Clinical Information

Thyroglobulin (Tg) is a highly thyroid-specific large homodimeric glycoprotein (approximately 660 KDa). It contains 8% to 10% of carbohydrates and iodine. Thyroxine (T4) and triiodothyronine (T3) are synthesized on Tg within the lumen of thyroid follicles. For T4 and T3 release, Tg is reabsorbed into thyrocytes and proteolytically degraded, liberating T4 and T3 for secretion.

Small amounts of intact Tg are secreted alongside T4 and T3 and are detectable in the serum of healthy individuals, with levels roughly paralleling thyroid size (0.5-1.0 ng/mL Tg per gram thyroid tissue, depending on thyroid-stimulating hormone [TSH] level). In situations of disordered thyroid growth (eg, goiter), increased thyroid activity (eg, Graves disease), or glandular destruction (eg, thyroiditis), larger amounts of Tg may be released into the circulation.

Clinically, the main use of serum Tg measurements is in the follow-up of differentiated follicular cell-derived thyroid carcinoma. Because Tg is highly organ-specific, serum Tg concentrations should be undetectable, or very low, after the thyroid gland is removed during primary treatment for thyroid cancer. Current clinical guidelines consider a serum Tg of >1 ng/mL in an athyrotic individual as suspicious of possible residual or recurrent disease. To improve diagnostic accuracy, it is recommended that at least initially this measurement is obtained after TSH stimulation, either following thyroid hormone withdrawal, or after injection of recombinant human TSH. Most patients will have a relatively low risk of recurrence, and will thereafter only require unstimulated Tg measurement. If unstimulated (on thyroxine) serum Tg measurements are <0.1 to 0.2 ng/mL, the risk of disease is <1%. Patients with higher Tg levels, who have no demonstrable remnant of thyroid tissue, might require additional testing, such as further stimulated Tg measurements, neck ultrasound, or isotope imaging. A stimulated Tg >2 ng/mL is considered suspicious.

There are 3 situations, when serum Tg measurement might be misleading:

1. Remnant thyroid tissue (see above, 0.5-1 ng/mL Tg per gram)
2. Antithyroglobulin autoantibodies (TgAB), which occur in 15% to 30% of thyroid cancer patients, can lead to false-low measurement in immunometric assays (most commonly used); in competitive assays they may cause false-high results.
3. Heterophile antibodies (HAB) are antibodies that are capable of interacting with the antibodies used in immunoassays, usually resulting in false-high measurements. Depending on the assay and the patient population, this can lead to erroneously high results in 0.1% to 3.0% of patients.

Traditionally, there have been no reliable means to obtain accurate Tg measurements in patients with TgAB or HAB. However, recently, trypsin...
digestion of serum proteins, which cuts both antibodies and Tg into predictable fragments, has allowed accurate quantification of Tg in samples with antibody interferences through measurement of Tg-specific tryptic peptides by mass spectrometry.

**Reference Values**

Healthy individuals with intact, functioning thyroid: < or =33 ng/mL

The reference ranges listed below, however, are for thyroid cancer follow up of athyrotic patients and apply to unstimulated and stimulated thyroglobulin measurements. Ranges are based on best practice guidelines and the literature, which includes Mayo studies, and represent clinical decision levels.

Decision levels for thyroid cancer patients, who are not completely athyrotic (ie, patient has some remnant normal thyroid tissue), have not been established, but are likely to be somewhat higher: remnant normal thyroid tissue contributes to serum Tg concentrations 0.5-1.0 ng/mL per gram of remnant tissue, depending on the thyroid-stimulating hormone (TSH) level.

Tg <0.5 ng/mL: Thyroglobulin (Tg) levels must be interpreted in the context of TSH levels, serial Tg measurements, and radioiodine ablation status.

Undetectable Tg levels in athyrotic individuals on suppression therapy indicate a minimal risk (<1%-2%) of clinically detectable recurrent papillary/follicular thyroid cancer.

Tg > or =0.5 ng/mL to 2.0 ng/mL: Thyroglobulin (Tg) levels must be interpreted in the context of TSH levels, serial Tg measurements, and radioiodine ablation status. Tg levels of 0.5-2.0 ng/mL in athyrotic individuals on suppressive therapy indicate a low risk of clinically detectable recurrent papillary/follicular thyroid cancer.

