Laboratory Procedure Manual

Analyte: **Iodine**

Matrix: **Urine**

Method: **Urine Iodine ICPMS** [Renamed from Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)]

Method No.: ITU004A

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as performed by: *Inorganic Toxicology and Nutrition Branch*
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Important Information for Users

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

Public Release Data Set Information

This document details the Lab Protocol for NHANES 2003–2004 data.

A tabular list of the released analytes follows:

File Name	Variable Name	SAS Label
l06uio_c	URXUIO	Urinary Iodine(ng/mL)

1. Clinical Relevance and Summary of Test Principle

    A. Clinical Relevance

    Iodine, an essential element for thyroid function, is necessary for normal growth, development, and functioning of the brain and body. Iodine-deficiency disorder (IDD) is a well documented global health problem, 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 toward 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. Because 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. This method achieves rapid and accurate quantification of iodine content in urine. (1)

    B. Test Principle

    Inductively coupled plasma-mass spectrometry (ICP-MS) is a multi-element analytical technique (2). Liquid samples are introduced into the ICP through a nebulizer and spray chamber carried by a flowing argon stream. By coupling radio-frequency power with flowing argon, plasma is created in which the predominant species are positive argon ions and electrons. The sample passes through a region of the plasma that has a temperature of 6000–8000 K. The thermal energy atomizes the sample and then ionizes the atoms. The ions and the argon enter the mass spectrometer through the interface that separates the ICP, which operates at atmospheric pressure, from the mass spectrometer, which operates at a pressure of 10^{-6} torr. The mass spectrometer permits rapid-sequence ion detection at each mass, which allows determination of individual isotopes of an element. Electrical signals from the ion detection are processed into digital information that is used to first indicate the intensity of the ions and then the concentration of the element. Urine samples are diluted 1+9 with 1% (v/v) tetramethylammonium hydroxide (TMAH) containing tellurium for internal standardization.

2. Safety Precautions

    PerkinElmer provides safety information that should be read before operating the instrument. This information is in the PerkinElmer ELAN 6000 ICP-MS System Safety Manual. Possible hazards include ultraviolet radiation, high voltages, radio-frequency radiation, and high temperatures.

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

    Exercise special care when handling and dispensing concentrated nitric acid. Always remember to add acid to water. Nitric acid is a caustic chemical that is capable of severe eye and skin damage. Wear powder-free gloves, a lab coat, and safety glasses.

> If nitric acid comes in contact with any part of the body, quickly wash the affected area of the body 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, whenever possible, eliminate hand entry of specimen identifiers or analytical results, proofread all transcripted data, and regularly defragment and back up the ICP-MS computer's hard drive.

A. Data Entry and Transfer

Use bar-code scanners whenever possible to enter sample identifiers into the ICP-MS computer software. Following this procedure will help avoid errors associated with the keyboard-entry process and will also speed up sample processing. When bar-code scanners cannot be used, proofread transcribed data after entry. Data handle and transfer electronically when reporting or moving to other computerized data-handling software. The Nutritional Biochemistry Branch, stores sample analysis results generated by this method in Microsoft Access or MS SQL Server 7 database software (including the analysis date, analytical-run number, quality control [QC] results for the run, specimen analytical results by specimen identification [ID] and method identifier).

B. Routine Computer Hard Drive Maintenance

Using software such as Norton Utilities, regularly defragment the computer hard drive to maximize computer performance and to 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, providing documentation of this step.

C. Data Backup

(1) Schedule of Backups

(a) Weekly full-data backups onto one recordable compact disk and onto one tape (active "elandata" directory and all subdirectories). Set aside two tapes for these full backups. Use the tapes on an alternate basis (e.g., week 1, use tape 1; week 2, use tape 2; week 3, use tape 1, etc.)

(b) Daily differential backups onto tape save all data files created or altered since the last full-tape backup. Only one daily backup tape is necessary. It will be overwritten each night when the backup is performed.

(2) Backups Procedures

When making a backup (daily or weekly, CD-R or tape) of the active "elandata" directory, include all subdirectories. Before making weekly backups, save a copy of the Windows-event log in the active "elandata" directory. This procedure will ensure that all recent software-system events have been archived (including communications between ICP-MS and ELAN software the numbered of times the hard drive has been defragmented and other Windows system events that have occurred).

Compact-Disk Backups

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

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

(c) Use Adaptec Easy CD Creator or similar software to make compact-disk backups. (For Easy CD Creator v.4 software, use the following settings: "Create Data CD", "Create CD" under "Create Options", "Track-At-Once" and "Close CD" under "Write Method").

