

## THYROID FUNCTION TESTS

### **Thyroid Stimulating Hormone (TSH):**

The thyroid stimulating hormone (TSH) assay measures the concentration of thyroid stimulating hormone in the serum. TSH assays have been classified by "generation" based on the functional sensitivity of the assay, i.e., the lowest concentration at which the assay is able to maintain an inter-assay precision, expressed in terms of percent coefficient of variation (%CV), of 20% or less. Virtually all TSH assays currently in use are either second or third generation, with functional sensitivities of 0.1 to 0.2  $\mu$ IU/mL and 0.01 to 0.02  $\mu$ IU/mL, respectively; thus third generation assays are an order of magnitude more sensitive than second generation assays (sTSH). TSH is recognized as an exquisitely sensitive indicator of thyroid status and thus TSH assays (second or third generation) have been widely adopted as the front-line thyroid function test. In ambulatory patients with intact hypothalamic and pituitary function, a normal TSH result excludes hypo- or hyperthyroidism; whereas elevated and suppressed TSH results are diagnostic of hypo- and hyperthyroidism, respectively. Abnormal TSH results are generally confirmed with a complementary determination of thyroid hormone levels as described below.

The third generation assays have been recognized as consistently superior to second generation assays with their ability to accurately distinguish between normal and suppressed results. Furthermore, third generation assays distinguish between mildly suppressed and profoundly suppressed states. These assays, therefore, provide a powerful tool for estimating the severity of hyperthyroidism, for distinguishing between frank hyperthyroidism (TSH values below 0.01 to 0.02  $\mu$ IU/mL) and the effects of non-thyroidal illness and certain drugs among hospitalized patients that suppress TSH levels in the absence of thyroid disease (sick euthyroid disease), and for optimizing suppressive therapies.

In normal individuals, TSH levels typically are between 0.3 and 5.0 mU/ml. TSH is under negative feed back control by the amount of free thyroid hormone (T4 and T3) in the circulation and positive control by the hypothalamic thyroid-releasing hormone (TRH). Thus in the case of thyroid hormone deficiency (hypothyroidism) the TSH level should be elevated. A value greater than 20 mU/ml is a good indicator of primary failure of the thyroid gland. A value of between 5 and 15 is a borderline value that may require more careful evaluation. If the hypothyroid state is due to failure of the pituitary gland (TSH) or the hypothalamus (TRH), the values for TSH may be low, normal or occasionally in the borderline range. Thus a TSH above 15 is very good evidence for primary hypothyroidism and a value below 5 is very good evidence against primary hypothyroidism. The presence of low Free T4 with a TSH of less than 10 strongly suggests a pituitary or hypothalamic etiology for the hypothyroidism (secondary hypothyroidism). The TSH alone cannot be used to screen for secondary hypothyroidism and usually requires a measurement of thyroid hormone levels to be adequately interpreted.

Because high levels of free thyroid hormone will suppress TSH levels, in almost all cases of hyperthyroidism the TSH values will be less than 0.3 and usually less than 0.1 mU/L. Though TSH is a very effective tool to screen for hyperthyroidism, the degree of suppression of TSH does not always reflect the severity of the hyperthyroidism. Therefore a measurement of free thyroid hormone levels is usually required in patients with a suppressed TSH level. If the Free T4 is normal, the free T3 should be checked as it is the first hormone to increase in early hyperthyroidism.

TSH levels can also be used to effectively monitor patients being treated with thyroid hormone. However, it should be noted that TSH results may be misleading during the several months required for full equilibration of thyroid physiology following initiation or significant alteration of a treatment regimen. Total or free T4 generally serve as the front-line assays during this period. Once equilibration has occurred, high TSH levels usually indicate under-treatment, while low values usually indicate over-treatment. Again, abnormal TSH values should be interpreted with the measurement of free thyroid hormone before modifying therapy because serum thyroid hormone levels change more quickly than TSH levels. Thus patients who have recently been started on thyroid hormone, or who have been noncompliant until shortly before an office visit may have normal T4 and T3 levels, though their TSH levels are still elevated. TSH levels may be affected by acute illness and several medications, including dopamine and glucocorticoids.

