Laboratory Procedure Manual

Analyte: **Selenium**

Matrix: **Serum**

Method: **Inductively Coupled Plasma-Dynamic Reaction Cell-Mass Spectrometry (ICP-DRC-MS)**

Revised: *August 24, 2004*

as performed by: *Trace Elements Laboratory*
Wadsworth Center
New York State Department of Health

contact: *Dr. Patrick J. Parsons*
Phone: 518-474-5475
Fax: 518-473-7586
E-mail: patrick.parsons@wadsworth.org

Important Information for Users

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

Procedure Change Log:

Date	Changes Made	By	Reviewed By	Date Reviewed
6/13/03	Now use 2% v/v ethanol and 1% v/v nitric acid in both the diluent and wash solution. The difference in ethanol concentration between the patient samples (10% v/v ethanol) and wash (2% v/v ethanol) was causing spray chamber equilibrium problems that resulted in sub-optimal peak shapes.	CP	PP	6/17/03
9/04/03	Addition of DRC Mode Optimization Directions	CP	PP	9/04/03
10/17/03	Minor SOPM edits	CP	PP	10/17/03
3/11/04	Gallium concentration in diluent increased from 5 µg/L to 25 µg/L. Some patient samples had high levels of barium in them, Ba++ ion is an interference on gallium, the instrument was over-correcting itself for samples that appeared to give higher Ga internal standard counts. An increase in the Ga concentration minimized the Ba++ interference.	ML	PP	3/11/04
3/11/04	Mass Flow Controller (MFC) B now used for the DRC cell gas. Optimal setting changed from 0.55 mL/min (setting for MFC A) to 0.35 mL/min. It turns out that each MFC is calibrated differently, cell gas flow should be re-optimized each time a different MFC, or instrument is used. “0.55 mL/min” on one instrument may not be the optimal setting for another instrument.	ML	PP	3/11/04
6/14/04	Changed optimal cell gas flow-rate from 0.35 mL/min to 0.2 mL/min. Signal to noise and detection limits are improved.	ML	PP	6/14/04
6/21/04	Changed Read Delay Time to 90 seconds.	ML	PP	6/21/04

Public Release Data Set Information

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

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label (and SI units)
l39_c	LBXSEL	Selenium (µg/dL)
	LBDSELSI	Selenium (µmol/L)

1. Summary of Test Principle and Clinical Relevance

The analytical method for serum selenium employed by the Trace Elements Lab is based on inductively coupled plasma mass spectrometry (ICP-MS) using matrix-matched calibration standards. This method was developed specifically for use with the Perkin-Elmer Sciex ELAN 6100 DRC Plus ICP-MS instrumentation as a method for Selenium in Serum (although a DRC II ICP-MS may also be used).

The method for serum selenium, as practiced in the Trace Elements Laboratory, has been based upon ICP-MS in the standard mode at mass 82, using a mathematical correction equation provided in the Perkin Elmer software to correct for the polyatomic interference from Ar_2H and the isobaric overlap from Kr. We have investigated using the Dynamic Reaction Cell with methane gas to capture selenium at mass 78 and/or mass 80 to meet the requirements of this NHANES project.

The proposed method is taken from the standard procedure used by the CDC's Elemental Analysis Laboratory for NHANES (1). Use of methane as the DRC gas for serum Se has also been proposed by Nixon et al. in a Perkin Elmer Application note (2).

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

B. Test principle
Inductively coupled plasma-mass spectrometry is a multielement analytical technique. Liquid samples are introduced into the ICP through a nebulizer and spray chamber carried by a flowing argon stream. By coupling radio frequency power into flowing argon, a plasma is created in which the predominate species are positive argon ions and electrons. The sample passes through a region of the plasma having a temperature of 6000 - 8000°C. The thermal energy atomizes the sample, then ionizes the atoms. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP, which is operating at atmospheric pressure, from the mass spectrometer, which is operating at a pressure of 10^{-6} torr. The mass spectrometer permits detection of ions at each mass in rapid sequence, allowing individual isotopes of an element to be firm. The dynamic reaction cell (DRC) component of the instrument is pressurized with an appropriate reaction gas and also contains a quadrupole. In the DRC, elimination or reduction of argon- based interferences take place through the interaction of the reaction gas with the interfering polyatomic species in the incoming ion beam. The quadrupole in the DRC allows elimination of unwanted reaction by-products, which would otherwise react to form new interferences. Electrical signals resulting from the detection of the ions are processed into digital information that is used to indicate the intensity of the ions and subsequently the concentration of the element. In this method, Se (isotope mass 78 and/or mass 80), and gallium (mass 69) are measured in serum by inductively coupled plasma dynamic reaction cell spectrometry using methane as reaction gas. Serum samples are diluted 100 µL + 100 µL with 18 M-ohm de-ionized water and + 2200 µL with diluent (1% v/v nitric acid, 0.01% v/v Triton X-100, 2% v/v ethanol containing gallium for internal standardization)

2. Safety Precautions

Use standard precautions when handling any bodily fluid. Wear gloves, a lab coat, and safety glasses. The hepatitis B vaccination series is recommended and is available via NYS Employee Health Services, for all analysts who work with human specimens. Place in a biohazard autoclave bag disposable plastic, glass, and paper (e.g. pipette tips, autosampler cups, gloves, etc.) that are contaminated with serum. Keep these bags in appropriate containers until they can be sealed and autoclaved. Wipe down all work surfaces with a 10% sodium hypochlorite (Bleach) solution when prep work is finished.

