MAYO CLINIC LABORATORIES

Test Definition: IETG

Interference Evaluation Heterophile, Thyroglobulin Tumor Marker, Serum

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 antithyroglobulin antibodies is evaluated.

Highlights

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

In the absence of antithyroglobulin 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

TGII: Medical Interpretation
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)

NY State Available

Yes

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, patient **should not** take multivitamins or dietary supplements (eg, hair, skin, and nail supplements) containing biotin (vitamin B7).
Supplies: Sarstedt Aliquot Tube 5 mL (T914)
Collection Container/Tube: Red top (serum gel/SST are **not** acceptable)
Submission Container/Tube: Plastic vial
Specimen Volume: 2.5 mL Serum
Collection Instructions: Centrifuge and aliquot serum into a plastic vial.

Specimen Minimum Volume
Serum: 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	
	Ambient	72 hours	
	Frozen	30 days	

Clinical & Interpretive

Clinical Information

Serum thyroglobulin (Tg) measurements are used in the follow-up of differentiated follicular cell-derived thyroid 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 antithyroglobulin 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 antimouse 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
< or =33 ng/mL

THYROGLOBULIN, MASS SPECTROMETRY
< or =33 ng/mL

THYROGLOBULIN ANTIBODY
<1.8 IU/mL
Reference values apply to all ages.

Interpretation

Specimens are evaluated for the presence of potential interfering antithyroglobulin (TgAb) and heterophile antibody

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 may interfere with the measurement of Tg. TgAbs should be measured in conjunction with every measurement of serum Tg to rule out potential interference. Anti-TgAbs 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 TgAbs is most likely to cause a reduction in measured Tg concentrations. Measurement of Tg by mass spectrometry is not affected by the presence of TgAbs.

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.

Thyroglobulin and anti-Tg 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.

Patients treated with desiccated thyroid extract (eg, Armour Thyroid) may have elevated Tg results due to antibody cross-reactivity with porcine Tg, affecting immunoassays.

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.

Clinical Reference

1. Barbesino G, Algeciras-Schimnich 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-Schimnich 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-Schimnich 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, Boscato L, Hickman PE. The investigation of interferences in immunoassay. Clin Biochem. 2017;50(18):1306-1311
6. Ponder M, Lamos E, Munir K. Two cases of Armour Thyroid interference in thyroglobulin monitoring for thyroid cancer. Case Rep Endocrinol, 2021;1152572. doi:10.1155/2021/115257

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, and serial dilution of the sample.

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

Thyroglobulin:
The Beckman Coulter UniCel DxI 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 4 monoclonal antithyroglobulin (Tg) antibodies (TgAb), streptavidin-coated paramagnetic particles, and monoclonal anti-Tg antibody-alkaline phosphatase conjugate. The biotinylated antibodies and the sample 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.; 09/2024)

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 sample TgAb binds to the thyroglobulin on the paramagnetic particles. 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. 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. Beckman Coulter Inc; 09/2024)

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 liquid chromatography tandem mass spectrometry.(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
Mayo Clinic Laboratories - Rochester Superior Drive

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.

Test Definition: IETG

Interference Evaluation Heterophile, Thyroglobulin Tumor Marker, Serum

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