C. Thyrotropin/Thyroid Stimulating Hormone (TSH)

For more than twenty-five years, TSH methods have been able to detect the TSH elevations that are characteristic of primary hypothyroidism. Modern-day TSH methods however, with their enhanced sensitivity are also capable of detecting the low TSH values typical of hyperthyroidism. These new methods are often based on non-isotopic immunometric assay (IMA) principles and are available on a variety of automated immunoasssay analyzer platforms. Most of the current methods are capable of achieving a functional sensitivity of 0.02mIU/L or less, which is a necessary detection limit for the full range of TSH values observed between hypo- and hyperthyroidism. With this level of sensitivity, it is possible to distinguish the profound TSH suppression typical of severe Graves' thyrotoxicosis (TSH < 0.01 mIU/L) from the TSH suppression (0.01 – 0.1 mIU/L) observed with mild (subclinical) hyperthyroidism and some patients with a non-thyroidal illness (NTI).

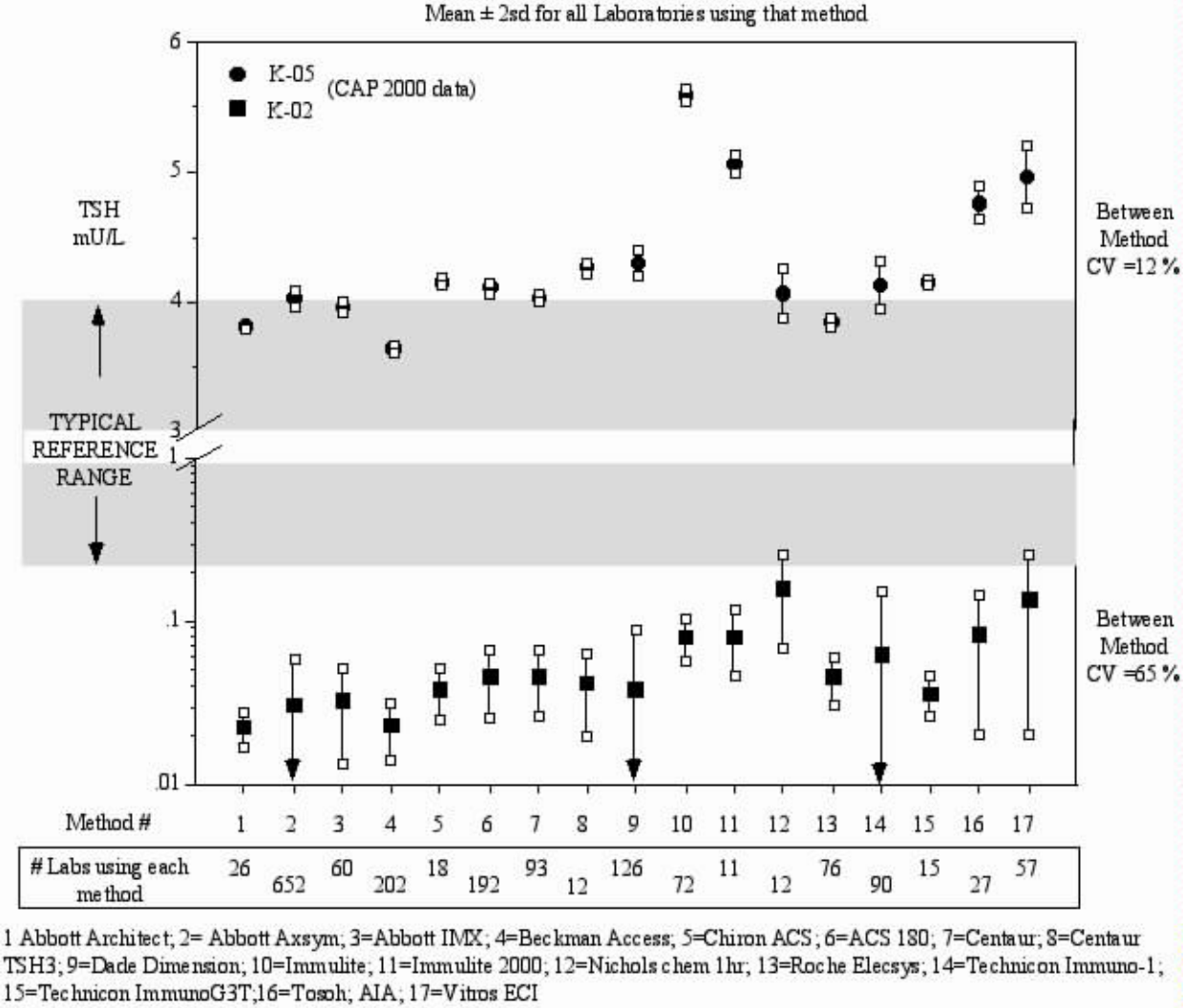
In the last decade the diagnostic strategy for using TSH measurements has changed as a result of the sensitivity improvements in these assays. It is now recognized that the TSH measurement is a more sensitive test than FT4 test for detecting both hypo- and hyperthyroidism. As a result, some countries now promote a TSH-first strategy for diagnosing thyroid dysfunction in ambulatory patients (provided that the TSH method has a functional sensitivity ≤ 0.02 mIU/L). Other countries still favor the TSH + FT4 panel approach, because the TSH-first strategy can miss patients with central hypothyroidism [Section 3C(d)vi] or TSH-secreting pituitary tumors [Section 3C(d)vii] (18,189-191). An additional disadvantage of the TSH-centered strategy is that the TSH-FT4 relationship cannot be used as a "sanity check" for interferences or unusual conditions characterized by a discordance in the ratio of TSH/FT4 (Table 1).

(a) Specificity

TSH is a heterogeneous molecule and there are different TSH isoforms found in blood as well as in pituitary extracts used for assay standardization (Medical Research Council (MRC) 80/558). In the future, recombinant human TSH (rhTSH) preparations might be used as primary standards for standardizing TSH immunoassays (190). Current TSH IMA methods use TSH monoclonal antibodies that virtually eliminate cross-reactivity with other glycoprotein hormones. These methods however, may detect differences in epitope recognition with abnormal TSH isoforms secreted by some euthyroid individuals, as well as some patients with abnormal pituitary disease conditions. For example, patients with central hypothyroidism caused by pituitary or hypothalamic dysfunction, secrete TSH isoforms with abnormal amounts of glycosylation and reduced biological activity. These isoforms are measured as paradoxically normal or even elevated serum TSH concentrations by most methods (189,191,192,194). Likewise, paradoxically normal serum TSH levels may be seen in patients with hyperthyroidism due to a TSH-secreting pituitary tumor, that appears to secrete TSH isoforms with enhanced biologic activity (190,195,196).

Sensitivity and/or specificity problems with a TSH IMA method typically results in falsely high rather than falsely low TSH values (197). This is because inadequate washing or the presence of an interfering substance results in a high background or false bridge between the capture and signal antibodies. This creates a high signal on the solid support which is read out as a falsely high result (115). The erroneous value may not necessarily be in the abnormal range but may be an inappropriately normal value in a hyperthyroid patient (197). In vitro heparin contamination of the specimen can result in erroneously low serum TSH values, whereas insensitivity or the presence of heterophilic antibodies (HAMA) are the most common cause of erroneously high serum TSH results (115,197). It should be noted that since antibodies cross the placenta, HAMA has the potential to influence a neonatal screening test result.

Figure 5. Serum TSH Measurement Using Different Methods



As shown in Figure 5, the between-method variability around the upper reference limit approximates 12%, whereas there is a much higher variability in method-to-method variability and within-method precision when measuring subnormal range values (CV 65% for values < 0.1 mIU/L).

The conventional laboratory approach to verify an analyte concentration such as dilution may not always detect an interference problem. Since methods vary in their susceptibility to most interfering substances, the most practical way to test for interference is to measure the TSH concentration in the specimen using a different manufacturer's method, and to check for a significant discordance between the TSH values. Figure 5 shows the expected variability between TSH methods. When the variability of TSH measurements made on the same specimen with different methods exceeds expectations, interference may be present. Biologic checks may also be useful to verify an unexpected result. Inappropriately low TSH values could be checked by a TRH-stimulation test, which is expected to elevate TSH more than more than 2-fold (≥4.0 mU/L increment) normal individuals (198). In cases where TSH appears inappropriately elevated, a thyroid hormone suppression test (1mg L-T4 or 200µg L-T3, po) would be expected to suppress serum TSH more than 90 % by 48 hours in normal individuals.

C.1 Guidelines for Investigating Discordant Serum TSH Values in Ambulatory Patients

A discordant TSH result in an ambulatory patient with stable thyroid status may be a technical error. Specificity loss can result from laboratory error, an interfering substance (i.e. heterophilic antibodies), or the presence of an unusual TSH isoform (see guideline 2.7 and Table 1). Physicians can request that their laboratory perform the

following checks:

- Confirm the specimen's identity (i.e. have laboratory check for a switched specimen in the run).
- When TSH is unexpectedly high, ask the laboratory to re-measure the specimen diluted, preferably in thyrotoxic serum, to check for parallelism.
- Request that the laboratory analyze the specimen by a different manufacturer's method. (Send to a different laboratory if necessary).
- Once a technical problems has been excluded, biologic checks may be useful:
  - Use a TRH stimulation test for investigating a discordant low TSH result, expect a 2-fold (≥4.0 mU/L increment) response in TSH in normal individuals.
  - Use a thyroid hormone suppression test to verify a discordant high TSH level, expect 1mg of L-T4 or 200µg L-T3, po to suppress the serum TSH more than 90 % by 48 hours in normal individuals.