All employees must complete the NY State series of safety training seminars prior to performing any work in the Lead Poisoning/Trace Elements Laboratory.

Note: Dispose of all biological samples and diluted specimens in a biohazard autoclave bag at the end of the analysis according to NYS guidelines for disposal of hazardous waste.

Exercise **caution** when handling and dispensing concentrated acids and bases. Always remember to add acid or base to water. Acids and bases are caustic chemicals that are capable of causing severe eye and skin damage. Wear powder-free gloves, a lab coat, and safety glasses. If the acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.

Perkin-Elmer provides safety information that should be read before operating the instrument. This information can be found in the Perkin-Elmer Elan DRC Plus ICP-MS System Safety Manual located in room D-146A. Possible hazards include ultraviolet radiation, high voltages, radio frequency radiation, and high temperatures.

3. Computerization; Data System Management

A. Maintain the integrity of specimen and analytical data generated by this method by proofreading all transcribed data and storing data in several computer systems. Store data files containing the date, analytical run identification (ID), specimen analytical results by specimen ID, and method code on the local hard drive of the ICP-MS PC. When a run is completed, the data file, is copied to a remote network directory.

B. Routine backup procedures on the networked drive include daily backup of data files. Contact either the supervisor or lab director for emergency assistance.

C. Accomplish statistical evaluation and calculation of the run with the calibration curve used by the ELAN 3.0 (Hotfix 1) software. Transfer these data into the NHANES folder located on the LEAD server.

D. New Born Screening support staff make sure that files stored on the network are automatically backed up to tape each night.

E. Both the laboratory research notebook and ELAN record book contain documentation for system maintenance and daily laboratory activities.

4. Serum or Plasma Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection

A. Special instructions prior to specimen collection are element specific (see Appendix C for details). Specimen type -serum, optimal amount of specimen required is 2-3 mL, minimum volume required for analysis is about 0.8 mL.

B. Acceptable containers include pre-screened vacutainers; 7-mL, Royal blue cap (unless Al is to be determined) or plastic 5-mL EDTA vacutainers (unless Zn is to be determined). Since no one specimen container can be used for all trace elements, consult contamination study data (see lab director) for details.

C. The criteria for unacceptable specimens are either a low volume (< 0.8 mL) or suspected contamination due to improper collection procedures or collection devices. In all cases, a second specimen should be requested.

D. Specimen characteristics that may compromise test results are as indicated above including contamination of serum by contact with dust, dirt, etc. from improper handling.

E. Use the Beckman 'GPR' Centrifuge to separate (i) the red blood cells (RBCs) from the plasma, or (ii) the clot from the serum. Load the blood tubes into the appropriate centrifuge adapter/holder and spin down at 1000-1200 g for 10 minutes, i.e. 2200 rpm for the large Beckman centrifuge. Ensure the centrifuge is properly balanced. Don't use the brake to stop the centrifuge but allow the timer to switch off after 10 minutes. Under a Biosafety hood, carefully transfer the serum layer (micropipet) into a pre-screened polypropylene or polyethylene vial.

F. In general, serum specimens should be transported and stored at 4 °C. Once received, they can be frozen at -20 °C or at -70 °C until time for analysis. Portions of the sample that remain after analytical aliquots are withdrawn and should be refrozen at -20 °C. Samples thawed and refrozen several times are not compromised.

5. Procedures for Microscopic Examinations; Criteria Rejection of Inadequately Prepared Slides

Not applicable for this procedure.

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

A. Reagent Preparation

(1) Diluent

The diluent used in this method is an aqueous solution of 25 µg/L Gallium, in 1% (v/v) double distilled nitric acid, 0.005% v/v Triton X-100, and 2% v/v ethanol. This solution will be added in the preparation of all standards and samples during the dilution process just prior to analysis. To prepare, acid rinse a 2 L Teflon PFA container, and partially fill with 18.2 MΩ.cm, ultrapure water. Add 20 mL of GFS double distilled (or similar trace-metal free purity) concentrated nitric acid, and 40 mL ethanol. Spike in 50 µL of 1000 mg/L Ga. Add 1 mL or a previously prepared solution of 10% v/v

SigmaUltra, high-purity Triton X-100. Make up to volume with 18.2 MΩ, ultrapure water. Store at room temperature and prepare as needed.

(2) Base Serum preparation.

The base serum matrix used in this method is a pool of commercial human serum collected from several donors. The base serum should ideally have low concentrations of the elements of interest (i.e. <50 µg/L Al, <100 µg/L Se, <1000 µg/L Cu, and <1000 µg/L Zn). This base serum will be combined with intermediate working standards during the dilution process just prior to analysis. The base serum may be stored at -80 °C, then removed from the freezer and stored at 4 °C whenever a vial of the base serum is needed to prepare standards and/or controls.

(3) ICP-MS Rinse Solution.

The rinse solution used in this method is an aqueous solution of 2% v/v ethanol, 0.005% v/v Triton X-100 and 1% v/v GFS double-distilled (or similar trace metal free level) concentrated nitric acid. This solution will be pumped into the sample introduction system between samples to prevent carry over of the analytes of interest from one sample measurement to the next. To prepare, acid rinse a 2-L polypropylene or Teflon PFA container, and partially fill with 18.2 MΩ, ultrapure water. Add 20 mL of GFS double distilled concentrated nitric acid, 40 mL of ethanol, and 1 mL of the 10% v/v Triton X-100 to 18.2 MΩ ultrapure water. Dilute to 2 L with 18.2 MΩ, ultrapure water. Store at room temperature and prepare as needed.

