Laboratory of Inorganic and Nuclear Chemistry
Division of Environmental Health Sciences
Wadsworth Center
Department of Health
State of New York

NYS CLEP Laboratory ID 1067
CLIA Laboratory ID 33D0654341

Standard Operating Procedure

Mercury in Urine by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

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Revision Record

Rev	Date	**Responsible Person**	**Description of Change**
1	4/26/04	Christopher Palmer	Initial release.
2	5/18/04	Christopher Palmer	Major revision.
3	11/03/06	Michael Minnich	Major revision.
4	1/29/08	Amy Steuerwald	Sulfamic acid solution preparation, addition to clinical specimens and diluent revised. Change data back up from Lead server to trel.
5	2/29/08	David Bellis	Addition of criteria for assessing repeat measurements.
6	4/21/08	David Bellis	Quality control data updated.
7	9/1/08	David Bellis	Major revision.
8	1/9/09	Amy Steuerwald	Minor revisions.
9	12/13/10	Amy Steuerwald	Minor revisions.

The following laboratory staff have read this Manual.
A copy of this page will be distributed to the employee training record file.

Name	Title	Date

1.0 Method capabilities

This method is used to achieve rapid and accurate quantitative determination of mercury (Hg) in urine. Protocols for specimen collection were developed based on Centers for Disease Control and Prevention (CDC) guidelines (1). The method is suitable for assessing exposure in occupationally and non-occupationally exposed subjects. This method has been used successfully in several biomonitoring studies of mercury exposure (2, 3).

2.0 Safety precautions

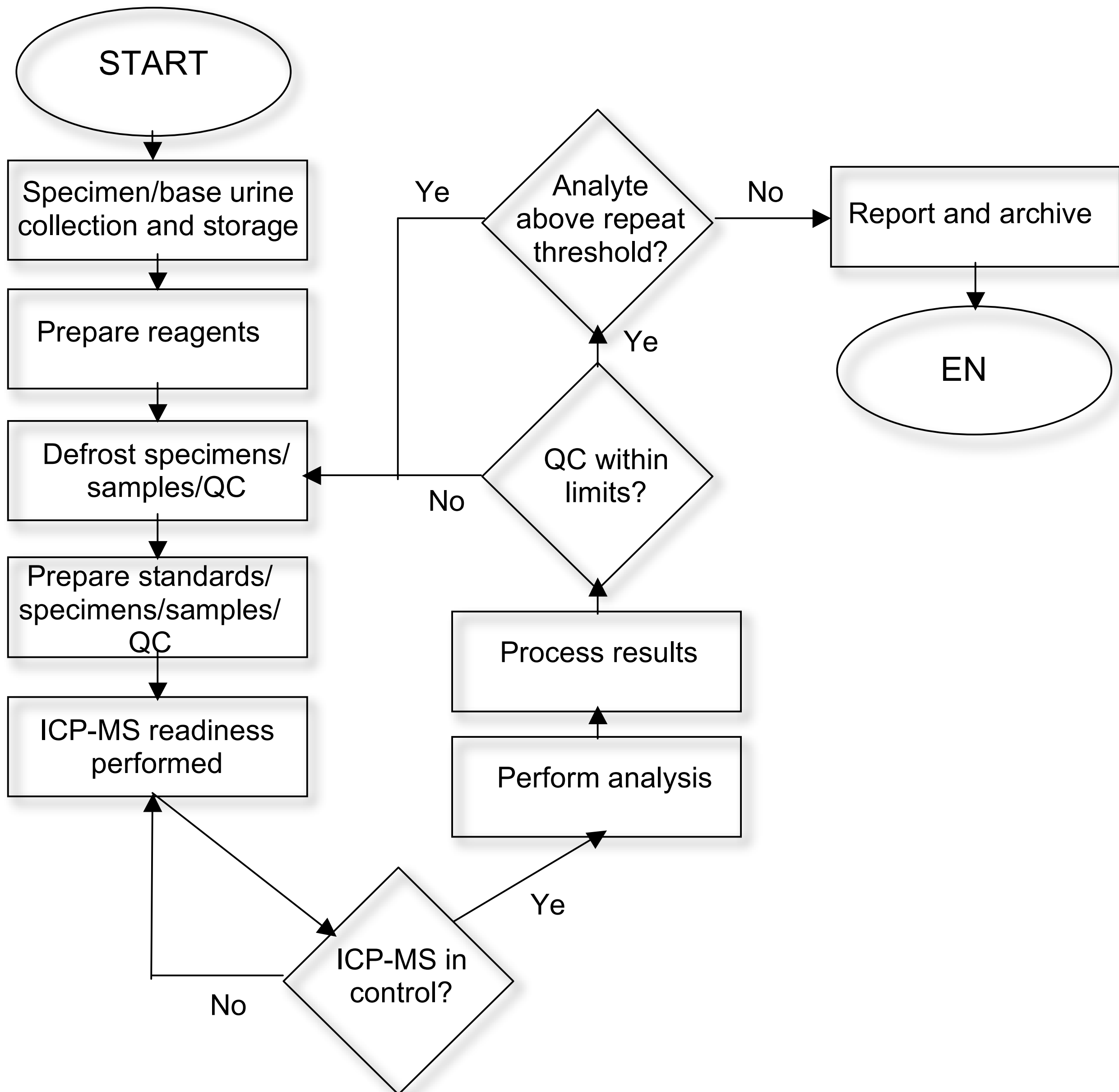
Before operating the instrument, read the information in the PerkinElmer® ELAN® ICP-MS System Safety Manual.

