# *E. Thyroglobulin (Tg)*

Thyroglobulin (Tg), the precursor protein for thyroid hormone synthesis is detectable in the serum of most normal individuals when a sensitive method is used. The serum Tg level integrates three major factors: (i) the mass of differentiated thyroid tissue present; (ii) any inflammation or injury to the thyroid gland which causes the release of Tg; and (iii) the amount of stimulation of the TSH receptor (by TSH, hCG or TRAb). An elevated serum Tg concentration is a non-specific indicator of thyroid dysfunction. Most patients with elevated serum Tg have benign thyroid conditions. The primary use of serum Tg measurements is as a tumor marker for patients carrying a diagnosis of differentiated thyroid cancer (DTC). Approximately two thirds of these patients have an elevated pre-operative serum Tg level that confirms the tumor's ability to secrete Tg, and validates the use of serum Tg measurements as a post-operative tumor marker *(307)*. In contrast, when the pre-operative serum Tg concentration is <u>not</u> elevated above normal, there is no evidence that the tumor is capable of Tg secretion, and the value of an undetectable post-operative serum Tg value is less reassuring. In such patients a detectable post-operative serum Tg could represent a large amount of tumor. In general, changes in serum Tg post-operatively represent changes in tumor mass, provided that a constant TSH level is maintained with L-T4 therapy.

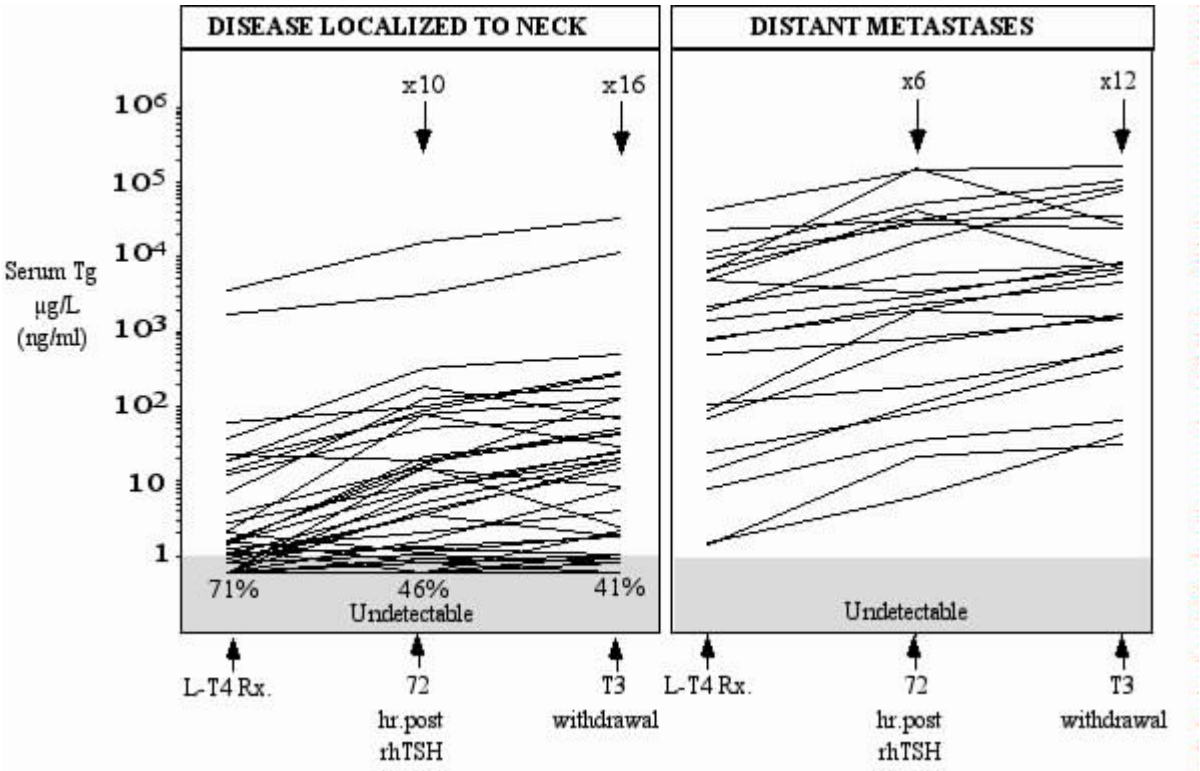


*Fig 6. Serum Tg responses after rhTSH administration or T3 withdrawal. Data from ref. 308.*

A serum Tg measured during TSH stimulation [endogenous TSH or recombinant human TSH (rhTSH)] is more sensitive for detecting residual or metastatic DTC than a basal Tg measurement made during L-T4 treatment (Figure 6) *(308)*. The magnitude of the serum Tg increase in response to TSH provides a gauge of the TSH sensitivity of the tumor. Well-differentiated tumors typically display a ~10-fold stimulation of serum Tg in response to a high TSH *(309)*. Poorly differentiated tumors that do not concentrate iodide may display a blunted response to TSH stimulation *(310)*.