B. Standards Preparation

(1) Selenium intermediate stock standard

The intermediate stock standard solution used in this method is an aqueous 0.1% v/v nitric acid solution spike with Se from SPEX 1,000 µg/L Se standard solution (or similar commercially available NIST traceable stock standard solution). The intermediate stock standard solution is the first dilution of the primary standard from which all intermediate working standards will be made. To prepare the intermediate stock solution, partially fill an acid-washed 10 mL volumetric flask (polypropylene or poly-methyl pentane flask preferred) with 0.1% nitric acid. For ease of preparation of standards solution first prepare 1 L of 0.1% nitric acid by diluting 1 mL of double distilled concentrated nitric acid with D.I. Water in a 1 L acid-rinsed polyethylene or Teflon bottle. Determine the mass of a 1 mL aliquot of SPEX Se standard addition to the flask. Next, dilute the solution in the flask to approximately 8.5 mL volume using 0.1% nitric acid. Mix the solution thoroughly, and carefully add the remaining little drops of 0.1% nitric acid needed to dilute to exact volume. The concentration (in µg/L of the resulting intermediate stock standard solution can then be calculated using the following formulas:

$$\text{Se Conc. (mg/L)} = \frac{\text{mass standard spike (g)} \times \text{conc. Standard (mg Se/g)}}{0.010\ \text{L}}$$

Se Conc. (µg/L) = Se conc. (mg/L) x 1,000 µg/mg

Store the solution in several smaller portions in acid-washed containers at room temperature, and prepare as needed.

(2) Selenium intermediate working standards.

The intermediate working standard solutions used in this method are a series of 5 aqueous dilutions of the selenium intermediate stock standard solution in 0.1% v/v double distilled nitric acid. These solutions will be used each day of analysis as the final working standards that will be placed in the autosampler of the ICP-DRC-MS. To prepare, acid rinse 5, 100 mL volumetric flasks, and partially fill those with D.I. water followed by 1 mL of the high purity doubly distilled nitric acid. Spike each flask with the appropriate volume of selenium intermediate stock standard solution, as is shown in Table 1.

Table 1. Preparation of Intermediate Working Calibration Standards

Calibration Standard	Spike volume of intermediate stock standard (µL)
0	0
1	20
2	50
3	100
4	200
5	300

Make each solution up to the mark on the volumetric flask with D.I. water. Mix the solution thoroughly, and carefully add the remaining little drops of water needed to dilute to exact volume. Dispense into smaller volume, acid washed tubes (i.e. 15 mL polypropylene Falcon tubes for daily use).

The final concentrations of selenium in each of the intermediate working standards are dependent on the concentration of the intermediate stock standard used. Each standard concentration can be calculated using the formula:

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

When a new set of intermediate calibration standards are prepared, the calibration standard concentrations in the ELAN ICP-DRC-MS software must be updated. The values entered into the software should be the concentration of the intermediate working standards. Store at room temperature and prepare as needed.

(3) Working Calibration Standards

The working calibration standard solutions are dilutions of the 5 intermediate working standards into a serum matrix for the purpose of external calibration of an analytical run. They are made up the day of the preparation and analysis of the patient samples. All calibration standards, and patient samples in the same analytical run must be prepared using the same diluent (see Sect. 6.A.1). To prepare the working calibration standards, transfer 100 µL of the appropriate aqueous intermediate working standard, 100 µL of base serum, and 2200 µL of diluent to a 15 mL polypropylene Falcon centrifuge tube (blue-topped) using the Digiflex autopipettor . Cap the tube and mix well before analysis.

To ensure a complete rinsing of the sample/standard with the diluent solution. A Digiflex method has been adopted where the serum volume is drawn up into the Digiflex's delivery tip, followed by 1100 µL, then aliquoted into a Falcon tube, followed by the acid/standard and another 1100 µL. This approach has been found to reduce memory effects associated with the delivery tip, and also minimizes sample and standard cross-contamination. A more detailed description of the use of the Digiflex, and maintenance information can be found in the "Instrumental Maintenance Records" binder.

(4) Preparation of Quality control materials

NY State trace elements control materials for the 23 elements in blood, urine and serum matrices are currently being characterized. Selenium in serum (caprine serum) has been characterized for 5 NYS control materials NYS SE02-01 - SE02-05, during part of an Interlabatory Study (results reported March 2003). Test materials from the Institute National de Santé Publique du Quebec ICP-MS Intercomparison Program are also routinely used for control purposes. The median and standard deviation values for each analyte concentration in these materials have been established. These materials are stored at – 80 °C until needed. Quality control materials are prepared in the same manner as patient samples (see Section 8. b.)

C. Other Materials

(1) Selenium stock solution, 1000 mg Se/L, Spex Claritas PPT CL2-179SE (or equivalent commercially available NIST traceable stock standard).

(2) Ultrex-II double-distilled Nitric Acid (J.T. Baker Chemical Co., Phillipsburg, NJ).

(3) Ultrapure water, 18.2 MΩ from the Milli-Q water purification system (Millipore Systems Inc., Bedford, MA).

(4) Liquid Argon equipped with approved gas regulator (Matheson Gas Products, Secaucus, NJ).

(5) High-purity methane used as the Dynamic Reaction Cell gas (Matheson Gas Products, Secaucus, NJ).