(b) Sensitivity

Historically, the "quality" of a serum TSH method has been determined from a clinical benchmark - the assay's ability to discriminate euthyroid concentrations (~ 0.3 to 4.0 mIU/L) from the profoundly low (<0.01 mIU/L) TSH level typical of overt Graves' thyrotoxicosis. Most TSH methods now claim a detection limit of 0.02 mIU/L or less ("third generation" assays).

C.2 Guideline for Laboratories and Manufacturers: Definition of Functional Sensitivity

Functional Sensitivity should be used to determine the Lowest Detection Limit of the assay.

- TSH assay functional sensitivity is determined from the 20 % between-run coefficient of variation (CV) determined by the recommended protocol (see Guideline C.3)

Manufacturers have largely abandoned the use of the "analytical sensitivity" parameter for determining the sensitivity of a TSH assay because it is calculated from the within-run precision of the zero calibrator which does not reflect the sensitivity of the test in clinical practice (120,121). Instead, a "functional sensitivity" parameter has been adopted (197). Functional sensitivity is calculated from the 20% between-run coefficient of variation (CV) for the method and is used to establish the lowest reportable limit for the test (199).

C.3 Recommended Protocol for Functional Sensitivity & Between –Run Precision

Measure human serum pools covering the assay range in at least 10 different runs. The lowest pool value should be 10% above the detection limit and the highest pool value should be 90% of the upper assay limit.

- Carry-over should be assessed by analyzing the highest followed by the lowest pool.
- Use the same test mode as for patient specimens (i.e. singlicate or duplicate).
- The instrument operator should be blinded to the presence of test pools in the run.
- Runs should be spaced over a clinically representative interval (i.e. 6 to 8 weeks for TSH in an outpatient setting).
- Use at least two different lots of reagents and two different instrument calibrations during the testing period.

C.4 Guidelines for Manufacturers of TSH Tests

- Manufacturers that market TSH tests with differing sensitivities are urged to discontinue marketing the less sensitive product.
- There is no justification for the pricing of TSH assays to be based on sensitivity!
- There is no scientific justification for reflexing from a less sensitive to a more sensitive TSH test
- Manufacturers should help laboratories independently establish functional sensitivity by providing appropriately

low TSH human serum pools when requested.
- Kit Package Inserts should:
  - Document the realistic functional sensitivity of the method using Guideline C.3
  - Cite the functional sensitivity that can be achieved across a range of clinical laboratories
  - Display the typical between-run precision profile expected for a clinical laboratory
  - Recommend the use of functional sensitivity not analytical sensitivity to determine the lowest reporting limit. (Analytical sensitivity prompts laboratories to adopt an unrealistic detection limit.)

Functional sensitivity should be determined by strictly adhering to the recommended protocol (Guideline C.3) which is designed to assess the minimum detection limit of the assay in clinical practice. It is necessary to follow a strict protocol for determining functional sensitivity to ensure that the parameter realistically represents the lowest detection limit of the assay. This protocol is designed to take into account a variety of factors that can influence TSH method imprecision in clinical practice. These include:

- Matrix differences between patient serum and the standard diluent
- Erosion of precision over time
- Lot-to-lot variability in the reagents supplied by the manufacturer
- Differences between instrument calibration and technical operators
- Carry-over from high to low specimens (200)

The use of the functional sensitivity limit as the lowest detection limit is a conservative approach to ensure that any TSH result reported is not merely assay "noise". Further, the 20% between-run CV approximates the maximum imprecision required for diagnostic testing (Table 5).

C.5 Guidelines for Laboratories Performing TSH Testing

Functional sensitivity is the most important performance criterion that should influence the selection of a TSH method. Practical factors such as instrumentation, incubation time, cost, and technical support though important, should be secondary considerations. Laboratories should use calibration intervals that optimize functional sensitivity, even if re-calibration needs to be more frequent than recommended by the manufacturer:

- Select a TSH method that has a functional sensitivity ≤ 0.02 mIU/L.
- Establish functional sensitivity of your method independent of the manufacturers by using Guideline C.3.
- There is no scientific justification for reflexing from a less sensitive to a more sensitive test. (Insensitivity causes falsely high not falsely low values that are missed by reflexing.)

(c) TSH Reference Intervals

Serum TSH levels exhibit a diurnal variation with the peak occurring during the night and the nadir, which approximates to 50% of the peak value, occurring between 1000 and 1600 hours (68,69). This biologic variation does not influence the diagnostic utility of the test since most clinical TSH measurements are performed on ambulatory patients between 0800 and 1800 hours and TSH reference intervals are established from specimens collected during this time period. Serum TSH reference intervals should be established using specimens from TPOAb-negative, ambulatory, euthyroid subjects who have no personal or family history of thyroid dysfunction and no visible goiter. The variation in the reference intervals for different methods reflects inter-method bias (Figure 5), differences in antibody epitope recognition by the different kit reagents and the rigor applied to the selection of appropriate normal subjects.

Arithmetic serum TSH concentrations determined in normal euthyroid subjects are skewed with a relatively long "tail" towards the higher values of the distribution. The values however, are normally distributed when log-transformed. Thus for reference range calculations, it is customary to log-transform the TSH results to calculate the 95% reference interval (typical population mean value ~1.5 mIU/L, range 0.3 to 4.0 mIU/L in iodine-sufficient

populations) (197,201). Although adult populations show slight age-related and ethnic-related differences in TSH levels, (NHANES III US survey) it is not considered necessary to adjust the reference interval for these factors in clinical practice (4).

(i) TSH Upper Reference Limits

Over the last two decades the upper reference limit for TSH has steadily declined from ~10 to approximately ~4.0-4.5 mU/L. This decrease reflects a number of factors including the improved sensitivity and specificity of current monoclonal antibody based immunometric assays, the recognition that normal TSH values are log-distributed and importantly, improvements in the sensitivity and specificity of the thyroid antibody tests that are used to pre-screen subjects. The recent follow-up study of the Whickham cohort found that individuals with a serum TSH >2.0 mIU/L at their primary evaluation had an increased odds ratio of developing hypothyroidism over the next 20 years, especially if thyroid antibodies were elevated (28). An increased odds-ratio for hypothyroidism was even seen in antibody-negative subjects. It is likely that such subjects did have low levels of thyroid antibodies present, which could not be detected by the insensitive agglutination tests used in the initial study (202). Furthermore, a recent study of a group of subjects with "high-normal" TSH levels (2.0-4.0 mU/L) revealed a higher probability of thyroid antibodies and higher lipid levels compared with a "low-normal" group of subjects (0.40-1.99 mU/L) (203). This study found that L-T4 treatment of "high-normal" TSH subjects with positive thyroid antibodies caused a lowering of lipids. This suggests the presence of occult mild (subclinical) hypothyroidism (203). These studies emphasize the importance of using sensitive thyroid antibody tests to exclude individuals with positive thyroid antibodies and occult thyroid dysfunction from the selection of a "normal" cohort for TSH reference range determinations. The majority (~95%) of healthy euthyroid subjects have serum TSH values below 2.5 mU/L. It follows that ambulatory patients with a serum TSH in the "upper normal range" (>3.0 mU/L) may be in the early phase of developing hypothyroidism and warrant having a repeat TSH measurement after 3 weeks and/or a reflex TPOAb measurement made.

C.6 Guideline: TSH Reference Intervals

The reference interval of a TSH method should be established by each laboratory, independent of the manufacturer's recommended range, from the 95 % confidence limits of the log-transformed values of at least 120 ambulatory euthyroid subjects who are:

- TPOAb-negative (by sensitive immunoassay)
- No personal or family history of thyroid dysfunction
- No visible or palpable goiter
- On no medications (except HRT)

(ii) TSH Lower Reference Limits

Before the immunometric assay era, TSH methods were too insensitive to detect values in the lower end of the reference range (204). Current methods however, are capable of measuring TSH at the lower end and now cite lower limits between 0.2 and 0.4 mIU/L (197). As the sensitivity of the methods have improved, there has been an increased interest in defining the true lower limit of normal to determine the clinical presence of mild (subclinical) hyperthyroidism. Current studies suggest that TSH values in the 0.1 to 0.4 mIU/L range may represent thyroid hormone excess and in elderly patients might be associated with an increased risk of atrial fibrillation, and cardiovascular mortality (29,30). It is therefore important to carefully exclude subjects with a goiter, any illness or stress in the normal cohort selected for reference range determinations.

