Laboratory Procedure Manual

Analyte: **Perchlorate, Nitrate and Thiocyanate**

Matrix: **Urine**

Method: **Ion Chromatography with Tandem Mass Spectrometry (IC-MS/MS)**

Method No: **2150.05 (modification)**

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as performed by:

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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 testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
PRNT_K_R	URXUP8	Perchlorate, urine (ng/mL)
	URXNO3	Nitrate, urine (ng/mL)
	URXSCN	Thiocyanate, urine (ng/mL)

1. Clinical Relevance and Summary of Test Principle

a. Clinical Relevance

Perchlorate, nitrate and thiocyanate are polyatomic anions that can disrupt thyroid function by competitively inhibiting iodide uptake at the sodium-iodide symporter (NIS).[1,2] Pharmacological doses of NIS-inhibitors or iodine deficiency can significantly reduce iodide uptake. Sufficient inhibition of iodide uptake can lead to decreased thyroid hormone production, and chronically impaired thyroid function can lead to hypothyroidism [3,4] and impaired neurodevelopment in infants [5]. Linkage between health effects and environmental exposure to NIS inhibitors requires improved exposure assessment. By assessing exposure to these toxicologically related analytes (perchlorate, nitrate, and thiocyanate) in one assay, the relative impact of each chemical on thyroid function can be estimated and thus provide useful information for assessing the potential association between exposure and health effects.

Nitrate is commonly found in physiological fluids resulting from both exogenous and endogenous sources including a variety of foods (green leafy vegetables, milk) and drinking water. Thiocyanate is commonly found in physiological fluids, primarily as a metabolite of cyanide exposure from tobacco smoke or diet [6-8]. Perchlorate exposure is widespread in the U.S. [9]. Perchlorate has been associated with decreased thyroid function in females with urinary iodine < 100µg/L [10], indicating the need to assess exposure to perchlorate, other iodide uptake inhibitors and iodide.

b. Test Principle

This method is a quantitative procedure for the measurement of perchlorate, thiocyanate, and nitrate in human urine using ion chromatography coupled with electrospray tandem mass spectrometry. Chromatographic separation is achieved using an Ion Pac AS16 column with potassium hydroxide as the eluent. The eluent from the column is ionized using an electrospray interface to generate and transmit negative ions into the mass spectrometer. Comparison of relative response factors (ratio of native analyte to stable isotope-labeled internal standard) of unknowns with known standard concentrations yields individual analyte concentrations.

2. Safety Precautions

a. Reagent toxicity or carcinogenicity

Perchlorate and other NIS inhibitors can reversibly inhibit thyroid function at doses of µg per kg body weight per day. Therefore, avoid intake of perchlorate (oral or inhalational). Additionally, some perchlorate salts (e.g., ammonium perchlorate) are strong oxidizers. Take special care to prevent contact of solid ammonium perchlorate salt with combustible or oxidizable material since this constitutes an extreme fire and explosion hazard. However, aqueous solutions of perchlorate do not present a fire or explosion hazard. Perchlorate solutions can irritate skin and mucous membranes, and thus avoid dermal exposure. Observe Universal Precautions (wear gloves, lab coat, and safety glasses) while handling all human urine. Place disposable supplies (pipette tips, autosampler tubes, gloves, etc.) contaminated with urine in a biohazard autoclave bag. Keep autoclave bags in appropriate containers until

sealed and autoclaved. Wipe down all work surfaces with a surface disinfectant/decontaminant when work is finished.

b. Radioactive hazards

None.

c. Microbiological hazards

Follow Universal Precautions. Because of the possibility of exposure to various microbiological hazards, take appropriate measures to avoid any direct contact with the urine specimen. Wear gloves, lab coats and safety glasses while handling all human urine products. A Hepatitis B vaccination series is recommended for health care and laboratory workers who are exposed to human fluids and tissues.

d. Mechanical hazards

There are only minimal mechanical hazards when performing this procedure using standard safety practices. Laboratorians should read and follow the manufacturer's information regarding safe operation of the equipment. Avoid direct contact with the mechanical and electronic components of the mass spectrometer unless all power to the instrument is off. Generally, mechanical, and electronic maintenance and repair should only be performed by qualified technicians. The autosampler and the mass spectrometer contain several areas which are hot enough to cause burns. Take precautions when working in these areas.

e. Protective equipment

Follow standard safety precautions when performing this procedure, including using a lab coat/disposable gown, safety glasses, appropriate gloves, and chemical fume hood. Refer to the laboratory Chemical Hygiene Plan and CDC Division of Laboratory Sciences safety policies and procedures for details related to specific activities, reagents, or agents.

f. Training

Formal training in the use of the ion chromatograph and mass spectrometer is necessary. Users are required to read the operation manuals and should demonstrate safe techniques in performing the method.

g. Personal hygiene

Follow Universal Precautions. Take care when handling chemicals or any biological specimen. Routinely use gloves and wash hands properly. Refer to the laboratory Chemical Hygiene Plan and CDC Division of Laboratory Sciences safety policies and procedures for details related to specific activities, reagents, or agents.

h. Disposal of waste

1) Dispose of waste materials in compliance with laboratory, federal, state, and local regulations.

2) Dispose of solvents and reagents in an appropriate container clearly marked for waste products and temporarily stored in a chemical fume hood.

3) Place all disposable items that come in direct contact with the biological specimens in a biohazard autoclave bag that is kept in an appropriate container until sealed and autoclaved.

4) Immediately place unshielded needles, pipette tips and disposable syringes into a sharps container and autoclave when this container becomes full.

5) Wipe down all surfaces with a surface disinfectant /decontaminant when work is finished.

6) Wash any non-disposable glassware or equipment that comes in contact with biological samples with a non-bleach disinfectant solution before reuse or disposal.

7) Wash, recycle, or dispose of any other non-disposable glassware in an appropriate manner.

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.

3. Computerization; Data-System Management

a. Software and knowledge requirements

This method has been validated using the Dionex IC system controlled by a compatible Chromeleon Software coupled with a Sciex mass spectrometer run with a compatible Analyst software. Results are exported from Analyst software to text files and uploaded into the STARLIMS relational database. Knowledge of and experience with these software packages (or their equivalent) are required to utilize and maintain the data management structure.

b. Sample information

Enter information pertaining to particular specimens into the database either manually or transfer electronically. Transfer the result file electronically into the database. Use no personal identifiers; reference all samples to a blind-coded sample identifier.

c. Data maintenance

Check all samples and analytical data prior to being uploaded into the STARLIMS database for transcription errors and overall validity. Routinely back up the database locally onto a computer hard drive through the standard practices of the NCEH network. Contact the local area network manager for emergency assistance.

d. Information security

Information security is managed at multiple levels. The information management systems that contain the final reportable results are restricted through user ID and password security access. The computers and instrument systems that contain the raw and processed data files require specific knowledge of software manipulation techniques and physical location. Site security is provided at multiple levels through restricted access to the individual laboratories, buildings, and site. Confidentiality of results is protected by referencing results to blind-coded sample IDs (no names or personal identifiers).

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

a. Special instructions

No special instructions such as fasting or special diets are required.

b. Sample collection

1) Collect urine specimens from subjects in polystyrene or polypropylene (PP) collection containers.

2) Lot screen specimen collection containers to ensure the absence of any analyte contamination.

3) Use sterile collectors for specimen acquisition.

c. Sample handling

Specimen handling conditions are outlined in the DLS protocol for urine collection and handling (copies available in the laboratory and specimen handling offices). Collection, transport, and special requirements are discussed in the division protocol.

1) Collaborators send urine specimens overnight in dry ice to CDC.

2) Urine specimens are deep freeze once received by Sample Logistics until time for analysis.

3) Refreeze portions of the sample that remain after analytical aliquots are withdrawn. Samples are not compromised by repeated freeze and thaw cycles. Preliminary experiments indicate that perchlorate is stable in urine samples for > 9 months when stored at or below freezer temperatures.

d. Sample quantity

The optimal amount of specimen required for analysis is 2 mL. If sample quantity is limited for the study, at minimum 0.50 mL is required for analysis.

e. Unacceptable specimens

1) Reject specimens if suspected of 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.

2) Reject samples with visible microbiological growth (e.g., mold, bacteria). In all cases, a second urine specimen should be requested.

3) Record on the sample transfer sheet a description of reasons for each rejected sample such as low sample volume, leaking or damaged container.

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

Not applicable for this procedure.

6. Preparation of Reagents, Calibration Materials, Control Materials, and all Other Materials; Equipment and Instrumentation

a. Reagents and sources

Reagents and sources used during the development, validation, and application of this method are listed in Table 1. All chemicals and solvents are used without further purification. Reagents procured from other sources should meet or exceed the listed requirements.

Table 1. Reagents and Sources.