(6) Base human serum, pooled from healthy human subjects (screened and found to be negative for HIV 1/2 and HIV-1 p24 Ag and non-reactive to HbsAg, HCV 3 and STS), provided by Tennessee Blood Services, Inc., Memphis, TN 38105. Used to matrix match standards, and as a NYS QC Lo.

(7) Base caprine serum, pooled from healthy caprine (goat) subjects, provided by Bioresource Technology, Inc., Fort Lauderdale, FL 33313. Used for NYS QC Med 1, QC Med 2 and QC HI materials.

(8) Gallium: SPEX PLGA2-2Y, 1000 mg/L (SPEX Industries Inc., Edison, NJ) or equivalent. Falcon 15-mL conical tubes (#2097) (Becton-Dickinson Labware, Franklin Lakes, NJ). High Purity Triton-X-100 (t-octylphenoxypolyethoxyethanol) (SigmaUltra purity).

(9) Sigma-Aldrich Co., St. Louis, MI, or any source found to be low in trace metal concentration.

(10) N-DEX 100% Nitrile examination gloves.

(11) Cotton swabs.

(12) Dehydrated alcohol, anhydrous reagent grade, J.T. Baker Chemical Co. or equivalent.

(13) Biohazard autoclave bags.

(14) Bleach (10% sodium hypochlorite solution) – any vendor.

(15) Reagent grade, concentrated nitric acid (J.T. Baker Chemical Co., or any source with comparable reagent) for use with acid washing. **Note:** This grade of acid is NOT to be substituted for the double distilled grade which is used for making standard solutions.

D. Instrumentation

Inductively Coupled Plasma-Mass Spectrometer fitted with a Dynamic Reaction Cell, ELAN DRC Plus (Perkin-Elmer Corp., Shelton, CT). Nebulizer gas flow rate and autolens voltage are optimized daily. Other instrumental parameters are given below.

Table 2. PE-Sciex ELAN DRC PLUS ICP-MS Operating Conditions

Parameter	Setting
RF power	1.3 kW
Ar nebulizer gas flow	Typically 0.8 – 1.0 LPM
Detector mode	Dual
Measurement Units	Cps
Autolens	On
Blank Subtraction	After internal standard
Curve Type	Simple Linear
Sample Units	µg/L
Sweeps/Reading	6
Readings/Replicate	1
Replicates	3

Dwell Time	500 ms
Integration Time	3000 ms
DRC gas (Se)	Methane
Cell gas flow-rate	0.20 mL/min (Dependent on instrument and Mass-Flow controller used for analysis).
Rpq	0.65
Rpa	0.00

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

7. Calibration and Calibration Verification Procedures

A. Calibration

A simple linear calibration curve for selenium is generated using a series of 5 external standards whose concentrations are defined in the calibration page of the quantitative analysis method software. The calibration curve plots the ratio of the observed intensities for selenium and the internal standard versus the concentration of the calibrator. The ratio of the observed intensities for selenium and the internal standard in the patient sample are compared to those obtained from the calibrators to determine the concentration of selenium in the sample.

B. Verification

In order to verify the calibration curve for NHANES serum selenium samples, four levels of control material must be run for every set of samples analyzed. NYS serum pools; NYS Base human serum (QC LO), SE02-03 (QC ME1), SE02-05 (QC ME2) and SE02-02 (QC HI), will be used for daily calibration verification. Calibration verification data is stored along with the NHANES Se cover sheets.

Agreement with certified or accepted values should be within the ± 2 S.D. limits.

8. Procedure Operating Instructions; Calculations; Interpretation of Results

A. Preliminaries

(1) For information regarding the range of linearity and how to handle results outside this range, refer to the Calculations section of this document (Sect. 10 - Remedial Action)

(2) Allow frozen serum/plasma specimens, quality control specimens, and base serum calibration material to reach ambient temperature. Mix each of the samples thoroughly, before taking an aliquot for analysis.

B. Sample Preparation

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

(2) Set up a series of pre-screened 15-mL polypropylene tubes (Falcon or similar) corresponding to the number of blanks, standards, QCs, and patient samples to be analyzed.

(3) Prepare the following solutions into the 15-mL Falcon tubes using the Digiflex.

Table 3. Preparation of samples for analysis (all volumes in µL)

ID	Water	Intermediate working standard	Base Serum	Serum Sample or QC	Diluent
Serum Blank	-	100	100	-	2200
Calib Stds	-	100	100	-	2200
Aqueous Blank	200	-	-	-	2200
Serum Sample or QC	100	-	-	100	2200

(a) Prepare an aqueous blank consisting of 200 µL of D.I. Water and 2200 µL diluent. The aqueous blank will be used as the blank for the quality control pools and patient samples.

(b) Prepare 8 serum blanks consisting of 100 µL of base serum (same material used for preparation of the serum calibration standards), 100 µL of 0.1% v/v nitric acid that was used for the calibration standards preparation (Standard 0), and 2200 µL of diluent. One of these serum blanks will be run as the blank for the calibration standards, and as a blank check after standard 5 and at the end of the run (as Serum blank). The others will be used to 'condition' the sample introduction system (nebulizer, torch and cones) before any analysis begins.

Also, prepare a serum blank that is three times the volume of the other blank Falcon tubes. This will be used during the daily optimization to optimize the reaction cell for an optimal selenium signal.

(c) Prepare the working calibration standards as described in section 6.B.3.

(d) Prepare dilutions of the quality control and patient serum samples consisting of 2200 µL diluent, 100 µL of D.I. Water, and 100 µL of the patient or quality control serum sample.

