THYROGLOBULIN QUANTITATIVE DETERMINATION IN HUMAN SERUM AND PLASMA BY THE ACCESS IMMUNOASSAY SYSTEMS

Table of Contents

Principle 2
Specimen Collection 2
Reagents and Equipment 3
Calibration Details 6
Quality Control 6
Procedure 6
Results 7
Procedural Comments 8
Limitations of the Procedure 8
References 9

This document is provided as an aid to writing laboratory procedures following CLSI guidelines but does not include all activities identified in CLSI GP2-A4 Clinical Laboratory Technical Procedure Manuals; Approved Guideline – Fourth Edition. Each laboratory is responsible for ensuring their procedures are comprehensive and complete.

Beckman Coulter, Inc. • Brea, CA 92821
**Principle**

**Principles of the Procedure**
The Access Thyroglobulin assay is a simultaneous one-step immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel, along with a biotinylated mixture of four monoclonal anti-Tg antibodies, streptavidin coated paramagnetic particles, and monoclonal anti-Tg antibody alkaline phosphatase conjugate. The biotinylated antibodies and the serum or plasma thyroglobulin binds to the solid phase, while the conjugate antibody reacts with a different antigenic site on the thyroglobulin molecule. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos® 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of thyroglobulin in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

**Summary and Explanation**
The thyroid is a small endocrine gland located in the base of the neck. It consists of two lateral lobes connected by an isthmus. The gland produces a variety of metabolic hormones in a negative biofeedback loop.

Thyroglobulin (Tg) is a large glycoprotein (MW = 660,000) that is stored in the follicular colloid of the thyroid gland. Thyroglobulin functions as a prohormone in the intrathyroid synthesis of T4 and T3. Lysosomes containing proteases cleave T4 and T3 from Tg, resulting in release of T4 and T3.

Thyroglobulin is present in the serum of normal healthy individuals and can be elevated in numerous disorders which disrupt thyroid tissue. Elevated circulating levels of Tg have been reported in a number of thyroid conditions including Hashimoto’s disease, Graves’ disease, thyroid adenoma, subacute thyroiditis and thyroid carcinoma (1).

Thyroid cancer is a relatively common form of cancer. It is not generally highly malignant, and normal life span can be obtained with appropriate follow-up and treatment. Females are affected 2 to 3 times more frequently than males. Thyroglobulin has become a useful tool in the follow-up of patients with differentiated thyroid carcinoma (i.e. papillary-follicular or follicular carcinoma of the thyroid). The thyroid is the only source of Tg; therefore, the serum Tg level will drop to a very low or undetectable level after total or near-total thyroidectomy and successful radioiodine ablation of the residual thyroid tissue. A rise in the serum level of Tg points to the recurrence of the disease. Thyroglobulin levels in patients who have undergone only a partial thyroidectomy will retain measurable levels of Tg, depending on how much tissue is remaining after surgery. These patients can be monitored by Tg measurement, but the post-surgical Tg level must be taken into account.

An additional monitoring tool used in conjunction with Tg is whole body scan (WBS) following a dose of $^{131}$I. Generally, both Tg and WBS can be used to follow newly diagnosed and treated patients.

A limiting factor in the use of serum Tg measurements is the presence of Tg autoantibodies found in some patients. These antibodies may interfere with the immunoassay used to measure Tg and can cause false high or false low values. It is important to determine the levels of Tg autoantibodies in patients requiring serum Tg measurements.

**Specimen Collection**

A. Serum and (heparinized) plasma are the recommended samples.

B. Observe the following recommendations for handling, processing, and storing blood samples (8):

1. Collect all blood samples observing routine precautions for venipuncture.

2. Allow serum samples to clot completely before centrifugation.

3. Keep tubes stoppered at all times.
4. Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.

5. Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.

6. If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.

7. If the assay will not be completed within 7 days, or for shipment of samples, freeze at -20°C or colder. Reference: The Effects of Preanalytical Variables on Clinical Laboratory Tests (Third edition, Young)

8. Thaw samples only once.

C. Use the following guidelines when preparing specimens:

1. Ensure residual fibrin and cellular matter has been removed prior to analysis.

2. Follow blood collection tube manufacturer’s recommendations for centrifugation.

D. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.

Refer to “Sample Integrity in Chemistry” write up in “Policies and Procedures” manual for additional information.

