Laboratory Procedure Manual

Analyte: **Mercury**

Matrix: **Urine**

Method: **Flow Injection Cold Vapor Atomic Absorption (CVAA)**

Method No.: **1180B/05-OD**

Revised:

as performed by: *Inorganic Toxicology & Nutrition Branch*
Division of Laboratory Sciences
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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 NHANES 1999–2000 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
lab06	URXUHG	Mercury, urine (ng/mL)

1. Clinical Relevance and Summary of Test Principle

Mercury in urine is measured by flow injection cold vapor atomic absorption analysis, which is based on the method that Guo and Bassner developed (1). Because mercury in urine is found almost entirely in the inorganic form, Guo and Bassner's method does not use microwave digestion, and decomposition of mercury compounds is achieved by manually adding mixed bromate-bromide reagent and concentrated hydrochloric acid (HCl). Further decomposition of mercury compounds is achieved by adding of potassium permanganate online. The mercury vapor (reduced from inorganic mercury compounds by sodium tetrahydroborate) is measured by the spectrophotometer at 253.7 nm (2–4).

Mercury analysis is performed to identify cases of mercury exposure or toxicity. The brain and kidneys are the main organs affected by mercury. Psychic and emotional disturbances are the initial signs of chronic intoxication by elemental mercury vapor or salts. Paresthesias 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. (5)

2. Safety Precautions

Follow universal precautions. Wear gloves, a lab coat, and safety glasses while handling all human urine products. Place in a biohazard autoclave bag disposable plastic, glass, and paper (e.g., pipette tips, gloves) that contact urine. Keep these bags in appropriate containers until they are sealed and autoclaved. Wipe down all work surfaces with a 10% sodium-hypochlorite solution when work is finished. Using the foot pedal on the Micromedic Digiflex reduces the analysts, contact with work surfaces that have been in contact with urine and also keeps the analyst's hands free to hold the specimen vials and to wipe the tip of Micromedic Digiflex.

Dispose all biological samples and diluted specimens in a biohazard autoclave bag at the end of the analysis.

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 severe eye and skin damage. Wear powder-free or "metal-free" gloves, a lab coat, and safety glasses. If 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 tetrahydroborate, potassium bromide, potassium bromate, and argon are available through the DLS computer network.

3. DLS Computerization; Data-System Management

A. Maintain the integrity of specimen and analytical data generated by this method by electronically uploading all the data onto a floppy disk and storing the analytical data in a master database system (custom database written in Microsoft Access). Store data files containing the date, analytical run identification (ID), specimen analytical results, specimen ID, and method code on a local hard drive and back up the files onto the floppy diskette that is used to upload the data to the database.

B. Routine backup procedures include weekly backup of data files, archival on 3.5" floppy diskettes, and archival on CD-ROM disk. Store off site those floppy disks or CD-ROM disks containing sensitive data.

C. Evaluate the analytical run by evaluating the calibration curve generated by the AA Winlab software. Use the AA Winlab Reformat Program to reformat data and save to 3.5" diskettes. Import these data into the Inorganic Toxicology & Nutrition Branch laboratory data management system for evaluation of QC parameters and for reporting and archiving.

D. NCEH LAN support staff will make sure that files stored on the NCEH network are automatically backed up to tape each night.

E. The laboratory research notebook and the maintenance 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, special diets are required.

B. The specimen type is urine with preservative, 50 µL of sulfamic acid with Triton X-100 solution (see 6a) per 5 mL of urine.

C. Optimal amount of specimen is 8-10 mL; minimum amount is about 1,000 µL (1.0 mL).

D. Acceptable containers include 15-mL plastic centrifuge tubes (Corning catalog # 25319) or polystyrene 15-mL centrifuge tubes (Falcon catalog # 2099). Use sterile collectors for specimen acquisition.

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

F. The criteria for unacceptable specimen are either a low volume (< 1.0 mL) or suspected contamination due to improper collection procedures or collection devices. Unpreserved specimens or specimens that have been stored at room temperature (20°C) are not acceptable. In all cases, request a second urine specimen.

G. Specimen characteristics that may compromise test results are indicated above and include high storage temperature or no preservative.

