



Laboratory Procedure Manual

Analyte: **Iodine & Mercury**

Matrix: **Urine**

Method: **Inductively Coupled Plasma Dynamic
Reaction Cell Mass Spectrometry
(ICP-DRC-MS)**

Method No: **ITU007B**

Revised:

as performed by:

Inorganic Toxicology and Nutrition Branch
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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.

Procedure Change Log

Procedure: Iodine and Mercury in Urine DLS Method Code:

Date	Changes Made	By	Rev By (Initials)	Date Reviewed
4/10/06	Section 7 revised to prepare intermediate standards using volumetric flasks rather than pipetting into disposable tubes.	JJ		
4/10/06	Corrected concentration listed for "Concentrated" hydrochloric acid (was 6M, but concentrated is actually 12M).	JJ		
9/27/06	Clarified that method can be run either as single element (I or Hg) or as a multi-element method.	JJ		
9/27/06	Added "failure to add preservative to collected urine" as a criteria for an unacceptable urine sample for mercury analysis.	JJ		
9/27/06	Added statement allowing use of vendor-purchased, NIST-traceable standards at the concentrations of the intermediate standards.	JJ		
9/27/06	Enhanced description of sulfamic acid preservative, Triton X-100, internal standard intermediate, and diluent solution preparations	JJ		
9/27/06	Concentration of gold in diluent and rinse solution reduced from 1000 ug/L to 500 ug/L.	JJ		
9/27/06	Changed expiration of intermediate working standards to 1 week from date of preparation.	JJ		
9/27/06	Clarified use of reference materials for periodic evaluation of method accuracy	JJ		
9/27/06	Added the ELAN DRC Plus as an acceptable system for method application	JJ		
9/27/06	Clarified when equivalent types of equipment could be substituted for those specified.	JJ		
9/27/06	Clarified requirements of calibration verification	JJ		
9/27/06	Clarified when and how to perform extra dilution of patient samples, based on implementation of periodic calibration verification	JJ		
9/27/06	Clarified the proper procedure for conditioning the instrument prior to DRC analysis	JJ		
9/27/06	Changed method file name to link to CLIA method name and explained acceptable reasons and formats for editing method file names.	JJ		
9/27/06	Expanded typical run size to 40-80 patient samples	JJ		
9/27/06	Clarified procedures for final review of data including treatment of results abnormally low or high or with inadequate precision as well as reporting procedures.	JJ		
9/27/06	Clarified quality control and corrective actions procedures	JJ		
12/29/06	Added 200s extra rinse after iodine measurements exceeding 2000 ug/L.	JJ		

Public Release Data Set Information

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

Data File Name	Variable Name	SAS Label
UHG_E	URXUHG	Urinary Mercury(ng/mL)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

A. Clinical Relevance

Iodine (I), an essential element for thyroid function, is necessary for normal growth, development, and functioning of the brain and body. Iodine-deficiency disorders (IDDs) are well-documented global health problems affecting more than a billion people worldwide. Consequences of IDD include goiter, cretinism, intellectual impairment, brain damage, mental retardation, stillbirth, spontaneous abortions, miscarriages, congenital deformities, and increased perinatal mortality. Progress toward eliminating IDDs has been substantial; an estimated 70% of the world's edible salt currently is iodized. Most excess iodine is excreted, and most people can tolerate fairly large amounts without experiencing problems. People with a tendency towards autoimmune thyroid disease are less tolerant of excess iodine. If a person has previously been iodine deficient, that person may be at risk for iodine-induced hyperthyroidism. Excessive iodine intake by a mother can pose a reproductive risk. Since urinary iodine values directly reflect dietary iodine intake, urinary iodine analysis is the recommended and most common method for biochemically assessing the iodine status of a population (1). On the other hand, Mercury (Hg) is a toxic non-essential element that can affect various organ systems within the body but especially the central nervous system. The main sources of mercury intake in humans are fish, dental amalgams, and occupational exposure. The main organs affected by mercury are the brain and the kidneys (2). Psychic and emotional disturbances are the initial signs of chronic intoxication by elemental mercury vapors or salts. Parasthesia, neuralgias, renal disease, digestive disturbances, and ocular lesions may develop (3). Massive exposure over a longer period of time results in violent muscular spasms, hallucinations, delirium, and death (4). The determination of total Hg in blood and urine are both used to assess the internal exposure. Since urine can be collected non-invasively, it is more commonly used to assess exposure to mercury, particularly in occupational health settings where biomonitoring of random spot urine samples is routinely practiced. This method is used to achieve rapid and accurate quantification of iodine (I) and / or mercury (Hg) in urine. The method can be used to analyze urine for both elements at the same time, or just one of the elements.

Mercury is widespread in the environment and originates from natural and anthropogenic sources. The general population may be exposed to three forms of mercury: elemental, inorganic, or organic (primarily methylmercury). The concentration of total mercury in urine is a biomeasure of exposure primarily to elemental and inorganic mercury. Elemental and inorganic mercury exposure can result from mercury spills, dental amalgams, and occupational exposures. Both elemental and inorganic mercury are nephrotoxic and neurotoxic. Health effects related to low exposure in the general population are not well defined. In the 1999-2002 NHANES, urine mercury levels were measured in all women aged 16-49 years. In 2003-2004, urine mercury levels are measured in a one-third subsample of participants aged 6 years and older.

B. Test Principle

Urine iodine and mercury concentrations are determined by ICP-DRC-MS (Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectroscopy). This multielement analytical technique is based on quadrupole ICP-MS technology (5) and includes DRC™ technology (6, 7). Coupling radio frequency power into a flowing argon stream seeded with electrons creates the plasma, the heat source, which is ionized gas suspended in a magnetic field. Predominant species in the plasma are positive argon ions and electrons. Diluted urine samples are converted into an aerosol using a nebulizer inserted within the spray chamber. A portion of the aerosol is transported through the spray chamber and then through the

central channel of the plasma, where it is exposed to temperatures of 6000-8000 K. This thermal energy atomizes and ionizes the sample. The ions and the argon enter the mass spectrometer through an interface that separates the ICP, which is operating at atmospheric pressure (approximately 760 torr), from the mass spectrometer, which is operating at approximately 10^{-5} torr. The mass spectrometer permits detection of ions at each mass-to-charge ratio in rapid sequence, which allows the determination of individual isotopes of an element. Once inside the mass spectrometer, the ions pass through the ion optics, then through DRC™, and finally through the mass-analyzing quadrupole before being detected as they strike the surface of the detector. The ion optics uses an electrical field to focus the ion beam into the DRC™. The DRC™ component is pressurized with an appropriate reaction gas and contains a quadrupole. Electrical signals resulting from the detection of the ions are processed into digital information that is used to indicate the intensity of the ions and subsequently the concentration of the element. Traditionally ICP-MS has been a trace analysis technique and the typical measurement ranges from < 1 µg/L to around 100 µg/L. DRC technology provides additional control of ICP-MS sensitivity; therefore appropriate adjustments of the reaction cell parameters can significantly extend the useful concentration measurement range. In this method, iodine (isotope mass 127), tellurium (isotope mass 130), mercury (isotope mass 202) and bismuth (isotope mass 209) are measured in urine by ICP-DRC-MS using 100% argon as the Dynamic Reaction Cell™ (DRC) gas utilizing collisional focusing. Urine samples are diluted 1+1+ 8 (sample+ water + diluent) with water and diluent containing tellurium and bismuth for internal standardization.

2. SAFETY PRECAUTIONS

Precautionary information that is important to protecting personnel and safeguarding equipment will be presented inside a box, such as this one, throughout the procedure where appropriate.

Follow universal precautions. Wear gloves, a lab coat, and safety glasses while handling human blood, plasma, serum, urine or other bodily fluid or tissue. Place disposable plastic, glass, and paper (e.g., pipette tips, autosampler tubes and gloves) that come in contact with human biological fluids, such as urine, in a biohazard autoclave bag. Keep these bags in appropriate containers until they are sealed and autoclaved. When work is finished, wipe down all work surfaces where human biological fluid was handled with a 10% (v/v) sodium hypochlorite solution or equivalent. The use of the foot pedal on the Micromedic Digiflex™ is recommended because it reduces analyst contact with work surfaces that have been in contact with human biological fluid and also keeps the hands free to hold specimen cups and autosampler tubes. Dispose of all biological samples and diluted specimens in a biohazard autoclave bag at the end of the analysis according to CDC/DLS guidelines for disposal of hazardous waste.

PerkinElmer provides safety information that should be read before operating the instrument. This information is found in the PerkinElmer ELAN ICP-DRC-MS System Safety Manual. Possible hazards include ultraviolet radiation, high voltages, radio-frequency radiation, and high temperatures.

Caution!

