Laboratory Procedure Manual

Analyte: **Selenium**

Matrix: **Serum**

Method: **Atomic Absorption Spectrometry**

Method No.:

Revised:

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

Contact:

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

Public Release Data Set Information

This document details the Lab Protocol for NHANES 1999–2000 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label (and SI units)
lab06	LBXSEL	Selenium (ng/mL)
	LBDSELSI	Selenium (nmol/L)

1.   SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Selenium is measured in serum by atomic absorption spectrometry in a procedure based on the methods described by Lewis et al. (1) and by Paschal and Kimberly (2). Quantification is based on the measurement of light absorbed at 196.0 nm by ground-state atoms of selenium from a selenium electrodeless discharge lamp (EDL) source. Serum samples, human serum quality control pools, and serum calibration standards are diluted with a matrix modifier (Triton X-100, nickel nitrate, and magnesium nitrate). The selenium content is determined by using a Perkin-Elmer model SIMAA 6000 transversely heated graphite furnace atomic absorption spectrophotometer with Zeeman background correction, or a Model 5100 GFAAS. The Zeeman system offers improved background correction over deuterium arc-corrected systems; use of the latter often results in over-correction caused by spectral interference from iron or phosphate in the serum (3,4).

Low selenium levels have been linked to Keshan's disease. This is a cardiomyopathy that has been observed in young children who live in areas where the soil is low in selenium, particularly in some regions of China. Selenium has been shown to affect drug metabolism and toxicity. In addition, selenium may have a role in cancer prevention.

2.   SAFETY PRECAUTIONS

All serum samples received for analysis must be considered potentially positive for infectious agents including HIV and hepatitis B viruses. Observe Universal Precautions. Wear gloves, lab coat, and safety glasses while handling all human blood products. We recommend the hepatitis B vaccine series for all analysts working with intact blood and serum sample materials. Place disposable plastic, glass, and paper (pipette tips, autosampler cups, gloves, etc.) that contact serum in a biohazard autoclave bag. Keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% bleach solution when work is finished.

Dispose of all biological samples and diluted specimens in a biohazard bag at the end of the analysis.

Special care should be taken when handling and dispensing concentrated nitric acid. Always remember to add acid to water. Nitric acid is a caustic chemical capable of causing severe eye and skin damage. Wear gloves, a lab coat, and safety glasses. If the nitric acid comes in contact with any part of the body, quickly wash with copious quantities of water for at least 15 minutes.

Material safety data sheets (MSDSs) for nitric acid, Triton X-100, magnesium nitrate hexahydrate, nickelous nitrate hexahydrate, and argon are available through the NCEH computer network.

3.   COMPUTERIZATION; DATA SYSTEM MANAGEMENT

a.   Statistical evaluation and calculation of the run are accomplished with a SAS program, "SELENIUM," which is run in ROSCOE on the CDC mainframe. (Although the SIMAA can be run in concentration mode, because the assay has to be run with standard addition to a base pool, additional external calculation must be performed to subtract the matrix blank for standard curve calculations.)

b.   Data are uploaded from calculated PC output into a holding file on the NHANES 1999+ Microsoft Access database located on the EHLS Local Area Network (LAN). The data are reviewed and corrected if necessary by the analyst; then the Access database performs an evaluation of the quality control pool data, calculating means and ranges for the pools, and comparing these values to built-in look-up tables for each QC pool. The supervisor receives an electronic notification of the file's readiness, and either approves and releases the data for upload into the final database, or notifies the analyst that re-analysis is required. Data transmission is accomplished several times weekly, and is verified by the supervisor and the computer specialist. Abnormal values are confirmed, and codes for missing data are entered by the analyst and are transmitted as part of the data file to the Westat ISIS

computer, and are eventually forwarded to NCHS.

c.   Files stored on the network or CDC mainframe are automatically backed up nightly to tape by EHLS, LAN support staff and CDC Data Center staff, respectively.

d.   Documentation for data system maintenance is contained in hard copies of data records, as well as in "system log" files on the local hard drives used for archiving data, and Access database transaction records.

4.   SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

a.   For best results, a fasting sample should be obtained. Collection of nonfasting specimens can result in samples that are lipemic and difficult to analyze.

b.   Specimens for selenium analysis should be fresh or frozen serum. Since selenium is not an ubiquitous contaminant, blood may be collected in a red-top Vacutainer brand tube by standard venipuncture procedures.

c.   A 1-mL sample of serum is preferable. The minimum sample volume required for analysis is 250 µL.

d.   The appropriate amount of serum is dispensed into a Nalge cryovial or other plastic screw-capped vial labeled with the participant's ID. The tubes and vials should be screened for the presence of selenium, although contamination from this element is not a common problem.

e.   Specimens collected in the field should be frozen, then shipped on dry ice by overnight mail. Once received, they should be stored at –20°C until analyzed. Portions of the specimen that remain after analytical aliquots are withdrawn should be refrozen at –20°C. Samples thawed and refrozen several times are not compromised.

f.   Specimens should generally arrive frozen. Refrigerated samples may be used provided they are brought promptly from the site where the blood was collected.

g.   Samples collected or processed with equipment contaminated with selenium will not give accurate results.

h.   Specimen handling conditions are outlined in the Division protocol for whole blood collection and handling (copies available in the NHANES laboratory). The protocol discusses collection and transport of specimens and the special equipment required. In general, serum specimens should be transported and stored at no more than 4–8 °C. Samples thawed and refrozen several times are not compromised. If there is more than one analyte of interest in the specimen and it needs to be divided, the appropriate amount of blood should be transferred into a sterile cryovial labeled with the participant's ID.

5.   PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6.   EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

a.   Instrumentation

(1)   Perkin-Elmer (Norwalk, CT) model 5100 graphite furnace atomic absorption spectrophotometer (GFAAS) with Zeeman background correction, including HGA 600 furnace, selenium EDL system 2 source lamp, EDL system 2 power supply, AS-60 autosampler, and Epson EX-800 printer; OR

(2)   Perkin-Elmer (Norwalk, CT) model SIMAA 6000 multi-element atomic absorption spectrometer, including THGA 600/700 furnace, furnace cooling system, selenium source lamp, EDL Power

Supply (if EDL sources are to be used), AS-72 Autosampler, and UV starter source; Hewlett-Packard LaserJet 6 printer, Dell Optiplex computer .

(3) Micromedic Digiflex Automatic pipettors equipped with a 2000-µL dispensing syringe, and 2000-µL or 200-µL sampling syringes (Titertek Instruments Inc., Huntsville, AL).

(4) Mettler PL 200 top-loading balance (Mettler Instrument Corp., Hightstown, NJ).

(5) Milli-Q ultra-pure water purification system (Millipore Corporation, Bedford, MA).

(6) Vortex-Genie vortex mixer (Fisher Scientific, Atlanta, GA).

