

**Overview****Useful For**

Evaluation of suspected interference from heterophile antibodies causing a falsely elevated thyroglobulin result

**Profile Information**

Test Id	Reporting Name	Available Separately	Always Performed
TGII	TG, Interference Interpretation	No	Yes
TGQN	Thyroglobulin, Tumor Marker, S	Yes, (Order HTG2)	Yes
TGABI	Thyroglobulin Antibody, S	Yes, (Order HTG2)	Yes
TGMS	Thyroglobulin, Mass Spec., S	Yes	Yes

**Testing Algorithm**

Heterophile antibody evaluation consists of comparison of thyroglobulin (Tg) concentrations obtained by immunoassay with the following:

- Tg concentrations following pretreatment with commercial heterophile blocking reagents
- Tg concentrations obtained by mass spectrometry
- Tg concentrations obtained by serial dilutions of the sample

In all samples the presence of anti-thyroglobulin antibodies are evaluated.

**Highlights**

The specimen will be evaluated for potential heterophile antibody interference in the Beckman Access Thyroglobulin (Tg) Immunoassay.

In the absence of anti-thyroglobulin antibodies, the presence of heterophile interference is not suspected when the Tg concentration in the alternate platform evaluation, dilutions, and heterophile blocking tube pretreatment are substantially altered.

**Method Name**

TGQN/TGABI: Immunoenzymatic Assay

TGMS: Tryptic Protein Fragmentation, purified with Immunocapture, Analysis by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

(This service is performed pursuant to an agreement with SISCAPA Assay Technologies Inc. covering US Patent 7,632,686)

TGII: Medical Interpretation

**NY State Available**

No

**Specimen****Specimen Type**

Serum Red

**Ordering Guidance**

If interference or heterophile testing is not required, order HTG2 / Thyroglobulin, Tumor Marker, Serum.

For fine-needle aspirate specimens, order TFNAB / Thyroglobulin, Tumor Marker, Fine-Needle Aspiration Biopsy Needle Wash.

**Specimen Required**

**Patient Preparation:** For 12 hours before specimen collection do not take multivitamins or dietary supplements containing biotin (vitamin B7), which is commonly found in hair, skin, and nail supplements and multivitamins.

**Collection Container/Tube:** Red top (serum gel/SST are not acceptable)

**Submission Container/Tube:** Plastic vial

**Specimen Volume:** 2.5 mL

**Collection Instructions:** Centrifuge and aliquot serum into a plastic vial.

**Specimen Minimum Volume**

2 mL

**Reject Due To**

Gross hemolysis	Reject
Gross lipemia	OK
Gross icterus	Reject

**Specimen Stability Information**

Specimen Type	Temperature	Time	Special Container
Serum Red	Refrigerated (preferred)	7 days	
	Frozen	30 days	
	Ambient	72 hours	

**Clinical & Interpretive****Clinical Information**

Serum thyroglobulin (Tg) measurements are used in the follow-up of differentiated follicular cell-derived thyroid

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carcinoma. Because Tg is thyroid specific, serum Tg concentrations should be undetectable or very low after the thyroid gland is removed during treatment for thyroid cancer.

Most often Tg is measured by immunometric assays as they are widely available in automated high-throughput instruments, have shorter turnaround times, and have functional sensitivities of 0.1 mcg/L or less. However, these immunoassays may be affected by the presence of both anti-thyroglobulin antibody (TgAb) and heterophile antibody interferences. The presence of TgAb might cause falsely low/undetectable Tg that can mask disease; whereas heterophile antibodies might cause falsely high Tg that can be mistaken for residual or recurrent disease.

Some patients, due to exposure to animal antigens, have developed heterophile antibodies, such as human anti-mouse antibodies, that can interfere with immunoassay testing by binding to the animal antibodies used in immunoassays. In some sandwich immunoassays, including those for Tg, the presence of heterophile antibodies in the patient's sample might lead to a false-positive result.

Although rare, false-negative assay results due to heterophile interference have also been reported in the literature. Manufacturers often add blocking agents to their reagents, but, occasionally, patient samples containing heterophile antibodies are incompletely blocked and exhibit heterophile antibody interference. Subsequent reporting of erroneous results can have adverse effects on patient management, especially with tumor marker assays.

