Thyroid Stimulating Immunoglobulins (TSI) assay

Background & Clinical indications

Grave's disease (GD) is the most common cause of hyperthyroidism. The clinical manifestations are protean and even to some extent shared with those of Hashimoto thyroiditis (HT) which is autoimmune hypothyroidism. The shared manifestations may include: muscle weakness, menstrual disturbances, decreased concentration, fatigue, goiter, depression, and hair and skin changes. The autoimmune nature of GD was established when the so-called long-activating thyroid stimulator (LATS) in sera from patients with GD was able to induce hyperthyroidism in experimental animals. LATS were later found to be Thyroid stimulating hormone receptor (TSHR)-stimulating antibodies. TSHR is a membrane receptor that belongs to superfamily that contains hormone receptors for LH, FSH, and HCG. The extracellular domain (ectodomain, subunit A) of TSHR has several leucine-rich repeats (LRDs) that create the binding site for TSH. Anti-TSHR stimulating and blocking autoantibodies bind to the same domain though with different Fab fragment orientations, the latter accounting for different downstream cellular events. TSHR stimulating antibodies (Abs) may have higher affinity for the TSH binding site on TSHR and even displace TSH, thereby inducing unremitting thyroid hormone production and thyrocyte proliferation without being controlled by the physiological feedback mechanism. The latter antibodies typically belong to IgG1 subclass.[1–10] A very well-characterized human monoclonal antibody (m22) from patients with GD was found to have TSHR-stimulating activity with very high affinity (circa 6.7×10^{-11} mol/L).[8] The tests based on this antibody offer very high clinical sensitivity and specificity.

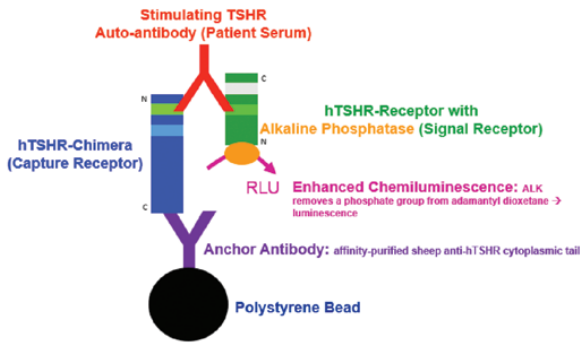
Diagnosis of GD is based on clinical grounds and on blood levels of free T3, free T4, and TSH. This, however, can be aided by the TSI test in certain circumstances such as: *i*) when Grave's orbitopathy (GO) or dermopathy is absent but other results point to GD; *ii*) in pregnant women with GD, and *iii*) in patients on antithyroid drugs (ATDs) to determine the need for discontinuation of ATDs. It is, however, important to note that GD and HT are two extremes of an autoimmune spectrum and over time patients may move from one end to another or at times manifest a fluctuating course. There are TSHR-blocking Abs as well as anti-TSHR Abs that only bind to other parts of TSHR with different affinities and they may be of additional subclasses such as IgG2 and IgG3. It is not uncommon to see patients with either GD or HT to have both stimulating and blocking antibodies at the same time. Apparently it is the titer and the avidity of the Ab population that determines the outcome, at least in some circumstances but it is pivotal to note that the final diagnosis and management decisions should not be solely based on TSI test results. What is more important than qualitative TSI test result is measuring TSI concentrations over time in patients, as for instance TSI is found in 7% of HT patients without GO but in 68% of HT patients with GO.[1–10]

It is important to note that by using blocking Ab bioassay, the prevalence of TSHR-blocking Abs among GD and HT patients is established around 4% and 9%, respectively.[11] This means ca. 96% of GD patients do not have TSHR-blocking Abs thus obviating the need for distinguishing between the stimulatory and blocking antibodies in these patients especially due to the costly and labor-intensive nature of bioassay without making a significant difference in patient management.

Methodology

The new test is FDA-approved for *in vitro* diagnostic purposes and is called Thyroid Stimulating Immunoglobulins (TSI) for use on IMMULITE®2000 systems (Siemens). The test principle is based on automated, random-access, two-cycle chemiluminescent bridging immunoassay. The solid phase (polystyrene beads) are coated, through a monoclonal Ab, with a chimeric human TSHR (Mc4). In Mc4 the TSH binding site is intact but its membrane-proximal part has been replaced by rat luteinizing hormone-choriogonadotropin receptor, to not only stabilize the molecule so it can maintain its native form but also to preclude "other" TSHR-binding (but non-stimulating) Abs of binding to this chimeric molecule. Mc4 is also used by a commercial bioassay. During the second step, the capture TSHR is added but the latter is conjugated to alkaline phosphatase (ALP) which will generate the chemiluminescent signal upon addition of the

Figure 1. TSI Immulite test principle. See text for details.