Exercise caution when handling and dispensing concentrated nitric acid and base Tetramethylammonium hydroxide (TMAH). Always remember to add acid to water. Nitric acid and TMAH are caustic chemicals that are capable of severe eye and skin damage. Wear powder-free gloves, a lab coat, and safety glasses. If nitric acid or TMAH comes in contact with any part of the body, quickly wash the exposed area with copious quantities of water for at least 15 minutes.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

To maintain the integrity of specimen and analytical data generated by this method, eliminate hand entry of specimen identifiers or analytical results whenever possible, proofread all transcribed data, and regularly defragment and back up the ICP-MS computer's hard drive.

A. Data Entry and Transfer

Whenever possible, use bar code scanners to enter sample identifiers into the ICP-DRC-MS computer software to avoid errors associated with the keyboard-entry process and to speed up sample processing. When bar code scanners cannot be used, proofread transcribed data after entry. Handle or transfer data electronically when reporting or moving data to other computerized data-handling software. In the Inorganic Toxicology and Nutrition Branch, sample analysis results generated by this method are stored for long periods in Microsoft Access™ or MS SQL Server database software. The results should include at least the analysis date, analytical run number, quality-control (QC) results for the run, results of specimen analysis by specimen identification (ID), and method identifier.

1. Routine Computer Hard Drive Maintenance

Defragment the computer hard drive regularly by using software such as Microsoft Windows® Disk Defragmenter (located in Start > Programs > Accessories > System Tools) or an equivalent backup program to maximize computer performance and maintain data integrity for files on the hard drive. An entry will automatically be made in the Windows™ system event log when this process is done and will provide documentation of this step.

2. Data Backup

Schedule of Data Backups

Weekly. Full data backups onto one or more recordable compact discs (CD-R) or digital video discs (DVD).

Daily. Full data backups onto an external hard drive.

(1) Backup Procedures

Whenever making a backup (daily or weekly) include the directories and subdirectories:
C:\elandata (include all subdirectories)

Before making weekly backups, saving a copy of the Windows™ event log in the active "elandata" directory will ensure archiving of all recent software system events (including communications between ICP-DRC-MS and ELAN® software, as well as times of hard drive defragmentation, and other Windows™ system events).

(a) External Hard Disk Backups

Connect the ELAN data system computer to an external hard disk with sufficient storage capacity to store several copies of the backup files (≥ 18 gigabytes).

Configure Microsoft Windows® Backup™ (located in Start > Programs > Accessories > System Tools) program to do a daily backup of the ELAN data system computer's data directories (see

(1) Backup Procedures)

(b) Compact Disc Backups

Use CD-R disks only (recordable compact disks), *not* CD-RW disks (rewritable compact disks).

Record the CD-R so that after creation the recordable compact disk cannot be written to again (to prevent any accidental over-writing of stored data).

Use Adaptec "Easy CD Creator"™ or equivalent software to backup.

(c) Removing Data from the ICP-DRC-MS Computer Hard Drive

When the active "elandata" directory on the ICP-DRC-MS computer hard drive becomes too large to fit onto a single recordable compact disk, remove the oldest data on the hard drive so that a regular backup can be done onto a single CD-R. Usually, this procedure can be done annually.

Back up the oldest data on the hard drive in duplicate onto two CD-R disks. Manually select each dataset folder (subdirectories under "C:\elandata\dataset" and other relevant files (i.e., optimization, tuning, and sample files) that are to be included on these backups. Verify that backup CD-R disks operate correctly before deleting any data from the hard drive. To verify the operation of a CD-R disk, open any file on the disk by using the appropriate computer software (ICP-DRC-MS software).

After verifying that all backups are operational, delete the original data from the hard drive. Keep one copy of the CD-R disk in a building other than the laboratory in case of fire (currently, the storage room is room 1011 in building 103). Keep the other near the ICP-MS laboratory.

(d) Backup of Sensitive Data

Make a backup for sensitive data on duplicate, recordable compact disk. Store the two CD-R disks in two different buildings.

B. Documentation of System Maintenance

Computer Maintenance: Record any maintenance of computer hardware and ICP-DRC-MS software in the instrument logbook. Place other electronic records relating to integrity of the data and hard drive in the Windows™ event log. Back up the event log on a regular basis by saving a copy in the active "elandata" directory. The event log will then be backed up along with the ELAN data when backup CD-R disks and tapes are made.

Instrument Maintenance: Document system maintenance in hard copies of data records (i.e., daily maintenance checklists, PerkinElmer service records, and instrument log book) as well as in electronic records relating to instrument optimization (default.dac), tuning (default.tun).

4. COLLECTING, STORING, AND HANDLING SPECIMENS; CRITERIA FOR REJECTING SPECIMENS

A. Specimen Type

Specimen type is urine. No special instructions for fasting or special diets are required of patient or study subjects.

B. Specimen Collection, Handling and Storage

1. No special instructions for fasting, special diets are required.
2. The specimen type is urine with preservative (for mercury). The preservative is a solution of approximately 2 M sulfamic acid and 0.1% Triton X-100™. It is added for the purpose of preventing loss of mercury from the urine before analysis. Urine should be mixed with the preservative as soon as possible after initial collection in the proportion of 10 µL of preservative solution per 1 mL of urine

(example: To a tube containing 50 µL of preservative, up to 5 mL of urine can be added for urine mercury analysis). Mix the urine well after addition of the preservative. See Section 6.b. for details on preparation of the preservative solution.

3. Optimal amount of specimen is 1.8 mL; minimum amount in a cryo-vial is about 0.75 mL. 500 µL needed for an analysis.
4. Acceptable containers for allotment of urine for this method include 15 mL PP centrifuge tubes (e.g., Becton, Dickinson and Company model number 352097). Use sterile collectors for specimen acquisition.
5. Screen specimen collection cups, containers and sample tubes for iodine and mercury contamination before use.
6. Specimen stability has been demonstrated for 1 year at $\leq -20^{\circ}\text{C}$.
7. Specimen characteristics that may compromise test results are indicated above and include high storage temperature or no preservative.
8. Specimen handling conditions are outlined in the division protocol for urine collection and handling. Copies are available in the branch, laboratory and special activities specimen-handling offices. The protocol addresses collection, transport, and special-equipment requirements. In general, transport and store urine specimens at $\leq -20^{\circ}\text{C}$. Upon receipt, freeze the specimens at $\leq -20^{\circ}\text{C}$ until time for analysis. The analyst puts the remaining samples in the freezer after analytical aliquots are done and refreezes them at $\leq -20^{\circ}\text{C}$. Samples that are thawed and refrozen several times will not be compromised.

C. Criteria for an Unacceptable Specimen

The criteria for an unacceptable specimen are either a low volume (< 0.75 mL), suspected contamination due to improper collection procedures or collection devices, or failure to add the proper preservative to urine to prevent the loss of mercury. The volume of urine used in a single analysis is 0.5 mL, but sample volumes < 0.75 mL may not allow for proper pipetting. Requested volume is > 1.8 mL to allow for repeat / confirmation analysis if necessary. Specimen contact with dust or dirt may compromise test results. In all cases, request a second urine specimen.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS

Not applicable for this procedure.

6. CHEMICALS, STANDARDS, AND QUALITY CONTROL MATERIAL

A. Chemicals

9. Water, high purity (≥ 18 M Ω -cm resistivity using a NANOpure Diamond Ultrapure Water System or equivalent).
10. TritonX-100™ ("Baker Analyzed," J.T. Baker Chemical Co. [www.jtbaker.com], or any source whose product is low in trace-metal contamination).
11. Sulfamic Acid ("Baker Analyzed," J.T. Baker Inc., Phillipsburg, NJ)
12. Double-distilled concentrated nitric acid (GFS Chemicals Inc., Columbus, OH or equivalent vendor. Concentrated nitric acid is 70% or 16M. If other stock concentrations are used, volumes must be adjusted accordingly).

13. Double-distilled concentrated hydrochloric acid (GFS Chemicals Inc., Columbus, OH) or equivalent vendor. Concentrated hydrochloric acid is 37% or 12M. If other stock concentrations are used, volumes must be adjusted accordingly.
14. Ethyl Alcohol (C₂H₅OH), USP dehydrated 200 proof (Pharmco Products, Inc. or equivalent).
15. Stock solution of mercury (Hg): SPEX, 1,000 mg/L in 10% HNO₃ (SPEX Industries, Inc. 3880 Park Ave., Edison, NJ 08820), or equivalent NIST traceable stock solution. Alternatively, NIST traceable mercury standards can be purchased from vendors at the concentrations of the intermediate standards listed in section 7.b.
16. Stock solution of iodide (I): SPEX, 1,000 mg/L in H₂O (SPEX Industries, Inc. 3880 Park Ave., Edison, NJ 08820) made from NaI, or equivalent NIST traceable stock solution. Alternatively, NIST traceable iodide standards can be purchased from vendors at the concentrations of the intermediate standards listed in section 7.b.
17. Bismuth (Bi): SPEX, 1,000 mg/L in 10% HNO₃ (SPEX Industries, Inc., Chemical Sales Dept. 3880 Park Ave, Edison, NJ, USA) or NIST traceable equivalent.
18. Tellurium (Te): SPEX, 1,000 mg/L in 10% HNO₃ (SPEX Industries, Inc., Chemical Sales Dept. 3880 Park Ave, Edison, NJ, USA) or NIST traceable equivalent.
19. Gold (Au): SPEX, 1,000 mg/L in 10% HCl (SPEX Industries, Inc., Chemical Sales Dept. 3880 Park Ave, Edison, NJ, USA) or NIST traceable equivalent.
20. Disodium Ethylenediamine Tetraacetate (EDTA) (Fisher scientific Comp., Chemical manufacture Division, Fair Lawn, NJ 07410 or equivalent).
21. Tetramethylammonium hydroxide (TMAH), 25% w/w, or equivalent (AlfaAesar, 30 Bond St., Ward Hill, MA 01835).
22. Bleach (10% sodium hypochlorite solution) or equivalent - any vendor.
23. Liquid argon (supplied by Speciality Gases or other contract agency) equipped with approved gas regulator (Matheson Gas Products, Secaucus, NJ – or equivalent).
- 24.