(7) Eppendorf fixed-volume pipettes, 20-, 100-, 250-, 400-, 500-, 600-, and 1000-µL (Brinkmann Instruments, Inc., Westbury, NY).

(8) Magnetic stirrer (Corning Glass Works, Corning, NY) and stirring bars (Fisher Scientific, Pittsburgh, PA).

b. Materials

(1) Stock solution of selenium: NIST SRM 3149, 10,000 mg/L, (National Institute of Standards and Technology, Gaithersburg, MD).

(2) Redistilled concentrated nitric acid (G. Frederick Smith Chemical Co., Columbus, OH).

(3) Triton X-100 (Fisher Scientific, Fairlawn, NJ).

(4) Nickelous nitrate, hexahydrate (J.T. Baker Chemical Co.).

(5) Magnesium nitrate, hexahydrate (J.T. Baker).

(6) Ultrapure water (from the Milli-Q water purification system).

(7) Argon, 99.996% purity (supplied as a compressed gas by Holox or other contract agency), equipped with approved gas regulator (Matheson Gas Products, Secaucus, NJ).

(8) Serum Standard Reference Material: NIST SRM 909 and 909b (National Institute of Standards and Technology). These materials are to be analyzed periodically as additional checks of accuracy.

(9) Bench and blind human-serum quality-control pools spiked with low and high levels of selenium.

(10) Low-level human serum used as a base for the preparation of standards (NH3 Selenium Base Pool, 9113, prepared by CDC 10/91 is an example).

(11) Pyrolytic graphite tubes, solid pyrolytic graphite L'vov platforms, insertion and alignment tools, and graphite contact rings (Perkin-Elmer).

(12) Small plastic weighing boats (Scientific Products, McGaw Park, IL).

(13) Pipette tips: 1- to 100-µL and 1- to 1000-µL sizes (Bio-Rad Laboratories, Richmond, CA).

(14) Acid-cleaned volumetric flasks, 1000-, 100-, and 10-mL volumes (Corning Glassworks, Corning, NY). The glassware is soaked in a soapy solution (2% solution of Isoclean detergent, Akron, OH) for at least 24 hours, rinsed, soaked in 25% nitric acid for 48 hours, rinsed with ultra-pure water, and dried under clean conditions.

(15) Acid-cleaned volumetric pipettes (1- , 5-, and 10-mL volumes).

(16) Conical-bottom 2-mL polystyrene autosampler cups (Lancer, St. Louis, MO).

(17) Snap-Cap Bio-Vials (Beckman Instruments, Inc., Somerset, NJ).

(18) Floppy disks for storage of analytical software and methods (Maxell Corporation of America, Moonachie, NJ).

(19) Cotton swabs (Hardwood Products Co., Guilford, Maine).

(20) Vinyl examination gloves (Travenol Laboratories, Inc., Deerfield, IL).

(21) Biohazard autoclave bags (Curtin-Matheson Scientific, Inc., Atlanta, GA).

(22) Bleach (10% sodium hypochlorite solution) (any vendor).

(23) Deionized water (Continental Water Systems, Inc., San Antonio, TX).

(24) Kay-Dry paper towels and Kim-Wipe tissues (Kimberly-Clark Corp., Roswell, GA).

(25) 50-mL graduated cylinder (Corning).

(26) Dehydrated alcohol, USP (Midwest Grain Products of Illinois, Pekin, IL).

(27) Epson EX-800 printer ribbons (Perkin-Elmer).

c. Reagent Preparation

- Matrix modifier for Model 5100: (1% (w/v) nickelous nitrate, 2% (w/v) magnesium nitrate, and 0.5% (v/v) Triton X-100) Dissolve 10.0 g nickelous nitrate hexahydrate – $Ni(NO_3)$ $6H_2O$ – and 20.0 g magnesium nitrate

- hexahydrate – $Mg(NO_3)$ $6H_2O$ – in about 500 mL of Ultra-pure water in a 1000-mL volumetric flask and

- mix until the salts are dissolved. Using a volumetric pipette, add 5.0 mL of Triton X-100. Add an acid-rinsed magnetic stirring bar; stir the solution gently on a magnetic stirring plate until the Triton X-100 is completely dissolved. Remove the stirring bar and bring the solution to 1000 mL with Ultra-pure water. Store at room temperature and prepare as needed in a flask dedicated to this solution.

- 0.1% (v/v) Triton X-100 - Using a volumetric pipette, dilute 1 mL of Triton X-100 to 1000 mL with ultra-pure water in a volumetric flask and mix well. Use the magnetic stirrer for adequate mixing. Store at room temperature and prepare as needed.

- Flushing liquid for AS-60 & AS–72 Autosamplers Fill the reservoir with deionized water from the appropriate tap at the laboratory sink. Using an Eppendorf pipette, add 1.0 mL of 0.1% Triton X-100. Cap the reservoir and mix the liquid. Prepare as needed.

- 10% (vol/vol) bleach solution In a 500-mL wash bottle, dilute 50 mL of bleach, added with a graduated cylinder, to 500 mL with deionized water. Store at room temperature and prepare as needed.

ci. Standards Preparations

(1) 1000 mg/L stock selenium standard - Dilute 1 mL NIST SRM 3149 (delivered with either a 1.0-mL Eppendorf pipette or the Micromedic Digiflex) to 10 mL with ultra-pure water in an acid-cleaned volumetric flask. Store at room temperature and prepare every 6 months in a flask dedicated to this solution.

(2) 10 mg/L stock selenium standard - Using either a 1.0 mL Eppendorf pipette or the Micromedic Digiflex, dilute 1.00 mL of the 1000 mg/L stock selenium standard to 100 mL with ultra-pure water in an acid-cleaned volumetric flask. Store at room temperature and prepare monthly in a flask dedicated to this solution.

(3) Working selenium standards - Using the Eppendorf pipette, transfer the volumes of intermediate standard as outlined in Table 3 to 10-mL volumetric flasks and dilute to volume with ultra-pure water. Store at room temperature and prepare daily in flasks dedicated to these solutions. (Note: the 25 ng/mL standard has been added for more accurate measurement of specimens from children 3-11 years old in NHANES 1999+.) This standard is also used for NCI studies such as those from areas of China with endemic Kenshan's disease.

Table 1. Preparation of Working Standards

Intermediate Stock (µL)	Working Standard Concentration (µg/L)	Sample Concentration (ng/mL)
125	125	25
250	250	50
500	500	100
750	750	150
1000	1000	200

(4)   Calibration standards (NOTE: This procedure is used with both instruments.)

(a) In a pre-cleaned (rinsed with ultra-pure water) Bio-Vial, and using the Eppendorf pipette, prepare a fivefold dilution (1+ 4) of the serum calibration material: dilute 600 µL of the serum calibration material with 2.40 mL of matrix modifier. Cap the vial and mix the serum well.