(e) Cap all of the blanks, standards, and samples and mix them with a vortex mixer for approximately 15 seconds. Uncap them and place them in the autosampler of the ELAN ICP-DRC-MS. If possible, allow 30 minutes for the reaction cell to equilibrate before beginning analysis with the cell gas flowing at 0.20 mL/min (mass-flow controller B, mass flow-controller A may require a different setting, 0.55 mL/.min was used in the past for MFC A). **Note:** the cell gas will automatically turn off after 1 hour if the analysis has not begun. Start the Workspace "Conditioning the Dynamic Reaction Cell," and scan for Se 78, Se 80 and Ga 69, ensure proper settings are in place (Rpq and cell gas). DO NOT save the Workspace. This will keep the cell gas running until the operator stops it.

C. Instrument and Software Set-Up for the ICP-MS

(1) Turn on the computer, log on to the LEAD server. Make sure the printer, peristaltic pump and autosampler are also turned on.

(2) Set up the peristaltic pump tubing on the pump. A daily visual inspection of the pump tubing is necessary to make sure that it is suitable for use and not overly worn. It may not be necessary to change the pump tubing everyday, in fact slightly worn pump tubing provides a smoother, less pulsed flow of sample to the nebulizer. If the pump tubing is changed, make a note in the *Daily Maintenance Log Book.* It is important to get the tension on the autosampler tubing correct, or it will adversely affect the precision of the ICP-DRC-MS measurements. In the ELAN software's METHOD/SAMPLING window, press the "Probe" button, then the "Goto Rinse" button to lower the autosampler probe into the rinse solution. Watch as the solution is taken up through the autosampler probe tubing. When the leading edge is visible, press STOP in the DEVICES window. The leading edge of solution in the autosampler tubing line should be moving. If not, tighten the tension screw for this line on the back of the peristaltic pump. Loosen the peristaltic pump tubing screw for the autosampler tubing until the leading edge of the solution in the autosampler tubing begins to move again, then tighten the screw just enough to make the solution edge stop. Tighten the screw another 1/8 to 1/4 of a turn. Next, start the peristaltic pump by pressing the appropriate arrow in the DEVICES window (make sure that the rotational direction is correct for the way that the tubing is set up in the peristaltic pump).

(3) Perform necessary daily maintenance checks as described in chapter 5 of the ELAN 6100 DRC Hardware Guide (i.e. argon supply, interface components cleanliness and positioning, interface pump oil condition). Record any routine maintenance that is performed in the *Daily Maintenance Log*. Note the base vacuum pressure in the INSTRUMENT window of the software. (Before igniting the plasma, the vacuum is typically 8 x 10^{-7} to 1.8 x 10^{-6} torr.) Record this pressure in the *Daily Maintenance Log Book*.

(4) Ensure that the 2-L rinse solution container is sufficiently full enough with rinse solution, so that there is enough solution to last through the duration of the analysis. Take the autosampler probe and make sure that it is in the ICP-MS rinse solution.

(5) Open up the INSTRUMENT window in the ELAN software. Press the START button to ignite the plasma.

(6) In the DEVICES window, select the AUTOSAMPLER tab to open up the autosampler window. Press the CONNECT button to establish communication between the computer and the autosampler, then start the peristaltic pump by pressing the appropriate arrow (make sure that the rotational direction is correct for the way that the tubing is set up in the peristaltic pump). Type in '-18' into the 'rpm' field of the DEVICES-AUTOSAMPLER window to set the pump head speed at -18 revolutions per minute.

(7) Allow at least 45 minutes warm-up time for the mass spectrometer. Complete daily optimization procedures as required according to the 'Tuning and Optimization' of the ELAN 6100 DRC Inductively Coupled Plasma Mass Spectrometer Software Guide (ELAN Version 3.0 Software Guide, 1006920) chapter. Prepare a 1 µg/L multielement solution for instrument tuning purposes;

an intermediate dilution of CLARITAS PPT 10 mg/L Tuning Solution 1; CL-TUNE1, typically 1 mL into a 100 mL PP volumetric flask with 1% v/v nitric acid - the resulting concentration in the flask will be 100 µg/L.

Further 1 + 99 dilution of this 100 µg/L tuning solution (1 mL into a 100 mL polypropylene volumetric flask, diluted with 1% v/v nitric acid) will result in a 1 µg/L multielement solution.

Record the results for the daily optimization procedures in the *Daily Maintenance Log Book.*

(8) Click on **Open Workspace** from the **File** menu. Select workspace file "NHANESserumSe." Select **Review Files** from the **File** menu. From this window you will be able to set up the correct files and directories for data for your analysis.

Method:	"NHANESserumSe"
Dataset:	If this is the first run of the day, create a new dataset using the date as the name (use the format 031202 for March 12, 2002). If a run has already been performed today, select the dataset for today's date.
Sample:	If an analysis has been performed that is similar to the one you are going to perform, select the sample file corresponding to it. This file will then need to be edited so that it contains sample information for the samples of the present analysis.
Report Template:	Select "NHANESserumSe.rop"
Tuning:	Save the present settings, using today's date as the file name (use the format 031202 for March 12, 2002).
Optimization:	Save the present settings, using today's date as the file name (use the format 031202 for March 12, 2002).
Calibration:	No need to select a file at the start of the analysis. However, one the calibration curves have been generated save them as a calibration file using today's date as the file name (use the format 031202 for March 12, 2002).
Polyatomic:	elan.ply

(9) In the SAMPLES/BATCH window, update the table to reflect the current sample set (autosampler locations, sample i.d., analysis methods). Typically the matrix-matched calibration standards will go in autosampler positions 9-14, the urine blank in autosampler location 15, and the aqueous blank in autosampler location 16.