**1. Current Status of Tg Methods**

Thyroglobulin is usually measured in serum, but measurements can also been made in thyroid cyst fluids and material obtained by fine needle biopsy of thyroid nodules *(311)*. The measurement of Tg in serum is

technically challenging. Currently, immunometric assays (IMA) are gaining in popularity over radioimmunoassay (RIA) methods. This is because IMA methods offer the practical advantage of a shorter incubation time, an extended dynamic range for the assay and a more stable labeled antibody reagent that is less prone to labeling damage than RIA *(312)*. Laboratories can now choose from a range of both isotopic (immunoradiometric, IRMAs) and nonisotopic, (primarily chemiluminescence, ICMA) IMA methods. However, IMA methods are more prone to interference by thyroglobulin autoantibodies (TgAb), which cause an underestimation of serum Tg levels. This has prompted some laboratories to choose RIA methods for measuring serum Tg in TgAb-positive patients and to restrict the use of IMA methods to TgAb-negative patients only. However, no method can claim to be totally unaffected by TgAb interference that can cause either an over- or underestimation of Tg RIA measurements. Apart from the problems with TgAb interference, current Tg IMA methods are also compromised by differences in standardardization and specificity and generally show poor sensitivity, sub-optimal between-run precision and the potential for high dose "hook" effects *(312)*.

(a) Standardization

Serum Tg concentrations measured by either RIA or IMA methods, vary widely *(312,313)*. A recent collaborative effort sponsored by the Community Bureau of Reference of the Commission of the European Communities has developed a new international Tg reference preparation, CRM-457 *(298,314)*. This material can be obtained from Dr. Christos Profilis, BCR, Rue de la Loi 200, B 1049 Brussels, Belgium.

**Guideline 42. For Manufacturers Developing Tg Methods**

- The diluent used for standards should ideally be Tg-free/TgAb-free human serum. Non-serum matrices should be selected to produce a signal (radioactive counts, relative light units etc) that is identical to Tg-free/TgAb-free human serum to avoid matrix-related biases.

The bias between different Tg methods may result from differences between the Tg-free matrix used to dilute standards and patient serum, or differences in the epitope recognition by the different Tg antibodies used by individual manufacturers. Ideally, the diluent used for standards should be Tg-free/TgAb-free human serum or alternatively, a non-serum matrix that has been selected to produce a signal (radioactive counts, relative light units etc) that is identical to Tg-free/TgAb-free human serum. It is critical that physicians be informed before the laboratory changes its Tg method to allow for a re-baselining of DTC patients.

The widespread adoption of the CRM-457 standard was projected to reduce, but not eliminate the significant method-to-method variability that exists with this procedure. It was hoped that worldwide standardization would facilitate better agreement in the literature from different studies as well as improve the clinical use of serial Tg monitoring of DTC patients who sometimes have serum Tg measurements determined by different laboratories. Unfortunately, the use of the new CRM-457 standard has not eliminated the problems of between-method variability as much as initially thought. Currently, serum Tg levels determined by methods that use CRM-457 standards can differ by as much as four-fold (Figure 7). These method-to-method differences are greater than the goal for maximum imprecision required for monitoring individual patients (Table 5) and precludes the interchangeable use of different Tg methods for long-term follow-up of thyroid cancer patients.