Reagent	Grade	Source *
Sodium Perchlorate	98%	Sigma Aldrich, St. Louis, MO
Ammonium Perchlorate	99.999%	Sigma Aldrich, St. Louis, MO
Potassium Thiocyanate		Sigma Aldrich, St. Louis, MO
Perchlorate 1,000µg/mL	Certified Solution	AccuStandard, New Haven, CT
Nitrate 1,000µg/mL	Certified Solution	AccuStandard, New Haven, CT
Potassium Thiocyanate 1,000 µg/mL	Certified Solution	Inorganic Ventures, Christiansburg, Virginia
Labeled Sodium Perchlorate ($^{18}O_4$)	98%	Isotec, Miamisburg, OH
Labeled Potassium Nitrate (^{15}N)	99%	Cambridge Isotope Lab, Andover, MA
Labeled Potassium Thiocyanate (^{15}N)	98%	Isotec, Miamisburg, OH
Deionized Water	18 MOhm-cm	Barnstead water purifier

* or equivalent

b. Preparation of Calibration Materials

1) Stock Solutions and dilutions

a) *Stock Solution*

Stock solutions are prepared by dilution of certified solutions (1,000 µg/mL) for each of the analytes into deionized (DI) water to give target concentrations of 100, 10, and 1 µg/L depending on the current needs. For example, prepare these stock solutions in volumetric flasks by diluting 10 mL into 100 mL total volume (100 µg/L), 1 mL into 100 mL total volume (10 µg/L), or 1 mL into 1000 mL total volume (1 µg/L). These stock solutions are used to prepare the working standard solutions as shown in Table 2-5.

b) *Labeled Internal Standard Solution*

1. Labeled Perchlorate

i. Weigh approximately 2.5 mg of ^{18}O-labeled sodium perchlorate, transfer to a 25-mL volumetric flask and take to volume with DI water to produce an approximate 100-ppm concentrated stock solution.

ii. Dilute the initial stock solution approximately 1:20 (1.25 mL of a 100-ppm stock into a 25-mL volumetric flask diluted with DI water) to produce a final concentration of 5 ppm.

2. Labeled Nitrate ($^{15}NO3$)

Weigh approximately 25 mg of $^{15}NO_3$, transfer to a 25-mL volumetric flask and dilute to volume with DI water to produce an approximate 1000 ppm solution.

3. Labeled Thiocyanate ($SC^{15}N$)

Weigh approximately 25 mg of $SC^{15}N$, transfer to a 25-mL volumetric flask and dilute to volume with DI water to produce an approximate 1000 ppm solution.

4. Internal Standard solution Mix

i. Prepare 1000 mL of the working labeled internal standard solution by adding 800 µL of 5 ng/µL labeled perchlorate, 10,000 µL of 1000 ng/µL labeled nitrate, and 1300 µL of 1000 ng/µL labeled thiocyanate into a 1000-mL volumetric flask.

ii. Dilute this solution to 1000 mL with DI water and transfer to a 1-L glass bottle.

iii. The concentration of the working solution for $Cl^{18}O_4$, $SC^{15}N$, and $^{15}NO_3$ is approximately 0.004, 10, and 1.3 ng/µL, respectively, from which 500 µL is added to the sample.

2) Working Standard Solutions

Prepare working standard solutions by aliquoting known amounts of each analyte from previously prepared stock solutions (6.b.1) and diluting to final volume with DI water in a volumetric flask. Standard solutions (1-9) are prepared as presented in Tables 2-5, which specifies stock solution to use, volume to aliquot and final volume for each standard.

Table 2. Perchlorate Calibration Standards

Standard ID*	Stock Solution		Final Solution	
	Concentration ng/µL (ppm)	Volume (µL)	Concentration in urine, ng/mL (ppb)	Total Volume (mL)
SS*mmyy*01	1.0	12.5	0.05	25
SS*mmyy*02	1.0	10	0.10	10
SS*mmyy*03	1.0	33	0.33	10
SS*mmyy*04	1.0	100	1.0	10
SS*mmyy*05	10	33	3.3	10
SS*mmyy*06	10	100	10	10
SS*mmyy*07	100	33	33	10
SS*mmyy*08	100	75	75	10
SS*mmyy*09	100	100	100	10

Table 3. Nitrate Calibration Standards

Standard ID*	Stock Solution		Final Solution	
	Concentration ng/µL (ppm)	Volume (µL)	Final Concentration in urine, ng/mL (ppb)	Total Volume (mL)
SS*mmyy*01	1000	125	500	25
SS*mmyy*02	1000	100	1,000	10
SS*mmyy*03	1000	250	2,500	10
SS*mmyy*04	1000	500	5,000	10
SS*mmyy*05	1000	1000	10,000	10
SS*mmyy*06	1000	2500	25,000	10
SS*mmyy*07	1000	5000	50,000	10
SS*mmyy*08	1000	7500	75,000	10
SS*mmyy*09	1000	X[a]	100,000	X[a]

[a]Nitrate in standard mix solution 9 is added separately when preparing calibration curve. See procedure below in section 8.a.3)

Table 4. Thiocyanate Calibration Standards

Standard ID*	Stock Solution		Final Solution	
	Concentration ng/µL (ppm)	Volume (µL)	Final Concentration in urine, ng/mL (ppb)	Total Volume (mL)
SS*mmyy*01	100	25	10	25
SS*mmyy*02	100	25	25	10
SS*mmyy*03	100	50	50	10
SS*mmyy*04	100	100	100	10
SS*mmyy*05	1000	25	250	10
SS*mmyy*06	1000	50	500	10
SS*mmyy*07	1000	100	1,000	10
SS*mmyy*08	1000	250	2,500	10
SS*mmyy*09	1000	500	5,000	10

* *mmyy* represents the month and year of standard preparation.

Aliquots of these solutions are store in 1.5-mL vials in the freezer until use. After the vial is used, store it in the refrigerator.

c. Preparation of Control Materials

1) Quality Control materials
   a) Prepare quality control (QC) materials by collecting human urine.

   b) Analyze urine samples collected and pool together to create two urine pools.

   c) Fortify each urine pool with the different analytes to achieve levels within the linear range of the method: typical target levels for low QC are 3, 2,000, and 45,000 µg/L for perchlorate, thiocyanate, and nitrate, respectively; and for a high QC 15, 4,000, and 75,000 µg/L for perchlorate, thiocyanate, and nitrate, respectively.

   d) After fortifying the urine to reach target concentrations, store the QC solutions overnight in the refrigerator for equilibration.

   e) Analyze the fortified urine pools after overnight equilibration to verify target levels.

   f) After overnight equilibration let QC solutions reach room temperature and aliquot into 2-mL cryovials.

   g) Store the 2-mL cryovials at deep frozen conditions until use.

2) Proficiency Testing materials

a) Prepare proficiency testing (PT) materials from certified 1,000 µg/L reference solutions for each of the analytes (from a second source).

b) Four target concentrations covering the linear range for each analyte are selected.

c) Dilute to final concentration with water in a 25-mL volumetric flask.

d) Blind-code aliquots and store in cryovials under deep frozen conditions until use.

e) Analyze PT samples twice a year as well as following any major maintenance on the instrumentation.

Proficiency testing samples are blind coded for analysis; results are evaluated by the PT coordinator and submit for further approval.

Note: Proficiency Testing materials are prepared by the team lead and blind to the analyst. Consult with the team lead when additional PT materials need to be prepared.

d. Other materials and supplies

Materials / supplies and sources used during the development, validation, and application of this method are listed below. Materials/supplies procured from other sources should meet or exceed these specifications. All materials that have direct contact with sample matrix were lot-screened to verify no perchlorate contamination.

- Nalgene 1.8-mL cryovials (Fisher Scientific, Fairlawn, NJ).
- Eppendorf Repeater Plus Pipette (Brinkmann Instruments Inc., Westbury, NY).
- Rainin Electronic Pipettes (100, 250, and 1000-µL; Rainin, California)
- Pasteur pipettes and bulbs (Kimble Glass, Inc., Vineland, NJ).
- VWR Brand Mini vortexer (The Lab Depot, Alpharetta, GA).
- 1.5-mL Vial Kit with Split Septum (Dionex, Sunnyvale, Ca)
- AERS 500, 2mm Suppressor (Dionex, Sunnyvale, Ca) or equivalent
- Ion Pac ® AS 16 Column (Dionex, Sunnyvale, Ca)
- Nalgene Sterilization filter unit (Fisher Scientific, Fairlawn, NJ)
- Envirocide Surface Disinfectant/ Decontaminant Cleaner

e. Instrumentation

Aliquoting of urine and quality control samples is conducted using a Hamilton Microlab Star workstation (Hamilton Robotics, Inc. Reno, NV) or individual pipettes. Analyses of samples are conducted with a Dionex ion chromatography system equipped with a GP50 gradient pump, AS50 autosampler, AS50 thermal compartment and a 2-mm anion self-regenerating suppressor (ASRS Ultra II) operated in the external water mode (Dionex Corp, Sunnyvale,

CA) or equivalent models. The Chromeleon software is used for system control. The separation is performed using an Ion Pac AS16 column (2 x 250mm, Dionex) with a 25-µL injection loop. A Sciex API4000 triple quadrupole mass spectrometer or equivalent (Foster City, CA) with electrospray interface is used for the detection of perchlorate and other anions.

