Laboratory Procedure Manual

Analyte: **Thyroglobulin**

Matrix: **Serum**

Method: **Siemens Immulite 2000 XPI**

As performed by: **University of Minnesota**
Advanced Research and Diagnostic Laboratory (ARDL)
1200 Washington Ave S, Suite 175
Minneapolis, MN 55415

Contact: **Anthony Killeen, MD, PhD, Laboratory Director**
Jennifer Peters, MT, ASCP, Laboratory Manager

Important Information for Users

The Advanced Research and Diagnostic Laboratory (ARDL) periodically refine 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 testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
THYROD_K	LBXTGN	Thyroglobulin (ng/mL)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

This is a Quantitative Chemiluminescent Immunometric Assay. Thyroglobulin (TG) is a heterogeneous iodoglycoprotein which has a molecular mass of approximately 660,000 daltons. Thyroglobulin is normally synthesized in the follicular cells of the thyroid gland, under the influence of thyrotropin, and represents the precursor to thyroxine and the other iodothyronines. The expected upper limit of normal for circulating thyroglobulin is approximately 40 to 60 ng/mL, with a median of 5 to 10 ng/mL. Somewhat higher values are encountered in newborns and during the third trimester of pregnancy. Thyroglobulin levels also tend to be elevated in regions of endemic goiter. The major clinical applications for measurement of this prohormone derive from the fact that functioning thyroid tissue appears to be the only source of circulating thyroglobulin. Accordingly, thyroglobulin determinations have been widely used to complement radioiodine scanning and other techniques (such as ultrasound or immunohistochemical staining) as an aid in identifying the presence or absence of functioning thyroid tissue, or an increase in such tissue relative to an individually established baseline. The differential diagnosis of congenital hypothyroidism is a well-established context of use for this application of serum thyroglobulin measurement.

Congenital Hypothyroidism:

Thyroglobulin determinations have been used, sometimes in conjunction with ultrasound and radioiodine scanning, to help clarify the type of thyroid defect in previously diagnosed congenital hypothyroidism. Very low or undetectable thyroglobulin levels are expected in infants born without thyroid tissue (thyroid agenesis), whereas higher, but widely varying levels are generally encountered in infants with hypoplastic thyroid glands, ectopic thyroid tissue, dyshormonogenic goiter, congenital TBG deficiency or transient hypothyroidism.

Other Applications: Thyroglobulin measurements may also be of value in helping to distinguish subacute thyroiditis from thyrotoxicosis caused by covert administration of thyroid hormones. In the latter event, low levels of thyroglobulin are expected due to thyroid hormone suppression of thyrotropin.

2. SAFETY PRECAUTIONS

Caution: This product is of human and animal origin. Handle as though capable of transmitting infectious disease. Wear appropriate PPE when handling equipment, reagents, and samples.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

ARDL utilizes a highly specialized Laboratory Information System (LIS) (STARLIMS, Abbott Informatics Corporation; Hollywood, FL, 33021-6755) for all lab functions. Major instrument platforms are interfaced directly to the LIS, allowing data to be electronically transferred directly to the main database. The system provides an extensive quality assurance package and data management tools. Numerous networked computer workstations are used in the laboratory for data management and transmission, and also include software for word and spreadsheet creation and manipulation, statistical analysis, report presentation, and electronic communication. All workstations are user password protected with job specific security access levels and have idle time out functionality. All systems are redundantly backed up on a real time basis.

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

a. **Specimen Type and Requirements:** Use serum that has been separated from the clot within one hour of collection. Serum or heparinized plasma are acceptable (EDTA plasma should not be used). Serum and plasma are stable for eight days at 2-8ºC, two months at -20°C, and longer at –70ºC. The Special Chemistry Lab validated that samples are stable over 3 freeze/thaw cycles at -70°C. Specimens must be at room temperature (20-25 ºC) prior to assay. Lipemic samples should be cleared by ultracentrifugation. Before assay, ensure that samples are free of clots, particulate matter, and bubbles Specimens with visible particulates should be centrifuged for 10 minutes at 1500xg before use. This specimen is received frozen and the test is analyzed from NHANES Vial 118.