Reagents and Equipment

Beckman Coulter, Inc.
250 S. Kraemer Blvd.
Brea, CA 92821

A. R1: Access Thyroglobulin Reagent Pack
Cat. No. 33860: 100 determinations, 2 packs, 50 tests/pack.

Provided ready to use. Store upright and refrigerate at 2 to 10°C. Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument. Stable until the expiration date stated on the label when stored at 2 to 10°C. Stable at 2 to 10°C for 28 days after initial use. Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range. If the reagent pack is damaged (i.e., broken elastomer), discard the pack.

1. R1a: Dynabeads** paramagnetic particles coated with streptavidin, suspended in a TRIS buffer with protein (bovine), < 0.1% sodium azide, and 0.1% ProClin*** 300.

2. R1b: Mouse monoclonal anti-thyroglobulin-alkaline phosphatase (bovine) conjugate in a TRIS buffer with protein (bovine, murine), < 0.1% sodium azide, and 0.1% ProClin 300.

3. R1c: Mouse monoclonal anti-thyroglobulin antibodies coupled to biotin in a HEPES buffer with protein (bovine and mouse), < 0.1% sodium azide, and 0.5% ProClin 300.

B. Access Thyroglobulin Calibrators
Cat. No. 33865: S0-S5, 2.0 mL/vial

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e. assay calibrators) are tested like patient samples to measure the response. The mathematical
relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

Provided lyophilized. Reconstitute each vial volumetrically with 2.0 mL distilled water. Allow 30 minutes for dissolution. Mix gently before use. Lyophilized calibrators are stable until the expiration date stated on the label when stored at 2 to 10°C. Reconstituted stability is 4 months at 2 to 10°C. Signs of possible deterioration are control values out of range or failure of calibrators to completely reconstitute.

Refer to calibration card and or vial labels for exact concentrations.

Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

1. **S0**: HEPES buffer with bovine serum albumin (BSA), < 0.1% sodium azide, and 0.5% ProClin 300. Contains 0.0 ng/mL thyroglobulin.

2. S1–S5: Human thyroglobulin at levels of approximately 1.0, 10, 100, 250, and 500 ng/mL, respectively, in HEPES buffer with BSA, < 0.1% sodium azide, and 0.5% ProClin 300.

3. **Calibration Card**: 1

C. **Access Substrate**
Cat. No. 81906: 4 x 130 mL

Provided ready to use. Refer to the following chart for storage conditions and stability. An increase in substrate background measurements may indicate instability.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unopened</td>
<td>2 to 8°C</td>
<td>Until expiration date stated on the label</td>
</tr>
<tr>
<td>Equilibration prior to use</td>
<td>15 to 30°C (room temperature)</td>
<td>Minimum 18 hours</td>
</tr>
<tr>
<td>(unopened)</td>
<td></td>
<td>Maximum 14 days</td>
</tr>
<tr>
<td>In use (opened)</td>
<td>External fluids tray substrate position</td>
<td>Maximum 14 days</td>
</tr>
</tbody>
</table>

R2 Substrate: Lumi-Phos 530 (buffered solution containing dioxetane Lumigen* PPD, fluorescer, and surfactant).

Refer to the appropriate system manuals and/or Help system for detailed instructions.

D. **Access, Access 2, SYNCHRON LXI:**
Access Wash Buffer II, Cat. No. A16792
**UniCel Dxi:**
Unicel Dxi: Wash Buffer II, Cat. No. A16793

Provided ready to use. Stable until the expiration date stated on the label when stored at room temperature (15 to 30°C). An increase in substrate background measurements or increased relative light units for the zero calibrators in “sandwich”-type assays may indicate instability.

R3 Wash Buffer: TRIS buffered saline, surfactant, < 0.1 sodium azide, and 0.1% ProClin 300.

Refer to the appropriate system manuals and/or Help system for detailed instructions.

E. **Quality control materials**: commercial control material
CONTROL NAME | SAMPLE TYPE | STORAGE
--- | --- | ---
Bio-Rad Liquichek Tumor Marker Control levels 1 and 3 vials in use kept refrigerated after thawing. Once thawed and mixed, it is ready to use and stable for 30 days at 2 to 8°C. Unopened Tumor Marker control vials kept frozen at -20 to -70°C and stable until the expiration date on the label. Control preparations and acceptance of QC results are in "Policies and Procedures" manual. Controls are run every Tuesday and Friday only on both DxI's by Dayshift. No controls should be run on Evening and Night shifts unless it’s needed.

F. Access Thyroglobulin Sample Diluent

The Tg level in patient samples may exceed the levels of the Access Thyroglobulin S5 calibrator. If a quantitative value is required, it will be necessary to dilute the samples in order to determine the Tg concentration.