B. Reagent Preparation

Preservative

For collected urine (approximately 2 M sulfamic acid, 0.1% Triton X-100™).

Partially fill a pre-screened or pre-acid-washed 50 mL polypropylene centrifuge tube with ≥18 Mohm cm⁻¹ water. Add 10 g of sulfamic acid and 5 mL of 1% Triton X-100™. Fill to the 50 mL mark with ≥18 Mohm cm⁻¹ water. Dissolve the sulfamic acid by mixing well (use of a vortexer, or warm water bath is helpful in this process). Store at room temperature. Expiration is 1 year from preparation.

For Intermediate Standards (approximately 2 M sulfamic acid).

Partially fill a pre-screened or pre-acid-washed 50 mL polypropylene centrifuge tube with ≥18 Mohm cm⁻¹ water. Add 10 g of sulfamic acid. Fill to the 50 mL mark with ≥18 Mohm cm⁻¹ water. Dissolve the sulfamic acid by mixing well (use of a vortexer, or warm water bath is helpful in this process). Store at room temperature. Expiration is 1 year from preparation.

Diluent (1% (v/v) TMAH, 0.02% Triton X-100™, 25 µg/L Te, 5 µg/ Bi, 5% (v/v) C₂H₅OH, 500 µg/L Au, 0.5 g/L EDTA).

Triton X-100 intermediate solution (1%)

For ease of daily preparation of the diluent solution, first prepare a 1% Triton X-100™ stock solution by adding 20 mL of Triton X-100™ to a preacid-washed 2 L container (Teflon™ preferred) that is partially filled with ≥18 MΩ·cm water. Fill to 2 L with ≥18 MΩ·cm water, add an acid-washed, Teflon™ coated stirring bar, and stir on a magnetic stirrer until the Triton X-100™ has completely dissolved into solution.

Internal Standard Intermediate Solution.

Partially fill a pre-screened or pre-acid-washed 50 mL polypropylene centrifuge tube with ≥ 18 Mohm cm^{-1} water. Add 5 mL Te stock (1000 mg/L) and 1 mL Bi stock (1000 mg/L), and then fill to the 50 mL mark with ≥ 18 Mohm cm^{-1} water. Mix well and store at room temperature. To prepare different volumes adjust the solution constituents proportionally.

Diluent.

Acid-rinse a 2 L container (Teflon™ preferred), and partially fill with ≥ 18 M Ω -cm water. Add the following, mixing in between each addition: 20 mL of 25% (v/v) TMAH, 40 mL of 1% Triton X-100™, 100 mL C₂H₅OH, 1 mL of 1,000 mg/L Au, 1 g EDTA, and 0.5 mL of internal standard intermediate solution (100 mg/L Te and 20 mg/L Bi). Dilute to 2 L with ≥ 18 M Ω -cm water. Store at room temperature and prepare as needed. To prepare larger volumes of diluent, add proportionally larger volumes of the solution constituents. Use this diluent to prepare all standards and samples during the dilution process, which should occur just before analysis. It is important to make all samples in a run from the same diluent solution so that the concentration of the internal standard is the same among all standards and samples in the run.

ICP-DRC-MS Rinse Solution (1% (v/v) TMAH, 0.02% Triton X-100™, 5% (v/v) C₂H₅OH and 500 $\mu\text{g/L}$ Au).

To prepare, acid-rinse a 2 L Teflon™ container and partially fill with ≥ 18 M Ω -cm water. Add 20 mL of 25% (v/v) TMAH and 40 mL of 1% Triton X-100™ (see section 6.b.2.a for preparation procedure), 100 mL C₂H₅OH and 1 mL of 1,000 mg/L Au. Dilute to 2 L with ≥ 18 M Ω -cm water. Store at room temperature and prepare as needed. To prepare larger volumes of rinse solution, add proportionally larger volumes of the solution constituents.

Pump this solution into the sample introduction system between samples to prevent carry over of the analytes of interest from one sample measurement to the next.

Base Urine Preparation

The base urine used in this method is pool of urine collected from anonymous donors. Collect urine in containers screened for iodine and mercury content. After receiving donations, analyze the urine to determine iodine and mercury concentrations. The final base urine pool should be ≤ 70 $\mu\text{g/L}$ iodine and ≤ 0.1 $\mu\text{g/L}$ mercury. If donated urine specimens have acceptable concentrations of iodine and mercury, they should be pooled and then dispensed into smaller-volume tubes (i.e., 50 mL polypropylene tubes) for daily use. For short-term storage (a few days), store at approximately 2-4°C. For long-term storage, store at $\leq -20^\circ\text{C}$. A 2 L base urine pool should be enough for ~350 analysis runs (~ 14,000 samples at 40 samples per run). Spike urine with intermediate working standards prior to analysis each day to prepare calibration standards for each run.

C. Standards Preparation

CAUTION!

Mercury compounds are toxic! Take extra care to avoid accidental ingestion or inhalation of these materials. **Wear appropriate personal protective gear. Above all, wear a laboratory coat and latex or nitrile gloves.** Clean up any spill that might occur according to applicable hazardous material spill procedures.

1. Materials:

Flasks: Need one 500 mL, six 100mL, and three 50mL flasks;
one 500 mL for 10% v/v HCl
one 100 mL for Hg Intermediate Stock Standard A (1mg/L)
one 50mL for Hg Intermediate Stock Standard B (0.1mg/L)
two 50 mL for I Intermediate Stock Standards A (100 mg/L) and B (10 mg/L)
five 100 mL for Intermediate Working Standards 1-5.

50 mL tubes: Need 16 total
ten for storage of prepared Intermediate Working Standards 1-5
six for storage of prepared solutions.

Pipette volumes: 80-1000 μ L

Stock solutions: iodine (1000 mg/L) & mercury (1000 mg/L).

Hydrochloric acid: double distilled, concentrated (12M or 37%).

2. Materials preparation

Clean the flasks and falcon tubes:

Rinsing: Double rinse each flask or tube vigorously with 5% v/v nitric acid followed by a double rinse with 18Mohm water. Fill each with 5% v/v nitric acid and allow them to soak for several hours or overnight. Follow with rigorous rinsing of each with 18Mohm water.

Monitoring: Compare analysis results of water poured out of these containers with results for water taken directly from the same water purification system to decide whether or not additional cleaning of the containers needed. Analysis of the water can be done directly without dilution or calibration using the normal ICP-DRC-MS method file. Acceptable counts for clean vessels should be negligibly different from water blank counts. Typical counts observed are Iodine <150cps and Mercury <30cps (counts vary between instruments due to sensitivity differences). If background counts are too high, repeat step 7.a.1.a.

Sulfamic Acid Preservative: Prepare approximately 2M sulfamic acid preservative as per Section 6.b.1.b.

Prepare 10% v/v HCl: Add 25mL of concentrated HCl (12 M or 37%) to approximately 400mL water in an acid-washed 500 mL volumetric flask (see step 7.a.1). Dilute to the mark with 18 Mohm water. Mix well. Check the Hg and I background in the solution per 7.a.1.b above.

3. Intermediate Stock Standards Preparation

NOTE: Manufactured intermediate stock standards for Hg and I may be procured and used in place of in-lab preparations.

Mercury Intermediate Stock Standards Preparation:

Current Hg standards purchased from High Purity Standards (1mg/L and 0.1 mg/L) have short expiration dates of 6 months and 3 months respectively.

Hg Intermediate Stock Solution A (1 mg/L mercury)

Add approximately 90mL of 10% v/v HCl to an acid-cleaned 100mL volumetric flask. Add 0.1 mL of 1000mg/L Hg Stock Solution. Dilute to 100 mL with 10% v/v HCl. Mix well. Store in acid-washed, labeled 50mL polypropylene tubes.