1) Ion chromatograph configuration

The ion chromatograph configuration is described in Table 6 below. The separation conditions were optimized to obtain resolution between perchlorate and other interferences present in urine (e.g., sulfate).

Table 6. Ion Chromatograph Configuration

Parameter	Setting
Column type	AS16 (2 x 250 mm)
Column temperature	30°C
Eluent	50 mM potassium hydroxide
Flow	0.5 mL/min
Injection Loop Volume	25 µL
Suppressor	AERS 500 or equivalent

2) Mass spectrometer SRM configuration

The following parameters were optimized for the ions of interest. These parameters should be re-optimized when transferring the method to another instrument. The mass spectrometer was operated under Multiple Reaction Monitoring (MRM) mode. The transitions of interest are presented in Table 7 and typical mass spectrometer parameters are presented in Tables 8 and 9.

Table 7. Perchlorate MRM Transitions

Analyte	MRM Transition
Perchlorate ClO_4^-	
Quantification	98.9 / 83.1
Confirmation	100.6 / 85.2
Labeled Perchlorate, $Cl^{18}O_4^-$	106.9 / 88.97
Nitrate, NO_3	
Quantification	62.0 / 45.8
Confirmation	62.0 / 62.0
Labeled Nitrate, $^{15}NO_3$	63.0 / 47.1
Thiocyanate, SCN	
Quantification	58.0 / 58.8
Confirmation	60.0 / 60.0
Labeled Thiocyanate, $SC^{15}N$	59.0 / 59.0

Table 8. Mass Spectrometer Configuration.

Parameter	Setting
Scan type	MRM
Polarity	Negative
Ion Source	Turbo Spray
Temperature	600ºC
IS	-4000 V
CAD	12
CUR	10
GSI	45 psi
GS2	45 psi
Dwell Time	400 ms
Probe Y distance	2.0 mm

Table 9. Mass Spectrometer Parameters Characteristics for each Analyte

Analyte	DP	EP	CE	CXP
Perchlorate				
Quantification	-55	-10	-45	-1
Confirmation	-60	-10	-38	-3
Nitrate				
Quantification	-40	-10	-40	-5
Confirmation	-40	-10	-35	-6
Thiocyanate				
Quantification	-76	-6	-55	-7
Confirmation	-35	-10	-25	-3

7. Calibration and Calibration Verification

a. Creation of curve

1) Calibration Data

   i. Prepare a fresh set of nine calibrators for each set of unknown samples.

   ii. Analyze calibrators along with unknown samples.

   iii. Generate a linear calibration curve with nine standards using the ratio of the peak area of the analyte to the labeled internal standard.

2) Calculation of curve statistics

Determine the slope, y-intercept and R-squared value for the nine-point calibration curve using a 1/x-weighted linear regression in compatible Analyst software.

3) Evaluation of curve statistics

Evaluate the calibration curve statistics to ensure that the R-squared value of the curve is equal to or greater than 0.997, and that the linearity of the standard curve extends over the entire standard range. If the calculated value of one calibrator deviates by greater than 20% from the actual value, then that one calibrator can be excluded. Only two calibrators can be excluded from the standard curve.

4) Calibration verification

Calibration is verified by analyzing a full set of calibrators twice with every run. Each calibration set consists of nine calibrators. Instrument performance is tested by analyzing proficiency testing materials twice a year. After instruments undergo preventive maintenance or any repair, calibrators and quality control materials are tested to verify instrument performance.

b. Use of the calibration curve

The lowest point on the calibration curve is the lowest reportable level and the highest point is above the expected range of results. The remaining points are distributed between these two extremes, with most points in the concentration range where typically unknowns fall.

8. Procedure Operation Instructions; Calculations; Interpretation of Results

An analytical run consists of a blank, 9 calibration standards, 2 low level QCs, 2 high level QCs and up to 75 unknown urine samples.

a. Sample preparation

1) Preliminary sample preparation steps

a) Allow frozen urine specimens, quality control materials, and calibration standards to reach ambient temperature.

b) Mix samples thoroughly by inversion or vortexing.

c) Set up and label a series of 1.5-mL autosampler vials corresponding to the number of blanks, standards, QCs, and samples to be analyzed.

2) Preparation of standards (1-8)

a) Using a 100-µL pipettor transfer 50 µL of the appropriate standard stock solution into the appropriately marked autosampler vial.

b) Using a 1000-µL pipettor add 450 µL of DI water.

c) Using a 1000-µL pipettor add 500 µL of the internal standard solution to make a final volume of 1 mL.

d) Cap the vial and mix for a few seconds using a vortex mixer.

3) Preparation of standard 9

a) Using a 100-µL pipettor transfer 50 µL of standard mix 9 into the appropriately marked autosampler vial.

b) Using a 100-µL pipettor add 50 µL of the 1000 ppm nitrate certified stock solution.

c) Using a 1000-µL pipettor add 400 µL of DI water.

d) Using a 1000-µL pipettor add 500 µL of the internal standard solution to make a final volume of 1 mL.

4) Preparation of the blank

a) Using a 1000-µL pipettor transfer 500 µL of DI Water into the appropriately marked autosampler vial.

b) Using a 1000-µL pipettor add 500 µL of the internal standard solution to make a final volume of 1 mL

c) Cap the vial and mix for a few seconds using a vortex mixer.

5) Preparation of the low Quality Control sample

a) Mix (either by vortexing or repetitive sample inversion) the QC sample.

b) Aliquot 250 µL of QC low stock solution into the autosampler vial using the Hamilton MicroLab Star (Appendix A) or a 300-µL pipettor.

c) Using a 300-µL pipettor add 250 µL of DI water.

d) Using a 1000-µL pipettor add 500 µL of the internal standard solution to make a final volume of 1 mL.

e) Cap the vial and mix for a few seconds using a vortex mixer.

6) Preparation of the high Quality Control sample

a) Mix (either by vortexing or repetitive sample inversion) the QC sample.

b) Aliquot 250 µL of QC high stock solution into the autosampler vial using the Hamilton MicroLab Star (Appendix A) or a 300-µL pipettor.

c) Using a 300-µL pipettor add 250 µL of DI water

d) Using a 1000-µL pipettor add 500 µL of the internal standard solution to make a final volume of 1 mL.

e) Cap the vial and mix for a few seconds using a vortex mixer.

7) Preparation of the unknown specimens

a) Mix (either by vortexing or repetitive sample inversion) the unknown sample.

b) Aliquot 250 µL of unknown into the autosampler vial using the Hamilton MicroLab Star (Appendix A) or a 300-µL pipettor.

c) Using a 300-µL pipettor add 250 µL of DI water.

d) Using a 1000-µL pipettor add 500 µL of the internal standard solution to make a final volume of 1 mL.

e) Cap the vial and mix for a few seconds using a vortex mixer.

Note: For the delivery of internal standard and DI water an Eppendorf Repeater Plus Pipette can be used.

b. Instrument and software setup for the IC-MS/MS

1) Preliminary system setup

a) Tuning and calibration of the mass spectrometer

i. Set the y-distance of the probe to 6 mm and infuse the PPG 3000 solution at a 10 µL/min flow rate.

ii. Using **Manual Tuning,** load the PPG 3000 calibration file. In the tuning window make sure that the mass spectrometer is showing peaks for each ion in the calibration file. This is to make sure that the tuning solution is constantly flowing into the mass spectrometer.

iii. Once checked, perform a **Resolution Optimization** with **Calibration** upon success.

iv. Make sure that the following specified parameters are met. For peak width, the resolution is set to 0.60 ± 0.05 mass units and sensitivity is met using the ion 933 m/z with an intensity of **2.0×10^7 minimum** (combined intensity of 10 scans).

v. Check the tune and mass calibration of the instrument biweekly.

b) IC system setup

i. Fill the mobile phase bottles with filtered and sonicated (5-min) fresh DI water.

ii. Ensure that the water reservoir for the suppressor is full.

iii. Replace the column frit before each run.

iv. Turn on the pump, EG, CR-TC, suppressor, and temperature sensors.

v. Allow the system to equilibrate for at least 30 min prior to starting a run.

vi. Once the total conductivity in the system reaches a value less than 3 µSiemens, the system is ready.

c) Performance evaluation

i. Allow the system to equilibrate with the method to be run (both MS and IC).

ii. To check the performance of the system, inject the lowest standard three times to ensure equilibration of the system.

iii. Examine the peak to ensure an acceptable signal–to-noise ratio (S/N >10 for the lowest standard).

iv. Once these limits are met the system is ready to start a run.

Note: Replacement of consumables may be needed if system set-up fails, or performance deviations were noted on previous runs. See Appendix B.