(b) Using an Eppendorf pipette, dispense 20 µL of each of the selenium working standards into separate autosampler cups.

(c) Using an Eppendorf pipette, add 500 µL of the diluted serum and matrix modifier to each of these autosampler cups as well as to an empty cup (to be used as a blank).

(d) Place the blank in position 1 and the standards, in ascending order, in positions 2–5 on the autosampler tray.

e.   Preparation of Quality Control Materials

Commercially collected serum that has been screened for hepatitis and HIV is obtained from the CDC Serum Bank and stored frozen until time for use. The serum is thawed, pooled in acid-cleaned 20-L glass carboys, and mixed well on a magnetic stirrer. The level of selenium in this pool is assessed by using the protocol described herein.

Prepare the materials as follows: Clean-filter the serum through Millipore filters in a sequential manner by using the following filters (each preceded by a pre-filter): 3.00-, 1.20-, 0.80-, 0.65-, 0.45-, 0.30-, and 0.22-µm. Divide the pooled serum into three parts. Retain one part for the normal pool (or "medium" or "base"). To prepare the low pool, dilute the serum by 25-30% with 0.9% NaCl and mix it well. To prepare the high pool, spike the serum with a small volume of 10 mg/L selenium solution and mix it well. Filter each pool through a sterile pre-filter and 0.22-:m filter combination.

Using sterile technique under a laminar-flow hood, dispense the serum in 1-mL aliquots into 2.5- or 5-mL wide-mouth tubing vials by using a Micromedic Digiflex dispenser. Cap the vials with rubber stoppers and seal them with tear-away aluminum crimped caps. Store the vials at –70°C. Select 20 vials of each level randomly for characterization of the quality control limits and for testing of homogeneity.

Two levels of blind quality control pools are prepared and similarly dispensed, except that vials and labels identical to NHANES specimen vials are used. Two levels (low-normal and high-normal selenium concentrations) of blind QC pools may be prepared from pooled, filter-sterilized human serum obtained from fasting donors with elevated or decreased ferritin levels. Through the use of sterile technique under a laminar-flow hood, dispense the serum in 1-mL aliquots with a Micromedic Digiflex dispenser into 2.0 mL Nalge cryovials. Cap and label the vials with NHANES bar-coded labels that have been specially prepared for the QC pools. Store the pools at –70°C at the CDC CASPIR Specimen Repository in Lawrenceville where they will be inserted randomly into the NHANES runs. Select 20 vials of each level at random for characterization of the quality control limits and for testing of homogeneity.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Reference materials (serum products with certified values assigned by independent reference methods) are used periodically as a check of accuracy. NIST SRM 909 or 909b should be analyzed once a week for this purpose (and at least once during specimen analysis for smaller non-NHANES studies) NIST SRMs come in lyophilized form with a vial of ultra-pure water for reconstitution. Once the reference material is reconstituted, it is stable indefinitely as long as it is kept refrigerated. This material should be prepared in the same manner as all other controls and participant samples and inserted in the run following the daily controls. If the stock of this material becomes low, more should be ordered in time for the new pool to be analyzed concurrently with the old one.

This method produces linear results from 0 to 400 ng/mL (2), which is beyond the normal range of values expected to be observed for human serum selenium concentration. The linear regression program ("SELENIUM") in ROSCOE is used to calculate the calibration curve and the specimen concentrations. This program generates slopes, intercepts, correlation coefficients, standardized residuals, and plotted and fitted curves. The correlation coefficient, R^2, for each curve should be 0.99 or higher. For optimum sensitivity on the Model 5100, slopes should be more than 0.00035 and intercepts should be less than 0.003. Process blanks should be less than 0.005 Abs-sec.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries

Allow frozen serum specimens, quality control serum specimens, and serum calibration material to reach ambient temperature.

b. Sample Preparation

(1) Prepare a process blank as follows: using the Micromedic Digiflex, dispense 100 µL of ultra-pure water and 400 µL of the matrix modifier into an autosampler cup.

(2) Dilute the specimens and controls fivefold (1+4) with the matrix modifier solution in the clean autosampler cups as follows: using the Micromedic Digiflex, dispense 100 µL of the serum control material or specimen and 400 µL of the matrix modifier into the autosampler cups. Prepare duplicate samples of the control materials.

(3) Place a process blank in positions 6 and 7 on the autosampler tray. Place the autosampler cups containing the controls and specimens (controls first) in positions 8 and following on the autosampler tray.

c. Instrument Setups

Table 2. Instrument Settings for the Model 5100 GFAAS

Parameter   Setting
Wavelength 196.0 nm EDL   VII-I-6
Power Supply 5 W

EDL Mode	Continuous
Slit	0.7 nm (low)
Signal	Peak Area

Table 3. Furnace Temperature Program for the Model 5100 GFAAS

Step	Temperature, °C Ramp (sec) Hold (sec)	Gas Flow, mL/min
Dry*	110 5 10	300
Dry*	150 5 25	300
Char	1180 5 25	300
Atomize	2400 1 5	0
Burnout	2400 1 3	300
Cool	20 1 5	300
Baseline: –1 sec (BOC set automatically)		
Read: 0 sec		

*Dry temperature and hold time may vary with different graphite tube and platform combinations.

Table 2a. Instrument Settings for the Model SIMAA 6000 GFAAS

Parameter	Setting
Wavelength	196.0 nm
EDL Power Supply	5 W
EDL Mode	Continuous
Slit	0.7 nm (low)
Signal	Peak Area

Table 3a. Furnace Temperature Program for the Model SIMAA 6000 GFAAS

Furnace Temperature Program - Furnace				
Step	Temp °C	Ramp (sec)	Hold (sec)	Gas Flow mL/min
Dry*	110	5	10	300
Dry*	160	5	25	300
Char	1180	1	25	300
Atomize	2400	0	5	0
Burnout	2000	1	3	300
Baseline: -1 sec (BOC set automatically)				
Read: 0 sec				

*Dry temperature and hold time may vary with different graphite tube and platform combinations.

The program for the atomize step includes instructions for the instrument to turn on the Zeeman magnet at the beginning of the step (Read) and to activate the record function (Recorder) during the step.

Operating Procedure Steps for the Model 5100 GFAAS

(1) Turn on the argon gas (The setting may vary from 40 to 50 psi, depending on instrument used. The furnace has a low pressure shutoff below 40 psi).

(2) Turn on the cooling water supply.

(3) Turn on the HGA 600 furnace.

(4) Turn on the Zeeman 5100.

(5) Turn on the spectrophotometer.

(6) Turn on the Epson EX-800 printer.

(7) Turn on the Epson CRT.

(8) Turn on the Epson Equity III and computer. The Desktop page will appear.

(9) Click twice on the file 5100 P.C.EXE. The Benchtop page will appear and the autosampler will flush.