All personnel must successfully complete the Hazard Communication / Lab Safety Training annually, either on-line or in-person. All personnel must abide by the regulations set forth in the Policies and Procedures Manual issued by the Wadsworth Center Safety and Security Office.

Wear gloves, a lab coat, and safety glasses while handling all human urine. Observe universal precautions. Place in a biohazard autoclave bag disposable plastic, glass and paper (e.g., pipette tips, autosampler tubes, gloves, etc.) that contact urine. Dispose of all biological samples and diluted specimens in a biohazard autoclave bag at the end of the analysis. Keep these bags in appropriate containers until they are sealed and sent to be autoclaved.

Exercise special care when handling and dispensing concentrated nitric acid. Always remember to add acid to water. 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.

3.0 Standard operating process and procedures



3.1 Specimen/base urine collection and storage

3.1.1 Specimen collection and storage

This method does not require any special instructions for the patient such as fasting or special diets. The specimen type is random spot urine. A sulfamic acid preservative solution (section 3.2.1) is required to prevent endogenous inorganic mercury loss in urine specimens as previously described (4).

The optimal amount of submitted specimen is 4–7 mL; the minimum amount of submitted urine is approximately 2 mL. The analysis requires at least 500 µL (0.5 mL) for a single assay. Typical specimen collection involves addition of 50 µL of sulfamic acid preservative solution for every 5 mL of urine collected. Acceptable containers include wide mouth specimen containers with screw lids (Kendall Precision Industries or equivalent), lot-screened 15-mL polypropylene centrifuge tubes, the Urine-Monovette® (Sarstedt Inc., Newton, NC) or Nalgene® cryovials. Use of sterile collectors for specimen acquisition is desirable, but not mandatory.

The criteria for unacceptable specimens are (a) insufficient volume (<0.5 mL), (b) lack of sulfamic acid preservative solution or (c) suspected contamination due to improper collection procedures or devices. Specimen characteristics that may compromise test results include contamination of urine by contact with dust, dirt, etc., from improper handling. A fresh urine specimen should be collected if the original specimen is believed to be unacceptable or compromised.

The laboratory protocol for urine collection and handling outlines specimen handling conditions. Collection, transport and special requirements are discussed. In general, urine specimens may be transported at ambient temperature, refrigerated or frozen and packed in dry ice during shipment. Once received, store long term at ≤-20°C until the analysis can proceed.

Short-term storage at approximately 2–4°C is acceptable. Refreeze at ≤-20°C portions of the sample that remain after analytical aliquots are withdrawn. Thawing and refreezing has not been found to compromise Hg determination in urine when the sulfamic acid preservative solution is added to specimens during collection.

For further information refer to the CDC instructional DVD, “Responding to a

chemical emergency, collection and handling of clinical specimens".