2) Final setup and operation

a) Create the run sequence

In the Chromeleon software of the IC system, create a sequence for the run using the wizard. Make sure that the appropriate number of samples is loaded, and the appropriate program is selected (*mmddyy* where *mmddyy* is the most recent date that the program was changed and/or saved). The last sample of the sequence should have the appropriate shutdown method. Queue the sequence.

b) Assign the acquisition and quantitation methods

i. Create a sequence in Analyst to include information of the standards, QCs, and unknowns to be analyzed.

ii. Select the acquisition method (*mmddyy*_INIS.dam; where *mmddyy* is the most recent date that the method was changed and / or saved) and the quantitation method (*mmddyy*_INIS.qmf; where *mmddyy* is the most recent date that the method was changed and / or saved).

iii. The letter "I" before the methods name (NIS) correspond to the first letter of the instruments name (J for Joker and M for McDreamy).

iv. Save the sequence using the instrument name and the Julian Calendar date (Iyyddd)

v. Ensure that the icons on the right corner of the window are green indicating that the system has equilibrated and is ready to start.

c) Submit and start batch in Analyst

i. Open and submit the **Equilibration** batch as well as the batch of the unknowns to be analyzed.

ii. Press the "Start Sample" icon on top of the window to start the run.

iii. The instrument waits for a sync signal from the IC to start the acquisition.

d) Start the sequence in IC

i. Click **Queue** in the main menu for the list of pending sequences.

ii. Once the window is open, corroborate the sequence list is starting with the equilibration sequence.

iii. Press **Start,** making sure that the MS is ready to start.

iv. The system will immediately start by turning green on the first sequence to run.

3) <u>System shutdown</u>

After the end of an analytical run the IC system is queue to a shutdown method to turn off all the modules. The MS system goes into standby mode.

c. Processing of data

1) Once the run has finished, note the final pressure as well as conductivity in the instrument maintenance book.

2) Quantify all raw data files using the quantitation capabilities of the Analyst software. The peaks are automatically integrated using the quantitation method created for the analysis.

3) Visually review the integration of each peak and manually correct when needed.

4) Generate a calibration curve from the calibrators; QCs, unknowns and blanks are quantified against the calibration curve.

5) Save the reviewed data files in a report file and export as a text file.

6) Open STARLIMS and run the text file through the macro. Save the macro file in the run batch folder.
7) Follow the data evaluation steps in STARLIMS.

9. Reportable Range of Results

a. Linearity Limits

The reportable range of results for perchlorate using this method is 0.05 to 100 µg/L. The lower reportable limit corresponds to the lowest standard 0.05 µg/L which is greater than the detection limit for the method. The upper reportable limit corresponds to the concentration of the highest standard 100 µg/L. In the case of nitrate and thiocyanate the lowest reportable levels are 500 and 10 µg/L respectively. The upper reportable limits are 100,000 and 5000 µg/L.

Table 10. Method Detection Limits, Lowest Reportable Values and Calibration Ranges

Compound	Linear Range (µg/L)	R^2	Limit of Detection (µg/L)	Lowest Reportable Level (µg/L)
Nitrate	500 – 100,000	0.9930	143	500
Perchlorate	0.05 - 100	0.9998	0.004	0.05
Thiocyanate	10 – 5,000	0.9992	0.681	10

b. Limit of Detection

The limit of detection was determined (using Taylor's method[11]) by calculating the standard deviation at different standard concentrations following repeated measurements of the concentration standards in urine. The absolute values of the standard deviations were then plotted versus concentration. The intercept of the least squares fit of this line is defined as S_0; $3S_0$ equals the limit of detection (LOD). Since the LOD is below the lowest standard, the lowest standard is used as the lowest reportable level.

c. Accuracy

The accuracy of the assay was established by analyzing certified perchlorate and other anions standards blind to the analyst (i.e., Proficiency Testing samples) and matrix spike samples. The accuracy of the method was obtained by comparing the concentration calculated from analyzing the samples to the theoretical concentration. The results of these measurements are given in Table 11.

Table 11. Method Accuracy and Precision

Analyte	Sample	Average %CV [a]	Average Absolute % Diff [b]
Nitrate	Proficiency Test (1,500 – 62,000 µg/L)	1.88	4.54
	Spiked Urine (5,000 – 75,000 µg/L)	3.98	2.39
Perchlorate	Proficiency Test (0.19 - 72.0 µg/L)	4.7	3.78
	Spiked Urine (4.0 - 75 µg/L)	2.14	3.59
Thiocyanate	Proficiency Test (100 – 4,000 µg/L)	0.48	5.08
	Spiked Urine (600 - 4,000 µg/L)	0.82	1.81

[a] Coefficient of Variation
[b] Average absolute value of % difference between theoretical and calculated amount

d. Precision

The precision of the method is reflected in the variance of quality control samples analyzed over time. The coefficient of variation (CV) of the method determined by analyzing 20 QC samples is listed in Table 12 below.

f. Analytical specificity

IC-MS/MS is the most selective analytical method in use for quantifying the target analytes in complex aqueous matrices. Ion chromatography produces reproducible chromatographic resolution of the target analytes, even in the most concentrated urine samples. The analyte peaks elute in well-defined regions of the chromatogram with no visible interferences and very low background. Tandem mass spectrometry provides a further degree of selectivity, by filtering out all ions except a specific transition of parent to daughter ion for each analyte. Additionally, qualifier ratios are determined by comparing the responses of the quantitation ion and the confirmation ion transitions over the standard and QC samples. The average value of this ratio ± 25% is used to confirm the analyte determined in unknown samples that are found at levels above the limit of detection.

g. Ruggedness Testing

Method ruggedness for the assay was tested by varying the following parameters: Internal standard volume, sample volume, sample mixing time, storage time after sample preparation and the position of the quality material in the analytical batch. See Appendix B for ruggedness testing results.

10. Quality Assessment and Proficiency Testing

a. Quality Assessment

Quality assessment procedures follow standard practices[12]. Daily experimental checks are made on the stability of the analytical system. Blanks and standards, as well as QC materials, are added to each day's run sequence. The QC blank is analyzed at the beginning of each run to check the system for possible contamination or in the spiking solutions and/or reagents. Relative retention times are examined for the internal standard to ensure

the choice of the correct chromatographic peak. A calibration curve is developed for the batch using a complete set of calibration standards. The calibration curve must be linear with an R^2 value of at least 0.997. The results from the analysis of a QC materials obtained using this calibration curve are compared with acceptance criteria given below to assure the proper operation of the analysis.

b. Quality Control Procedures

1) Establishing QC limits

Quality control limits are established by characterizing assay precision with 20 distinct analyses of each QC pool. Two different pools of quality control material are used. Different variables are included in the characterization analyses (e.g., different analysts, columns, reagents) to capture realistic assay variation over time. The mean, standard deviation, coefficient of variation, and confidence limits are calculated from this QC characterization data set. Individual quality control charts for the characterization runs are created, examined, and quality control limits are used to verify daily assay precision and accuracy. Typical QC characterization statistics are listed in Table 12. Limits are based on statistical calculation accounting for 2 QCs analyzed in each analytical run.

Table 12. NIS Quality Control Samples QC 0707

Analyte ID	QC ID	Count	Mean	σ	% CV	Mean - 3σ	Mean - 2σ	Mean + 2σ	Mean + 3σ
Nitrate	QH0707	182	95,332	1,452	1.5	90,657	92,225	98,438	100,006
	QL0707	182	33,459	1,052	3.1	30,072	31,208	35,709	36,845
Thiocyanate	QH0707	186	3,532	142.9	4.1	3,072	3,226	3,837	3,992
	QL0707	186	1,927	73.57	3.8	1,690	1,769	2,084	2,163
Perchlorate	QH0707	188	72.54	2.16	3.0	65.58	67.92	77.17	79.50
	QL0707	188	3.2	0.11	3.4	2.85	2.96	3.43	3.55

σ = standard deviation, %CV = % coefficient of variation

2) Quality Control evaluation

After the completion of a run, the calculated results from the analysis of quality control samples are compared to the established quality control limits to determine if the run is "in control". The quality control results are evaluated according to the DLS Policies and Procedures Manual using the DLS SAS QC Program. The average quality control results for each QC pool are evaluated according to Westgard[12] rules:

a) If both the low and the high QC results are within the 2 **α** limits, then accept the run.

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

i. $\mathbf{1_{3\alpha}}$ – Average of both low QC OR average of both high QC is outside of a 3**α** limit.

ii. **$2_{2\alpha}$** – Average of both low QC AND average of both high QC is outside of 2 **α** limit on the same side of the mean.

iii. **$R_{4\alpha}$ sequential** – Average of both low QC AND average of both high QC is outside of 2 **α** limit on opposite sides of the mean.

iv. **10_x sequential** – The previous 9 average QC results (for the previous 9 runs) were on the same side of the mean.

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

c. Proficiency Testing (PT)

1) Scope of PT

Target analytes for this assay are not included in a Centers for Medicare and Medicaid Services (CMS) PT Program. Thus, an in-house PT scheme is administered by a PT coordinator. Certified analyte standards from a second vendor are purchased, diluted and blind-coded by the laboratory team lead. The samples are analyzed, and results evaluated by the PT coordinator for further approval.

2) Frequency of PT

Five samples of unknown PT concentrations are analyzed twice a year using the same method described for unknown samples.

3) Documentation of PT

Analytical PT data is reviewed by the Analyst and submitted to the PT coordinator for testing evaluation. The analysis passes proficiency testing if ≥ 80% of the results for each analyte deviate ≤ 25% from the known value. A summary report of the PT evaluation is maintained in the DLS PT Plans and Records in STARLIMS and in the laboratory quality control manual.

