Laboratory Procedure Manual

Analyte: **Thyroid Stimulating Hormone**

Matrix: **Serum**

Method: **Chemiluminescent Immunoassay**

Method No.:

Revised:

as performed by: *Collaborative Laboratory Services*
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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.

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
l40_b	LBXTSH	Thyroid Stimulating Hormone

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The Access hTSH Assay is a two-site (sandwich), paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of human thyroid-stimulating hormone (hTSH) 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 then added to the reaction vessel, and light generated by the reaction is measured with a luminometer. The photon production is proportional to the amount of enzyme conjugate bound to the solid support. The amount of analyte in the sample is determined by means of a stored, multi-point calibration curve.

The major use of the TSH assay has been in the differential diagnosis of primary hypothyroidism from secondary and tertiary hypothyroidism. In primary hypothyroidism, TSH levels are significantly elevated, while in secondary and tertiary hypothyroidism, TSH levels are low or normal. The use of TSH assays for the recognition of primary hyperthyroidism has been made possible by the development of very sensitive assays which can detect very low levels of TSH.

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 Sani-Cloth HB, 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 (LIS) are used to manage the data. The test is analyzed on a Beckman Access2 Immunoassay System. When ordered tests are completed for each sample, the results are printed out by Beckman Access2 instrument.

B. A statistical evaluation of the runs is accomplished with Microsoft Excel software on a PC. Completed sample data is entered into an Excel spreadsheet for evaluation. The Excel spreadsheet results file data are copied to the shipment file and saved as a comma delimited file (CSV) and e-mailed to Westat within 21 days of sample receipt.

C. The Excel files containing all raw data and results are backed up once a week using a CD writer or Zip drive for storage.

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 samples should not be used.

B. Separated serum or plasma should not remain at +15–30°C longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2–8°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 0.3 mL serum is needed for the TSH.

E. Sample volume for individual test is 55 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 2 mL Sample Cups (Cat. #81902)

(2) Access Immunoassay Reaction Vessels (Cat. #81901)

(3) 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.

Beckman Access Hypersensitive hTSH Calibrators (Cat. #33825).

E. Bio-Rad Immunoassay Plus Control Materials (Levels 1, 2, and 3) (Cat. #371, 372, 373).

(1) Reconstitute each vial with 5 mL of deionized water using a volumetric pipette. Replace the stopper and let control stand for 15 minutes. Before using, invert vial several times to mix.

(2) Reconstituted control is stable for 7 days when stored at 2–8°C.

(3) At least three levels of control should be analyzed in a 24-hour time period.

(4) Ensure that assay control values are within the concentration ranges stated in the package insert or calculated from cumulative data at CLS.

(5) 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

Program requested tests on Access instrument using sample I.D. and slot I.D. (See Attachment G).

B. Sample Preparation

Thaw samples and vortex, mixing well.

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.

(2) Check supplies.

(3) Program the requested tests.

(4) Prepare the primary sample tubes or sample cups and load them on the sample trays.

(5) 110 µl of sample is required for each determination in addition to the sample cup or sample tube dead volume.

(6) Load the trays onto the instrument.

(7) Press RUN.

D. Recording of Data

(1) Operator will review results.

(2) Operator will place printouts in file labeled for NHANES samples.

(3) Results and information about the run are entered into an Excel spreadsheet on a PC and copied into another Excel file to further evaluate the data.

(4) A printout of the Excel spreadsheet for each container ID results is made and comments noted.

(5) Project supervisor reviews the results. If problems noted with patient 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.

(7) Repeat values are used when they match the original results within 3 cv’s.

E. Replacement and Periodic Maintenance of Key Components

(See Attachment AC) for the Access Maintenance Schedule.

F. Calculations

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 four-parameter logistic curve math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration curve.

9. REPORTABLE RANGE OF RESULTS

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 <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, and 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 includes 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 nonconfirmatory 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)

Table 1. 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 Clin Lab Med. 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, and then transferred to sample 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)

Test results 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 a comma delimited file, either through electronic mail or other electronic means.

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

Internets FTP transfer of files or dial up modem transfer options are available.