On receipt of a sample, check if "chain of custody" form is included. Sign and date form if present and file.

Accession samples (i.e., record in the order of acquisition) in the "Laboratory accessioning log book". Record pertinent specimen information including identifiers and the date of receipt. Provide and record a unique New York State Identification number for each specimen.

3.1.2 Base urine collection and storage

The base urine used in this method is a pool of human urine collected from volunteer donors. The pooled urine is acidified to 1% (v/v) using double-distilled nitric acid and 1% (v/v) sulfamic acid preservative solution (section 3.2.1). The base urine is frozen at ≤-20ºC, defrosted and centrifuged to remove precipitated urate salts. The supernatant urine is used to prepare matrix-matched calibration standards and quality control (QC) materials (section 3.4).

Collect urine in lot-screened or acid-rinsed sample collection cups or 24-hour urine collection containers. Once the urine is collected from donors, it should be analyzed to ensure that the Hg concentration is below the suggested maximum analyte concentration (Table 1). For short-term storage, store at approximately 2–4°C. For long-term storage, store at ≤-20°C.

Table 1. Suggested maximum analyte concentration for the matrix-match base urine.

Analyte	Concentration (µg/L)
Hg	<0.11

3.2 Prepare reagents

Reagents should be prepared under class 100 clean room conditions (i.e., in a Clean Room or Biological Safety Cabinet) using ≥18 MΩ·cm double-distilled deionized water (Barnstead Nanopure; Millipore Corporation, Bedford, MA or comparable) and high purity acids that are purchased from a manufacturer or generated in-house through use of an acid still. Certified and periodically calibrated pipettors should be used at all times. Label reagents with contents, date prepared, date of expiration and initials.

3.2.1 Reagent preparation

(1) Triton X-100™

A solution of 10% Triton X-100™ is made by adding 10 mL of concentrated Triton X-100™ stock (t-octylphenoxypolyethoxyethanol, SigmaUltra; Sigma-Aldrich Company, St. Louis, MO) to 90 mL of ≥18 MΩ·cm water. Mix the solution for several hours on a rotator mixer (Orbitron Rotator 1, Boekel Scientific, or similar) until the Triton X-100™ has dissolved. Store at room temperature and prepare as needed.

(2) Sulfamic acid preservative solution

To prepare, acid rinse or use a dedicated 125 mL Teflon™ container. Use an acid rinsed 100 mL plastic graduated cylinder to fill the Teflon™ container with 90 mL of ≥18 MΩ·cm water. Weigh 20 g of sulfamic acid (99.3% ACS reagent; Sigma–Aldrich Company, St. Louis, MO) into a weigh boat and add to the Teflon™ container. Dissolve the solid completely by agitating overnight on a rotator mixer or heating in warm water. Add 10 mL of concentrated Triton X-100™ stock solution. Mix thoroughly until completely dissolved. Store at room temperature and prepare as needed. Preservative solution shelf-life is 1 month.

(3) Diluent

The diluent used in this method is an aqueous solution of 1% (v/v) double-distilled nitric acid, 1% (v/v) sulfamic acid preservative solution, 0.005% Triton® X-100™, 1 mg/L Au and 10 µg/L Ir as an internal standard. This solution is used to prepare

the 1+19 dilution of all specimens, standards and QC materials. To prepare, acid rinse or partially fill a dedicated 2 L Teflon™ container with ≥18 MΩ·cm water. Add 20 mL double-distilled, concentrated nitric acid and 20 mL of sulfamic acid preservative solution. Add 20µL of 1000 mg/L Ir, 200 µL of 10000 mg/L Au and 1 mL of 10% Triton X-100™. Dilute to volume (2 L) with ≥18 MΩ·cm water. Store at room temperature and prepare as needed. Diluent expires 1 month from preparation.

(4) ICP-MS Rinse solution

The rinse solution used in this method is an aqueous solution of 2% (v/v) double-distilled nitric acid, 1000 µg/L Au, and 0.005% Triton X-100™ solution. This solution is used to prevent carry-over of analyte between samples. To prepare, acid-rinse or partially fill a dedicated 2 L Teflon™ container and with ≥18 MΩ·cm water. Add 40 mL double-distilled, concentrated nitric acid. Add 1 mL of 10% Triton X-100™ to the rinse solution and 200 µL of 10000 mg/L Au. Dilute to 2 L using ≥18 MΩ·cm water. Store at room temperature and prepare as needed.