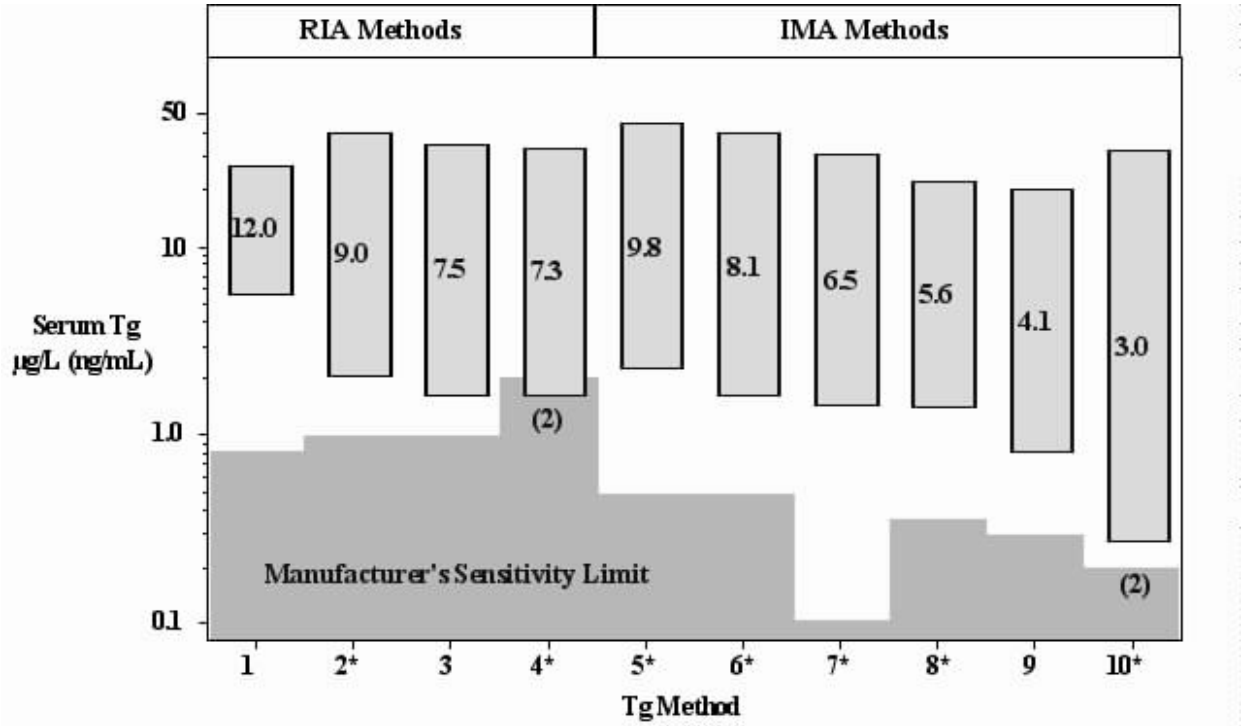


*Fig 7. Mean ± 2sd values for measuring 20 TgAb-negative normal sera by 10 different Tg methods. Method #1= Diagnostic Systems Laboratories, Webster, TX, USA; Method #2=University of Southern California RIA, Los Angeles, CA, USA; RIA #3= Kronus RIA, Boise ID, USA; Method #4= Endocrine Sciences RIA, Calabasas, CA, USA; Method #5=Nichols Institute Diagnostics ICMA, San Juan Capistrano, CA, USA; Method #6= Endocrine Sciences ICMA, Calabasas, CA, USA; Method #7=Sanofi Pasteur IRMA, Marnes-La-Coquette, France; Method #8=Kronus OptiQuant IRMA, Boise ID, USA; Method #9=Brahms DynoTest TgS IRMA, Berlin, Germany; Method #10=Diagnostic Products Immulite ICMA, Los Angeles, CA, USA. An asterisk denotes assays claiming CRM-457 standardization.*

**Guideline 43. For Laboratories Considering Changing their Tg Method**

*Select a Tg method on the basis of its performance characteristics not cost or expediency. Before changing the Tg method the laboratory should consult with physician users and compare results between the old and proposed new method using specimens from both TgAb-negative and TgAb-positive patients.*

- *TgAb-negative patients:* If the bias between the old and new method results is > 10%, physicians should be informed and given sufficient time to re-baseline critical patients.
- *TgAb-positive patients:* The laboratory should warn physicians about the likely direction of interference in the presence of TgAb.
- If serum Tg values are to be reported for TgAb-positive specimens, an appropriate cautionary comment should be displayed on each laboratory report:

- **FOR IMA METHODS*:***

*IMA methods may give inappropriately low or underestimate serum Tg levels when TgAb is present. Undetectable serum Tg results cannot be used to indicate the absence of tumor in a TgAb-positive patient. A detectable Tg level indicates that Tg is present, but concentrations may be underestimated.*

- **FOR RIA METHODS:**

*RIA methods may give inappropriately higher- or underestimated serum Tg values when TgAb is present (depending on the method). Detectable serum Tg results should not be used as the sole factor for determining the presence of residual thyroid tissue or tumor.*

(b) Sensitivity

Some Tg methods are too insensitive to detect the lower euthyroid reference limit that approximates 1-3 µg/L (ng/mL) (depending on the assay). Methods that are unable to detect Tg in normal sera are usually too insensitive for monitoring DTC patients for recurrence. As with TSH, Tg assay functional sensitivity is determined by the 20% CV between-run precision [Section-3 C2]. The protocol used to determine Tg assay functional sensitivity is the same as described for TSH (Guideline 20) with the three stipulations described in Guideline 44.

(c) Precision

Both within-run and between-run precision, expressed as percent coefficient of variation (% CV) are important parameters for validating the performance of a Tg assay. Precision should be established using TgAb-negative serum pools with target Tg values at three different levels (see Guideline 44).

The within-run precision for immunoassay methods is better than between-run precision as would be expected. This is because measurements made within a single run are not subject to the variability introduced by using batches of reagents and different instrument calibrations. Within-run precision may be the more relevant parameter when assessing the serum Tg response to rhTSH stimulation *(308)*. In this setting, a basal and rhTSH-stimulated specimen are drawn 3 to 5 days apart and usually measured in the same run (Figure 6) *(308,309)*. In contrast, when using Tg measurement for serial monitoring, the longer the interval between runs the greater the variability and the worse the between-run precision. Non-human matrices used to determine low-range precision may produce unrealistic functional sensitivity limits compared with measurements made in TgAb-free human serum. It is important to establish functional sensitivity and between-run precision from data spanning a 6 to 12 month period, since this is the typical clinical interval used for monitoring DTC patients.