Tape Backups

(a) Use Microsoft Backup Exec or similar software to make tape backups.

(b) Set up the differential backup to run automatically when the instrument is not in use (e.g., 2:00 a.m.). This procedure reduces the possibility of the backup software and the ICP-MS software interfering with one another.

Removing Data from the ICP-MS Computer Hard Drive

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

(a) Back up the oldest data on the hard drive onto two CD-R disks and two tapes. Manually select each dataset folder (subdirectories under "elandata"/dataset) and other relevant files (i.e., optimization, tuning, and sample files) that are included on these backups in the CD-R software. Back up the entire "elandata" directory (and all subdirectories) to tape because tape capacity is much larger than CD-R capacity.

(b) Before deleting any data from the hard drive verify that backup CD-R disks and tapes are operating correctly.

(i) To verify the operation of a CD-R disk, use the appropriate computer software (ICP-MS software or otherwise) to open any file on the disk.

(ii) To verify the operation of a tape, copy a file from the tape back onto a location on the computer hard drive, then use the appropriate computer software (ICP-MS software or otherwise) to open it.

(iii) After verifying backup operation, delete the original data from the hard drive.

(iv) Keep one copy of the CD-R disk and tape in a building other than the laboratory building (in case of fire in one structure). Keep the other copy near the ICP-MS laboratory.

Backup of Sensitive Data

(a) Make duplicate, recordable compact-disk backups of sensitive data and store the two CD-R disks in two different buildings.

D. Documentation of System Maintenance

Computer maintenance Record all maintenance of computer hardware or ICP-MS software in the instrument log book. Other electronic records relating to integrity of the data and hard drive are in the Windows system-event log. Back up the event log on a regular basis by saving a copy of it 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 checklist, PerkinElmer service records, and instrument log book) as well as in electronic records relating to instrument optimization (default.dac), tuning (default.tun).

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

No special instructions for fasting or special diets are required.

A.   The specimen type is urine

B.   The optimal amount of specimen required is 4–7 mL; the minimum required volume is about 1.5 mL.

C.   Acceptable containers include prescreened, 15-mL polypropylene (PP) tubes and 5.0-mL cryovials. Use sterile collections containers for urine specimen acquisition.

D.   The criteria for unacceptable specimens are either a low volume (< 1.5 mL) or suspected contamination due to improper collection procedures or collection devices. In all cases, request a second urine specimen.

E.   Specimen characteristics that may compromise test results include contamination of urine through contact with dust, dirt, etc., from improper handling.

F.   The division protocol for urine collection and handling outlined specimen-handling conditions. 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 4°C. Upon receipt, freeze the specimens at –20°C until time for analysis. The analyst puts the remaining samples in the freezer after analytical aliquots are done and refreezes them at –20°C. Samples that are thawed and refrozen several times will not be compromised.

5.   Procedures for Microscopic Examinations; Criteria for Rejection 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)   Diluent (1% TMAH, 0.01% Triton X-100 plus 10 µg Te/L)

The diluent used in this method is an aqueous solution of 1.0% (v/v) TMAH, 0.01% Triton X-100 and 10 µg/L Te. To prepare, acid-rinse a 2-L Teflon container and partially fill with ultrapure, 18 M-ohm water. Add 80 mL of 25% (v/v) TMAH 10 mL of 2% Triton X-100, and 20 µl of Te. Dilute to 2 L with ultrapure water. Store at room temperature and prepare as needed.

(2)   ICP-MS Rinse Solution (1% TMAH plus 0.1% Triton X-100)

The wash solution used in this method is an aqueous solution of 0.1% Triton X-100 and 1.0% TMAH. 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. To prepare, acid-rinse a 2-L Teflon container and partially fill with ultrapure18 M-ohm water. Add 80 mL of 25% (v/v) TMAH and 100 mL of 2% Triton X-100/ 5% GFS double-distilled, concentrated nitric-acid solution. Dilute to 2 L with ultrapure water. Store at room temperature and prepare as needed.

(3)   Base Urine

The base urine used in this method is a pool of the urine collected from several donors. Combine this base urine with intermediate working standards during the dilution process just prior to analysis. Initially, collect urine in 1-L, acid-rinsed, wide-mouth bottles. After the urine is collected from donors, mix together into a Teflon bottle. Aliquot into 15-mL PP centrifuge tubes; use one tube at a time. Store at 4°C between uses.

B.   Standards Preparation

(1)   Stock standard: Iodide stock standard 1,000 µg/mL.

(2)   Working stock standard: 100 µg/mL.

(3)   Intermediate standards: 0, 10, 50, 100, 500, 1,000. (Dilute with ultrapure 18 M-Ω water.)