3.3 Defrost specimens, samples and QC materials

All specimens, samples and QC materials should be stored at ≤-20°C until batched analysis can be arranged. Place all frozen urine specimens, previously prepared and frozen base urine for calibration curve matrix matching, QC materials and samples for analysis on a rotator mixer. Start rotation and allow specimens to reach ambient temperature.

3.3.1 Internal QC materials

Internal QC materials may be prepared by spiking prepared base urine (section 3.1.2) with Hg, stirring for 24 hours, aliquoting and freezing. QC materials should be prepared under Class 100 Clean Room conditions.

3.3.2 External QC samples

Samples certified for the analytes of interest (e.g., NIST Standard Reference Material (SRM) 2670a Toxic Elements in Urine (National Institute of Standards and

Technology, Gaithersburg, MD)) may be used for method validation and should be analyzed periodically to ensure method accuracy. External Quality Assessment (EQA) scheme (e.g., L'Institut National de Santé Publique du Québec (INSPQ), Centre de Toxicologie du Québec (CTQ), Canada) or Proficiency Testing (PT) samples (i.e., New York State Department of Health, Albany, New York) may also be used during validation and for ongoing QC procedures.

3.4 Prepare standards, specimens, samples and QC materials

Standards should be prepared under class 100 clean room conditions (i.e., in a Clean Room or Biological Safety Cabinet) using ≥18 MΩ·cm double-distilled deionized water and high purity acids that are purchased from a manufacturer or generated in-house through the use of an acid still. Caps should only be removed from standards, specimens, samples and QC under class 100 clean room conditions. Certified and periodically calibrated pipettors should be used at all times. Label reagents with contents, date prepared, date of expiration and initials.

3.4.1 Standard preparation

(1) Hg Stock standard

The stock standard solution is a NIST traceable aqueous solution of 1000 mg/L of inorganic Hg. This solution is a single element ICP standard prepared by GFS Chemicals (GFS Chemicals, Powell, OH). The shelf life of the stock standard is certified by the manufacturer and should be stored according to manufacturer instructions. The Hg stock standard is used to prepare the Hg intermediate stock standard.

(2) Hg intermediate stock standard

The Hg intermediate stock standard is an aqueous solution of 8 mg/L Hg in 1% (v/v) nitric acid and 1% (v/v) sulfamic acid preservative solution. Prepare by acid rinsing or using a dedicated 100 mL polypropylene (PP) volumetric flask and partially filling it with ≥18 MΩ·cm water. Add 1 mL of double-distilled, concentrated nitric acid and 1 mL of the sulfamic acid preservative solution. Add 800 μL of the 1000 mg/L Hg stock solution. Bring to volume with ≥18 MΩ·cm water. Table 2

shows the Hg intermediate stock standard preparation scheme.

Table 2. Hg intermediate stock standard preparation.

Reagent	Volume (µL)
Hg stock standard (1000 µg/L)	800
Concentrated HNO_3	1000
Sulfamic acid preservative solution	1000
≥18 MΩ·cm water	Balance to 100 mL final volume

(3) Hg Intermediate working standards

The intermediate working standard solutions used in this method are a series of six aqueous dilutions of the Hg intermediate stock standard solution and one aqueous blank in 1% (v/v) double-distilled nitric acid and 1% (v/v) sulfamic acid preservative solution. These solutions are used each day of analysis to prepare the final working standards. To prepare, acid-rinse or partially fill seven dedicated 100 mL PP volumetric flasks and partially fill them with ≥18 MΩ·cm water. To each 100 mL flask, add 1 mL of double-distilled, concentrated nitric acid and 1 mL of sulfamic acid preservative solution. Add the appropriate aliquot of the Hg intermediate stock standard solution (Table 3) and bring to volume with ≥18 MΩ·cm water. One of the volumetric flasks will not have an aliquot of the intermediate stock standard and serves as the 1% (v/v) nitric acid and 1% (v/v) sulfamic acid blank. Store solutions at room temperature in the dedicated 100 mL volumetric flasks. Intermediate working standards expire 1 week from the preparation date. The final concentration of Hg in each standard is listed in Table 4.

