Laboratory Procedure Manual

Analyte: **Thyroid Stimulating Hormone (TSH)**

Matrix: **Serum**

Method: **Access 2 (Beckman Coulter)**

Method No:

Revised:

as performed by:

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Important Information for Users

University of Washington 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.

Thyroid Stimulating Hormone (TSH) in Serum
NHANES 2009- 2010

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

File Name	Variable Name	SAS Label
THYROD_F	LBXTSH1	Thyroid Stimulating Hormone (mcIU/mL)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The Access HYPERsensitive human thyroid-stimulating hormone (hTSH) assay is a 3rd generation two-site immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel with goat anti-hTSH-alkaline phosphatase conjugate, buffered protein solution, and paramagnetic particles coated with immobilized mouse monoclonal anti-hTSH antibody. (Goat anti-mouse antibody is used to immobilize the mouse anti-hTSH antibody.) The hTSH binds to the immobilized monoclonal anti-hTSH on the solid phase while the goat anti-hTSH-alkaline phosphatase conjugate reacts with a different antigenic site on the hTSH. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos™ 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of human thyroid-stimulating hormone in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Human TSH is one of several glycoproteins hormones consisting of two non-covalently bound peptide chains: a α-chain, which is nearly identical in all, and a β-chain, which is responsible for immunological and biological specificity. These similarities result in varying degrees of cross-reactivity of different antisera. The Access HYPERsensitive hTSH assay has virtually no cross-reactivity with other peptide hormones. The sensitivity and specificity of this test enable better discrimination between hyperthyroid and euthyroid patients.

TSH stimulates the production and secretion of the metabolically active thyroid hormones, thyroxine (T4) and triiodothyronine (T3), by interacting with a specific receptor on the thyroid cell surface. T3 and T4 are responsible for regulating diverse biochemical processes throughout the body that are essential for normal metabolism and neural activity. In normal individuals, the levels of thyroid hormone in the blood are maintained in dynamic equilibrium by a sensitive negative feedback control on the pituitary. The pituitary and TSH occupy a central position in this negative feedback scheme that regulates T3 and T4 secretions. The synthesis and secretion of TSH is stimulated by the hypothalamic tripeptide thyrotropin-releasing hormone (TRH) in response to low levels of circulating thyroid hormones. In contrast, elevated levels of T3 and T4 suppress the production of TSH.

The principal clinical use for hTSH measurement is for the assessment of thyroid status. In patients with intact hypothalamic-pituitary function, hTSH is measured to: 1) exclude hypothyroidism or hyperthyroidism; 2) monitor T4 replacement treatment in primary hypothyroidism or ant thyroid treatment in hyperthyroidism; 3) follow T4 suppression of the tropic influence of hTSH in "cold nodules" and non-toxic goiter; and 4) assess the response to thyrotropin-releasing hormone (TRH) stimulation testing. As more sensitive and precise methods become available, hTSH measurements are also increasingly used to identify subclinical or latent hypothyroidism or hyperthyroidism.

2. SAFETY PRECAUTIONS

Consider all samples received for analysis potentially positive for infectious agents including HIV and the hepatitis B virus. Observe universal precautions. Wear gloves, lab coat, and safety glasses when handling all human blood products and infectious viruses. Place disposable plastic, glass, paper, and gloves that contact blood in a biohazard bag or discard pan to be autoclaved. Disinfect all work surfaces with Vephene solution.

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

Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wash hands thoroughly after removal or personal protective devices used in handling specimens and kit reagents.

Material safety data sheets for all reagents used in the performance of this assay are kept in the Immunology Division, University of Washington Medical Center (UWMC).

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

A. Each shipment of specimens received from the NHANES mobile unit arrives with a corresponding transmittal sheet and an electronic version of the shipping/resulting file. The file structure is determined by NHANES and is described in the National Health and Nutrition Examination Survey (NHANES) Contract Laboratory Manual.

B. After the testing is completed results from the Access 2 are transferred to the laboratory server system, which is backed up daily. This instrument file contains the following information for each sample, control and calibrator tested.

Patient ID
Sample ID
Rack
Verify
Test Name
Interpretation
Result
Units
Comp. Time
Flags
LIS
Instrument
RLU
Pipettor
Sample Type
Sample Priority
Test ID
Reagent Pack Lot #
Reagent Pack Serial #
Dilution
Calibrator level
Comments
Load Date/Time

C. QC results are transferred to an Excel file using laboratory-developed software. This file calculates the QC statistics, plots Levey-Jennings charts, displays relevant instrument flags, tracks reagent lots and recent calibrations. QC results are reviewed prior to resulting samples.