b. **Specimen Volume:** The required dead volume is 150uL when the specimen is stored in a 2-mL screw cap conical microvial (e.g. Sarstedt #72.664 or Fisher #0554166). Use of larger vials or round/flat-bottomed vials will increase the dead

volume requirement. Test sample volume is 50 µL for serum or plasma.**Dead volume is not recoverable if sample is transferred into a sample cup.

c. **Acceptable Specimens/Unacceptable Specimens:** Serum or heparinized plasma are acceptable. Other anticoagulants are not acceptable.

d. **Specimen Stability and Storage:** Separated serum or plasma should be removed from the cells within one hour of collection. Serum and plasma are stable for eight days at 2-8ºC, two months at -20°C, and longer at –70ºC. The Special Chemistry Lab validated that samples are stable over 3 freeze/thaw cycles at -70°C. Specimens must be at room temperature (20-25 ºC) prior to assay. Lipemic samples should be cleared by ultracentrifugation. Before assay, ensure that samples are free of clots, particulate matter, and bubbles Specimens with visible particulates should be centrifuged for 10 minutes at 1500xg before use.

e. **Interferences or limitations:**

Because anti-thyroglobulin antibody (TgAb) can interfere with both competitive immunoassays and immunometric assays for thyroglobulin, all patients should be screened for TgAb by a sensitive immunoassay: recovery studies are not adequate for ruling out interference by TgAb. Thyroglobulin antibodies TgAb are present in the majority of patients with Hashimoto's thyroiditis, but also in approximately 5% of healthy individuals. At least 6 weeks should elapse after thyroidectomy or 131I treatment. Some reports have indicated that thyroglobulin levels may remain elevated for several months following successful treatment. In this case, serial determinations assessed relative to a post-treatment baseline established for the patient may still be of value in monitoring. It is important to obtain thyroglobulin measurements before administering 131I for scanning, since the scan may result in the release of large amounts of thyroglobulin from even a very small number of cells. Likewise, samples for thyroglobulin assay should not be collected prematurely following procedures such as needle biopsy that are likely to cause a transient elevation in the thyroglobulin level. Although thyroglobulin measurements are of established value in the differential diagnosis of congenital hypothyroidism, they are not recommended for use in screening for this condition since a wide spectrum of thyroglobulin levels are obtained for this condition. EDTA Plasma

has an effect on the measurement of thyroglobulin in the procedure. Immunologically inactive thyroglobulin may not be determined by this assay. In patients receiving thyroid suppression therapy, thyroglobulin may not be reliably detected. Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with in vitro immunoassays. Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Specimens that contain biotin at a concentration of 5 ng/mL demonstrate a less than or equal to 10% change in results. Biotin concentrations greater than this may lead to falsely depressed results for patient samples. Results from patients taking biotin supplements or receiving high-dose biotin therapy should be interpreted with caution due to possible interference with this test.

f. Specimen Handling and Transport: Mix specimens well, allow clot to fully form (if serum), and centrifuge 10 minutes at 2000 x g before use. Aliquot a minimum of 0.150 mL. Freeze sample until shipment. Ship frozen on dry ice.

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. Reagents and Supplies

- Thyroglobulin Bead Pack (L2TY12) reagent kit with barcode (200 tests): 200 beads, coated with anti-ligand
- Thyroglobulin Reagent Wedge (L2TYA2). With barcode. 11.5 mL of ligand-labeled anti- thyroglobulin murine monoclonal antibody and alkaline

phosphatase (bovine calf intestine) conjugated to sheep polyclonal anti-thyroglobulin antibody in buffer, with preservative. Stable at 2–8°C until expiration date.

- Thyroglobulin Adjustors (LTYL, LTYH). Two vials (Low and High) of lyophilized thyroglobulin in a nonhuman serum matrix, with preservative.
- Thyroglobulin Sample Diluent (L2TYZ): For the on-board dilution of high samples.

b. Reagent Preparation (* Thyroglobulin bead pack and reagent wedge are ready to use; no preparation required)

• Thyroglobulin Bead Pack (L2TY12) and Thyroglobulin Reagent Wedge (L2TYA2)- Before use, tear off the top of the label at the perforations, without damaging the barcode. Remove the foil seal from the top of wedge; snap the sliding cover down into the ramps on the reagent lid.