(d) Clinical Use of Serum TSH Measurements

(i) Screening for Thyroid Dysfunction in Ambulatory Patients

Most professional societies recommend that TSH should be used for case finding or screening for thyroid dysfunction in ambulatory patients, provided that the TSH assay used has a functional sensitivity at or below 0.02 mU/L (5,10,205). The TSH assay sensitivity stipulation is critical for the reliable detection of subnormal values, since less sensitive TSH assays are prone to produce false negative (normal range) results on specimens with subnormal TSH concentrations (197). The log/linear relationship between TSH and FT4 dictates that serum TSH is the preferred test, since only TSH can detect mild (subclinical) degrees of thyroid hormone excess or deficiency (Figure 1)[Section 2A(a)]. Mild (subclinical) thyroid dysfunction, is characterized by an abnormal TSH associated with a normal range FT4 and has a reported prevalence of ~10% and 2% (mild (subclinical) hypo- and hyperthyroidism, respectively) by most population studies (10,23,24). Despite the clinical superiority of TSH, the TSH-centered strategy has two principle limitations. Firstly, it assumes that hypothalamic-pituitary function is intact and normal. Secondly, it assumes that the patients thyroid status is stable, i.e. no recent therapy for hypo-or hyperthyroidism [Section 2A(a) and Figure 2] (18). If either of these criteria is not met, serum TSH results can be diagnostically misleading (Table 1).

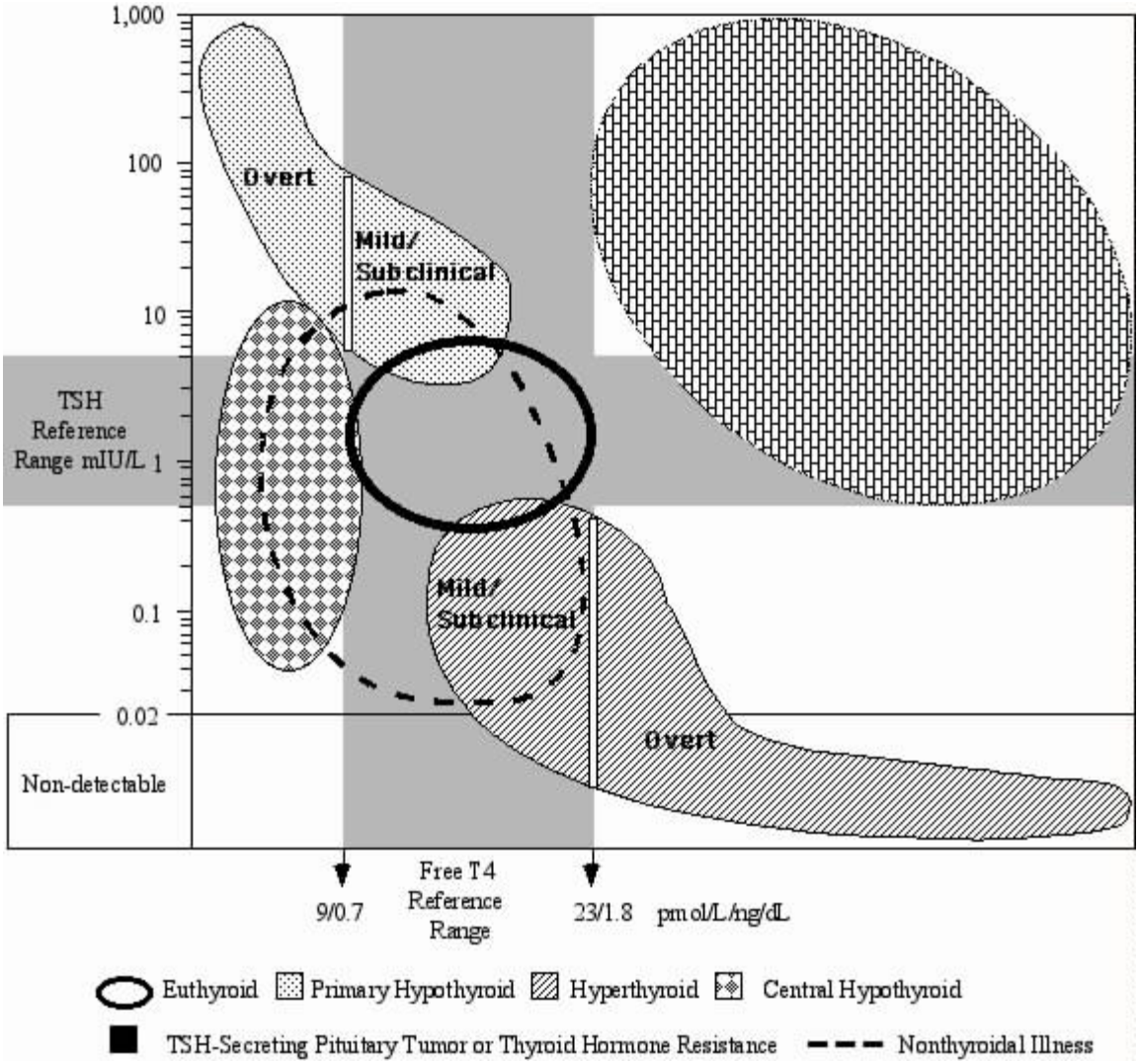
Most studies support screening for thyroid dysfunction in the elderly (8,10,206). However, there is no consensus regarding the optimal age to begin the screening. American Thyroid Association guidelines recommend screening at age 35 and every 5 years thereafter (10). Decision analysis appears to support the cost-effectiveness of this strategy, especially for women (207). The strategy for using TSH to screen for mild (subclinical) hypo- and hyperthyroidism will remain in dispute until there is more agreement on the clinical consequences of having a chronically abnormal TSH. Also there needs to be agreement as to the level of TSH abnormality that should indicate the necessity for treatment (208,209).

There is mounting evidence to suggest that patients with a persistent TSH abnormality may indeed be exposed to greater risk if left untreated. Specifically, a recent study reported a higher cardiovascular mortality rate when patients have a chronic low serum TSH abnormality (30). Further, there are an increasing number of reports which indicate that mild hypothyroidism in early pregnancy increases fetal wastage and impairs the IQ of the offspring (60-63). Such studies support the efficacy of early thyroid function screening, especially in women during their childbearing years.

(ii) Elderly Patients

The prevalence of both low and high TSH is increased in the elderly compared with younger patients. Hashimotos' thyroiditis associated with a high TSH and detectable TPOAb is encountered with increasing prevalence with age (28). The incidence of a low TSH is also increased in the elderly (28). This abnormality may be transient but is a persistent finding in approximately 2 % of elderly subjects, with no apparent thyroid dysfunction (29,206). A recent study by Parle et al showed a higher cardiovascular mortality rate in such patients suggesting that the cause of a persistently low TSH level should be investigated (30). The presence of a multinodular goiter should be ruled out as the cause of a low TSH in older individuals, especially in areas of iodine deficiency (210). Medication history should be thoroughly reviewed and serum TSH levels should be rechecked in such patients together with TPOAb measurements after 4 to 6 weeks.

Figure 6. TSH/FT4 Relationship in Different Clinical Conditions


1,000
100
10
1
0.1
0.02
Non-detectable
TSH
Reference
Range mIU/L
Overt
Mild/
Subclinical
Mild/
Subclinical
Overt
9/0.7
Free T4
Reference
Range
23/1.8
pmol/L/ng/dL
Euthyroid
Primary Hypothyroid
Hyperthyroid
Central Hypothyroid
TSH-Secreting Pituitary Tumor or Thyroid Hormone Resistance
Nonthyroidal Illness

(iii) L-T4 Replacement Therapy

It is now well documented that patients on thyroxine therapy require higher levels of serum TT4 and FT4 to restore the serum TSH and T3 levels to that of euthyroid control subjects (211,212). A reduction in thyroid gland T3 may partially explain these observations. Levothyroxine (L-T4) and not dessiccated thyroid, is the preferred long-term replacement medication for hypothyroidism. A euthyroid state is usually achieved in adults with a L-T4 dose averaging 1.6 μg/kg bw/day. Children require higher doses (up to 4.0μg/kg bw/day) and older individuals require lower doses (1.0 μg/kg bw/day). The initial dose and the optimal time needed to establish the full replacement dose should be individualized relative to age, weight and cardiac status. The requirements for thyroxine increase during pregnancy [Section 2A(c)] and in post-menopausal women just starting hormone replacement therapy (HRT) (213).

A serum TSH result between 0.5 and 2.0 mU/L is generally considered the therapeutic target for a standard L-T4 replacement dose for primary hypothyroidism. A serum FT4 concentration in the upper third of the reference interval is the therapeutic target for L-T4 replacement therapy when patients have central hypothyroidism due to pituitary and/or hypothalamic dysfunction. A typical schedule for gradually titrating to a full replacement dose involves giving L-T4 in 25 μg increments each 6 –8 weeks until the full replacement dose is achieved (serum TSH 0.5-2.0 mU/L). As shown in figure 2, TSH is slow to re-equilibrate to a new thyroxine level. Patients with chronic, severe hypothyroidism may develop pituitary thyrotroph hyperplasia which can mimic a pituitary adenoma, but which resolves after several months of L-T4 replacement therapy. (214).

