Laboratory Procedure Manual

Analyte: **Free Triiodothyronine, Free T3**

Matrix: **Serum**

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

Method No:

Revised:

as performed by:

Collaborative Laboratory Services
Ottumwa, Iowa

contact: Dr. Quackenbush, M.D

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.

Free T3 (FT3) in Serum
NHANES 2011-2012

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_G	LBXT3F	Free Triiodothyronine (pg/mL)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The Access Free T3 assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of free triiodothyronine levels in human serum and plasma using the Access Immunoassay Systems. The Free T3 Assay is a competitive binding immunoenzymatic assay. Sample is added to a reaction vessels with an anti-T3 monoclonal antibody conjugated to alkaline phosphatase. During the incubation, free T3 in the sample reacts with the anti-T3 antibody. Particles coated with streptavidin and biotinylated T3 analog are then added to the mixture. Unoccupied binding sites on the anti-T3 antibody are bridged to the particle through the T3 analog. After incubation, materials bound to the solid phase are held in a magnetic field while the unbound materials are washed away. 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 inversely proportional to the concentration of Free T3 in the sample. The amount of analyte in the sample is determined by means of a stored calibration curve.

Free T4 and Free T3 regulate normal growth and development by maintaining body temperature and stimulating calorigenesis. Free T4 and Free T3 affect all aspects of carbohydrate metabolism as well as certain areas of lipid and vitamin metabolism.

With normal levels of thyroid binding proteins, free T3 levels correlate with Total T3. Measuring free T3 is useful when altered levels of total T3 occur due to changes in thyroid hormone binding proteins, especially in cases with altered TBG or low albumin concentrations. Free T3 is elevated alone (T3 toxicosis) in about 5% of hyperthyroids.

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 is 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 <20 mg/dL bilirubin or <3000 mg/dL triglycerides or <500 mg/dL cholesterol.
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 0.5 mL serum is needed for FT3.

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 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 Free T3 Reagent Pack (*Cat. #A13422*), 100 determinations, 50 tests/pack. Contains the following components.

R1a: Dynabeads® paramagnetic particles coated with streptavidin in TRIS buffer with protein (aves), surfactant and <0.1% NaN_3.

R1b: MES buffer.

R1c: Biotinylated T3 analog in a TRIS buffer with protein (aves), surfactant, and <0.1% NaN_3.

R1d: TRIS buffer containing animal protein (goat, bovine, aves), surfactant, <0.1% NaN_3, and 0.5% ProClin™300

R1e: Monoclonal antibody-alkaline phosphatase conjugate in an ACES buffer with protein (aves), surfactant and <0.1% NaN_3.

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, 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 Free T3 Calibrators (*Cat. #A13430*).

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: Access Free T3 Calibrators (*Cat. #A13430*).
   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 Free T3 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.
   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) The analytical range for Free T3 is 0.88 - the value of the highest calibrator (~30) pg/mL.
   2) A result over range high should be reported as ">30". Samples cannot be diluted for Free T3 determinations...
   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 Free T3 determination is 0.88 pg/mL.
   5) 0 is not a reportable value. Report results below 0.88 as "<0.88".

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 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. <20 mg/dL bilirubin has no significant interference.
c. Lipemia has no significant interference in samples containing equivalent of 3000 mg/dL triglycerides or up to 500 mg/dL cholesterol.
d. Patient with biotin (vitamin H) treatment may have elevated free T3 levels due to displacement of the biotinylated T3 analog from paramagnetic particles coated with streptavidin.
e. Substances added to serum samples observed per-cent changes as follows:

Substance	Analyte Added (mg/dl)	% change
Aspirin	75	1.3
Sodium Salycilate	75	13.9
Ibuprophen	150	17.2
Acetaminophen	200	-5.0
Phenylbutazone	7.5	3.9
Thiouracil	5	2.1
Phenytion	10	-0.1

Furosemide	2	15.9
Carbamazeprine	12	12.7
Methimazol	0.4	-0.1
Oleic Acid	283	11.4
Linoleic Acid	280	0
D-biotin	0.001	-7.3

f. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the sample. Individuals who have been regularly exposed to animals or who have received immunotherapy or diagnostic 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, or human anti-triiodothyronine antibodies may be present in patient samples.

g. Free T3 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.

13. REFERENCE RANGES (NORMAL VALUES)

Free T3

	pg/mL
Serum	2.5-3.9

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, and 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.