- Decreased (low to undetectable) in Grave's Disease
- Increased in TSH-secreting pituitary adenomas (secondary hyperthyroidism), PRTA and in hypothalamic disease with increased thyrotropin (tertiary hyperthyroidism)
- Elevated in hypothyroidism (along with decreased T<sub>4</sub>) except for pituitary and hypothalamic disease
- Mild to modest elevations in patients with normal T<sub>4</sub> and T<sub>3</sub> levels indicates impaired thyroid hormone reserves and incipient hypothyroidism (subclinical hypothyroidism)
- Mild to modest decreases in patients with normal T<sub>4</sub> and T<sub>3</sub> levels indicates subclinical hyperthyroidism

### **Thyrotropin releasing hormone (TRH)**

Prior to the availability of sensitive TSH assays, thyrotropin releasing hormone (TRH) stimulation tests were relied upon for confirming and assessing the degree of suppression in suspected hyperthyroidism. Typically, this stimulation test involves determining basal TSH levels and levels 15 to 30 minutes after an intravenous bolus of TRH. Normally, TSH would rise into the concentration range measurable with less sensitive TSH assays that could provide useful information from the profile of increase even if they were not sensitive enough to measure baseline values. Third generation assays do not have this limitation and thus TRH stimulation is generally not required when third generation assays are used to assess degree of suppression. TRH-stimulation testing however continues to be useful for the differential diagnosis of secondary (pituitary disorder)

and tertiary (hypothalamic disorder) hypothyroidism. Patients with these conditions appear to have physiologically inactive TSH in their circulation that is recognized by TSH assays to a degree such that they may yield misleading, "euthyroid" TSH results. The TRH-stimulation test produces a very characteristic sluggish rise in TSH values.

- Helpful in diagnosis in patients with confusing TFTs. In primary hyperthyroidism TSH are low and TRH administration induces little or no change in TSH levels
- In hypothyroidism due to end organ failure, administration of TRH produces a prompt increase in TSH
- In hypothyroidism due to pituitary disease administration of TRH does not produce an increase in TSH
- In hypothyroidism due to hypothalamic disease, administration of TRH produces a delayed (60-120 minutes, rather than 15-30 minutes) increase in TSH

### **Total T4 (TT4) and Free T4 (FT4)**

T4 assays complement TSH assays, and are used to confirm a thyroid disorder when this is suggested by an abnormal TSH result. Furthermore, T4 assays may become the front-line assays in conditions that are known to possibly compromise the reliability of TSH results. Several months may be required for the dynamics of the regulatory mechanism (along the hypothalamic-pituitary-thyroid axis) to fully equilibrate after a treatment regimen is initiated or significantly altered; during this time TSH results may be misleading. Secondary (hypothalamic disorder) and tertiary (pituitary disorder) hypothyroidism are other conditions in which TSH results may be misleading, and the differential diagnosis is likely to rely on T4 (Free T4) results complemented by the characteristic profile of TSH results obtained during a TRH-stimulation testing procedure. (See TSH).

The total T4 test measures the concentration of thyroxine in the serum, including both the protein bound and free hormone. The total (but not the free) hormone concentration is dependent on the concentration of thyroid transport proteins, specifically thyroid binding globulin (TBG), albumin, and thyroid binding prealbumin (transthyretin). Thus any conditions that affects levels of thyroid binding proteins will affect the total (but not the free) T4 hormone levels. For example, estrogens and acute liver disease will increase thyroid binding, while androgens, steroids, chronic liver disease and severe illness can decrease it. Also, while TT4 is usually elevated in hyperthyroidism, it misses 5% of cases that are due to triiodothyronine (T3) toxicosis (see below).