Patients taking Rifampin and anticonvulsants that influence the metabolism of L-T4 may also need an increase in their dose of L-T4 to maintain the TSH within the therapeutic target range. Both free T4 and TSH should be used for monitoring hypothyroid patients suspected of intermittent or non-compliance of their L-T4 therapy. The paradoxical association of a high FT4 + high TSH is often an indication that compliance may be an issue. Specifically, acute ingestion of missed L-T4 before a clinic visit will raise the FT4 but fail to normalize the serum TSH because of the “lag effect” (Figure 2). In essence, the serum TSH is analogous to the hemoglobin A1c as a long-term free T4 sensor! At least 6 weeks is needed before retesting TSH following a change in the dose of L-T4 or brand of thyroid medication. Annual TSH testing of patients receiving a stable dose of L-T4 is recommended. The optimal time for TSH testing is not influenced by the time of day the L-T4 dose is ingested (127). However, the daily dose should be withheld when FT4 is used as the therapeutic endpoint, since serum FT4 is significantly increased (15-20%) above baseline for 9 hours after ingesting the last dose (215). Ideally L-T4 should be taken before eating; at the same time of day and at least 4 hours apart from any other medications or vitamins. Many of these medications can influence T4 absorption/metabolism (especially Cholestyramine, Ferrous Sulfate, Soy Protein, Sucralfate, antacids containing Aluminum Hydroxide, anticonvulsants or Rifampin) (5,216).

C.7 Guidelines: Levothyroxine (L-T4) Replacement Therapy for Primary Hypothyroidism

- L-T4, not dessiccated thyroid, is preferred medication for long-term replacement therapy for hypothyroidism.
- A euthyroid state is usually achieved with an average L-T4 dose of 1.6 µg/kg body weight/day. The initial dose and time to achieve full replacement should be individualized relative to age, weight and cardiac status. An initial L-T4 dose is normally 50-100 µg daily. Serum TSH measurement after six weeks will indicate the need for dose adjustment by 25-50 µg increments.
- Children require higher doses of L-T4, up to 4.0µg/kg bw/day, due to rapid metabolism. Serum TSH and FT4 values should be assessed using age-specific and method-specific reference ranges (Table 3).
- Serum TSH levels between 0.5 and 2.0 mU/L is generally considered the optimal therapeutic target for the L-T4 replacement dose for primary hypothyroidism.
- TSH is slow to re-equilibrate to a new thyroxine status (Guideline 2.2). Six to 8 weeks is needed before retesting TSH after changing the L-T4 dose or brand of thyroid medication.
- Intermittent or non-compliance with levothyroxine (L-T4) replacement therapy will result in discordant serum TSH and FT4 values (high TSH/high FT4) because of a persistently unstable thyroid state (Guideline 2.2). Both TSH and FT4 should be used for monitoring such patients.
- Thyroxine requirements decline with age. Older individuals may require less than 1.0 µg/kg bw/day and may need to be titrated slowly. Some physicians prefer to gradually titrate such patients. An initial dose of 25 µg is recommended for patients with evidence of ischemic heart disease followed by dose increments of 25 µg every 3-4 weeks until the full replacement dose is achieved. Some believe that a higher target TSH (0.5-3.0 mU/L) value is appropriate for the elderly patient.
- Thyroxine requirements increase during pregnancy. Thyroid status should be checked with TSH + FT4 during each trimester of pregnancy. The L-T4 dose should be increased (usually by 50 µg/day) to maintain a serum TSH between 0.5 and 2.0 mU/L and a serum FT4 in the upper third of the normal reference interval.
- Post-menopausal women starting hormone replacement therapy (HRT) may need an increase in their L-T4 dose to keep the serum TSH within the therapeutic target.
- TSH testing of patients receiving a stable L-T4 dose is recommended on an annual basis. The best time for TSH testing is not influenced by the time of day the L-T4 dose is ingested.
- Ideally L-T4 should be taken before eating, at the same time of day, and at least 4 hours apart from any other medications or vitamins.
- Patients beginning chronic therapy with Cholestyramine, Ferrous Sulfate, calcium carbonate, Soy protein, Sucralfate and antacids containing Aluminum Hydroxide that influence L-T4 absorption may require a larger L-T4 dose to maintain TSH within the therapeutic target range.
- Patients taking Rifampin and anticonvulsants that influence the metabolism of L-T4 may also need an increase in their dose of L-T4 to maintain the TSH within the therapeutic target range.

(iv) L-T4 Suppression Therapy

L-T4 administration designed to suppress serum TSH levels to subnormal values is typically reserved for patients with well differentiated thyroid carcinoma for which thyrotropin is considered a trophic factor (217). The efficacy of L-T4 suppression therapy has only been determined from uncontrolled retrospective studies that have yielded conflicting results (218,219). It is important to individualize the degree of TSH suppression by weighing patient factors such as age, clinical status including cardiac factors and DTC recurrence risk against the potentially deleterious effects of iatrogenic mild (subclinical) hyperthyroidism on the heart and bone (29). Many physicians use a serum TSH target of 0.05-0.1 mU/L for low-risk patients and a TSH of <0.01 mU/L for high-risk patients. Some physicians reduce the L-T4 dose to give low-normal TSH values when patients have undetectable serum thyroglobulin (Tg) levels and no recurrences 5-10 years after thyroidectomy. Suppression therapy for non-endemic goiters is generally considered ineffective (220). Furthermore, patients with nodular goiters often already have suppressed TSH concentrations as a result of thyroid autonomy (210).

C.8 Guidelines for L-T4 Suppression Therapy

- Serum TSH is considered a growth factor for differentiated thyroid cancer (DTC). The typical L-T4 dose used to suppress serum TSH in DTC patients is 2.1µg/kg body weight/day.
- The target serum TSH level for L-T4 suppression therapy for DTC should be individualized relative to the patient's age and clinical status including cardiac factors and DTC recurrence risk.
- Many physicians use a serum TSH target value of 0.05-0.1 mU/L for low-risk patients and a TSH of <0.01 mU/L for high-risk patients.
- Some physicians use a low-normal range therapeutic target for TSH when patients have undetectable serum Tg levels and have had no recurrence 5-10 years after thyroidectomy.
- If iodine intake is sufficient, L-T4 suppression therapy is rarely an effective treatment strategy for reducing the size of goiters.
- Over time, multi-nodular goiters typically develop autonomy that is characterized by a subnormal serum TSH level. Serum TSH should be checked before initiating L-T4 suppression therapy in such patients.

(v) Serum TSH Measurement in Hospitalized Patients with NTI

Although most hospitalized patients with NTI have normal serum TSH concentrations, transient TSH abnormalities in the 0.02 – 20 mIU/L range are commonly encountered in the absence of thyroid dysfunction (19,90,95,96). It has been suggested that the use of a wider reference range (0.02 –10 mU/L) would improve the positive predictive value of TSH measurements for evaluating the sick hospitalized patient (19,95,96,221). TSH should be used in conjunction with a FT4 estimate (or TT4) test for evaluating hospitalized patients with clinical symptoms or patients with a history of thyroid dysfunction (Guidelines 2.6 and C.9). Sometime the cause of the TSH abnormality in a hospitalized patient is obvious, such as in the case of dopamine or glucocorticoid-therapy (90,95). In other cases the TSH abnormality is transient and appears to be caused by NTI per se, that resolves with recovery from the illness. It is common to see a transient minor suppression of TSH into the 0.02-0.2 mIU/L range during the acute phase of an illness, followed by a rebound to mildly elevated values during recovery (107). It is important to use a TSH assay with a functional sensitivity ≤ 0.02 mIU/L in the hospital setting in order to be able to reliably determine the degree of TSH suppression. Specifically, the extent of TSH suppression can be used to discriminate sick hyperthyroid patients with profoundly low serum TSH values (< 0.02 mIU/L) from patients with a mild transient suppression caused by a NTI (19).

Diagnosing hyperthyroidism in NTI patients can be a challenge because current FT4 methods can give both inappropriately low and high values in euthyroid NTI patients (105,222). Serum TT4 and TT3 measurements may be useful for confirming a diagnosis of hyperthyroidism if assessed relative to the severity of the illness (Guideline 2.6). A suppressed serum TSH below 0.02mU/L is less specific for hyperthyroidism in hospitalized individuals, compared with ambulatory patients. One study found that 14% of hospitalized patients with TSH < 0.005 mU/L were euthyroid. However, such patients had a detectable TRH-stimulated TSH response whereas patients truly hyperthyroid with NTI did not (19).
Mild (subclinical) hypothyroidism cannot be easily diagnosed during a hospitalization, because of the frequency of high TSH abnormalities associated with NTI. Provided that the thyroid hormone (FT4 or TT4) concentration is within normal limits, any minor abnormality in serum TSH (0.02-20.0 mU/L) arising from a mild (subclinical) thyroid condition is unlikely to affect the outcome of the hospitalization, and can be deferred for evaluation 2-3 months after discharge. In contrast, sick hypothyroid patients typically exhibit the combination of low FT4 and elevated TSH (>20 mIU/L) (95).

C.9 Guidelines for TSH Measurement in Hospitalized Patients

- TSH + T4 (FT4 or TT4) is the most useful test combination to detect thyroid dysfunction in a sick hospitalized patient.
- It is more appropriate to use a widened TSH reference interval (0.05 and 10.0 mIU/L) in the hospitalized setting.
- A serum TSH value between 0.05 and 10.0 mIU/L is usually consistent with a euthyroid state, or only a minor thyroid abnormality that can be evaluated by retesting after the illness subsides. (This only applies to patients

not receiving medications such as dopamine that directly inhibit pituitary TSH secretion.)
- A low-normal TSH level in the presence of a low TT4 and low TT3 may reflect central hypothyroidism as a result of a prolonged illness; whether or not this condition requires immediate treatment remains uncertain and is currently controversial.
- When thyroid dysfunction is suspected, a thyroid peroxidase antibody (TPOAb) test may be useful to differentiate autoimmune thyroid disease from NTI.