Table 3. Preparation of Hg single element intermediate working standards.

Hg Intermediate Stock Standard	Volume of Hg Intermediate Stock Standard (µL)						
	Blank	Std. 1	Std. 2	Std. 3	Std. 4	Std. 5	Std. 6
8 mg/L	0	25	63	125	250	375	500

Table 4. Concentration of the intermediate working standards.

Analyte	Intermediate Working Standard Concentration (µg/L)					
	Std. 1	Std. 2	Std. 3	Std. 4	Std. 5	Std. 6
Hg*	2.0	5.0	10	20	30	40

* Ir-193 internal standard.

Note: Enter the Table 4 concentrations into the method calibration page of the ELAN® software.

3.4.2 Standard, specimen, sample and QC dilution

Class 100 clean room conditions are required for dilutions. Prepare test samples, including urine specimens, QC materials and working calibration standards with the Digiflex dispenser (ICN Biomedicals Inc., refer to manufacturer's handbook for more operational guidelines) or similar. Table 5 provides a summary of the sample preparation process.

Table 5. Preparation scheme for the NYS urine Hg method.

	Volume (µL)				
	1% HNO_3 / 1% Sulfamic Acid	Base Urine	Intermediate Working Standards	Patient or QC Urine	Diluent
Reagent blank	1000	0	0	0	2x4500
Urine blank	500	500	0	0	2x4500
Standards	0	500	500	0	2x4500
Specimens/ Samples and QCs	500	0	0	500	2x4500

Note: 9000 µL diluent is best dispensed from the Digiflex™ as two 4500 µL portions (i.e., when preparing a Standard dilution, dispense 4500 µL diluent + 500 µL intermediate working standard in one cycle of Digiflex™, then 4500 µL diluent + 500 µL base urine in the next cycle of the Digiflex™ to prepare a 10 mL total volume dilution).

(1) Reagent blanks

Prepare three reagent blanks consisting of 1000 µL of 1% (v/v) nitric acid, 1% sulfamic acid preservative solution and 9000 µL of diluent. These will be used as the blank for any urine based QC materials, external reference samples and patient specimens.

(2) Urine blank

Prepare one urine blank dilution consisting of 500 µL of base urine (i.e., same material used to prepare the urine calibration standards), 500 µL of reagent blank,

and 9000 µL of diluent. The urine blank will be the blank for the calibration standards.

(3) Working calibration standards

Prepare the working calibration standards as 500 µL of the appropriate aqueous intermediate working calibration standard, 500 µL of base urine, and 9000 µL of diluent.

(4) Urine specimens, samples and urine-based QC materials

Prepare the urine specimens, samples and the urine-based QC material by diluting 500 µL of urine with 500 µL of reagent blank and 9000 µL of diluent.

Prepare a single sample from each specimen. For research or biomonitoring studies, prepare a minimum of 2% random repeats for quality assurance purposes.

If (i) results are needed as-soon-as-possible or (ii) results are likely to exceed the repeat threshold (Table 9), it is acceptable to prepare specimens in duplicate and perform two consecutive runs on separate calibration curves.

At least 2 levels of urine QC materials must be analyzed with specimens, one with typical element concentrations and one with elevated concentrations. QC materials must be present at the beginning and end of each analytical run. QC materials should also be analyzed between approximately every 10–20 specimens. Additional QCs can be prepared if thought necessary.

Cap and mix well all diluted urine materials.

3.5 ICP-MS readiness performed

Total urine Hg (*m/z*=202) is determined by this method using a Perkin Elmer Sciex ELAN DRC II inductively coupled plasma-mass spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, CT) or similar, with analyses performed in standard mode as previously described (5). The instrument should be equipped with an automated sample introduction system, such as the CETAC ASX500 autosampler,

a Meinhard® concentric quartz nebulizer (Meinhard Glass Products, Golden, CO) and a baffled quartz cyclonic spray chamber (Glass Expansion, Pocasset, MA). Comparable hardware may be substituted.