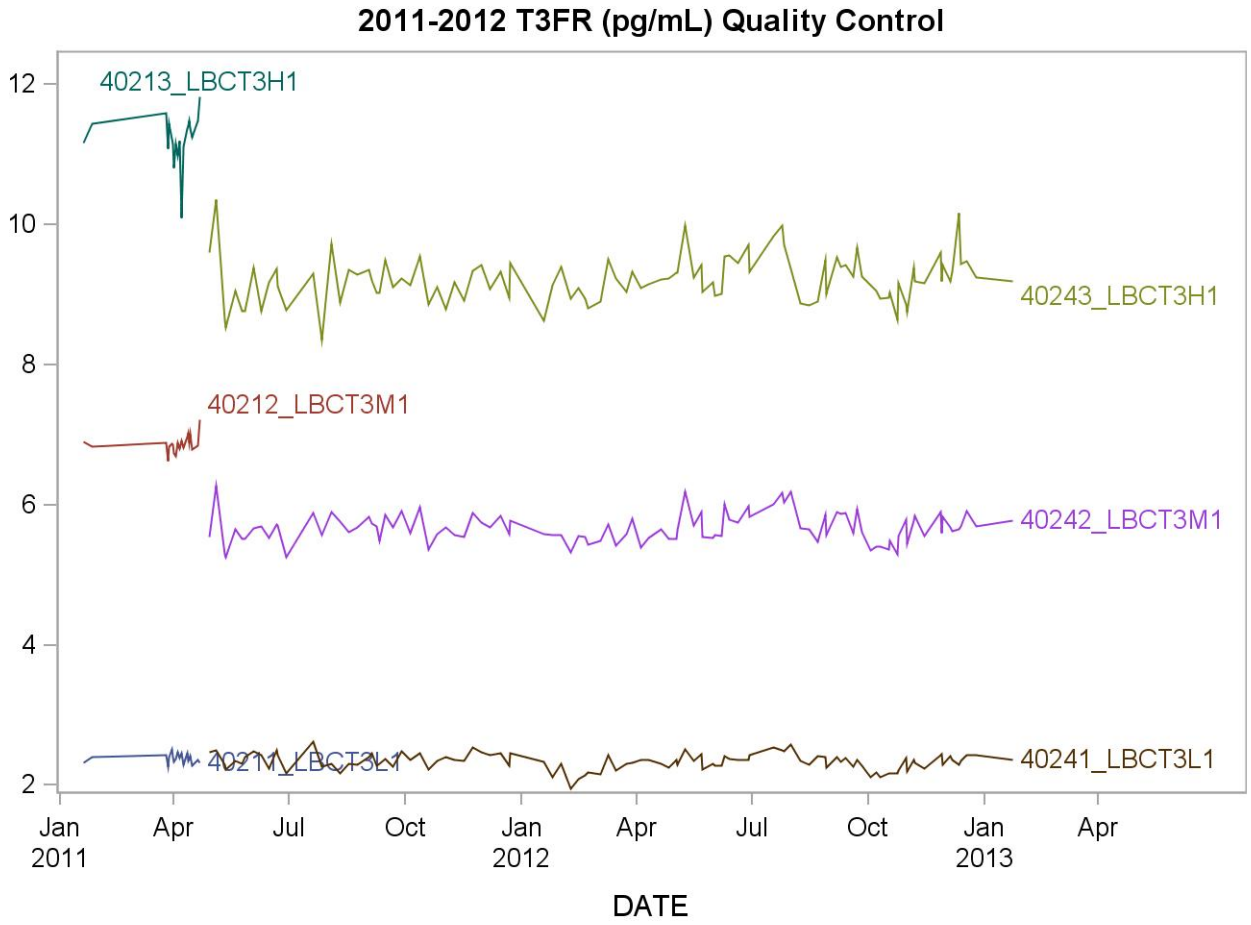
The Project supervisor is responsible for keeping a logbook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. It is recommended that records, including related QA/QC data, be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

19. SUMMARY STATISTICS AND QC GRAPHS

See next page.

Summary Statistics for T3FR (pg/mL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
40213_LBCT3H1	20	19JAN11	21APR11	11.229	0.351	3.1
40211_LBCT3L1	20	19JAN11	21APR11	2.382	0.066	2.8
40212_LBCT3M1	20	19JAN11	21APR11	6.870	0.126	1.8
40243_LBCT3H1	106	29APR11	23JAN13	9.212	0.334	3.6
40241_LBCT3L1	106	29APR11	23JAN13	2.338	0.117	5.0
40242_LBCT3M1	106	29APR11	23JAN13	5.673	0.208	3.7



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