Hg Intermediate Stock Solution B (0.1 mg/L mercury)

Add approximately 40mL of 10% v/v HCl to an acid-cleaned 50mL volumetric flask. Add 5 mL of 1mg/L Hg Intermediate Stock Solution A. Dilute to 50 mL with 10% v/v HCl. Mix well. Store in acid-washed, labeled 50mL polypropylene tubes.

Iodine Intermediate Stock Standards Preparation:

a. Iodine Intermediate Stock Solution A (100 mg/L iodine)

Add approximately 40mL of 18Mohm water to an acid-rinsed 50mL volumetric flask. Add 5 mL of 1000mg/L stock standard. Dilute to 50 mL with 18 Mohm water. Mix well. Store in acid-washed, labeled 50mL polypropylene tubes.

b. Iodine Intermediate Stock Solution B (10 mg/L iodine)

Add approximately 40mL of 18Mohm water to an acid-cleaned 50mL volumetric flask. Add 5 mL of 100mg/L stock standard. Dilute to 50 mL with 18 Mohm water. Mix well. Store in washed, labeled 50mL polypropylene tubes.

4. Intermediate Working Standards Preparation

Fill five labeled, acid-washed 100mL volumetric flasks with approximately 90mL of 18Mohm water each. Add 1mL of the approximately 2 M sulfamic acid preservative solution to each flask. Mix well. Spike each flask with the appropriate volume of the Intermediate Stock Standards Solutions (see the table below). Smaller or larger volumes of intermediate working standards can be prepared including proportionally smaller or larger additions of all components.

Mix well. Dilute to 100mL with 18Mohm water. Mix well and pour out into dry, water rinsed, labeled 50mL polypropylene tubes.

Intermediate Working Standards Preparation volumes (µL).				
Working Standard Number	Hg Intermediate Stock STD C (0.1 mg/L)	Hg Intermediate Stock STD B (1 mg/L)	I Intermediate Stock STD B (10 mg/L)	I Intermediate Stock STD A (100 mg/L)
STD 1	80		80	
STD 2	240		240	
STD 3	800		800	
STD 4		240		240
STD 5		800		800

Pour out small amounts of each calibrator into separate, clean 15mL polypropylene tubes for daily use in preparing working standards. The final concentrations of iodine and mercury in each of the intermediate working standards can be calculated by the formula below (see table below for final concentrations). The values entered into the ICP-DRC-MS software should be the concentrations of the intermediate working standard.

$$\text{Int. Work. Std. Conc. (}\mu\text{g/L)} = \frac{\text{Int. Stock Std. Conc. (}\mu\text{g/L)} \times \text{Int. Stock Std Spike (L)}}{0.100 \text{ L}}$$

Intermediate Working Standards Concentrations (ug/L)		
	Mercury	Iodine
STD 1	0.08	8
STD 2	0.24	24
STD 3	0.8	80
STD 4	2.4	240
STD 5	8.0	800

Store at room temperature and prepare a fresh set of standards weekly. Test each fresh set of intermediate working standards for acceptability by analyzing prepared working standards prior to use on actual analysis runs.

5. Working Standards (Calibrators)

The working calibrators are dilutions of the five intermediate working standards into a urine matrix for the purpose of external calibration of an analytical run (i.e. the run calibrators). Make them up the day of the preparation and analysis of the patient samples. Prepare all calibration standards and patient samples in the same analytical run by using the same diluent (See Section 10.b.3.). To prepare the working calibration standards, transfer 500 µL of the appropriate aqueous intermediate working standard, 500 µL of base urine, and 4,000 µL of diluent to a 15 mL polypropylene centrifuge tube by using the Micromedic Digiflex™. Cap the tube and mix well before analysis.

D. Quality Control Material

1. Bench QC Materials

Analyze low and high bench QC material in each run to determine the validity of the concentration measurements being made. Quality control (QC) materials are made by spiking human urine collected from anonymous donors (see section 6.b.3) with single element iodide and mercury standards. Prepare these pools periodically, as supply dictates, by spiking base urine to desired concentrations. Prepare new pools far enough in advances so that both old and new pools can be analyzed together for a period of time (preferably at least 20 runs) before switching to the new QC materials.

The two urine QC pools made for iodine and mercury assay are designated as:

Where substitutions are: yy = the last two digits of production year, and ### = assigned pool identification number.

QC material that is to be used for bench quality control purposes will need to be “characterized” as described in the section A. *Establish QC limits for each QC pool.*

2. NIST SRM 2670a (“Elevated”)

The NIST SRM 2670a (elevated) is freeze-dried urine, which has a certified concentration value of $88.2 \pm 2.2 \mu\text{g/L}$ for iodine and $95.1 \pm 1.96 \mu\text{g/L}$ for mercury. It, along with other reference materials (i.e. from proficiency testing or interlaboratory comparison programs), can be used for periodic external calibration verification, and testing the accuracy of the analytical method. To reconstitute, add 20.0 mL of $\geq 18 \text{ M}\Omega\text{-cm}$ water to the SRM vial; close and swirl the vial to make sure that all freeze-dried material is reconstituted. Transfer all of the reconstituted urine to a 50 mL acid-washed polypropylene centrifuge tube. Store in smaller volumes for daily use (i.e., four 5 mL aliquots in acid-washed 15 mL polypropylene centrifuge tubes). Store at $\leq -20^\circ\text{C}$. Reconstitute new vials as needed, but allow for overlap of use of new vials (i.e., analyze both old and new preparations in one or more analysis runs to compare).

7. INSTRUMENT & SOFTWARE FOR THE ICP-DRC- MS

A. ICP-DRC-MS System

25. Inductively Coupled-Plasma Dynamic-Reaction Cell Mass Spectrometer ELAN[®] DRC Plus or DRC II (PerkinElmer Instruments, Headquarters Office, 710 Bridgeport Ave., Shelton, CT 06484-4794). Parameters of x-y alignment, mass calibration, autolens voltages, and nebulizer gas flow rates are optimized regularly. Other DRC[™] parameters are optimized for each specific instrument.

26.

N[®] ICP-DRC-MS Method Parameters	
Parameter	Setting
RF Power	1.45 KW
nebulizer gas flow is the same as the RC mode nebulizer gas flow	Approx 0.9-1 LPM
Detector mode	Pulse
Measurement units	Cps
Autolens	On
Blank subtraction	After internal standard
Curve type	Simple Linear
Sample units	$\mu\text{g/L}$
Sweeps/reading	90
Readings/replicate	1
Replicates	3
Dwell time	30 ms for iodine and 50 ms for Hg
Cell gas	Argon
Cell gas flow rate	0.5 mL/min (optimize as needed)
RPQ	~0.75 for iodine and ~0.35 for Hg (optimize as needed)

Quadrupole Voltages (optimized as needed)

	<u>CPV</u>	<u>CRO</u>	<u>QRO</u>
Standard	-17.0	-9.0	0
DRC	-15.0	-1.5	-6.5

27.

28. **B. ELAN instrument** control and data handling software, version 3.0 with service pack 2 or equivalent (PerkinElmer Instruments, Shelton CT).

29. Cyclonic spray chamber (PerkinElmer Instruments, Shelton CT), or equivalent.

30. Concentric glass nebulizer, (P/N SB-50-A2, J. E. Meinhard Associates, CA) or equivalent.

31. External peristaltic 4-channel peristaltic pump, "Minipuls 3" (Gilson, Middleton, WI) or equivalent.

C. Other equipment

32. 1. Water purification system (NANOpure Diamond Ultrapure Water System, Barnstead International, Bedford, MA or equivalent) for providing ultrapure water with a resistivity $\geq 18 \text{ M}\Omega\text{-cm}$.

33. 2. Analytical balance for routine weighing of material to the nearest hundredth of a gram and with a loading capacity of at least 200 g.

34. Micromedic Digiflex™ automatic pipette (or equivalent) equipped with 10.0 mL dispensing syringe, 2.0 mL sampling syringe, 0.75 mm tip, and foot pedal (LABREPO, Inc., 101 Witmer Rd., Suite 700, Horsham, PA 19044).

D. Supplies

1. 5-100 μL pipette tips, 960 tips per case (Eppendorf® catalogue # 2235137-1, distributed by Brinkmann Instruments, Westbury NY), or equivalent.

2. 20-300 μL pipette tips, 960 tips per case (Eppendorf® catalogue # 2235144-3, distributed by Brinkmann Instruments, Westbury NY), or equivalent.

3. 1,000 μL pipette tips, 960 tips per case (Eppendorf® catalogue # 2249044-3, distributed by Brinkmann Instruments, Westbury NY), or equivalent.

4. 5 mL pipette tips, 500 tips per case (Eppendorf® catalogue # 2235081-1, distributed by Brinkmann Instruments, Westbury NY), or equivalent.

5. 6 Acid-cleaned volumetric flasks, 100 mL for standards preparation (polypropylene or polymethylpentene flasks preferred). To acid-wash flasks, rinse with 5% (v/v) reagent-grade nitric acid, followed by rigorous rinsing with $\geq 18 \text{ M}\Omega\text{-cm}$ water. Repeat this process several times depending on prior use of the containers.