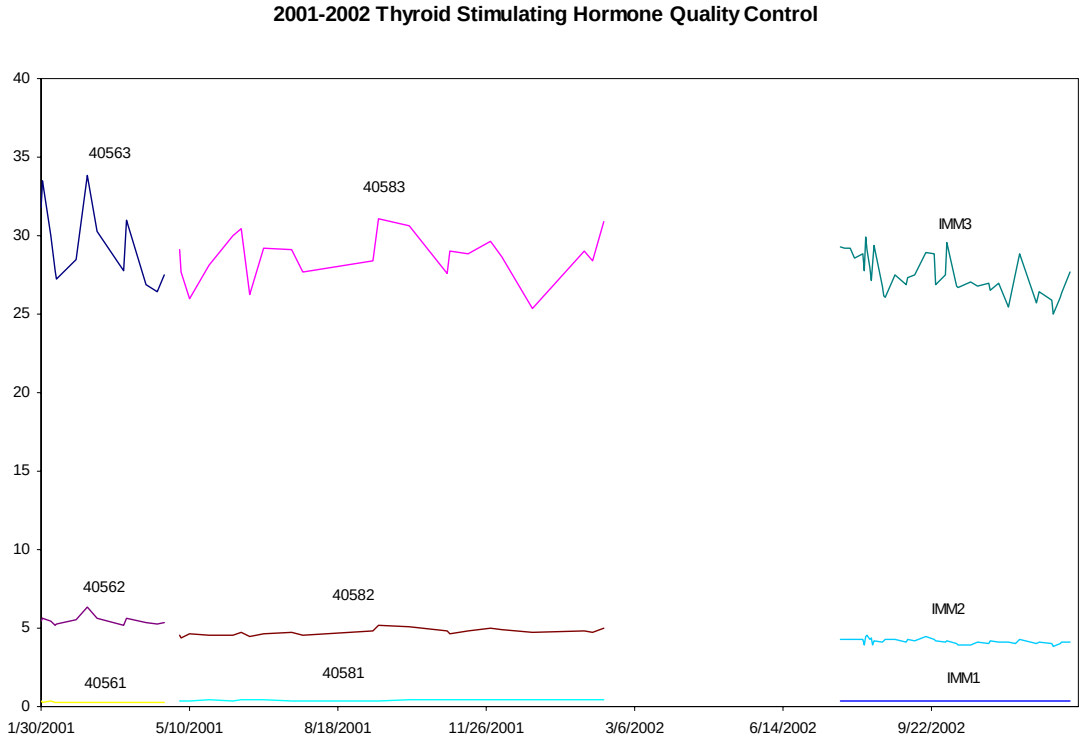
18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Microsoft Excel spreadsheets are used to keep records and track specimens analyzed on the Access. 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

Summary Statistics for Thyroid Stimulating Hormone by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
40561	13	1/30/2001	4/23/2001	0.305	0.011	3.7
40562	13	1/30/2001	4/23/2001	5.471	0.305	5.6
40563	13	1/30/2001	4/23/2001	29.425	2.504	8.5
40581	22	5/3/2001	2/13/2002	0.407	0.021	5.2
40582	22	5/3/2001	2/13/2002	4.738	0.206	4.4
40583	22	5/3/2001	2/13/2002	28.688	1.522	5.3
IMM1	41	7/23/2002	12/24/2002	0.36	0.01	3.5
IMM2	41	7/23/2002	12/24/2002	4.17	0.16	3.8
IMM3	41	7/23/2002	12/24/2002	27.49	1.27	4.6



REFERENCES

1. Beckman Access Assay Manual, 1998.
2. Tietz, NW. Textbook of Clinical Chemistry. Philadelphia: W.B. Saunders;1986.
3. Tietz, NW. Specimen Collection and Processing; Sources of Biological Variation. In: Textbook of Clinical Chemistry. 2nd Ed. Philadelphia:W.B. Saunders;1994.
4. National Committee for Clinical Laboratory Standards. Procedures for the Handling and Processing of Blood Specimens, Approved Guideline. Publication H18-A. NCCLS:Villanova, PA;1990.
5. Tietz, NW, ed. Clinical Guide to Laboratory Tests. 3rd Ed. Philadelphia:W.B. Saunders;1995.
6. National Committee for Clinical Laboratory Standards. How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory, Approved Guideline. Publication C28-A. NCCLS:Villanova, PA;1995.
7. Tietz, NW, ed. Fundamentals of Clinical Chemistry. 3rd Ed. Philadelphia:W.B. Saunders;1987.
8. Henry, JB, ed. Clinical Diagnosis and Management by Laboratory Methods. 18th Ed. Philadelphia:W.B. Saunders;1991.
9. Young, DS. Effects of Drugs on Clinical Laboratory Tests. 4th Ed. Washington, DC:AACC Press;1995.
10. Friedman, RB, DS Young. Effects of Disease on Clinical Laboratory Tests. 3rd Ed. Washington, DC:AACC Press;1997.
11. Young, DS. Effects of Preanalytical Variables on Clinical Laboratory Tests. 2nd Ed. Washington, DC:AACC Press;1997.
12. National Committee for Clinical Laboratory Standards. Method Comparison and Bias Estimation Using Patient Samples, Approved Guideline. Publication EP9-A. Villanova, PA:NCCLS;1995.
13. National Committee for Clinical Laboratory Standards. Precision Performance of Clinical Chemistry Devices, Tentative Guideline. 2nd Ed. Publication EP5-T2. Villanova, PA:NCCLS;1992.
14. Bio-Rad Immunoassay Plus Package Insert, July 1999.