Prepare the intermediate standards as follows:

S1 Partially fill a 100-mL, acid-washed, PP volumetric flask with ultrapure water. Then add 10 μL of the initial dilution of intermediate stock standard. Bring to a final volume of 100 mL with ultrapure18 M-Ω water. Pour desired amount into cleaned and labeled 50-mL tubes.

S2 Partially fill a 100-mL, acid-washed, PP volumetric flask with ultrapure water. Then add 50 μL of the initial dilution of intermediate stock standard. Bring to a final volume of 100 mL with ultrapure18 M-Ω water. Pour desired amount into cleaned and labeled 50-mL tubes.

S3 Partially fill a 100-mL, acid-washed, PP volumetric flask with ultrapure water. Then add 100 μL of the initial dilution of intermediate stock standard. Bring to a final volume of 100 mL with ultrapure18 M-Ω water. Pour desired amount into cleaned and labeled 50-mL tubes.

S4 Partially fill a 100-mL, acid-washed, PP volumetric flask with ultrapure water. Then add 500 μL of the initial dilution of intermediate stock standard. Bring to a final volume of 100 mL with ultrapure18 M-Ω water. Pour desired amount into cleaned and labeled 50-mL tubes.

S5 Partially fill a 100-mL, acid-washed, PP volumetric flask with ultrapure water. Then add 1,000 μL of the initial dilution of intermediate stock standard. Bring to a final volume of 100-mL with ultrapure18 M-Ω water. Pour desired amount into cleaned and labeled 50-mL tubes.

Freeze the unused tubes at –20°C in order to maintain integrity of iodide. Use one tube at a time.

Urine Iodine Standards:

The urine iodide standard solutions used in this method are a series of 1:50 dilutions of the corresponding intermediate standards. In this step, add the aqueous intermediate working standards to the diluent and base urine to matrix matches the working standards with the urine samples being analyzed. Run these solutions along with the diluted donor urine samples for the purpose of external calibration. To prepare, transfer 50 mL of the appropriate aqueous intermediate standard, 50 mL of base urine, and 2,400 mL of diluent to a 15-mL tube using the Micromedic Digiflex. See Appendix A.

C. Preparation of Quality Control Materials

The QC material used in this method is a pool of the urine collected from several donors. Combine this base urine with intermediate working standards during the dilution process just prior to analysis. Initially, collect urine in 1-L, acid-rinsed PP or polymethylpentene (PMP) wide-mouth bottles. Mix together into a 2-L Teflon bottle. Store at 4°C between uses.

D. Other Materials

(1) Primary stock solution: Iodide 1,000 μg/mL, SPEX VCDCGA-1 (SPEX Industries, Inc., Edison, NJ) or equivalent.

(2) GFS double-distilled nitric acid (GFS Chemicals Inc., Columbus, OH) or equivalent.

(3) Tetramethylammonium hydroxide (TMAH; Alfa Aesar, Johnson Matthey Co., Ward Hill, MA) or equivalent.

(4) Ultrapure water (18 M-Ω from the Milli-Q water-purification system) Millipore Corporation, Bedford, MA.

(5) Liquid argon (Holax or other contract agency) equipped with approved gas regulator (Matheson Tri-Gas Products, San Jose, California).

(6) Base urine pooled from healthy donors.

(7) Tellurium: SPEX PLTE2-2Y. 1,000 mg/L (SPEX Industries, Inc., Edison, NJ) or equivalent.

(8) Pipette tips: 1-200 μL (#RT-20, fits up to 100 μL pipettes) and 200-1000 μL (#RT-200, fits between 100 μL and 1,000 μL pipettes) sizes (Rainin Instrument Co., Inc., Woburn, MA) or equivalent.

(9) Acid-cleaned PP and PMP volumetric flasks (100-mL, 2,000-mL volumes) for standards and QC preparation. One 10-mL acid-cleaned volumetric flask. Three 2-mL acid-cleaned Teflon bottles (one for diluent, one for base urine pool, and one for the 2% Triton X-100/5% GFS double-

distilled, concentrated nitric-acid solution). Five 1-L acid-cleaned, wide-mouth PP (or PMP), (four for base urine collection from donors, and one for rinse solution). Rinse containers and flasks with reagent-grade, concentrated nitric acid, then rinse rigorously with 18 M-ohm Milli-Q water.

(10) 15-mL conical tubes (#2097) and 50-mL conical tubes (#2098): (Becton, Dickinson Labware, Franklin Lakes, NJ) or equivalent.

(11) Triton X-100 ("Baker Analyzed," J. T. Baker Chemical Co., or any source whose product is low in trace metal contamination.).