6. 2 Acid-cleaned 2 liter PE bottles. To acid-wash containers, rinse with 5% (v/v) reagent-grade nitric acid, followed by rigorous rinsing with $\geq 18 \text{ M}\Omega\text{-cm}$ water. Repeat this process several times depending on prior use of the containers.

7. 2 acid-cleaned 4 liter PE bottles. To acid-wash containers, rinse with 5% (v/v) reagent-grade nitric acid, followed by rigorous rinsing with $\geq 18 \text{ M}\Omega\text{-cm}$ water. Repeat this process several times depending on prior use of the containers.

8. Kay-Dry™ paper towels and Kim-Wipe™ tissues (Kimberly-Clark Corp., Roswell GA, or equivalent vendor).

9. Teflon™-coated magnetic stir bars (2) (Catalog Number 58948-974 or equivalent), VWR Scientific Products, Buffalo Grove, IL.
10. Cotton swabs (Hardwood Products Co. ME, or equivalent vendor).
11. 15 mL (# 352097) and 50 mL (#352098) polypropylene centrifuge tubes or equivalent: (Becton Dickinson Labware, 1 Becton Drive, Franklin Lakes, New Jersey 07417 or equivalent).
12. Nitrile or Latex, powder-free examination gloves (N-Dex®, Best Manufacturing Co., Menlo, GA, or equivalent vendor).

8. CALIBRATION AND CALIBRATION-VERIFICATION PROCEDURES

A. Calibration Curve

Generate a simple linear calibration curve for iodine & mercury by using a series of five external calibrators whose concentrations are defined in the calibration page of the quantitative analysis method software. The calibration curve plots the ratio of the observed intensities for iodine & mercury and the internal standards versus the concentration of the calibrators. Compare the ratio of the observed intensities for iodine & mercury and the internal standards in the patient sample to those obtained from the calibrators to determine the concentration of iodine & mercury in the sample.

1. Calibration Verification

a. Bi-annual tests as defined in the DLS Policy and Procedures manual: CLIA requires the verification of accuracy of instrument response to analyte concentration be completed at least every 6 months. NIST traceable calibrators are analyzed in each run to define this response up to the concentration of the highest calibrator in the run. To verify accuracy of instrument response at concentrations higher than the highest calibrator in each run, analyze a NIST traceable standard with 3000 ug/L I and 300 ug/L Hg at least every 6 months. Prepare the Calibration Verification Standard for analysis just as a working calibrator is prepared. Use the "Urine Blank" as the blank when it is analyzed. If the observed concentrations for the Calibration Verification Standard are not within 10% of the target value the lab supervisor should be notified and the issue should be investigated. Do not substitute external reference materials (i.e. biological samples from a PT program) for the Calibration Verification Standard when performing this. Solutions needed for the Calibration Verification checks can be purchased from standards vendors (i.e. SPEX, High Purity Standards, etc . . .) or prepared in-house from NIST traceable single element standards. Always verify that normal background levels have been re-achieved through adequate rinse time following analysis of elevated standards for calibration verification.

b. As-needed confirmations (per supervisor discretion): When a sample result is greater than the highest calibrator in the run, the supervisor may request that the result be confirmed in an analysis run which includes a standard or external reference material with equivalent (within 10%) or greater concentration than the sample. In order to avoid needless contamination of the instrument with high concentrations of analytes, the analyst should use the lowest appropriate calibration verification solution concentrations to meet the need.

For infrequent verification needs, the calibration verification stock solutions can be used to prepare verification standards to appropriate concentrations. This will, however, introduce elevated concentrations of all elements in the method to the sample introduction system. Frequent measurement of these very high concentrations can result in high background levels in the instrument which are difficult to rinse out and which may limit the ability to measure low concentrations.

For frequent verification needs (i.e. when certain studies have many elevated results on particular elements) or when a concentration higher than the normal testing range for linearity needs to be verified, use NIST-traceable single element stock standards to prepare single element verification standards. This will limit the exposure of the instrument to elevated concentrations of only the elements needing verification.

Always verify that normal background levels have been re-achieved through adequate rinse time following analysis of elevated standards for calibration verification. An external reference material (i.e. historical proficiency testing sample) can be substituted in place of the Calibration Verification Standard sample in these situations IF

- (a) The target value has been assigned by an external source (i.e. NIST, or the proficiency testing program).
- (b) The concentration of the external reference material is within 10% or is higher than the concentration of the material you need it to confirm.
- (c) There is confidence that there is no contamination of previously used external reference material.
- (d) A note to file is made that this was done.
- (e) If the observed concentrations are not within 10% of the target value the lab supervisor should be notified and the issue should be investigated.

10. OPERATING PROCEDURES; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preliminaries

1. For information about the reportable range of results and how to handle results outside this range, refer to the Reportable Range of Results section of this document (section 16).
2. Allow frozen urine specimens, QC specimens, and base urine to reach ambient temperature. Vortex the sample well so that no particulates remain on the bottom of the tube before taking an aliquot for analysis.

B. Sample Preparation

1. Thaw the frozen urine specimens; allow them to reach ambient temperature (about 20°C).
2. Set up a series of 15 mL polypropylene centrifuge tubes corresponding to the number of blanks, standards, QCs, and patient samples to be analyzed.
3. Prepare the following solutions into the 15 mL polypropylene centrifuge tubes by using the Micromedic Digiflex™.

Preparation of Samples for Analysis (All Volumes in μL)					
ID	Water	Intermediate Working Std.	Base Urine	Urine Sample or QC	Diluent
Urine blank	500	-	500	-	4,000
Calibration standards	-	500	500	-	4,000
Aqueous blank	1,000	-	-	-	4,000
Urine sample or QC	500	-	-	500	4,000
2x dilution of Urine sample*	750	-	-	250	4,000

Note: These volumes are used because the total volume each sample this method consumed for is around 3,000 μL

* Volumes listed here are an example of how to combine the correct proportions of water, urine, and diluent in making a 2x dilution. Other volumes of the same proportions can also be used. Other dilutions can be prepared as needed by adjusting the proportion of urine to the total volume of diluted sample.

- a. Prepare an aqueous blank that consists of 1,000 μL of $\geq 18 \text{ M}\Omega\text{-cm}$ water and 4,000 μL diluent. Use the aqueous blank for the QC pools and patient samples.
- b. Prepare five urine blanks that consist of 500 μL of base urine (same material used for preparation of the urine calibration standards), 500 μL of $\geq 18 \text{ M}\Omega\text{-cm}$ water, and 4,000 μL of diluent. Run one of these as the blank for the calibration standards. Run two urine blanks after standard 5 (as sample IDs UrBlkChk1 and UrBlkChk2, respectively). Analyze the fourth and fifth urine blanks before the calibration blank to condition the system.
- c. Prepare the working calibration standards as described in section 7.d.
- d. Prepare dilutions of the QC and patient urine samples consisting of 500 μL of $\geq 18 \text{ M}\Omega\text{-cm}$ water, 4,000 μL of the diluent and 500 μL of the patient or QC urine sample.
- e. Cap all of the blanks, standards, and samples and mix them well. Uncap and place them in the autosampler of the ELAN[®] ICP-DRC-MS. It may be necessary to operate the instrument with cell gas flowing at the method flow rate for at least 30 minutes before the run begins. This is to allow the conditions within the reaction cell to equilibrate before the run begins. Note: The cell gas will automatically turn off after 1 hour of no ICP-MS DRC mode analysis.

C. Instrument Setup and Configuration

1. Turn on the computer, printer, peristaltic pump, and autosampler. Log into the computer operating system.
2. Start the ELAN[®] ICP-DRC-MS software from Windows[™] and note whether all graphical indicators of instrument readiness are green. If not, take the appropriate actions described in the instrument's software and hardware manual.
3. Perform necessary daily maintenance checks as described in Chapter 5 of the *ELAN[®] ICP-DRC-MS Hardware Guide* (e.g., argon supply, interface components, cleanliness, positioning and interface pump oil condition). Note the base vacuum pressure in the INSTRUMENT window of the software (Before igniting the plasma, the vacuum is typically between 8×10^{-7} and 1.8×10^{-6} torr). Record any maintenance procedures along with the base vacuum pressure in the *Daily Maintenance Checklist* (See example of daily checklist in the [Appendix](#)).
4. Set up the peristaltic pump tubing for the autosampler, rinse station, and spray-chamber waste line. Position the tubing and close the pump clamps. Adjust the tension on the pump tubing later.
5. In the INSTRUMENT window of the software, press the "Start" button to ignite the plasma.
6. Start the peristaltic pump by pressing the appropriate arrow in the DEVICES window (Make sure that the rotational direction is correct for the way the tubing is set up in the peristaltic pump). Fill the rinse station reservoir quickly by pressing the "Fast" button in the DEVICES window. After the rinse station is filled with the rinse solution, type in "20" in the rpm field of the DEVICES window to set the pump speed. If the spray chamber rinse line is not draining the spray chamber correctly or the rinse solution is not flowing properly to the rinse station, adjust the tension screws on the peristaltic pump.
7. *Read this step through entirely before proceeding. It is important to get the tension on the autosampler tubing correct, or it will adversely affect the precision of the ICP-DRC-MS measurements.* Through the METHOD/SAMPLING window in the software, press the "Probe" button, then the "Go to Rinse" button to lower the autosampler probe into the rinse solution. Watch as the solution is taken up through the autosampler probe tubing. When the leading edge of the solution is visible, press "Stop" in the DEVICES window. The leading edge of solution in the autosampler tubing line should stop moving. If it does not stop, tighten the tension screw for this line on the back of the peristaltic pump. Loosen the peristaltic pump tubing screw for the autosampler tubing until the leading edge of solution in the autosampler tubing begins to move

again, then tighten the screw just enough to make the solution edge stop. Tighten the screw another eighth to a quarter of a turn. Next, start the peristaltic pump by pressing the appropriate arrow in the DEVICES window (make sure that the rotational direction is correct for the way the tubing is set up in the peristaltic pump).