Tg 2.1 ng/mL to 9.9 ng/mL: Thyroglobulin (Tg) levels must be interpreted in the context of TSH levels, serial Tg measurements and radioiodine ablation status. Tg levels of 2.1-9.9 ng/mL in athyrotic individuals on suppression therapy indicate an increased risk of clinically detectable recurrent papillary/follicular thyroid cancer.

Tg > or =10 ng/mL: Thyroglobulin (Tg) levels must be interpreted in the context of TSH levels, serial Tg measurements and radioiodine ablation status. Tg levels of > or =10 ng/mL in athyrotic individuals on suppressive therapy indicate a significant (>25%) risk of clinically detectable recurrent papillary/follicular thyroid cancer.
Interpretation

Current guidelines recommend measurement of thyroglobulin (Tg) with a sensitive immunoassay limit of quantification <1 ng/mL; for measurements of unstimulated Tg, the detection limit should be in the 0.1 to 0.2 ng/mL range. In all cases, serum antithyroglobulin autoantibodies (TgAB) should also be measured, preferably with a method that allows detection of low concentrations of TgAB (< or = 20 kIU/L). If TgAB are detected, the laboratory report should alert the ordering provider to the possibility of false-low Tg results. If the apparent Tg concentration is <1 ng/mL, the sample should be remeasured by mass spectrometry. This will allow confident detection of Tg in the presence of TgAB down to 0.5 ng/mL (risk of residual/recurrent disease <1-3%).

Samples from patients with Tg concentrations >1 ng/mL (or 2 ng/mL; there is some discussion in the literature) might not require Tg measurement by mass spectrometry, because current guidelines suggest further work-up might be necessary above this threshold. However the positive predictive value for residual/recurrent disease is modest at best when Tg is just above this threshold (3%-25%, rising in parallel with Tg concentrations up to 10 ng/mL) in athyrotic patients. Above 10 ng/mL, the risk of residual/recurrent disease is at least 25%, with many studies showing 60% to >90% risks. In selected patients, it might therefore also be useful to test TgAB positive samples by mass spectrometry, even if the Tg concentration is >1.0 ng/mL, but has not yet passed the 10 ng/mL threshold. These considerations are even more relevant in patients with a known thyroid remnant of a few grams, who may always have serum Tg concentrations of 1.0 to 10 ng/mL, owing to remnant Tg secretion, regardless of the presence or absence of residual/recurrent cancer.

There are no routine tests that can detect heterophile antibodies in patient samples. An unexpected high result is usually the tip-off in this case, and should prompt remeasurement by mass spectrometry, which will provide a reliable result.

It has been determined that the presence of Tg autoantibodies in serum can lead to underestimation of Tg concentration by immunoassay methods. When antibodies are present in samples with detectable Tg, the Tg values may be underestimated by up to 60% in immunoassays. In addition, 20% of specimens containing antibodies that are negative for Tg by immunoassay tested positive by liquid chromatography-tandem mass spectrometry (LC-MS/MS); no results over 3 ng/mL by LC-MS/MS were observed.

In rare cases, when Tg is measured in patients with an intact thyroid gland who do not have thyroid cancer, substantial elevations will primarily be observed in very large goiters, highly active Graves disease, and, most pronounced, in the early phase of acute thyroiditis, when follicular destruction releases massive amounts of stored Tg into the circulation. Levels are often well above 100 ng/mL.
Cautions

The test is most sensitive for detection of thyroid cancer recurrence when patients are off thyroid replacement long enough to have an elevated thyroid-stimulating hormone (TSH) prior to drawing the specimen. This test also can be used to follow patients with normal TSH; however, thyroglobulin (Tg) values from specimens with high TSH should not be compared with values with normal TSH, because TSH stimulation may change the baseline determinations, if any residual benign thyroid tissue is still present. 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, our 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. Therefore, if the results of the mass spectrometry measurement are much lower than those obtained previously (within 3-6 months) with an immunometric immunoassay, this possibility should be considered. In this event, we recommend alerting us as soon as possible, and we will attempt to resolve the discrepancy.

Clinical Reference