• Thyroglobulin Adjustors (LTYL, LTYH)- At least 30 minutes before use: Reconstitute each vial with 4.0 mL distilled or deionized water. Mix by gentle swirling or inversion until the lyophilized material is fully dissolved. Make eight 0.5 ml aliquots of each level in 12X75 tubes, using the supplied barcode labels for identification. Use immediately or freeze. Stable at –20°C for 2 months after reconstitution. Discard aliquots after use.

• Thyroglobulin Sample Diluent (L2TYZ): For the on-board dilution of high samples. 25 mL of concentrated (ready-to-use) thyroglobulin-free nonhuman serum matrix. Storage: 30 days (after opening) at 2–8°C or 6 months (aliquotted) at –20°C. Barcode labels are provided for use with the diluent. Before use, place an appropriate label on a 16 × 100 mm test tube, so that the barcodes can be read by the on-board reader.

c. Equipment/Instrumentation-

Immulite 2000 XPI Analyzer (Siemens Healthcare Diagnostics, Products Ltd., Glyn Rhonwy, Llanberis, Gwynedd LL55 4EL, United Kingdom)

d. Specimens are run in singleton

e. Quality Control

- Bio-Rad Lyphochek Tumor Marker Plus Controls Levels 1 & 3 (Ref #367 and 369). To prepare, reconstitute each vial with 2.0 mL distilled or deionized water. Replace the stopper and allow the control to stand at room temperature for approximately 15 minutes, swirling occasionally. Before sampling, invert the vial several times to ensure homogeneity. Divide freshly reconstituted controls into five 400 µL aliquots in 12x75 tubes. Use immediately or freeze at -20ºC. Thaw frozen aliquots, mix, use, and discard; do not refreeze. Stable 30 days frozen.Both levels of quality control are analyzed at the start of the day and results are verified for acceptability prior to testing specimens. Quality control is also analyzed with change in reagent, after major maintenance, or as needed for troubleshooting.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Thyroglobulin Adjustors (LTYL, LTYH). Two vials (Low and High) of lyophilized thyroglobulin in a nonhuman serum matrix, with preservative. At least 30 minutes before use: Reconstitute each vial with 4.0 mL distilled or deionized water. Mix by gentle swirling or inversion until the lyophilized material is fully dissolved. Make eight 0.5 ml aliquots of each level in 12X75 tubes, using the supplied barcode labels for identification. Use immediately or freeze. Stable at –20°C for 2 months after reconstitution. Discard aliquots after use.

Traceability: Adjusters are standardized against Certified Reference Material for human thyroglobulin (CRM 457) in terms of the Community Bureau of Reference of the European Commission.

Calibration range is up to 300 ng/mL.

Calibration frequency:

Each lot of reagent must be adjusted only with the lot of adjustors supplied in the kit, in order to ensure the applicability of the lot-specific stored Master Curve. While the same lot remains in use, adjustment should be repeated every 2

weeks. Adjustment is also required when replacing major system components or as indicated by quality control results.

If calibration fails perform the following corrective action steps in sequence:

- Check reagent and calibrator for appropriate lot numbers, expiration dates, preparation, and storage conditions.
- Repeat calibration with new calibrator.
- Repeat calibration with new reagent and new calibrator
- If successful calibration is not achieved, discontinue testing and notify the supervisor.

8. OPERATING PROCEDURE INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. **Instrument Operation:** The IMMULITE 2000 is a continuous random-access Instrument that performs chemiluminescent immunoassays.
The Instrument uses assay-specific antibody or antigen-coated polystyrene beads as the solid phase. A bead is dispensed into a specially designed Reaction Tube, which serves as the vessel for the incubation, wash, and signal development processes. After the sample is incubated with an alkaline phosphatase-labeled reagent, the reaction mixture is separated from the bead by spinning the Reaction Tube at high speed along its vertical axis. The fluid is transferred to a Coaxial Sump Chamber, which is integral to the Bead/Tube Wash Station. Four discrete washes occur within seconds, allowing the Reaction Tubes to be processed sequentially with uniform timing. The bead remains in the ReactionTube with no residual unbound label.The bound label is then quantified using the dioxetane substrate to produce light. Light is emitted when the chemiluminescent substrate reacts with the alkaline phosphatase label bound to the bead. The amount of light emitted is proportional to the amount of analyte originally present in the sample. This light emission is detected by the Photomultiplier Tube (PMT) and results are calculated for each sample.

b. **Professional Judgement:** Check results for error flags and take appropriate corrective action. Investigate alert values and delta checks.

c. **Result Entry**

This assay is currently being used for research samples only. A Special Chemistry technical specialist will provide the results to the research coordinator.