A typical sample file for this method will look like:

A/S	Sample ID	Measurement Action	Method
146	*Conditioning Blank 1*	*Run Sample*	*NHANESserumSe.mth*
147	*Conditioning Blank 2*	*Run Sample*	*NHANESserumSe.mth*
148	*Conditioning Blank 3*	*Run Sample*	*NHANESserumSe.mth*
149	*Conditioning Blank 4*	*Run Sample*	*NHANESserumSe.mth*
150	*Conditioning Blank 5*	*Run Sample*	*NHANESserumSe.mth*
15	Base Serum Blank	Run Stds and Sample	NHANESserumSe.mth
16	Reagent Blank	Run Sample	NHANESserumSe.mth
17	NYS SE03-01 QC LO	Run Sample	NHANESserumSe.mth
18	NYS SE02-02 QC HI	Run Sample	NHANESserumSe.mth
19	NYS SE02-03 QC ME1	Run Sample	NHANESserumSe.mth
20	NYS SE02-05 QC ME2	Run Sample	NHANESserumSe.mth
21	*Sample 1*	Run Sample	NHANESserumSe.mth
22	*Sample 2*	Run Sample	NHANESserumSe.mth
23	*Sample* 3	Run Sample	NHANESserumSe.mth
	etc…..		

(10) Close out samples with the NYS Low and High Controls at the end of the sample run. QC material should be run repeatedly throughout the run (1 QC control for every ten patient samples).

(11) The following settings should be used for uptake and rinse times for all samples (these values are already stored in the method files for blanks and standards).

	Pump speed (rpm)	Duration (sec)
Sample flush	-24	35
Read delay and analysis	-18	90
Wash	-24	120

Note: negative values for pump speed indicate direction of pump rotation. Make sure that pump tubing is set up appropriately to match the direction of pump rotation.

(12) Highlight the samples that you want to measure; then click on **Analyze Batch.**

(13) Recording of data: Back up data on to the LEAD server; record also onto a CD- CD- Rom using the PD's writable CD drive.

(14) Replacement and periodic maintenance of key components (part nos. given are from Perkin Elmer Atomic Spectroscopy Supplies Catalog).

(a) Nickel Skimmer (# WE02-1137) and sampler cones (part # WE02-1140), or the Spectron equivalent: at least 2 of each on hand.
(b) Skimmer and sampler cone o-rings (# N812-0512 and N812-0511, respectively): at least 5 for each on hand.
(c) Quartz torch: at least two spare torches should be on hand (# N812-2006), or the Spectron equivalent.
(d) RF coil (# WE02-1816): one spare should be on hand.
(e) Injector Support / Torch Base (#N812-0116): one spare should be on hand.
(f) Torch 0-ring Kit (# N812-0100): one spare kit should be on hand.

(g) Ryton Spray Chamber kit (# N812-0124): one spare kit should be on hand. ((#WE01-3060), retaining ring (#WE01-4081), and right angled drain connector (#WE01-3ll9) can be ordered individually. One spare of each should be on hand.)
(h) Crossflow Nebulizer Body (# N058-0613): at least one on hand.
(i) Crossflow Nebulizer tips (# N058-0624): at least 2 spare pairs on hand (1 pair = 1 tip for liquid + 1 tip for gas).
(j) Crossflow Nebulizer 0-ring kit (# N930-0067): at least 2 spare kits on hand.
(k) Peristaltic pump tubing for sample (0.03-in i.d., #0990-8587), and for waste (0.125-in i.d., #N812-2012): Keep at least 2 packages of 12 on hand of the sample tubing, and 1 package of 12 on hand of the waste tubing. Other suppliers may offer the same size/type of peristaltic tubing.
(l) Nebulizer Capillary tubing (used to connect the nebulizer and the peristaltic pump tubing, #0990-8265, or any source of polyethylene tubing, 0.6-mm i.d. x 0.97-mm o.d): one pack (10 ft) on hand.
(m) Autosampler probe (# B300-0161): one spare should be kept on hand.
(n) Leybold pump oil for the roughing pump (# N810-2201): Should keep one gallon bottle on hand. A similar supplier such as Spectron can be used only if the instrument is no longer under warranty. Using oil other than Leybold voids the warranty on the instrument while the instrument is still under warranty.
(o) Neslab chiller coolant (PE Sciex Coolant, #016558A): two 1-L bottles should be on hand.

(15) Calculations

The ELAN has two on-board computers that work with the external system computer. The computers interface with other electronic components within the system to convert the detector signals to digital ion intensity values. As standard solutions are analyzed, the software plots the measured intensity versus the concentration of each element in the standard solution. These individual calibration curves are updated as each subsequent standard is analyzed. Gallium as an internal standard is added to the diluent, the internal standard provides a means to correct for changes in instrument response. The software uses the ratio of analyte and the internal standard intensities to determine the actual intensities for the analyte. Because the responses to instrumental effects for all the elements in a standard group are assumed to be similar to the response for the internal standard, the ratio of each element's intensity to the internal standard's intensity is used for each element.