D. Sample results are transferred to an Excel file using laboratory-developed software that enters results after matching sample identifiers from the instrument file with those provided in the NHANES shipping/resulting file. This Excel file is formatted to match the NHANES shipping/resulting file and the program uses the conventions outlined in the NHANES Contract Laboratory Manual.

E. Data entry is checked for errors.

F. After the TSH testing has also been completed, resulted, and checked, the result file is transmitted electronically to NHANES WESTAT. Electronic and hard copies of the files are kept in the laboratory.

G. Technical support for this system is provided by Westat, Rockville, MD (1-301-294-2036)

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

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

B. Serum is the preferred specimen type. Heparin plasma is acceptable. If testing is to be done within 48 hours, samples can be refrigerated at 2 to 8°C. Freeze at -20ºC or colder for longer storage.

C. Blood should be collected aseptically and the serum separated by standard laboratory techniques. Specimens may be collected by using regular or serum-separator Vacutainers. Serum should be separated from the cells within 2 hrs of collection.

D. The requested sample volume for the assay is 1.0 mL, and the minimum sample volume is 0.3 mL.

E. Specimens may be stored in glass or plastic vials, as long as the vials are tightly sealed to prevent desiccation of the sample.

F. Turbid samples or those with particulate matter should be centrifuged prior to assay.

G. More than one freeze-thaw cycles is not recommended.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR 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. Instrumentation

1. Beckman Access or Access II Immunoassay System (Beckman Coulter, Fullerton, CA.)

   The Beckman Access is a fully automated, random access, instrument that

features on-board storage of reagent packs in a refrigerated compartment; an ultrasonic probe tip for level sense detection, sample and reagent delivery, mixing, and probe cleaning to minimize carryover; barcode identification of specimens and reagent packs; temperature controlled reaction reactions; and measurement and analysis of the light signal generated by the chemiluminescent reaction (RLU) using a weighted four parameter logistic curve math model.

The HYPERsensitive hTSH assay parameter settings for the instrument are as follows:

Parameter	Setting
Sample Volume Requirements Minimum sample volume Sample volume used for testing	 300 ul 110 ul
No. of Standard Points	6
Calibration curve calculation	Weighted four parameter logistic curve math model
Standard Curve Measuring Range (At initial dilution; approximate values, range is dependent upon standard value)	0 – 100 uIU/mL (mcIU/mL)

2. Hewlett Packard DeskJet printer (Hewlett Packard, Boise, ID)

3. Computers (Dell Computer Corporation, Round Rock, Texas).

4. Centrifuge (Jouan Inc., Winchester, VA)

B. Equipment

1. Reaction Vessels (Beckman Coulter, Fullerton, CA)

2. Sample Cups (Fisher Scientific, Pittsburgh, PA)

3. Latex gloves, disposable (Any manufacturer).

4. Pipettes and tips (Rainin, Emeryville, CA)

C. Reagents

All reagents are purchased from Beckman Coulter, Fullerton, CA.

1. R1: Access HYPERsensitive hTSH Reagent Pack Cat. No.33820:
100 determinations, 50 tests/pack

Provided ready to use. Store upright and refrigerate packs at 2 to 10°C. Packs must be refrigerated at 2 to 10°C for a minimum of two hours before use on the instrument. Stable until the expiration date stated on the label when stored at 2 to 10°C. After initial use, the pack is stable at 2 to 10°C for 28 days. Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range. All antisera are polyclonal unless otherwise indicated.

R1a:Paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-hTSH complexes suspended in TRIS buffered saline, with surfactant, bovine serum albumin (BSA), 0.1% sodium azide, and 0.1% ProClin™ 300.

R1b: TRIS buffered saline with surfactant, BSA, protein (murine, goat), <0.1% sodium azide, and 0.1% ProClin™ 300.

R1c: Goat anti-hTSH-alkaline phosphatase (bovine) conjugate in TRIS buffered saline, with surfactant, BSA, protein (goat), < 0.1% sodium azide, and 0.1% ProClin™ 300.

2. Access Substrate Cat. No. 81906: 4 x 130 ml

   Lumi-Phos*530 (buffered solution containing dioxetane), Lumigen* PPD, fluorescer, and surfactant. Bring to room temperature (15 – 30 ºC) at least 18 hours before use. Stable for 14 days at room temperature or after bottle has been opened.

3. Access Wash Buffer II: Cat # A16792
   Provided ready to use. Store at room temperature (15 – 30 ºC), stable until expiration date on label.