8. Allow at least 45 minutes warm-up time for the ICP-DRC-MS (with plasma running). After this warm-up time, complete the appropriate daily optimization procedures as described in Chapter 3 of the *ELAN[®] DRCII Software Guide*. Include beryllium (m/z 9) in the mass calibration, autolens optimization, and daily performance check by using a 1 µg/L multielement solution. Fill in the *Daily Maintenance Checklist* according to the completed optimization procedures. Save new tuning (mass-calibration) parameters to the file "default.tun." Periodically, save these parameters also in a separate file containing the analysis date "default_YYMMDD.tun". Save new optimization parameters (i.e., detector voltages, autolens values and nebulizer gas flow rate) to the file "default.dac". Periodically, save these parameters also in a separate file containing the analysis date "default_YYMMDD.dac" (where YY=year, MM=month and DD=day).
9. To set up the run in the software, click on "Open Workspace" from the "File" menu. Select the workspace file "CDC_Urine_I_Hg.wrk.". Select "Review Files" from the "File" menu. From this window, you will be able to set up the correct files and directories for data for your analysis. Select the method, report template, tuning, and optimization files later. There is no need to select a calibration or polyatomic file (If this workspace has not been created on the instrument computer being used, follow the directions in the ELAN ICP-DRC-MS software manual to set it up using the parameters described in this write-up).
 - **Data set:** If this is the first run of the day, create a new data set by using the date as the name (Use the format 20050801 for August 1, 2005). If a run has already been performed today, select the data set for today's date.
 - **Sample:** If an analysis has been performed that is similar to the one you are going to do, select the sample file corresponding to it. Edit it later for the present analysis.
10. In the SAMPLES/BATCH window, update the table to reflect the current sample set (e.g., autosampler locations, sample identification (ID), analysis methods and peristaltic pump speeds). Two method files (CDC_UIHg_methITU007B_urblk.mth and CDC_UIHg_methITU007B_aqblk.mth) will be used. *These two methods differ only in the autosampler locations of the blank and calibration solutions.* Use the "UR" method file to run the base urine blank and the calibration standards at the very beginning of the run. Because of the autosampler positions defined in the method file (these are editable), the urine blank must go in autosampler location 11 and the urine calibration standards 1-5 must go in autosampler locations 12-16, respectively. Use the "AQ" method file to run the aqueous blank before the first sample. Because of the autosampler positions defined in the method file (these are editable), the aqueous blank must go in autosampler location 19. Except for defining the blank and calibration standards' autosampler locations, it does not matter which of these files is used when analyzing a sample since all other analysis parameters are identical in the method files. A typical SAMPLE/BATCH window for this method will look like Table below. (Note: All other autosampler positions besides those specified above are arbitrary.)

Typical Sample File Setup for a Urine Iodine & Mercury Analysis Run

A/S Location	Sample ID	Measurement s Action	Method File*
37	DRC Rinse Delay	Run sample	CDC_UIHg_methITU007B_urblk.mth
38	DRC Rinse Delay	Run sample	CDC_UIHg_methITU007B_urblk.mth
39	Conditioning Urine Blank	Run sample	CDC_UIHg_methITU007B_urblk.mth
40	Conditioning Urine Blank	Run sample	CDC_UIHg_methITU007B_urblk.mth
100	UrBlkChk1	Run blank, standards, and sample	CDC_UIHg_methITU007B_urblk.mth

101	UrBlkChk2	Run sample	CDC_UIHg_methITU007B_urblk.mth
20	AqBlkChk	Run blank and sample	CDC_UIHg_methITU007B_aqblk.mth
23	Low-bench QC	Run sample	CDC_UIHg_methITU007B_aqblk.mth
24	High-bench QC	Run sample	CDC_UIHg_methITU007B_aqblk.mth
35	Sample 1	Run sample	CDC_UIHg_methITU007B_aqblk.mth
36	Sample 2	Run sample	CDC_UIHg_methITU007B_aqblk.mth
37	Sample 3	Run sample	CDC_UIHg_methITU007B_aqblk.mth
Etc.		
74	Sample 40	Run sample	CDC_UIHg_methITU007B_aqblk.mth
25	Low-bench QC	Run sample	CDC_UIHg_methITU007B_aqblk.mth
26	High-bench QC	Run sample	CDC_UIHg_methITU007B_aqblk.mth

*(Where 20050510 is the starting date of the method being used in the analysis)

Modification of the method file is allowed for situations such as running only Hg or only I (and the appropriate internal standard) analyzing calibrators at different autosampler locations (when you want to perform multiple runs with different sets of calibrators).

In such cases, any new file created should be renamed to have the original filenames (above) at the beginning. Examples include CDC_UIHg_methITU007B_urblk_Hg.mth (when only analyzing for Hg) or CDC_UIHg_methITU007B_urblk_calset2.mth (when performing a second run with a different set of calibrators).

The autosampler positions of QCs and patient samples do not have to be those shown above, but the order in which these are run (DRC mode delay time of approximately 1 hour with rinse solution aspirating, followed by 2 UrBlkChk conditioners, a urine blank for the calibrators, calibration standards 1-5, urine blank checks 1 and 2, low-bench QC, high-bench QC, 40-80 patient samples including 1 blind QC sample, low-bench QC, and high-bench QC) should be as shown in Table above.

The settings in Table below should be used for uptake and rinse times for all samples, QC's, and standards. (These values are already stored in the method files for the blanks and standards.)

Sample File Timing Parameters for a Urine Iodine & Mercury Analysis Run		
	Pump Speed*	Duration
Sample flush	-20 rpm	45 s
Read delay and analysis	-20 rpm	50 s
Wash	-48 rpm	100 s

The QC tab/sample tab in the method should be setup so that a 200s rinse time will occur after any sample whose Hg concentration exceeds 30 µg/L or whose I concentration exceeds 2000 µg/L.

* Note: Negative values for pump speed indicate direction of pump rotation. Make sure that pump tubing is set up appropriately to match the direction of pump rotation.

If using the ITN database for long-term recording and handling of data (Section 10.d.(2)(d)), do not use the Elan[®] software to automatically correct for sample dilutions. When dilutions of any sample are run, the sample ID should be edited to reflect the level of dilution being performed (A two-fold dilution of "Sample 1" could be recorded in the sample ID as "Sample 1 (2X)". The exact wording is not critical). Edit this sample ID during the data-import process to the database

so that it is recognized as the appropriate sample. (See Section 10.d.(2)(d)).

Before beginning the analysis run, start the flow of the reaction-cell gas (argon) and allow the cell conditions to equilibrate. Make sure that the reaction-cell gas pressure to the instrument is approximately 7 psi on the cell-gas cylinder regulator. In the “Manual Adjust” page of the “Optimization” window, enter a value of “0” in the appropriate cell-gas field (cell-gas A for this method). Then enter 0.001mL/min cell gas flow rate in the same field. A clicking should be heard from the ICP-DRC-MS cell-gas solenoid as the flow turns on. Flush the cell gas for 60 seconds by lifting the flush level at the front of the instrument (The flush step may not be necessary if this same gas cell was used recently and no gas tubing has since been disconnected). After the cell gas flush, enter the method-appropriate cell gas flow rate in the same field. Monitor the flow in the ELAN software, on the Instrument window (diagnostics tab). It is usually necessary to operate the instrument with cell gas flowing at the method flow rate for 30 minutes to 1 hour before the run begins. This is to allow the conditions within the reaction cell to equilibrate before the run begins. Necessity of this equilibration time can be determined by monitoring stability of the observed iodine and mercury concentration of a standard analyzed multiple times within a run. **Note:** The cell gas will automatically turn off after 1 hour of no DRC-mode analysis.