Start the Elan software and ensure that the instrument is in ready mode. Check argon supply, that water circulator is on and that vacuum pressure is within limits. Inspect sample introduction tubing, nebulizer, spray chamber and injector. Clean or replace any sample introduction parts if necessary. Inspect sampling and skimmer cones for accumulation and clean or replace as needed.

In the case of serious problems, contact a supervisor. Telephone Perkin-Elmer Technical support: 1-800-762-4000) if necessary.

Turn on plasma and allow at least 30 minutes stabilization prior to optimization.

Follow the optimization steps below using the Tuning Solution as described in the ELAN Software Guide.

1. Optimization of nebulizer gas flow rate for 3% oxides (Section 4-72)
2. Optimization of lens voltage and autolens (Sections 4-60 and 4-61)
3. Daily Performance (Section 4-39), see Table 6 below for typical acceptable criteria.

3.6 ICP-MS in control?

Generate “Daily performance report”.
Inspect report to see if it meets the requirements given in Table 6.
If performance is acceptable, then proceed. File the printed “Daily performance report” in the appropriate folder and fill in “Daily performance summary” document.

If performance is unacceptable, take the recommended corrective actions.

Table 6. Assessment of daily performance results.

Parameter	Acceptable level	Possible corrective actions
Sensitivity of Mg	>1,000 cps	Make new Daily performance solution
		Perform x-y adjustment
Sensitivity of In, U	>10,000 cps	Adjust nebulizer gas flow
		Clean lens
		Optimize detector voltage
Oxide CeO/Ce	≤0.03	Adjust nebulizer gas flow
		Etch spray chamber
Lens voltage (In)	<10	Clean lens

If problems persist contact a supervisor. Telephone Perkin-Elmer Technical support: 1-800-762-4000 if necessary.

3.7 Perform analysis

Refer to the manufacturer handbook and for further guidelines on ICP-MS operation.

Open the workspace “analysis.wrk”.
Open method template“xxxxxx NYS Urine Hg.mth”.
Save method as “mmddyy NYS Urine Hg.mth”, where mmddyy represents the date of the analysis.
In the method-report window, define report filename as mmddyy NYS Urine Hg.
Create new sample file and save as “mmddyy NYS Urine Hg.sam”.
Create new dataset folder “mmddyy NYS Urine Hg” and load.
Open Report Template file: “NYS Report.rop.”
Load Tuning file: “default.tun” (Note: The setting in the method file used during

analysis will supersede your selection here).
Load Optimization file “default.dac” (Note: The setting in the method file used during analysis will supersede your selection here).
No Calibration is file needed.
Load Polyatomic file: elan.ply.
Review and save all files.

Typical ICP-MS parameters are listed in Table 7.

Table 7. ICP-MS settings.

Parameter	Setting
RF power	1100-1400 W
Nebulizer gas flow rate	0.67 – 0.99 L/min
Sweeps/reading	30
Readings/replicate	1
Replicates	3
Autolens	On
Detector mode	Dual
Measurement units	Counts per second (cps)
Blank subtraction	After internal standard
Curve type	Simple linear
Dwell time	50 ms
Sample units	µg/L

Load prepared standards, specimens, samples and QC materials into the Autosampler. Autosampler position numbers are dependent on the type of autosampler being used.

For a CETAC ASX 500 series autosampler (tray B), calibration solutions 1–6 will be in positions 11–16, the urine blank will be in position 17 and the reagent blanks will be placed in positions 18-20.

Place start QCs and urine specimens in order from position 21. The run will end with the end normal and elevated QC materials.

In the sample window, enter the Autosampler locations in ascending order and input sample name, analysis method file, and peristaltic pump speeds (Table 8).

For the first sample (i.e., position 17 Urine Blank), select “Run Standards and Sample” in the measurement action field. For all further samples select “Run Sample”.

Table 8. Pump speed and duration for sample analysis and sample rinse-out.

Action	Pump Speed (rpm)	Duration (seconds)
Sample Flush	-18	90
Read Delay and Analysis	-18	30
Wash	-24	0-240

In the method window, check that the information entered for calibration standards (i.e., concentration level, Autosampler position, pump speeds) are correct.