- Results are reported to one decimal place, as in x.x, in ng/mL.
- Report low results as <0.9 ng/mL.
- Report high results as >30,000 ng/mL.
- Check results for error flags and take appropriate corrective action.
- Investigate alert values and delta checks.

9. REPORTABLE RANGE OF RESULTS

Out of Range results: Certain tests have pre-programmed limits that trigger an automatic re-analysis. The Immulite will not trigger a repeat analysis if the result is within the analytical range. These limits may be low-end values or high-end values (but within technical range). If the duplicate value is in agreement with the initial value, then the initial value is reported.

Results are reported to one decimal place, as in x.x, in ng/mL. Report low results as <0.9 ng/mL.

a. Reportable Range of Test Results: Reportable Range 0.9-30,000 ng/mL

Intra-assay %CV (10 within-day replicates at a concentration of 3.3 ng/dL) 4.6%

Intra-assay %CV (10 within-day replicates at a concentration of 118.4 ng/dL) 4.9%

Inter-assay %CV (49 between day replicates at a concentration of 3.3 ng/dL) 5.9%

Inter-assay %CV (49 between day replicates at a concentration of 119.4 ng/dL) 5.9%

Dilutions: Samples with results >300 ng/mL should be repeated on a 1:100 auto-dilution, which will be automatically ordered by the instrument.

Reference Ranges:

≤55 ng/mL ng/mL

Critical Results: None

Analytical Measurement Range: 0.9-30,000 ng/mL

Reportable Range: 0.9-30,000 ng/mL

Limit of Detection (standard 1 + 2 SD): 0.9 ng/mL

10. QUALITY CONTROL (QC) PROCEDURE

Bio-Rad Lyphochek Tumor Marker Plus Controls Levels 1 & 3 (Ref #367 and 369). To prepare, reconstitute each vial with 2.0 mL distilled or deionized water. Replace the stopper and allow the control to stand at room temperature for approximately 15 minutes, swirling occasionally. Before sampling, invert the vial several times to ensure homogeneity. Divide freshly reconstituted controls into five 400 µL aliquots in 12x75 tubes. Use immediately or freeze at -20ºC. Thaw frozen aliquots, mix, use, and discard; do not refreeze. Stable 30 days frozen.Both levels of quality control are analyzed at the start of the day and results are verified for acceptability prior to testing specimens. Quality control is also analyzed with change in reagent, after major maintenance, or as needed for troubleshooting.

The analytical measurement range (AMR) must be validated every 6 months.

Siemens Immulite Thyroglobulin Calibration Verification Material:

a. Follow package insert instructions to prepare CVM.
b. Run all levels (4 per assay) in triplicate, programmed as Verifiers.
c. Evaluate data using EP Evaluator Linearity and Calibration Verification module. In the Parameters window, enter Units and Analyst fields and select Confirm Linearity. In the Specimens and Assigned Values section, click the Edit button. Enter the verifier levels in the Spec ID field and the target mean in the Assigned Value field. In the Allowable Error Criteria section, enter the following:
    - Allowable Total Error (Tea) Conc: Difference of mean and the upper end of 2SD range of level 1 verifier.
    - Allowable Total Error (Tea) Pct: (2 X SD of highest verifier X 100) ÷ mean of highest verifier.

- % for Systematic Error: 100

d. Enter assay results and print a report. Retain report, instrument printouts and verifier insert for a minimum of 2 years.
e. AMR check must meet the following criteria:
   - Mean of all 3 verifier replicates must be within the guideline 2SD range.
   - Evaluation on EP Evaluator report must state that results are linear

If the above criteria are not met, re-assay the verifiers, readjust the assay, or troubleshoot as necessary until acceptable results are obtained.

New Lot Verification: Each new reagent lot must be verified for acceptability before being placed into use. Calibration, quality control, and comparison of at least 5 patient samples on the old and new lots must be performed and found to be within acceptable limits before a new lot can be placed into use.