If the assay fails proficiency testing, then the sample preparation and instrumentation are thoroughly examined to identify and correct the source of assay error. Unknown specimens are not analyzed until the method successfully passes proficiency testing.

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

In the instance of calibration curve linearity and/or QC result outside of the method specifications and QC criteria the analytical system conditions are verified. If the source of failure is easily identifiable and corrected, reinjection of the sample batch is acceptable. Otherwise, a new batch of calibrators, QCs and unknowns needs to be prepared.

If these steps do not result in correction of the "out of control" values for QC materials, the supervisor should be consulted for other appropriate corrective actions. Analytical results are not reported for runs that are out of statistical control.

12. Non-conforming events (NCEs), corrective and preventive actions (CAPAs)

a. General

NCEs are defined as events that occur that are outside acceptable performance specifications and/or acceptable laboratory operations. The DLS Policies and Procedures Manual defines the process for the identification of risk and to address potential sources of nonconformities and improvements. Also, the process for identifying, recording, and investigating nonconforming events (NCEs) using root cause analysis and for implementing corrective actions.

An NCE is to be handled commensurate with its risk of influencing reported laboratory results. **Risk is defined as the product of the likelihood of reoccurrence and the amount of impact the NCE would have on reported laboratory results.** NCEs with medium or high likelihood of reoccurrence with medium or high amount of impact must have a corrective action in place within 3 weeks of detection of the NCE. NCEs with low likelihood of reoccurrence with low amount of impact must have corrective action in place within 5 weeks of detection of the NCE.

b. Procedure for responding to NCEs and implementing CAPAs

Response to NCEs and implementing CAPAs is assigned to general supervisors with review by a technical supervisor. NCEs and CAPAs must be documented in STARLIMS which requires approval at the technical supervisor level. The NCE and CAPA procedure is detailed in the Policies and Procedures Manual in STARLIMS.

c. NCEs and corrective actions

Technical supervisors may establish SOPs that address common types of NCEs and corrective actions. NCEs include, but are not limited to the following:

- Analytical methods perform outside criteria specified in DLS QA Program. Quality control specimens that show results outside DLS QC criteria (see sections 8.7 – 8.9 in Policies and Procedures Manual), should be reviewed for likely causes by a general supervisor. DLS criteria have a false positive rate of about 5%. Mild deviations from QC criteria may be addressed by repeating the run and do not need to have a separate NCE and CAPA. If the general supervisor assesses that persistent failures are occurring, then NCE and CAPA is needed.

- Patient test results are outside the reportable range. Dilution of the sample until it is within the reportable range is acceptable unless the method procedure specifies that it is not. The fact that the sample was diluted to run it is to be noted in sample information in STARLIMS.

- The laboratory cannot report patient test results within its established time frame. If a specified time frame for reporting lab results is required by the requestor, it is to be agreed upon in advance of the laboratory analyses. If the reporting of data will exceed the agreed upon time frame, the Laboratory Chief must contact the requestor or assure that the requestor is contacted to inform the requestor of the delay, discuss any appropriate actions which may be needed, and document both the reason for delay and the communication with the requestor. This NCE should be entered into STARLIMS along with the root cause analysis and corrective action and, if needed, preventive actions.

- Errors in the reported patient test results are detected. When errors are detected in the reporting of patient results, the Laboratory Chief is to notify the requestor by phone, followed by the issuance of a corrected report within a time suitable to the requestor, but not to exceed one week later. The corrected report must clearly show in the title that the new results are corrected results. Exact duplicates of the original as well as the corrected report are to be maintained for at least two years. The corrected report is to be approved and signed by the Division Director. This NCE should be entered in STARLIMS along with the root cause analysis and corrective and, if needed, preventive actions.

- Less than 80% satisfactory performance in a proficiency testing challenge

- Unauthorized departures from policies, procedures, or other written requirements

- Use of equipment or instrumentation that is out of calibration/verification/PM

13. Limitations of Method, Interfering Substances and Conditions

The described method is highly selective. Due to excellent chromatographic and mass spectrometric resolution, we have not found any substances that have similar chromatographic and mass spectrometric characteristics. In less than 1% of urine samples the presence of an unknown compound does distort perchlorate chromatography. This problem is resolved by diluting the sample 5-fold and re-analyzing it.

14. Reference Ranges (Normal Values)

Reference ranges for perchlorate, nitrate and thiocyanate are presented in Table 13, as derived from NHANES 2001-2002 data for study participants ages 6+.

Table 13. Geometric mean and selected percentiles of urinary perchlorate, nitrate, and thiocyanate levels.

Analyte	Sample Size	GM	5th Percentile	50th Percentile	95th Percentile
Perchlorate	2820	3.54	0.78	3.6	14
		(3.29-3.81)[a]	(0.68-0.91)	(3.4-3.9)	(11-17)
Nitrate	2815	45857	11000	51000	120000
		(43919-47881)	(9700-11000)	(49000-53000)	(12000-13000)
Thiocyanate	2817	1446	230	1300	9900
		(1366-1530)	(190-270)	(1300-1500)	(8500-11000

[a] 95% Confidence Interval.

15. Critical Call Results ("Panic Values")

The health effects of chronic exposure to trace levels of perchlorate are unclear. Therefore, a definitive panic value has not been established. The National Academy of Sciences has reviewed the toxicological literature for perchlorate and recommended 0.0007 mg/Kg-day as a reference dose. This dose correlates to a urinary perchlorate excretion rate of 35 µg per g creatinine and would be flagged as a "high exposure level". Greer et al reported possible inhibition of thyroid hormones at a dose of 0.5 mg/Kg-day of perchlorate [13]. This dose correlates to a urinary perchlorate excretion rate of 24,000 µg/g creatinine, which would be set as the "Critical Call Value".

16. Specimen Storage and Handling During Testing

Specimens may reach and maintain ambient temperature during analysis. Perchlorate, nitrate, and thiocyanate are stable in urine at room temperature. If the measurement is delayed until the next day, refrigerate the samples.

17. Alternate Methods for Performing Test or Storing Specimens if Test System Fails

Alternate validated methods have not been evaluated for measuring perchlorate in urine. If the analytical system fails, refrigerate the samples overnight until the analytical system is restored to functionality. If long-term interruption is anticipated, store urine specimens at deep frozen conditions.

18. Test Result Reporting System; Protocol for Reporting Critical Calls (if Applicable)

Results are reported to three significant figures based on assay sensitivity calculations. Study subject data is reported in both concentration units (ng/mL) and adjusted based on creatinine excretion (µg/g creatinine).

Once the validity of the data is established by the QC/QA system outlined above, these results are verified by a DLS statistician, and the data reported in an electronic copy. This data, a cover letter, and a table of method specifications and reference range values will be routed through the appropriate channels for approval (i.e., supervisor, branch chief, division director). After approval at the division level, the report will be sent to the contact person who requested the analyses.

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

If greater than 1 mL of sample remains following successful completion of analysis, this material should be returned to deep frozen storage conditions in case reanalysis is required. These samples shall be retained until valid results have been obtained and reported and sufficient time has passed for review of the results.

Standard record keeping (e.g., database, notebooks, and data files) is used to track specimens. Records are maintained for 3 years, including related QA/QC data, and duplicate records will be kept off-site in electronic format. Study subject confidentiality is protected by providing personal identifiers only to the medical officer.