(vi) Central Hypothyroidism

A diagnosis of central hypothyroidism will usually be missed using a "TSH first" strategy (18). Central hypothyroid conditions are characterized by paradoxically normal or slightly elevated serum TSH levels (223). The log/linear relationship between TSH and FT4 dictates that patients with primary hypothyroidism and a subnormal FT4 should have a serum TSH value > 10mU/L (Figure 1) [Section 2A(a)]. When the degree of TSH elevation in response to a low thyroid hormone level appears inappropriately low, pituitary insufficiency should be excluded.

C.10 Guidelines: Levothyroxine (L-T4) Replacement Therapy for Central Hypothyroidism

- A serum FT4 level in the upper third of the reference interval is the therapeutic target for the L-T4 replacement dose used to treat central hypothyroidism due to pituitary or hypothalamic dysfunction.
- When using FT4 as the therapeutic endpoint for central hypothyroidism, the daily dose of L-T4 should be withheld on the day of the FT4 measurement. (Serum FT4 is increased (15-20%) above baseline for 9 hours after ingesting L-T4).

In one study of central hypothyroid patients, 35% had subnormal TSH values but 41% and 25% had inappropriately normal and elevated TSH values, respectively (224). It is now well documented that the paradoxically elevated serum TSH level seen in central hypothyroid conditions is caused by the measurement of biologically inert TSH isoforms that are secreted when the pituitary is damaged or when hypothalamic TRH stimulation is deficient (191). The inappropriate TSH values arise because the monoclonal antibodies used in current TSH assays cannot distinguish between TSH isoforms of different biological activity, since TSH biological activity is determined not by the protein backbone but by the degree of glycosylation, specifically sialylation, of the TSH molecule. It appears that a normal secretion pattern of TRH is essential for TSH sialylation and the normal association of TSH subunits to form mature, biologically active TSH molecules (191,223,225). The biological activity of TSH in central hypothyroid conditions appears inversely related to the degree of sialylation as well as the FT4 level in the circulation (223). TRH-stimulation testing may be useful for specifically diagnosing central hypothyroidism (226). Typical TSH-responses in such conditions are blunted (<2-fold rise/ ≤4.0 mU/L increment) and sometimes delayed (191,198,226,227). In addition, the T3-responses to TRH-stimulated TSH are blunted and correlate with TSH biological activity (191,228,229).

(vii) Inappropriate TSH Secretion Syndromes

The apparent paradoxical dissociation between high levels of thyroid hormone and a non-suppressed serum TSH has led to the widespread use of the term "inappropriate TSH secretion syndromes" to describe these conditions. Specimens that display a discordant TSH/FT4 relationship are increasingly being identified now that sensitive TSH assays can reliably detect subnormal TSH concentrations and are in widespread use (Section 3C(b)]. As shown in Table 1, it is critical to first exclude likely causes of a discordant TSH/FT4 ratio, i.e technical interference and/or binding protein abnormality. This confirmatory testing should be performed on a fresh specimen by measuring TSH together with free and total thyroid hormone with a different manufacturer's method. Only after the more common causes of discordance have been eliminated should rare conditions such as a TSH-secreting pituitary tumor or thyroid hormone resistance be considered.

• ***TSH-Secreting Pituitary Tumors***

Pituitary tumors that hypersecrete TSH are rare, representing <1% of cases (230,231). These tumors often present as a macroadenoma with symptoms of hyperthyroidism associated with a non-suppressed serum TSH and MRI evidence of a pituitary mass (231).

After excluding a technical reason for the paradoxically elevated TSH level (i.e. HAMA), the diagnosis of TSH-secreting pituitary tumor is usually made on the basis of:

- A lack of TSH response to TRH stimulation
- An elevated serum TSH alpha subunit
- A high alpha subunit/TSH ratio
- The demonstration of a pituitary mass on MRI

• Thyroid Hormone Resistance

Thyroid hormone resistance (THR) is usually caused by a mutation of the thyroid hormone (TR), TR-beta receptor gene that occurs in 1: 50,000 live births (233-236). Although the clinical presentation can be variable, patients have a similar biochemical profile. Specifically, serum FT4 and FT3 are typically elevated (from a minimal degree to a 2-3-fold elevation above the upper normal limit) associated with a normal or slightly elevated serum TSH that responds to TRH stimulation (236,237). However, it should be recognized that TSH secretion is <u>not</u> inappropriate given the fact that the tissue response to thyroid hormone is reduced, requiring higher thyroid hormone levels to maintain a eumetabolic state. THR patients typically have a goiter as a result of chronic hypersecretion of a TSH isoform that has increased sialylation and consequently increased biologic potency (194,238). The clinical manifestation of thyroid hormone excess covers a wide spectrum. Some patients appear to be eu-metabolic with a near-normal serum TSH and whose receptor defect appears to be compensated for by high levels of thyroid hormone (Generalized THR). Other patients appear to be hypermetabolic and appear to have a defect that selectively affects the pituitary (Pituitary THR).

The distinctive features of THR are the presence of a non-suppressed TSH, together with an appropriate response to TRH despite elevated thyroid hormone levels (236,239). Although rare, it is important to consider the diagnosis of THR when encountering a patient with elevated thyroid hormone levels associated with a paradoxically normal or elevated TSH (236,240). Such patients have often been misdiagnosed as having hyperthyroidism and subjected to inappropriate thyroid surgery or radioiodine ablation (236). As shown in Table 1, binding protein abnormalities or assay technical problems are the most common causes for a discordant FT4/TSH relationship. Repeat testing of TSH as well as free and total thyroid hormone should be made, preferably with a second fresh specimen. Reanalysis of the test results using a different manufacturer's method will often reveal whether there are technical problems or binding protein abnormalities. If the biochemical profile is confirmed, the possibility that a TSH-secreting pituitary tumor is the cause of the paradoxical TSH should be eliminated first before assigning the diagnosis of THR. It should be noted that both conditions can coexist (241). As described above, a TSH-secreting pituitary tumor produces a similar biochemical profile but can be distinguished from THR by TSH-alpha subunit testing and radiographic imaging. Additionally, TRH-stimulation testing may be useful in developing the differential diagnosis. Specifically, a blunted TRH-stimulation test and T3-suppression test is characteristic of most TSH-secreting pituitary tumors whereas a normal response is seen in most cases of THR (239).

C.11 Clinical Utility of TSH Measurements (Functional Sensitivity ≤ 0.02 mU/L)

- The majority (~95%) of healthy euthyroid subjects have a serum TSH value below 2.5 mU/L. A repeat TSH measurement after 3 weeks and/or with a reflex TPOAb measurement may be appropriate when an ambulatory patient has a serum TSH concentration in the "upper normal range" (>3.0 mU/L). These individuals may be in the early stages of developing hypothyroidism.
- A serum TSH measurement is the most diagnostically sensitive test for detecting mild (subclinical) as well as overt primary hypo- and hyperthyroidism in ambulatory patients.
- A serum TSH measurement is the therapeutic endpoint for titrating the L-T4 replacement dose for primary hypothyroidism (see Guideline C.7) and for monitoring L-T4 suppression therapy for differentiated thyroid

carcinoma (see Guideline C.8).
- Serum TSH measurements are more reliable than FT4 in hospitalized patients with non-thyroidal illness not receiving dopamine. Serum TSH should be used in conjunction with T4 (TT4 or FT4) testing of hospitalized patients (Guidelines 2.6 and C.9).
- TSH cannot be used to diagnose central hypothyroidism because current TSH assays measure biologically inactive TSH isoforms.
- Central hypothyroidism is characterized by an inappropriate normal or slightly elevated serum TSH level and a blunted (<2-fold rise/ ≤4.0 mU/L increment) TRH response.
- When serum FT4 is low and yet the serum TSH is only minimally elevated (<10 mU/L), a diagnosis of central hypothyroidism should be considered.
- Serum TSH measurements are an important pre-natal and first trimester screening test to detect mild (subclinical) hypothyroidism in the mother (see Guideline 2.4).
- A low TSH in the setting of a multinodular goiter suggests the presence of mild (subclinical) hyperthyroidism due to thyroid autonomy (see Guideline C.8).
- Serum TSH measurement is required test for confirming that an elevated thyroid hormone level is due to hyperthyroidism and not a thyroid hormone binding protein abnormality (such as FDH).
- A serum TSH measurement is the primary test for detecting Amiodarone – induced thyroid dysfunction (see Guideline 2.5).