In the sample window, select the sample rows to be analyzed.

Select the “Analyze Batch” operation to start measuring the calibration standards and samples.

Monitor the calibration curves to check linearity. If 2 or more calibration points are not co-linear, the run can be stopped at this point for remedial action (i.e., remake standards and re-start run).

If desired select "auto off" option to shut down plasma after completion of the samples.

Allow analytical run to complete unless serious problems occur.

3.8 Process results

Examine the calibration curve. If a standard gives anomalous results (i.e., not co-linear with the other standards), then that result may be rejected. Select and reject the point in the calibration window of the Elan software. Reprocess the data to produce a new results file. No more than 1 calibration point can be rejected.

Refer to the manufacturer's guidelines for further instructions on Microsoft Excel™ (or similar) operations to process results.

Open the "Report Output" folder and locate file "mmddyy NYS Urine Hg.csv". Copy columns A&B from "mmddyy NYS Urine Hg.csv" to columns A&B in the "Raw Data" worksheet in the "xxxxxx NYS Urine Hg.xls" results template. Save as the appropriate "mmddyy NYS Urine Hg.xls" file.

The worksheets "Organized data", "Blank Corrected data", and "Data corrected for dilution" will update automatically.

Select and copy the data in "Data corrected for dilution" worksheet.

In the "Paste Special_values, transpose" worksheet, select cell A2, select "paste special" from edit menu, select "values" and "transpose" and click "OK".

Select, copy, and paste QC results to QC table starting in column F.

3.9 QC within limits?

The analyst and supervisor are responsible for review of QC results. For each QC pool, the acceptable range is the mean ± 2 standard deviations of the results determined in at least 20 characterization runs ($\pm 2S_m$).

The following modified Westgard QC rules are applied to the data to establish acceptance/rejection:

(a) If the QC means are within $2S_m$ limits, and individual results are within $2S_i$ limits, then accept the run (S_w = Within-run standard deviation).

(b) If 1 of the mean QC is outside the $2S_m$ limit, then reject the run if:
i. Extreme Outlier – Run mean is beyond the characterization mean ± $4S_m$
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

(c) If one of the 4 QC individual results is outside a $2S_i$ limit, then reject the 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.
S_m = Standard deviation of the run means.
S_w = Within-run standard deviation.

If the criteria defined above for evaluating the QC results are not satisfied, then the results for all patient specimens analyzed during that run are invalid for reporting. Repeat the analysis using freshly prepared calibration standards and QCs.

3.10 Analyte above repeat threshold?

Refer to the Third National Report on Human Exposure to Environmental Chemicals for selected percentiles for concentrations of National Health and

Nutrition Examination Survey (NHANES) elements in urine (6). Table 9 includes the 95th percentile concentration for Hg in urine for the U.S. population from the survey years 2001-2002.

If the concentration of Hg is greater than the repeat upper boundary concentration listed in Table 9, perform a confirmatory analysis. An additional QC material with similarly high levels of Hg can be included in the repeat analysis if necessary. The repeat threshold may be changed based on results from research studies to reflect the 99th percentile of the study population or at the discretion of the Laboratory of Inorganic and Nuclear Chemistry Chief.

NOTE: If a specimen or sample is believed to have a Hg concentration above the repeat threshold, then two identical runs may be performed with independent calibration curves.

If the repeats are in agreement with 4 standard deviations of the long-term precision of the method as established from the QC plots, then report the mean value of the repeats (i.e. the repeats lie within 2 standard deviations of the mean). If the repeats do not meet this criteria perform a third analysis. If agreement is achieved between 2 of the three analyses, report that mean. If agreement is not achieved, consult with the Laboratory of Inorganic and Nuclear Chemistry Chief.

Table 9. Analyte reference ranges, and repeat upper boundaries.

