

Laboratory Procedure Manual

Analyte: **Thyroxin (T4)**

Matrix: **Serum**

Method: **Hitachi 704 Analyzer**

Method No.:

Revised:

as performed by: *Collaborative Laboratory Services
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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 2001–2002 data.

Two laboratories performed this testing during 2001–2002. In order to maintain confidentiality of the participants, the quality control summary statistics and graphs were combined to mask the individual analysis dates from the two laboratories. Methods for both labs are included in this release. Most methods for Lab18 analytes are in one combined file. Methods Lab40 are described in a separate file for each analyte tested.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label (and SI units)
lab40	LBXT4	Thyroxin (T4) (ug/dL)
	LBDT4SI	Thyroxin (T4) (nmol/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The Access Total T4 assay is a paramagnetic particle, chemiluminescent, competitive binding enzyme immunoassay for the quantitative determination of total thyroxine (T4) in human serum, using the Access Immunoassay System. A sample is added to a reaction vessel with anti-thyroxine antibody, thyroxine-alkaline phosphatase conjugate, and paramagnetic particles coated with goat anti-mouse capture antibody and a stripping agent to dissociate all T4 from serum-binding proteins. Thyroxine in the sample competes with the thyroxine-alkaline phosphatase conjugate for binding sites on a limited amount of specific anti-thyroxine antibody. Resulting antigen: antibody complexes bind to the capture antibody on the solid phase. 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 photon production is inversely proportional to the concentration of T4 in the sample. The amount of analyte in the sample is determined by means of a stored calibration curve.

Total T4 measurements are used alone, or in conjunction with other thyroid tests, in the diagnosis and treatment of thyroid disorders.

Elevated levels of T4 occur in Graves' disease, subacute thyroiditis, toxic nodule or secondary hyperthyroidism. Decreased levels occur in primary hypothyroid diseases such as Hashimoto's thyroiditis and neonatal hypothyroidism due to defects at the hypothalamic-pituitary level.

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 (L.I.S.) 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.

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. Manufacturer recommends frozen specimens can be stored up to six months before testing. 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 Total T4.

e. Sample volume for individual test is 30 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 Total T4 Reagent Pack (Cat. #33800), 100 determinations, 50 tests/pack. Contains the following components.
 - R1a: Paramagnetic particles coated with goat anti-mouse IgG suspended in Tris buffered saline, with surfactant, bovine serum albumin, 8-anilino-1-naphthalencsulfonic acid, <0.1% sodium azide, and 0.1% ProClin 300.
 - R1b: Mouse monoclonal antibody to thyroxine diluted in Tris buffered saline, with surfactant, protein, <0.1% sodium azide, and 0.1% ProClin 300.
 - R1c: Thyroxine-alkaline phosphatase conjugate diluted in Tris buffered saline, with surfactant, protein, <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 stable until expiration date when stored as directed.
 - e) After initial use, pack is stable for 14 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, fluorescer, 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 Total T4 Calibrators (Cat. #33805).
- e. Control Material: 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 three 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: Access Total T4 Calibrators (Cat. #33805).
- 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 T4 reagent is loaded, when the calibration curve expires (curve stability is 21 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) 55 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 match the original results within 3 CSV'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) The analytical range for Total T4 is 0.5 - the value of the highest calibrator (~30) µg/dL.
- 2) A result over range high should be reported as ">30". To obtain a numerical answer, the specimen may be diluted with an equal volume of the T4 0.0 Calibrator. After assaying the diluted sample, multiply the printed values by two to obtain the reportable answer.
- 3) Limits of detection (LOD) are established by Beckman Coulter and linearity data verifies the reportable range. Detection of results below the reportable range is not relevant and formal limit of detection study is unnecessary.
- 4) Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the Total T4 determination is 0.50 µg/dL.
- 5) 0 is not a reportable value. Report results below 0.5 as "<0.5".

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 (See Attachment I).