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

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

(14) Vinyl examination gloves (Travenol Laboratories, Inc., Deerfield, IL) or equivalent.

(15) Biohazard autoclave bags (Curtin-Matheson Scientific, Inc., Atlanta, GA) or equivalent.

(16) Bleach (10% sodium-hypochlorite solution) from any vendor.

(17) Reagent-grade, concentrated nitric acid (J.T. Baker Chemical Co. or any source with comparable reagent).

E. Instrumentation

(1) Use any reliable, inductively coupled plasma-mass spectrometer that is capable of rapid monitoring of selected masses (peak hop mode). Inductively Coupled Plasma-Mass Spectrometer ELAN 6100 (PerkinElmer, Norwalk, CT). Optimize settings daily for iodine.

Table 1. Optimal Daily Settings for Iodine

Parameter	Setting
RF Power	1.2 KW
Ar Nebulizer Gas Flow	1.01 LPM
Detector Mode	Pulse
Measurement Units	Cps
Replicates	3
Readings/replicate	1
Sweeps/reading	25
Replicate time	20.3 sec
Dwell time	200 ms; Te: 200ms

(2) Micromedic Digiflex automatic pipette equipped with 10.0-mL dispensing syringe, 2,000 μL sampling syringe, 0.75-mm tip, and the foot pedal, if desired (Micromedic Systems, Inc., Horsham, PA).

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

(4) Eppendorf fixed-volume micropipettes: 1,000, 500, 100, 50, and 10 μL

(5) volumes (Brinkmann Instruments, Inc., Westbury, NY) or equivalent brand.

7. Calibration and Calibration-Verification Procedures

A. Calibration Curve

Using a series of five external standards with concentrations that are defined in the calibration page of the quantitative analysis method software, generate a simple linear calibration curve for iodine. The calibration curve plots the ratio of the observed intensities for iodine and the internal standard versus

the concentration of the calibrator. Compare the ratio of the observed intensities for iodine and the internal standard obtained from the patient sample to those obtained from the calibrators to determine the concentration of iodine in the sample.

B.   Calibration Verification

There are no primary or secondary matrix-matched reference materials available to measure urinary iodine. In order to verify the concentrations of the calibrators being used in this method, urine iodine materials having concentrations assigned by a consensus mean of laboratories involved in the EQUIP (Ensuring the Quality of Iodine Procedures) programs are analyzed periodically (at least every 6 months) and the results are compared to their target values. Agreement of results should be within 3 standard deviations (SD) of the median value for the participating laboratories and within 2 SD of the mean of the ICP-MS-participating laboratories. Store calibration-verification data in the Laboratory Calibration Verification Log. Place copies in the ICP-MS Maintenance Log in the laboratory housing the instrument.

8.   Operating Procedure; Calculations; Interpretation of Results

A.   Preliminaries

(1)   For information regarding 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 9).

(2)   Allow frozen urine specimens, QC specimens, and base urine calibration material to reach ambient temperature. Before taking an aliquot for analysis, mix the sample so that no particulates remain on the bottom of the tube.

B.   Sample Preparation (See Appendix A)

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

(2)   Set up a series of 15-mL PP centrifuge tubes corresponding to the number of blanks, standards, QCs, and patient samples to be analyzed.

(3)   Using the Micromedic Digiflex, prepare the following solutions in the 15-mL tubes.

(4)   Prepare an aqueous blank consisting of 400 μL of ultrapure 18 M-Ω water, and 3600 μL of diluent.

(5)   Prepare urine blank consisting of 300 μL of base urine (same material used for preparation of the urine calibration standards), 100 μL of ultrapure water, and 3600 µL of diluent.

(6)   Prepare the patient urine samples consisting of 3600 μL of diluent, 100 μL of the patient urine sample, 300 µL ultrapure 18 M-ohm water

(7)   Prepare working QC samples consisting of 100 μL of QC sample 300 μl ultrapure 18 M-Ω water and 3600 μL of diluent.

(8)   Mix samples well by vortexing for at least 30 seconds.

C.   Instrument and Software Setup for the ICP-MS

(1)   Turn on the computer, printer, peristaltic pump, and autosampler. Log in as "Administrator." (Press the "Enter" key rather typing in a password.)

(2)   Start the ELAN ICP-MS software from Windows and verify that 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 6100 Hardware Guide (i.e., Argon supply, interface components, cleanliness and 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 Book.