H. The division protocol outlines specimen-handling conditions for urine collection and handling. (Copies are available in the branch laboratory and special activities specimen-handling office.) The protocol discusses collection, transport, and special equipment requirements. In general, transport urine specimens and stored at –80°C to –20°C. Once received, store them at –80°C to –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 samples; however, keep them frozen whenever possible.

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 Tetrahydroborate Solution (0.2% sodium tetrahydroborate in 0.05% NaOH). Weigh out 2.0 g sodium tetrahydroborate and 0.5 g NaOH, and dissolve in 1 L of 3-megaOhm (MΩ) water. Add 1 mL of Dow Corning DB-110A antifoam agent to 1 L of reduction solution and mix by shaking the container. Prepare this solution daily.

(2) Potassium Permanganate Solutions (0.25% [w/v]). Dissolve 2.5 g of potassium permanganate in 1 L of 3 MΩ water. Make sure that the potassium permanganate crystals are dissolved completely. Store all the solutions in dark bottles and prepare weekly.

(3) Oxidation reagent Dissolve 4.0 g of potassium bromide and 1.1 of potassium bromate in 50 mL of 3 MΩ water. Add 0.6 mL of 100% Triton X-100 and mix well. Prepare solution weekly.

(4) Hydrochloric acid solution(s) For 5% (v/v), add 50 mL of concentrated hydrochloric acid (HCl) to several hundred of mL 3 MΩ water, then make up to 1 L with more water. For 32% (v/v), concentrated HCl no dilution.

(5) Triton solution (0.12% [v/v]). Dissolve 0.6 mL of 100% Triton X-100 in 500 mL of 3 MΩ water, then mix well.

(6) Carrier Solution Use 5% HCl.

(7) Preservative In 50 mL of 3 MΩ water, add 10 g of sulfamic acid and 5 mL of Triton X-100. Dissolve well, using a stirrer (hot plate might help dissolve the acid). Use 50 μL of preservative per 5 mL of urine.

B. Standards Preparation

(1) 1,000-mg/L Stock Mercury Standard (Inorganic). Using National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 3133 (10 mg/mL) or a NIST-traceable equilivant standard solution, dilute 1.00 mL of the standard solution using a calibrated micropipette, to 10 mL with 3 MΩ water in an acid-cleaned volumetric flask. Store solution in refrigerator. Prepare every 6 months in a flask dedicated to this solution.

(2) 10-mg/L Intermediate Mercury Standard Using a calibrated micropipette, dilute 1.00 mL of the 1,000-mg/L stock mercury standard to 100 mL with 3 MΩ water in an acid-cleaned volumetric flask. Store solution in refrigerator. 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 10% of HCl.

Table 1. Dilutions for Working Mercury Standards

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

(4) Calibration Standards

(a) Fill a series of 50-mL volumetric flasks with 10% HCl. Dispense mercury standards that will correspond to concentration levels of 1, 2, 5, 10, 20, and 30 μg/L.

(b) Using a Micromedic Digiflex, dispense 500 μl of the above standards into 15-mL tubes and dilute with 2 mL of 3 MΩ water.

(c) Using a calibrated Eppendorf pipette, dispense into each of the calibration tubes 0.1 mL of oxidation reagent ($KBr/KBrO_3$) and 0.25 mL of concentrated HCl (32%). Position

calibration standards in the autosampler (including a blank tube containing both the oxidant and concentrated HCl).

(d) The calibration curve (absorbance versus µg/L mercury) is calculated and plotted automatically by AA WinLab software (aqueous calibration using zero intercept: linear calibration).

C. Preparation of Quality Control (QC) Materials

Collect and preserve two pools of urine with sulfamic-acid solution and add inorganic mercury to make “bench” and “blind” QC materials. Dispense the QC pools into prescreened vials and stored frozen at –20°C.