The free T4 (FT4) assay measures the concentration of free thyroxine, the only biologically active fraction, in the serum (about 0.05% of the total T4). The free thyroxine is not affected by changes in concentrations of binding proteins such as TBG and thyroid binding prealbumin. Thus such conditions as pregnancy, or estrogen and androgen therapy do not affect the FT4. Thus the FT4 assays generally are considered to provide the more reliable indication of true thyroid status because only the free hormone

is physiologically active. In developing hypothyroidism, T4 (free T4) is the more sensitive indicator of developing disease than is T3 (Free T3), and is therefore preferred for confirming hypothyroidism that has already been suggested by an elevated TSH result.

TT4 and FT4 are not always reliable indicators of thyroid disease. For example, a substantial proportion of seriously ill patients will have abnormal thyroid function in the absence of true thyroid disease, due to "sick euthyroid syndrome." Also, screening with TT4 or FT4 will generate many false-positive results in healthy populations. And, because TT4 and FT4 are normal by definition in subclinical thyroid dysfunction, they are not useful as screening tests for this condition.

### **Total and Free Triiodothyronine (T3)**

The total T3 test measures the concentration of triiodothyronine in the serum. The T3 is increased in almost all cases of hyperthyroidism and usually goes up before the T4 does. Thus T3 levels are a more sensitive indicator of hyperthyroidism than the total T4, and T3 levels are therefore preferred for confirming hyperthyroidism that has already been suggested by a suppressed TSH result. T3 assays are also useful for the differential diagnosis of T3 thyrotoxicosis, a variant of hyperthyroidism that manifests itself with abnormally elevated T3 and suppressed TSH levels, while T4 levels remain within euthyroid (normal) limits. In hypothyroidism the T3 is often normal even when the T4 is low. The T3 is decreased during acute illness and starvation, and is affected by several medications including Inderal, steroids and amiodarone. This test measures both bound and free hormone. And only the free hormone is biologically active. Since free T3 accounts for only about 0.5% of the total T3, measurement of free hormone is generally considered to provide the more reliable indication of true thyroid status. As noted above for T4 levels, anything which effects thyroid binding globulin (TBG), or albumin will effect the total T3 levels.

### **Resin Thyroid Uptake (T-uptake)**

These assays have been variously referred to as T3-uptake, T4-uptake and thyroid-uptake tests, depending on the assay design. All are used in exactly the same manner and for the same purpose, not as stand-alone assays, but in combination with total T4 or total T3 assays. Matched T-uptake and total T4 results are used to calculate a free thyroxine index (FT4I or FT3I). The FT4I serves as an indirect estimate of free T4 levels, and were heavily relied upon historically, particularly before direct free T4 assays became available. The resin T3/T4 uptake is used to assess the binding capacity of the serum for thyroid hormone. This is used to help determine if the total T4 is reflecting the free T4, or if abnormalities in binding capacity are responsible for changes in T4 values and thus this test is only useful in conjunction with Total T4 or Total T3. In the Resin T3 Uptake test, labeled hormone is added to the patient's serum. If there is an increase in binding capacity, more labeled hormone will be bound to the binding proteins and thus less will be left free in the serum. The free labeled hormone in the serum is measured and usually reported as a percent of the total labeled hormone added. If a patient has a high total T4,

it may be due to overproduction of thyroid hormone (Hyperthyroidism) or to an excess of one of the thyroid binding proteins, usually thyroid binding globulin (TBG). If the high Total T4 is secondary to high TBG, the Resin T3 will be low, otherwise it will be normal or elevated. Another way of putting this is that if the Total T4 or Total T3 deviates from normal in one direction and the Resin T3 uptake deviates in the opposite direction, then the abnormality is due to changes in binding capacity, otherwise it is secondary to a true change in thyroid function (i.e. Hyper- or Hypothyroidism). For example, if the binding capacity is increased because of high estrogens, the free labeled hormone will be decreased and the Resin T3 uptake will be decreased.