(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) After the plasma ignites, press the "Connect" button (in the DEVICES window of the software) to establish communication between the computer and the autosampler. 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.) Fill the rinse-station reservoir quickly by pressing the "Fast" button in the DEVICES window. When the rinse station is filled with the rinse solution, type in "12" 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 if the rinse solution is not flowing properly to the rinse station, adjust the tension screws on the back of 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-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 moves 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, 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 mass spectrometer. Complete daily optimization procedures as required according to Chapter 3 of the ELAN 6100 Inductively Coupled Plasma-Mass Spectrometer Software Guide. Use PerkinElmer, P/N N812-2014 10-ppb multi-element solution (added to 10-ppb Beryllium). Record the results for the daily optimization procedures in the Daily Maintenance Log Book.

(9) Click on "Open Workspace" from the "File" menu. Select workspace file "urinary iodine.cdc.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.

Table 2. Setup of Files and Directories for the Data

Method:	"urinary iodine.cdc.wrk"
Dataset:	If this is the first run of the day, create a new dataset using the date as the name. (Use the format 010202 for January 2, 2002). If a run has already been performed today, select the dataset for today's date.
Sample:	If a similar analysis has already been performed, 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 (autosampler locations, sample identification and analysis methods). Use two method files ("urinary i.mth" and "urinary i aq blank.mth"), which differ only in the autosampler locations of the calibration solutions. Use the "urinary i.mth" method file for the first three or four samples, which are only used to condition the sample introduction components, base urine, urine blank, and calibration standards. (Urine blank goes in AS location 9, standards 1–5 go in locations 10–14, respectively; and the base urine goes in AS location 1 and 16.) Use the "urinary i aq blank.mth" method for the aqueous blank (AS location 17) and all other samples. A typical sample file for this method looks like the following:

Table 3. Typical Sample File for the urinary.i.aq.blank.mth Method

A/S Loc.	Sample ID	Measurements Action	Method
1	Base urine	Run sample	urinary.i.mth
1	Base urine	Run sample	urinary.i.mth
1	Base urine	Run sample	urinary.i.mth
16	Base urine	Run blank, standards, and sample	urinary.i.mth
18	UILP2	Run blank and sample	urinary.i.aq.blank.mth
19	UIHP2	Run sample	urinary.i.aq.blank.mth
20	Sample 1	Run sample	urinary.i.aq.blank.mth

Use the following settings for uptake and rinse times for all samples. (These values are already stored in the method files for the blanks and standards.)

Table 4. Sample File Timing Parameters for a Urine Iodine Analysis Run

	Pump Speed*	Duration
Sample flush	–48 rpm	20 s
Read delay and analysis	–12 rpm	75 s
Wash	–21 rpm	100 s
* 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.		

(11) Edit the sample file, and then place solutions in the autosampler tray according to the sample and method files. Highlight the samples that you want to measure, then click on "Analyze Batch."

D.   Recording of QC Data

Store the results of the QC samples analyzed in each run in the Microsoft Access (or MS SQL Server 7) database when all other data for the run are imported from the ELAN software. The database allows several types of QC reports to be printed. Print one of these reports and keep it in the analyst's laboratory analysis notebook for future reference. Store or place another copy with the analysis printouts from the run.

(1)   Storing Quality Control Data in Microsoft Access

(a)   Analysis Printouts and Analyst-Run Report

Bind the analysis printouts and a printout of the calibration curve and curve statistics and place them in the study folder(s). Store the results of the patient samples analyzed in each run in the Microsoft Access (or MS SQL Server 7) database when all other data for the run are imported from the ELAN software. The database allows run summary reports to be printed. These reports can provide such information as whether patient results were outside of the normal concentration reference range or whether any measurements failed precision limits. Print one of these reports and keep it in the analyst's laboratory analysis notebook for future reference. Keep another copy with the analysis printouts from the run.

(b)   Supervisor Review

Use the Microsoft Access or MS SQL Server 7 database. The supervisor can review the QC and sample results in the database. After the supervisor reviews the data, file the paper printouts from the analysis run in the study folder(s).

(c) Plotting Quality Control (QC) Results

The Microsoft Access or MS SQL Server 7 database, automatically updates QC plots when the data are imported into the database. Monitor these plots regularly for any trends in the bench QC results. If trends are observed, contact the laboratory supervisor.

(d) Using the Microsoft Access Database

After an analysis run, export the results to a .TXT file, and then import them into the Microsoft Access or MS SQL Server 7 database that handles data for the Nutritional Biochemistry Branch. Print report summaries for QC and sample results and keep them with the hard copies of the data printout from the ICP-MS in the study folder.