4. Access Sample Diluent A – Cat. No. 81908
   Used to dilute high TSH results. Provided ready to use. Allow the contents to stand for 10 minutes at room temperature. Mix gently by inverting before use. Avoid bubble formation. Stable until the expiration date stated on the vial label when stored at 2 to 8°C.

D. Standards/Calibration Preparation

Access HYPERsensitive hTSH Calibrators – Cat. No. 33825
S0-S5, 4.0 mL/vial. Provided ready to use. Store upright and refrigerate at 2 to 10°C. Mix contents by gently inverting before use. Avoid bubble formation. Stable until the expiration date stated on the label when stored at 2 to 10°C. Signs of possible deterioration are control values out of range. Refer to calibration card and or vial labels for exact concentrations

S0: Buffered bovine serum albumin (BSA) matrix with surfactant, <0.1% sodium azide, and 0.5% ProClin™ 300 Contains 0.0 mcIU/mL hTSH.

S1–S5: Approximately 0.1, 0.5, 4.0, 10.0, and 100.0 mcIU/mL hTSH, respectively, in buffered BSA matrix with surfactant, < 0.1% sodium azide, and 0.5% ProClin™ 300.

Calibration Cards: 2.

These calibrators are used for both the hYPERsensitive hTSH and the Fast hTSH assay. Separate calibration curves are required to run the hYPERsensitive hTSH and Fast hTSH assays. Each has a separate calibration card and must be calibrated accordingly. Only the HYPERsensitive hTSH assay has been defined on the instrument used to perform NHANES testing.

E. Preparation of Quality Control Materials

Three different levels of serum controls are run with each run. The controls are purchased from BioRad Laboratories (Hercules, CA) or prepared in-house. Commercial controls are stored and used according to the manufacturer's recommendations. In house controls are stored frozen (-20ºC or colder). Once thawed, the controls are stored at 2-8 ºC. All controls are used within their stated expiration dates.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibration Curve

TSH concentrations are calculated by using a calibration curve. This method utilizes a four parameter logistic curve with a direct relationship of measured light produced (RLU) to concentration of TSH in the serum sample. Serum results are expressed as mcIU/mL.

Calibrators are traceable by comparison with a set of primary reference calibrators standardized to the WHO 2nd International Reference Preparation 80/558.

An active calibration curve is required for all tests. For the Access TSH assay, calibration is required every 28 days or whenever new lot numbers of reagents are placed into use. Refer to the Operator's Guide and Reference Manual for complete instructions on calibration procedures.

B. Verification

1. Three levels of control are run for each test series. If, within a testing series, these controls do not conform to specifications as defined in the quality control manual, the entire series is invalidated.

2. New lot numbers of calibrator are verified by running 100 or more samples tested on the previous lot number. The correlation is analyzed using one or more linear regression formulas.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preliminaries

1. Bring all controls and patient specimens to room temperature before use. Mix any specimens or controls that have been frozen. Centrifuge samples with particulate matter prior to testing.

2. Prime system: pipettor - 1 time, and substrate - 4 times

3. Check reagent, substrate, wash buffer, and reaction vessel status. Load any needed supplies onto the instrument. Mix reagent pack contents by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs — mix reagents by swirling gently.

B. Instrument Operation (see operator's manual for details).

1. Check sample volume to make sure that there is sufficient volume to perform testing. Gently mix, uncap and load specimens into specimen racks, with the barcode in the open slot. Make sure there are no bubbles. Alternately, use the barcode wand to identify the specimens. If the barcode is not reading properly, sample IDs can be entered manually. Load the racks onto the instrument.

2. Select the HYPERsensitive TSH test. Note: if other thyroid testing is also ordered, the entire 8 test panel can be ordered as a group. Testing is done in singlicate. Select the sample(s) to be used for the random repeat testing.

3. The instrument automatically calculates all results. After testing is completed, results are printed and review by the technologist.

4. Remove specimens and controls soon after the instrument finishes pipetting from the sample. Return controls to the refrigerator and refreeze specimens.

5. Perform scheduled instrument maintenance (daily, weekly, and monthly) as outlined on the maintenance log. See the operator's manual for specific instructions.

C. Recording of Data

1. Using a lab developed program, specimen results are transferred from the instrument data file into the assay specific results table created from the send file corresponding to the specific sample box. The file format is Excel (Microsoft Corporation, Redmond WA). A copy of this file is printed out and checked for accuracy of data entry.

2. Control results are entered to the Assay Specific QC/Levy-Jennings Table using the Excel program. Compliance with the Westgard rules is evaluated. A copy of this table is printed out and checked for accuracy of data entry.