D. Other Materials

(1) Stock solution of mercury: NIST SRM 3133, 10,000 mg/L, (National Institute of Standards and Technology, Gaithersburg, MD) or a NIST-traceable reference material.
(2) Ultrapure, concentrated hydrochloric acid (Ultrex J.T. Baker Chemical Co. [www.jtbaker.com]), or equivalent.
(3) Triton X-100 ("Baker Analyzed," J.T. Baker Chemical Co.,[www.jtbaker.com] or source whose product is be low in mercury contamination).
(4) Deionized, 3 MΩ water (house water).
(5) Potassium permanganate (any source low in mercury.)
(6) Sodium tetrahydroborate (any source low in mercury).
(7) Potassium bromide (any source low in mercury).
(8) Potassium bromate (any source low in mercury).
(9) 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.
(10) NIST SRM 2672a (urine mercury) or SRM 2670 (trace metals in urine). Run this periodically to verify accuracy.
(11) Urine QC pools with various levels of mercury that have reference values established by CVAAS or equivalent.
(12) Pipette tips: 1- to 200 µL and 200- to 1,000 µL sizes.
(13) Acid-cleaned volumetric flasks (1,000-, 100-, 50-, and 10-mL volumes).
(14) Kay-Dry paper towels and Kim-Wipe tissues (Kimberly-Clark Corp., Roswell, GA, or equivalent vendor).
(15) Powder-free nitrile, medical-examination gloves (N-Dex Best Manufacturing Co., Menlo, GA, or equivalent vendor.
(16) Biohazard autoclave bags (Curtin-Matheson Scientific, Inc., Atlanta, GA).
(17) Bleach (10% sodium-hypochlorite solution) from any vendor.
(18) Cotton swabs (Hardwood Products Co., ME or equivalent vendor).
(19) Small plastic weighing boats.
(20) 15-mL and 50-mL polystyrene conical tubes with caps (Becton, Dickinson Labware, Franklin Lakes, NJ).

E. Instrumentation

(1) PerkinElmer Flow Injection Mercury System 400 (FIMS-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), Flow Injection Analysis System 400 (FIAS 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).

(b) The AS-91 autosampler is a computer-controlled, multipurpose sampling system with 152 locations for 15-mL sample vessels.

(c) The FIAS 400 is a flow injection system used to transport various liquids. Pump 1 feeds the sample solution through the sample loop. Pump 2 feeds carrier solution (5% HCl) through the sample loop to carry the sample into the first manifold block, where the sample mixes with the potassium-permanganate solution. The reductant solution is introduced into the system at the second manifold block, where the mercury is reduced. The reaction mixture interacts with carrier gas at the third manifold block and is fed to the gas/liquid separator, where the elemental mercury vapor separates from the liquid. The carrier gas carries the mercury vapor to the FIMS cell. Recommended tubing configuration for the FIMS 400 is described in the book FIMS Installation, Maintenance, System Description.

Table 2. Analytical Parameters for FIMS 400

Parameter	Setting
Wavelength	253.7 nm
Slit width	0.7 nm
Signal type	AA
Signal measurement	Peak height
Smoothing point	9
Read time	20 (sec)
Read delay	0
BOC time	5

Table 3. Injection Program: Urine Mercury Measurement*

Step	Time (sec)**	Pump 1 Speed	Pump 2 Speed	Valve Position	Read Step
Prefill	5	100	120	Fill	
1	10	100	120	Fill	
2	5	0	120	Inject	X
3	1	0	120	Fill	

* Instrument model: FIAS 400. Sample volume: 500 µL (no amalgam)

** Flow time for each step may need to be adjusted 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).

(1) 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).

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

(3) Milli-Q water-purification system (Millipore Corporation, Bedford, MA).

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

(5) Eppendorf fixed-volume micropipettes: 1,000-, 500-, 250-, 200-, 100, 50-, 40- and 10-µL volumes (Brinkmann Instruments, Inc., Westbury, NY).

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

(7) Flow meter (Cole-Parmer Instrument Co., Vernon Hills, IL).

7. Calibration and Calibration-Verification Procedures

A. Use an aqueous, linear, zero-intercept calibration method. Prepare calibration solutions at different mercury levels using various mercury standard solutions. Construct a calibration curve with AA WinLab software using the measured mean values of absorbance of standards at 1, 2, 5, 10, 20, and 30 µg/L, plotted versus concentration.

B. Once the instrument calibration has been completed, 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 0.0019-0.0035.

C. Verify calibration using QC material certified for mercury concentration. Suitable material is NIST SRM 2672a (or an equivalent SRM). Run this control material once each month. Agreement with certified values should be ± 10%.