9. Quality Control (QC) Procedures

The bench quality control pools used in this method comprises four levels of concentration spanning the low to high ranges for selenium. NYS serum pools; NYS Base Human serum (QC LO), SE02-03 (QC ME1), SE02-03 (QC ME2) and SE02-02 (QC HI), will be used for daily calibration verification. Agreement with certified or accepted values should be within the ± 2 S.D. limits. These pools are analyzed after the calibration standards, but before any patient samples are analyzed so that judgments on the analyte calibration curves may be made prior to analysis of patient samples. One of these controls is then analyzed again after approximately each 10 patient samples, and then all QC controls are run at the end of each day's run.

Quality Control Results Evaluation

After the completion of a run, consult the QC limits to determine if the run is in control. The following QC rules apply to the average of the beginning and ending analyses of each of the QC pools:

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

If one of two QC results is outside the 2s limits, then apply the rules below and reject the run if any condition is met.
1_{3S} – Average of both low QC results OR average of both high QC results is outside of a 3s limit.
2_{2S} – Average of both low QC results AND average of both high QC results is outside of 2s limit on the same side of the mean.
R_{4S} sequential – Average of both low QC results AND average of both high QC results is outside of 2s limit on opposite sides of the mean.
10_X sequential – The previous nine average QC results (for the previous nine runs) were on the same side of the mean for either the low OR high QC results.

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

If one or more quality control samples fall outside of 2 standard deviations of the mean analyte value established by the NYS or CTQ proficiency-testing program, then the following steps should be taken:

A. Fresh working serum selenium calibration standards should be prepared (see section 6.B.4), and the entire calibration curve be run using freshly prepared standards;

B. Fresh working serum multielement QC samples should be prepared (see section 6.B.5), and re-analyzed.

If these two 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. No analytical results should be reported for runs not in statistical control.

11. Limitations of the Method; Interfering Substances and Conditions

In the trace element method for serum, the DRC mode of operation is required to monitor Se at mass 78 and mass 80. In the 'DRC mode' of operation (i.e. the cell is pressurized with the DRC gas which for Se is methane, and the bandpass filter applied) determination of $^{78}Se^{+}$ and $^{80}Se^{+}$ are made along with a determination of $^{69}Ga^{+}$. Without the DRC, i.e. if the instrument was running in the Standard Mode, the major interferences on $^{78}Se^{+}$ and $^{80}Se^{+}$ are the Ar-Ar dimer, Kr, and Br-H ($^{80}Se^{+}$ only). It is important to make sure that the correction equations for the Krypton interference on both selenium isotopes are removed from the method prior to analysis. These equations, which automatically appear in the software, were installed by the manufacturer for operation of the instrument in the standard mode. Operation of the instrument with these equations in place will result in a high bias for selenium.

12. Reference Ranges (Normal Values) Adapted from NCCLS 38A(3):

Selenium

Age	µg/L	µmol/L
Preterm	35 - 94	0.44 – 1.19
Term	57 - 96	0.72 – 1.21
1 – 5 years	96 - 144	1.22 – 1.82
6 – 9 years	101 - 162	1.28 – 2.05
10 – 16 years	103 - 186	1.31 – 2.35
Adult	109 - 181	1.38 – 2.29

13. Critical Call Values (“Panic Values”)

When a serum selenium value >300 µg/L or <50 µg/L is found and confirmed upon repeat analysis, the authorized person (i.e., patient’s physician, study PI) who ordered the test should notified by telephone as quickly as possible. Ensure all state confidentiality regulations and privacy regulations under federal HIPAA are followed, (see lab policy on confidentiality of patient data and HIPAA privacy).

14. Specimen Storage and Handling during Testing

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

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

If the analytical system fails, then store all specimens at -70 °C until the analytical system is restored to functionality. If long term interruption (greater than 1 week) is anticipated, then consult with lab director about moving the analysis to the second ELAN DRC II instrumentation.

16. Test-result Reporting System; Protocol for Reporting Results

A. Quality Control Data

The reporting sheet has self-explanatory blanks for the means and ranges of duplicate determinations of QC pools. Put a copy of this form in the study folder(s).

B. Analytical Results

Reformat the data file by using the Transposer Software (coverts the data to an easily tabulated ASCII file), and then download the data file for calculation or reporting. Record the results in µg/L. If a result is below the detection limit of the method, write "ND" (for nondetectable) or "< MDL" in the blank. For problematic samples, a list of NHANES codes (Specimen Comment Codes 5/16/01) is available, and the specific code relating to the problem specimen should be entered in to the NHANES reporting Excel Spreadsheet.

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

The analyst who receives specimen/samples delivered to the Trace Elements Lab sets up a "Specimen Accessioning Sheet." Fill out an accessioning sheet and place it in the folder to be given to the analyst performing the analysis. The accession sheet tracks location, status, and final disposition of the specimens. When sample analysis is completed, place the accession sheet in the ICP-MS Accessioning Sheets Log Book located in the Trace-Elements Lab.

Use standard record keeping means (e.g., electronic – Microsoft Excel) to track specimens. A NY State Accessioning number will be assigned to each specimen vial. All electronic records must be backed up on the LEAD server. Maintain all records indefinitely. Include related Quality Assurance (QA/QC) data. Keep duplicate records (off site, if sensitive or critical) in electronic or hardcopy format. Use only numerical identifiers (e.g., case ID numbers). Any personal identifiers and/or demographics are available only to the medical supervisor and/or project coordinator, lab director, bench supervisor and analyst to safeguard confidentiality.