(10) Two boxes will appear at the bottom of the page (AS-60 and HGA). Six boxes will appear at the right of the page. When the two bottom boxes turn blue, click twice on the AS-60 box in the top right corner of the page.

(11) Click once on ELEMENT FILE. The "Item Selector Page" will appear. Click once on SE, then once on OK.

(12) The lamp carousel will rotate to SELENIUM EDL.

(13) Plug in the EDL and turn on the power supply. Turn up the power to three quarters of maximum and wait for the EDL to spontaneously light. (If the lamp does not light, expose it to a portable igniter until it lights.) After the lamp lights, turn it down to the recommended voltage.

(14) Four windows will appear on the screen:
    a. AS-60 CONTROL (green)
    b. DISPLAY CALIBRATION
    c. DISPLAY DATA
    d. DISPLAY PEAKS

    A maximum of five windows can appear at one time.

(15) Click once on the DISPLAY CALIBRATION window. Click once on the bow tie in the left corner of the window to remove the window from the screen.

(16) Click once on the DISPLAY PEAKS window. Click and hold on the top portion of the box and move the entire box up into the right top of screen. Shrink the box to fit the space.

(17) Choose the WINDOWS option at the top of the screen. Click once on HGA CONTROL. Move the HGA CONTROL window (yellow) into the bottom right corner of screen.

(18) Choose the WINDOWS option again. Click on ELEMENT PARAMETER.

(19) When the element parameter page appears, click on the HGA box. The furnace program will appear. Check the parameters and update them if necessary. Click on the bow tie (left corner of window) to remove the page from the screen.

(20) Choose the WINDOWS option. Click on ID/WEIGHT parameter.

(21) Type your initials for analyst, then click on the first box under "Position" to bring the cursor down to this location.

(22) Choose the EDIT option. Click on GLOBAL ENTRY. Type in the starting and ending positions (e.g., starting position 1, ending position 30).

(23) Type "blank" under "Sample ID" for positions 6 and 7. Positions 8 through 13 are controls, and position 14 is another blank. After the above information is entered, click on the bow tie in the left

corner to remove the screen.

(24) Install a new graphite tube and platform after 2 full days of runs (or sooner if deteriorated peak shape or increased matrix blank absorbance indicate the need to do so). Open the furnace by pressing the FURNACE key on the HGA 600. Install a L'vov platform in the furnace using the insertion tool. Make sure that the platform is properly seated in the tube by holding the tube on end and gently tapping it on a hard surface. If it falls out, reinsert it using more pressure. Install the tube in the furnace with the platform at the bottom of the tube. Use the alignment tool inserted in the sample port while pressing the FURNACE key again to close the furnace. After the furnace closes, press the furnace together to ensure that it has properly closed.

(25) Check the quartz windows of the furnace to make sure that they are clean. If there is evidence of sample spattering on the windows, remove the windows and clean them using a cotton swab soaked in dehydrated alcohol. Wipe the windows dry with a soft tissue (Kim-Wipe) and carefully reinstall them.

(26) Click on SAMPLE STANDBY; the arm of the autosampler will lift out of the wash cup. Check the alignment of the sampling tip by manually moving the arm to the sample port. If the alignment needs adjustment, unlock the autosampler base by turning the knob on the center front of the base. The knobs on the back left and front left sides then can be used to move the base to the necessary position. Lock the base in place with the center front knob. Move the mirror down so that the tip can be observed. Check the depth of the autosampler tip in the graphite tube. Use the front adjustment knob to the right of the sampling arm to raise or lower the tip as necessary. Move the mirror back to the upright position.

(27) Click on SAMPLE STANDBY again. The sampling arm will return to its position in the wash cup and flush for several seconds.

(28) Click on the HGA CONTROL window (yellow). Condition the graphite tube. Type in the temperatures listed below, click on the MANUAL TEMP after each entry, and wait for about 5 seconds. Click the MANUAL TEMP again to turn off the furnace. Wait about 10 seconds before typing in the next temperature.

<div align="center">1000 °C   500 °C   2000 °C   400 °C   2650 °C</div>

(29) Click on the AS-60 CONTROL window (green). Go to the bottom of the AS-60 CONTROL page and type 20 after LOCATION and again after VOLUME (:L).

(30) Check the drying step of the furnace program: click on RUN SPECIAL. Lower the mirror and observe the sample tip as it deposits the sample on the platform. Continue to observe as the sample dries. (Return the mirror to upright, out of the light path, after the sample dries.) The drying should be complete 5-10 seconds before the char step begins. If it is dry sooner, decrease the hold time of the dry step appropriately. If it is not dry in time, two options are available: the temperature of the drying step may be increased or the hold time may be increased. Increase the hold time only if increasing the temperature will cause the sample to boil and splatter during the dry step.

(31) If the parameters in the dry step need to be changed, choose WINDOWS. Then when the ELEMENT PARAMETER page appears, click on the HGA box. Change the parameters as necessary.

(32) Continue to check the dry step as in (30) until a successful step is observed.

(33) When the appropriate dry temperature is determined (even if it is the one already in the program), make sure that the blank is low (less than 0.005 Abs-sec).

d. Operation

(1) On the AS-60 Control page, click on PRINTER ON/OFF, and then click on RUN STANDARDS.

(2) The Model 5100 will run the calibration curve.

(3) When the curve is finished, run the quality control materials. Type in 6-14 under SAMPLE LOCATIONS. Next click on RUN SAMPLES. Verify that the quality control materials are within the specified limits.

(4) If the values observed for this analytical run are in control, proceed with the analysis of the diluted

serum specimens. (See the Quality Control System section of this document for criteria.)

(5) After 20 samples (this includes calibration standards and controls) have been run through the graphite tube, halt the model 5100 from proceeding with analyses. Open the graphite furnace and remove the tube and platform. Clean the charred carbonaceous material from the platform with a cotton swab. First use the cotton end to remove the excess, then use the wooden end to gently scrape off the remaining material. Perform this procedure before each new tray of samples is placed on the autosampler. This procedure should also be done before the system is turned off for the day. Before proceeding with another rack of specimens, run a furnace blank and a process blank to recondition the graphite and ensure that the tube and platform have not been contaminated.

(6) Turn off the system in reverse order.

(7) Turn off the argon gas.

(8) To bleed gas from the line, click on the HGA CONTROL page. Click on the HGA ON/OFF box. The model 5100 will start to run through one cycle. An ERROR window will appear when the gas pressure is low. Click on OK.

(9) Choose the FILE option.

(10) Click on EXIT TO DOS.

(11) A window will appear asking whether you want to save the ID weight parameters. If you do not want to save them, click on OK.

(12) A window will appear asking "Is it OK to exit software?" Before you answer, turn off the EDL power supply and unplug the EDL.