D. Thyroid Autoantibodies (TPOAb, TgAb and TRAb)

Autoimmune thyroid disease (AITD) causes cellular damage and alter thyroid gland function by humoral and cell-mediated mechanisms. Cellular damage occurs when sensitized T-lymphocytes and/or autoantibodies bind to thyroid cell membranes causing cell lysis and inflammatory reactions. Alterations in thyroid gland function results from the action of stimulating or blocking autoantibodies on cell membrane receptors. Three principal thyroid autoantigens are involved in AITD. These are thyroperoxidase (TPO), thyroglobulin (Tg) and the TSH receptor. Other autoantigens, such as the Sodium Iodine Symporter (NIS) have also been described, but as yet no diagnostic role in thyroid autoimmunity has been established (242). TSH receptor autoantibodies (TRAb) are heterogeneous and may either mimic the action of TSH and cause hyperthyroidism as observed in Graves' disease or alternatively, antagonize the action of TSH and cause hypothyroidism. The latter occurs most notably in the neonate as a result of a mother with antibodies due to AITD. TPO antibodies (TPOAb) have been involved in the tissue destructive processes associated with the hypothyroidism observed in Hashimoto's and atrophic thyroiditis. The appearance of TPOAb usually precedes the development of thyroid dysfunction. Some studies suggest that TPOAb may be cytotoxic to the thyroid (243,244). The pathologic role of TgAb remains unclear. In iodine sufficient areas, TgAb is primarily determined as an adjunct test to serum Tg measurement, because the presence of TgAb can interfere with the methods that quantitate Tg [Section 3E(a)v]. In iodine deficient areas, serum TgAb measurement may be useful for detecting autoimmune thyroid disease in patients with a nodular goiter and for monitoring iodine therapy for endemic goiter.

Laboratory tests that determine the cell-mediated aspects of the autoimmune process are not currently available. However, tests of the humoral response, i.e. thyroid autoantibodies, can be assessed in most clinical laboratories. Unfortunately, the diagnostic and prognostic use of thyroid autoantibody measurements is hampered by technical problems as discussed below. Although autoantibody tests have inherent clinical utility in a number of clinical situations, these tests should be selectively employed.

(a) Clinical Significance of Thyroid Autoantibodies

TPOAb and/or TgAb are frequently present in the sera of patients with AITD (245). However, occasionally patients with AITD have negative thyroid autoantibody test results. TRAb are present in most patients with a history of or who currently have Graves' disease. During pregnancy, the presence of TRAb is a risk factor for fetal or neonatal dysfunction as a result of the transplacental passage of maternal TRAb (246,247). The prevalence of thyroid autoantibodies is increased when patients have non-thyroid autoimmune diseases such as type 1 diabetes and pernicious anemia (248). Aging is also associated with the appearance of thyroid autoantibodies (249). The clinical

significance of low levels of thyroid autoantibodies in euthyroid subjects is still unknown (250). However, longitudinal studies suggest that TPOAb may be a risk factor for future thyroid dysfunction, including post-partum thyroiditis (PPT) as well as the development of autoimmune complications from treatment by a number of therapeutic agents (251-253). These include amiodarone therapy for heart disease, interferon therapy for chronic hepatitis C and lithium therapy for psychiatric disorders (81,254-257). The use of thyroid autoantibody measurements for monitoring the treatment for AITD is generally not recommended (258). This is not surprising since treatment of AITD addresses the consequence (thyroid dysfunction) and not the cause (autoimmunity) of the disease.

(b) Nomenclature of Thyroid Antibody Tests

The nomenclature used for thyroid autoantibodies has proliferated, particularly in the case of TSH receptor antibodies (LATS, TSI, TBII, TSH-R and TRAb). The terms used in this monograph, TgAb, TPOAb and TRAb are those recommended internationally. These terms correspond to the molecular entities (immunoglobulins) which react with the specified autoantigens recognized by the laboratory test. Method differences may bias the measurement of these molecular entities, e.g.: methods may detect only IgG or IgG plus IgM; TPOAb or Ab directed to TPO and other membrane autoantigens; TSH inhibiting and/or TSH stimulating TRAb.

(c) Specificity

The use of thyroid autoantibody measurements has been hampered by specificity problems. Studies show that results vary widely depending on the method used. This is due to differences in both the sensitivity and specificity of the methods and the absence of adequate standardization. In the past few years, studies at the molecular level have shown that autoantibodies react with their target autoantigens, by binding to " conformational " domains or epitopes. The term "conformational" refers to the requirement for a specific three- dimensional structure for each of the epitopes recognized by the autoantibodies. Accordingly, assay results critically depend on the molecular structure of the autoantigen used in the test. Small changes in the structure of a given epitope may result in a decrease or a loss in autoantigen recognition by the autoantibodies targeted to this epitope. Recently, dual specificity TGPO antibodies, that recognize both Tg and TPO, have been demonstrated in the blood of patients with AITD (259). It has been known for years that autoantibodies are directed against few epitopes as compared to heterologous antibodies. Current methods differ widely in epitope recognition. Specificity differences can result from misrecognition of an epitope that leads to a bias regarding the autoantibody population tested. This results in vastly different reference intervals, even when methods are standardized to the same international reference preparation. Whatever the targeted autoantigen, thyroid autoantibodies are clearly not unique molecular entities but, rather, mixtures of immunoglobulins that only have in common their ability to interact with Tg, TPO or the TSH receptor.

D.1 Guidelines: Thyroid Antibody Methods Differ in Sensitivity & Specificity

Laboratories should consider changing their method when physicians express concerns regarding method specificity or sensitivity

- Recognize that the results of thyroid antibody tests are method-dependent.
- Thyroid antibody methods recognize different epitopes in the heterogeneous antibody populations present in serum.
- Thyroid antibody assay differences reflect different receptor preparations (receptor assays) or cells (bioassays) used in the assay.
- Assay differences can result from contamination of the antigen reagent with other autoantigens.
- Assay differences can result from the inherent assay design (i.e. competitive versus non-competitive immunoassay formats as well as the signal used.
- Assay differences can result from the use of different secondary standards.

Differences in the sensitivity of autoantibody tests may arise from the design of the assay (e.g. competitive RIA versus two-site IMA) as well as the physical method used for the signal (e.g. radioisotope versus chemiluminiscence). Differences in specificity may occur as a result of contamination of the autoantigen preparation by other autoantigens (e.g. thyroid microsomes versus purified TPO). Further, misrecognition of an epitope may lead to an underestimation of the total amount of circulating autoantibody present, resulting in decreased sensitivity.

D.2 Guidelines for Establishing the Functional Sensitivity of Thyroid Antibody Tests

Functional sensitivity assessment of thyroid autoantibody tests should:

- Be determined with human serum pools containing a low autoantibody concentration
- Be determined using the same protocol as described for TSH (guideline C.3) but with the between-run precision assessment made over a 6 to 12 month time-period to represent an appropriate clinical assessment interval.

Functional sensitivity should be determined with human serum pools containing a low autoantibody concentration. The protocol for functional sensitivity should be the same protocol as described for TSH (Guideline C.3) except that the between-run precision for autoantibody tests should be assessed across a longer time-period (6 to 12 months), consistent with the longer monitoring interval typically used in clinical practice.

(d) Standardization

Standardization of thyroid autoantibody tests is currently inadequate. International Reference Preparations, MRC 65/93 for TgAb, MRC 66/387 for TPOAb are available from the National Council for Biological Standards and Control in London, UK (www.mrc.ac.uk). These preparations were made from a pool of serum from patients with autoimmune thyroid disease and were prepared and lyophilized 35 years ago! It is well known that lyophilized antibodies are prone to degradation over time. Degradation of the antibodies could have introduced a bias in the binding activity of these reference preparations towards the less stable thyroid antibodies of unknown clinical relevance. Due to the scarcity of these preparations, they are only used as primary standards for calibrating assay methods. Commercial kits contain secondary standards that differ for each method. Currently, assay calibrations vary with the experimental conditions as well as the antigen preparation used by the manufacturer. This may introduce another bias in detecting the heterogeneous antibodies present in patient specimens. In the case of TRAb, the reference preparation MRC 90/672 is more recent (1990) but not currently used by manufacturers.

D.3 Guidelines for Manufacturers Standardizing Thyroid Antibody Assays

- Assays should be standardized against MRC International Reference Preparations:- MRC 65/93 for TgAb, MRC 66/387 for TPOAb and MRC 90/672 for TRAb
- New International Reference Preparations should be prepared for TgAb and TPOAb.
- Secondary standards should be fully characterized to avoid bias between different methods.
- Reference preparations or recombinant antigen preparations should be used when available.

(e) TPOAb Methods

Thyroid Peroxidase (TPO) is a 110 kD membrane bound hemo-glycoprotein with a large extracellular domain, and a short transmembrane and intracellular domain. TPO is involved in thyroid hormone synthesis at the apical pole of the follicular cell. Several isoforms related to differential splicing of TPO RNA have been described. TPO molecules may also differ with respect to their three-dimensional structure, extent of glycosylation and heme binding. Most of the TPO molecules do not reach the apical membrane and are degraded intracellularly.

TPO autoantibodies were initially described as anti-microsomal autoantibodies (AMA) since they were found to react with crude preparations of thyroid cell membranes. The microsomal antigen was later identified as TPO (260).