(i) Data-Export Process (from ELAN software to .TXT file). In the ELAN ICP-MS software, select "Review Files" from the "File" menu. From this window, open the files and directories that you used to collect exportable data. (If the analysis has just ended, all of these files and directories still will be open.) Note: A second copy of the ELAN software can be run as an "Edit/Reprocess" copy without affecting an ongoing analysis being conducted by the first copy of the software. After opening 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 "database_output.rep" and type in a report file name by using a format such as "08022001a_group55.txt" to designate data from analysis of group 55 from August 2, 2001, run #1. Select the "Report Format" option "Use Separator" and the "File Write" option "Append." Finally, reprocess the data of interest (see the PerkinElmer ELAN 6100 Software Manual). Apply the correct blank to the correct samples and QCs. (Use the urine blank for all of the calibration standards. Use the aqueous blank for all analyses of patient samples and QC samples.)

(ii) Data-Import Process (from .TXT file to Microsoft Access database). Transfer the .TXT 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 identification, such as I:/Instruments/ELAN 6100A /2001/08/Study 2001-01.) Using your user ID, log into a computer that has access to Microsoft Access or MS SQL Server 7 database to track data. Once logged into the database, open the ELAN 6100 A directory in the GO TO window. Select "Import Instrument File." Enter the appropriate information in the "Instrument", "Analyst", "Assay", "StudRefID", and "Run Number" fields 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 are not recognized as valid QC-pool IDs or sample IDs 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 (i.e., correction of transcription errors and adjustment for level of dilution). If samples were diluted for analysis, edit both the sample IDs and the dilution factor in this table before transferring the values to the database. First, change the dilution factor to reflect the way that the sample was analyzed, and 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 for this.) After making any corrections to sample IDs, 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.

E. Replacement and Periodic Maintenance of Key Components (The part numbers are PerkinElmer numbers from the 1996-1997 Atomic Spectroscopy Supplies Catalog.)

(1) Nickel skimmer (#WE02-1137) and sampler cones (#WE02-1140). Keep at least two of each on hand.

(2) Skimmer and sampler cone O-rings (#N812-0512 and #N812-0511, respectively). Keep at least five of each on hand.

(3) Quartz torch. At least two spare torches should be on hand (#N812-2006) or equivalent.

(4) RF coil (#WE02-1816). Keep one spare should be on hand.

(5) Injector support/torch base (#N812-0116) or equivalent . One spare should be on hand.

(6) Torch O-ring kit (#N812-0100) or equivalent. One spare kit should be on hand.

(7) Alumina 2.0-mm inner diameter (i.d.), sample injector (#N812-6041) or equivalent. At least two spare injectors should be on hand.

(8) Ryton spray-chamber kit (#N812-0124). One spare kit should be on hand. Large O-ring (#WE01-3060), retaining ring (#WE01-4081), and right-angled drain connector (#WE01-3119) can be ordered individually or equivalent. One spare of each should be on hand.

(9) Crossflow nebulizer body (#N058-0613) or equivalent. Keep at least one on hand.

(10) Crossflow nebulizer tips (#N058-0624) or equivalent. Keep at least two spare pairs on hand (one pair = one tip for liquid + one tip for gas).

(11) Crossflow Nebulizer O-ring kit (part # N930-00667) or equivalent: at least 2 spare kits on hand.

(12) Peristaltic-pump tubing for sample (0.03-inch i.d., #0990-8587) and for waste (0.125-inch i.d., #N812-2012). Keep at least two packages of 12 on hand of the sample tubing, and one package of 12 on hand of the waste tubing. Other suppliers may offer the same size or type of peristaltic tubing.

(13) Nebulizer capillary tubing used to connect the nebulizer and the peristaltic-pump tubing, #0990-8265 or any source of polyethylene tubing, 0.6-mm i.d. x 0.97-mm outer diameter (o.d.). Keep one pack (10 feet) on hand.

(14) Autosampler probe (#B300-0161). One spare should be kept on hand.

(15) Pump oil for the roughing pump (#N810-2201) or equivalent. Keep four bottles on hand.

(16) Neslab chiller coolant (PE Sciex coolant, #016558A) or equivalent. Keep two 1-L bottles on hand.

F. Calculation

(1) The ELAN ICP-MS has two onboard microcomputers that work with the external-system computer. The computers interface with other electronic components within the system to convert the detector signals to digital-ion-intensity values. As standard solutions are analyzed, the software plots the measured intensity versus the concentration for each element in the standard solution. These individual calibration curves are updated as each subsequent standard is analyzed. Internal standards are used (Te), which allows for the correction of changes in instrument response. The software uses the ratio of analyte and internal-standard intensities to determine the actual intensities for the analyte. Because the responses to instrumental effects for all the elements in a standard group are assumed to be similar to the response for the internal standard, the ratio of iodine's intensity to the internal-standard's intensity is used for each element. Concentrations for the elements from the printout equal the concentrations of the elements detected in the urine sample.