11. After the parameters in the SAMPLE/BATCH window are edited for the run, place the solutions in the autosampler tray according to the setup of the SAMPLE/BATCH window and method files. Highlight (click and drag with the mouse) the table rows of the samples that are to be included in the run, and then click on “Analyze Batch.” If sample analysis is to finish the run unattended after normal laboratory hours, the AutoStop (Instrument window- AutoStop Tab) may be enabled to shut off the plasma when the analysis is completed. If the AutoStop is not enabled, the DRC gas will shut off approximately 1 hour after the last sample analysis in DRC mode, but the plasma will remain lit. If the plasma is to remain operating unattended, ensure that adequate rinse solution is available for the time that the instrument will be unattended.
12. Instrument Shut Down
 35. a. Shut off ICP-DRC-MS plasma if it has not already done so. Stop the peristaltic pump and loosen tensioning bars and tubing.
 36. b. At the controller computer, visit the ELAN Instrument Control Session application and open the “Dataset” window. Confirm that all samples ran successfully and that the corresponding data for each sample is listed in this window.

D. Recording of Data

1. QC Data

Store the results of the QC samples analyzed in each run in the Microsoft Access™ (or MS SQL Server) database when all other data for the run is imported from the ELAN® software See Section 10.d.(2)(d) for a description of how to import data into the Microsoft Access™ database).

2. Analytical Results

a. Analysis Printouts and Analyst Run Report

Bind the analysis printouts with a printout of the calibration curve and curve statistics as the top page and place them in the study folder(s). Write the following information on the cover sheet of the analysis printouts: Run date, run number, study ID, and analyst ID (the Run ID from the database is also helpful). Store the results of the patient samples analyzed in each run in the Microsoft Access™ (or MS SQL Server) database when all other data for the run is imported from the ELAN® software. See Section 10.d.(2)(b) for a description of how to import data into the ITN database. If the database allows for the printing of a run summary report that indicates whether any particular patient-sample results are outside of the normal concentration reference range or whether any measurement failed precision limits, it may be helpful to print it out after each analysis. These reports can be helpful to keep in a

notebook for future reference (See Section 10.d.(2)(b) for a description of how to import data into the database to print out a customized sample report).

b. **Using the Microsoft Access™ Database**

After an analysis run, export the results to a .TXT file and then import into the Microsoft Access™ or MS SQL Server database that handles data for the Inorganic Toxicology and Nutrition Branch.

(1) Data Export Process (from ELAN® software to .TXT or .CSV file)

In the ELAN® ICP-DRC-MS software, select “Review Files” from the “File” menu. From this window, you must open the files and directories that were used when collecting the data of the run that you wish to export (If the analysis has just ended, all of these files and directories will still be open). NOTE: A second copy of the ELAN® software can be run as an Edit/Reprocess copy without affecting an ongoing analysis by the first copy of the software running in Windows. After you open the relevant files, go to the “Report” page in the METHOD window. Deselect the box that prints a paper copy of data and select the box that sends data to a file. Select the “Report Options Template” named “CDC_Database_output.rop” and type in a report filename using a format such as “20050801a_group55.txt” (or 20050801a_group55.csv) to designate data from analysis of group 55 from August 1, 2005, run #1. Under “Report Format”, choose the “Use Separator” option, and under the “File Write” section choose “Append.” Finally, reprocess the data of interest (See *PerkinElmer ELAN® II Software Manual*). Make sure you apply the correct blank to the correct samples and QCs (use the urine blank for all of the calibration standards, UrBlkChk1, and UrBlkChk2. Use the aqueous blank for all analyses of patient samples and QC samples).

(2) Data Import Process (from .TXT or .CSV file to Microsoft Access™ database).

Transfer the .TXT or .CSV file to the appropriate subdirectory on the network drive where exported data are stored (Note that directories are named according to instrument/year/month/ and study name or ID, such as I:/Instruments/ELAN® DRCIIIG/2005/08/Study 2005-xx). From a computer that has access to Microsoft Access™ or MS SQL Server database used for tracking data, log in using your user ID#. After you log into the database, open the select “Import Instrument File” from the “Front End Set”. Enter the appropriate information to identify the run, assay, study, instrument, and analyst and press the “Import” button. Select the location of the data file on the network drive and press the “Open” button. In the “Imported Results” table, pressing the “Find X’s” button will show only those samples whose sample ID is not recognized as a valid QC pool ID or sample ID for this study (Sample IDs are set up when the study is logged into the database). Corrections to sample IDs and dilution factors can be made in this table (e.g., correction of transcription errors and adjustment for level of dilution). If samples were diluted for analysis (Section 10.c.(10)), both the sample ID and the dilution factor need to be edited in this table before the values are transferred to the database. First, change the dilution factor to reflect the way that the sample was analyzed then edit the sample ID to remove any comments about the level of dilution at which the sample was analyzed (The replace command is useful here). When corrections to sample IDs are made, press the “Recheck” button to evaluate the sample IDs. Any sample or analyte row marked “Not Recognized” will not be transferred to the database when the “Transfer” button is pressed. From this point, the data should be labeled with the appropriate settings for QC accept / reject, final value status, and comment.

11. FINAL REVIEW OF THE DATA

A. Abnormal Patient Results:

Boundaries Requiring Confirmatory Measurement:

a. Results Lower than the First Lower Boundary (1LB): If a result for a patient sample is below 50 ug/L for I (the 1LB in the laboratory database), the analyst should confirm the result by analysis of a new sample preparation (dilution using the Digiflex). Report the original result, as long as the confirmation is within 10% of the original. Continue repeat analysis until a concentration can be confirmed. As a toxic element, there is no lower confirmation boundary for mercury.

1. **Results Greater than the First Upper Boundary (1UB):** If a result for a patient sample is above the 800 ug/L for I or 5 ug/L for Hg (the 1UB in the laboratory database), the analyst should confirm the result by analysis of a new sample preparation (dilution using the Digiflex). Report the original result, as long as the confirmation is within 10% of the original. Continue repeat analysis until a concentration can be confirmed.
2. **Results Greater Than Highest Calibrator:** When a sample result is greater than the highest calibrator in the run, the supervisor may request that the result be confirmed in an analysis run which includes a standard or external reference material with equivalent (within 10%) or greater concentration than the sample.
3. **Results Greater Than Range of Linearity Tested (RLT):** Perform an extra dilution on any urine sample whose concentration is greater than those listed in section 9.b.1 which is tested at least every 6 months to verify the linearity of the method. See table in section 10.b for details of preparing a sample with extra dilution.
4. **Inadequate Precision in Confirmation of a Measurement:** If a sample is reanalyzed to obtain a confirmation of an initially elevated result, the confirmation should be within 10% of the original result.
5. **Inadequate Precision Within One Measurement:** If the range of the three replicate readings (maximum replicate concentration value - minimum replicate concentration value) for a single sample analysis is greater than 30 ug/L for I or 1 ug/L for Hg (" $>$ Lim Rep Delta" in the database) and the range of the three replicate readings is greater than 10% of the observed concentration, do not use the measurement for reporting. Repeat the analysis of the sample.

B. Evaluating Bench QC Results: See sections 16.b-c for how to apply division QC rules and take corrective actions if necessary.

C. Submitting Final Work for Review

1. Once results have been imported, reviewed, and set as final in the database by the analyst,
2. Submit an email to the QC reviewer informing them of the readiness of the data for final review. The email should include
 37. a. Instrument ID, run Date, run number, study ID, group ID.
 38. b. Any bench QC failures (include reasons if known).
 39. c. Any patient sample iodine result less than 10 ug/L or greater than 2000 ug/L should be reported in the email as an abnormal concentration (these represent the 2LB and 2UB boundaries in the database). For mercury, report any sample result greater than 10 ug/L as an elevated result (this represents the 2UB boundary in the database).

40. d. If any patient sample results are greater than the action levels listed in section 18, report these immediately to the supervisor.
 41. e. Anything out of the ordinary about this analytical work which could have a bearing on the availability (i.e. insufficient sample to analyze), accuracy, or precision of the results.
3. Include all items called for by the study folder cover sheet in the study folder (i.e. printouts from the ICP-MS, bench QC evaluation) together in the study folder before submitting the folder for review when analysis is complete.

12. REPLACEMENT AND PERIODIC MAINTENANCE OF KEY COMPONENTS

Part numbers listed below are PerkinElmer part numbers from their *2005 Consumables Catalog*. Equivalent parts may be substituted.