8. Operating Procedures; Calculations; Interpretation of Results

A. Preliminaries and Sample Preparation

(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 urine specimens, QC specimens, and calibration material to reach ambient temperature, then mix on a vortex mixer for 10 seconds.

(3) While the specimens are thawing, turn on the FIMS computer, printer, and the FIMS instrument and allow the instrument to warm up a minimum of 30 minutes.

(4) Prepare the following solutions: 0.2% sodium tetrahydroborate in 0.05% sodium hydroxide (NaOH) and 0.25% potassium permanganate.

(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 flow meter to 20 mL/min.

(3) Change the filter paper in the separator block.

(4) The FIMS computer automatically controls the FIMS/FIAS. 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.” Note that five windows will appear on the screen: AUTOMATED ANALYSIS CONTROL, EXAMINE PEAK, RESULTS, EXAMINE CALIBRATION, and FIAS CONTROL.

(5) Click on the FIAS CONTROL window to make it active. Start pump 1 and pump 2 to pump ultrapure water through the FIAS system, making sure that 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 filled with water.)

(6) Select “Open Method” from the “File” menu. Select the method for urine mercury measurement.

(7) Select “New Sample Info File” from the “File” menu. Input the sample information as the order of sample loaded on the autosampler. 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. Type in the data file name.

(9) Fill the blank container/wash beaker at autosampler position “0” with ultrapure water. Check the waste container and empty it, if necessary.

C. Instrument Operation

(1) For preparation of calibration standard solutions, see Section 6.B.(4)(a)–(c).

(2) Pipette 500 µL of QCs and unknown specimens into 15-mL conical tubes and dilute with 2.0 mL of ultrapure, 3 MΩ water (using Digiflex).

(3) Add 0.1 mL of oxidation solution (KBr/KBrO3) and 0.25 mL of concentrated HCl to each tube. Vortex for 5 seconds.

(4) Load QCs and unknowns onto AS-91 autosampler starting at A/S Location #9.

(5) Click on the FIAS window to activate it. Start pump 1 at 100 and pump 2 at 120.

(6) Place the carrier tubing inlet in a 5% HCl solution, the reductant tubing inlet in a 0.2% sodium-tetrahydroborate solution, and then the potassium-permanganate tubing inlet in a 0.25% potassium-permanganate solution.

(7) In the AUTOMATED ANALYSIS CONTROL window, click on the “Analyze” tab at the bottom of this window. Click on the button marked “Calibrate.” The software will run the calibration blank and each standard in duplicate. If the blank’s absorbance is > 0.0003, wash the system with 50% HCl and try again. If a high blank remains, stop the run and call the supervisor.

(8) While the program is running, access the DISPLAY CALIBRATION window. Notify the supervisor if the correlation coefficient is < 0.990 or if the slope is outside 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 make sure the results are displayed. If they are not, notify the supervisor.

(9) When a run is finished, the following analysis progress information will be displayed on the AUTOMATED ANALYSIS CONTROL window; “Sample Complete . . . 100%,” “Current Status . . . Idle.” If more than one set of samples are to be analyzed, 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.

(10) When all analyses have been completed, let both pumps continue to pump. Start the rinsing procedure in the following order:

(a) Remove the inlet of potassium permanganate tube from the brown bottle and place it in a wash container (3 MΩ water), and pump until the tube is clear.

(b) Place the reductant-reagent tubing inlet in the same wash container, and pump for 30 seconds.

(c) Place the carrier in the same container, and pump all the tubes for 5 minutes. Within this wash period, access FIAS AUTOMATED ANALYSIS CONTROL window and 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.

(d) Rinse all the tubes with a 10% HCl solution for 5 minutes.

(e) Rinse all the tubes with a solution of HCl:H_2O = 50:50 for 3-5 minutes.

(f) Place all the tubes back in the wash container and rinse them for 10 minutes.

(11) 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.

(12) Exit from the program (under "File"). Turn off the Argon gas, instrument, and computer. Put the standards, QCs, and urine samples back into the refrigerator.

(13) Place the printed output of the runs in a data file folder. Make a record of the runs in the laboratory research notebook. Also, enter any other maintenance items or problems in notebook. Back up and archive the data files weekly.