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

If QC values are outside of specified ranges, do the following, in order, until QC is acceptable:

1. Repeat the analysis using fresh QC material.
2. Perform a calibration.
3. Check for system problems.
4. Contact Roche Technical Support for assistance and possible service dispatch. Phone: 1-800-428-2336; account number: 55042919

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

a. Limit of Detection (standard 1 + 2 SD): 0.9 ng/mL
b. Analytical Measurement Range: 0.9-30,000 ng/mL
c. Interfering Substances and Conditions

Because anti-thyroglobulin antibody (TgAb) can interfere with both competitive immunoassays and immunometric assays for thyroglobulin, all patients should be screened for TgAb by a sensitive immunoassay: recovery studies are not adequate for ruling out interference by TgAb. Thyroglobulin antibodies TgAb are present in the majority of patients with Hashimoto's thyroiditis, but also in approximately 5% of

healthy individuals. At least 6 weeks should elapse after thyroidectomy or 131I treatment. Some reports have indicated that thyroglobulin levels may remain elevated for several months following successful treatment. In this case, serial determinations assessed relative to a post-treatment baseline established for the patient may still be of value in monitoring. It is important to obtain thyroglobulin measurements before administering 131I for scanning, since the scan may result in the release of large amounts of thyroglobulin from even a very small number of cells. Likewise, samples for thyroglobulin assay should not be collected prematurely following procedures such as needle biopsy that are likely to cause a transient elevation in the thyroglobulin level. Although thyroglobulin measurements are of established value in the differential diagnosis of congenital hypothyroidism, they are not recommended for use in screening for this condition since a wide spectrum of thyroglobulin levels are obtained for this condition. EDTA Plasma has an effect on the measurement of thyroglobulin in the procedure. Immunologically inactive thyroglobulin may not be determined by this assay. In patients receiving thyroid suppression therapy, thyroglobulin may not be reliably detected. Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with in vitro immunoassays. Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings. Specimens that contain biotin at a concentration of 5 ng/mL demonstrate a less than or equal to 10% change in results. Biotin concentrations greater than this may lead to falsely depressed results for patient samples. Results from patients taking biotin supplements or receiving high-dose biotin therapy should be interpreted with caution due to possible interference with this test.

13. REFERENCE RANGES (NORMAL VALUES)

≤55 ng/mL ng/mL

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable for this procedure.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens are frozen at -70°C between sample receipt and analysis on the instrument. Specimens must be at room temperature prior to assay. Specimens are returned to refrigerated or frozen temperature post analysis depending on the study specific requirements.

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

Should the testing system become inoperable, discontinue testing and notify the supervisor. While instrument trouble-shooting or repair occurs; keep specimens at refrigerated or frozen temperature depending on study specific requirements.

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

All data is reported electronically via an eFile that is uploaded to the WESTAT secure website within 21 days of receipt of specimens.

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

Specimen Receipt:

Shipments for NHANES generally will arrive on Tuesdays and/or Wednesdays. These shipments are recorded on the Log of Quality Assurance located in a binder labeled NHANES Shipping Log in the receiving area. The specimen barcode numbers in the boxes are checked against the manifests. The receipt date is written on top of the boxes. The frozen samples (vial 118) are placed in the designated -70°C freezer until analysis. The manifests are filed in a binder labeled NHANES Shipping Manifests located in the receiving area. All labels are removed from the shipping box and the provided airbill is attached for return shipment.

Quality Assurance Log:

A Quality Assurance Specimen Receipt and Specimen Return Log is maintained by laboratory staff. The following parameters are tracked: NHANES shipper I.D., NHANES Container I.D., Vial #, Date Received, Specimen Receipt Conditions, Number of Specimens Received, 2.5% QC Repeats, Total Number of Specimens, 21 Day Due Date, Analysis Date, Date Results Sent, Number of Days For Result Return, Thaw Date (if applicable), Return To Freezer Date, 1 Year Discard or Return Date, and NHANES Quarterly Report Date.