(2) The detection limits for elements in urine specimens are based on 3 times the concentration SD at a low-normal concentration base urine that is run a minimum of 20 times. (6). See results below the detection limit are reported as no detectable (less than the calculated lower detection limit [LDL]) refer to Section 9 in this document).

G. Special Procedure Notes – CDC Modifications

None applicable for this operation.

9. Reportable Range of Results

    Urine iodine values are reportable in the range LDL < urine iodine < 1,000 µg/L without dilution, where LDL= the calculated (3 SD) lower detection limit. If a urine iodine value is < 1.0 µg/L (the approximate LDL of this method), report it as < 1.0 µg/L; if the volume is greater than 1,000 µg/L, dilute the specimen to (1.1 or whatever is appropriate) and reanalyze it.

    Typical correlation coefficients for the calibration curves will have three 9s or better.

10. Quality Control (QC) Procedures

    The Inorganic toxicology and Nutrition branch uses the method described in this protocol for nutritional-health screening studies. The method is accurate, precise, and reliable.

    This analytical method utilizes two types of QC systems. With one type of QC system the analyst inserts "bench" QC specimens at the start of each run, before and after every set of 20 samples, and at the end in each analytical run (a set of consecutive assays performed without interruption) so that judgments can be made on the day of analysis. With the other type of QC system, "blind" QC samples are placed in vials and are then labeled and processed so that they are indistinguishable from the subject samples. The supervisor decodes and reviews the results of the blind specimens. With both systems, assess all levels of the analyte concentrations by taking these samples through the complete analytical process. Then use the data from these materials 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 for the analytes of interest; they are named UIL2 and UIH2, respectively. Analyze both of these pools after the calibration standards but before analyzing any patient samples (Section 8.C.(9)) so that judgments on the analyte calibration curves can be made prior to analyzing patient samples. Analyze both the UIL2 and UIH2 QC samples again after every 20 patient samples and also at the end of the run.

    Establish QC limits for each QC pool. Perform an analysis of variance for each pool after performing at least 20 characterization runs in which previously characterized QC and bench QC pools are used for evaluation. In addition to providing QC limits, the characterization runs can also serve to establish homogeneity of the pools. After establishing the homogeneity of the bench and blind materials, have them analyzed by another independent reference method, e.g., standard additions, if possible.

    Precision and Accuracy

    A. Quality Control Results Evaluation. After the completion of a run, consult the QC limits to determine if the run is "in control." The QC rules apply to the average of the beginning and ending analyses of each of the bench QC pools. The 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 QCs OR average of both high QCs is outside of a 3s limit).
            (b) 2_{2s} – Average of both low QCs AND average of both high QCs is outside of 2s limit on the same side of the mean.
            (c) R_{4s} sequential – Average of both low QCs AND average of both high QCs 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.

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

B.   Sample-Results Precision Evaluation

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% of the mean of the three replicates, repeat the analysis of that sample.

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

If one or more QC samples fall outside 95% limits for mean or range of the three replicate values, do the following steps:

A.   Prepare fresh working urine iodine calibration standards (Section 6.B.) and run the entire calibration curve using freshly prepared standards;

B.   Prepare fresh working urine iodine QC standards (Section 6.C.) and reanalyze.

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

12.   Limitations of Method; Interfering Substances and Conditions

No limitations or interfering substances or conditions to this method (other than those mentioned in Section 4.E. and 4.F.).

13.   Reference Ranges (Normal Values)

The Mayo Clinic defines normal values as falling between 42 and 350 µg/L for spot urine samples and between 100 and 460 µg/L for 24-hour urine samples.

14.   Critical-Call Results (Panic Values)

For urinary iodine levels in excess of 10,000 µg/L, report the levels by facsimile, telephone, or e-mail to the supervising physician or principal investigator.

15.   Specimen Storage and Handling During Testing

Specimens may reach and maintain ambient temperature during analysis. Take stringent precautions to avoid external contamination by the iodine to be determined.

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

No backup method is available. If the analytical system fails, then store urine specimen at 4ºC until the analytical system is restored to functionality. If long-term interruption (longer than 4 weeks) is anticipated, then store urine specimens at –20°C.