The suggested goal for maximum imprecision of serum Tg measurements for monitoring patients should be <5% (Table 5). It is unlikely that current Tg assays can maintain such tight precision over the typical 6 to 12 month time-span used for monitoring DTC patients. This precision problem can be overcome by measuring archived, stored samples from the patient in the same run as the current specimen *(9)*.

**Guideline 44. Tg Assay Functional Sensitivity & Between –Run Precision**

*Functional sensitivity and between-run precision should be established using the same protocol as for TSH (Guideline 20) with three important stipulations:*

- Use human serum pools that contain no TgAb, determined by a sensitive TgAb immunoassay.
- Target values are recommended for low, medium and high pools:
  Low Pool (used to determine functional sensitivity) should have a serum Tg value that is 30 to 50 % higher than the expected functional sensitivity (FS) limit.
  [If FS = 1.0 µg/L (ng/ml) the low pool target should be 1.3 to 1.5 µg/L (ng/ml)]
  Medium Pool target = ~10 µg/L (ng/ml) i.e. close to the mid-normal range.
  High Pool target = ~90% of the upper reportable limit suggested by manufacturer.
- The test period used for assessing between-run precision should be at least 6 months. This is more representative of the clinical interval used for monitoring DTC patients than the 6-8 week interval recommended for TSH in Guideline 20.

(d) High Dose Hook Effect

A high dose hook effect affects primarily IMA methods. Falsely low values due to a "hook effect" are especially problematic for tumor-marker tests like Tg, because it is not unusual to encounter very high values when patients have advanced metastatic disease *(307,310,315)*. A hook effect occurs when an excessive amount of antigen overwhelms the binding capacity of the capture antibody. This results in an inappropriately low signal that translates into an inappropriately low or paradoxically normal range result for a patient with an excessively elevated serum Tg concentration (>1000 µg/L (ng/mL)) *(312)*.

Manufacturers of IMA methods attempt to overcome the hook effect problem by one of two approaches:
• Two-step assay design. The serum specimen is first reacted with the capture antibody before unbound constituents are washed away and the labeled antibody is introduced, followed by a second incubation.
• Two dilutions (usually undiluted and 1/10) are made for each specimen To detect any "hook".

A 'hook" is suspected when the dilution tube has a higher result than the undiluted specimen. Further dilutions are made until the result in the dilution tube decreases and the serum Tg concentrations of the two dilutions are in agreement.

**Guideline 45. Testing for "Hook" Effects**

- A two-step design is recommended to minimize hook problems. "One-step" assays that are more prone to hook effects should measure every specimen at two dilutions (undiluted and 1:10) to check for a discrepancy in the two results.
- All assays (two-step or one-step) should be validated for a hook effect before manufacturer release.
- To check for a hook effect, measure serial 10-fold dilutions of ~ 20 different TgAb-negative specimens with serum Tg concentrations above 10,000 µg/L (ng/ml) and ~ 20 different TgAb-negative specimens with serum Tg values above 100,000 µg/L until parallelism is demonstrated.

(e) Thyroglobulin Autoantibody (TgAb) Interference

Thyroglobulin autoantibodies (TgAb) are detected in a higher percentage of DTC patients than the general population (~20 versus ~10 %, respectively) *(276)*. Serial serum TgAb measurements may be an independent prognostic indicator of the efficacy of treatment for, or recurrence of, DTC in TgAb-positive patients *(276-278,316)*. Any TgAb present in the specimen has the potential to interfere with any Tg method *(317,318)*. Because TgAb is heterogeneous, neither the measured TgAb concentration nor an exogenous Tg recovery test is 100% reliable for predicting whether the TgAb in a specimen will cause interference *(276,317,318)*). Probably the most reliable hallmark of TgAb interference is the presence of RIA/IMA discordance. Specifically, Tg measured by RIA is typically higher than Tg measured by IMA if the specimen contains interfering TgAb *(276,309)*. There is now consensus that Tg recovery tests are an unreliable approach for detecting TgAb and should be eliminated *(276,318)*. Early studies that reported low recoveries in the absence of TgAb in some sera were flawed by the insensitivity of early TgAb methods. When a sensitive immunoassay is used, TgAb is always detected when recovery is low.

Non-competitive immunometric assay (IMA) methods appear to be more prone to TgAb interference than RIA methods, as evidenced by the finding of undetectable Tg values in Graves disease subjects *(319,318)*. It appears that IMAs fail to quantify the Tg that is complexed with TgAb in some cases, and this can result in an underestimation of the total Tg concentration. In contrast, RIA methods appear capable of quantifying both the free and TgAb-bound Tg moieties in the specimen, and typically produce higher values than IMA methods when TgAb is present *(276,309)*. The sensitivity and specificity of different TgAb tests is highly variable [Section-3 D6(b)]. It is essential that the TgAb measurement be made by the laboratory performing the Tg testing because that laboratory is responsible for selecting the TgAb method most suited for detecting TgAb interference with the Tg methods it uses.