20. Method Performance Documentation

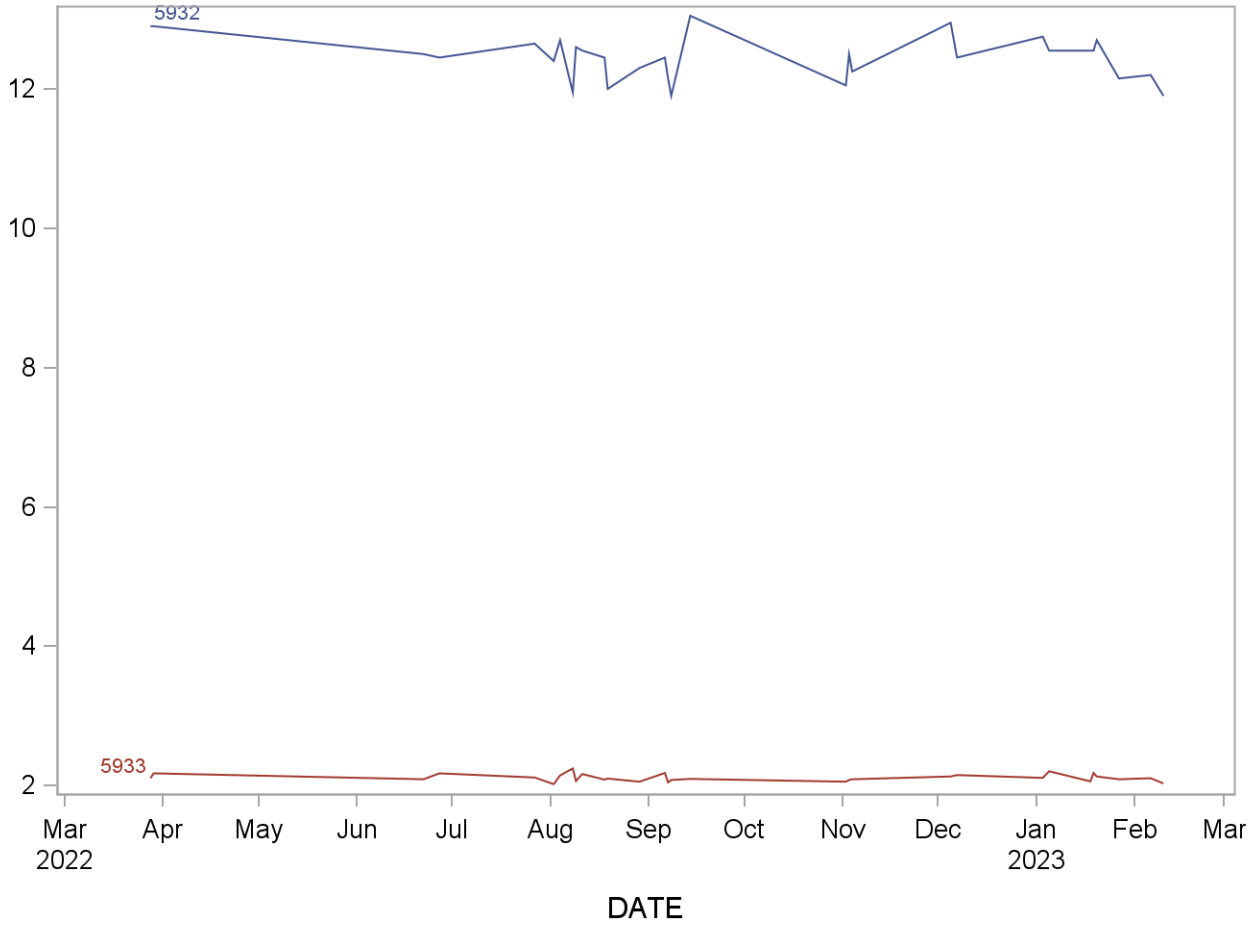
Method performance documentation for this method including accuracy, precision, sensitivity, specificity, and stability is provided in Appendix C of this method documentation. **The signatures of the branch chief and CLIA laboratory director on the first page of this procedure denote that the method performance is fit for the intended use of the method**.

21. Summary Statistics and QC Graphs

Please see following pages

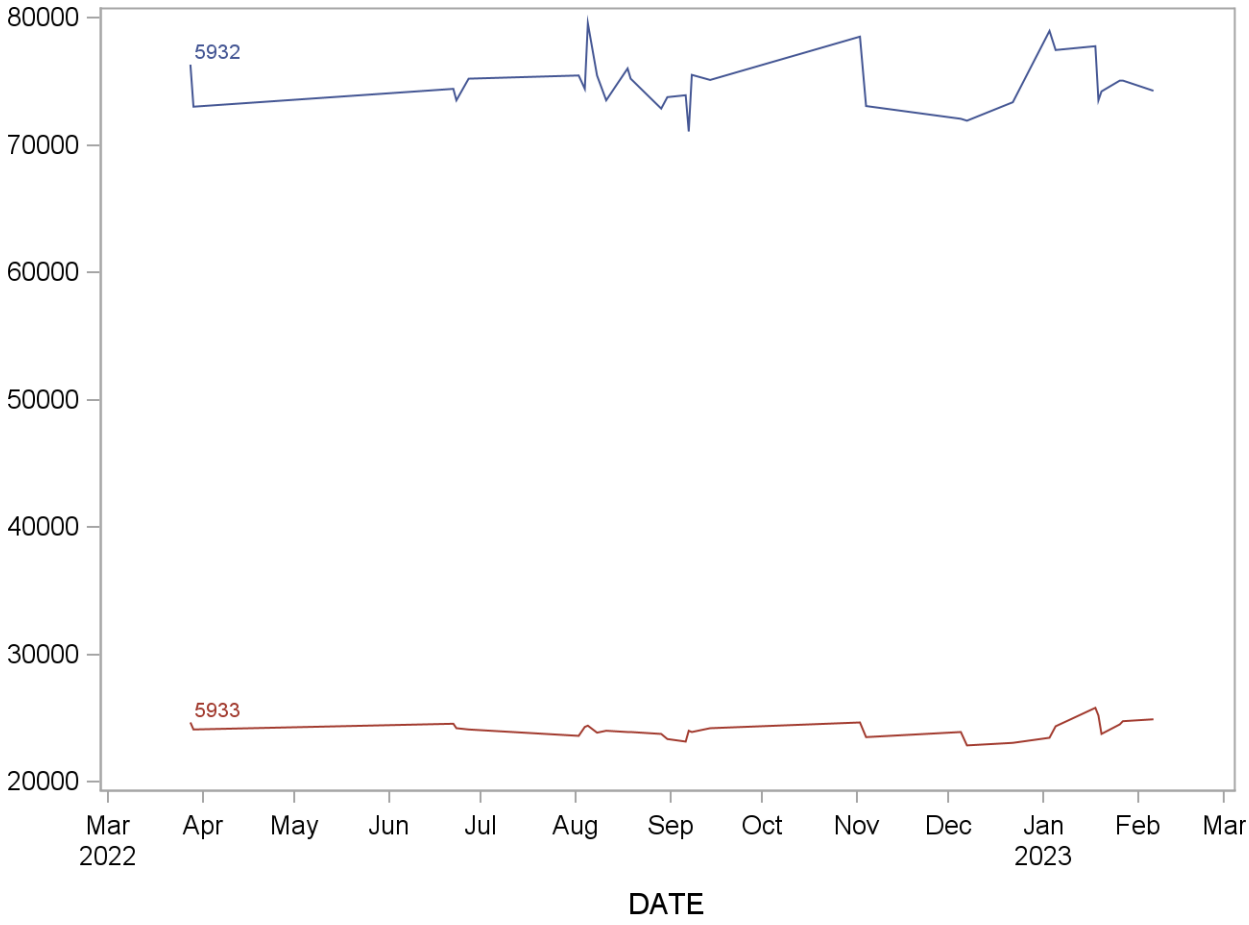
2019-2020 Summary Statistics and QC Chart
URXUP8 (Perchlorate, urine (ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
5932	30	28MAR22	10FEB23	12.44833	0.31417	2.5
5933	30	28MAR22	10FEB23	2.11183	0.05426	2.6



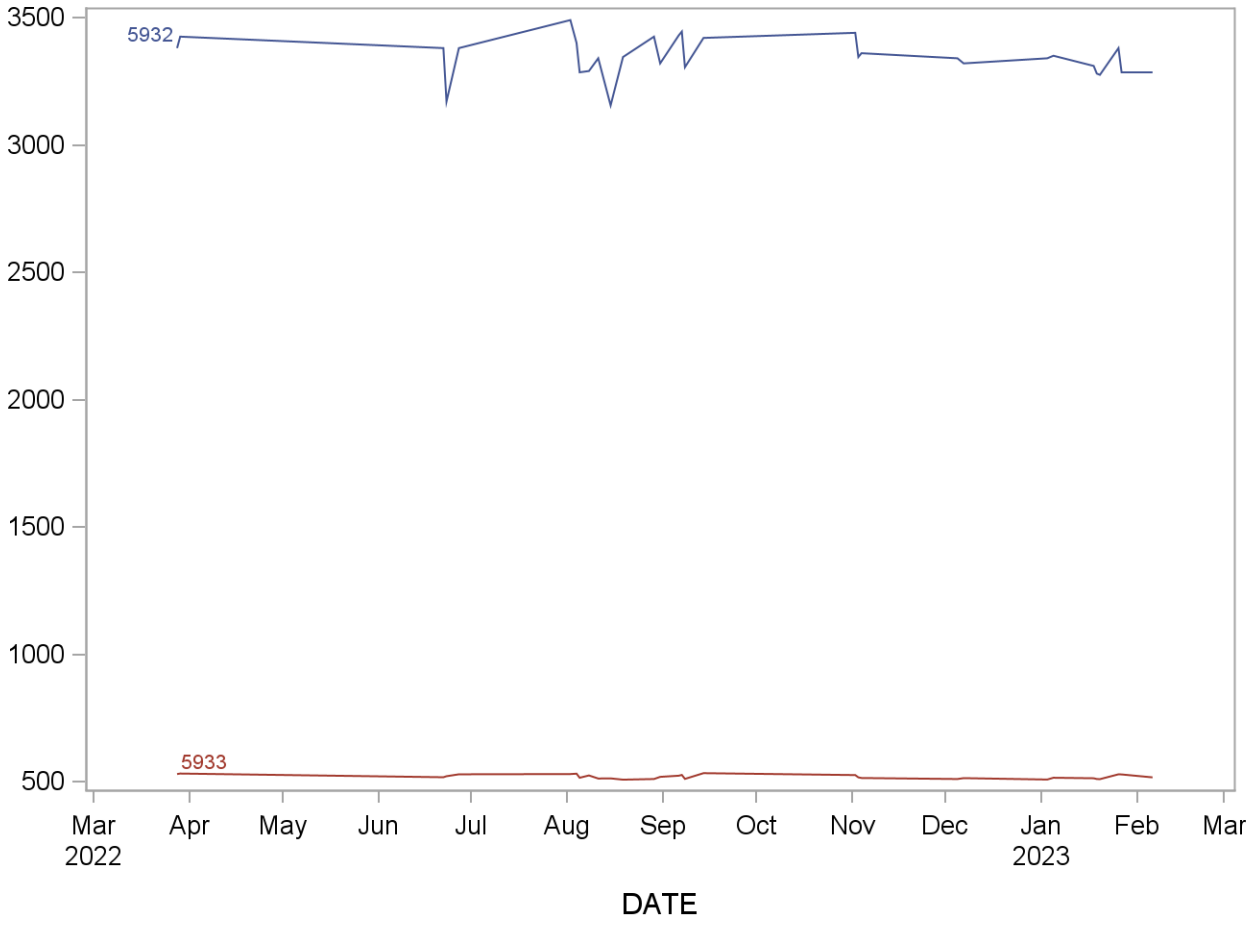
2019-2020 Summary Statistics and QC Chart
URXNO3 (Nitrate, urine (ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
5932	31	28MAR22	06FEB23	74811.3	2040.9	2.7
5933	31	28MAR22	06FEB23	24082.3	632.0	2.6