Older AMA immunofluorescence assays as well as passive tanned red cell agglutination tests are still currently in use in addition to the newer, more sensitive competitive and non-competitive TPOAb immunoassays. These new immunoassay methods are currently replacing the older AMA agglutination tests because they are quantitative, more sensitive and can easily be automated. However, there is wide variability in the sensitivity and specificity of these new TPOAb methods. Some of this variability stems from differences in the TPO preparations used in the various assay kits. When extracted from human thyroid tissue, TPO may be used as a crude membrane preparation or may be purified by different methods. The assay specificity may also differ because of contamination by other thyroid autoantigens – notably Tg and/or variations in the three-dimensional structure of TPO. The use of recombinant human TPO (rhTPO) eliminates the risk of contamination but does not solve the problem of the differences in TPO structure that depend upon the technique used to isolate TPO. Most current TPOAb assays are quantitated in international units using the reference preparation MRC 66/387. Unfortunately, the use of this primary standard does not alleviate between-method variations as is evident from the broad variability in sensitivity limits claimed by the different kit manufacturers (range <0.3 to <20 kIU/L) and the differences in normal reference intervals.

D.4 Guideline: TPOAb Methods

- Sensitive, specific TPOAb immunoassays, using suitable preparations of highly purified native or recombinant human TPO as the antigen, should replace the older insensitive, semi-quantitative anti-microsomal antibody (AMA) agglutination tests.

  (Consensus Level 75%)

- The clinical significance of a low TPOAb concentration requires more study.

(i) TPOAb Prevalence & Reference Intervals

The estimate of TPOAb prevalence depends on the sensitivity and specificity of the method employed. The recent NHANES III United States survey of ~17,000 subjects without apparent thyroid disease, reported detectable TPOAb levels in 12 % of subjects using a competitive immunoassay method (4). Whether low levels of TPOAb detected in healthy individuals and/or patients with non-thyroid autoimmune diseases reflect normal physiology, the prodrome of AITD, or an assay specificity problem, remains unclear.

Normal reference values for TPOAb assays are highly variable and often arbitrarily established, so that a large majority of patients with AITD test positive, and most subjects without clinical evidence of AITD test negative. The lower normal limit appears to relate to technical factors. Specifically, assays citing a low detection limit (<10 kIU/L) typically report undetectable TPOAb levels in meticulously selected normal subjects. Such methods suggest that the presence of TPOAb is a pathologic finding. In contrast, TPOAb assays reporting higher detection limits (>10kIU/L) typically cite a TPOAb "normal reference range". Since such methods appear to have no enhanced sensitivity for detecting AITD, these "normal range" values may represent non-specific assay "noise" and are not pathologically meaningful.

The recent 20 year follow-up study of the Whickham cohort reported that detectable TPOAb titers (measured as AMA) was not only a risk factor for hypothyroidism but that a detectable AMA preceded the development of an elevated TSH (Figure 7) (28). This suggests that a detectable TPOAb is a risk factor for AITD (Guideline D.6). However, individuals with low TPOAb levels would have had undetectable AMA by the older methods used in this study (28). The clinical significance of low TPOAb levels that are not detectable by AMA agglutination methods remains to be established through longitudinal studies. Thus, whether individuals with low levels of TPOAb and/or TgAb should be considered normal remains in question until long-term follow-up studies on such individuals show that they do not have an increased risk for developing thyroid dysfunction.

The criteria employed for selecting subjects for the normal cohort used to establish an autoantibody normal reference range, is critical. Such a cohort should be comprised of young, biochemically euthyroid (TSH 0.5 to 2.0 mIU/L) male subjects with no goiter and no family history of AITD. This rigorous selection process would be least likely to include subjects with a predisposition to AITD.

D.5 Guidelines for Determining Reference Intervals for Thyroid Antibody Tests

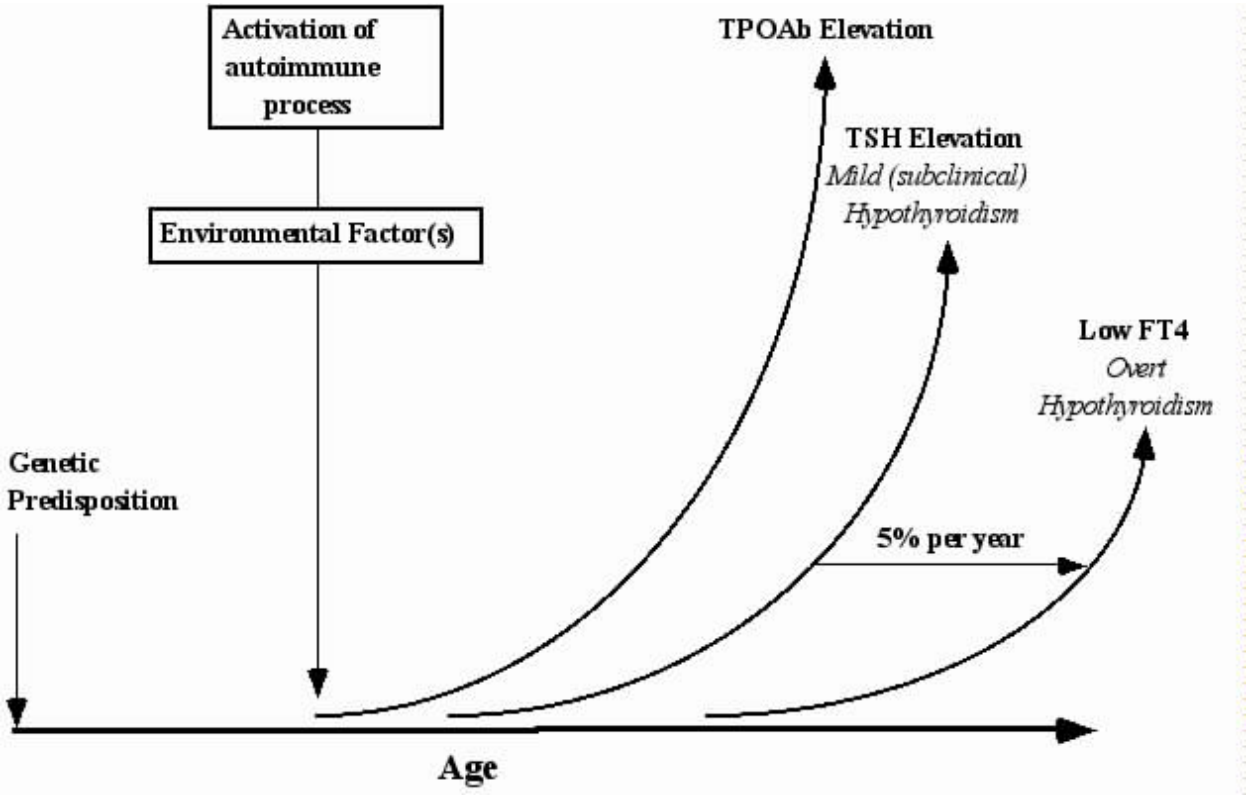
120 "Normal" subjects should be used to establish reference intervals for thyroid antibody tests: Subject selection should minimize the inclusion of persons with a predisposition for autoimmune thyroid disease. Normal subjects should be selected to be:

- Male
- Young (< 30 years of age)
- Have serum TSH levels between 0.5 and 2.0 mIU/L
- No goiter
- No personal or family history of thyroid disease
- No non-thyroid autoimmune diseases
- No systemic diseases (e.g. lupus or diabetes)

(ii) Clinical Uses of TPOAb Measurements

TPOAb is the most sensitive test for detecting autoimmune thyroid disease (261). As shown schematically in Figure 7, TPOAb is typically the first abnormality to appear in the course of developing hypothyroidism secondary to Hashimotos' thyroiditis. In fact, when TPOAb is measured by a sensitive immunoassay, >95% of subjects with Hashimotos thyroiditis have detectable levels of TPOAb. Such methods also detect TPOAb in most (~85%) patients with Graves' disease (248). Patients with TPOAb detected in early pregnancy are at risk for developing post-partum thyroiditis (253). Recent reports have suggested that the IQ of children born to mothers with increased TSH and/or detectable TPOAb during pregnancy may be compromised (60-62). This has prompted recommendations that all pregnant women should have TSH and TPOAb levels measured in the first trimester of their pregnancy [Section 2A(c) and Guideline 2.4)]. Further, TPOAb measurements may have a role in infertility, since high TPOAb levels are associated with a high risk of miscarriage and failure with in-vitro fertilization (262).

Figure 7. TPOAb Changes with Developing Autoimmune Thyroid Disease



The presence of TPOAb is well established as a risk factor for thyroid dysfunction when patients are being treated with lithium (254), amiodarone (81), interleukin-2 (263) or interferon-alpha (255,256). During interferon-alpha treatment, a preexisting thyroid autoimmune disorder or positive TPOAb titer are predisposing factors for the development of thyroid disease during therapy (257). The presence of TPOAb before therapy had a sensitivity of 20%, a specificity of 95% and a predictive value of 66.6% for the development of thyroid dysfunction (264).