D. Replacement and Periodic Maintenance of Key Components

(1) Keep a spare FIAS valve on hand. If there is any leakage, remove the valve from the pump unit and clean the individual parts of the valve with deionized water.

(2) Keep a spare manifold and gas/liquid separator on hand. The manifold blocks are blocked easily by reductant solutions. Clean them with soapy water.

(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 all the pump tubes after every 7-day run period.

(5) Keep a spare sample probe on hand. Clean the probe with cleaning wire for AS 91 Probe, if necessary.

E. Calculations

(1) The method described here is linear up to at least 30 µg/L (the highest concentration point in the calibration standards). The calibration curve (linear regression) is generated by the AA WinLab software. 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, the slope should be greater than 0.0019. The software also calculates the standard concentration and sample concentration (blank corrected) of each replicate and mean, standard deviation (SD), and % Relative Standard Deviation for each sample, and more.

(2) 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) Reanalyze specimens containing more than 20 µg/L of mercury for confirmation. For a specimen with a concentration greater than 30 µg/L, dilute the sample with 3 MΩ water and reanalyze it. Multiply the result by the appropriate dilution factor.

(4) The detection limit, based on 3 times the SD of 11 repeat measurements of a sample with low mercury concentration, is 0.1 µg/L. Report results below the detection limits as nondetectable (ND; refer to Section 17.B., “Test-Result Reporting System,” in this document).

9. Reportable Range of Results

Urine mercury values are reportable in the range LDL < mercury urine (HgU) < 30 µg/L without dilution, where LDL = the calculated (3 SD) lower detection limit. If a urine mercury value is less than 0.1 µg/L (the approximate LDL of this method), it should be reported as < 0.1 µg/L. If greater than 30 µg/L, the sample should be diluted with deionized water and the specimen should be reanalyzed. The result must then be multiplied 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 purpose of environmental and occupational health studies. The method is accurate, precise, and reliable. The primary standard is a NIST SRM. Estimates of imprecision can be generated from long-term, QC pool results.

This analytical method utilizes two types of QC systems. With one of the QC system, the analyst inserts "bench" QC specimens several 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 results of the blind specimens are decoded and reviewed by the supervisor. With both systems, all levels of mercury concentration are assessed by taking these samples through the complete analytical process. The data from these materials are then used to estimate methodological imprecision and to assess the magnitude of any time-associated trends.

The method utilizes three bench QC pools. The mercury level of these QC pools ranges from 2 µg/L to 20 µg/L. Periodically use reference materials (urine 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 QC pool. An analysis of variance is performed for each pool after characterization runs have been performed in which previously characterized 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 standards and bench QC materials are analyzed (at the beginning of an analytical run), evaluate QC materials to determine if the system is "in control." Two types of charts are 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:

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 quality control rules apply to the average of the beginning and ending analyses of each of the bench QC pools. The quality control rules are as follows:

(1) If both the low and the high 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) 1_{3s} – Average of both low QC results OR average of both high QC results is outside of a 3s limit).

(b) 2_{2s} – 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) R_{4s} 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) 10_x 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 the analysis results for all patient samples analyzed during that run are invalid for reporting.

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, then take the following steps in succession:

A. Prepare fresh dilutions of all urine QC samples and reanalyze them.

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

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. Report no analytical results for runs not in statistical control.

12. Limitations of Method; Interfering Substances and Conditions

This method has been validated with urine specimens with target values obtained from reference (expert) laboratories (NIST SRM 2672a or SRM 2670 and Quebec Toxicology Center (CTQ).) 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 urine mercury values below about 0.1 µg/L.

13. Reference Ranges (Normal Values)

A. CDC Recommendations

CDC recommendations have not been determined.

B. Other References

(1) Of 1,107 urine specimens in a multinational survey (15 countries), 95% were 20 µg/L or less (LDL = 0.5 µg/L) (3).

(2) Reference values for urine mercury excretion are given as < 5 µg/L or 1–2 µg mercury/day (4,5).

14. Critical-Call Results (Panic Values)

A. Pediatric > 200 µg/L

B. Adult > 200 µg/L – Occupational Safety and Health Administration (OSHA) has set an 8-hour exposure limit of air mercury levels of > 0.05 mg/m^3 inorganic mercury and > 0.01 mg/m^3 organic mercury.