### **Reverse T3 (RT3):**

Reverse T3 (RT3) is formed when T4 is deiodinated at the 5 position. RT3 has little or no biological activity and serves as a disposal path for T4. During periods of starvation or severe physical stress, the level of RT3 increases while the level of T3 decreases. In hypothyroidism both RT3 and T3 levels decrease. Thus RT3 can be used to help distinguish between hypothyroidism and the changes in thyroid function associated with acute illness, such as euthyroid sick syndrome.

### **Antithyroid Antibodies (Autoantibodies):**

Autoantibodies of clinical interest in thyroid disease include thyroid-stimulating antibodies (TSAb), TSH receptor-binding inhibitory immunoglobulins (TBII), antithyroglobulin antibodies (Anti-Tg Ab) and the antithyroid peroxidase antibody (Anti-TPO Ab). Of these, anti-TPO Ab has emerged as the most generally useful marker for the diagnosis and management of autoimmune thyroid disease.

The Anti-TPO Ab was historically referred to as the antimicrosomal antibody. The thyroid peroxidase enzyme (responsible for iodinating tyrosine residues in the thyroglobulin molecule) was subsequently identified as the major microsomal component recognized by these autoantibodies. New, improved assays, designed in the wake of this insight, have been rapidly replacing the older antimicrosomal antibody assays.

Anti-TPO Abs mediate antibody-dependent thyroid cell destruction; levels correlate with the active phase of the disease. Measurement of this autoantibody is useful for resolving the diagnostic dilemma presented by the apparent inconsistency between elevated TSH and normal free T4 results. Given abnormally elevated TSH and euthyroid T4 results, a positive anti-TPO Ab test provides strong evidence for early, subclinical autoimmune disease. This assay is also used to monitor response to immunotherapy, to identify at-risk individuals (with family history of thyroid disease), and as a predictor of postpartum thyroiditis. Approximately 10 percent of asymptomatic individuals have elevated levels of Anti-TPO Ab that may suggest a predisposition to thyroid autoimmune disease. Elevated levels are found in virtually all cases of Hashimoto's thyroiditis and in approximately 85 percent of Graves' disease cases.

Historically, Anti-TG Ab determinations were used in tandem with antimicrosomal Ab determinations to maximize the probability of a positive result in patients with autoimmune disease. Although the prevalence of Anti-TG Abs in thyroid autoimmune disease is significant (85 percent and 30 percent in Hashimoto's thyroiditis and Graves' disease, respectively), it is much lower than the prevalence of the Anti-TPO Abs. The diagnostic information provided by Anti-TPO assays is rarely improved upon by the addition of an Anti-TG determination. The growing trend is to adopt the anti-TPO Ab test as the front-line test for autoimmune disease and no longer to routinely use the anti-TG assay routinely for this purpose. Because anti-TG Abs constitute an interference in thyroglobulin (TG) assays, another major use of the anti-TG test is to screen samples that have been submitted for thyroglobulin determinations.

Thyroid-stimulating antibodies (TSAb) are present in more than 90% of Grave's Disease. TSH receptor-binding inhibitory immuno-globulins (TBII) are present in atrophic form of Hashimoto's Disease, maternal serum of pregnant women (predictive of congenital hypothyroidism) and myxedema

### **Thyroid Binding Globulin (TBG)**

TBG remains an esoteric thyroid function test that is useful for the differential diagnosis of patients presenting with significantly abnormal levels of total thyroid hormone levels but no other clinical signs or symptoms of thyroid disease. Depending on genetic determinants, the patient's health status (including pregnancy), and medication, TBG levels can vary widely from very elevated to very low. Total hormone levels also adjust accordingly, to maintain free thyroid hormone levels within the euthyroid range. In certain situations, the knowledge that grossly abnormal thyroid hormone levels are not the consequence of a thyroid disorder may be very reassuring. Although not widely used, there has been some interest in the ratio of total T4 to TBG (thyroid hormone bonding ratio) as an index of free T4 levels.