When serum containing TgAb are measured by both an RIA and IMA method, an RIA:IMA discordance [Tg RIA = ≥2 µg/L (ng/mL): Tg IMA = undetectable] is frequently observed. This discordance appears to characterize TgAb interference with one or both classes of method. Since the current threshold for a positive rhTSH-stimulated Tg response is 2 µg/L (ng/mL), this degree of discordance has the potential to influence clinical decision-making *(308)*. Some believe that RIA measurements produce more clinically valid serum Tg results for TgAb-positive patients than IMA measurements, as judged by correlations with clinical status and parallellism with serial TgAb measurements *(276,320)*. However, it should be stressed that no RIA method is immune to TgAb interference in all TgAb-positive sera and the influence of TgAb on different RIA methods is quite variable and relates to the assay components and incubation conditions. Specifically, the quality of the $^{125}$I-Tg tracer, together with the specificity of the Tg polyclonal antibody reagent, determines the propensity of

the method for TgAb interference *(275,321,322)*.

**Guideline 46. TgAb Interference and Recovery Tests**

- Recovery tests do not reliably detect TgAb and should be discouraged and eliminated. Previous studies have shown that low recoveries are sometimes seen in the absence of TgAb were flawed by the insensitivity of early TgAb methods. When sensitive immunoassays are used, TgAb can always be detected when recovery is low.
- Discordance between IMA and RIA Tg measurements for TgAb-positive specimens suggests TgAb interference (if values are typically concordant for TgAb-negative specimens).
- Laboratories should not report undetectable serum Tg values for TgAb-positive patients if the method produces inappropriately low or undetectable serum Tg values for TgAb-positive DTC patients with documented disease.

Although no current Tg method is guaranteed free from interference by TgAb, the underestimation of serum Tg concentrations typical of TgAb interference with IMA methodology is the most serious direction of interference, since underestimation has the potential to mask metastatic disease. It follows that laboratories should not report undetectable serum Tg values for TgAb-positive patients if that method produces inappropriately low or undetectable serum Tg values for TgAb-positive DTC patients with documented disease.

**Guideline 47. For Manufacturers and Laboratories**

*Tg method package inserts should cite realistic performance characteristics for the method (i.e. performance that can be reproduced across a range of clinical laboratories).*

- Assays should be standardized against the CRM-457 reference preparation. Assays <u>not</u> standardized against CRM-457 should provide a correction factor.
- The mean Tg level and the 2sd limits of the reference range for TgAb-negative normal euthyroid subjects (established using Guideline 48) should be cited in all publications to allow comparison of absolute values.
- Assays that cannot detect Tg in all normal sera have suboptimal sensitivity for monitoring DTC patients.
- The matrix used to dilute the standards should be checked for bias (Guideline 42).
- Functional sensitivity and within and between-run precision should be established using the protocols described in Guideline 44.
- TgAb interference should be assessed by checking for RIA:IMA discordances in TgAb-positive sera [TgAb levels 100 to >1000 kIU/L (IU/ml)].
- TgAb immunoassay measurements and <u>not</u> exogenous Tg recovery studies should be used to detect TgAb interference (see Guideline 46).
- Serum Tg values for TgAb-positive specimens should not be reported if the method gives inappropriately undetectable values in TgAb-positive DTC patients with documented disease.

**2. Tg Messenger RNA (mRNA) Testing**

The clinical value of Tg mRNA measurements in peripheral blood has yet to be established. Before Tg mRNA testing can be used to facilitate the therapeutic decision-making for DTC, questions regarding the sensitivity and tissue specificity of Tg mRNA in peripheral blood need to be resolved *(323-325)*.

Reverse transcriptase-polymerase chain reaction (RT-PCR) amplification of tissue specific mRNA has been used to detect circulating cancer cells in the peripheral blood of patients with melanoma, prostate and breast malignancies *(326-328)*. The availability of Tg-specific primers now allows the application of this technique to the detection of Tg mRNA transcripts in blood. The use of RT-PCR to detect recurrent thyroid cancer was first reported in 1996 *(329)*. Subsequently, the technique has been applied to cervical lymph node metastases and has been found to be more sensitive than the measurement of Tg in the aspirate *(330)*.

A number of groups have now developed quantitative RT-PCR methods to detect Tg mRNA transcripts in

blood *(323-325,331-333)*). These studies generally find detectable Tg mRNA in all normal subjects but with a poor correlation with serum Tg as measured by immunoassay *(331,332)*. The correlation between Tg mRNA and tumor burden also differs. Some studies have reported that the amount of Tg mRNA correlates with the presence or absence of metastases while others report no such correlation *(324,331,333)*. These discrepancies likely reflect differences in the sensitivity and specificity of the Tg primers and RT-PCR systems used, differences in the sensitivity of the imaging techniques and Tg immunoassays used as well as differences in the TSH status of the patient. Specificity problems (false positives) are a recognized limitation of RT-PCR methodology *(328,334)*. Further studies are needed to determine whether the detectable Tg mRNA levels reported for athyreotic patients without known metastases reflect clinically occult disease, assay artifact or illegitimate transcription.