D.6 Guidelines: Clinical Uses of TPOAb Measurement

- Diagnosis of Autoimmune Thyroid Disease
- Risk factor for Autoimmune Thyroid Disease
- Risk factor for hypothyroidism during Interferon-alpha, Interleukin-2 or Lithium therapy
- Risk factor for thyroid dysfunction during Amiodarone therapy (see Guideline 2.5)
- Risk factor for thyroid dysfunction during pregnancy and for post-partum thyroiditis
- Risk factor for miscarriage and in-vitro fertilization failure
- Risk factor for neonatal hypothyroidism

(f) Thyroglobulin Autoantibodies (TgAb)

Thyroglobulin (Tg), the prothyroid globulin, is a high molecular weight (660 kDa) soluble glycoprotein made up of two identical subunits. Tg occurs with a high degree of heterogeneity due to differences in post-translational modifications (glycosylation, iodination, sulfation etc). During the process of thyroid hormone synthesis and release, Tg is polymerized and degraded. Consequently, the immunologic structure of Tg is extremely complex. The characteristics of Tg preparations may vary widely depending on the starting human thyroid tissue and the purification process used. This is the first clue that explains why TgAb assays, as well as Tg assays [Section 3E(a)] are so difficult to standardize.

D.7 TgAb Methods

- Serial TgAb measurements in thyroid cancer patients have clinical significance regarding disease course. Physicians should be informed in advance of any change in method to allow time to re-baseline patients.
- Understanding the sensitivity and specificity of the TgAb method being used is critical, since very low levels of TgAb may interfere with serum Tg measurement.

(i) TgAb Methodology

As with TPOAb methods, the design of TgAb assays has evolved from immunofluorescence of thyroid tissue sections, to passive tanned red cell agglutination methods and to the more current, competitive and non-competitive immunoassays. This technical evolution has improved both the sensitivity and specificity of serum TgAb measurements. However, because the older and newer methods are still being used concurrently in clinical laboratories, the sensitivity and specificity of available methods can vary widely depending on the laboratory. Assays are calibrated with purified or crude preparations of TgAb by pooling patient sera or blood donor material. These various secondary standards are often, but not always, calibrated against the primary standard (MRC 65/93). However, standardization with MRC 65/93 does not ensure that different methods are quantitatively or qualitatively similar. Other reasons for method differences relate to the heterogeneity of the TgAb itself. The heterogeneity of TgAb is restricted in patients with AITD compared with other thyroid disorders such as differentiated thyroid carcinomas (DTC) in which the heterogeneity of TgAb appears less restricted (265). This reflects differences in the expression of the different autoantibodies that may be normally expressed at very low levels in healthy subjects (266). The inter-method variability of serum TgAb values may also reflect qualitative differences in TgAb affinity and epitope specificity in different serum samples from patients with different underlying thyroid and immunological conditions. Another reason for inter-method differences is that assay designs are prone to interference by high levels of circulating antigen (Tg), as can be the case with Graves' disease and metastatic DTC (267).

D.8 Guideline for Manufacturers Developing TgAb Methods

- The epitope specificity of TgAb methods should be broad not restricted, since TgAb epitope specificity may be wider for TgAb-positive patients with DTC compared to patients with autoimmune thyroid disease.

(ii) TgAb Prevalence & Reference Intervals

As with TPO antibodies, the prevalence and normal cut-off values for thyroglobulin antibodies depends on the sensitivity and specificity of the assay method (268). The NHANES III survey reported a TgAb prevalence of ~10% for the general population, measured by competitive immunoassay (4). The TgAb prevalence appears to be two-fold higher than normal for patients diagnosed with DTC (~20 %) (268). As with TPOAb, the clinical significance of low TgAb levels that would be undetectable by the older agglutination methods remains unclear. It has been suggested that low levels may represent " natural " antibody in normal individuals or a " scavenger " antibody response to antigen release following thyroid surgery or radioactive iodide therapy. Alternatively, low levels might represent underlying silent AITD (250). Different TgAb methods report different normal threshold values, as discussed for TPOAb [Section 3D(e)i]. Specifically, some TgAb methods report that normal subjects should have values below the assay detection level, other methods report a "normal range". When TgAb measurements are used as an adjunct test to serum Tg measurements, the significance of low TgAb levels relates less to the pathophysiology of its presence but more to the potential for low TgAb levels interfering with the serum Tg method.

(iii) Sensitivity and Precision of TgAb Measurement

Sensitivity is critical when serum TgAb measurements are used as an adjunct test to serum Tg for monitoring patients with DTC. This is because even very low levels of TgAb can interfere with Tg measurements causing falsely low or high serum Tg values (267,268). It is also essential that TgAb tests demonstrate good between-run precision (<10% CV across a one-year period) because serial TgAb measurements are used to monitor TgAb-positive patients with DTC (268-270). Specifically, TgAb-positive patients who are rendered athyreotic by their initial treatment typically display a progressive decline in TgAb levels during their early post-operative years. In fact, most patients who are TgAb-positive at the time of thyroidectomy become TgAb-negative after 2-4 years. In contrast, a rise or appearance of serum TgAb is often the first indication of a recurrence.

(iv) Clinical Uses of TgAb Measurement

The United States NHANES III study reported that 3 % of subjects with no risk factors for thyroid disease had detectable TgAb without associated presence of TPOAb (4). Since this cohort had no associated TSH elevation, TgAb measurements do not appear to be a diagnostic test for AITD in areas of iodine sufficiency (250,271). In iodine deficient areas however, TgAb is believed to be useful for detecting AITD, especially for patients with a nodular goiter. TgAb measurements are also useful for monitoring iodine therapy for endemic goiter, since iodinated Tg molecules are more immunogenic. The majority of serum TgAb measurements are made as an adjunct test for serum Tg measurements because low TgAb levels can interfere with Tg measurements made by most methods [Section 3E(a)v] (267,268).

D.9 Guidelines: Use of TgAb Measurement in Non-Neoplastic Conditions

- In iodine sufficient areas, it is not usually necessary or cost-effective to order both TPOAb and TgAb, because TPOAb-negative patients with detectable TgAb rarely display thyroid dysfunction.
- In iodine deficient areas, serum TgAb measurement may be useful for detecting autoimmune thyroid disease in patients with a nodular goiter.
- Monitoring iodine therapy for endemic goiter.

D.10 Guidelines: Use of TgAb Measurement in Differentiated Thyroid Carcinomas (DTC)

- The serum TgAb level should be measured in every specimen sent for Thyroglobulin (Tg) testing.
- The laboratory performing the Tg testing should also perform the TgAb test.
- The TgAb method should be immunoassay not agglutination, because low levels of TgAb can interfere with the accuracy of measuring serum Tg by most methods.
- Serial measurements of serum TgAb have prognostic value for monitoring the treatment response of TgAb-positive DTC patients.
- Before changing the TgAb method, the laboratory should consult with physician users and compare the results between the old and proposed new method. Patients should be re-baselined if the differences between the methods is >10%.

(g) TSH Receptor Autoantibodies (TRAb)

The TSH receptor is a member of the superfamily of receptors with seven transmembrane domains linked to G proteins. The 60kb TSH receptor gene located on the long arm of chromosome *14q31* has been cloned and sequenced (272). Exons 1-9 code for the extracellular domain of the receptor (397 amino acids) and exon 10 codes for the transmembrane region (206 amino acids). Activation of G proteins by the hormone receptor complex results in stimulation of cAMP production by adenylate cyclase and inositol phosphate turnover by phospholipases (273). Site-directed mutagenesis has shown that the 3-dimensional receptor structure is important for the interaction with TSH and/or TRAbs. There are three broad types of TRAb measured by either bioassay or receptor assay (Table 6). Receptor, or TSH Binding Inhibitory Immunoglobulin (TBII) assays do not measure biologic activity directly but assess whether the specimen contains immunoglobulins that can block the binding of TSH to an in vitro receptor preparation. TSH stimulating antibodies (TSAb) appear to bind the N-terminal portion of the extracellular domain

and mimic the actions of TSH by inducing post-receptor signal transduction and cell stimulation. In contrast, the C-terminal region is more important for TSH receptor blocking antibodies (abbreviated TBAb or TSBAb) which block stimulation by either TSAb or TSH causing hypothyroidism (274). Thyroid growth-stimulating immunoglobulins (TGI) are less well characterized in this regard.

It has now been shown that the lack of correlation between TRAb levels and the clinical status of patients is largely because of circulating TRAb's that are heterogeneous. The fact that TRAb heterogeneity can coexist within an individual patient and change over time is one reason why it has been difficult to develop diagnostically accurate TRAb tests (275,276). Indeed, the clinical presentation of Graves' patients who exhibit both TSAb and TBAb/TSBAb will likely depend on the relative concentration and affinity of the predominant antibody. A shift from stimulating to blocking TRAb may explain the spontaneous remission of Graves' disease during pregnancy as well as radioiodine induction of transient hypothyroidism (274,277). It is important to note that bioassays that use cell preparations to measure the biologic effects of TRAb (stimulation, inhibition of TSH activity or growth) can detect functional changes in TRAb heterogeneity. In contrast, the receptor, or TSH Binding Inhibitory Immunoglobulin (TBII) type of assay, which are used by many clinical laboratories, merely measures the ability of a serum or IgG preparation to block the binding of a TSH preparation and does not measure the biological response (Table 6). This fundamental difference in assay design explains why bioassays and receptor assays typically display a weak correlation (r = 0.31-0.65) (276,278).

(i) TRAb Methodology

The first report that that there was a thyroid stimulator that differed from TSH with respect to its longer half-life (Long Acting Thyroid Stimulator or LATS) was published in 1956 using an in vivo bioassay (279). LATS was later identified as an immunoglobulin. