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

Collaborative Laboratory Services 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 (hTSH) 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 hTSH Assay is a two-site immunoenzymatic ("sandwich") assay, for the quantitative determination of human thyroid-stimulating hormone in human serum, using the Access Immunoassay System. 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 serum 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 serum hTSH. Separation in a magnetic field and washing removes materials not bound to the solid phase. A chemiluminescent substrate, Lumi-Phos® 530, is added to the reaction 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 by means of a stored, multi-point calibration curve.

The major use of the hTSH assay 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 antithyroid treatment in hyperthyroidism; 3)follow T4 suppression in "cold nodules" and non-toxic goiter; 4)assess the response to TRH stimulation testing. hTSH measurements are also used to identify subclinical and latent hypothyroidism or hyperthyroidism.

2. SAFETY PRECAUTIONS

Consider all plasma or serum specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves, laboratory coats. Place disposable plastic, glass, and paper (pipette tips, gloves, etc.) that contact plasma and any residual sample material in a biohazard bag and keep these bags in appropriate containers until disposal by maceration chlorination. Wipe down all work surfaces with Germicidal Disposable Wipe when work is finished.

Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood.

Reagents and solvents used in this study include those listed in Section 6. Material safety data sheets (MSDSs) for these chemicals are readily accessible as hard copies in the lab.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

a. Microsoft Excel software on a PC and our Laboratory Information Systems (L.I.S.) are used to manage the data. The test is analyzed on a Beckman Coulter Access2 Immunoassay System. The Access2 is interfaced to the Laboratory Information Systems (L.I.S.) with a bi-directional interface. After tests are completed, the results will go to the L.I.S. Host Computer Interface to be verified by qualified analyst.
b. Reflex testing is set up in the L.I.S. to order a repeat of any critical result, to verify abnormal values.
c. Statistical evaluation of the runs are accomplished with Microsoft Excel software on a PC.
d. A result file is generated in the L.I.S. database. The file is opened and copied to an Excel spreadsheet for evaluation. The run numbers, and date specimens were received are entered into the Excel file. The Excel spreadsheet results file data are copied to the shipment Excel file and sent using Internet FTP transfer of files or e-mailed to Westat within 21 days of sample receipt.
e. The Excel files containing all raw data and results are backed up once a week using a CD writer or External drive for storage. Files stored on the L.I.S. network are automatically backed up nightly to tape.
f. Documentation for data system maintenance is contained in printed copies of data records, as well as in "system log" files on the local hard drives used for the archival of data.

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

a. Interferences:
   1) No interference from <10 mg/dL bilirubin or <1800 mg/dL triglycerides.
   2) No interference from <500 mg/dL hemoglobin. Grossly hemolyzed should not be used.

b. Separated serum or plasma should not remain at +15°C to +30°C longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

c. Fasting is not required.

d. A minimum of 0.5 mL serum is needed for the TSH.

e. Sample volume for individual test is 110 µL.

f. Sample is run singly.

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: Beckman Access2 Immunoassay System

b. Materials:
   1) Access Immunoassay 1.0 mL Insert Cups (*Cat. #81915*)
   2) Access Immunoassay 3.0 mL Sample Container (*Cat. #81914*)
   3) Access Immunoassay Reaction Vessels (*Cat. #81901*)
   4) Stockwell Scientific Tubes, 13x100mm, polystyrene, (Prod #8570)
   5) S/P Plastic Transfer Pipette (*Cat. #P5214-10*)

c. Reagent Preparation:
   1) Access HYPERsensitive hTSH Reagent Pack (*Cat. #33820*): 100 determinations, 50 tests/pack. Contains the following components:

   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.
      a) Provided ready to use.
      b) Store upright at 2-10°C.
      c) Packs must be refrigerated at 2-10°C for two hours before loading on instrument.
      d) Unopened packs are stable until expiration date when stored as directed.
      e) After initial use, pack is stable for 28 days at 2-10°C.
      f) CAUTION: Sodium azide may react with lead and copper plumbing. On disposal of liquid, flush drain with large volume of water. ProClin is a potential skin sensitizer, in case of contact with reagent, thoroughly flush with water.

   2) Access Substrate (*Cat. #81906*)
      a) Lumi-Phos 530 (buffered solution containing dioxetane Lumigen PPD, flourescer, and surfactant).
      b) Allow substrate to equilibrate, unopened at room temperature for a minimum of 18 hours (maximum 14 days) prior to use.
      c) Unopened substrate is stable until expiration date when stored at 2-10°C

d) Opened substrate on board in external fluids tray is stable for 14 days.
e) Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.

3) Access Wash Buffer (*Cat. #81907*).
a) Tris buffered saline, surfactant, 0.1% sodium azide and 0.1% ProClin 300.
b) Stable until expiration date when stored at room temperature.

d. Standards Preparation: No preparation required.
1) Beckman Access HYPERsensitive hTSH Calibrators (*Cat. #33825*).

e. Control Material:
1) Bio-Rad Immunoassay Plus Controls (Levels 1, 2, and 3) (*Cat. #371, 372, 373*).
a) Reconstitute each vial with 5 mL deionized water using a volumetric pipette. Replace the stopper and let control stand for 15 minutes. Before using, invert vial several times to mix.
b) Reconstituted control is stable for 7 days when stored at 2-8°C.
c) At least two levels of control should be analyzed in a 24 hour time period.
d) Ensure that assay control values are within the concentration ranges stated in the package insert or calculated from cumulative data at CLS.
e) Refer to Quality Control Flow Chart for action decision guidelines.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Calibrators: Beckman Access HYPERsensitive hTSH Calibrators (*Cat. #33825*).
1) Six levels of calibrator.
2) Provided ready to use.
3) Mix contents by gently inverting prior to use.
4) Stable until expiration date when stored at 2-10°C.
5) Refer to calibration card enclosed with each set of calibrators for actual concentrations.

b. Calibration:
1) Calibration is required when a new lot of hTSH reagent is loaded, when the calibration curve expires (curve stability is 28 days), or when controls are out of range.
2) Refer to Access2 Quick Reference Guide or Access2 “help” icon for detailed instructions on programming a calibration.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries
1) Enter test in L.I.S. as a part of a panel according to procedure listed in this document.

b. Sample Preparation
1) Thaw samples and vortex, mixing well. .
2) Specimen handling, labeling and transferring serum.

c. Operation
1) For detailed instructions on operating the Access, refer to the Access2 Quick Reference Guide, or use the “help” icon on the instrument screen.

d. Recording of Data
1) Operator will review and verify results in the L.I.S.
2) The L.I.S. reorders tests to verify any critical results. These results are stored in the L.I.S. along with the original results. Original values are used when repeat results match the original within 3 cv’s.
3) Project supervisor will export data from the L.I.S. into an Excel file. The data is copied in into another Excel file for further evaluation.
4) An Excel spreadsheet printout of the results for each container ID is made and comments noted.
5) Project supervisor reviews the results. If problems noted with results or QC, Project Supervisor investigates and discusses issues if necessary with Laboratory Director. Repeat samples if necessary.
6) Daily log sheets are completed and any problems or issues noted.

e. Replacement and Periodic Maintenance of Key Components

f. Calculations:
   1) The Access Immunoassay System performs all calculations internally to produce the final reported result. Patient test results are determined automatically by the system software using a weighted four parameter logistic curve (4PLC) math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data.

9. REPORTABLE RANGE OF RESULTS

a. Analytical Range:
   1) 0.01 -The value of the highest calibrator (~100) µIU/mL.
   2) A result over range high should be reported as ">100". To obtain a numerical answer, the specimen may be diluted one volume of sample to four volumes of 0.0 Calibrator or Access Sample Diluent A (*Cat. #81908*). After assaying the diluted sample, multiply the printed value by 5 to obtain the reportable answer.
   3) Beckman defines sensitivity as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the hTSH determination is 0.003 µIU/mL.
   4) The literature suggests functional (clinical) sensitivity for hTSH assays is defined in terms of precision. Dose responses of 0.01-0.02 µIU/mL with interassay (between run) Cvs of $\leq$20% are considered to demonstrate "Third Generation" functional sensitivity performance.
   5) CLS will periodically monitor low TSH reproducibility between runs by repeating patient samples. Previously repeated analysis within 1 day of samples with initial values between 0.01 and 0.03 yielded 8 results with no difference and two that differed by 0.01.
   6) 0 is not a reportable value. Report results below 0.01 as <0.01.

10. QUALITY CONTROL (QC) PROCEDURES

a. Blind QC Specimens are included in the samples received from NHANES.
b. Bio-Rad Immunoassay Plus Controls levels 1,2,3 are assayed prior to running CDC-NHANES samples and after running CDC-NHANES samples.
c. Acceptable Answer:
   1) Controls must be within ±2 S.D.
   2) Refer to Quality Control Flow Chart for action decisions guidelines.

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

Remedial action for out of control conditions include examination of the pipetting and detection equipment and examination of reagent materials. The QC parameters are compared to the patient means to look for confirmatory or disconfirmatory evidence. When the 2 2s and/or 1 3s rules are violated, samples are repeated following corrective maintenance or reagent changes.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

a. Hemolyzed samples with up to 500 mg/dL hemoglobin have no significant interference.
b. <10 mg/dL bilirubin has no significant interference.
c. Lipemia has no significant interference in samples containing equivalent of 1800 mg/dL triglycerides.
d. This assay has been formulated to minimize the effect of human anti-mouse antibodies or heterophile antibodies which may be present in some patient samples.
e. TSH levels obtained during the first trimester of pregnancy or whenever very high hCG levels are present should be interpreted with caution.

13. REFERENCE RANGES (NORMAL VALUES)

TSH

Serum	*µIU/mL*
Normal TSH	0.24-5.4
Equivocal	5.5-10.0

Adult reference Range values were established from wellness participants with an age mix similar to our patients. These data were analyzed using non-parametric techniques described by Reed (Clin Chem 1971;17:275) and Herrara (J Lab Clin Med 1958;52:34-42) which are summarized in recent editions of Tietz' textbook. Descriptions appear in Clin Chem 1988;34:1447 and Clinics in Laboratory Medicine June 1993;13:481.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

There are no critical call back values.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens arrive frozen with dry ice. Specimens are kept frozen at -70°C until ready to analyze. Sample is thawed, mixed well by vortexing, then transferred to sample cup or sample insert cup on the Access.

Specimen vials are returned to container and refrigerated after transfer of aliquot and double checking of Sample I.D. Specimen vial container is placed in -70°C freezer after testing is complete.

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

Samples will remain in -70°C freezer until instrument is back in operation.

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

The collaborating agency with access to patient identifiers or the responsible medical officer receives an Excel file with all results for a specimen with any critical values. These files with critical values are sent in advance of results that are not abnormal, unless all results are ready to send at the same time. The earliest reporting of results would be the day after arrival of specimens. More frequently two to three days after receiving specimens.

Test results that are not abnormal are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an Excel file, either through Internet FTP transfer of files or electronic mail or other electronic means.

All data are reported electronically to Westat within 21 days of receipt of specimens.

Internet FTP transfer of files is available and is preferred for data transfer.