> *When turning off the EDL power supply, first turn off the power switch. When the power indicator decreases to zero, turn off the variable power knob.*

(13) Now click on OK. The lamp carrousel will rotate and the autosampler will flush.

(14) The computer screen will be blank except for the C:\ in the left corner. Type in HDSIT. Wait for three paragraphs to appear on the screen explaining the procedure.

(15) Turn off the printer.

(16) Turn off the CRT and computer.

(17) Turn off the spectrophotometer.

(18) Turn off the Zeeman 5100.

(19) Turn off the HGA-600.

(20) Turn off the cooling water.

Operating Procedure Steps for the SIMAA 6000 GFAAS

e. Instrument setup

(1) Turn on the argon gas tank and verify that the tank pressure is $100 psi; the outlet pressure should be 40-50 psi. Turn on the SIMAA 6000, computer, monitor, and printer, in that order. Verify the water level in the cooling system. The level should be close to the maximum point.

(2) The SIMAA 6000 AA WinLab software controls the AA automatically. On boot up, the software loads the operating system (Windows '95) under which the AA control program runs and opens to the SIMAA 6000 window. Using the mouse, double click on the AA WinLab Analyst icon in the SIMAA 6000 window. The system will check for the furnace and initiate the optical system as well as perform a rinse of the autosampler. When finished, the Multi-element AA WinLab window will be open and the dialogue box of the Perkin-Elmer AA WinLab software will be displayed at the bottom of the screen. This box has choices of select workspace, technique, tutorials and references, and menus and toolbars. The options in this dialogue box are also available through the Multi-element AA WinLab toolbar. For more information, see Chapter 5 "Performing Analyses"

in the Perkin-Elmer SIMAA 6000 "Setting Up & Performing Analyses" guidebook (P/N 0993-5219).

(3)   To check the condition of the furnace tube, in the MultiElement AA WinLab window, click on Tools, then Diagnostics, and select the Furnace tab at the bottom-right. The Furnace Diagnostics dialogue box appears, the tube cycle and cylinder cycle tabs are shown at the bottom of the screen, and the reset button can be used to set the counts to zero if a new furnace tube will be installed.

(4)   Note the number of firings performed with the current tube. If the number of firings is >400, change the tube-and-platform combination: (see pp. 1-10 to 1-12, in "Setting Up and Performing Analyses", P/N 0993-5219).

(a)   Unscrew the autosampler support door lock in the front of the SIMAA and swing away from the furnace. Click the Furnace icon on the menu bar, click the OPEN/CLOSE button to unlock the furnace in the Furnace Control window. Swing the furnace lock to the left. Pull the forward tube support down.

(b)   Remove the graphite tube-and-platform and inspect the condition. Discard if visibly pitted or contains noticeable deposits.

(c)   Clean the furnace before inserting the new tube: Wipe the graphite contact cylinders with methanol and a Kimwipe™. Use a cotton-tipped stick to clean the hole in the rear graphite cylinder. Stubborn deposits may sometimes be removed with 1% (v/v) HNO_3. Replace cylinders, following instructions in the User's Manual, if worn or pitted. Worn contact cylinders will result in skewed peaks and poor precision.

(d)   Handling with plastic tweezers, wood grips or a Kimwipe, orient the graphite tube so that the injection port is on top. The side of the tube where the platform is attached is on the left towards the rear of the tube. Place the graphite tube in the rear graphite cylinder.

[Note: Do not touch the graphite tube with your fingers.]

(e)   Swing the forward tube support up. Swing the furnace lock to the right, under the forward tube support. Click the Open/Close button in the Furnace Control window to close the furnace.

(f)   Swing the autosampler support door back into position and screw the lock closed.

(g)   Click on Condition Tube in the Furnace window to condition the tube. When the conditioning program is finished, close the furnace window.

(5)   Double-click on the Lamps icon on the menu bar of the Multi-element Automated Analysis window to make it active.

(6)   If the selenium lamp is installed in the turret, the instrument will automatically recognize the lamp and its position.

(7)   Check the autosampler's reservoir and waste container. If necessary, empty the latter and fill the former with approximately 20 mL of matrix modifier dissolved in 2 L of ultrapure 18MS water. Fill the blank container at position 71 with fresh ultrapure 18MS water. Install the carousel with dustcover in the autosampler.

(8)   Autosampler Alignment: (see pp. 1-14 to 1-21, in "Setting Up and Performing Analyses", P/N 09935219).

NOTE: DO NOT ATTEMPT TO MOVE THE AS-72 ARM MANUALLY WITHOUT FIRST DISABLING THE SOFTWARE CONTROL VIA THE AUTOMATED ANALYSIS OR FURNACE CONTROL WINDOWS.

(a)   Click on the Automated Analysis window to make it active. Click on the button marked Align Tip. The autosampler control dialogue box will open and be displayed on the screen. Click on Unlocked and the autosampler arm will rise to permit manual rotation.

(b)   Examine the tip of the capillary tubing. If it is bent or discolored, prepare a fresh one: Pull more tubing through the sleeve using a pair of pliers. Measure 6-7 mm beyond the plastic sleeve and cut with a razor blade held vertically to produce a 45/ angle. NOTE: If the tubing is cut too short, the sleeve will hit the graphite tube.

(c) Using the knob on the left hand side of the autosampler control box, manually swing the arm through its normal cycle. With a flashlight or other local light source illuminating the furnace opening, check that it enters the graphite tube without touching the wall. If not, adjust the in/out (forward/backward) or left/right control knobs as necessary.

(d) Now that the tip enters the tube, adjust the depth. Swing down the mirror and backstop. Rotate the arm to its deepest penetration. Turn the depth adjustment knob (the one farther from the furnace) until the angled point of the tip is just above the platform. Recheck the alignment and click on Unlocked again to lock the autosampler into place. The autosampler will reset and rinse the autosampler.

(e) Click on "Align in Tube" and the autosampler will cycle through and place the tip into the tube. Recheck the alignment and click on "Align in Tube" again to remove the autosampler from the tube and return the autosampler to the rinse location. The autosampler will reset and rinse the autosampler tip. Swing the mirror and backstop up.

(f) Check the proper alignment of the autosampler: In the bottom of the Automated Analysis window, click on "Select Location", click in the dialogue box that appears, enter 37, and click "OK", to run a blank. Wait a few seconds for the lamp to peak, then swing the mirror down. Carefully observe the sample deposition. THIS IS CRITICAL FOR UNATTENDED OPERATION. The tip must deposit the entire sample onto the platform. If the tip is too high in the tube, the sample will creep up the tip and crawl onto the wall. Realign and recheck. Do not continue until the sample is deposited onto the platform and **only** onto the platform.