Provided ready to use. Allow the contents to stand for 10 minutes at room temperature. Mix gently by inverting before use. Avoid bubble formation. Stable until the expiration date stated on the vial label when stored at 2 to 10°C.

Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.1 to 500 ng/mL). If a sample contains more Tg than the stated value of the S5 calibrator, start diluting 1 volume of sample with 1 (1:2) or 4 (1:5) or up to 9 (1:10) volumes of Access Thyroglobulin Sample Diluent until an accurate number will be achieved. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.

Sample Diluent: HEPES buffer with bovine serum albumin (BSA), < 0.1% sodium azide, and 0.5% ProClin 300. Contains 0.0 ng/mL thyroglobulin.

G. Access Immunoassay System and supplies

H. Warnings and Precautions

1. For U.S.A. only, Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by or on the order of a physician.

2. The presence of serum autoantibodies to thyroglobulin (TgAb) can interfere with assays for thyroglobulin (Tg). Therefore, sera which contain TgAb, even at very low levels, should not be tested for Tg.

The concentration of thyroglobulin in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the Tg assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining Tg levels serially is changed, additional sequential testing should be carried out to confirm baseline values.

3. For in vitro diagnostic use.

4. Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
5. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.

6. Xi. Irritant: 0.5% ProClin 300.
R 43: May cause sensitization by skin contact.
S 28–37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

7. Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease (10).

8. Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.

9. The Material Safety Data Sheet (MSDS) is available upon request.

**Calibration Details**

An active calibration curve is required for all tests. For the Access Thyroglobulin assay, calibration is required every 56 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

The Access Thyroglobulin Calibrators are provided at six levels - zero and approximately 1.0, 10, 100, 250, and 500 ng/mL. Calibrators are prepared gravimetrically from purified human thyroglobulin and buffered BSA based matrix. Assay calibration data are valid up to 56 days.

Calibrators run in duplicate.

**Calibrator location:** Chemistry section, room L568. Refer to reagent “map” on Chemistry refrigerator #8.

**Quality Control**

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a “random access” format rather than a “batch” format, quality control materials should be included in each 24-hour time period (11). Include commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

**Procedure**

**Important Note:**

Two aliquot tubes will be generated on all TGA test ordered (TGLU for Parnassus and TGAB for China Basin) if loaded on the automation line or manually aliquot the sample using the false bottom tubes (if necessary). All TGLU aliquot samples should be stored refrigerated. A designated rack has been specifically created with a label "TGA (TGLU) Chem Parnassus" and placed on the top shelf of the refrigerator #3 in the Central Processing area (the same shelf where we store our rack for Lithium and
MTX samples). However, completed samples should be stored frozen on the second shelf by the door of the freezer #8 in Chemistry for one week.

This assay will be run every Tuesday and Friday on DxI #1 by dayshift. The cut-off time will be 10 am. Samples will then be checked for fibrin clot before re-spinning. Since download and upload features are active, all samples should only be manually loaded on DxI #1 without manual test order entry by the DxI bench CLS for that day. This is an approximately 42 minute assay for each sample. Once completed, the results will then be uploaded in the system. The device code is DL3 for OEM function. The worksheet is TGLU. This worksheet has been added to the ACH and PCH worksheets. The pending log/ worksheet should be monitored by the designated DxI bench CLS for all shifts and filed accordingly on the provided folder until completed. Refer to “Thyroglobulin Procedure Attachment A” for additional information.

A. Access Instrument

Refer to the appropriate system manuals and/or Help system for preparation and operation.

B. Assay Procedure

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

Results

| Linearity of Assay: 0.1 to ~500.0 ug/L. Dilute with the Access Thyroglobulin Sample Diluent if result is greater than the highest calibrator (approximately 500 ug/L) until an accurate number will be achieved. |

A. 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. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

B. Expected Values  (Note on units: 1ng/mL = 1 ug/L)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1.4-29.2 ug/L</td>
</tr>
<tr>
<td>Female</td>
<td>1.5-38.5 ug/L</td>
</tr>
</tbody>
</table>

Note: Normal reference ranges were adopted from the literature and verified in house by measuring thyroglobulin in serum from 138 healthy blood donors with normal TSH levels and negative for anti-thyroglobulin antibodies and anti-TPO antibodies (Giovanella et al. Clin Chem Lab Med, 2011).

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.

2. Thyroglobulin concentrations were measured in sera from 152 apparently healthy TgAb negative individuals using the Access Thyroglobulin method. The results ranged from 1.15 to 130.77 ng/mL, with a median of 9.08 ng/mL, and 2.5th and 97.5th percentiles of 1.59 and 50.03 ng/mL.