Dilution of the specimen prior to assay performance often yields unexpected nonlinear results in the presence of interfering substances such as heterophile antibodies and/or TgAb. Heterophile blocking tube treatment is also utilized for troubleshooting samples that exhibit potential heterophile interference. Finally, assessment of an analyte such as Tg with an alternative assay will often lead to apparent discrepant results in the presence of heterophile antibodies and/or TgAb interference.

Measurement of Tg by liquid chromatography-tandem mass spectrometry (Tg-MS) has been introduced as a method for accurate Tg quantitation in the presence of TgAb and heterophile antibodies. Tg-MS assays are based on peptide quantitation after tryptic digestion and immunocapture of Tg-specific peptides. The advantage of trypsin digestion is that all proteins are cleaved, including both TgAb and heterophile antibodies, thus eliminating them as interferences.

**Reference Values**

## THYROGLOBULIN TUMOR MARKER

Athyrotic: &lt;0.1 ng/ml

Intact thyroid: &lt; or =33 ng/mL

## THYROGLOBULIN, MASS SPECTROMETRY

Athyrotic: &lt;0.2 ng/mL

Healthy individuals with intact, functioning thyroid: &lt; or =33 ng/mL

## THYROGLOBULIN ANTIBODY

&lt;1.8 IU/mL

Reference values apply to all ages.

**Interpretation**

Specimens are evaluated for the presence of potential interfering anti-thyroglobulin (TgAb) and heterophile antibody

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interference in the Beckman Access thyroglobulin (Tg) immunoassay. While the presence of TgAb can result in falsely low Tg concentrations in the Beckman immunoassay, the presence of heterophile antibodies can result in falsely elevated Tg concentrations in the Beckman immunoassay. Following investigation of the presence of TgAb, heterophile antibody evaluation consists of pretreatment with commercial heterophile antibody blocking reagents, serial dilutions of the sample, and testing on an alternate platform generally unaffected by the presence of heterophile antibodies or TgAb (ie, Tg liquid chromatography-tandem mass spectrometry [Tg-MS]). The presence of heterophile antibody interference in the Beckman Access Tg immunoassay is not suspected when the results from the pretreatment, serial dilutions, and the alternative platform (Tg-MS) agree with the original result.

The presence of heterophile antibody interference in the Beckman Access Tg immunoassay is suspected when 1 or more of the following are observed: a significant decrease in Tg concentration (>20%) upon treatment of the sample with heterophile antibody blocking reagents, lack of linearity upon serial dilutions, or a significant difference in Tg concentration on the alternate platform (Tg-MS). When a heterophile antibody interference affecting the Beckman Access immunoassay is suspected, the Tg result from this assay is considered false positive and should not be used in clinical management.

#### Thyroglobulin Antibody:

TgAb may interfere with the measurement of Tg. TgAb should be measured in conjunction with every measurement of serum Tg to rule out potential interference. Anti-Tg antibodies greater or equal to 1.8 IU/mL are likely to cause interference in the Tg immunoassay. In the Beckman Access Tg immunoassay utilized in this interference evaluation, the presence of TgAb is most likely to cause a reduction in measured Tg concentrations. Measurement of Tg by mass spectrometry is not affected by the presence of TgAb.

#### Cautions

This heterophile antibody interference evaluation does not rule out the presence of other types interfering substances such as biotin.

There may be some samples with extremely strong heterophile interference. In such cases heterophile blocking reagents may not be able to block all the assay interference.

Specimens with thyroglobulin (Tg) concentrations greater than 250,000 ng/mL may "hook" and appear to have markedly lower levels.

Tg and anti-thyroglobulin (TgAb) values determined by different methodologies might vary significantly and cannot be directly compared with one another. Some patients might have antibody-positive results by some methods and antibody-negative results by others. Comparing values from different methods might lead to erroneous clinical interpretation.

Rare normal amino acid sequence variations within Tg can cause a false-low result in the Tg mass spectrometry assay, if they happen to be present in the Tg proteotypic peptides that are used for Tg quantification. While the exact prevalence of such changes is unknown, validation data on large sample numbers indicate that this affects less than 1% of samples. In the heterozygote state, the result would be an apparent reduction in Tg concentration by about 50%, while the homozygous state (<0.01%) is predicted to result in total loss of signal.

