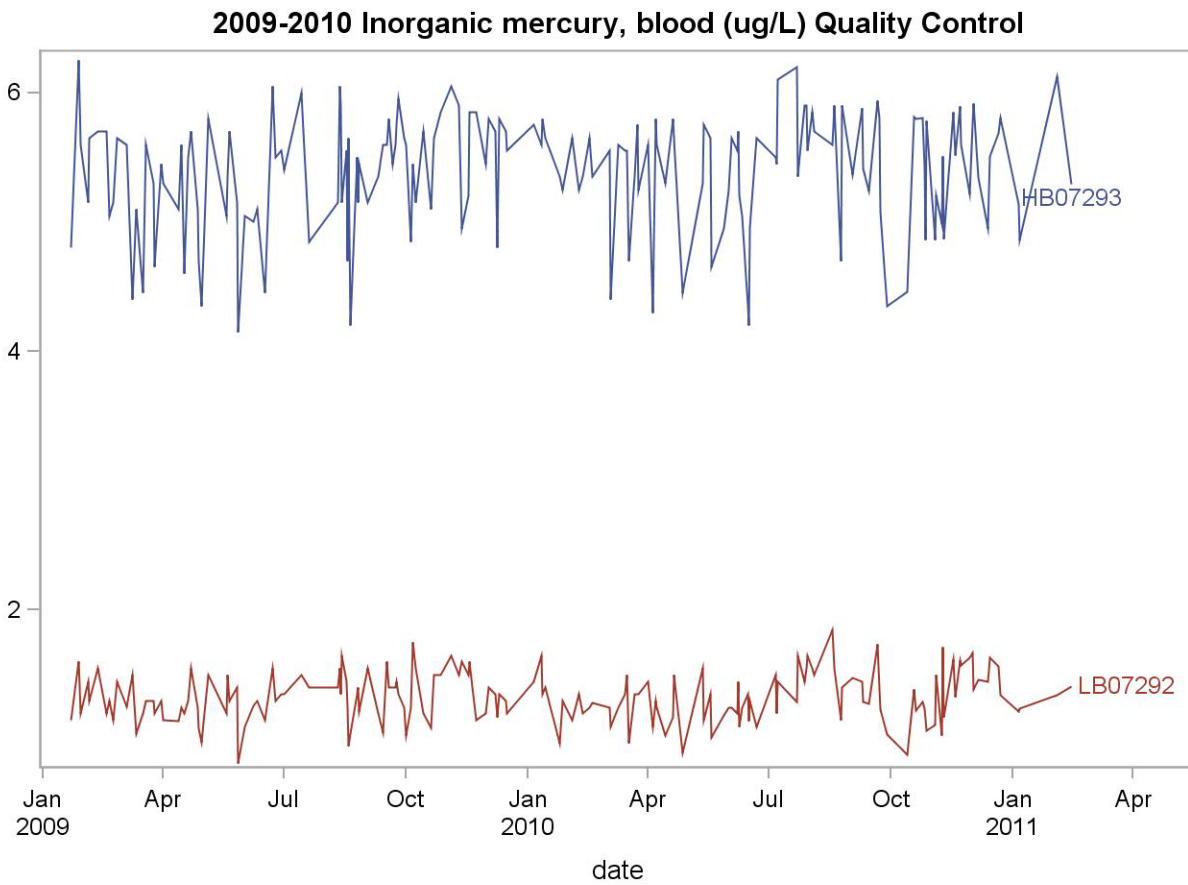
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HB03362	247	22JAN09	11FEB11	5.857	0.134	2.3
LB03361	248	22JAN09	11FEB11	0.516	0.078	15.1



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B. Inorganic Mercury

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HB07293	458	22JAN09	14FEB11	5.051	0.542	10.7
LB07292	449	22JAN09	14FEB11	1.205	0.215	17.8



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