2019-2020 Summary Statistics and QC Chart
URXSCN (Thiocyanate, urine (ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
5932	31	28MAR22	06FEB23	3345.000	74.76630	2.2
5933	31	28MAR22	06FEB23	519.4677	8.17408	1.6



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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.

1. APPENDIX A: Automated Sample Aliquoting Technique

Instrument used: Hamilton MicroLab Star, (Hamilton Robotics, Inc. Reno, NV)

a) Remove urine samples from the ultralow freezer and place them in the refrigerator to thaw overnight.

b) On the morning of aliquoting, remove samples from the refrigerator and allow them to get to room temperature.

c) Invert each box of samples 7 times and allow samples to stand for 10 minutes.

d) When removing caps, make sure they stay in the proper order.

e) Place cryovial tubes containing unknown urine samples and blank Dionex vials in the Hamilton racks (The urine samples will be aliquoted into the Dionex vials).

f) Please note: There are 32 holders in each Hamilton rack; and the holders are numbered in order from 1-32. Place the tubes in numerical order (left to right).

g) Load tubes and dispensing tips onto the Hamilton instrument.

h) The Hamilton mixes each sample five times (Aspirating and dispensing 1mL of sample each time).

i) When finish mixing, the Hamilton dispenses 250 µL of urine into its corresponding Dionex vial.

j) Cap the Dionex vials and recap the urine samples.

k) Store samples in the ultralow freezer until analysis.

2. Appendix B: Replacement of IC-MS/MS system Consumables

All consumables are replaced as needed. Basic considerations for consumables replacement are described below. Discuss with your team lead before replacing a consumable.

a) **Analytical column** – chromatographic separation issues (e.g., broad peaks, noise), pressure (high/low), analyte retention time shift, number of injections (~1500 injections)

b) **Suppressor** - chromatographic separation issues (e.g., broad peaks, noise), number of injections (~1500 injections)

c) **Eluent generator (EG) cartridge** – chromatographic separation issues (e.g., broad peaks, noise), ion count is ~10%

d) **MS probe electrode** – clog, uneven spray, loss of sensitivity

Manufacturer's instructions for replacement of consumables listed above are in the lab network folder Consumables Replacement Manuals.

3. APPENDIX C: Ruggedness Testing for the detection of perchlorate, nitrate, and thiocyanate in urine

Table 1. Ruggedness Testing Results for Perchlorate

	Method Specification		Parameters Adjustment			
Parameters Tested	*Value*	*Analyte Result*	*Low level*	*Analyte Result*	*Higher Level*	*Analyte Result*
Internal Standard Volume	500 µL	2.88	480 µL	3.07	520 µL	2.83
Sample Volume	250 µL	2.96	240 µL	2.84	260 µL	3.14
Sample Mixing Time	15 seconds	2.88	Invert once	3.15	30 seconds	3.03
Storage Time (4˚C)	run when prepared	2.96	1 day	3.09	4 days	3.05
QC Position	before/after unknowns	3.10	before unknowns	3.09	before/after unknowns	3.11

Table 2. Ruggedness Testing Results for Nitrate

	Method Specification		Parameters Adjustment			
Parameters Tested	*Value*	*Analyte Result*	*Low level*	*Analyte Result*	*Higher Level*	*Analyte Result*
Internal Standard Volume	500 µL	27,833	480 µL	29,800	520 µL	31,067
Sample Volume	250 µL	31,000	240 µL	28,600	260 µL	31,133
Sample Mixing Time	15 seconds	27,833	Invert once	31,250	30 seconds	29,050
Storage Time (4˚C)	run when prepared	31,000	1 day	30,600	4 days	31,200
QC Position	before/after unknowns	30,425	before unknowns	30,600	before/after unknowns	30,250

Table 3. Ruggedness Testing Results for Thiocyanate

	Method Specification		Parameters Adjustment			
Parameters Tested	*Value*	*Analyte Result*	*Low level*	*Analyte Result*	*Higher Level*	*Analyte Result*
Internal Standard Volume	500 µL	1,778	480 µL	1,843	520 µL	1,693
Sample Volume	250 µL	1,803	240 µL	1,717	260 µL	1,803
Sample Mixing Time	15 seconds	1,778	Invert once	1,755	30 seconds	1,730
Storage Time (4˚C)	run when prepared	1,803	1 day	1,940	4 days	1,875
QC Position	before/after unknowns	1,918	before unknowns	1,940	before/after unknowns	1,895

4. APPENDIX D: Method Performance Documentation

Accuracy using Spike Recovery - Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: Perchlorate, Nitrate, and Thiocyanate in Urine
Method #: 2150
Matrix: Urine
Units: µg/L
Analyte: Perchlorate

Replicate		Sample 1					Sample 2					Mean recovery (%)	SD (%)
		Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0.00878	0.0389	0.0128		0	0.319	0.346	0.335		103	7
	2		0.00119	0.0130				0.322	0.346				
	3		0.00710	0.00811				0.317	0.362				
Sample + Spike 1	1	0.3	0.353	0.348	0.355	114	0.3	0.623	0.647	0.635	100		
	2		0.353	0.337				0.613	0.629				
	3		0.391	0.350				0.650	0.647				
Sample + Spike 2	1	3.3	3.49	3.06	3.32	100	3.3	3.86	3.72	3.88	107		
	2		3.36	3.25				3.88	3.81				
	3		3.48	3.28				4.13	3.87				
Sample + Spike 3	1	33	32.7	30.9	31.9	97	33	31.6	31.4	32.4	97		
	2		32.9	30.7				31.7	31.9				
	3		30.8	33.3				35.1	32.5				

Method name: Perchlorate, Nitrate, and Thiocyanate in Urine
Method #: 2150
Matrix: Urine
Units: µg/L
Analyte: Thiocyanate

Replicate		Sample 1					Sample 2				
		Spike concentration	Measured concentration			Recovery (%)	Spike concentration	Measured concentration			Recovery (%)
			Day 1	Day 2	Mean			Day 1	Day 2	Mean	
Sample	1	0	26.7	24.6	25.6		0	2.21	2.50	2.35	
	2		25.1	26.4				2.19	2.17		
	3		25.5	25.0				3.04	1.97		
Sample + Spike 1	1	50	76.9	73.7	76.3	102	50	62.7	57.1	56.4	108
	2		77.3	77.3				54.6	56.3		
	3		74.6	78.1				49.4	58.4		
Sample + Spike 2	1	250	287	295	294	107	250	319	274	285	113
	2		300	281				271	299		
	3		294	304				261	287		
Sample + Spike 3	1	1000	1040	1010	1030	100	1000	1070	1020	1068	107
	2		1060	1060				1250	1060		
	3		998	1010				960	1050		

Mean recovery (%)	SD (%)
106	5

Method name: Perchlorate, Nitrate, and Thiocyanate in Urine
Method #: 2150
Matrix: Urine
Units: µg/L
Analyte: Nitrate

Replicate		Sample 1					Sample 2					Mean recovery (%)	SD (%)
		Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	291	0	147		0	413	0	221		108	4
	2		250	0				481	0				
	3		340	0				430	0				
Sample + Spike 1	1	2500	2940	2510	2810	107	2500	3070	2510	2927	108		
	2		3050	2550				3300	3040				
	3		3250	2560				2840	2800				
Sample + Spike 2	1	10000	11500	11200	11383	112	10000	10900	11800	11283	111		
	2		11400	11600				10400	12100				
	3		11100	11500				11000	11500				
Sample + Spike 3	1	50000	48400	53400	51883	103	50000	49500	53400	52433	104		
	2		51500	54100				50100	54200				
	3		50500	53400				53300	54100				