The correlation between Tg mRNA test results and clinical recurrence, especially in patients with positive Tg mRNA and undetectable serum Tg levels, would need to be shown before the Tg mRNA test becomes widely used in clinical practice. Since the Tg mRNA test is more expensive than a serum Tg measurement, it is likely that if Tg mRNA measurements are shown to be clinically useful, these tests will be reserved for high-risk or TgAb-positive patients in whom serum Tg measurements are diagnostically unreliable.

**3. Serum Tg Reference Values**

(a) Normal Euthyroid Subjects

Serum Tg concentrations are log-normally distributed in euthyroid individuals. Values tend to be slightly higher in women, but gender-related reference ranges are unnecessary *(335)*. Cigarette smoking is a factor associated with goiter and higher serum Tg values *(336)*. Tg reference ranges are geographically sensitive, since serum Tg is influenced by iodide availability and intake *(337,338)*. Subject selection for the normal cohort for Tg reference range evaluation should have the following exclusion criteria:

- Goiter
- Cigarette smoking
- Personal or family history of thyroid disease
- Presence of thyroid autoantibodies (TgAb and/or TPOAb)
- Serum TSH < 0.5 mU/L or >2.0 mU/L

(b) Serum Tg Values following Thyroid Surgery

As indicated by Guideline 48, the Tg reference interval cited on laboratory reports does not apply to patients who have had thyroid surgery! In the first few weeks after surgery, the serum Tg will be determined by the completeness of the surgery, the degree of leakage of Tg from the surgical margins, and most importantly whether thyroid hormone has been given to prevent the expected rise in TSH. In fact, the serum TSH concentration is such a powerful modulator of the serum Tg level that it is usually necessary to know the TSH status of the patient before assessing the significance of any serum Tg measurement.

In the early weeks following thyroidectomy, serum Tg concentrations typically fall with a half-life approximating 2-4 days, when thyroid hormone administration prevents TSH from rising *(340,341)*. In this setting, the relationship between the pre-operative and 6-8 week post-operative serum Tg values can provide information that could influence the treatment plan. During long-term monitoring, serum Tg concentrations measured on and off L-T4 treatment (low or high TSH, respectively) provide different information. The pattern of change in serum Tg values (on L-T4 treatment) is a better indicator of a change in tumor burden than any single serum Tg value *(122)*. The serum Tg concentration during L-T4 treatment is a more stable indicator of tumor mass than a serum Tg measured when the TSH is high (L-T4 withdrawal or rhTSH administration) prior to a radioiodine (RAI) scan. This is because the magnitude of the TSH-stimulated serum Tg elevation is influenced by the extent and chronicity of the TSH elevation, which can vary from scan to scan. However, as shown in Figure 6, because TSH usually stimulates serum Tg more than five-fold, TSH-stimulated serum Tg measurements are more sensitive for detecting disease confined to the neck, than serum Tg levels measured

during TSH suppression *(308,309)*. The magnitude of the TSH-stimulated serum Tg response provides a gauge of the TSH sensitivity of the tumor. Poorly differentiated metastatic tumors that are RAI-scan negative have blunted (less than three-fold) TSH-stimulated serum Tg responses *(310)*.

**Guideline 48. Serum Tg Normal Reference Intervals**

- Tg reference ranges should be determined locally because serum Tg concentrations are influenced by iodide intake:

<u>*Countries with adequate iodide intake:*</u> The serum Tg reference interval for a TgAb-negative euthyroid population using CRM-457-standards approximates 3 to 40 µg/L (ng/ml).
<u>*Countries manifesting iodide deficiency:*</u> The population mean Tg value and the upper Tg reference limit may be elevated relative to the degree of iodide deficiency.

- Laboratories should validate their Tg normal reference interval independent of the manufacturer.
- Tg reference ranges should be established from the log transformed values of 120 normal, non-smoking, euthyroid (TSH 0.5 to 2.0 mIU/L) subjects less than 40 years of age with no personal or family history of thyroid disease and with no evidence of TgAb or TPOAb.
- It is misleading to cite the normal euthyroid reference range when reporting serum Tg values for thyroidectomized DTC patients. Reference values should be related to the euthyroid reference limits for the method, the thyroid mass and TSH status.

*For example, the reference ranges below would be appropriate for a Tg method with a euthyroid reference range of 3-40 µg/L (ng/ml):*

| Tg µg/L (ng/ml) | Condition |
|---|---|
| 3 – 40 | Normal thyroid gland reference (TSH 0.4-4.0 mIU/L) |
| 1.5 – 20 | Normal thyroid gland reference (TSH <0.1 mIU/L) |
| < 10 | Thyroid lobectomy (TSH < 0.1 mIU/L) |
| < 2 | Near-total thyroidectomy (TSH < 0.1 mIU/L) |

**4. Clinical Uses of Serum Tg Measurement**

The serum Tg concentration reflects thyroid mass, thyroid injury and TSH receptor stimulation *(122)*. It follows that an elevated serum Tg is a non-specific finding associated with virtually any thyroid pathology.