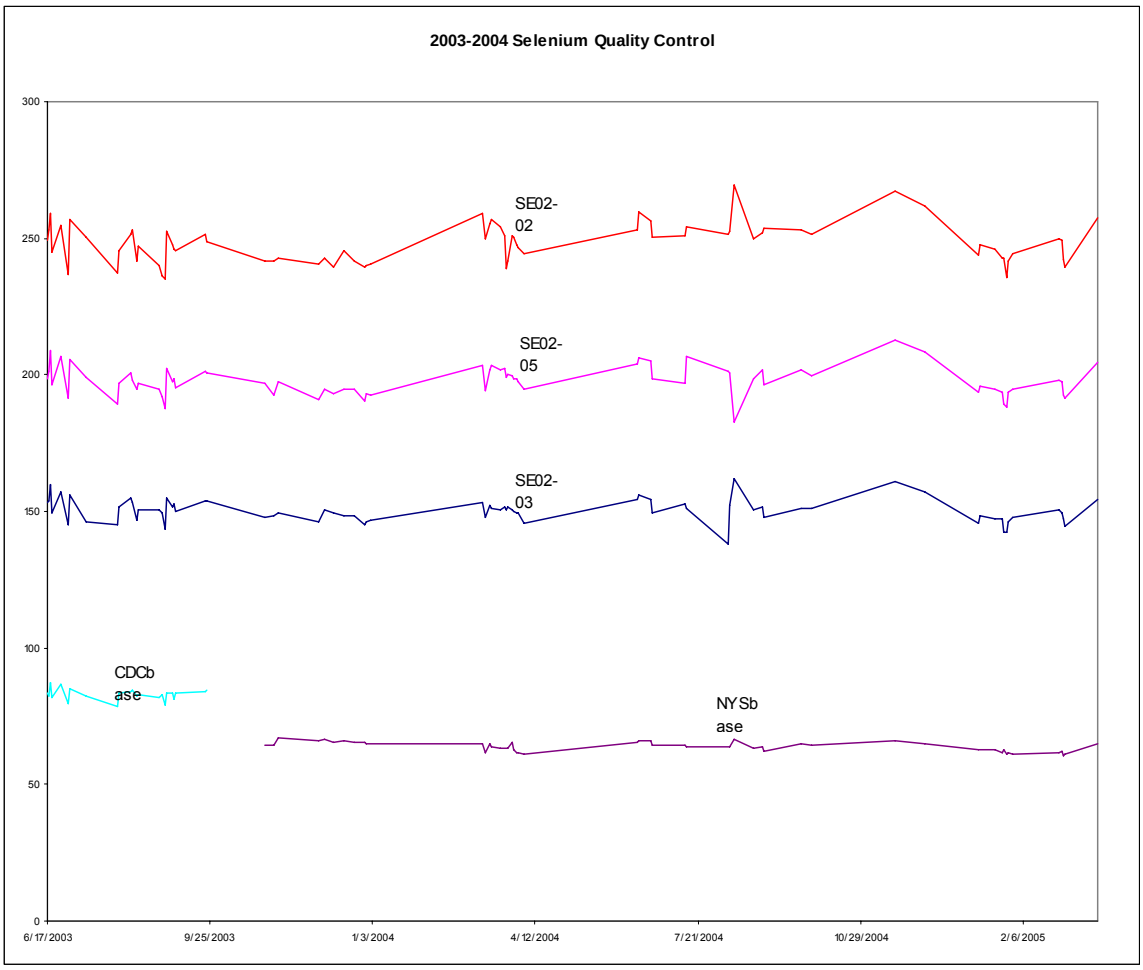
18. Data Processing

Clinical specimens received into the Trace Elements Lab for analysis are pre-accessioned for analysis by assigning each one a unique code number based on the calendar year (four digits) + the Julian Day (three digits) + a four digit number for the day's sample, for a total of 11 digits. Bar-coded specimen labels are generated and affixed to each sample. Results must be reviewed and released by the technician and then reviewed and released by the supervisor before a report can be generated. For NHANES, a mailed hard copy report is unnecessary given the absence of demographics but, in the case of the EP and Se projects, we have found it preferable to transfer analytical data into an Excel spreadsheet from which ASCII files are generated for e-mail transfer to Westat. For the purposes of this proposal, QC data for each batch of results generated for NHANES would be reviewed by the Trace Elements Lab Director before results are released.

19. Summary Statistics and QC Graphs

Summary Statistics for Selenium by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
CDCbase	23	6/17/2003	9/23/2003	83.0	2.1	2.5
SE02-03	76	6/17/2003	3/24/2005	150.3	4.2	2.8
SE02-05	76	6/17/2003	3/24/2005	197.8	5.4	2.7
SE02-02	76	6/17/2003	3/24/2005	248.1	7.2	2.9
NYSbase	53	10/29/2003	3/24/2005	63.8	1.7	2.7



References

1. CDC, DLS, NCEH Laboratory Procedure Manual for Serum Selenium, September 21, 2001.

2. Nixon et al., Determination of selenium in serum and urine using the ELAN DRC ICP-MS, Perkin Elmer Sciex ICP Mass Spectrometry Application Note.

3. Lockitch, G, Fassett JD, Gerson B, Nixon DE, Parsons PJ and Savory J. Control of Pre-Analytical Variation in Trace Element Determinations; Approved Guideline. NCCLS document C38-A, (ISBN 1-56238-332-9), National Committee for Clinical Laboratory Standards Wayne, PA. 1997. 30 pgs. (Subcommittee Member)

4. NYS-DOH, Wadsworth Center, Standard Operating Procedure Manual for trace Elements in Serum, revised December 6th, 2002.