Precision – Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name: Perchlorate, Nitrate, and Thiocyanate in Urine
Method #: 2150
Matrix: Urine
Units: µg/L
Analyte: Perchlorate

Quality material 1						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	2.08	2.07	2.08	2.50E-05	2.50E-05	8.61
2	2.18	2.09	2.14	0.00203	0.00202	9.12
3	2.03	2.03	2.03	0	0	8.24
4	2.03	2.04	2.04	2.50E-05	2.50E-05	8.28
5	2.05	2.06	2.06	2.50E-05	2.50E-05	8.45
6	2.13	2.05	2.09	0.00160	0.00160	8.74
7	2.13	2.01	2.07	0.00360	0.00360	8.57
8	2.09	1.98	2.04	0.00302	0.00303	8.28
9	1.99	2.04	2.02	0.000625	0.000625	8.12
10	2.04	2.04	2.04	0	0	8.32
Grand sum	41.2	**Grand mean**	2.06			

	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**
Within Run	0.0219	0.00219	0.046797436	2.27
Between Run	0.02282	0.002535556	0.013144496	0.64
Total	0.04472		0.048608413	**2.36**

Quality material 2						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	10.2	10.0	10.1	0.0100	0.0100	204
2	10.1	10.2	10.2	0.00250	0.00250	206
3	9.48	9.43	9.46	0.000625	0.000625	179
4	10.2	9.61	9.91	0.0870	0.08703	196
5	9.84	10.0	9.92	0.00640	0.00640	197
6	9.59	9.54	9.57	0.000625	0.000625	183
7	9.80	9.73	9.77	0.00123	0.00123	191
8	9.47	9.46	9.47	2.50E-05	2.50E-05	179
9	9.50	9.67	9.59	0.00723	0.00722	184
10	10.1	10.0	10.1	0.00250	0.00250	202
Grand sum	196	**Grand mean**	9.80			

	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**
Within Run	0.236	0.0236	0.1537	1.57
Between Run	1.27	0.141	0.242	2.47
Total	1.50		0.287	**2.93**

Method name: Perchlorate, Nitrate, and Thiocyanate in Urine
Method #: 2150
Matrix: Urine
Units: µg/L
Analyte: Thiocyanate

Quality material 1						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	360	348	354	36.0	36.0	250632
2	369	363	366	9.00	9.00	267912
3	375	364	370	30.3	30.3	273061
4	370	373	372	2.25	2.25	276025
5	364	365	365	0.250	0.250	265721
6	346	357	352	30.3	30.3	247105
7	364	348	356	64.0	64.0	253472
8	353	351	352	1.00	1.00	247808
9	361	360	361	0.250	0.250	259921
10	369	353	361	64.0	64.0	260642
Grand sum	7213	**Grand mean**	361			

	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**
Within Run	475	47.5	6.89	1.91
Between Run	928	103	5.28	1.46
Total	1403		8.68	**2.41**

Quality material 2						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	4500	4360	4430	4900	4900	39249800
2	4500	4320	4410	8100	8100	38896200
3	4590	4400	4495	9025	9025	40410050
4	4560	4450	4505	3025	3025	40590050
5	4490	4480	4485	25.0	25.0	40230450
6	4280	4350	4315	1225	1225	37238450
7	4260	4340	4300	1600	1600	36980000
8	4350	4220	4285	4225	4225	36722450
9	4370	4370	4370	0	0	38193800
10	4460	4600	4530	4900	4900	41041800
Grand sum	88250	**Grand mean**	4413			

	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**
Within Run	74050	7405	86.1	1.95
Between Run	149925	16658	68.0	1.54
Total	223975		110	**2.49**

Method name: Perchlorate, Nitrate, and Thiocyanate in Urine
Method #: 2150
Matrix: Urine
Units: µg/L
Analyte: Nitrate

Quality material 1						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	28200	28200	28200	0	0	1590480000
2	28000	29400	28700	490000	490000	1647380000
3	27400	28400	27900	250000	250000	1556820000
4	28200	28800	28500	90000	90000	1624500000
5	27700	27900	27800	10000	10000	1545680000
6	26500	27200	26850	122500	122500	1441845000
7	27000	27100	27050	2500	2500	1463405000
8	27100	28100	27600	250000	250000	1523520000
9	27000	27100	27050	2500	2500	1463405000
10	27600	28100	27850	62500	62500	1551245000
Grand sum	555000	**Grand mean**	27750			

	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**
Within Run	2560000	256000	506	1.82
Between Run	7030000	781111	512	1.85
Total	9590000		720	**2.59**

Quality material 2						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	75300	77200	76250	902500	902500	11628125000
2	78500	82500	80500	4000000	4000000	12960500000
3	74900	75900	75400	250000	250000	11370320000
4	76000	76600	76300	90000	90000	11643380000
5	79200	79100	79150	2500	2500	12529445000
6	72200	71800	72000	40000	40000	10368000000
7	71400	71400	71400	0	0	10195920000
8	74900	75900	75400	250000	250000	11370320000
9	73100	75100	74100	1000000	1000000	10981620000
10	78300	79600	78950	422500	422500	12466205000
Grand sum	1518900	**Grand mean**	75945			

	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**
Within Run	13915000	1391500	1180	1.55
Between Run	160974500	17886056	2872	3.78
Total	174889500		3105	**4.09**

Stability - All stability sample results should be within ±15% of nominal concentration

- Freeze and thaw stability -three times frozen at -80°C and then thawed (3 freeze-thaw cycles)
- Bench-top stability - Assess short-term stability original samples stored at room temperature for 1 day
- Processed sample stability - Assess short-term stability of processed samples, including resident time in autosampler
- Long-term stability - Assess long-term stability samples stored at -80°C for 2 years

Method name: Perchlorate, Nitrate, and Thiocyanate in Urine
Method #: 2150
Matrix: Urine
Units: µg/L
Analyte: Perchlorate

Quality material 1								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	2.13	2.11	2.13	2.17	2.18	2.09	2.13	2.24
Replicate 2	2.13	1.99	2.13	2.07	2.03	2.03	2.13	2.09
Replicate 3	2.13	2.04	2.13	2.13	2.03	2.04	2.13	2.02
Mean	2.13	2.05	2.13	2.1	2.08	2.05	2.13	2.1
% difference from initial measurement	--	**-3.9**		**-0.3**		**-1.3**		**-0.8**

Quality material 2								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	1.08	1.16	9.88	9.72	10.1	10.2	9.88	9.80
Replicate 2	1.08	1.04	9.88	9.57	9.48	9.43	9.88	10.1
Replicate 3	1.08	1.07	9.88	7.37	10.2	9.61	9.88	9.73
Mean	1.08	1.1	9.88	8.89	9.93	9.75	9.88	9.86
% difference from initial measurement	--	**0.9**		**-10.1**		**-1.8**		**-0.2**

Method name: Perchlorate, Nitrate, and Thiocyanate in Urine
Method #: 2150

Matrix: Urine
Units: µg/L
Analyte: Thiocyanate

Quality material 1								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	371	337	371	362	369	363	371	357
Replicate 2	371	356	371	366	375	364	371	368
Replicate 3	371	336	371	327	370	373	371	361
Mean	371	343	371	352	371	367	371	362
% difference from initial measurement	--	**-7.6**		**-5.3**		**-1.3**		**-2.5**

Quality material 2								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	869	831	4473	4190	4500	4320	4473	4420
Replicate 2	869	823	4473	4160	4590	4400	4473	4395
Replicate 3	869	833	4473	3220	4560	4450	4473	4370
Mean	869	829	4473	3857	4550	4390	4473	4395
% difference from initial measurement	--	**-4.6**		**-13.8**		**-3.5**		**-1.7**

Method name: Perchlorate, Nitrate, and Thiocyanate in Urine
Method #: 2150
Matrix: Urine

Units: µg/L
Analyte: Nitrate

Quality material 1								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	29934	28500	29934	30400	28000	29400	29934	30100
Replicate 2	29934	31400	29934	30200	27400	28400	29934	27900
Replicate 3	29934	30700	29934	28600	28200	28800	29934	27050
Mean	29934	30200	29934	29733	27867	28867	29934	28350
% difference from initial measurement	--	**0.9**		**-0.7**		**3.6**		**-5.3**

Quality material 2								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	27929	27800	77157	80000	78500	82500	77157	78600
Replicate 2	27929	27000	77157	70000	74900	75900	77157	75000
Replicate 3	27929	26900	77157	57400	76000	76600	77157	74100
Mean	27929	27233	77157	69133	76467	78333	77157	75900
% difference from initial measurement	--	**-2.5**		**-10.4**		**2.4**		**-1.6**

LOD, Specificity and Fit for Intended Use

Method name: Perchlorate, Nitrate, and Thiocyanate in Urine
Method #: 2150
Matrix: Urine

Units: µg/L

Analytes	Limit of Detection (LOD)	Interferences successfully checked in at least 50 human samples	Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use
Perchlorate	0.004	yes	yes
Thiocyanate	0.681	yes	yes
Nitrate	143	yes	yes