(9) Once the alignment is correct, check for proper drying. The sample must dry evenly, without boiling or sputtering. This is especially important when a new tube is installed. Check drying cycle first with blank (matrix modifier), and then with a diluted serum sample. If sample has not dried before the start of step 3 (Char), increase the drying temperature by 10/C and re-check. If sample sputters during step 1 or 2 (Dry), *decrease* the drying temperature by 10/C and re-check. THE OPTIMUM DRYING PARAMETERS ARE CRITICAL FOR ACCURATE AND PRECISE OPERATION. Do not continue until the sample dries evenly. If you cannot get the sample to dry in a controlled manner, ask the supervisor for assistance.

f. Instrument operation

(1) In the Automated Analysis window, click on the box marked Calibrate. The software will proceed with running the blank and each standard in duplicate. If the blank's absorbance is >0.005 selenium, stop the run and call the supervisor.

(2) When standards have finished running, the calibration results will print. Notify the supervisor if the correlation coefficient is <0.99, or if the slope is outside the [to be established] range for selenium using the EDL lamp.

(3) After the calibration curve is generated, prepare the sample information file. First call up the Sample Information File by clicking File, Open, Sample Info File. The Open window will be displayed, scroll down to select a sample information file from the available *.SIF list. Double click on the file to open. The Sample Information Editor window is now open and changes can be made to the file. Enter the sample ID's for the corresponding samples in the same number position in the autosampler tray. Finally, select Save As from the File menu to name the sample information file (.SIF) file using the following standard convention: Enter the date as MM/DD/YY plus a letter (A, B, C, ...) if two or more carousels are set up for that day, e.g., 020398A for tray #1 of February 3, 1998. Make a separate sample information file (.SIF) for each carousel. Each carousel holds up to 65 samples, in autosampler positions 6 through 70.

(4) Proceed with the analysis by clicking "Analyze Samples". Monitor the first run of reference samples to ensure results are in the acceptable range. If they are not, notify the supervisor.

(5) While the PE SIMAA 6000 is operating, check each run at least every 30 minutes for problems, such as:

 (a) Error messages on the screen, e.g., "Timeout error", "Printer not on", "Gas pressure is low".

 (b) Premature completion, if recalibration does not fix an out-of-range reference.

 (c) Jammed printer.

(d) Crooked or melted tip.

(6) The run can be stopped manually at any time during a run by clicking on the highlighted button in the Automated Analysis window. A dialogue box will appear giving you the option of stopping (i) immediately (default), (ii) after the current sample replicate, or (iii) after all replicates of the current sample. Press return to stop immediately or click to select the appropriate choice.

(7) When a run is finished, all the terms of the buttons in the Automated Analysis window go from grey to blue, and the Analyze Samples button is no longer highlighted in green. If a second carousel is to be run, open the appropriate sample information file, place the carousel in the autosampler, and execute Analyze Samples again.

(8) When all analyses have been completed, click on the Lamps icon and turn off each lamp by clicking the green highlighted button. Next, click on File, select Exit to exit from the program; turn off gas, and instrument. Close all windows and exit from the Windows Software before turning off the computer and printer.

(9) Enter results for each reference material in the PC database files for plotting. For each level, enter the duplicate concentration values. Notify supervisor of any unusual trends or biases. Record number of cycles for the current tube on the SIMAA 6000 Routine Maintenance sheet. Make a record of the slope of the standard line and the lamp energy (available from the Align Lamps window). Any other maintenance items or problems noted should also be entered onto the Instrument Log.

g.   Replacement and Periodic Maintenance of Key Components For Both the Models 5100 and SIMAA 6000 (in addition to preventive maintenance performed by the service engineer).

(a) Source lamp: Ensure that a spare selenium source lamp is available. Order another lamp if the spare has been used for replacement.

(b) Printer ribbon: Keep a supply of printer ribbons on hand. Order more when the last is installed. OR

(c) Printer cartridges: a supply of desk jet printer cartridges should be on hand. Order more when the last is installed.

(d) Graphite tubes and pyrolytic platforms: Keep at least 3 months' supply on hand. Order more when the inventory falls below this quantity.

(e) Graphite contact rings: approximately every six months, the graphite contact rings of the furnace housing will need replacement. Indications that this procedure needs to be performed are an apparent loss of temperature control or sounding of the alarm by the instrument indicating a problem with the tube. It is useful to maintain a log book to help keep track of when these replacements occur. At least one spare set of graphite contact rings should be kept on hand.

(f) Reposition and trim the sampling tip of the autosampler every few weeks, depending on how the dispensing is proceeding.

h.   Calculations

At present, because the selenium method cannot be satisfactorily be run on the SIMAA with aqueous calibrators, we will continue to operate in absorbance mode and perform final standard-addition corrections off-line. The program "SELENIUM" run in ROSCOE on the CDC mainframe will correct for the dilution discrepancy between the standard blank and the spiked standards using the dilution factor 1.04. The program will also adjust the integrated absorbance values--measured as peak areas--of the serum standards by subtracting the integrated absorbance of the unspiked serum blank from the integrated absorbances of the selenium-spiked serum standards. It will also adjust the integrated absorbances of the control and specimen samples by subtracting the integrated absorbance of the process blank.

i. Modifications in the Selenium Method Used for NHANES 1999+

These modifications were made for NHANES III. Other changes for NHANES 1999+ will relate only to switching to the SIMAA 6000 THGFAAS, such as reducing the nickelous nitrate concentration in the matrix modifier to 0.33%.

j. A Micromedic Digiflex automatic dispenser is used for many of the dispensing needs in this method because it is easier to use and provides better precision and accuracy than micropipettes.

k. The matrix modifier is added as part of the standard and specimen preparation procedure rather than by separate autosampler addition to save time during the analytical run. Magnesium nitrate is used in the matrix modifier to aid in charring of the sample during the furnace program. The standards and samples are diluted fivefold with matrix modifier (rather than twofold) to reduce matrix effects.

l. Since the original publication of the serum selenium method, NIST has made available SRM 3149, which is used in this version for the preparation of the standards.

9. REPORTABLE RANGE OF RESULTS

a. The method described here is linear between 0 and 400 ng/mL (2), which is beyond the normal range of values expected to be observed for human serum selenium concentration.

b. Duplicate measurements of integrated absorbance or concentration should not differ by more than 10% (± 7 at 70 ng/mL or ± 15 at 150 ng/mL). If they do the analysis is repeated.

c. The detection limit, based on 3 times the standard deviation of 10 repeat measurements of the blank, is 8 µg/L. This value is well below that expected in the normal human adult population. Infants and children may have serum selenium concentrations as low as 20 µg/L, which is detectable by the method described here. Samples with a selenium level >170 ng/mL or < 50 ng/mL are reanalyzed for confirmation before results are released. (Samples with concentration levels >200 ng/mL are diluted 1:1 with DI water and reanalyzed.)