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 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. Measurement of Total T4 gives a reliable reflection of clinical thyroid status in the absence of binding abnormalities. Changes in binding proteins can occur which affect the level of Total T4 but leave the level of unbound hormone unchanged.
- e. Thyroid status should not depend on results from a single Total T4 test. Complete thyroid status evaluation should include additional thyroid function test and should be interpreted in light of the total clinical picture.

13. REFERENCE RANGES (NORMAL VALUES)

Total T4

	µg/dL
Serum	5.4-12.8

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 Herrera (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. June 1993;13:481.

Concentrations of total T4 were measured in sera from 533 consecutive presumably healthy wellness participants. The median was 8.33 µg/dL and the 1st and 99th percentiles were 5.44 and 12.85. CLS will use 5.4 to 12.8 as the reference range.

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.

Internet FTP transfers 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.

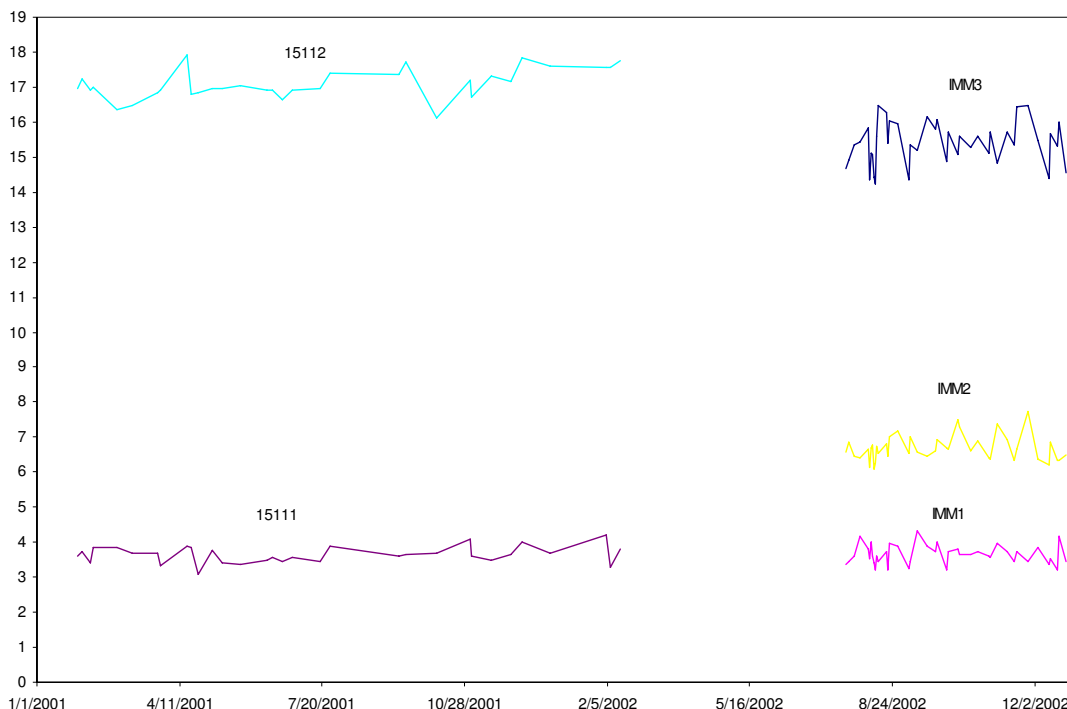
The Project supervisor is responsible for keeping a logbook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. It is recommended that records, including related QA/QC data, be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

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Thyroxine by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
15111	32	1/30/2001	2/14/2002	3.646	0.241	6.6
15112	32	1/30/2001	2/14/2002	17.085	0.427	2.5
IMM1	41	7/23/2002	12/24/2002	3.64	0.28	7.7
IMM2	41	7/23/2002	12/24/2002	6.68	0.37	5.5
IMM3	41	7/23/2002	12/24/2002	15.39	0.61	4.0

2001-2002 Thyroxine Quality Control



REFERENCES

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