

Laboratory Procedure Manual

Analyte: **Thyroglobulin Antibodies**

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.

**Thyroglobulin Antibody (TGAB) 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
THYROID_G	LBXATG	Thyroglobulin Antibodies (IU/mL)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The Access Thyroglobulin Antibody II (TgAb) assay is a sequential two-step immunoenzymatic ("sandwich") assay. The sample is added to reaction vessel with paramagnetic particles coated with Thyroglobulin protein. The serum or plasma Thyroglobulin Antibody binds to the thyroglobulin. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. The thyroglobulin-alkaline phosphatase conjugate is added and binds to the TgAb. After the second incubation, materials bound to the solid phase are held in a magnetic field while 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 directly proportional to the concentration of Thyroglobulin Antibody in the sample. The amount of analyte in the sample is determined by means of a stored, multi-point calibration curve.

Thyroglobulin (Tg) is produced by the thyroid gland. It is a large, water soluble glycoprotein that is a major component of the thyroid follicular colloid and is present in small amounts in serum. The principal role of thyroglobulin is the storage and synthesis of thyroid hormones of T4 and T3.

Thyroglobulin autoantibodies (TgAb) are often present in patients with autoimmune thyroid disease. Approximately 10% of healthy individuals have TgAb at measurable levels. TgAb can be detected in 30% of patients with Graves' disease and in 85% of patients with Hashimoto's thyroiditis. However, elevated levels of autoantibodies to thyroid peroxidase (TPO autoantibodies) occur more frequently than high TgAb levels in these diseases.

Sensitive TgAb methods are needed to identify patient sera that contain thyroglobulin autoantibodies that may interfere with serum thyroglobulin measurements.

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 <40 mg/dL bilirubin or <500 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 no more than two times. 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 TgAb.
- e. Sample volume for individual test is 10 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 Thyroglobulin Antibody II Reagent Pack (*Cat. #A32898*), 100 determinations, 50 tests/pack. Contains the following components.

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- R1a: Dynabeads® paramagnetic particles coated with streptavidin, and coupled to biotinylated human thyroglobulin, suspended in TRIS buffer with protein (bovine), <0.1% sodium azide, and 0.1% ProClin 300.
- R1b: Human thyroglobulin-alkaline phosphatase (bovine) conjugate in TRIS buffer with protein (bovine), <0.1% sodium azide, and 0.1% ProClin 300.
- R1c: TRIS buffer with <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 56 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.
- 1) Beckman Access Thyroglobulin Antibody II Calibrators (*Cat. #A36920*).
- e. Control Material:
 - 1) Bio-Rad Liquichek Specialty Immunoassay Control (Levels 1, 2, and 3) (*Cat. #364, 365, 366*).
 - a) Unopened, stable until expiration date when stored at -20°C.to -70°C
 - b) Thawed, UNOPENED, stable for 20 days at 2-8°C.
 - c) Thawed, and opened, stable for 3 days at 2-8°C.
 - d) Frozen aliquots in tightly capped aliquot vials, will be stable for 30 days at -20°C.to -70°C. After each use, discard any remaining thawed aliquot.
 - e) Allow frozen control to stand at room temperature until completely thawed, swirl gently several times to ensure homogeneity.
 - e) At least two levels of control should be analyzed in a 24 hour time period.
 - f) Ensure that assay control values are within the concentration ranges stated in the package insert or calculated from cumulative data at CLS.
 - g) Refer to Quality Control Flow Chart for action decision guidelines.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

- a. Calibrators: Access Thyroglobulin Antibody II Calibrators (*Cat. #A36920*).
 - 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 Thyroglobulin Antibody II reagent is loaded, when the calibration curve expires (curve stability is 56 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 (*See Attachment A*).
- b. Sample Preparation
 - 1) Thaw samples and vortex, mixing well.
 - 2) Specimen handling, labeling and transferring serum (*See Attachment B*)
- 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 (*See Attachment C* for specific procedures for NHANES samples)
- 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:
(*See Attachment D*) for the Access Maintenance Schedule.
- 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 smoothing spline 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 Thyroglobulin Antibody is 0.9 - the value of the highest calibrator (~2500) IU/mL.
 - 2) A result over range high should be reported as ">2500". Samples are not diluted for Thyroglobulin Antibody 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 Thyroglobulin Antibody determination is 0.9 IU/mL.
 - 5) 0 is not a reportable value. Report results below 0.9 as "<0.9".

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10. QUALITY CONTROL (QC) PROCEDURES

- a. Blind QC Specimens are included in the samples received from NHANES.
- b. Bio-Rad Liquichek Specialty Immunoassay Control 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 F*).