10. QUALITY CONTROL (QC) PROCEDURES

The method described in this protocol has been used since 1987 in the Nutritional Biochemistry Branch for NHANES III and other epidemiological health studies. The method has proven to be accurate, precise, and reliable. The instrumentation used is "state-of-the-art." The primary standard used is a NIST SRM. Estimates of imprecision can be generated from long-term quality control pool results.

Two types of quality control systems are used in this analytical method. One system involves "bench" quality control specimens that are inserted by the analyst two times in each analytical run (a set of consecutive assays performed without interruption) so that judgments may be made on the day of analysis. The other system involves "blind" quality control samples that are placed in vials, labeled, and processed so that they are indistinguishable from the subject samples. The results of the blind specimens are decoded and reviewed by the supervisor. With both systems, all levels of selenium concentration are assessed by taking these samples through the complete analytical process. The data from these materials are then used in estimating methodological imprecision and in assessing the magnitude of any time-associated trends.

Two levels of blind quality control pools are used. These pools are prepared in sufficient quantity to last throughout the survey. The levels chosen are in the "low-normal" (approximately 100 ng/mL) and "high-normal" (approximately 200 ng/mL) ranges so as not to be obvious to the analyst. The pools are prepared in the same way as the bench pools, but they are dispensed in vials identical to those used in the field for NHANES subject samples, labeled with pseudo-participant numbers corresponding to each geographical location of the survey, and stored at <–70°C. At least one blind sample is randomly incorporated with every 20 NHANES samples and analyzed according to the method protocol.

The bench quality control pools comprise three levels of concentration spanning the "low-normal" (approximately 75 ng/mL), "normal" (approximately 100 ng/mL), and high (approximately 160 ng/mL)

ranges for selenium.

Quality control limits are established for each pool. An analysis of variance (ANOVA) is performed for each pool after 20 characterization runs have been performed in which previously characterized reference materials (a NIST SRM and Nyegaard RM) and bench quality control pools are used for evaluation. In addition to providing quality control limits, the characterization runs also serve to establish the homogeneity of the pools. Once the homogeneity of these bench and blind materials has been established, it is useful to have them analyzed by another independent reference method (e.g., IDMS).

The precision and accuracy of the method are reflected in these long-term quality control pool results:

Table 4. Representative Quality Control Pool Results (ng/mL) from NHANES III

Pool	Mean	95% limits	99% limits	N	Total SD	Total CV (%)
SRM 909	103.2	95.85-110.45	93.54-112.76	29	4.90	4.75
9110	69.6	64.26-74.86	62.59-76.54	55	4.36	6.26
9111	99.2	92.35-106.06	90.18-108.23	55	4.86	4.90
9112	153.5	141.37-165.73	137.51-169.58	55	7.53	4.90
1188	87.3	81.18-93.46	79.24-95.40	96	4.56	5.23
1288	107.1	99.75-114.48	97.42-116.81	96	5.02	4.69
1388	148.5	139.15-157.85	136.20-160.81	96	5.75	3.87

After the standards and bench quality control materials are analyzed (at the beginning of an analytical run), the long-term quality control charts for each control material are consulted to determine if the system is "in control." Two types of charts are used. The first chart plots the means of the duplicate determinations and compares them with the 95% and 99% confidence limits and with the center line (the overall mean of the characterization runs). The system is out of control if any of the following events occur for any one of the quality control materials:

- The mean from a single pool falls outside the 99% confidence limits.
- The means from two pools fall either above or below the 95% confidence limits.
- Two successive run means for a single pool fall either both above or both below the lower 95% limit.

- The means from eight successive runs for one pool fall either all above or all below the center line.

The second type of quality control chart plots the range of the duplicate determinations and compares them with the 95% and 99% limits and with the center line. The system is out of control if any of the following events occur for any one of the quality control materials:

- The range from a single pool falls above the upper 99% limit.
- The ranges from two pools fall above the upper 95% limit.
- Two successive within-run ranges for a single pool fall above the upper 95% limit.
- The ranges from eight successive runs for a single pool fall above the center line.

If the run is declared "out of control," the system (instrument, calibration standards, etc.) is investigated to determine the root of the problem before any analysis of specimens occurs.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

If one or more of the quality control samples fail to meet acceptable criteria for mean or range of duplicate values, take the following steps in succession.

a. Prepare and re-analyze fresh dilutions of all quality control samples.
b. Prepare fresh calibration standards and run the entire calibration curve using the freshly prepared standards.
c. Using a new (unused) graphite furnace and/or pyrolytic graphite L'vov platform, re-analyze the entire sequence of calibrators, blanks, and quality control samples.

If the steps outlined above do not result in correction of the "out of control" values for QC materials, consult the supervisor for other appropriate corrective actions. Do not report analytical results for runs not in statistical control.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

a. The range of linearity and limits of detection were previously mentioned in Section 9 of this method.
b. No known chemical or physicochemical interferences have been documented for this analytical method; however, it is very important to avoid external contamination.
c.

13. REFERENCE RANGES (NORMAL VALUES)

a. The average serum selenium value for 20 (non-U.S.) adults was listed as 75.3 ± 19.9 ug/L (5).
b. The preliminary unweighted serum selenium concentrations for 14,000 NHANES III participants ages 12+, all races, and both sexes have a 95% confidence interval of 95-150 ng/mL.

The NHANES IV data will establish the normal ranges of the U.S. population for children 3-11 years of age.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Although toxicity is not well defined in humans, the supervisor or designated analyst should routinely report serum selenium values >300 ng/mL by FAX for follow up in non-NHANES studies. Since the NHANES data are routinely uploaded several times weekly to the Westat ISIS computer, abnormal results are immediately transferred to the NCHS survey physician for follow-up.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens are allowed to remain at ambient temperature during analysis.

16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Because the analysis of serum for selenium is inherently complex and challenging, there are no acceptable alternative methods of analysis in the NHANES laboratory, although either the SIMAA 6000 or the model 5100 GFAA is available as a backup should the primary instrument fail If the analytical system fails, then store specimens and sample materials at –20°C until the analytical system is restored to functionality.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

  a. Use the "NHANES LABORATORY SERUM SELENIUM STANDARD AND QUALITY CONTROL REPORTING SHEET" to record QC data. This reporting sheet has self-explanatory blanks for the standard absorbance data, run information, and quality control pool results. Prepare this form in duplicate, using carbon paper.

  b. Use the "NHANES 1999+ Standard and Quality Control Sheet" to record quality control data. The entry of standard concentrations and quality control pool results on this reporting sheet is self explanatory. Prepare this form in duplicate. (The original copy is kept by the supervisor; the carbon copy is retained by the analyst, in dedicated notebooks for the survey duration. These records are later transferred to long-term storage.)