<u>(a) Non-Neoplastic Conditions</u>

Serum Tg is elevated when patients have a goiter or in most hyperthyroid conditions. A low serum Tg concentration can be a useful parameter for confirming the diagnosis of thyrotoxicosis factitia and/or investigating the etiology of congenital hypothyroidism *(342,343)*.

**Guideline 49. Serum Tg Measurement for Non-Neoplastic Conditions**

*Abnormally high serum Tg concentrations result from abnormalities in thyroid mass, excessive thyroidal stimulation, or physical damage to the thyroid secondary to surgery, FNA or thyroiditis.. Serum Tg measurements are useful:*

- For diagnosing thyrotoxicosis factitia which is characterized by a non-elevated serum Tg.
- To investigate the etiology of congenital hypothyroidism in infants detected by neonatal screening.
- To assess the activity of inflammatory thyroiditis, eg subacute thyroiditis, or amiodarone-induced thyroiditis.

Serum Tg measurements are also sometimes useful to confirm a past history of thyroiditis, in which the serum Tg concentration is typically the last biochemical parameter to normalize (up to 2 years) *(344)*. Recent studies propose the use of serum Tg measurement as a parameter to reflect the iodide status in a given population

*(337,338)*.

(b) Differentiated Thyroid Carcinomas (DTC)

In the setting of DTC, the serum Tg concentration reflects thyroid mass (tumor or normal remnant), thyroid injury (surgery or FNA) and TSH receptor stimulation (endogenous or rhTSH) *(122)*. Since the TSH level is a major regulator of serum Tg concentrations, it is difficult to interpret serum Tg values without knowing the TSH status of the patient. Although there is no "normal Tg reference range" for treated DTC patients, the normal relationship between thyroid mass and serum Tg provides an important reference point. Specifically, one gram of normal thyroid tissue releases ~1 µg/L (ng/mL) Tg into the circulation when the serum TSH is normal and ~0.5 µg/L (ng/mL) when the serum TSH is suppressed below 0.1 mU/L.

**Guideline 50. Serum Tg Measurements for Differentiated Thyroid Carcinoma (DTC)**

***TgAb-Negative patients:***

- Pre-operative serum values (drawn before or >2 weeks after FNA) are useful for determining the Tg-secretion capacity of the tumor.
- The acute post-operative decline in serum Tg reflects the completeness of surgery with the serum Tg half-life of 3-4 days. (If thyroid hormone is given to prevent a rise in TSH).
- There is no "normal range" for a thyroidectomized patient! Completely athyreotic patients should have no Tg detectable in their serum, even if the TSH is elevated.
- Useful reference point: one gram of normal thyroid tissue releases ~ 1 µg/L (ng/ml) Tg into the serum when TSH is normal, and ~0.5 µg/L (ng/ml) when TSH is suppressed < 0.1 mU/L.
- When serum Tg is detectable during L-T4 treatment (stable TSH), changes in tumor burden can be monitored by serial serum Tg measurements without thyroid hormone withdrawal or rhTSH.
- When serum Tg is undetectable during L-T4 treatment (and TgAb is absent) a TSH-stimulated serum Tg is more sensitive for detecting disease localized to the neck than serum Tg measured during TSH suppression.
- There is typically a >5-fold increase in serum Tg above basal L-T4 Rx. values following TSH stimulation (endogenous or rhTSH). Paired studies show that rhTSH-stimulated Tg responses are approximately half those seen with endogenous TSH following thyroid hormone withdrawal.

***TgAb-Positive Patients:***

- Typically display blunted or absent TSH-stimulated serum Tg responses.
- Serial TgAb measurements (by immunoassay) are valuable as a surrogate tumor marker test

***(i) Pre-operative Serum Tg***

Some thyroid tumors lack the ability to secrete thyroglobulin. An elevated pre-operative serum Tg level is seen in 2/3 of patients with DTC indicating that their tumors have the capacity for Tg secretion and by inference, post-operative serum Tg monitoring can be used clinically in these patients *(307)*. This information is key to the interpretation of post-operative serum Tg results. If the pre-operative serum Tg level is within normal limits, an undetectable post-operative serum Tg value is less reassuring because it is unclear whether the tumor originally secreted Tg. The sensitivity of post-operative serum Tg monitoring for detecting recurrence will be highest when the tumor is relatively small (≤2cm diameter) and the pre-operative serum Tg value is high. (Note: pre-operative specimens should be drawn before FNA, and held to await the cytologic diagnosis, or can be drawn >2 weeks following FNA.)

***(ii) Serum Tg Measurement 1-2 months after Thyroid Surgery***

Following thyroid surgery, serum Tg concentrations fall rapidly with a half-life of ~2-4 days *(340)*. Any Tg released from surgical margins should largely resolve within the first two-month period after surgery. During this time TSH will be the dominant influence on the serum Tg level. If thyroid hormone therapy is initiated immediately after surgery to prevent the rise in TSH, the serum Tg concentration will decline to a level that

reflects the size of the normal thyroid remnant plus any residual or metastatic tumor. Since the thyroid remnant left after near-total thyroidectomy typically approximates 2 grams of tissue, a serum Tg concentration < 2 µg/L (ng/mL) is expected when the patient has undergone successful near-total thyroidectomy and has serum TSH maintained below 0.1 mU/L.