- A. Autosampler probe assembly (part # B300-0161). Keep one spare on hand.
- B. Peristaltic pump tubing for sample (0.03 inch i.d., part #09908587), rinse station (can use either same tube type as for sample or 0.045 inch i.d., part #N0680375) and for waste (0.125 inch i.d., part #N8122012): Keep at least 6 packages of 12 on hand of the sample tubing, 6 for rinse station and 2 packages of 12 on hand of the waste tubing. Other suppliers may offer the same size/type of peristaltic tubing.
- C. Nebulizer capillary tubing (used to connect the nebulizer and the peristaltic pump tubing, part #09908265 or any source of polyethylene tubing, 0.6 mm i.d. x 0.97 mm o.d.). Keep one pack (10 feet) on hand.
- D. Injector Support/Torch Base (part #N8120116). Keep one spare on hand.
- E. Torch O-Ring Kit (packages of four, part #N8120100). Keep four spare packages on hand.
- F. Quartz torch. At least two spare torches should be on hand (part #N8122006).
- G. Quartz 2mm Bore Injector (part #WE023948).
- H. RF coil Assembly, self aligning (part #WE021816). One spare should be on hand.
- I. Nickel Skimmer (part #WE021137) and sampler cones (part #WE021140). Keep at least two spares of each on hand.
- J. Skimmer and sampler cone O-rings (part #N8120512 and #N8120511, respectively). Keep at least 10 spares of each on hand.
- K. Series II replacement Ion lens (part #WE018034). Keep two spares on hand.
- L. Pump oil for the roughing pump (part #N8122004). Keep four bottles on hand.
- M. Polyscience chiller coolant (PE Sciex Coolant, part #WE016558): Two 1 L bottles should be kept on hand.
- N. If possible, have a backup ASX 500 autosampler and Polyscience chiller. See a PerkinElmer sales representative for part numbers.
- O. Meinhard nebulizer type TQ-30-A3 (From Meinhard, part#TQ-30-A3).
- P. Meinhard nebulizer fit kit #3 (From Meinhard, catalog number is Fit kit 3).
- Q. Cyclonic spray chamber-Quartz type (From Meinhard, part#ML130019MSQ)

13. LIMIT OF DETECTION

The limits of detection (LOD) for iodine and mercury in urine specimens is based on three times the standard deviation of approximately 20 or more low concentration samples, each analyzed in a separate run. This represents the method detection limit. Report results below the detection limit as "< LOD" (where "LOD" is the calculated lowest detection limit). The LOD calculation is reevaluated at least once a year.

14. REPORTABLE RANGE OF RESULTS

Urine Iodine & Mercury results are reportable in the range of greater than the LOD, where LOD is the calculated lower detection limit.

15. SPECIAL PROCEDURE NOTES – CDC MODIFICATIONS

None applicable for this operation.

16. QUALITY CONTROL PROCEDURES

The Inorganic Toxicology and Nutrition Branch uses the method described in this protocol for environmental and occupational health screening studies.

This analytical method uses two types of Quality Control (QC) systems: With one type of the 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, labeled, and processed so that they are indistinguishable from the subject samples (as much as possible). If it is not possible to have the blind QC inserted into the sample group before receipt into the lab, an additional low and high QC pool should be made available to the analyst so that they can manually insert the material into the run. This type of "blind QC" should match the matrix of the patient samples as much as possible and the acceptable concentration limits (characterized limits) should be unknown by the analyst(s). 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 the analyte concentrations. 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 used in this method comprise two levels of concentration spanning the "low-normal" and "high-normal" ranges. Both of these pools are analyzed after the calibration standards are analyzed but before any patient samples are analyzed so that judgments on the iodine and mercury calibration curves may be made before analysis of patient samples. These bench QCs should be analyzed again at the end of the run.

A. Establish QC limits for each QC pool.

Perform an analysis of the mean and standard deviation for each pool from the concentration results observed in at least 20 characterization runs. During the 20 characterization runs, previously characterized QCs or pools with target values assigned by outside laboratories to evaluate each run's QC. In addition to providing QC limits, the characterization runs also serve to establish homogeneity of the pools.

B. Evaluating the Quality Control of a Run.

After completing a run, and importing the results into the database, export the QC results to the SAS program where the run will be judged to be in or out of control. The QC limits are based on the average and standard deviation of the beginning and ending analyses of each of the bench QC pools, so it will not be possible to know if the run is officially accepted or rejected until it is completed. The following is an explanation of the division QC rules which will be applied by the SAS program.

42. 1. If both QC run means (low & high bench QC) are within $2S_m$ limits and individual results are within $2S_i$ limits, then accept the run.
43. 2. If 1 of the 2 QC run means is outside a $2S_m$ limit - reject run if:
 - a. Extreme Outlier – Run mean is beyond the characterization mean $\pm 4S_m$
 - b. 1 3S Rule - Run mean is outside a $3S_m$ limit
 - c. 2 2S Rule - Both run means are outside the same $2S_m$ limit
 - d. 10 X-bar Rule – Current and previous 9 run means are on same side of the characterization mean
44. 3. If one of the 4 QC individual results is outside a $2S_i$ limit - reject run if:
 - a. R 4S Rule – Within-run ranges for all pools in the same run exceed $4S_w$ (i.e., 95% range limit)
45. Note: Since runs have multiple results per pool for 2 pools, the R 4S rule is applied within runs only.
46. Abbreviations:
47. S_i = Standard deviation of individual results (the limits are not shown on the chart unless run results are actually single measurements).
48. S_m = Standard deviation of the run means (the limits are shown on the chart).
49. S_w = Within-run standard deviation (the limits are not shown on the chart).

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

- A. If the division SAS program declares the run out of control” for any analyte, ONLY the analytes which were “out of control” are invalid for reporting from the run. Set all run results for those 1 or 2 analytes as “QC Rejected” in the database. Evaluate the reason for QC failure and take corrective action. Below is a list of areas to evaluate.
- B. Check the calibration curve(s) for linearity and for an intercept near zero. Calibration points not falling closely to the regression line may indicate a calibrator which was improperly prepared, analyzed, or needs to be made new. Be sure to use freshly prepared calibrators and QC material. Typical correlation coefficients (r^2) are > 0.999 . If possible, prepare new dilutions or preparations of calibrators which are outliers and reanalyze with the run to replace the original calibrator analysis. An individual calibration point may be removed from the curve if it is obviously an outlier. If the highest calibration point is removed, the highest calibrator used in the analysis should be specified in the laboratory database when the results are imported. If a certain calibrator is problematic repeatedly, investigate the problem and take corrective action to prevent the problem from continuing.
- C. Check for high blanks which lead to over-subtraction from analysis results.
- D. Check the ICP-DRC-MS stability during the run by examining the degree of variability and drift in internal standard raw peak areas over the course of the run. Irreproducibility that exceeds 15% and drift $>20\%$, or sudden large changes in internal standard peak area, likely indicates that there was a problem in plasma stability.

If these steps do point to appropriate corrective action, for the out-of-control values for QC materials, consult the supervisor for other appropriate corrective actions. No analytical results should be reported for runs that are not in statistical control.

18. REFERENCE RANGES

Reference Ranges for Elements Measured (all units $\mu\text{g/L}$)

Element/ Isotope Monitored	Reference Ranges (10th- 95th Percentile, $\mu\text{g/L}$) (weighted, non-creatinine corrected NHANES 2001 & 2002 results)
I-127*	41- 803 ⁽⁹⁾
Hg-202 [†]	< 4.63 ⁽⁸⁾

* Te-130 used as internal standard

[†] Bi-209 used as internal standard

19. ACTION-LEVEL RESULTS

If a patient sample has a non-dietary iodine concentration greater than 10,000 $\mu\text{g/L}$, report the levels telephone or E-mail to the supervising physician or principal investigator.

If a patient sample has a mercury concentration greater than 200 $\mu\text{g/L}$, report the levels by telephone or E-mail to the supervising physician or principal investigator.

20. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may reach and maintain ambient temperature during analysis. Take stringent precautions to avoid external contamination. After the samples are analyzed, return them to $\leq -20^{\circ}\text{C}$ freezer storage as soon as possible.

21. ALTERNATE METHODS FOR PERFORMING TEST AND STORING SPECIMENS IF TEST SYSTEM FAILS

If the analytical system fails, then store urine specimen at $\leq 4^{\circ}\text{C}$ until the analytical system is restored to functionality. If long-term interruption (longer than 4 weeks) is anticipated, then store urine specimens at $\leq -20^{\circ}\text{C}$. There are backup methods available. FIMS (Flow Injection Mercury System) is the alternate method for mercury analysis and spectrophotometric analysis is the alternate method for iodine determination.

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

Report test results as outlined in the *DLS Policies and Procedures Manual*. For critical calls, the supervisor should notify the supervising physician or principal investigator as soon as possible. The most expeditious means should be used (e.g., telephone or E-mail).

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

The analyst who receives specimens or samples delivered to Inorganic Toxicology and Nutrition Branch (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 sample analysis is completed, place the tracking form in the *Specimen Tracking Record Log Book* located in the trace-metals library.

Use standard electronic record keeping means (e.g., Microsoft Access™, optical disk, or tape backup) to track specimens. Maintain records, including related quality assurance (QA) and QC data, for 3 years or longer. 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.

24. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Urinary Mercury by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
LU-03250_UIHG	163	1/25/2007	2/27/2009	0.220	0.037	16.6
HU-03251_UIHG	163	1/25/2007	2/27/2009	2.297	0.108	4.7



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