Laboratory Procedure Manual

Analyte: **Total Arsenic**

Matrix: **Urine**

Method: **Urine Arsenic ICPDRCMS**
Renamed from "Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectrometry (ICP-DRC-MS)"

as performed by: Inorganic Toxicology and Nutrition Branch
Division of Laboratory Sciences
National Center for Environmental Health

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Important Information for Users

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

Public Release Data Set Information

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

A tabular list for the released variable follows:

File Name	Variable Name	SAS Label (and SI units)
l06uas_c	URXUAS	Total Arsenic, urine(µg/L)

Modifications/Changes: see Procedure Change Log

Procedure Change Log

Procedure: Urine Arsenic ICPDRCMS DLS Method Code: ITU002A

Date	Changes Made	By	Rev By (Initials)	Date Reviewed
10/21/02	Method analysis parameters changed for operation on a PerkinElmer ELAN® 6100 DRC Plus or ELAN® DRC II ICPMS. Sweeps per reading was 6, now 20 Dwell time was 500ms, now 50ms Cell Gas flow rate was 0.2 mL/min Now 0.2 – 0.3 mL/min (optimized per instrument) RPQ was 0.65, now 0.65 - 0.75 (optimized per instrument) Original method was written for operation on a PerkinElmer ELAN® 6100 DRC.	Jeff Jarrett		
10/21/02	Daily backups of computer data files to magnetic tape computer backups replaced with daily backups to external hard disk drives.	Jeff Jarrett		
10/21/02	Analysis of urine blank check “UrBlkChk2” moved from the end of the analysis run to immediately after “UrBlkChk1”.	Jeff Jarrett		
10/21/02	Duration of sample flush time changed from 30s to 35s.	Jeff Jarrett		
10/21/02	The QC tab in the method should be setup so that an additional 180s rinse time will occur after any sample whose As concentration exceeds 300 ppb.	Jeff Jarrett		
10/21/02	The rinse times on the calibration standards in the method should be set to 120s for standards 4 and 5, and 90s for all others.	Jeff Jarrett		
3/1/04	The rinse times on for all standards and samples were changed to 60s.	Jeff Jarrett		
5/24/04	Prepare and use an extended calibrator set (or parts of it) to avoid the need for diluting patient samples more than 1+1 during analysis. Dilutions of patient samples greater than 1+1 relative to the typical dilution scheme have been observed to result in significantly lower analysis results for urine arsenic relative to results from undiluted samples. This is thought to be due to a reduction of the ‘carbon effect’ on arsenic from the urine matrix in the sample with extra dilution (relative to the	Jeff Jarrett		

	typical 1+9 dilution of the urine). The 'carbon effect' is well reported in the literature for arsenic and other elements with high ionization potentials (see references 1&2 below). (1) Arsenic Stock Standard The stock standard solution described in these instructions is a single-element 1000 ug/mL aqueous solution of arsenic. It may be purchased from any vendor as long as the standard is traceable to the National Institute for Standards and Technology (NIST). It is not advised to use the multi-element intermediate standard described in the CDC urine multielement ICPDRCMS method ITU001A because spiking volumes would be too large and resulting concentrations of other elements in the working standards would be high enough to cause significant background problems to persist in the instrument for those elements. This could make switching methods on the instrument on short notice very difficult. (2) Arsenic Intermediate Working Standards To prepare, acid-rinse one 100-mL PP (or PMP) volumetric flask for each calibrator to be prepared and fill to the neck of the flask with >18 Mega-ohm·cm water. To each 100-mL flask add 2 mL of double-distilled, concentrated nitric acid. Mix well, then add the appropriate aliquot of the intermediate stock standard solution: 0.65mL to prep 6500ug/L (650ug/L working) 1.2mL to prep 12000ug/L (1200ug/L working) 2.1mL to prep 21000ug/L (2100ug/L working) 3.8mL to prep 38000ug/L (3800ug/L working) 6.8mL to prep 68000ug/L (6800ug/L working) Bring to volume, mix well, and store at room temperature in acid-cleaned, labeled, 50-mL containers (PP, PMP, or Teflon™). Once prepared, use any or all of these extended calibrators along with the normal 5 calibration standards. Use them each day of analysis to prepare the final working standards that you will place in the autosampler of the ELAN® ICPMS. When using any of the extended calibration curves, the curve type specified on the calibration page of the method should be "weighted linear". It is typically "simple linear"			

	during a normal run calibrated up to 200 ug/L. References 1. Campbell, M. J., Demesmay, C., Olle, M., J. Anal. At. Spectrom., 1994, Vol. 9, pp 1379-1384. 2. Larsen, E. H., Sturup, S., J. Anal. At. Spectrom., 1994, Vol. 9, pp 1099-1105.			

1. Clinical Relevance and Summary of Test Principle

A. The method described in this manual assesses arsenic exposure by analyzing urine through the use of inductively coupled-plasma dynamic reaction cell-mass spectrometry (ICP-DRC-MS). Urine is analyzed because urinary excretion is the major pathway for eliminating arsenic from the mammalian body (4). This method achieves rapid and accurate quantification of total urinary arsenic. Although this method does not reveal the chemical form of arsenic to which a person is exposed, it is sensitive enough to screen urine specimens rapidly from people thought to be exposed to arsenic or to evaluate total environmental or other total non-occupational exposure to arsenic.

Total urine arsenic concentrations are determined by using ICP-DRC-MS. This multielement analytical technique is based on quadrupole ICP-MS technology (5) and includes DRC™ technology (6), which minimizes or eliminates much argon-based polyatomic interference. 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 by using a nebulizer inserted within a 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. In the DRC™, elimination or reduction of argon-based polyatomic interferences takes place through the interaction of the reaction gas with the interfering polyatomic species in the incoming ion beam. The quadrupole in the DRC™ allows elimination of unwanted reaction by-products that would otherwise react to form new interferences. 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. In this method, arsenic (isotope mass 75) and gallium (isotope mass 71) or tellurium (isotope mass 126) is measured in urine by ICP-DRC-MS using argon/hydrogen (90%/10%, respectively) as a reaction gas (7). Urine samples are diluted 1+9 with 2% (v/v) double-distilled nitric acid containing gallium or tellurium for internal standardization.

2. Safety Precautions

PerkinElmer provides safety information that should be read before operating the instrument. This information is found in the PerkinElmer ELAN® 6100 ICP-DRC-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 disposable plastic, glass, and paper (e.g., pipette tips, autosampler tubes, and gloves) that come in contact with 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 urine was handled with a 10% (v/v) sodium hypochlorite solution. 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 urine 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.

Exercise caution 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 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 7™ 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.

B. Routine Computer Hard Drive Maintenance

Defragment the computer hard drive regularly by using software such as Norton Utilities™ 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.

C. Data Backup

1. Schedule of Backups

Weekly. Perform a full data backup onto a recordable compact disc (active "elandata" directory and all subdirectories).

Daily. Daily backups onto a physically separate hard disk drive saves all data files related to the ICPMS instrument.

2. Backups Procedures

Whenever making a backup (daily or weekly, hard drive or CD-R) include the active "elandata" directory with 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. 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 similar software to backup. For "Easy CD Creator"™ v.4 software, use the following settings: "Create Data CD", "Create CD" under "Create Options" and "Track-At-Once" and "Close CD" under "Write Method".
- Store CDs in a building other than the laboratory, or in a fireproof room within the building (in case of fire in one structure).

b. Secondary Hard Drive Backups
- Use Microsoft "Backup"™ or similar software for backup to a hard drive which is physically separate from the one onto which the data was written during analysis.
- Set up the backup to run automatically at a time when the instrument is not in use (e.g., 4:00 a.m.) This reduces the possibility of the backup software and the ICP-DRC-MS software interfering with each other.

- Save to the same file daily as backups are done, overwriting the last complete backup. Either complete, differential, or incremental backups can be used. The NCEH / DLS / ITN metals group currently uses complete backup daily since computer speed allows for this task to be accomplished quickly.

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 to compact disk can be done onto a single CD-R. Usually, this procedure can be done annually so that data can be removed from the computer in yearly groupings. Before deleting any group of data from the original hard disk, make a full backup of all data onto the external hard drive (directions above) in case of problems during this procedure.

- Back up the oldest data on the hard drive in duplicate onto two CD-R disks. Manually select each dataset folder (subdirectories under "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 (i.e. – PerkinElmer ELAN ICP-DRC-MS software).
- After verifying that all backups are operational, delete the original data from the primary hard drive.
- Keep one copy of the CD-R disk in a building other than the laboratory, or in a fireproof room within the building (in case of fire in one structure). Keep the other near the ICP-MS laboratory.

d. Backup of Sensitive Data
Make back up for sensitive data on duplicate recordable compact disks, and store the two CD-R disks in two different buildings (or store one of the copies in a fire-proof room).

D. Documentation of System Maintenance

Computer Maintenance: Record any maintenance of computer hardware or ICP-DRC-MS software in the instrument logbook. Electronic records relating to software operations ensuring the integrity of the data and hard drive will be automatically logged into the Windows™ event log. Back up the event log on a regular basis by saving a copy in the active "elandata" directory prior to making a weekly CD-R. The event log will then be backed up along with the ELAN® data when backup CD-R disks 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. Procedures for Collecting, Storing, and Handling Specimens; Criteria for Rejecting Specimens

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

   B. The specimen type is urine.

   C. Optimal amount of specimen is 1.75 mL; minimum is about 0.75 mL.

   D. Use sterile specimen cups for specimen acquisition. Acceptable containers for allotment of urine for this method include 15-mL polypropylene centrifuge tubes (e.g., Becton, Dickinson and Company model number 352097 or equivalent). Screen specimen collection cups and sample tubes for arsenic contamination before use.

   E. The criteria for an unacceptable specimen are either a low volume (< 0.75 mL) or suspected contamination due to improper collection procedures or collection devices. In all cases, request a second urine specimen.

   F. Specimen characteristics that may compromise test results are indicated above and include contamination of urine by contact with dust or dirt from improper handling.

   G. Specimen handling conditions are outlined in the division protocol for urine collection and handling. (Copies available in branch, laboratory and special activities specimen-handling offices.) The protocol discusses collection, transport, and special requirements. In general, transport and store urine specimens at $\leq 4°C$. Upon receipt, they can be frozen at $\leq -20^{o}C$ until time for analysis. Refreeze at $\leq -20°C$ portions of the sample that remain after analytical aliquots are withdrawn. Samples thawed and refrozen several times are not compromised.

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

   Not applicable for this procedure.

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

   A. Reagent Preparation

      1. Diluent
         The diluent used in this method is an aqueous solution of 10 μg/L internal standard(s) (gallium and/or tellurium) in 2% (v/v) double-distilled nitric acid. Add this solution when preparing 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. To prepare the solution, acid-rinse a 2-L container (Teflon™ preferred), and partially fill with18-MegaOhm

(M-Ω) water. Add 40-mL of double-distilled concentrated nitric acid. Spike 0.2 mL of 1,000-mg/L gallium (Ga) and/or 0.2 mL of 1,000 mg/L tellurium (Te) into the container. (Alternatively, an intermediate solution containing both internal standards can be prepared and used.) Dilute to volume (2 L) with18 M-Ω water. Store at room temperature and prepare as needed. To prepare larger volumes of diluent, add proportionally larger volumes of the solution constituents.

2. Base Urine Preparation
The base urine used in this method is an acidified pool of urine collected from anonymous donors. Collect urine in containers screened for arsenic (As) content. After receiving donations, analyze the urine to determine arsenic, gallium, and tellurium concentrations are low enough. The final base urine pool should be ≤ 5-10-μg/L As and ≤ 5-μg/L Ga, and Te. If donated urine specimens have acceptable concentrations of As, Ga, and Te, they should be pooled and acidified to 1% (v/v) double-distilled nitric acid, 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 ≤ -20°C. A 2-L base urine pool should be enough for ~250 analysis runs (~ 15,000 samples at 60 samples per run) Spike urine with intermediate working standards prior to analysis each day to prepare calibration standards for each run.

3. ICP-DRC-MS Rinse Solution
The rinse solution used in this method is an aqueous solution of 5% (v/v) double-distilled nitric acid and 0.002% Triton X-100™. Pump this solution through the sample introduction system between samples to prevent carry-over of arsenic and the internal standard(s) from one sample measurement to the next. For ease of daily preparation of the rinse solution, first prepare a 2%-Triton X-100™, 5% (v/v) double-distilled nitric acid stock solution by adding 40 mL of Triton X-100™ and 100 mL of double-distilled concentrated nitric acid to a preacid-washed 2-L container (Teflon™ preferred) that is partially filled with 18 M-Ω water. Fill to 2 L with 18 M-Ω 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. To prepare the final rinse solution, acid-rinse a 1-L container (polypropylene or Teflon™ preferred), and partially fill with18 M-Ω water. Add 50 mL of double-distilled concentrated nitric acid and 1 mL of the 2%-Triton X-100™, 5% (v/v) double-distilled nitric-acid stock solution. Dilute to 1-L with 18 M-Ω water. Store at room temperature and prepare as needed. To prepare larger volumes of diluent, add proportionally larger volumes of the solution constituents.
Intermediate stock standard and intermediate working standard solutions may be prepared by and purchased from an external laboratory, which then provides target concentration values to be used in the analysis.

Note: The concentrations of some stock standards, such as some National Institute of Standards and Technology (NIST) standard reference materials (SRMs), are certified relative to the mass of the solution, while others are certified relative to the volume of the solution. Refer to the certificate of analysis to determine this (specific for the lot number of the standard being used). If the standard was certified relative to mass, perform concentration calculations based on the weight measurements

recorded during standards preparation. Update the calibration information in the ELAN® ICP-DRC-MS software to reflect the new calibrator concentrations each time the calibrators are remade. This is necessary because the same mass of solution will not be pipetted each time the dilutions are made, resulting in different calibration standard concentrations. Instructions here are for a stock solution that is certified relative to the mass of the solution.

a. Arsenic Intermediate Stock Standard

The intermediate stock standard solution used in this method is an aqueous 2% (v/v) nitric acid solution spiked with arsenic from NIST SRM 3103a arsenic stock standard solution. (Alternatively, another NIST-traceable, arsenic-standard solution may be used as the beginning stock standard). The intermediate stock standard solution is the first dilution of the primary stock standard from which all intermediate working standards will be made. To prepare the intermediate stock solution, partially fill a 100-mL acid-washed volumetric flask (polypropylene or polymethylpentene flask preferred) with 18 M-Ω water and add 2 mL of double-distilled concentrated nitric acid. Determine the mass of a 1-mL aliquot of NIST SRM 3103a addition to the flask. Next, bring to volume in a 100-mL volume flask with 18 M-Ω water. Mix the solution thoroughly and carefully add the remaining little drops of water needed to dilute to exact volume. The concentration (in μg/L) of the resulting intermediate stock standard solution can then be calculated by using the following formulas:

$$\text{As Conc. (mg/L)} = \frac{\text{mass SRM 3103a spike (g)} \times \text{conc. SRM3103a (mg As/g)}}{0.100\ \text{L}}$$

$$\text{As Conc. (μg/L)} = \text{As Conc. (mg/L)} \times 1{,}000\ \text{μg/mg}$$

Store the solution in several smaller portions (i.e., four-25mL portions) in acid-washed containers at room temperature and prepare as needed.

b. Arsenic Intermediate Working Standards

The intermediate working standard solutions used in this method are a series of five aqueous dilutions of the arsenic intermediate stock standard solution in 2%(v/v) double-distilled nitric acid. Use these solutions each day of analysis to prepare the final working standards that will be placed in the autosampler of the ICP-DRC-MS. To prepare, acid rinse five 100-mL volumetric flasks and partially fill them with 18 megaOhm water. Add 2 mL of double-distilled concentrated nitric acid to each flask. Spike each flask with the appropriate volume of arsenic intermediate stock standard solution as shown in Table 1.

Table 1. Preparation of Intermediate Working Calibration Standards

Calibration Standard	Spike Volume of Intermediate Stock Standard Solution (mL)
1	0.05
2	0.5

3	1.0
4	2.0
5	3.0

Next, bring to volume in a 100-mL flask with 18 M-Ω water. Mix the solution thoroughly and carefully add the remaining little drops of water needed to dilute to exact volume. Dispense into smaller volume, acid-washed tubes (i.e., 15-mL polypropylene centrifuge tubes) for daily use.

The final concentrations of arsenic in each of the intermediate working standards depend on the concentration of the intermediate stock standard. Use the following formula to calculate each standard concentration:

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

After preparing a new set of calibration standards, update the calibration standard concentrations in the ELAN® ICP-DRC-MS software. The values entered into the software should be the concentration of the intermediate working standard divided by 10 (to account for the ten-fold dilution of the intermediate stock standard solution, relative to the urine samples, that takes place when preparing the working urine calibration standards). Store at room temperature and prepare as needed.

4. Working Calibration Standards

The working calibration standard solutions are dilutions of the five intermediate working standards into a urine matrix for the purpose of external calibration of an analytical run. 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 6.a.1.) To prepare the working calibration standards, transfer 100 μL of the appropriate aqueous intermediate working standard, 900 μL of base urine, and 9,000 μL of diluent to a 15-mL polypropylene centrifuge tube by using the Micromedic Digiflex™. Cap the tube and mix well before analysis. **Note:** These volumes are used because the 2 mL Micromedic Digiflex™ sample syringe is most accurate at volumes ≥ 100 μL.

B. Preparation of Quality Control (QC) Materials

1. NIST SRM 2670 (“Elevated”)

The NIST SRM 2670 (elevated) is freeze-dried urine, which has a certified concentration value of 480 ± 100 μg/L. Use it for external calibration verification. To reconstitute, add 20.0 mL of 18 M-Ω 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, add 20 μL of double-distilled nitric acid and mix well. Store in smaller volumes for daily use (i.e., four 5-mL aliquots in acid-washed 15-mL polypropylene centrifuge tubes). Store at ≤ -20°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).

2. Bench QC Materials

Analyze low- and high-bench QC material in each run to determine the validity of the concentration measurements being made. Prepare these pools periodically, as supply dictates, by spiking base urine. Prepare new pools far enough in advance 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.

To prepare new bench QC materials, collect urine (from anonymous volunteers or purchase from a vendor) and screen it for As, Ga, and Te concentrations. Two liters of a bench QC pool should be enough for approximately 2 years analysis with this method. Collected urine should have an As concentration less than the desired low bench pool. Ga and Te concentrations in the collected urine should be $\leq$ 5 ppb. If the urine has acceptable concentrations of As, Ga, and Te, it should be acidified to 1% (v/v) double-distilled nitric acid and divided into different containers for the two different pools. On the basis of the measured arsenic concentration, the volume of urine in the pool, and the concentration of arsenic stock standard solution being used (use NIST or NIST-traceable standards), spike the appropriate volume of arsenic standard solution into the two urine pools to produce a low-normal concentration urine arsenic pool (5–15 μg/L) and a high-normal concentration urine arsenic pool (100-200 μg/L). After spiking, mix the pool well (use a stirring plate and an acid-washed Teflon™ stir bar) and dispense (while being stirred) into appropriate-sized acid-washed tubes (i.e., 15-mL polypropylene tubes) for daily use. During the dispensing step, mark tubes periodically that can later be pulled from the racks and analyzed to determine the homogeneity of the arsenic concentration throughout the pools. If the analysis of these samples reveals a pronounced drift in arsenic concentration from the beginning to the end of the racks of dispensed urine, re-pool the urine and dispense with adequate stirring to make sure that homogeneity is preserved across all of the dispensed tubes. Store at $\leq$- 20°C.

C. Other Materials

1. Primary arsenic stock standard solution is National Institute for Standards and Technology Standard Reference Materials (NIST SRM) 3103a, which can be ordered from NIST, Gaithersburg, MD). Alternatively, NIST-traceable stock standard solutions of arsenic can be used from other vendors.

2. Pipette tips: 1-200 μL (#RT-20, fits up to 100-μL pipettes) and 200-1,000 μL (#RT-200, fits between 100-μL and 1,000-μL pipettes) sizes (Rainin Instrument Co., Inc., Woburn, MA or equivalent vendor). Pipette tips should be acid-rinsed with 1% (v/v) double-distilled nitric acid immediately before use.

3. Double-distilled nitric acid (GFS Chemicals Inc., Columbus, OH) or equivalent vendor.

4. Eighteen-Mega-Ohm water (from Milli-Q Academic™ and Elix 5™ reverse osmosis water purification system or equivalent vendor). Millipore Corporation, Bedford, MA.

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

6. Argon/hydrogen reaction gas for DRC™ (90% argon, 10% hydrogen) equipped with approved gas regulator (Matheson Tri-Gas Products, San Jose, California, or equivalent vendor).

7. Base urine pooled from anonymous donors or purchased from vendor.

8. Teflon™-coated magnetic stirs bars (2). (Catalog Number 58948-974 or equivalent), VWR Scientific Products, Buffalo Grove, IL.

9. Gallium: SPEX PLGA2-2Y. 1,000 mg/L (SPEX Industries, Inc., Chemical Sales Dept., Edison, NJ, or equivalent vendor).

10. Tellurium: SPEX PLTE2-2Y. 1,000 mg/L (SPEX Industries, Inc., Chemical Sales Dept., Edison, NJ or equivalent vendor).

11. Acid-cleaned volumetric flasks, 100-mL (6) for standards preparation (polypropylene or polymethylpentene flasks preferred). To acid-wash flasks, rinse with 10% (v/v) reagent-grade nitric acid, followed by rigorous rinsing with 18 M-Ω water. Repeat this process several times depending on prior use of the containers.

12. Acid-cleaned 2-L bottles (two: Teflon™ preferred). To acid-wash containers, rinse with 10% (v/v) reagent-grade nitric acid, followed by rigorous rinsing with 18 M-Ω water. Repeat this process several times depending on prior use of the containers.

13. Fifteen-(#352097) and 50-mL (#352098) milliliter polypropylene centrifuge tubes or equivalent: (Becton, Dickinson Labware, Franklin Lakes, NJ).

14. TritonX-100™ (“Baker Analyzed,” J.T. Baker Chemical Co. [www.jtbaker.com], or any source whose product is low in trace-metal contamination).

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

16. Cotton swabs (Hardwood Products Co., ME or equivalent vendor).

17. Nitrile, powder-free examination gloves (N-Dex®, Best Manufacturing Co., Menlo, GA, or equivalent vendor).

18. Biohazard autoclave bags (Curtin-Matheson Scientific, Inc., Florence, KY, or equivalent vendor).

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

D. Instrumentation

1. Inductively Coupled-Plasma Dynamic-Reaction Cell Mass Spectrometer ELAN® 6100 DRC ^{Plus} or ELAN® 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.

Table 2. ELAN® ICP-DRC-MS Method Parameters

Parameter	Setting
RF Power	1.45 KW
Argon 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	ppb
Sweeps/reading	20
Readings/replicate	1
Replicates	3
Dwell time	50 ms
Cell gas	10% hydrogen, 90% argon
Cell gas flow rate	0.2 – 0.3mL/min
RPQ	0.65 - 0.75

2. Milli-Q Academic™ and Elix 5™ reverse osmosis membrane water purification system (Millipore Corporation, 80 Ashby Rd., Bedford, MA 01730) or equivalent.

3. Eppendorf® fixed-volume pipettes (or equivalent): 1,000-, 500-, 50-μL volumes (Brinkmann Instruments, Inc., One Cantiague Rd., Westbury, NY 11590-0207).

4. Ohaus AP310™ analytical balance™, or equivalent (Ohaus, P.O. Box 900, 19A Chapin Rd. Pine Brook, NJ 07058).

5. Micromedic Digiflex™ automatic pipette (or equivalent) equipped with 10.0-mL dispensing syringe, 2,000-μL sampling syringe, 0.75-mm tip, and foot pedal (LABREPO, Inc., 101 Witmer Rd., Suite 700, Horsham, PA 19044).

7. Calibration and Calibration-Verification Procedures

A. Calibration Curve

Generate a simple linear calibration curve for arsenic by using a series of five external standards 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 arsenic and the internal standard versus the concentration of the calibrator. Compare the ratio of the observed intensities for arsenic and the internal standard in the patient sample to those obtained from the calibrators to determine the concentration of arsenic in the sample.

B. Calibration Verification

To verify the stability of the calibration of this test system and the accuracy of the concentrations of the calibrators, urine arsenic materials having concentration values assigned by the NIST SRM 2670 elevated level and the Center of Toxicology for Quebec (urine arsenic materials prepared for their urine arsenic proficiency testing and ICP-MS laboratory comparison programs) are analyzed at least every 6 months and the results compared with their target values. Agreement of results for the NIST SRM 2670 elevated level should be within the certified concentration range. Agreement of results for the Center of Toxicology for Quebec materials should be within three standard deviations of the median value for the participating laboratories. Store calibration verification data in the Laboratory Calibration Verification Log. Place copies in the ICP-DRC-MS maintenance log in the laboratory with the instrument.

8. 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 9).

2. Allow frozen urine specimens, QC specimens, and base urine calibration material to reach ambient temperature. Mix the sample 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™.

Table 3. Preparation of Samples for Analysis (All Volumes in μL)

ID	Water	Intermediate Working Std.	Base Urine	Urine Sample or QC	Diluent
Urine blank	100	-	900	-	9,000
Calibration standards	-	100	900	-	9,000
Aqueous blank	1,000	-	-	-	9,000
Urine sample or QC	-	-	-	250	2,250

Note: These volumes are used because the 2 mL Micromedic Digiflex™ sample syringe is most accurate at volumes ≥ 100 μL.

a. Prepare an aqueous blank that consists of 1,000 μL of 18 M-Ω water and 9,000 μL diluent. Use the aqueous blank for the QC pools and patient samples.

b. Prepare three urine blanks that consist of 900 μL of base urine (same material used for preparation of the urine calibration standards), 100 μL of 18 M-Ω water, and 9,000 μL of diluent. Run one of these as the blank for the calibration standards. Run one of these urine blanks twice after standard 5 (as sample IDs UrBlkChk1 and UrBlkChk2, respectively). Use the third urine blank to condition the sample introduction system before any analysis begins.

c. Prepare the working calibration standards as described in Section 6.b.(3).

d. Prepare dilutions of the QC and patient urine samples consisting of 2,250 μL of the diluent and 250 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. Necessity of this equilibration time should be determined by monitoring stability of the observed arsenic concentration of a standard analyzed multiple times within a run. Note: The cell gas will automatically turn off 1 hour after the last sample analysis has been performed.

C. Instrument and Software Setup for the ICP-MS

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. When 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. After 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 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® 6100 DRC 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_MMDDYY.tun" (where MM=month, DD=day, and YY=year). 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_MMDDYY.dac" (where MM=month, DD=day, and YY=year).

9. To set up the run in the software, click on "Open Workspace" from the "File" menu. Select the workspace file "total_urine_arsenic.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 010102 for January 1, 2002.) 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 (total_UAs_MMDDYYstds_UR_blk.mth and total_UAs_MMDDYYstds_AQ_blk.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 9 and the urine calibration standards 1-5 must go in autosampler locations 10-14, 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 16. 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 5. (Note: All other autosampler positions besides those specified above are arbitrary.)

Table 5. Typical Sample File Setup for a Total Urine Arsenic Analysis Run

A/S Location	Sample ID	Measurements Action	Method File*
37	Conditioning	Run sample	total_UAs_MMDDYYstds_UR_blk.mth
37	Conditioning	Run sample	total_UAs_MMDDYYstds_UR_blk.mth
37	Conditioning	Run sample	total_UAs_MMDDYYstds_UR_blk.mth
37	Conditioning	Run sample	total_UAs_MMDDYYstds_UR_blk.mth
36	UrBlkChk1	**Run blank, standards, and sample**	total_UAs_MMDDYYstds_UR_blk. mth
36	UrBlkChk2	Run sample	total_UAs_MMDDYYstds_UR_blk.mth
15	High-bench QC	Run sample	total_UAs_MMDDYYstds_AQ_blk.mth
17	Sample 1	Run sample	total_UAs_MMDDYYstds_AQ_blk.mth
18	Sample 2	Run sample	total_UAs_MMDDYYstds_AQ_blk.mth
Etc.			
86	Sample 70	Run sample	total_UAs_MMDDYYstds_AQ_blk.mth
87	Low-bench QC	Run sample	total_UAs_MMDDYYstds_AQ_blk.mth
88	High-bench QC	Run sample	total_UAs_MMDDYYstds_AQ_blk.mth

*(Where MMDDYY is the preparation date of the calibration standards being used in the analysis. This date is written on the calibration standard containers.)

The autosampler positions of QCs and patient samples do not have to be those shown above, but the order in which these are run (four conditioning samples, urine blank, calibration standards 1-5, urine blank check 1, low-bench, high-bench, 70 samples including 1 blind, low-bench, high-bench, urine blank check (2) should be as shown in Table 5.

The settings in Table 6 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.)

Table 6. Sample File Timing Parameters for a Total Urine Arsenic Analysis Run

	Pump Speed*	Duration
Sample flush	-24 rpm	35 s
Read delay and analysis	-12 rpm	50 s
Wash	-24 rpm	90 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.

- The rinse times on the calibration standards in the method should be set to 120s for standards 4 and 5, and 90s for all others.
- The QC tab/sample tab in the method should be setup so that an additional 180s rinse time will occur after any sample whose As concentration exceeds 300 ppb.
- The QC tab/sample tab in the method should be setup so that a rinse delay of 90s and a repeat sample analysis will be performed if the RSD of the 3 replicates in the measurement is greater than 30%.

If using the Microsoft Access™ database for long-term recording and handling of data (Section 8.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 (1 to 2 dilution)." 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 8.d.(2)(d)).

11. Before analyzing the samples, start the flow of the reaction-cell gas (10% hydrogen, 90% 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 or B, depending on how the instrument is set up). Then enter the method 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. Monitor the flow on the mass-flow controller display panel behind the large cabinet door on the front of the instrument. (Press #3 on the panel. The flow should quickly reach 200 and fluctuate slightly around this value. On some ELAN ICP-DRC-MS models, there may not be a separate mass flow controller visible at the front of the instrument. For these instruments, the gas flow rate may be monitored in the ELAN software, on the Instrument window (diagnostics tab)). Flush the cell gas for 30 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. 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. Necessity of this equilibration time should be determined by monitoring stability of the observed arsenic concentration of a standard analyzed multiple times within a run. Note: The cell gas will automatically turn off after 1 hour if the analysis has not begun.

12. 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, then click on "Analyze Batch."

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 7™) database when all other data for the run is imported from the ELAN® software. (See Section 8.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 7™) database when all other data for the run is imported from the ELAN® software. See Section 8.d.(2)(d) for a description of how to import data into the Microsoft Access™ 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 8.d.(2)(d) for a description of how to import data into the database to print out a customized sample report.)

b. Supervisor Review
The Microsoft Access™ or MS SQL Server 7™ database allows the supervisor to review the QC and sample results directly in the database. After the supervisor reviews the data, file the paper printouts from the analysis run in the study folder(s).

c. Plotting QC Results
When the Microsoft Access™ or MS SQL Server 7™ database is used QC plots are updated automatically when the data are imported into the database. Alternatively, the division SAS program can be used to assess quality control issues. 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 into the Microsoft Access™ or MS SQL Server 7™ database that handles data for the Inorganic Toxicology and Nutrition Branch.

1) Data Export Process (from ELAN® software to .TXT 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 "Total_UAs_database_output.rop" and type in a report filename using a format such as "08022001a_group55.txt" to designate data from analysis of group 55 from August 2, 2001, 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® 6100 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 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 ID, such as I:/Instruments/ELAN® 6100 DRC-A/2001/08/Study 2001-01.) From a computer that has access to Microsoft Access™ or MS SQL Server 7™ 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 8.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.

E. Replacement and Periodic Maintenance of Key Components (Part numbers listed below are PerkinElmer part numbers from their 2000/2001 Consumables Catalog.)

1. Autosampler probe assembly (part # B300-0161.) Keep one spare on hand.

2. Peristaltic pump tubing for sample (0.03 inch i.d., part #0990-8587), 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 #N812-2012): 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.

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

4. GemTip Cross-Flow II Ryton Nebulizer Assembly (part #N812-0516). Keep at least one spare on hand. (If using a Meinhard concentric nebulizer, part number is WE02-4371).

5. Cross-Flow II Replacement GemTips (part #N812-0515). Keep at least two spare pairs on hand (one pair = one tip for liquid + one tip for gas). Not needed if using the Meinhard concentric nebulizer.

6. Cross-Flow II Replacement Liquid and Gas Tip Ferrules (part #09920518 and #09920515, respectively). Keep at least two spares on hand. Not needed if using the Meinhard concentric nebulizer.

7. Cross-Flow II Replacement Liquid and Gas O-Rings (part # 09921045). Keep at least five spare pairs on hand. Not needed if using the Meinhard concentric nebulizer.

8. Ryton Spray Chamber Kit (part #N812-0124). One spare kit should be on hand; the large O-ring (part #WE01-3060), retaining ring (part #WE01-4081), and right-angled drain connector (part #WE01-3119) can be ordered individually. One spare of each should be kept on hand). If Meinhard concentric nebulizer is used, need cyclonic spray chamber (part number WE02-5221).

9. Injector Support/Torch Base (part #N812-0116). Keep one spare on hand.

10. Torch O-Ring Kit (packages of four, part #N812-0100). Keep four spare packages on hand.

11. Quartz torch. At least two spare torches should be on hand (part #N812-2006).

12. Alumina 2.0-mm i.d. sample injector (part #N812-6041). At least two spare injectors should be on hand. If using the cyclonic spray chamber, need the quartz injector (part number WE02-3948).

13. RF coil (part #WE02-1816). One spare should be on hand.

14. Nickel Skimmer (part #WE02-1137) and sampler cones (part #WE02-1140). Keep at least two spares of each on hand.

15. Skimmer and sampler cone O-rings (part #N812-0512 and #N812-0511, respectively). Keep at least 10 spares of each on hand.

16. Series II replacement Ion lens (part #WE018034). Keep two spares on hand.

17. Pump oil for the roughing pump (part #N812-2004). Keep four bottles on hand.

18. Polyscience chiller / recirculator coolant (PE Sciex Coolant, part #016558A): Two 1-L bottles should be kept on hand.

19. If possible, have a backup A/S 93 autosampler and Polyscience chiller / recirculator. See PerkinElmer sales representative for part numbers.

F. Calculations

1. Calibration
The ELAN® has two on-board microcomputers that work with the external system computer. The computers interface with the 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 ratio of the measured intensities of arsenic and internal standard versus the concentration for arsenic in the standard solution. Use the resulting calibration curve as a reference point to determine the concentration of arsenic in each patient sample based on the ratio of the intensities of arsenic and the internal standard observed in the samples. An internal standard Gallium or Tellurium allows for the correction of changes in instrument response during the run. The responses to instrumental effects for arsenic are assumed to be similar to the response for the internal standard, so basing the analysis on the ratio of the two should reduce effects of differing sample matrices and instrumental variations during the analysis run. The concentration for arsenic from the printout equals the concentration of arsenic detected in the urine samples. Typical correlation coefficients for the calibration curves will be ≥ 0.999.

2. Limit of Detection
The limit of detection (LOD) for arsenic in urine specimens is based on three times the concentration standard deviation of at least ten to twenty urine blanks, each analyzed in a separate run. This represents the method detection limit. Use UrBlkChk2, since it will be more certain to be free of carry-over effects from the

calibration standards. Report results below the detection limit as < LOD (where LOD = the calculated lower detection limit). Redo the LOD calculation at least twice a year to ensure that it has not changed.

The LOD for arsenic in urine by this method is 0.6 μg/L.

G. Special Procedure Notes – CDC Modifications

None applicable for this operation.

7. Reportable Range of Results

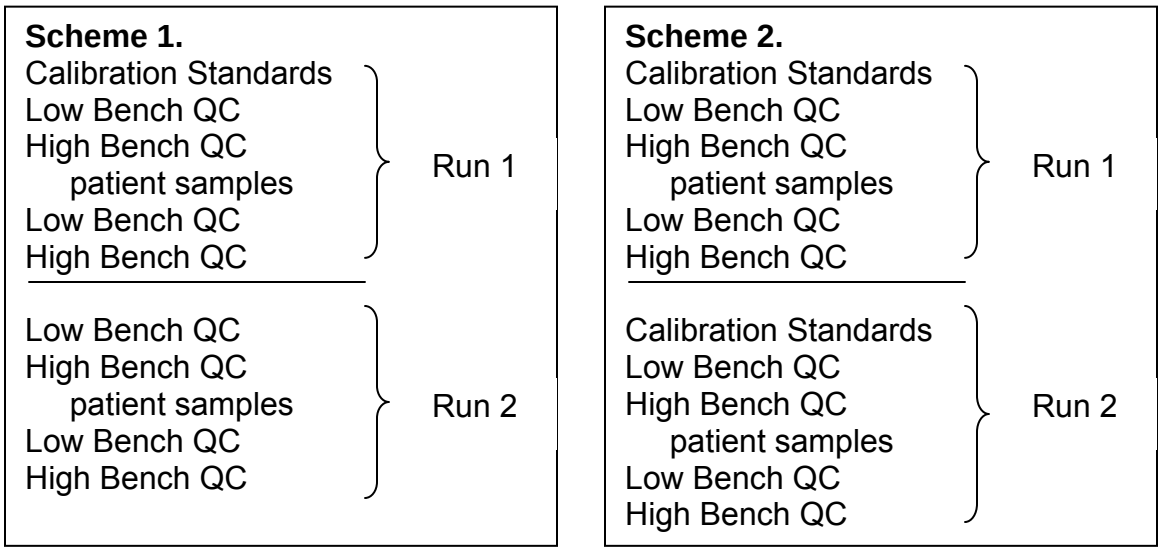
Urine arsenic results are reportable in the range of greater than the LOD, where LOD is the calculated lower detection limit. (See Section 8.f.(2))

Dilute results greater than the range of the calibration standard concentrations (approximately 300 μg/L) appropriately and reanalyze so that the results fall within the concentration range covered by the calibration standards. Dilute urine samples by adding the appropriate volume of 18MΩ water and diluent (e.g., for a 1:2 dilution, prepare the sample by adding 500 µL of urine, 500 µL of 18MΩ water, and 9,000 µL of diluent).

8. QC 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 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. 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 of arsenic. Both of these pools are analyzed after the calibration standards are analyzed but before any patient samples are analyzed so that judgments on the arsenic calibration curve may be made before analysis of patient samples. These bench QCs should be analyzed again at the end of the run (approximately 70 patient samples total). If more patient samples are analyzed on the same calibration curve after the second run of the bench QC (after approximately 70 patient samples), both the low-normal and high-normal bench QC should be reanalyzed both before and after the additional samples. For example, the following (schemes 1 and 2 shown below are acceptable ways to analyze more than one set patient samples in one day.



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. After the homogeneity of the bench materials is established, analysis by another independent reference method (e.g., isotope dilution mass spectroscopy) is useful.

A. Precision and Accuracy

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

   a. If both the low-and the high-QC results are within the 2s limits, accept the run.

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

      1) 1_{3s} – Average of both low QCs <u>OR</u> average of both high QCs is outside of a 3s limit.

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

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

      4) 10_X sequential – The previous nine average QCs results (for the previous nine runs) were <u>on the same side of the mean</u> for either the low <u>OR</u> high QC.

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

2. 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, the analysis of that sample should be repeated.

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

If an analyte result for a QC material falls outside of the 99% mean limits, the following steps should be taken, if possible:

A. If a particular calibration standard is obviously in error, remake a new dilution of that calibration standard (Section 6.b. (3)), reanalyze it, and reprocess the sample analyses by using this new result as part of the calibration curve.

B. Prepare a fresh dilution of the failing QC material (working QC standard) and reanalyze it.

C. Prepare fresh dilutions of the calibration standards (working urine multielement standards, Section 6.b. (3)), and reanalyze the entire calibration curve by using the freshly prepared standards.

If these three steps do not result in correction of 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.

10. Limitations of Method; Interfering Substances and Conditions

The argon chloride ($^{40}Ar^{35}Cl$) interferences on arsenic (^{75}As) are eliminated by the operation of the DRC™ under the parameters noted in the sections above during the urine arsenic analysis.

11. Reference Ranges

Table 7. References to Normal Total Urine Arsenic Concentrations

Reference	Concentration (μg/L)
Stokinger, 1981 (8)	<100
Fowler, 1977 (9)	15
Iffland, 1994 (10)	1 – 80 (Generally < 10)

Table 8. References to Elevated Total Urine Arsenic Concentrations

Reference	Concentration (μg/L)	Group Type Sampled
Iffland, 1994 (10)	300	After seafood consumption
	200	Copper smelter workers
	5 - 952 vs. 5 - 365	Wood treatment workers vs. comparison group (11)
	25.9 – 667	Seafood-preferring population
	74.1	Low-Inhalation exposure
	378.1	High-Inhalation exposure (12)
Gerhardsson et al., 1996 (13)	50 – 100	High intake of seafood or increased exposure of inorganic arsenic from food or air

12. Critical-Call Results ("Panic Values")

    If a patient sample has an arsenic concentration greater than 500 μg/L, conduct a test for contributions from dietary arsenic, if possible. Report the levels by fax, telephone, or E-mail to the supervising physician or principal investigator.

13. 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 ≤ -20°C freezer storage as soon as possible.

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

    If the analytical system fails, freezer storage (≤ -20°C) is recommended until the analytical system is restored functionality.

15. 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, FAX, or E-mail).

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

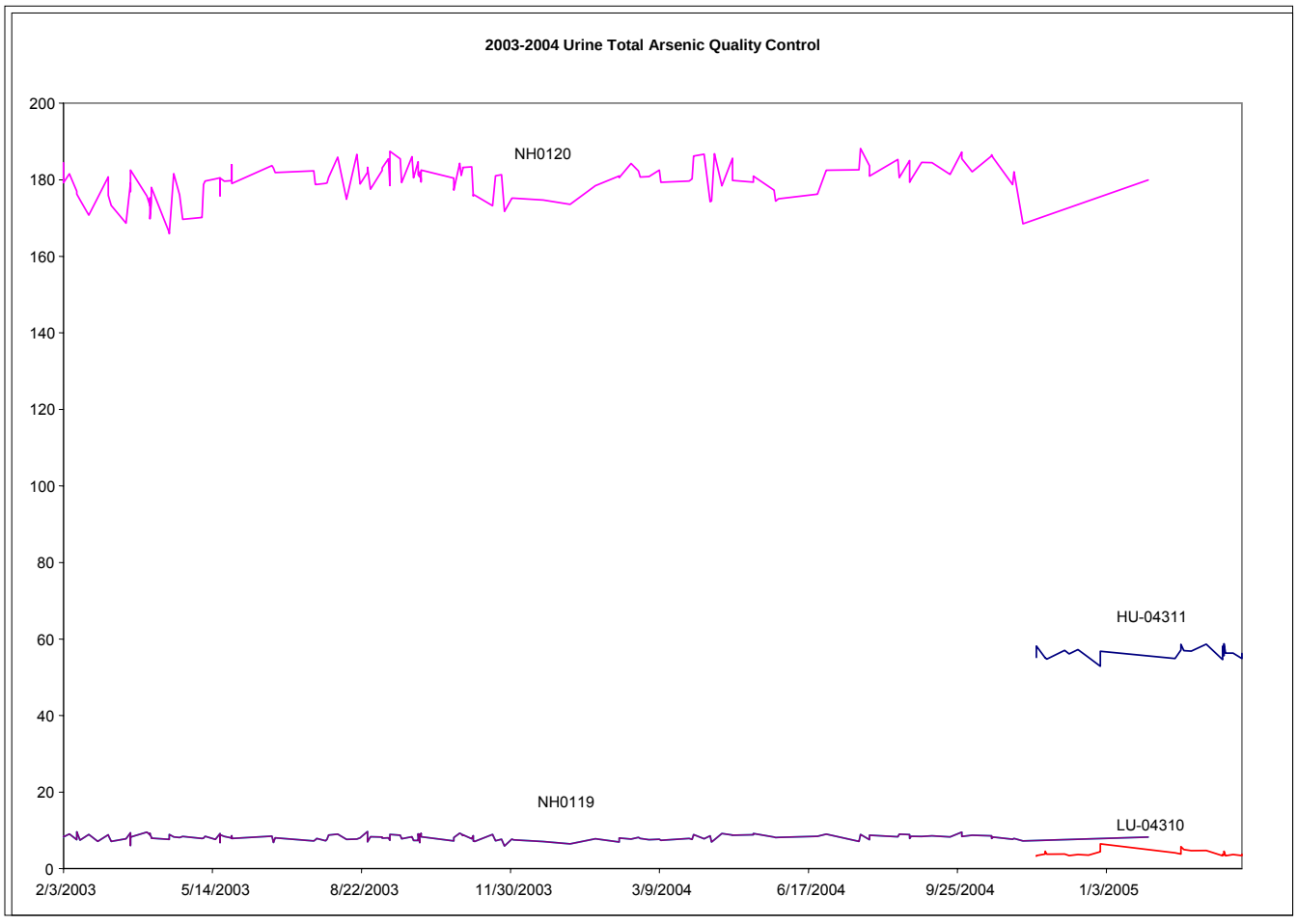
The analyst who receives specimens or samples delivered to Inorganic Toxicology and Nutrition Branch (ITN) sets up a "Specimen Folder." Fill out a tracking form and place it in the folder to be given to the analyst performing the analysis. The form tracks location, status, and final disposition of the 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.

17. Summary Statistics and QC Graphs

Summary Statistics for Urine Total Arsenic by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH0119	135	2/3/2003	1/31/2005	8.1517	0.7489	9.2
NH0120	135	2/3/2003	1/31/2005	179.6843	4.5996	2.6
LU-04310	25	11/17/2004	4/4/2005	4.0722	0.7908	19.4
HU-04311	25	11/17/2004	4/4/2005	56.2929	1.4897	2.6



References

1. Toxicological profile for arsenic. Atlanta, GA: Dept. of Health and Human Services (US), Agency for Toxic Substance and Disease Registry; 2000.

2. National Research Council (US.) Arsenic in drinking water. Washington: National Academy Press; 1999.

3. Peters HA, Croft WA, Woolson EA, Darcey B, Olson M. Hematological, dermal and neuropsychological disease from burning and power sawing chromium-copper-arsenic (CCA)-treated wood. Acta Pharmacol Toxicol (Copenh) 1986; 59(7):39-43.

4. Vahter ME. Arsenic. In: Clarkson T W, Friberg L, Nordberg G F, Sager P R, editors. Biological monitoring of toxic metals. New York: Plenum Press, 1988. p.303-21.

5. Date AR, Gray AL. Applications of inductively coupled plasma-mass spectrometry. New York: Chapman and Hall; 1989.

6. Tanner SD, Baranov VI. Theory, design and operation of a DRC™ for ICP-MS. Atomic Spectroscopy 1999; 20(2):45-52.

7. Neubauer K. Vollkopf U. The benefits of a DRC™ to remove carbon-and chloride-based spectral interferences by ICP-MS. Atomic Spectroscopy 1999; 20(2):64-8.

8. Stokinger HE. The metals. In: Patty's industrial hygiene and toxicology. 3rd edition Clayton GD, Clayton FE, editors. New York: John Wiley and Sons, 1981. p.1493-2060.

9. Fowler BA. In: Toxicology of trace elements. Goyer RA, Mehlman MS, editors. New York: John Wiley and Sons; 1977. p.79.

10. Iffland R. Arsenic. In: Handbook on metals in clinical and analytical chemistry. Seiler HG, Sigel A, Sigel H, Editors. Marcel Dekker, Inc.; New York: 1994. p.238-50.

11. Takahashi W, Pfenniger K, Wong L. Arch Environ Health, 1983; p.38:209.

12. Feldman RG, Niles CA, Kelly-Hayes M, Sax DS, Dixon WJ, Thompson DJ, Landau E. Neurology 1979; 23:939.

13. Gerhardsson L, Skerfving S. Concepts on biological markers and biomonitoring for metal toxicity. In: Toxicology of metals. Chang LA, editor. Boca Raton, Florida: CRC Press, 1996. p. 98.