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

Conduct test-result reporting as outlined in the Division of Laboratory Sciences Policies and Procedures Manual. (As stated in Section 14, the supervisor should notify the supervising physician or principal
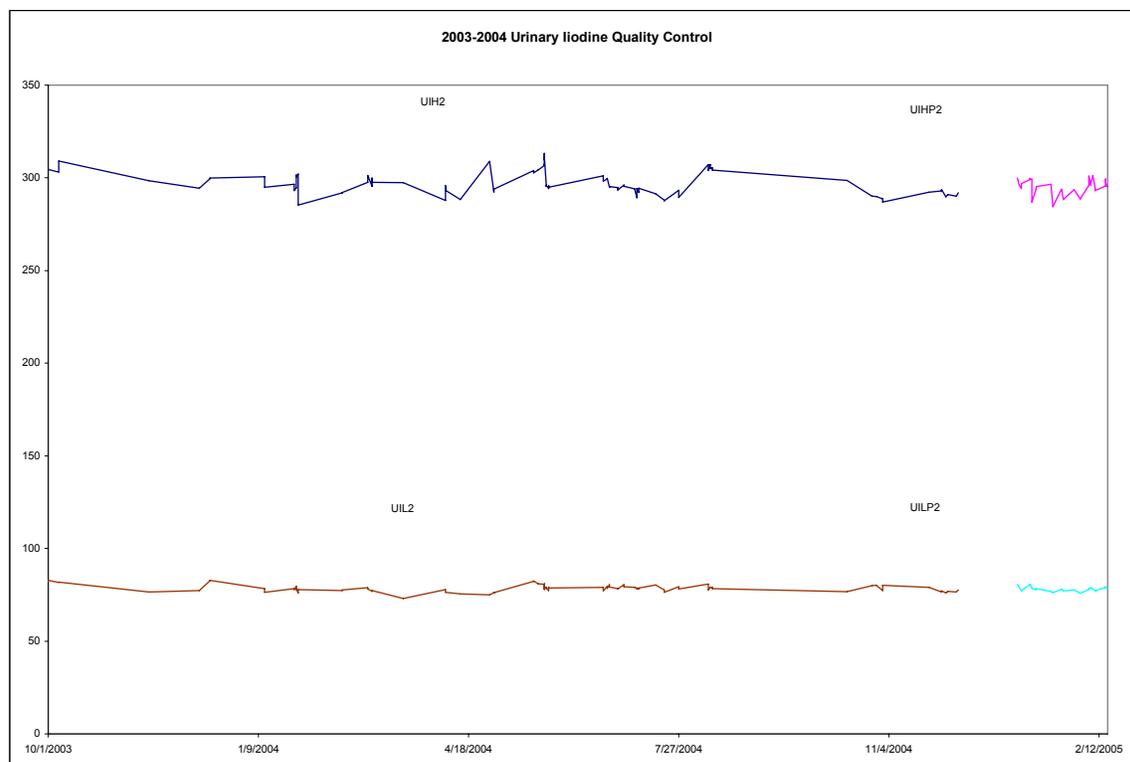
investigator as soon as possible for critical calls). Utilize the most expeditious means; telephone, facsimile, etc.)

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

The analyst who receives specimen/samples delivered to Nutritional Biochemistry Branch sets up a "Specimen Folder." Fill out a tracking form and place it in the folder for the analyst performing the analysis. The form tracks location, status, and final disposition of the specimens. After completion, 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 - Rbase, server-data files, optical disk or floppy disk) to track specimens. Maintain records for 3 years, including related Quality Assurance/QC data; keep duplicate records (off site, if sensitive or critical) in electronic or hardcopy format. Use only numerical identifiers (e.g., case ID numbers). All personal identifiers are available only to the medical supervisor or project coordinator. In order to safeguard confidentiality, provide only the MS or PJ with personal identifiers.

19. Summary Statistics and QC Graphs

Summary Statistics for Urinary Iodine by Lot						
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
UIL2	94	10/1/2003	12/7/2004	78.61398	1.86312	2.4
UIH2	94	10/1/2003	12/7/2004	297.554	6.45225	2.2
UILP2	27	1/4/2005	2/16/2005	78.25092	1.20603	1.5
UIHP2	27	1/4/2005	2/16/2005	295.30849	4.2217	1.4

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Appendix A

Prepare the following solutions in the 15-mL polypropylene centrifuge tubes using the Micromedic Digiflex

Identifier (ID)	Base Urine	18 Mega-Ohms (MΩ) Milli-Q Water	Intermediate Standards	Sample	Diluent (1% TMAH, 10 ppb Te, 18 MΩ Milli-Q Water)
Aqueous (Aq) blank		400 µl			3600 µl
Urine blank	300 µl	100µl			3600 µl
Working standards (stds)	300 µl		100 µl		3600 µl
Quality-Control (QC) / Samples		300µl		100 µl	3600 µl