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. <40 mg/dL bilirubin has no significant interference.
- c. Lipemia has no significant interference in samples containing equivalent of 500 mg/dL triglycerides.
- d. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the sample. Individuals who have been regularly exposed to animals or received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies (e.g. HAMA), that interfere with immunoassays. Additionally, other heterophile antibodies, such as human anti-goat antibodies, may be present in patient samples.
- e. Thyroglobulin Antibody 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)

Thyroglobulin Antibody

	<i>IU/mL</i>
Serum	<4.0

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 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.

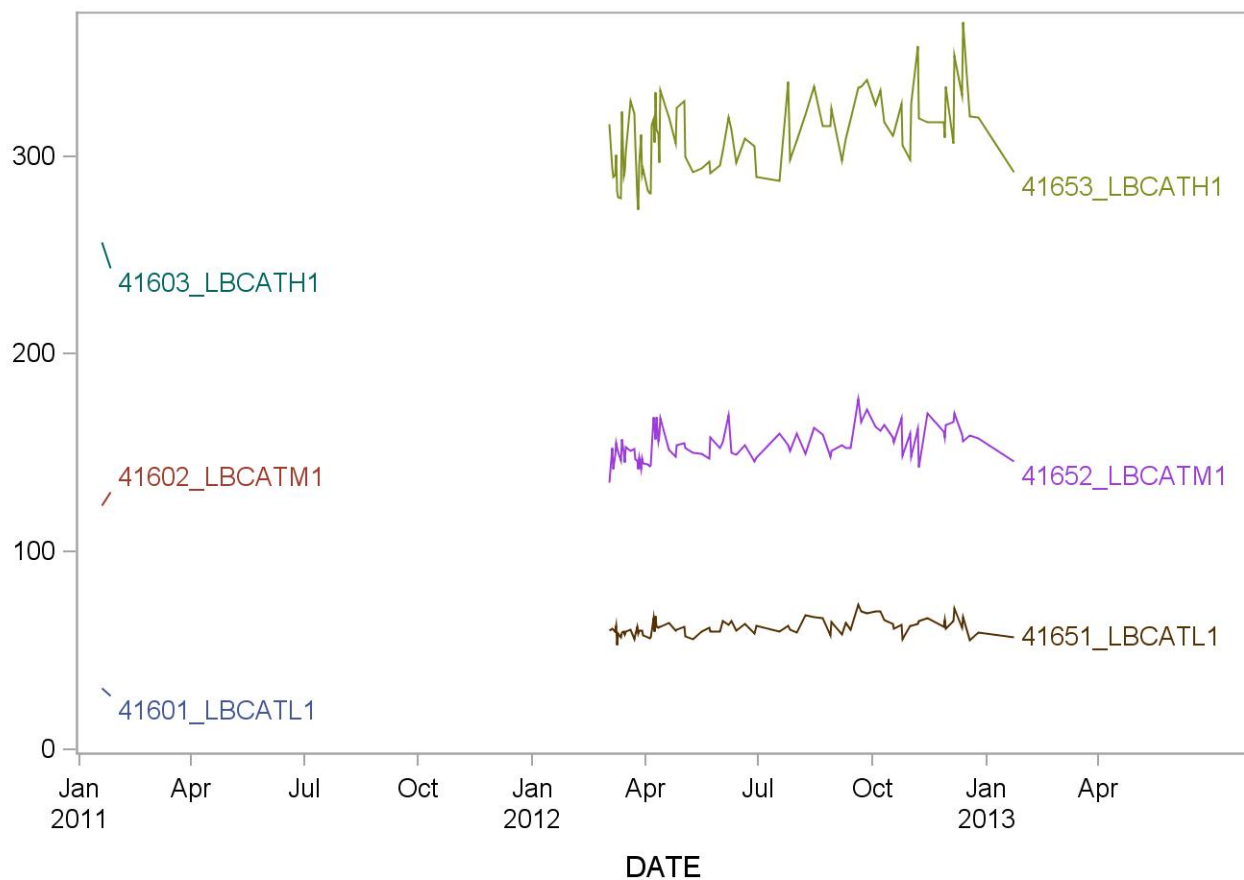
The Project supervisor is responsible for keeping a logbook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. It is recommended that records, including related QA/QC data, be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

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for aTG (IU/mL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
41603_LBCATH1	2	19JAN11	26JAN11	250.10	9.19	3.7
41601_LBCATL1	2	19JAN11	26JAN11	29.15	2.83	9.7
41602_LBCATM1	2	19JAN11	26JAN11	126.85	5.02	4.0
41653_LBCATH1	86	03MAR12	23JAN13	310.18	18.80	6.1
41651_LBCATL1	86	03MAR12	23JAN13	61.86	3.95	6.4
41652_LBCATM1	86	03MAR12	23JAN13	154.57	8.21	5.3

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