D. Replacement and Periodic Maintenance of Key Components

1. Daily Maintenance:
   Start-up:
   Inspect fluidics module.
   Check system supplies and replace as needed.
   Clean exterior of substrate, dispense, and aspirate probes.
   Prime pipettor – 1X and substrate - 4X.
   Verify temperature.
   Shut-down:
   Check waste containers, empty if needed
   Perform clean

2. Weekly Maintenance:
   Change probes and clean them
   Clean exterior of the analyzer
   Clean upper portion of the main pipettor with alcohol wipe
   Inspect waste filter bottle for fluid
   Run system check

3. Periodic Maintenance to be performed by the manufacturer's service engineer.

E. Calculations

Patient test results are determined automatically by the system software. The amount of analyte in a sample is determined from the measured light production by means of a stored nonlinear calibration curve. Patient test results can be reviewed using the Sample Results screen. Refer to the Operator's Guide for complete instructions on reviewing results.

9. REPORTABLE RANGE OF TEST RESULTS

Results are reported to the nearest thousandth (0.001). The lowest reportable TSH result is 0.003 mcIU/mL. Results above the top standard (generally near 100 mcIU/mL) is repeated after a 1:10 dilution with sample diluent A. Further dilutions may be needed for samples with extremely high levels. Estimates of imprecision can be generated from long-term quality control pool results.

10. QUALITY CONTROL (QC) PROCEDURES

A. Bench quality controls are used in this analytical method. Bench quality control specimens are tested with each analytical run (a set of consecutive assays performed without interruption) so that judgments may be made on the day of analysis. The data from these materials are then used in estimating methodological imprecision and in assessing the magnitude of any time-associated trends.

B. The bench controls are purchased in sufficient quantity to provide serum samples for all the assays for approximately 1 year. Ranges are established after 20 parallel runs with previously established controls. The quality control pools comprise three levels of concentration spanning the low, borderline and high ranges for TSH.

C. Bench quality controls are placed at the beginning of each analytical run. After

analysis, the long-term quality control charts (Levey-Jennings) for each control material are consulted to determine if the system is "in control." The Levey Jennings chart plots the quality control material observations on the y-axis and the date of the observation on the x-axis. Quality control material observations are compared with the 95% and 99% confidence limits as well as with the center line (the overall mean of the characterization runs) prior to reporting any results. The system is out of control if any of the following events occur for any one of the quality control materials:

The observation from a single pool falls outside the 99% confidence limits.
The observations from two pools fall either both above or both below the 95% confidence limits.
The observations from eight successive runs for one pool fall either all above or all below the center-line and the current result is above or below the 95% confidence limits.

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

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 results are released. Consult with the supervisor for appropriate actions.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

A. The lowest reportable value is approximately 0.003 mcIU/mL. According to the manufacturer, this is the lowest detectable level of TSH distinguishable from zero with 95% confidence. The manufacturer states that the functional sensitivity for the 3rd generation HYPERsensitive hTSH assay is 0.015 mcIU/mL.

B. There is no upper limit of the reportable values since high samples are diluted until they contain a measureable TSH level.

C. The TSH results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information. Evaluate hTSH levels carefully when the hTSH result is inappropriate for the apparent state of thyroid function.

D. This assay is not validated for testing neonatal serum hTSH levels.

E. According to the manufacturer the following substances do not interfere with the assay:

Hemoglobin up to 500 mg/dL
Bilirubin up to 10 mg/dL
Triglycerides up to 1800 mg/dl
Human albumin levels up to 9 g/dL

F. The manufacturer performed testing to determine the cross reactivity of the assay to substances which are similar in structure to hTSH. Cross reactants were spiking into the Access HYPERsensitive hTSH calibrator with a dose of 4.0 mcIU/mL.

Substance	Analyte Added (mIU/mL)	Cross Reactivity (%)
hLH	3,000	0.01
hFSH	1,000	0.09
hCG	1,000	Not detectable
h β-TSH	200	-0.8
h α-TSH	200	5.63

G. For assay employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients that have been regularly exposed to animals or have received immunotherapy or diagnostics procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interferes with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may also be present in patient samples. Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

13. REFERENCE RANGES (NORMAL VALUES)

0.34 – 5.60 mcIU/mL Based on manufacturer's studies using non-parametric analysis of the results measured in 217 human serum samples from apparently healthy male and female subjects with normal thyroid profiles.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable to this procedure.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens should be maintained at 20-25 ^{o}C during testing. After testing, the samples are stored at -70 ^{o}C or colder.

