



Laboratory Procedure Manual

Analyte: **Multiple Toxic Elements**

Matrix: **Urine**

Method: **Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)**

Method No.: **MTE-1.01**

Revised: **July 9, 2003**

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 1999–2000 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
lab06	URXUBA	Barium, urine (ng/mL)
	URXUBE	Beryllium, urine (ng/mL)
	URDUCD	Cadmium, urine (ng/mL)
	URXUCO	Cobalt, urine (ng/mL)
	URXUCS	Cesium, urine (ng/mL)
	URXUMO	Molybdenum, urine (ng/mL)
	URXUPB	Lead, urine (ng/mL)
	URXUPT	Platinum, urine (ng/mL)
	URXUSB	Antimony, urine (ng/mL)
	URXUTL	Thallium, urine (ng/mL)
	URXUTU	Tungsten, urine (ng/mL)

1. Clinical Relevance and Summary of Test Principle

A. Clinical Relevance

This method is used to achieve rapid and accurate quantifications of multiple elements of toxicological and nutritional interest. The method is sensitive and rapid enough to screen urine specimens from subjects suspected to be exposed to a number of important toxic elements or to evaluate environmental or other nonoccupational exposure to these same elements.

B. Test Principle

Inductively coupled plasma-mass spectrometry (ICP-MS) is a multi-element analytical technique (1). Liquid samples are introduced into the ICP through a nebulizer and spray chamber carried by a flowing argon stream. By coupling radio-frequency power into 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, along with the argon, enter the mass spectrometer through an interface that separates the ICP, from the mass spectrometer, which is operating at an atmospheric pressure of 10^{-5} torr. The mass spectrometer permits ions at each mass to be detected in rapid sequence, allowing individual isotopes of an element to be determined. Electrical signals resulting from the detection of the ions are processed into digital information that is used to indicate first the intensity of the ions and then the concentration of the element. The ICP-MS method is used to measure the following 12 elements in urine: Beryllium (Be), Cobalt (Co), Molybdenum (Mo), Cadmium (Cd), Antimony (Sb), Cesium (Cs), Barium (Ba), Tungsten (W), Platinum (Pt), Thallium (Tl), Lead (Pb), and Uranium (U). This method is based on the method by Mulligan et al. (2) Urine samples are diluted 1+ 9 with 2% (v/v), double-distilled, concentrated nitric acid containing both iridium (Ir) and rhodium (Rh) for multi-internal standardization. This procedure can be used for all 12 elements or subsets of the 12 elements.

2. Safety Precautions

Before operating the instrument, read the information in the PerkinElmer ELAN[®] ICP-MS System Safety Manual. Possible hazards include ultraviolet radiation, high voltages, radio-frequency radiation, and high temperatures.

Observe all necessary radiation safety considerations for radioactive isotope management and disposal indicated in the CDC Radiation Safety Manual (3). In addition, all personnel must successfully complete CDC's Radiation Safety training course in the laboratory or demonstrate equivalent instruction. Wear gloves, a lab coat, and safety glasses while handling all human urine. Place in a biohazard autoclave bag disposable plastic, glass, and paper (pipette tips, autosampler tubes, gloves, etc.) that contact urine. Keep these bags in appropriate containers until they are sealed and autoclaved. Dispose of all radioactive waste and contaminated material according to radiation safety guidelines. Wipe down all work surfaces with 10% sodium-hypochlorite solution when work is finished. 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 urine and also keeps the analyst's hands free to hold the specimen cups and autosampler tubes and to wipe off the tip of Micromedic Digiflex.

Observe universal precautions. 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 causing severe eye and skin damage. Wear metal-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 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 MS Windows Disk Defragmenter 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).

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

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-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 Roxio "Easy CD Creator" or similar software to backup. For "Easy CD Creator" v.5 software, use the following settings: "Create Data CD", and "Track-At-Once" and "Finalize CD" under "Record 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-MS software interfering with each other.
- It is suggested to have two or three separate backup files which are overwritten on a rotating basis (Monday to file 1, Tuesday to file 2, Wednesday to file 1, Thursday to file 2, Friday to file 1, etc . . .). 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-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-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

(1) Computer Maintenance

Record any maintenance of computer hardware or ICP-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.

(2) 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 Specimen Rejection

- A. This method does not require any special instructions, such as fasting or special diets.
- B. The specimen type is urine.
- C. The optimal amount of required specimen is 4–7 mL; the minimum amount is about 2 mL.
- D. Acceptable containers include lot screened 15-mL polypropylene (PP) centrifuge tubes. Use sterile collectors for specimen acquisition.
- E. The criteria for unacceptable specimen are either a low volume (< 2.0 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, dirt, etc., from improper handling.
- G. The division protocol for urine collection and handling outlines specimen-handling conditions. Copies are available in branch, laboratory, and special activities specimen-handling offices. Collection, transport, and special requirements are discussed. In general, urine specimens should be transported frozen (packed in dry ice during shipment is preferred when possible). Once received, store long term at $\leq -20^{\circ}\text{C}$ until time for analysis. Short-term storage at $2-4^{\circ}\text{C}$ is acceptable. Refreeze at $\leq -20^{\circ}\text{C}$ portions of the sample that remain after analytical aliquots are withdrawn. Thawing and refreezing samples has not been found to compromise sample results.

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 $\mu\text{g/L}$ Rh and 10 $\mu\text{g/L}$ Ir in 2% (v/v) double-distilled nitric acid. Add this solution when preparing samples during the dilution process, just prior to analysis. To prepare, acid-rinse a 2-L container (polypropylene (PP), polymethylpentene (PMP), or Teflon) and partially fill it with $>18\text{ M}\Omega\text{-cm}$ water. Add 40 mL of double-distilled, concentrated nitric acid, and add 1 mL each of 20 mg/L Ir and Rh (can be from one stock solution of Ir and Rh made up in 2% (v/v) double-distilled nitric acid). Make up to volume (2 L) with $>18\text{ M}\Omega\text{-cm}$ 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

The base urine used in this method is a pool of urine collected from anonymous donors and acidified to 1% (v/v) double-distilled nitric acid. Intermediate working standards are added to the base urine during the dilution process, just prior to analysis. Collect urine in lot screened or acid-rinsed sample collection cups. Once the urine is collected from donors, it should be analyzed to ensure that concentrations of the analytes in this method are relatively low, so as to not interfere with the proper measurement of calibration standards (see Table 1 for suggested maximum base

urine concentrations). Once screened, mix the urine collections together in a larger container (i.e. – acid washed polypropylene (PP), polymethylpentene (PMP), or Teflon) and acidify to 1% (v/v) double distilled nitric acid. For short term storage, store at 2–4°C. For long-term storage, dispense into smaller-volume tubes (i.e., 50-mL acid-washed or lot screened polypropylene tubes) and store at $\leq -20^{\circ}\text{C}$.

Table 1. Suggested maximum analyte concentrations for base urine

Analyte	Concentration ($\mu\text{g/L}$)
Be	0.5
Co	0.25
Mo	30
Cd	0.25
Sb	0.2
Cs	3
Ba	2
W	0.2
Pt	0.25
Tl	0.2
Pb	0.75
U	0.03

(3) ICP-MS Rinse Solution

The rinse solution used in this method is an aqueous 0.02% Triton X-100 and 5% (v/v) double-distilled nitric acid solution. 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 ease the day-to-day preparation of the rinse solution, first prepare a 2% Triton X-100/5% (v/v) double-distilled, nitric-acid solution by adding 40 mL of Triton X-100 and 100 mL of double-distilled, concentrated nitric acid to a 2-L, acid-washed bottle (PP, PMP, or Teflon) that is partially filled with $>18\text{ M}\Omega\cdot\text{cm}$ water. Fill to 2 L, add an acid-washed, Teflon-coated stirring bar, and stir on a magnetic stirrer until the Triton X-100 has completely dissolved in the solution. Prepare the final rinse solution by acid-rinsing a 1-L container (PP, PMP, or Teflon) and partially filling it with $>18\text{ M}\Omega\cdot\text{cm}$ water. Add 50 mL of double distilled concentrated nitric acid and 10 mL of the 2% Triton X-100/ 5% (v/v) double-distilled nitric-acid solution. Dilute to 1 L using $>18\text{ M}\Omega\cdot\text{cm}$ water. Store at room temperature and prepare as needed. To prepare larger volumes, add proportionally larger volumes of the solution constituents.

B. Standards Preparation

Intermediate stock standard solutions can be prepared by and purchased from an external laboratory, or made in-house. Calibration standards must always be traceable to the National Institute for Standards and Technology (NIST). Anytime new calibration standards are used, verify that the calibration information in the ELAN software (method window, calibration page) reflects the actual calibrator concentrations.

(1) Multi-element Intermediate Stock Standard

The intermediate stock standard solution used in this method is an aqueous solution either purchased from an external laboratory, or made in-house from NIST single element standard reference materials (SRMs).

The CDC laboratory currently purchases the intermediate stock standard from High Purity Standards (Item number SM-2107-001. Lot numbers used have included 223414, 223436, 223501, and 223518). Sub-boiled high purity acids were used to put the high purity metal, salts, or oxides into solution and to stabilize the standard. The solution matrix is 2% nitric acid with traces of HF and HCl in >18 M Ω -cm water. The standard was made gravimetrically by weighing the reference material to 5 significant figures. Volumetric glassware was calibrated gravimetrically to 5 significant figures. The concentrations of the 12 elements in the intermediate stock standard are listed in Table 5 in the Appendix. It is stored at room temperature. Due to the uranium content of the solution, and in keeping with the guidance of the CDC radiation safety manual (3), the intermediate stock standards must be kept in a lockbox (provided by CDC Radiation Safety Office).

(2) Multi-element Intermediate Working Standards

The intermediate working standard solutions used in this method are a series of five aqueous dilutions of the multi-element intermediate stock standard solution in 2% (v/v) double-distilled nitric acid and 0.1% (v/v) hydrochloric acid. Use these solutions each day of analysis to prepare the final working standards that you will place in the autosampler of the ELAN ICPMS. To prepare: acid-rinse five 100-mL PP (or PMP) volumetric flasks and partially fill them with >18 M Ω -cm water. To each 100-mL flask add 2 mL of double-distilled, concentrated nitric acid and 0.1 mL of double-distilled, concentrated hydrochloric acid. Add the appropriate aliquot of the multi-element intermediate stock standard solution (see Table 6 in the Appendix for volumes) and bring to volume with >18 M Ω -cm water. Transfer to acid-cleaned, labeled, 50-mL containers (PP, PMP, or Teflon) for storage. The final concentrations of the 12 elements are listed in Table 6 in the Appendix. Store at room temperature.

(3) Working Urine Multi-element Standards

The working urine multi-element standard solutions used in this method are a series of 1:100 dilutions for the corresponding five intermediate working standards. These are prepared at the time of analysis by adding the aqueous intermediate working standards to diluent and base urine. These dilutions are analyzed before the diluted patient urine samples for the purpose of matrix-matched external calibration.

C. Preparation of Quality Control (QC) Materials

(1) Urine-Based Internal (“Bench”) QC materials

Quality control materials can be either prepared by and purchased from an external laboratory or prepared within the CDC laboratories. Quality control must always be traceable to the National Institute for Standards and Technology (NIST). The CDC laboratory currently prepares its own bench QC materials. The preparation process used is the following:

(a) Collection of urine

Collect urine through an anonymous collection process into lot screened or acid-rinsed sample collection cups. Screen each collected sample for metal content before mixing together to make 2 separate base urine pools (for preparing the low and high bench QC materials). Final metal concentrations for the base urine collection for the low bench QC pool should be in the low normal population range. Final metal concentrations for the base urine collection for the high bench QC pool should be less than some pre-selected target concentration values in the high normal population range. See the Second National Report on Human Exposure to Environmental Chemicals for estimations of the normal population ranges for metals (<http://www.cdc.gov/exposurereport/metals/>). Volume of urine to collect is dependent on the desired pool size. This write-up will assume a 10-L pool size for both the low and high bench QC. Pools should be mixed into acid-washed 10-L carboys (PP or PMP).

(b) Spiking of urine

Analyze a sample of each pre-spiked urine pool. Determine target concentrations for the analytes in both pools. Based on the analysis results of the pools and the target concentrations spike each pool using single element stock standards (all standards used must be traceable to NIST). Mix pools well after spiking, then reanalyze. Continue to spike, mix, and analyze until all analytes reach target concentrations.

(c) Storage of urine

Urine pools should be stored long term in small volumes at $\leq -20^{\circ}\text{C}$ (i.e. in 5 or 15 mL polypropylene tubes). Storage containers must be lot screened and labeled. Dispensing can be accomplished most easily using a Digiflex automatic pipettor in continuous cycling dispense mode. Urine must be mixed well during dispensing using an acid washed Teflon stir bar and large stir plate. After dispensing, check homogeneity of analyte concentrations in pool aliquots by analysis of every Nth sample dispensed (where $N \sim 20-50$ depending on the pool size). Keep samples pulled for homogeneity analysis in the sequence that they were dispensed for the purpose of looking for trends in concentrations. Once dispensed and homogeneity has been shown to be good throughout the tubes of a pool, store tubes at $\leq -20^{\circ}\text{C}$ and pull tubes out as needed for analysis.

(2) Alternative Internal (“Bench”) QC Materials (Aqueous-Based Approach)

If it is not possible to make or obtain urine pools, as described in Section 6.C, where the analytes are stored in a urine matrix before analysis, these pools can be made and stored in an acidified water matrix and spiked into a base urine matrix during preparation of dilutions for analysis just as described for the calibration standards in Section 6.B. The following procedures detail a way in which to prepare and use these alternate bench QC materials.

The intermediate stock QC standard and / or intermediate working QC standard solutions can be prepared by and purchased from an external laboratory (i.e.- SPEX CertiPrep Inc. custom mix item number XCDCGA-3 purchased used as intermediate stock QC standard), or made in-house. If making these solutions in-house from NIST single element SRMs, keep in mind that some SRM concentrations are certified relative to the mass of the solution, while others are certified relative to the volume of the solution. Refer to the NIST certificate of analysis (specific for the lot number of the SRM being used) to determine this. If the SRM concentrations are certified relative to mass, calculate the appropriate sample size prior to standards preparation in order to prepare the desired concentration of QC material.

(a) Multi-element Intermediate Stock Bench QC Standards
(Alternative Aqueous-Based Approach)

The intermediate stock QC solution used in this method has been prepared in-house in the past as an aqueous solution spiked with aliquots of the 12 NIST SRMs and acidified with 10% (v/v) double-distilled nitric and 5% (v/v) double-distilled hydrochloric acids. This solution has also been prepared for CDC in the past by SPEX CertiPrep Inc. (custom mix item number XCDCGA-3, lot number 24-89AS) with an acidification of 1% (v/v) nitric acid and 10% (v/v) hydrochloric acid. Other external laboratories or vendors may suggest different acid content than is detailed below. This solution will be the first dilution from the NIST SRMs from which the intermediate working low and high bench QC standards will be made. Before making this intermediate stock QC standard solution, make an additional dilution for TI and U in order to reach the very low concentrations needed for the final working low and high bench QC standards. Prepare the initial TI and U dilution by acid-rinsing a 5-mL volumetric flask and

partially filling it with >18 M Ω -cm water. Add 0.5 mL of double-distilled, concentrated nitric acid but do not add hydrochloric acid to this initial dilution. Add the appropriate amounts of the NIST TI and NIST U SRMs (see Table 9, Standard A in the Appendix for aliquot sizes) and bring to volume with >18 M Ω -cm water. Prepare the complete intermediate stock QC standard by acid-rinsing a 250-mL PP (or PMP) volumetric flask, and partially filling it with >18 M Ω -cm water. Add 25 mL of double-distilled, concentrated nitric acid and 12.5 mL of double-distilled, concentrated hydrochloric acid. Add an aliquot of the initial TI and U dilution as well as aliquots of the other NIST elemental standards (see Table 9, Standard B in the Appendix for aliquot volumes) and bring to volume with >18 M Ω -cm water. The final concentrations of the 12 elements in the intermediate stock QC standard are listed in Table 9 in the Appendix. Transfer to 50-mL, acid-cleaned tubes (PP or PMP) for long-term storage. Store at room temperature.

(b) Multi-element Intermediate Working Low and High Bench QC Standards
(Alternative Aqueous-Based Approach)

The low and high intermediate working QC solutions used in this method can be two aqueous dilutions of the multi-element intermediate stock QC solution in 10% (v/v) double-distilled nitric acid and 5% (v/v) hydrochloric acid. If these solutions are purchased from an external laboratory, they may suggest a different acid content than is detailed here. Use these solutions on each day of analysis to prepare the final working bench QCs that you will place in the autosampler of the ELAN ICPMS. Prepare by acid-rinsing two 100-mL PP (or PMP) volumetric flasks and partially fill them with >18 M Ω -cm water. Add 10 mL of double-distilled, concentrated nitric acid and 5 mL of double-distilled, concentrated hydrochloric acid. Add the appropriate aliquot of the multi-element intermediate stock QC solution (see Table 10 in the Appendix for aliquot sizes), and bring to volume with >18 M Ω -cm water. Transfer to 50-mL, acid-cleaned falcon tube containers for storage. The final concentrations of the 12 elements are listed in Table 10 in the Appendix. Store at room temperature.

(c) Working Urine Multi-element QC Standards
(Alternative Aqueous-Based Approach)

When using the alternative, aqueous-based approach for bench QC pools, the working urine multi-element QC solutions used in this method are 1:100 dilutions of the intermediate working QC standards (low and high). The aqueous intermediate working QCs are diluted with diluent and base urine just prior to analysis. The base urine used for preparing these bench QC samples should be the same as is used for preparing the working calibration standards [see Section 6.B.(3)]. By spiking the bench QC materials into base urine, they are matrix-matched to the calibrators and patient urine samples. Prepare by transferring 100 μ L of the appropriate aqueous intermediate working QC standard, 900 μ L of base urine, and 9,000 μ L of diluent to a 15-mL tube (PP) using the Micromedic Digiflex. Mix well before analysis.

During analysis, bench QC which has been spiked into base urine must use the urine blank as the analytical blank. Initial analysis of the bench QC should be done before analysis of the aqueous blank so that data reprocessing will not be necessary for these initial QC checks. The ending bench QC will need to be reprocessed post-analysis using the urine blank as the analytical blank (reprocess the urine blank analyzed before the calibration curve, then reprocess the ending bench QC). Do not re-analyze (re-sample) the urine blank prior to the ending QC to avoid the data reprocessing step. Beginning and ending QC should have the same urine blank analysis subtracted.

(3) NIST SRM 2670

The 2670 and 2670A SRMs from the National Institute for Standards and Technology are freeze-dried urine, which have suggested values for some of the elements in this method. These materials are used as part of the external calibration verification of this method. There is both a normal and an elevated level. To reconstitute, add 20.0 mL of >18 M Ω ·cm water to the SRM vial, close, and swirl the vial to make sure that all freeze-dried material is reconstituted. Divide up the reconstituted SRM into smaller 5–10 mL portions for later use. All tubes used for storage must be either acid washed or lot screened. Store the reconstituted SRM for short-term at 2–8°C and long term at $\leq -20^{\circ}\text{C}$.

D. Other Materials

- (1) Bleach (10% sodium-hypochlorite solution) – any vendor.
- (2) Symbol LS-3000 bar code scanner, or equivalent (Symbol Technologies, Inc. Holtsville, NY).
- (3) Falcon 15-mL conical tubes (#2097) and 50-mL conical tubes (#2098) (Becton, Dickinson Labware, Franklin Lakes, NJ).
- (4) Polypropylene urine collection cup (4.5 oz, catalog # 354013, Becton Dickinson, Labware, Franklin Lakes, NJ or equivalent).
- (5) Ir: SPEX PLIR3-2Y. 1,000 mg/L (SPEX Industries, Inc., Edison, NJ,) or equivalent.
- (6) Rh: SPEX PLRH3-2Y. 1,000 mg/L (SPEX Industries, Inc., Edison, NJ,) or equivalent.
- (7) GFS double-distilled nitric acid (GFS Chemicals Inc. Columbus, OH) or equivalent.
- (8) GFS double-distilled hydrochloric acid (GFS Chemicals Inc. Columbus, OH) or equivalent.
- (9) >18 M Ω ·cm water from a NANOpure Diamond Ultrapure Water System (Barnstead International, Bedford, MA).
- (10) Teflon 2-L container, catalog# 02-923-30E (Fisher Scientific. Pittsburgh, PA.) or equivalent. Alternative is 4-L Nalgene polypropylene (PP) jug, catalog# 02-960-10A (Fisher Scientific. Pittsburgh, PA.) or equivalent.
- (11) Teflon 1-L container, catalog#02-923-30D (Fisher Scientific. Pittsburgh, PA.) or equivalent. Alternative is 1-L Nalgene Polypropylene (PP) container, catalog# 02-925F (Fisher Scientific. Pittsburgh, PA.)
- (12) Triton X-100 (“Baker Analyzed,” J.T. Baker Chemical Co. or any source whose products is low in trace-metal contamination).
- (13) Multi-element intermediate stock standard, item number SM-2107-001. Lot numbers used have included 223414, 223436, 223501, and 223518. (High Purity Standards, Charleston, SC.)
- (14) Multi-element intermediate stock bench QC solution, item number XCDCGA-3. Lot number used previously was 24-89AS. (SPEX CertiPrep, Inc., Metuchen, NJ.)
- (15) Volumetric flasks, 100mL (polypropylene (catalog # 10-198-50B), or polymethylpentene (catalog # 10-198-52C), Fisher Scientific, Pittsburgh, PA or equivalent).
- (16) Polypropylene 10-L carboy (catalog # 02-960-20C, Fisher Scientific, Pittsburgh, PA) or equivalent.
- (17) Primary stock solutions of 12 elements: National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs) 3105a (Be), 3113 (Co), 3134 (Mo), 3108 (Cd), 3102a (Sb), 3111a (Cs), 3104a (Ba), 3163 (W), 3128 (Pb), 3140 (Pt), 3158 (Tl), and 3164 (U) (National Institute of Standards and Technology (NIST), Office of Standard Reference Materials, Gaithersburg, MD). Other sources of standards can be used if they are NIST traceable.
- (18) High Purity liquid argon (Specialty Gases Southeast or other contract agency) equipped with approved gas regulator (Matheson Gas Products, Secaucus, NJ, or equivalent).

- (19) Kay-Dry paper towels and Kim-Wipe tissues (Kimberly-Clark Corp., Roswell, GA) or equivalent.
- (20) Cotton swabs (Hardwood Products Co., Guilford, ME) or equivalent.
- (21) Dehydrated alcohol, USP (Midwest Grain Products of Illinois, Pekin, IL), or equivalent.
- (22) Vinyl examination gloves (Travenol Laboratories, Inc., Deerfield, IL) or equivalent.
- (23) Biohazard autoclave bags (Curtin-Matheson Scientific, Inc., Atlanta, GA), or equivalent.

E. Instrumentation

- (1) Inductively Coupled Plasma-Mass Spectrometer ELAN ICPMS, ELAN 6100 DRC or ELAN DRC II (PerkinElmer Norwalk, CT). Parameters of x-y alignment, mass calibration, autolens voltages, and nebulizer gas flow rates are optimized regularly.

Table 2. Multi-element ICP-MS ELAN ICPMS Settings

Parameter	Setting
RF power	1.45 KW
Ar nebulizer gas flow	0.90-1.0 LPM
Detector mode	Pulse
Measurement units	Cps
Replicates	4
Readings/replicate	1
Autolens	On
Blank subtraction	After internal standard
Curve type	Simple Linear
Sample units	µg/L
Sweeps/reading	180
Replicate time	69 sec
Dwell time	Be 75 ms; all others 15 ms

- (2) ASX500 series autosampler (preferred substitution of PerkinElmer AS-93plus autosampler). Cetac Technologies, Omaha, Nebraska.
- (3) Micromedic Digiflex-CX Automatic pipette equipped with 10.0-mL dispensing syringe, 2,000-µL sampling syringe, 0.75-mm tip, and foot pedal (Titertek, Huntsville, AL).
- (4) Brinkmann Research Pro Electronic pipettes (Brinkmann Instruments, Inc., Westbury, NY): 5-100 µL (catalog # 022461362), 20-300 µL (catalog # 022461371), 50-1000 µL (catalog # 022461389), 100-5000 µL (catalog # 022461397). Note: pipette catalog numbers are without individual chargers. Can purchase individual chargers (pipette catalog numbers will differ) or a charging stand that will hold four pipettes (catalog # 022461559). When purchasing pipette tips (epTips), purchase one or more boxes, then reloads for those boxes after that: 5-100 µL (box catalog # 022491334, reload catalog # 022491539), 20-300 µL (box catalog # 022491342, reload catalog # 022491547), 50-1000 µL (box catalog # 022491351, reload catalog # 022491555), 100-5000 µL (box catalog # 022491385, reload not available, bulk bag catalog # 022492080). Equivalent pipettes and tips can be substituted.
- (5) NANOpure Diamond Ultrapure Water System (Barnstead International, Dubuque, Iowa) or equivalent water purification unit capable of producing >18 MΩ·cm water.

6. Calibration and Calibration-Verification Procedures

A. Calibration Curve

Generate a simple linear calibration curve for each of the 12 elements in this method using a series of external standards whose concentrations are defined in the calibration page of the quantitative analysis method software. The ratio of the analyte isotopes listed in Table 6 versus the internal standards is calculated as the net intensities of the analytes. Blank subtraction is performed after the analyte / internal standard ratio is calculated.

B. Calibration Verification

In order to verify that the calibration of this test system is accurate throughout reportable range, use external reference materials such as NIST SRM 2670, NIST SRM 2670A, and / or proficiency testing samples (such as those from the Center of Toxicology of Quebec) at least once every 6 months. Verification needs to be done at three points across the calibration range (low, medium, and high concentrations). If external reference materials such as these are not available for each analyte in the method, follow CLIA instructions of inter-laboratory sample exchanges.

7. Operating Procedures; 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 “Reportable Range of Results” in this document (See Section 9.).
- (2) Allow frozen and refrigerated 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

- (1) Thaw the frozen urine specimens; allow them to reach ambient temperature.
- (2) Set up a series of 15-mL polypropylene tubes corresponding to the number of blanks, standards, QCs, and patient samples to be analyzed.
- (3) Prepare the following solutions in the 15-mL falcon tubes using the Micromedic Digiflex™ (see Table 3 below for summary).
 - (a) Prepare an aqueous blank consisting of 1,000 μL of $>18 \text{ M}\Omega\cdot\text{cm}$ water and 9,000 μL of diluent. This will be used as the blank for any urine based QC materials, external reference materials, and urine patient samples.

Table 3. Dilution preparation summary

Dilution ID	Water (μL)	Base Urine (μL)	AQ Intermediate Working Standard (μL)	Patient or QC urine sample (μL)	Diluent (μL)
AQ Blank	1000	-	-	-	9000 *
Urine Blank and UrBikChk	100	900	-	-	9000 *

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Working Calibration Standards	-	900	100	-	9000 *
Patient Urine or Urine-Based QC	-	-	-	500	4500
Patient Urine 2x Dilution ^H	500	-	-	500	9000 *
Patient Urine 10x Dilution ^H	900	-	-	100	9000*

* 9000 μ L diluent is best dispensed from the Digiflex™ as 2 4500- μ L portions (i.e.- When preparing a Working Calibration Standard dilution, dispense 4500 μ L diluent + 100 μ L water in one cycle of Digiflex™, then 4500 μ L diluent + 900 μ L base urine in the next cycle of the Digiflex™ to prepare a 10 mL total volume dilution.)

^H Extra dilution is performed on urine samples whose concentration is greater than 110% of the concentration of the highest calibration standard. Any extra level of dilution can be prepared as long as the 9:10 ratio of diluent to total dilution volume is maintained. Use of the lowest possible dilution level is preferred because matrix differences may lead to different observed concentration results as the sample dilution becomes greater (i.e. 2x dilution is preferred over 10x if 2x is sufficient to dilute analyte into calibration curve range).

- (b) Prepare two urine blank dilutions consisting of 900 μ L of base urine (same material used to prepare the urine calibration standards), 100 μ L of >18 M Ω -cm water, and 9,000 μ L of diluent. One of these urine blanks will be the blank for the calibration standards; the other will be analyzed twice after standard 5 as UrBlkChk1 and UrBlkChk2, respectively. Results for 'UrBlkChk2' will be used to determine the method limit of detection.

Note: If the alternate, aqueous-based approach to bench QC materials is used, the urine blank will also be used as the blank for the aqueous-based bench QC pools.

- (c) Prepare the working calibration standards as 100 μ L of the appropriate aqueous intermediate working calibration standard, 900 μ L of base urine, and 9,000 μ L of diluent.

Note: If the alternate, aqueous-based approach to bench QC materials is used, prepare the working bench QC as 100 μ L of the appropriate aqueous intermediate working bench QC material, 900 μ L of base urine, and 9,000 μ L of diluent. See Table 11 in the Appendix for a modified Dilution preparation summary.

- (d) Prepare the patient urine sample and the urine-based bench quality control (QC) sample dilutions as 4,500 μ L of diluent and 500 μ L of the patient urine sample or bench QC material.

- (e) Cap all of the blanks, standards, and samples and mix them well. Uncap them and place them in the autosampler of the ELAN ICPMS.

C. Instrument and Software Setup for the ICP-MS

- (1) Turn on the computer, printer, peristaltic pump, and autosampler, and log into the operating system.
- (2) Set up the peristaltic pump tubing for the sample rinse station, positioning the tubing and closing the pump clamps.

- (3) Start the ELAN ICPMS software from Windows
- (4) Perform necessary daily maintenance checks as described in the ELAN ICPMS Hardware Guide (i.e., Ar supply pressure and tank level, interface components cleanliness and positioning, interface pump oil condition, etc.). 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.
- (5) In the INSTRUMENT window of the software, press the “Start” button to ignite the ELAN ICPMS plasma.
- (6) After lighting the plasma, go to the DEVICES window of the software and press the “Connect” button 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). Set the pump speed to 12 rpm in the DEVICES window.
- (7) 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.
- (8) Allow at least 45 minutes warm-up time for the ICP-MS. After this warm-up time, complete the appropriate daily optimization procedures as described in the ELAN ICPMS Inductively Coupled Plasma-Mass Spectrometer Software Guide. Include Be (m/z 9) in the daily performance check. Fill in the Daily Maintenance Checklist according to the optimization procedures performed. Save new tuning (mass calibration) parameters to the file “default.tun.” Save new optimization parameters (i.e., detector voltages, autolens values, nebulizer gas flow rate) to the file “default.dac.” Monthly, or any time large changes are made in optimization parameters, save a separate copy of these optimization files under a different name (i.e. default_070703.dac).
- (9) To set up the run in the software, click on “Open Workspace” from the “File” menu. Select the workspace file “CDC_urine multi-element.wrk” (or one customized for user preferences). Select “Review Files” from the “File” menu. From this window, you will be able to set up the correct files and data directories for your analysis.
Method: “nhanes_multi_XXXXXXstds_ur_blk.mth” (where XXXXXX is the preparation date of the calibration standards you are using in the analysis. Write this date written on the calibration standard containers.) You will set this up for each individual sample in the software. (Note: The setting in the sample files / batch mode set up for the analysis supersede the selection here.)

Dataset: If this is the first run of the day, create a new dataset using the date as the name (use the format 060102 for June 1, 2002). If a run has already been performed on the day of analysis, select the dataset for today’s date.

Sample: If an analysis has been performed that is similar to the one being set up, select the sample file corresponding to it and edit it for the present analysis.

Report Template: Select “Quant Comprehensive_CDC.rop.” (Note: The setting in the method file used during analysis will supersede your selection here.)

Tuning: “default.tun” (Note: The setting in the method file used during analysis will supersede your selection here.)

Optimization: “default.dac” (Note: The setting in the method file used during analysis will supersede your selection here.)

Calibration: No file needed.

Polyatomic: elan.ply

(10) NOTE: Autosampler position numbers are dependent on the type of autosampler being used.

For a PerkinElmer AS-93plus (tray F), urine blank will be position 9; urine calibrators 1-5 will be positions 10-14; and aqueous blank will be position 16. For a Cetac ASX 500 series autosampler (tray B), urine blank will be position 11; urine calibrators 1-5 will be positions 12-16; and aqueous blank will be position 19. Instructions below are written for the use of the PE AS-93plus (tray F).

In the SAMPLES/BATCH window, update the window to reflect the current sample set (i.e., autosampler locations, sample identification (id), analysis methods, peristaltic pump speeds, etc.). There are two method file nhanes_multi_XXXXXXstds_UR_blk.mth and nhanes_multi_XXXXXXstds_AQ_blk.mth) you will use that differ only in the autosampler locations of the blank and calibration solutions. The “UR Method” file must be used to analyze the base urine blank calibration standards at the very beginning of the run. The urine blank method (set up for AS-93plus, tray F) defines the urine blank in autosampler location 9 and the urine calibration standards 1-5 in autosampler locations 10-14, respectively. The “AQ Method” file must be used to analyze the first QC or patient sample (anything besides calibration standards or UrBlkChk). The aqueous blank method (set up for AS-93plus, tray F) defines the aqueous blank in autosampler location 16. Apart from 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 other words, when the “Measurements Action” is only ‘run sample’, it does not matter whether the urine blank or aqueous blank method file is used. CDC typically follows the pattern below, however, for the sake of consistency and as a reminder of which blank must be used for which type of sample. A typical SAMPLE/BATCH window for this method will look like the following:

A/S Location*	Sample ID	Measurements Action	Method^H
80	UrBlkChk1	Run blank, standards, sample	nhanes_multi_XXXXXXstds_UR_blk.mth
80	UrBlkChk2	Run sample	nhanes_multi_XXXXXXstds_UR_blk.mth
17	L bench QC	Run blank and sample [‡]	nhanes_multi_XXXXXXstds_AQ_blk.mth
18	H bench QC	Run sample	nhanes_multi_XXXXXXstds_AQ_blk.mth
19	Sample 1	Run sample	nhanes_multi_XXXXXXstds_AQ_blk.mth
20	Sample 2	Run sample	nhanes_multi_XXXXXXstds_AQ_blk.mth
21	Sample 3	Run sample	nhanes_multi_XXXXXXstds_AQ_blk.mth
71	L bench QC	Run sample	nhanes_multi_XXXXXXstds_AQ_blk.mth
72	H bench QC	Run sample	nhanes_multi_XXXXXXstds_AQ_blk.mth

- * The autosampler positions of QCs and patient samples do not have to be those shown above, but the order in which these are run should be as shown above.
- ^H “XXXXXX” represents the preparation date of the calibration standards being used in the analysis. Write this date on the calibration standard containers.
- [‡] If the alternative preparation of bench QC materials is used (preparation in aqueous matrix described in the Appendix), change the following in the example above:
 - the “Measurements Action” for the ‘L bench QC’ and ‘H bench QC’ samples in the example above should both be “Run Sample”
 - the method used for analysis of ‘L bench QC’ and ‘H bench QC’ samples should be the urine method (nhanes_multi_XXXXXXstds_UR_blk.mth).
 - the “Measurements Action” for ‘Sample 1’ should be changed to “Run Blank and Sample” since this will now be the first sample analyzed with the aqueous blank being subtracted.

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. Settings for Uptake and Rinse Times

	Pump Speed	Duration
Sample flush	-24 rpm	40 seconds
Read delay and analysis	-12 rpm	30 seconds
Wash	-24 rpm	300 seconds

If using the Microsoft Access database for long-term recording and handling of data, do not use the ELAN software to automatically correct for sample dilutions. Rather, when running dilutions of any sample, edit the sample ID to reflect the level of dilution being performed (i.e., A 1:2 dilution of sample 1 would be reflected in the sample ID “sample 1 (2x dilution). This sample ID will be edited during the data-import process to the database so that it is recognized as the appropriate sample.

- (11) Once you have edited the parameters in the SAMPLE/BATCH window for your run, place 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 should be included in the run, and then click on “Analyze Batch.”

D. Recording of Data

(1) Quality Control (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.

(2) Analytical Results

(a) Analysis Printouts

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. 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.

(b) Supervisor Review

The Microsoft Access or MS SQL Server 7 database allows the supervisor to export the QC data to SAS for review and also to review blind QC results. The paper printouts from the analysis run should be stored in the study folder(s). Supervisor review is documented in the database as to name, date, and time of review for each run.

(c) Plotting QC Results

When the Microsoft Access or MS SQL Server 7 database is used QC trend plots are updated automatically in the database. However, official pass / fail status of a run's QC is determined by the division SAS program. The database allows for exporting to this SAS program for evaluation of data. 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.

(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, 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 "database output report.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 ICPMS Software Manual.) Make sure you apply the correct blank to the correct samples and QCs. (Be careful to use the urine blank for all of the calibration standards, UrBlkChk1, and UrBlkChk2 [and any aqueous-based bench QC, if the alternative bench QC preparation procedure outlined in Section 6.C.(2) is used]. Use the aqueous blank for all analyses of patient samples and urine-based 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: 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# and password. Next, 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, 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 "check" button to re-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. Once transferred into the database, the data should be evaluated for QC pass / fail, then set with the with the appropriate settings for QC accept / reject, final value status, and comment(s).

F. 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 (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 (preferred alternative supplier is Kendall Healthcare).
- (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 autosampler and Polyscience chiller / recirculator. See Cetac and / or PerkinElmer sales representative for part numbers.

F. Calculations

- (1) The ELAN has two onboard 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. Internal standards are used in the analysis (Rh and Ir), which allows for the correction of changes in instrument response and sample matrix. The software uses the ratio of analyte and internal standard intensities to determine the net intensities for the analyte. As standard solutions are analyzed, the software plots the net intensity versus the concentration for each element in the standard solution. These individual calibration curves are updated as each subsequent standard is analyzed. Concentrations for the 12 elements from the printout equal the concentrations of the 12 elements detected in the urine sample.
- (2) The detection limits for elements in urine specimens are based on 3 times the concentration standard deviation of urine blanks (named UrBlkChk2) analyzed in at least 10-20 separate runs. Results below the detection limit are reported as nondetectable (< LDL where LDL = the calculated lower detection limit; refer to Section 9). Results of all the detection limits are listed in the Appendix, Table 7.

G. Special Procedure Notes: CDC Modifications

None applicable for this operation.

8. Reportable Range of Results

Urine multi-element values are reportable in the range between the LDL (see Appendix, Table 7) and the highest calibration standard (see Appendix, Table 6). For example, if a urine U value is < 0.004 µg/L (the approximate LDL of this method), report it as < 0.004 µg/L.

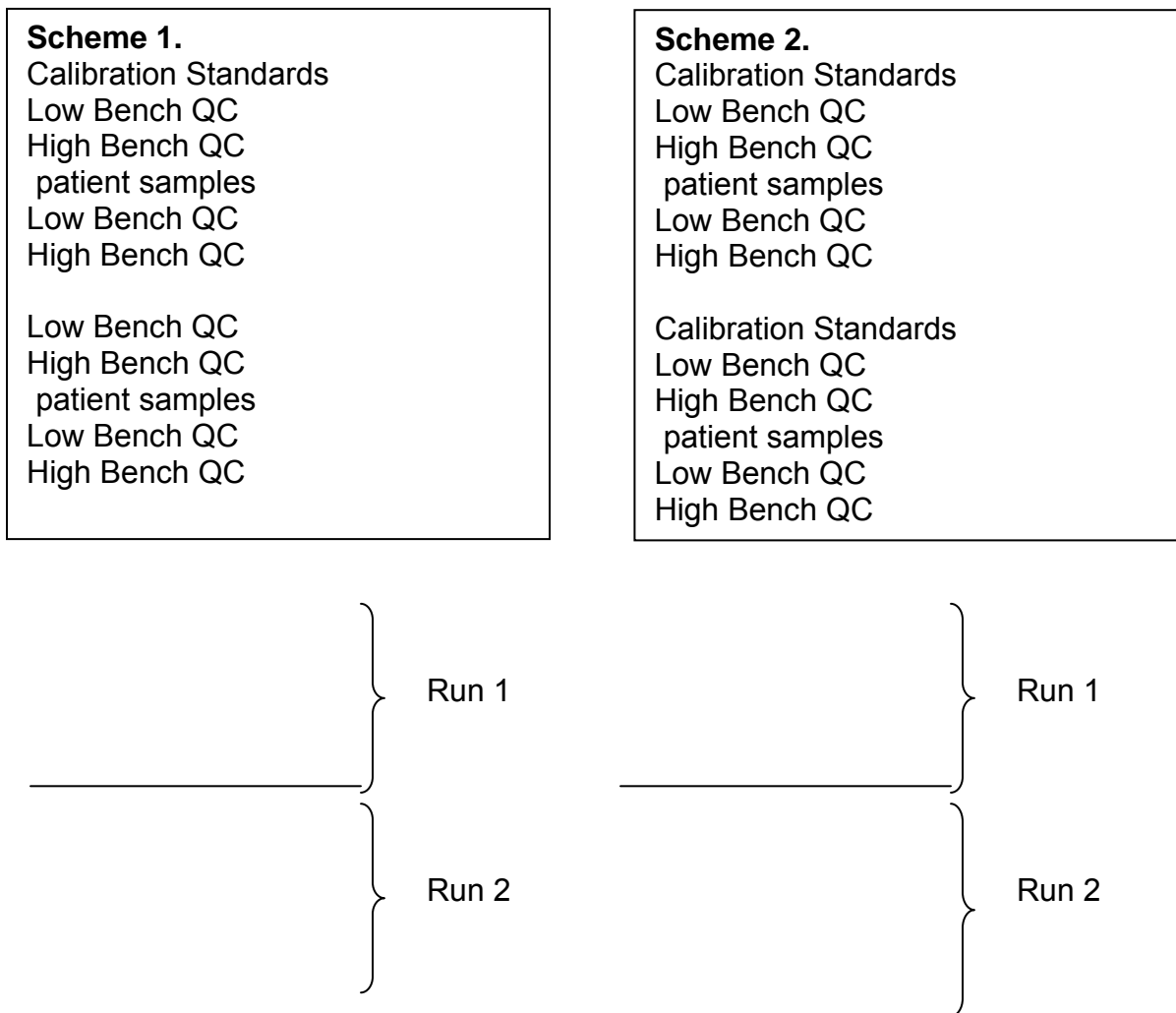
In this method the 95% reference ranges (see Appendix, Table 7) for these elements in urine fall within the linear range of the standard curves. Dilute results that fall above the range of the calibration standard concentrations (see Appendix, Table 7) with >18 MΩ·cm water and reanalyze them so that the results fall within the concentration range covered by the calibration standards.

Typical correlation coefficients for the calibration curves will be ≥ 0.999 .

9. Quality Control (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 the analyte of interest. Both of these pools are analyzed after the calibration standards are analyzed but before any patient samples are analyzed so that judgments on the calibration curves may be made before analysis of patient samples. These bench QCs should be analyzed again at the end of the run (approximately 20 patient samples total). If more patient samples are analyzed on the same calibration curve after the second run of the bench QC (after approximately 20 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 shown below are both acceptable ways to analyze more than one set of patient samples in one day. CDC typically uses scheme 2:



Before bench QC materials can be used, QC limits must be established for each pool. This is done by performing statistical calculations on 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 should be included to evaluate the analysis. In addition to providing QC limits, the characterization runs can also serve to establish homogeneity of the pools.

A. Precision and Accuracy

(see Appendix, Table 8)

(1) QC Results Evaluation

After completing a run, export the run from the ELAN software to the Database, then export it to the SAS program where the run will be judged to be in or out of control. The QC limits are based on the average and standard deviation of the beginning and ending analyses of each of the bench QC pools, so it will not be possible to know if the run is officially accepted or rejected until it is completed. The SAS program applies the DLS QC rules to the data as follows:

- (a) If both QC run means are within $2S_m$ limits and individual results are within $2S_i$ limits, then accept the run.
- (b) If 1 of the 2 QC run means is outside a $2S_m$ limit - reject run if:
 - (i) Extreme Outlier – Run mean is beyond the characterization mean $\pm 4S_m$
 - (ii) 1 $3S$ Rule - Run mean is outside a $3S_m$ limit
 - (iii) 2 $2S$ Rule - Both run means are outside the same $2S_m$ limit
 - (iv) 10 \bar{X} -bar Rule – Current and previous 9 run means are on same side of the characterization mean
- (c) If one of the 4 QC individual results is outside a $2S_i$ limit - reject run if:

R 4S Rule – Within-run ranges for all pools in the same run exceed $4S_w$ (i.e., 95% range limit)

Note: Since runs have multiple results per pool for 2 pools, the R 4S rule is applied within runs only.

Abbreviations:

S_i = Standard deviation of individual results (the limits are not shown on the chart unless run results are actually single measurements).

S_m = Standard deviation of the run means (the limits are shown on the chart).

S_w = Within-run standard deviation (the limits are not shown on the chart).

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 four replicate readings (maximum replicate concentration value - minimum replicate concentration value) for a single sample analysis is greater than the criteria listed in Table 8, then repeat the analysis of that sample.

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

- A. If an analyte result for the beginning QC material(s) falls outside of the 99% mean limits, then the following steps are recommended:
- (1) If a particular calibration standard is obviously in error, remake a new dilution of that calibration standard, reanalyze it, and reprocess the sample analyses using this new result as part of the calibration curve.
 - (2) Prepare a fresh dilution of the failing QC material, and reanalyze it.
 - (3) Prepare fresh dilutions of all of the calibration standards [working urine multi-element standards; reanalyze the entire calibration curve using the freshly prepared standards.
- B. 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. Do not report analytical results for runs that are not in statistical control.

11. Limitations of Method; Interfering Substances and Conditions

The tin isotope ^{114}Sn overlaps the cadmium isotope ^{114}Cd . Use the correction equation $(-0.026826 \cdot \text{Sn}118)$ to correct the counts observed as m/z 114 to exclude counts due to ^{114}Sn .

12. Reference Ranges (Normal Values)

See Appendix, Table 7.

13. Results Beyond Upper Boundaries / Action Levels

If the concentration of any element is beyond the repeat upper boundary concentrations listed in Table 7 (Appendix), perform a confirmatory analysis for that particular element, with an alternative method if one is available. Note: Because the observed concentrations for Be and Pt in urine are typically at or below the method LOD, a confirmatory analysis is only necessary for Be results ≥ 0.2 $\mu\text{g/L}$, and Pt results ≥ 0.1 $\mu\text{g/L}$.

There is no routine notification for elevated levels for the metals determined with this method. Report highly elevated levels to the supervising physician via fax, telephone, or E-mail. Use the Nuclear Regulatory Commission (NRC) recommendation for depleted U (U-238) in urine for urine U. The present NRC standard for workplace removal is 15 $\mu\text{g/L}$ of U in urine (6).

14. Specimen Storage and Handling During Testing

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

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

If the analytical system fails, then store the specimens at 4°C until the analytical system can be restored to functionality. If you anticipate long-term interruption (longer than 4 weeks), then store urine specimens at $\leq -20^\circ\text{C}$.

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

Conduct test-result reporting as outlined in the DLS Policies and Procedures Manual. As stated in Section 14, the supervisor should notify the supervising physician or principal investigator as soon as possible. Use the most expeditious means possible; (telephone, fax, etc.).

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

The designated person who receives specimen/samples delivered to Inorganic Toxicology and Nutrition Branch (ITN) sets up a "Specimen Folder." This person should 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. When the sample analysis is completed, place the tracking form in the Specimen Tracking Record Log Book located in the trace-metals library.

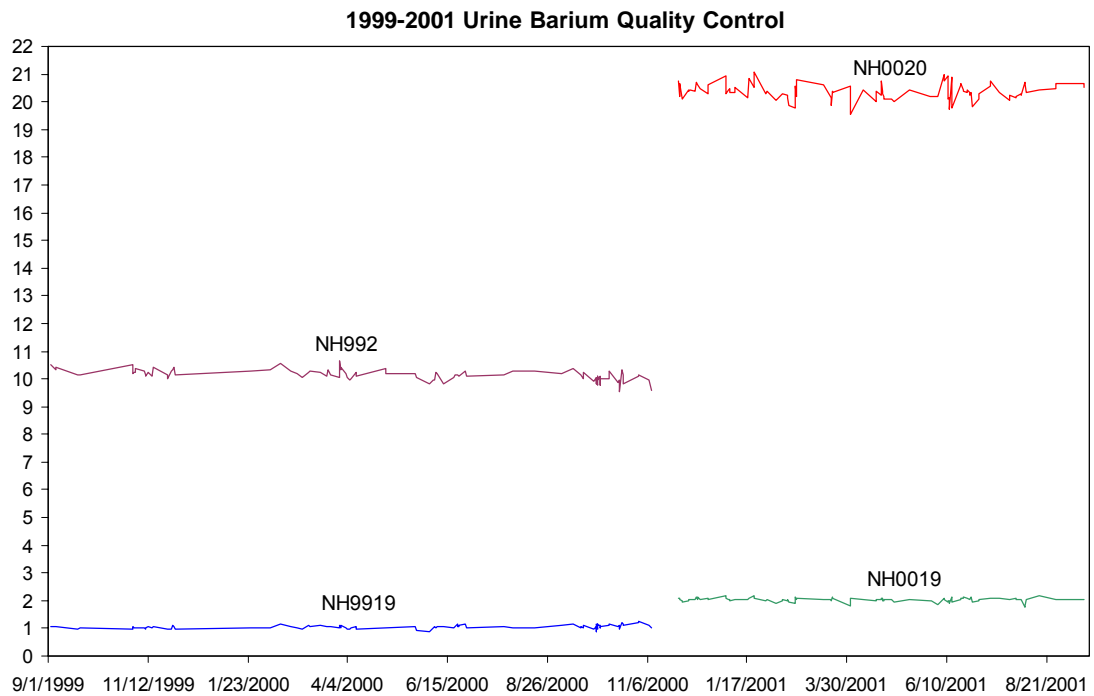
Use standard record-keeping means (e.g., electronic –Microsoft Access, optical disk or floppy disk) to track specimens. Maintain records for 3 years, including related Quality Assurance/QC data. Keep duplicate records in electronic or hardcopy format, and keep them off site if they are sensitive or critical. Use only numerical identifiers (e.g., case ID numbers). To safeguard confidentiality, provide only the medical supervisor (MS) or project coordinator (PC) with personal identifiers.

18. Summary Statistics and QC Graphs

A. Barium

Summary Statistics for Urine Barium by Lot

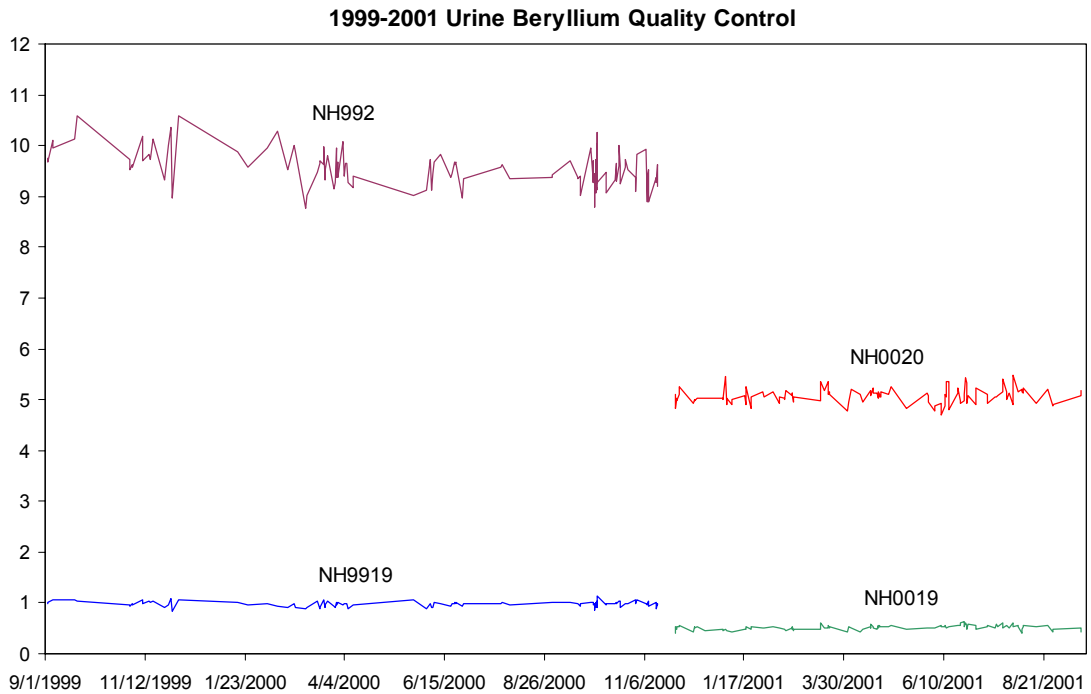
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH9920	96	9/3/1999	11/9/2000	10.145	0.196	1.934
NH9919	96	9/3/1999	11/9/2000	1.050	0.065	6.229
NH0020	98	11/29/2000	9/17/2001	20.365	0.288	1.413
NH0019	98	11/29/2000	9/17/2001	2.030	0.073	3.603



B. Beryllium

Summary Statistics for Urine Beryllium by Lot

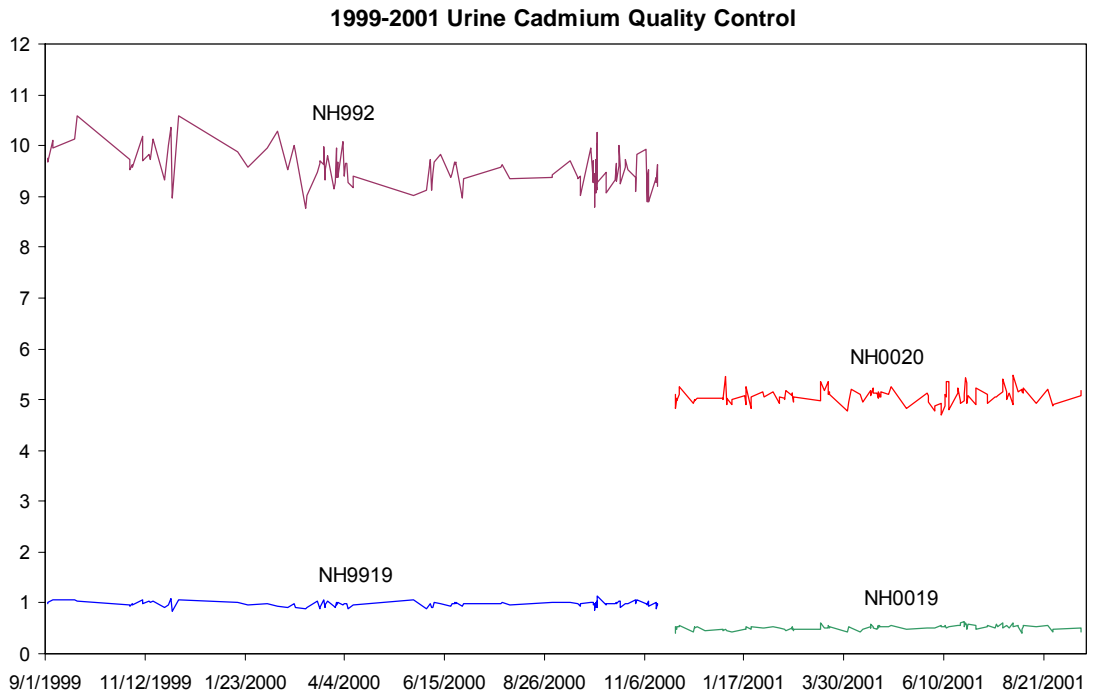
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH9919	119	9/3/1999	11/16/2000	0.981	0.052	5.281
NH9920	119	9/3/1999	11/16/2000	9.548	0.363	3.799
NH0019	112	11/29/2000	9/17/2001	0.513	0.045	8.827
NH0020	112	11/29/2000	9/17/2001	5.073	0.150	2.950



C. Cadmium

Summary Statistics for Urine Cadmium by Lot

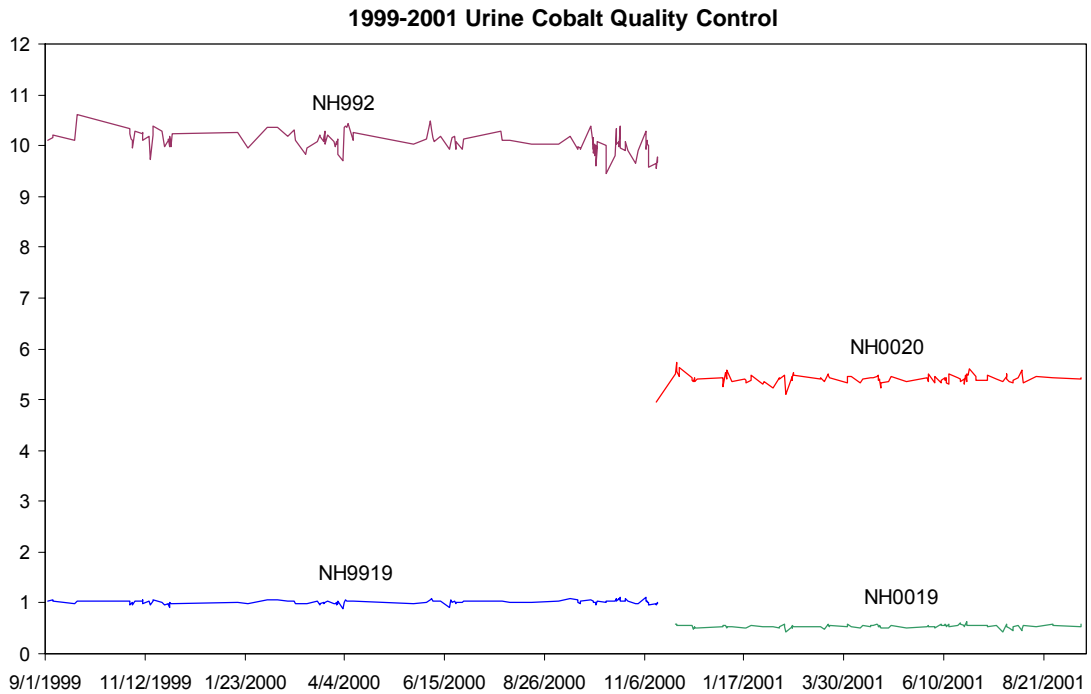
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH9919	109	9/3/1999	11/16/2000	0.990	0.036	3.591
NH9920	109	9/3/1999	11/16/2000	9.730	0.223	2.296
NH0019	105	11/29/2000	9/17/2001	0.523	0.025	4.712
NH0020	105	11/29/2000	9/17/2001	5.217	0.067	1.278



D. Cobalt

Summary Statistics for Urine Cobalt by Lot

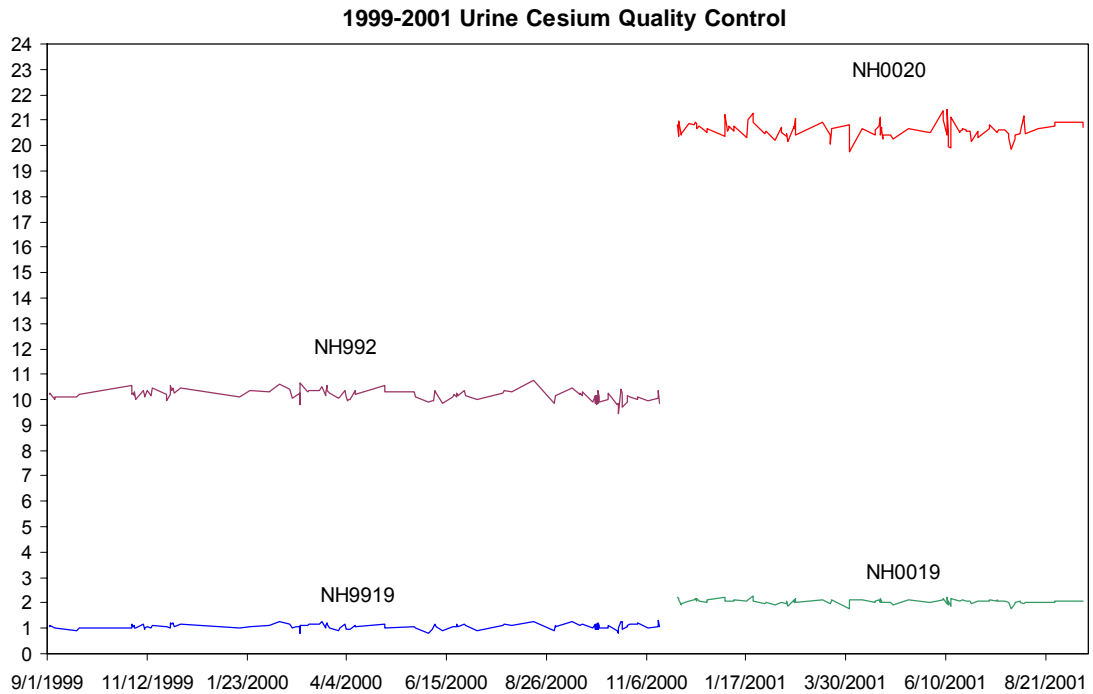
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH9919	122	9/3/1999	11/16/2000	1.018	0.037	3.646
NH9920	122	9/3/1999	11/16/2000	10.069	0.203	2.013
NH0020	110	11/15/2000	9/17/2001	5.413	0.095	1.762
NH0019	109	11/29/2000	9/17/2001	0.540	0.032	5.982



E. Cesium

Summary Statistics for Urine Cesium by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH9919	118	9/3/1999	11/16/2000	1.073	0.098	9.176
NH9920	118	9/3/1999	11/16/2000	10.171	0.215	2.115
NH0019	99	11/29/2000	9/17/2001	2.059	0.082	3.969
NH0020	99	11/29/2000	9/17/2001	20.607	0.307	1.491

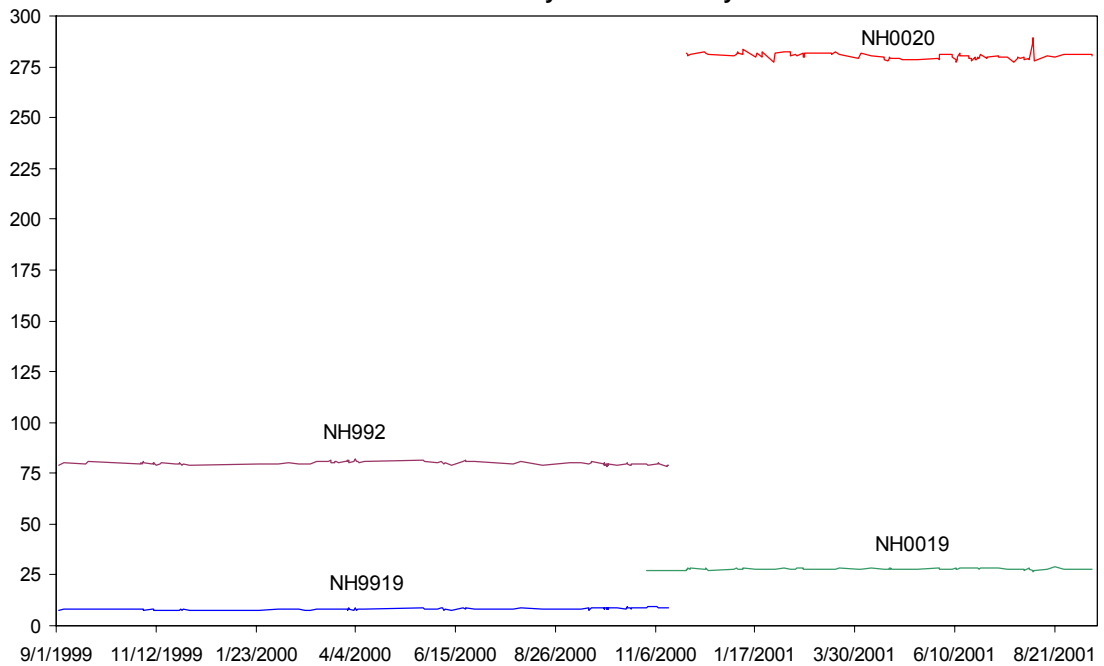


F. Molybdenum

Summary Statistics for Urine Molybdenum by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH9919	107	9/3/1999	11/16/2000	8.292	0.468	5.647
NH9920	107	9/3/1999	11/16/2000	79.971	0.747	0.934
NH0019	98	10/31/2000	9/17/2001	27.955	0.361	1.293
NH0020	97	11/29/2000	9/17/2001	280.331	1.769	0.631

1999-2001 Urine Molybdenum Quality Control

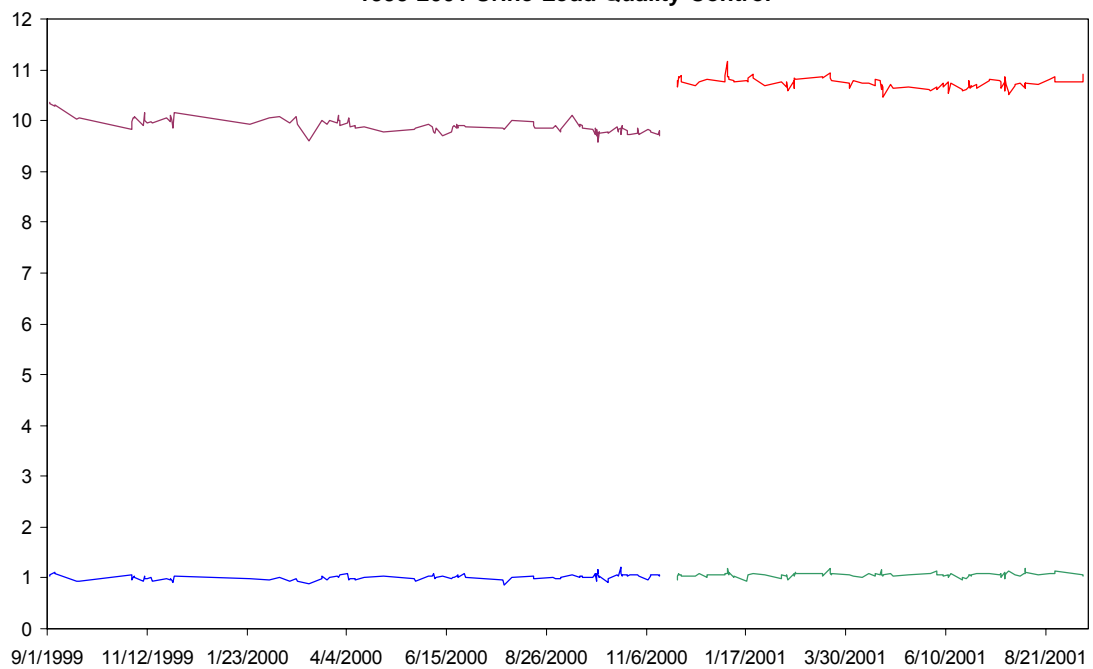


G. Lead

Summary Statistics for Urine Lead by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH9919	115	9/3/1999	11/16/2000	1.016	0.054	5.311
NH9920	115	9/3/1999	11/16/2000	9.894	0.144	1.451
NH0019	103	11/29/2000	9/17/2001	1.068	0.046	4.265
NH0020	103	11/29/2000	9/17/2001	10.736	0.104	0.973

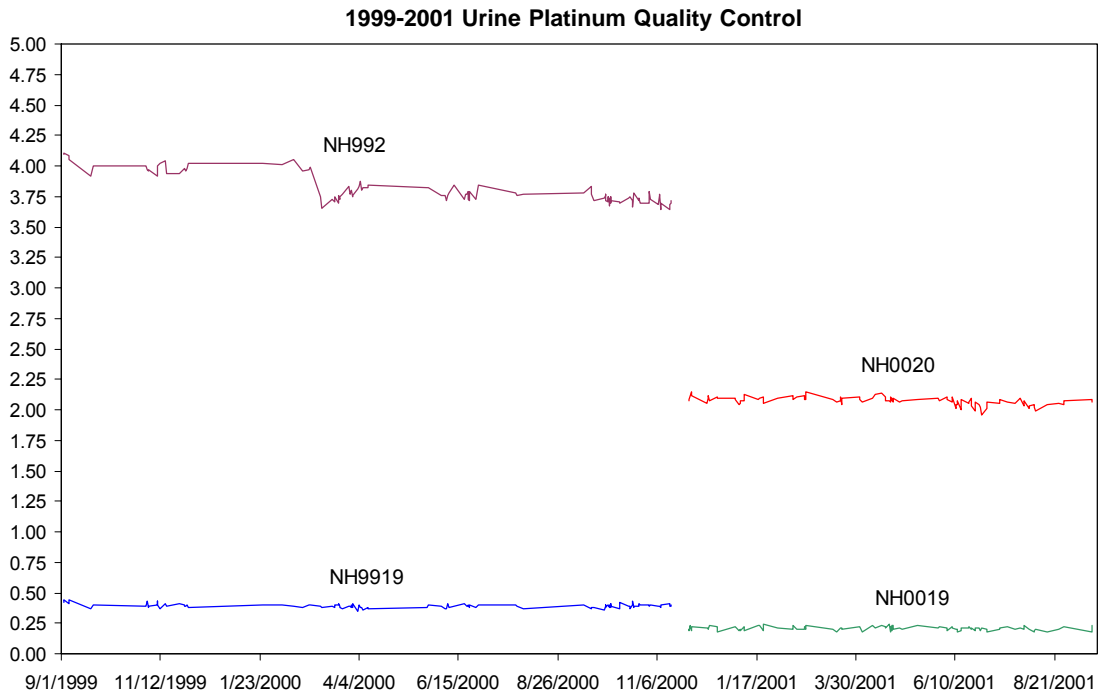
1999-2001 Urine Lead Quality Control



H. Platinum

Summary Statistics for Urine Platinum by Lot

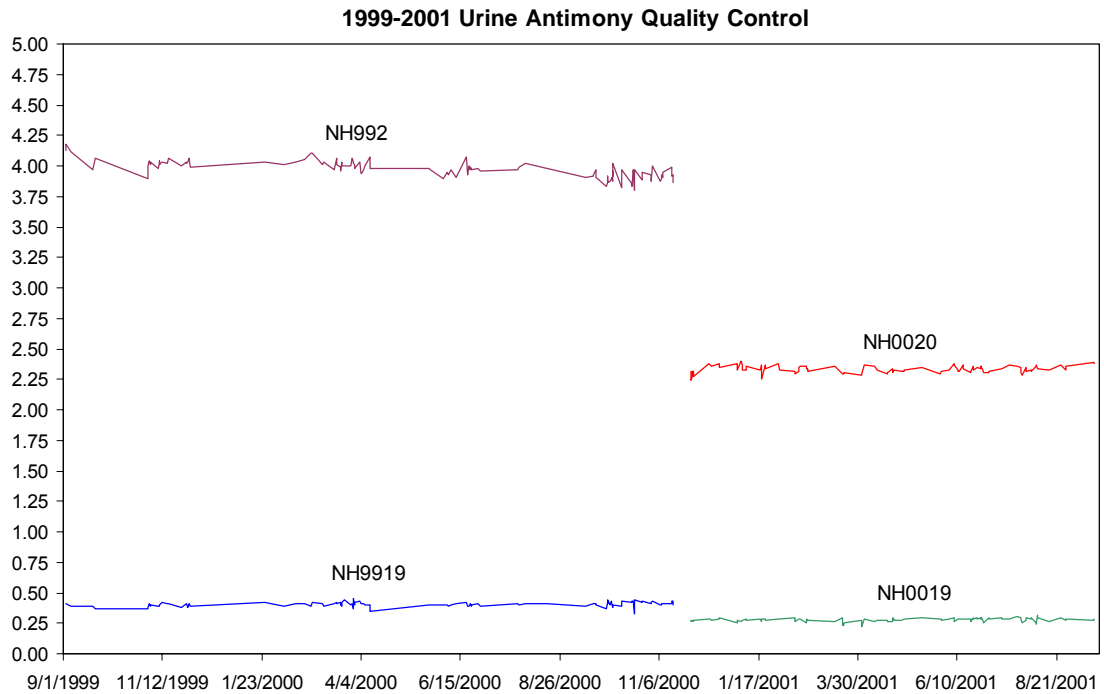
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH9919	116	9/3/1999	11/16/2000	0.392	0.016	4.149
NH9920	116	9/3/1999	11/16/2000	3.807	0.117	3.080
NH0019	99	11/29/2000	9/17/2001	0.208	0.016	7.698
NH0020	99	11/29/2000	9/17/2001	2.074	0.034	1.646



I. Antimony

Summary Statistics for Urine Antimony by Lot

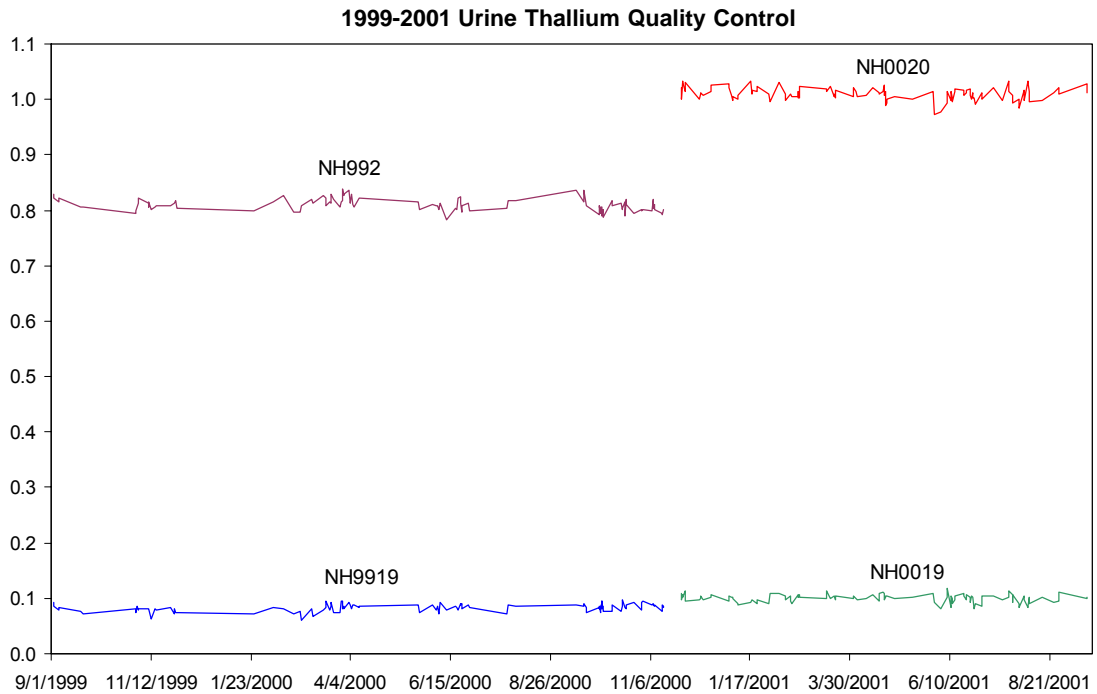
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH9919	110	9/3/1999	11/16/2000	0.405	0.019	4.769
NH9920	110	9/3/1999	11/16/2000	3.973	0.070	1.761
NH0019	100	11/29/2000	9/17/2001	0.277	0.015	5.510
NH0020	100	11/29/2000	9/17/2001	2.333	0.030	1.282



J. Thallium

Summary Statistics for Urine Thallium by Lot

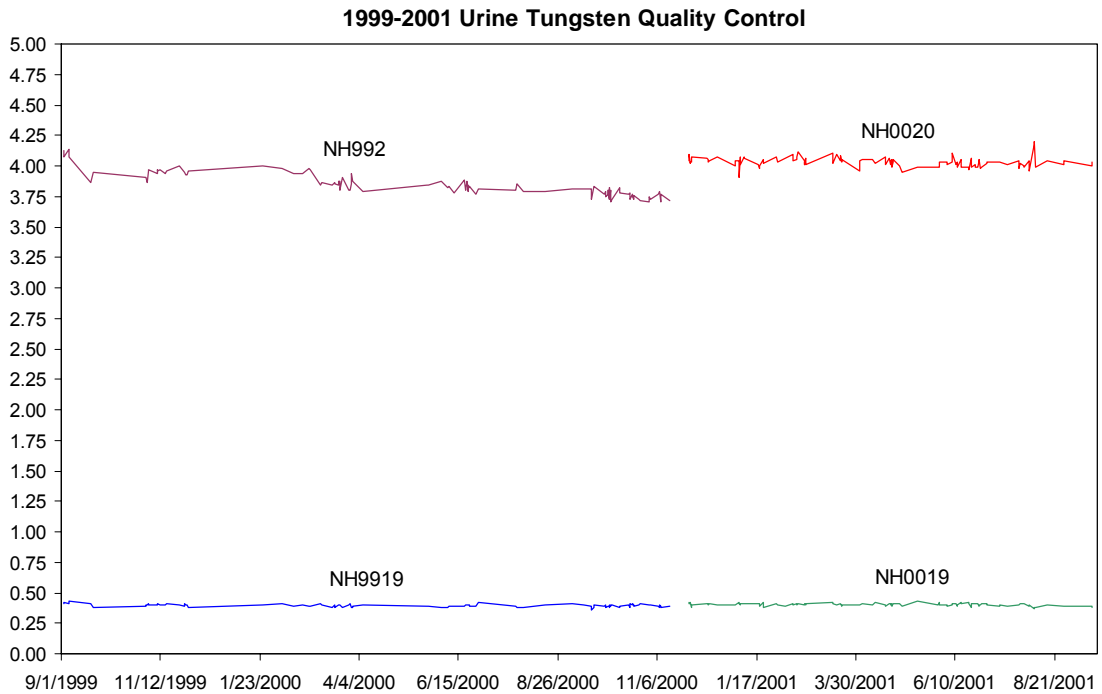
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH9919	111	9/3/1999	11/16/2000	0.083	0.007	8.462
NH9920	111	9/3/1999	11/16/2000	0.810	0.011	1.418
NH0019	106	11/29/2000	9/17/2001	0.100	0.007	7.399
NH0020	106	11/29/2000	9/17/2001	1.010	0.012	1.158



K. Tungsten

Summary Statistics for Urine Tungsten by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH9919	106	9/3/1999	11/15/2000	0.395	0.011	2.800
NH9920	106	9/3/1999	11/15/2000	3.845	0.094	2.447
NH0019	105	11/29/2000	9/17/2001	0.403	0.011	2.699
NH0020	105	11/29/2000	9/17/2001	4.032	0.043	1.057



Use of trade names is for identification only and does not imply endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

References

1. Date AR, Gray, AL. Applications of inductively coupled plasma-mass spectrometry. New York: Chapman and Hall; 1989.
2. Mulligan KJ, Davidson TM, Caruso JA. Feasibility of the direct analysis of urine by inductively coupled argon plasma-mass spectrometry for biological of exposure to metals. J Anal AT Spectrom; 1990; 5:301.
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5. Jarrett JM, Caldwell KL. Unpublished study, 2002.
6. U.S. Nuclear Regulatory Commission. Regulatory guide 8.22 (revision 1). Bioassay at uranium mills. Atlanta Ga: 1988.
7. Anderson I, Torjussen W, Zacgaruaseb H. Clin Chem 1978; 24:1198.Basekt RC.
8. Biological monitoring methods for industrial chemicals. Littleton MA: PSG/Biomedical; 1980.

Appendix

Table 5. Multi-element Intermediate Stock Standard

Analyte	Intermediate Stock Standard Concentrations (mg/L) High Purity Standards Item # SM-2107-001
Be	10
Co	5
Mo	300
Cd	5
Sb	4
Cs	20
Ba	20
W	4
Pt	5
TI	2
Pb	15
U	0.5
	Lot numbers used have included 223414, 223436, 223501, and 223518

Table 6. Preparation of Multi-element Intermediate Working Standards

Standard #	1	2	3	4	5
Volume of Flask (mL)	100	100	100	100	100
Volume Spike of Int. Stock Std. (mL)	0.05	0.15	0.30	0.75	1.00
	Concentrations (ug/L) ‡				
Be*	5	15	30	75	100
Co*	2.5	7.5	15	37.5	50
Mo*	150	450	900	2250	3000
Cd*	2.5	7.5	15	37.5	50
Sb*	2	6	12	30	40
Cs*	10	30	60	150	200
Ba*	10	30	60	150	200
W†	2	6	12	30	40
Pt†	2.5	7.5	15	37.5	50
Tl†	1	3	6	15	20
Pb†	7.5	22.5	45	112.5	150
U†	0.25	0.75	1.5	3.75	5

* Rh-103 used as internal standard

† Ir-193 used as internal standard

‡ A further 1:10 dilution occurs when added to base urine. Enter the table 2 concentrations divided by 10 into the ELAN software (method window, calibration page).

Table 7. Reference Ranges and Detection Limits for Elements Measured (All units µg/L)

Element/ Isotope Monitor ed	Reference Ranges (95 th Percentile, µg/L) (weighted, non-creatinine corrected NHANES 1999 & 2000 results) ⁴	Repeat Upper Boundary (µg/L) ⁵	CDC Method DL (µg/L) (May 2000)
Be-9*	< 0.13	0.2	0.13
Co-59*	1.32	2.83	0.07
Mo-98*	178	293.5	0.8
Cd-114*	1.36	2.54	0.06
Sb-121*	0.42	0.80	0.04
Cs-133*	11.4	16.5	0.14
Ba-138*	6.80	17.1	0.12
W-184†	0.500	1.38	0.04
Pt-195†	< 0.04	0.1	0.04
Tl-205†	0.45	0.62	0.02

Multiple Toxic Elements In Urine
NHANES 1999–2000

Pb-208 [†]	2.90	7.8	0.1
U-238 [†]	0.046	0.277	0.004

* Rh-103 used as internal standard

[†] Ir-193 used as internal standard

**Table 8. Multi-element Urine Sample Precision Evaluation
(Values Empirically Determined)**

Analyte	Range Maximum* (µg/L)
Be	0.3
Co	0.3
Mo	4.0
Cd	0.3
Sb	0.2
Cs	0.5
Ba	0.4
W	0.2
Pt	0.2
Tl	0.2
Pb	0.3
U	0.03

* Range maximum is the range of the four replicate readings for a single sample analysis (see Section 10.B.). This value is also called the “RepDelta” in the Microsoft Access database that handles data for the Inorganic Toxicology and Nutrition Branch.

Table 9. Alternative Preparation of Multi-Element Intermediate Stock QC Standard *

Element	Source (All SRMs from NIST)	NIST SRM Conc. (mg/L)	SRM Aliquot Volume (mL) per 5 mL for Standard A	SRM Aliquot Volume (mL) per 250 mL for Standard B	Intermediate Stock Standard Concentration (mg/L)
Be	SRM 3105A	10,000		0.25	10
Co	SRM 3113	10,000		0.25	10
Mo	SRM 3134	10,000		2.0	80
Cd	SRM 3108	10,000		0.25	10
Sb	SRM 3102a	10,000		0.1	4
Cs	SRM 3111	10,000		0.25	10

Multiple Toxic Elements In Urine
NHANES 1999–2000

Ba	SRM 3104a	10,000		0.25	10
W	SRM 3163	10,000		0.1	4
Pt	SRM 3140	10,000		0.1	4
TI	SRM 3158	10,000	1.0	0.1 [†]	0.8
Pb	SRM 3128	10,000		0.25	10
U	SRM 3164	10,000	0.1	0.1 [†]	0.08

* This solution has been prepared for CDC in the past by SPEX CertiPrep Inc. (custom mix item number XCDCGA-3, lot number 24-89AS).

[†] Aliquot taken from intermediate stock standard A

Table 10. Alternative Preparation of Multi-element Intermediate Working QC Standards

QC Solution	Working Low Bench QC	Working High Bench QC
	100 µL of Intermediate Stock QC Standard: per 100 mL	1000 µL of Intermediate Stock QC Standard: per 100 mL
Analyte	Aqueous QC Standard Concentrations (ug/L) [‡]	
Be*	10	100
Co*	10	100
Mo*	80	800
Cd*	10	100
Sb*	4	40
Cs*	10	100
Ba*	10	100
W [†]	4	40
Pt [†]	4	40
TI [†]	0.8	8
Pb [†]	10	100
U [†]	0.08	0.8

* Rh used as internal standard

[†] Ir used as internal standard

[‡] A further 1:10 dilution occurs when added to base urine

Table 11. Dilution preparation summary
(Including Alternative, Aqueous-Based Bench QC).

Dilution ID	Water (µL)	Base Urine (µL)	AQ Intermediate Working Standard (µL)	Patient or QC urine sample (µL)	Diluent (µL)
AQ Blank	1000	-	-	-	9000 *
Urine Blank and UrBlkChk	100	900	-	-	9000 *

Multiple Toxic Elements In Urine
NHANES 1999–2000

Working Calibration Standards or Working Bench QC (Aqueous-Based) †	-	900	100	-	9000 *
Patient Urine or Urine-Based QC	-	-	-	500	4500
Patient Urine 2x Dilution ‡	500	-	-	500	9000 *
Patient Urine 10x Dilution ‡	900	-	-	100	9000*

9000 µL diluent is best dispensed from the Digiflex as 2 4500-µL portions (i.e.- When preparing a Working Calibration Standard dilution, dispense 4500 µL diluent + 100 µL water in one cycle of Digiflex™, then 4500 µL diluent + 900 µL base urine in the next cycle of the Digiflex™ to prepare a 10 mL total volume dilution.)

Aqueous-based bench QC materials are the alternative way to prepare bench QC when large urine collection and spiking is not feasible. The working bench QC dilutions are prepared just as the working calibration standards are. By spiking these materials into base urine, they are matrix-matched to the patient urine samples. Currently, CDC primarily uses urine-based bench QC materials (preparation is described in Section 6.C).

Extra dilution is performed on urine samples whose concentration is greater than 110% of the concentration of the highest calibration standard. Any extra level of dilution can be prepared as long as the 9:10 ratio of diluent to total dilution volume is maintained. Use of the lowest possible dilution level is preferred because matrix differences may lead to different observed concentration results as the sample dilution becomes greater (i.e. 2x dilution is preferred over 10x if 2x is sufficient to dilute analyte into calibration curve range).