***(iii) Serum Tg Measurement during Long-term Monitoring on L-T4 Rx.***

When the TSH level is stable during L-T4 therapy, any change in the serum Tg level will reflect a change in tumor mass. Clinical recurrence in tumors judged to be "poor Tg secretors" (normal range pre-operative Tg value) may be associated with low or undetectable post-operative serum Tg values. In contrast, recurrence of tumors considered as "good Tg secretors" (elevated pre-operative Tg values) is usually associated with a progressive rise in serum Tg *(122)*. The pattern of serial serum Tg measurements, made when the patient has a stable TSH, is more clinically useful than an isolated Tg value. However, it is possible to interpret the significance of an isolated Tg value by knowing the normal reference range of the Tg assay, the extent of thyroid surgery and the serum TSH level (at steady state), as shown in Figure 8.

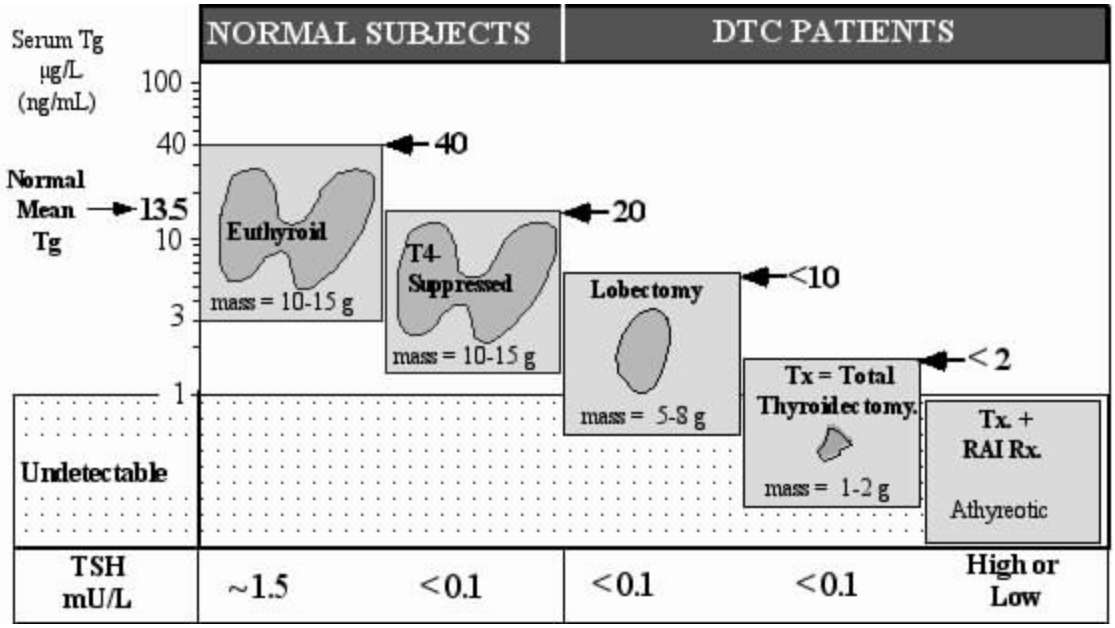


*Fig 8. Expected serum Tg values relative to thyroid mass and TSH status. (For methods with a different normal reference range than shown in Figure 8, adjust the absolute values by applying a correction factor based on the mean normal value of the method. (i.e. for methods with a mean normal value of 6.2 µg/L (ng/mL) correct the values shown by 50%).*

***Assumptions:***

- *No recent thyroid injury (surgery or FNA )*
- *Using Guideline 48, euthyroid normal control mean Tg = 13.5, range 3-40 (2sd) µg/L (ng/mL)*
- *Mass of normal thyroid tissue = 10-15 grams*
- *One gram of normal thyroid tissue produces ~1µg/L (ng/mL) Tg in serum @normal TSH*
- *One gram of normal thyroid tissue produces ~0.5µg/L (ng/mL) Tg @ TSH < 0.1 mIU/L*

***(iv) Serum Tg Responses to TSH Stimulation***

The magnitude of the rise in serum Tg in response to either endogenous TSH (thyroid hormone withdrawal) or recombinant human TSH (rhTSH) administration, provides a gauge of the TSH sensitivity of the tumor *(308,309)*. Typically, TSH stimulation of normal thyroid remnants or a well-differentiated tumor produces a >3-fold increase in serum Tg above basal (TSH-suppressed) levels, in TgAb-negative patients (Figure 6). The

serum Tg response to an endogenous TSH rise is typically greater than for rhTSH *(308,345)*. Moreover, poorly differentiated tumors, display a blunted (< 3-fold) increase in serum Tg in response to TSH stimulation *(310)*. It should be noted that TgAb-positive patients typically show a blunted or absent rhTSH-stimulated Tg response by most assays, even when the basal serum Tg concentration is detectable.