Analyte	LOD[a] (µg/L)	Geometric Mean[b] (µg/L)	95th Percentile[b] (µg/L)	Repeat Threshold[c] (µg/L)	Call Value[d] (µg/L)
Hg	0.09	0.606	3.99	10	20

[a] Limit of detection (LOD), calculated according to Clinical Laboratory Standards of Practice (CLEP), Trace Elements Standard 1, TE1 as 3 times the standard deviation of a blank or low concentration sample.
[b] Third National Report on Human Exposure to Environmental Chemicals, Centers for Disease Control and Prevention (July 2005), survey years 2001-2002.
[c] Set at 10µg/L below the required report value to NYS DOH Heavy Metals Registry

for elevated reportable metals in urine.
[d] Reportable Hg concentration to NYS DOH Heavy Metals Registry as provided in 10NYCRR, Sections 22.6 and 22.7.

3.11 Reporting and archiving

This method is currently fully validated for Hg. Hg can be reported for identifiable patient specimens, mandatory PT and EQA.

Urine Hg values are reportable in the range between the method limit of detection (MDL), and the highest calibration standard. If a specimen has a concentration higher than the highest calibration standard, it can be reported if
(1) the value is within the periodically assessed extended calibration range and (2) a internal or external QC material with similar concentration is analyzed and within acceptable limits. Accuracy for results above the highest calibration standard may also be demonstrated by satisfactory performance in PT and EQA samples at concentrations above 40 µg/L, the highest Hg standard.

The LOD is calculated according to Clinical Laboratory Standards of Practice (CLEP), Trace Elements Standard 1, TE1 as 3 times the standard deviation of a blank or low concentration sample for a minimum of 10 independent runs.

The extended calibration range is the maximum linear range of the method established annually by preparing additional calibration solutions with high analyte concentrations.

Documentation of the current LOD and extended calibration range is kept with these procedures.

Results should be reported using the “Internal Results Sheet Urine Hg.xls” template.

Note: This form automatically calculates the combined uncertainty of the method and indicates whether repeats are within acceptable limits.

The uncertainty of the results of sample measurements made by this method

should be calculated in a manner consistent with the ISO *Guidelines for the expression of uncertainty in measurement (GUM)*. These guidelines are based on the principle that uncertainty should be expressed as a standard deviation or expanded uncertainty that provides the best estimate of all possible sources of uncertainty in the result. Potential sources of uncertainty in this method are listed in Table 10. It should be noted that many of these component affect multiple stages in the method.

Table 10. Uncertainty components for method

Component	Description
Calibration stock solutions	Manufacturers stated uncertainty in element concentration
Base urine	Uncertainty in the element concentration
Blanks (reagent and calibration)	Uncertainty in the element concentration
Pipetting	Uncertainty in the volume dispensed by pipettes
Dilution into volumetric flasks	Uncertainty in final volume of intermediate standard solutions
Digiflex dispense/dilute	Uncertainty in the Digiflex procedure, including uncertainty in dispensed volume and in diluted volume
Internal standard	Uncertainty in the amount of internal standard added to samples and solutions
ICP-MS signal for analyte/internal standard	Uncertainty in the recorded ICP-MS signal

Calibration curve	Uncertainty in the linear regression
Method bias	Uncertainty of any bias in the method
Operator skill	Different operators may perform to different standards

A typical approach to evaluating uncertainty is to quantify the uncertainty of each individual component and combine those uncertainties into a 'combined uncertainty' based on the measurement equation. In the case of an externally calibrated method with multiple standards, defining a single measurement equation and combing individual uncertainty components is extremely complex.

The uncertainty for this method is thus calculated from (i) the best estimate of the long-term precision of the method and (ii) the best estimate of method bias.

The best estimate of the long-term precision (u_p) of the method (i) is gained from the long term QC data. It is reasoned that the standard deviation of multiple measurements performed over a long period of time accounts for all possible variation in the individual uncertainty components.

The best estimate of method bias (u_b), is obtained from repeated measurements over time of certified reference materials for trace metals in urine (e.g., NIST SRM 2670a or similar). No systematic bias has been established for this method. The bias uncertainty is thus the uncertainty in the certified values quoted on the certificate. In the absence of certified values for a particular element, the uncertainty in reference values may be used. In the absence of reference values for a particular element, scientific judgment can be used to assign an uncertainty.

The combined uncertainty u_c is thus calculated as:

$$u_c = \sqrt{u_p^2 + u_b^2}$$

END

REFERENCES

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