QUALITY ASSURANCE SPECIMEN RECEIPT					# received, analyzed and transmitted		
NHANES shipper I.D.	NHANES container I.D. #	vial #	date rec'd	receive spec. cond.	spec.	qc rpts COBAS & IMMULITE	total
774903957877	401219	118	4/9/2019	Ok	30	2	32
774965044160	401275	118	4/16/2019	Ok	8	2	10
774965218515	402034	118	4/16/2019	Ok	37	2	39
775015603793	401319	118	4/22/2019	Ok	5	2	7
775021014447	402098	118	4/23/2019	Ok	25	2	27
775078062025	402152	118	4/30/2019	Ok	30	2	32
775078790984	403034	118	4/30/2019	Ok	14	2	16
775135902127	402211	118	5/7/2019	Ok	20	2	22
775137049487	403077	118	5/7/2019	Ok	20	2	22
775195775497	403126	118	5/14/2019	Ok	14	2	16
775196146284	402259	118	5/15/2019	Ok	10	2	12
775255488432	403175	118	5/21/2019	Ok	21	2	23

QUALITY ASSURANCE OF SPECIMEN RETURN								
	COBAS	IMMULITE (TGN)	date	# days		return to	1 year discard	NHANES
21-day due date	analyzed date	analyzed date	result sent	results return	thaw date	ARDL freezer date	or return date	Quarterly Report
4/30/2019	5/22/2019	5/24/2019	5/28/2019	49	5/22/2019	6/12/2019		
5/7/2019	5/28/2019	5/29/2019	5/31/2019	45	5/28/2019	6/12/2019		
5/7/2019	5/30/2019	6/4/2019	6/11/2019	56	5/30/2019	6/12/2019		
5/13/2019	5/29/2019	5/31/2019	6/5/2019	44	5/29/2019	6/12/2019		
5/14/2019	6/3/2019	6/5/2019	6/11/2019	49	6/3/2019	6/12/2019		
5/21/2019	6/4/2019	6/6/2019	6/11/2019	42	6/4/2019	6/12/2019		
5/21/2019	6/4/2019	6/6/2019	6/11/2019	42	6/4/2019	6/12/2019		
5/28/2019	6/5/2019	6/7/2019	6/13/2019	37	6/5/2019	7/11/2019		
5/28/2019	6/3/2019 & 6/4/2019	43621	6/11/2019	35	6/3/2019	6/12/2019		
6/4/2019	6/5/2019	6/7/2019	6/13/2019	30	6/5/2019	7/11/2019		
6/5/2019	6/10/2019	6/12/2019	6/19/2019	35	6/10/2019	7/11/2019		
6/11/2019	6/11/2019	6/14/2019	6/19/2019	29	6/11/2019	7/11/2019		

Specimen Ordering/Labeling:

Electronic files for all NHANES specimens are sent via email from Westat, Inc to the NHANES contact person shortly before they are to be received. These files include the Sample ID, Analyte Type, Slot No, Sample Collection Date, Sample Comment, Age Grouping, Astro ID, Receipt Date, Analysis Date, Run Number, Tech ID, Analyte Result,

Result Comment, Adjusted Result, QC Repeat, LOD, Change Reason, and Change Reason Other. The first seven columns are protected and cannot be altered. The files are saved on the laboratory's common S drive in the NHANES Thyroid folder. After analysis, the contact person returns the completed files via their website to Westat, Inc.

The NHANES spreadsheets are used to set up pending batches for batch accession upload in the Laboratory Information system (STARLIMs). New labels are generated out of the Laboratory Information System (STARLIMs). The new bar-coded labels are attached to a carrier tube. The Cobas analyzer reads the bar-coded label for the sample ID and test information.

Specimen Storage:

The temperatures for all freezers and refrigerators are monitored 24 hours a day/ 7 days a week. If the temperature for any unit falls outside the allowable range, action is taken to resolve the problem. If the temperature cannot be corrected, the contents are moved to a different unit.

Specimen Handling/Specimen Return:

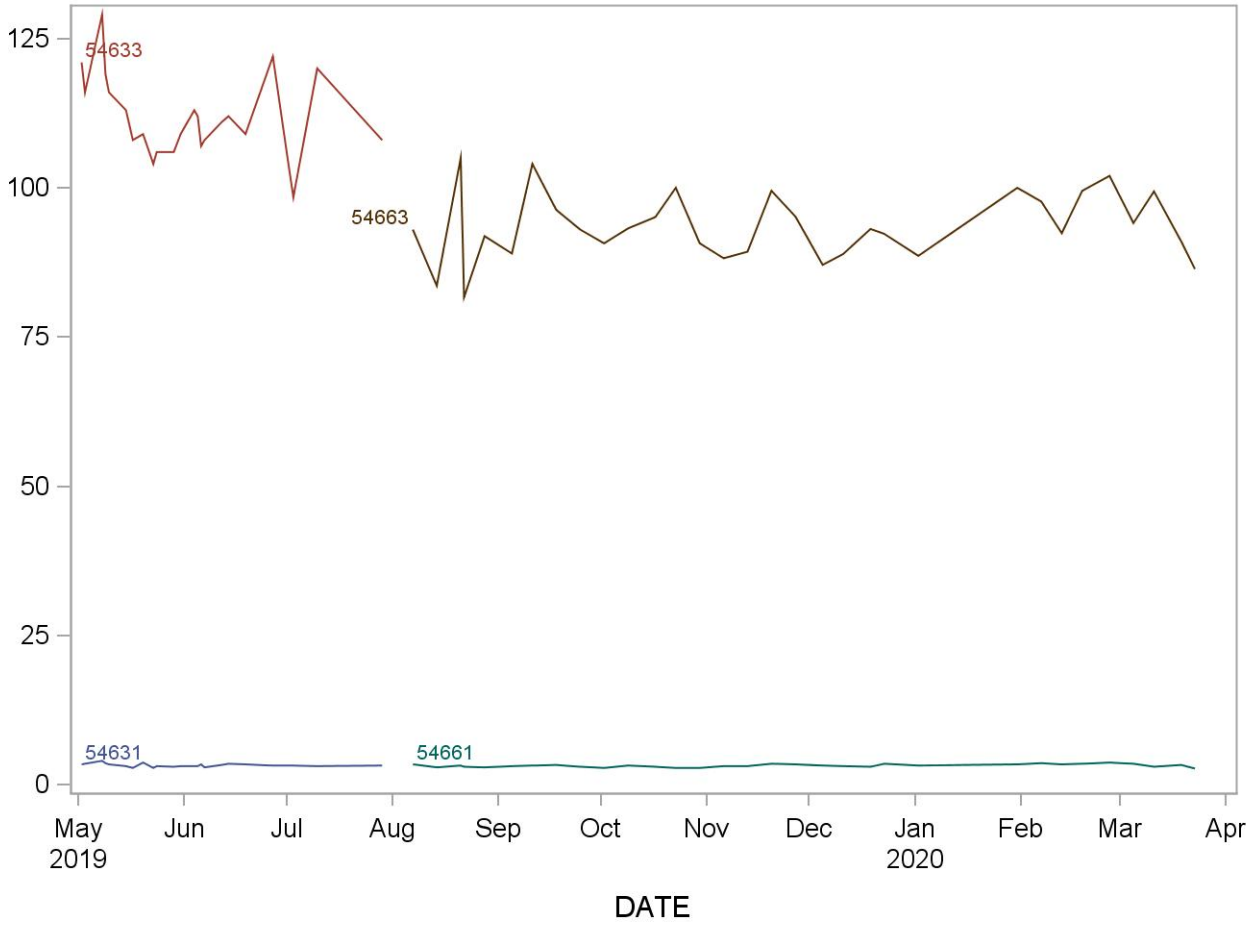
Prior to analysis, the specimens are stored in the designated -70°C freezer. On the day of analysis, the specimens are selected and thawed by the technician operating the COBAS. After analysis and the QC repeats have been run, the specimens are refrozen. After 1 year, the specimen vials that have at least 0.2ml of sample remaining will be shipped to SriSai Biopharmaceuticals in Frederick, MD. These specimens will be shipped on dry ice via Federal Express.

19. SUMMARY STATISTICS AND QC GRAPHS

See following page(s).

2019-2020 Summary Statistics and QC Chart
LBXTGN (Thyroglobulin (ng/mL))

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
54633	23	02MAY19	29JUL19	112.02	6.87	6.1
54631	23	02MAY19	29JUL19	3.26	0.29	8.9
54663	32	07AUG19	23MAR20	93.50	5.69	6.1
54661	32	07AUG19	23MAR20	3.18	0.26	8.2



REFERENCES

- Immulite 2000 Thyroglobulin package Insert, Siemens Healthcare Diagnostics, Products Ltd., Glyn Rhonwy, Llanberis, Gwynedd LL55 4EL, United Kingdom. Version (PIL2KTYD-25, 2017-11-24).