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

There are no acceptable alternative methods of analysis. Specimens may be stored at 4-8 ^{o}C for no longer than 2 days. Otherwise, specimens should be stored -70 ^{o}C or colder until the system is returned to functionality.

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

Not applicable to this procedure. However, e-mail notification is sent to NHANES for results that are above 10.0 mcIU/mL.

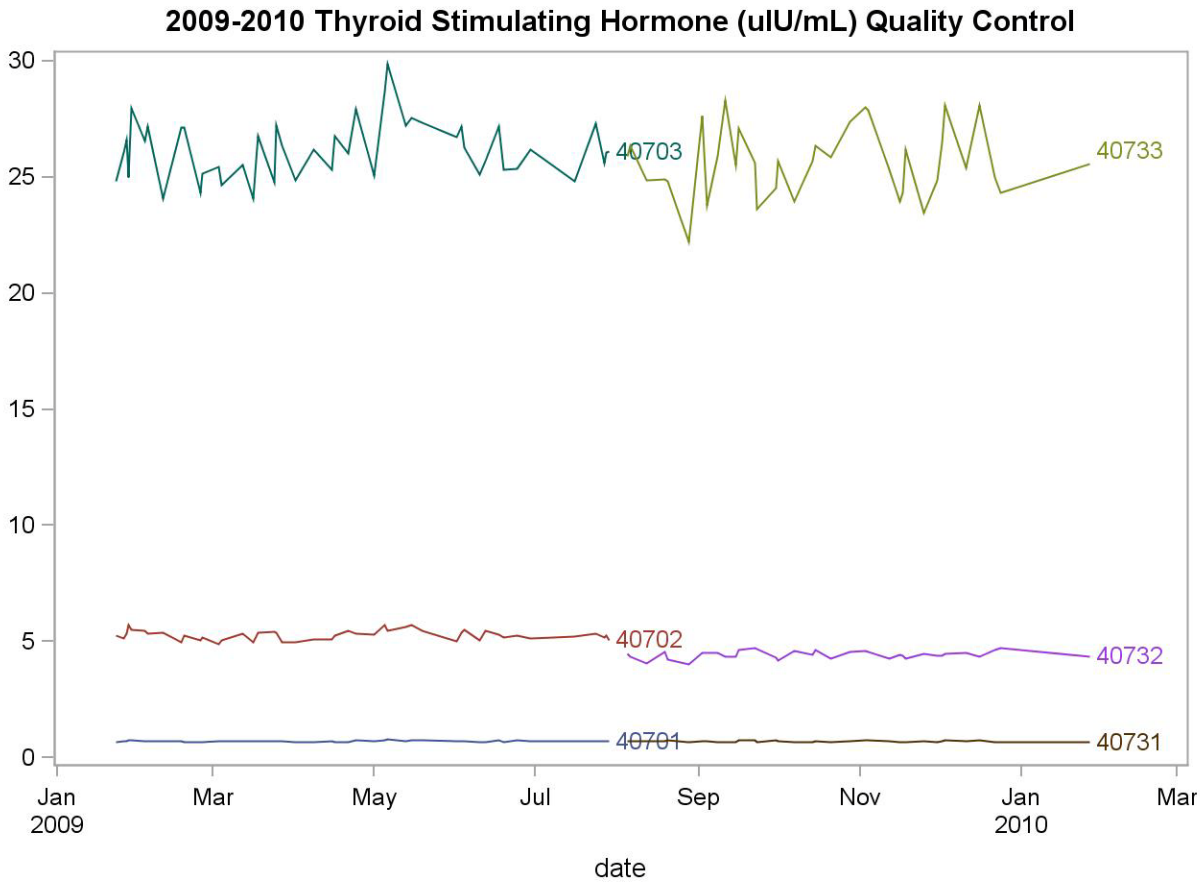
18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Standard record keeping should be used for tracking specimens. Samples are inspected upon arrival and new boxes are added to an Excel worksheet (sample log) used to track boxes. This sample log is used to track the status of testing and resulting.

The residual serum is stored at $\leq$ -70 ^{o}C for 6 months after analysis, then it is returned to the NHANES Repository in Rockville, MD for long-term storage.

19. Summary Statistics and QC Graphs

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
40703	46	23JAN09	29JUL09	26.1892	1.2453	4.8
40701	46	23JAN09	29JUL09	0.7088	0.0285	4.0
40702	46	23JAN09	29JUL09	5.2813	0.2146	4.1
40733	36	05AUG09	27JAN10	25.6407	1.4947	5.8
40731	36	05AUG09	27JAN10	0.7024	0.0283	4.0
40732	36	05AUG09	27JAN10	4.4381	0.1740	3.9



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