substrate. The generated photons are read as relative light units (or counts per second) for calculations (Figure). The kit uses two levels (low, high) of "adjustors" in replicates of four (logistical model). These are m22 Abs as described above, and are used as calibrators based on which the final TSI concentration is calculated in international units per liter (IU/L). The calibration is based upon a lot-specific master curve generated by the manufacturer. By using such adjustors and the test design, it is the stimulatory antibodies that are typically measured. Furthermore, it has been demonstrated that stimulatory antibodies such as m22 are able to bind to concave region of TSHR LRDs where TSH also binds whereas the blocking antibodies such as K1-70 bind to the receptor more N-terminally and 155° away from where stimulatory antibodies bind.[12] This key difference helps this kit to significantly avoid bridging the receptors by blocking antibodies due to steric hindrance, the latter is further accentuated by the upside-down nature of the signal receptor and presence of the conjugated ALP. According to the manufacturer, this assay is traceable to WHO 2nd international standard for thyroid stimulating antibody, national institute for biological standards and control (NIBSC), code: 08/204. The kit also has three controls (negative, low and high positive) using m22 Ab. The reportable range is 0.10-40 IU/L but instrument can do further dilutions to calculate the final concentration. The manufacturer recommends 0.55 IU/L as the positivity cutoff based on which the clinical sensitivity and specificity are 98.6% and 98.5%, respectively. This is congruent with the most recent American Thyroid Association (ATA) guideline that states: *In the setting of overt thyrotoxicosis, newer TRAb binding and bioassays have a sensitivity of 96–97% and a specificity of 99% for GD* (7). It is noteworthy to mention that ATA did not specifically mention this test and by "TRAb", they referred to the two commercial ELISAs (TBI). TSI Immulite received FDA approval after the guideline was finalized. It was also previously shown that TSI Immulite test had higher sensitivity than bioassay in patients with GD on ATDs.[9] Furthermore, the manufacture claims that TSI Immulite test results are not affected in sera spiked by high concentrations of: FSH, LH, TSH, HCG, anti-thyroglobulin, and anti-thyroid peroxidase. Last but not least, bioassay only measures cyclic adenosine monophosphate (cAMP), but other TSHR activation pathways that do not result in cAMP production, such as phospholipase C cascade, are not assessed using current bioassays; this is even of more importance when in GO, IGF-1 receptor forms a complex with TSHR to trigger orbital fibroblasts for glycosaminoglycan production and lipogenesis.[6]

Advantages

The TSI Immulite test while analytically and clinically maintains high levels of quality and performance characteristics but significantly lowers the reagent cost and technologist's time compared with the bioassay which requires cell culture, using multiple reagents and ancillary tests and manual calculations that predispose this test to analytical and post-analytical errors, beside QC failures, lack of amenability to automation, and extreme labor-intensiveness, the latter adversely affecting cross-training of new technologists. The TSI Immulite assay is done on a random-access platform, from Monday to Friday, tests can be added at any time during working hours without the need for batch-testing, obviating the prolonged bioassay turnaround time as the former only takes 65 minutes to completion. It is a walk-away system meaning technologist's time is immensely spared. The lab currently has the Immulite instrument using it for other tests. Another random-access platform available commercially is Elecsys® Anti-TSHR (Roche). Although published reports[9, 10] showed relatively poor performance but the test principle makes this test a suboptimal choice as well: porcine TSH instead of the Mc4 construct, murine monoclonal antibodies that make heterophile Ab interference likely, using biotin for conjugation of the latter Abs that make the test prone to falsely-elevated

results due to dietary biotin, all combined argue against its usefulness for TSI test. Regarding TSI Immulite test, upon discussion with several other labs in the United States that have adopted it, they expressed their high satisfaction with this test.

Validation summary

To verify the manufacturer's claims, the TSI Immulite test was validated in-house at Immunopathology laboratory at the main campus. To summarize, the linearity and accuracy study used a neat sample and multiple dilutions up to 1:250 in triplicate. The recovery rate, slope, intercept, and observed error were all within acceptable and established limits. For precision study, the inter- and intra-run simple and complex precision was done using low, medium, and high level samples with %CV values all were very well within acceptable limits confirming very high precision which is ideal for monitoring patients overtime. Method comparison study was done in two parts: one was done using known positive sera (70% female, age: 24–78 years) received from ARUP lab using their in-house developed bioassay. Their test uses 123% as the positivity cutoff and they sent us samples within 128-480% (low to high positive). The second part was done using sera received from Ohio State University Laboratories as they also use TSI Immulite. Both panels showed 100% categorical agreement confirming comparability. According to our alternate proficiency testing results, our in-house bioassay has always had 100% concordance with the ARUP lab bioassay. Carryover study used three replicates in low, high, low order, and also used thee replicates of a high positive sample with 1:10 dilution. The results confirmed no significant instrument carryover. The reference range study used apparently healthy subjects. None tested positive for TSI and their TSI results were all <0.10 IU/L. This verified the manufacturer positivity cutoff of 0.55 IU/L.

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Test Overview

Test Name	Thyroid Stimulating Immunoglobulin
Ordering Mnemonic	TSIGIM
Methodology	Chemiluminescent Immunoassay (CLIA)
Specimen Requirements	Primary container SST (Gold) 0.5 mL Alternate container lithium heparin PST (Green) 0.5 mL
Minimum Specimen Requirements	Minimum volume 0.35 mL
Stability	24 hours (Ambient), 7 days (Refrigerated), 1 year (frozen ≤-20°C)
Clinical Information	Thyroid Stimulating Immunoglobulin test is used as an aid in diagnosis of autoimmune hyperthyroidism especially in patients with Grave's orbitopathy and dermopathy. Low positive TSH receptor stimulating antibody levels may occasionally be found in patients with autoimmune hypothyroidism. Clinical correlation is required.
Reference Range	<0.55 IU/L
Linear Range	0.10–40.00 IU/L
CPT Code	84445
Days performed	Monday to Friday

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