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**Clinical Reference**

1. Barbesino G, Algeciras-Schimmich A, Bornhorst JA. False positives in thyroglobulin determinations due to the presence of heterophile antibodies: an underrecognized and consequential clinical problem. *Endocr Pract.* 2021;27(5):396-400. doi:10.1016/j.eprac.2020.10.011
2. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper DS, Doherty GM, et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid.* 2009;19(11):1167-1214
3. Netzel BC, Grebe SKG, Algeciras-Schimmich A. Usefulness of a thyroglobulin liquid chromatography-tandem mass spectrometry assay for evaluation of suspected heterophile interference. *Clin Chem.* 2014;60(7):1016-1018
4. Algeciras-Schimmich A. Thyroglobulin measurement in the management of patients with differentiated thyroid cancer. *Crit Rev Clin Lab Sci.* 2018;55(3):205-218
5. Ward G, Simpson A, Boscatto L, Hickman PE. The investigation of interferences in immunoassay. *Clin Biochem.* 2017;50(18):1306-1311

**Performance****Method Description**

The specimen will be evaluated for potential heterophile antibody interference in the Beckman Coulter Access Thyroglobulin Immunoassay. Heterophile antibody evaluation will consist of measurement of thyroglobulin antibody, pretreatment with commercial heterophile antibody blocking reagents, testing on an alternate platform liquid chromatography-tandem mass spectrometry (LC-MS/MS), and serial dilution of the sample.

Heterophile blocking agents consisted of HBT (Heterophile Blocking Tube) - Scantibodies Incorporated for the Beckman Assay. This blocker contains murine (HBT) derived proteins in a buffered salt solution.

**Thyroglobulin, Tumor Marker:**

The Beckman Coulter UniCel Dxl 800 is the instrument used for thyroglobulin tumor marker testing. The Access Thyroglobulin (2) assay is a simultaneous one-step immunoenzymatic ("sandwich") assay. The sample is added to a reaction vessel, along with a biotinylated mixture of four monoclonal anti-thyroglobulin (Tg) antibodies (TgAb), streptavidin-coated paramagnetic particles, and monoclonal anti-Tg antibody alkaline phosphatase conjugate. The biotinylated antibodies and the serum thyroglobulin bind 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 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. (Package insert: Access Thyroglobulin. Beckman Coulter Inc.; 06/2021)

**Thyroglobulin Antibody:**

The Access Thyroglobulin Antibody II assay is a sequential 2-step immunoenzymatic ("sandwich") assay. The sample is added to a reaction vessel with paramagnetic particles coated with Tg protein. The serum TgAb binds to the thyroglobulin. After incubation in a reaction vessel, materials bound to the solid phase are held in place by a magnetic field, while unbound materials are washed away. The Tg-alkaline phosphatase conjugate is added and binds to the TgAb.

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After the second incubation, materials bound to the solid phase are held in place by a magnetic field, while unbound materials are washed away. Then, the chemiluminescent substrate 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 TgAb in the sample. The amount of analyte in the sample is calculated by means of a stored, multi-point calibration curve. (Package insert: Thyroglobulin Antibody II Instructions for Use. Beckman Coulter Inc; 2019)

**Thyroglobulin, Alternative Method:**

Serum is fractionated by a salting out method. Fractionated serum is then reduced, alkylated, and digested with trypsin. Tryptic fragments are further purified by immunocapture with antibodies specific to the individual fragments. Finally, these fragments are analyzed by LC-MS/MS. (Unpublished Mayo method)

**Thyroglobulin, Interference Interpretation:**

A laboratory director will review the results and provide an interpretation.

**PDF Report**

No

**Day(s) Performed**

Monday through Saturday

**Report Available**

3 to 6 days

**Specimen Retention Time**

6 months

**Performing Laboratory Location**

Rochester

**Fees & Codes****Fees**

- Authorized users can sign in to [Test Prices](#) for detailed fee information.
- Clients without access to Test Prices can contact [Customer Service](#) 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact [Customer Service](#).

**Test Classification**

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

**CPT Code Information**

84432

86800

84432

**LOINC® Information**

Test ID	Test Order Name	Order LOINC® Value
IETG	Interference Eval, Heterophile, TG	101626-0

Result ID	Test Result Name	Result LOINC® Value
62749	Thyroglobulin, Mass Spec., S	3013-0
35998	Interpretation	59462-2
TGABI	Thyroglobulin Antibody, S	56536-6
TGQN	Thyroglobulin, Tumor Marker, S	96460-1
TGIF	TG, Interference Heterophile	101627-8
TGIN	TG, Interpretation	101628-6