3. Serum samples from patients with Graves’ disease, Hashimoto’s disease and other benign conditions were tested in the Access Thyroglobulin assay and gave results ranging from undetectable to 804 ng/mL.

4. In patients who have undergone total or near-total thyroidectomy, with or without ¹³¹I radioablation, the Tg concentration should approach zero or the functional sensitivity of the assay.

5. A total of 155 samples were obtained from 127 subjects, originally diagnosed with thyroid
cancer (papillary or follicular carcinoma) who had undergone near-total or total thyroidectomy, using surgery (with or without radioactivity). Of these samples, 88 had a clinical diagnosis of cancer recurrence and 67 samples were diagnosed with no recurrence.

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>n Samples</th>
<th>n Subjects</th>
<th>Mean Tg (ng/mL)</th>
<th>Standard Deviation</th>
<th>Median Tg (ng/mL)</th>
<th>Range Tg (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca Positive Cancer recurrence</td>
<td>88</td>
<td>65</td>
<td>76.2</td>
<td>115.8</td>
<td>35.8</td>
<td>0.58–625.1</td>
</tr>
<tr>
<td>Ca Negative No evidence of recurrence</td>
<td>67</td>
<td>62</td>
<td>2.83</td>
<td>4.66</td>
<td>1.88</td>
<td>&lt; 0.1–36.8</td>
</tr>
</tbody>
</table>

6. Any changes in serum Tg concentrations should be interpreted in light of the total clinical presentation of the patient, including clinical history, data from additional testing and other appropriate information. Single measurements of thyroglobulin are of minimal value in assessing disease status. Serial determinations are required, and should be referenced to the post-surgical baseline Tg result.

**Procedural Comments**

A. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.

B. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.

C. Use forty (40) µL of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.

D. The system default unit of measure for sample results is ng/mL.

**Limitations of the Procedure**

A. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.1–500 ng/mL).

- If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e. < 0.1 ug/L).
- If a sample contains more than the stated value of the highest Access Thyroglobulin Calibrator (S5) approximately 500 ug/L, start diluting one volume of sample with 1 (1:2) or 4 (1:5) or up to 9 (1:10) volumes of Access Thyroglobulin Sample Diluent until an accurate number will be achieved. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.

| Linearity of Assay: 0.1 to 500.0 ug/L. Dilute with Access Thyroglobulin Sample Diluent if result is greater than the highest calibrator (approximately 500.0 ug/L) until an accurate number will be achieved. |

B. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have 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.
Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

C. The Access Thyroglobulin 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.

D. Samples containing up to 10 mg/dL (171 µmol/L) bilirubin, lipemic samples containing the equivalent of 1800 mg/dL (20.32 mmol/L) triolein (triglycerides) and hemolyzed samples containing up to 1 g/dL (10 g/L) hemoglobin do not affect the concentration of thyroglobulin assayed. In addition, samples with 5 g/dL (50 g/L) human serum albumin added to the endogenous albumin in the samples do not affect the concentration of thyroglobulin assayed.

E. Samples containing up to 50 mg/dL Aspirin, 20 mg/dL Acetaminophen, 40 mg/dL Ibuprofen, and 218.5 µg/dL of Thyroxine do not affect the concentration of thyroglobulin assayed.

F. The lowest detectable level of Tg distinguishable from zero (Access Thyroglobulin Calibrator S0) with 95% confidence is 0.1 ng/mL.

G. The Access Thyroglobulin assay does not demonstrate any “hook” effect up to 40,000 ng/mL.

H. Samples containing thyroglobulin antibodies (TgAb) cannot be reliably measured. All samples should be screened for Tg antibodies, and samples which are TgAb antibody positive should be interpreted with caution as the true value may be higher than that obtained.

References

Beckman Coulter, Inc. Access Thyroglobulin product insert, Brea, CA 92821, P/N A34085.

Beckman Coulter, Inc. Access Substrate product insert, Brea, CA 92821, P/N 386966.

Beckman Coulter, Inc. Access Wash Buffer II product insert, Brea, CA 92821, P/N A16534.

Beckman Coulter, Inc. UniCel DxI Wash Buffer II product insert, Brea, CA 92821, P/N A16543.


Beckman Coulter, Inc. does not automatically distribute revised CLSI procedures. If you receive a revised copy of the assay insert, call Technical Support to determine if the CLSI procedure has also been revised.

Printed in U.S.A.