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

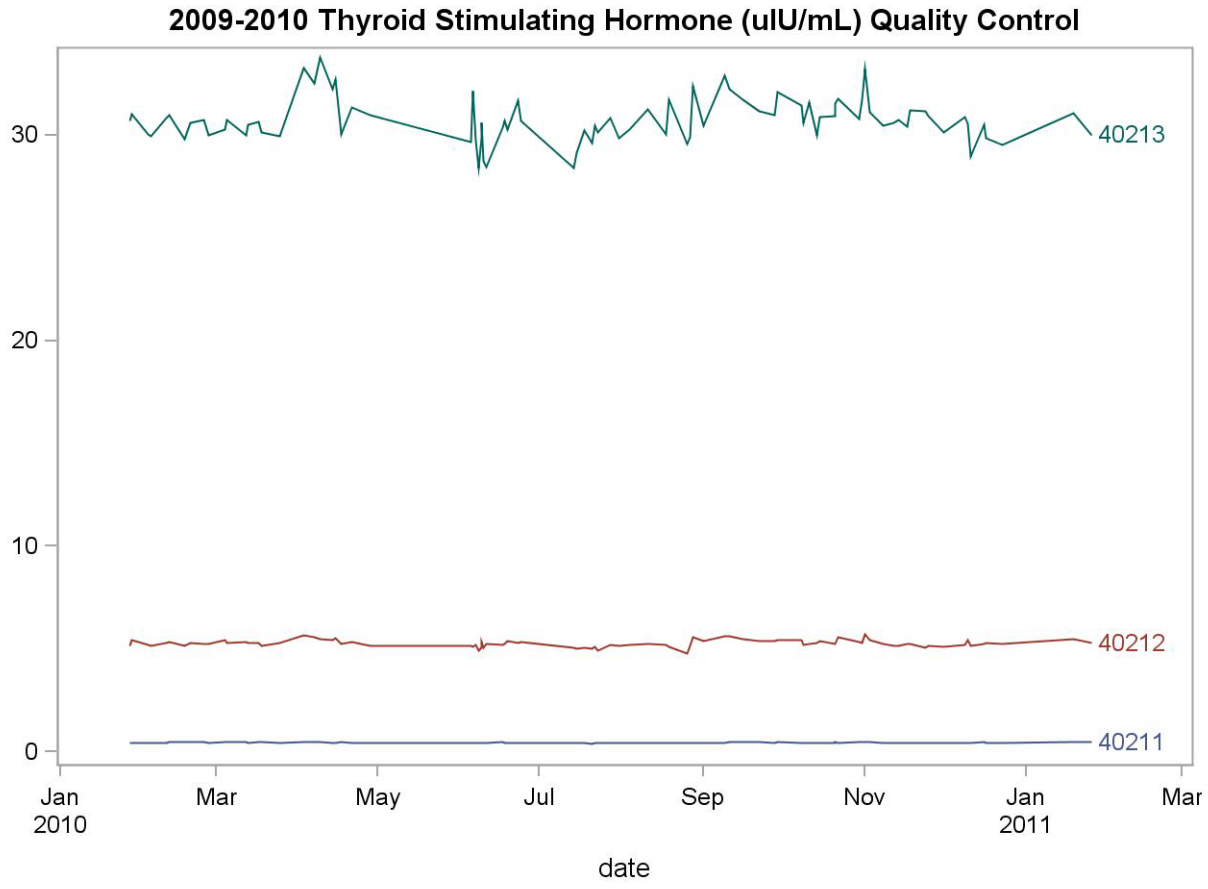
In general, when specimens are received, the specimen ID number, and a name identifying the container ID and slot number is entered into the Laboratory Information System (L.I.S.) database. New barcodes are printed and the specimens stored in a refrigerator. Samples are aliquoted to a sample cup or sample insert cup with the new barcodes. The specimen ID is read off of the tube by a barcode reader. Tracked in the database are the date and time of entry into the L.I.S., date and time analysis completed, and who certified the results.

Microsoft Excel spreadsheets are used to keep records and track specimens with the data taken from the Laboratory Information System. Logs are kept including information of when samples arrive, are processed and tested, when frozen after testing, and when returned to NHANES for long term storage.

The Project supervisor is responsible for keeping a logbook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. It is recommended that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study.

19. SUMMARY STATISTICS and QC GRAPHS

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
40213	88	27JAN10	26JAN11	30.7164	1.0577	3.4
40211	88	27JAN10	26JAN11	0.4418	0.0182	4.1
40212	87	27JAN10	26JAN11	5.2720	0.1682	3.2



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