C. Notify the principal investigator or supervising physician of laboratory results if either situation occurs.

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.

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

Since the analysis of urine mercury is inherently complex and challenging, there are no acceptable alternative methods of analysis and no backup system. 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 urine specimens between –80°C and –20°C.

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

The principal investigator or supervising physician should be notified as soon as possible if urine mercury results are 200 µg/L (pediatric) or > 200 µg/L (adults). Utilize the most expeditious means of notifying personnel; e.g., telephone, facsimile, email.

A. Quality Control Data

Use the ITN Access database to evaluate the analytical run. If the analyst determines that the run is in control, the data is relayed to the supervisor, who approves the data so that it can be reported. Place a copy of the QC report from the database in the study folder(s).

B. Analytical Results

Reformat the data file by using the Reformat AA WinLab program, then download the data file for calculation or reporting. Record the results for urine 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).

C. Reporting

Give both types of forms to the supervisor along with the hard copy of the data printout. Once the data is calculated and the final values are approved for release by the reviewing supervisor. The supervisor will keep the original copies of the reporting sheets.

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

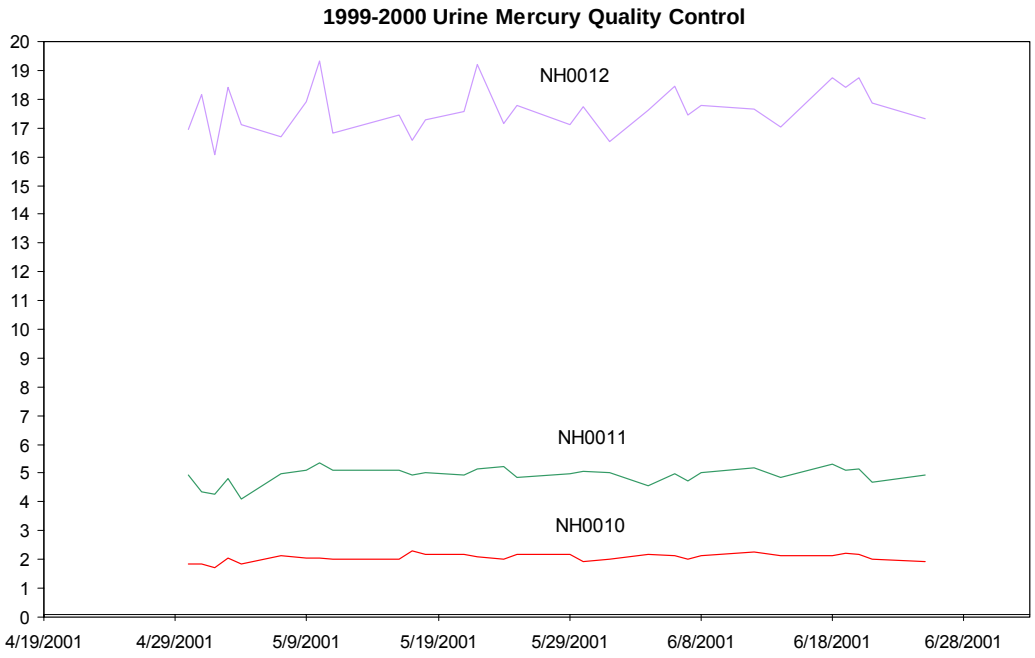
    The analyst who receives specimen/samples delivered to ITN sets up a “Specimen Folder.” Fill out a tracking form and place it in the folder to be given to the analyst performing the analysis. The form tracks location, status, and final disposition of the specimens. When the sample analysis is completed, 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, tape backup) to track specimens. Maintain records for at least 3 years, including related Quality Assurance (QA)/QC data; keep duplicate records (offsite, if sensitive or critical) in electronic or hardcopy format. Use only numerical identifiers (e.g., case ID numbers); In order to safeguard confidentiality, provide only the medical supervisor or the project coordinator with personal identifiers.

19. Summary Statistics and QC Graphs

Summary Statistics for Urine Mercury by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH0010	30	3/26/2001	6/25/2001	2.06	0.025	1.20
NH0011	30	3/26/2001	6/25/2001	4.93	0.053	1.07
NH0012	30	3/26/2001	6/25/2001	17.63	0.145	0.82



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