### **Thyroglobulin (TG)**

TG is only produced by thyroid tissue and this makes it an extremely specific marker for functioning thyroid tissue. The complete absence of TG provides strong evidence for the absence of any functioning tissue. Thus tests for remaining thyroid tissue are particularly important for monitoring thyroid cancer patients for residual, metastasized, and recurring thyroid tissue after the thyroid has been completely removed. Historically, the only procedure available for this purpose has been the total body scan. An appropriately sensitive TG assay offers a powerful complementary procedure that may in certain situations reduce reliance on the far more invasive total body scans. Anti-Tg antibodies interfere in the TG assay, and TG results may therefore not be reported for serum samples that are positive for these antibodies.

### **Radioactive iodine uptake (RAIU)**

RAIU primarily measures the activity of the thyroid's active iodine pump which is regulated by TSH. It is not used for initial documentation of hyperthyroidism, but as a secondary test to differentiate between "true" and "other" forms of hyperthyroidism. Thus thyroidial RAIU is elevated in "true" hyperthyroidism including TSH-secreting primary adenomas and PPTH, Graves Disease, trophoblastic disease, toxic adenoma and multinodular goiter. In hypothyroidism RAIU may be elevated, low or normal, and change over time due to the transient nature of some forms of some of the disease states

### Common Thyroid Function Tests

Test	Measures	Normal Values	Assay Interference	Comments
<b><i>Measurements of circulating thyroid hormone levels</i></b>				
FT <sub>4</sub>	Direct measure of free T <sub>4</sub>	0.7-1.9 ng/mL (Analog)	Altered TBG do not interfere	Most accurate measure of free T <sub>4</sub>
FT <sub>4I</sub>	Calculated free T <sub>4</sub> level	6.5-12.5 T <sub>4</sub> (1.3-3.9)	Euthyroid sick syndrome	Estimates direct free T <sub>4</sub> , compensates for altered TBG
TT <sub>4</sub>	Total free + bound T <sub>4</sub>	5.0-12 mg/dL	Alterations of TBG	Adequate if TBG is not altered
TT <sub>3</sub>	Total free + bound T <sub>3</sub>	70-132 ng/dL	Alterations of TBG; Euthyroid sick syndrome	Useful to detect early, relapsing and T <sub>3</sub> toxicosis
RT <sub>3U</sub>	Indirect measure of TBG saturation	26-35%	Alterations of TBG	Used to calculate FT <sub>3I</sub> and FT <sub>4I</sub>
<b><i>Tests of Thyroid Gland Function</i></b>				
RAIU	Thyroid uptake of iodine	24 hr: 15-35%	< with Excess Iodine and > with iodine deficiency	Different. of hyperthyroidism
Scan	Size, shape & activity	-----	Thyroid and antithyroid drugs	Detect “Hot” vs “cold” nodules
<b><i>Test Hypothalamic-Pituitary-Thyroid Axis</i></b>				
TSH	Pituitary TSH levels	0.5-4.7 U/L	DA, glucocorticoids, TH, amiodarone	Most sensitive index for hyperthyroidism & to monitor therapy
<b><i>Tests of Autoimmunity</i></b>				
ATgA	Antibodies to thyroglobulin	<8%	Non-thyroidal immune disease	Present in auto-immune thyroid disease; not present in remission
TPO	Thyroperoxidase antibodies	<100IU/mL	Non-thyroidal immune disease	More sensitive test; detectable during remission
TRab	Thyroid receptor IgG antibody	Titers negative	-----	Confirms Graves' incl. neonatal
Thyroglobulin	Colloid protein of gland	5-25 mg/dL	Goiters, Inflamm thyroid	Thyroid cancer marker