  c. Use the "NHANES 1999+ Analytical Worksheet" to record the specimen results. These have been prepared with sample IDs for each pre-racked run. If a sample is missing from the rack, enter the appropriate code. If a sample is not satisfactory (i.e. cannot be analyzed), enter the appropriate codes (hemolysis, lipemic). Enter codes for confirmatory repeat analysis of abnormal values. Prepare this form in duplicate. These codes are also entered into the database, and are transmitted to NCHS all with the analytical results.

  d. Quality control evaluation of each run will be initiated by the Microsoft Access database when the data are uploaded by the analyst. A separate module of the database will collect the QC data from each run and provide Levy-Jennings means and ranges charts on demand.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

The Microsoft Access database is used to keep records and track specimens for NHANES 1999+. If selenium analyses are used for smaller, non-NHANES studies, records are kept on files in L:\link\nbb on the EHLS LAN. We recommend that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study. Only numerical identifiers should be used (e.g., case ID numbers). All personal identifiers should be available only to the medical supervisor or project coordinator. Residual serum from these analyses for non-NHANES studies may be discarded at the request of the principal investigator, or may be transferred to the CDC CASPIR facility for use by other investigators. Very little residual material will be available after NHANES analyses are completed, and these vials may be routinely autoclaved.

19. Summary Statistics and QC Graphs

Two instruments were used during this 3-year period.

Summary Statistics for Selenium by Lot						
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
9918	93	1/27/1999	6/29/2000	44.1	3.1	6.9
9904	97	1/27/1999	6/29/2000	73.4	3.7	5.0
9905	96	1/27/1999	6/29/2000	93.8	3.4	3.6
9906	94	1/27/1999	6/29/2000	161.2	8.2	5.1
LP	56	10/3/2001	2/4/2002	82.3	1.5	1.9
HP	56	10/3/2001	2/4/2002	150.7	2.9	1.9

1999-2002 Selenium Quality Control
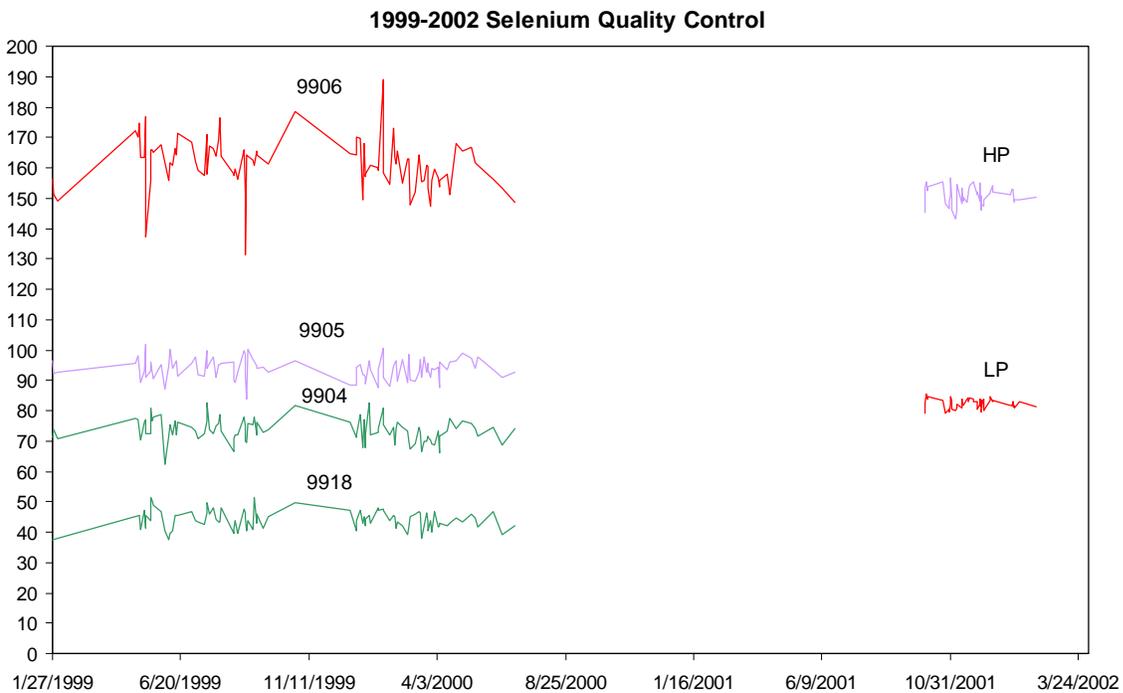
Summary Statistics for SEL by Lot						
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HP	56	10/3/2001	2/4/2002	150.7102083	2.937358018	1.949010655
LP	56	10/3/2001	2/4/2002	82.26264881	1.540874072	1.873115071

2001-2002 SEL Direct Quality Control
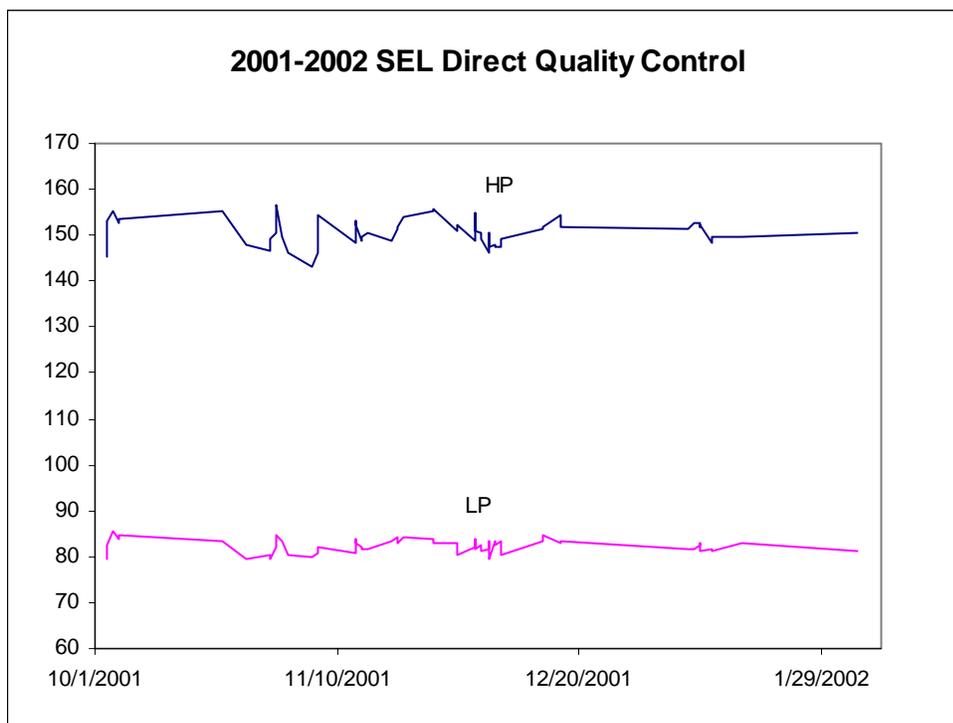
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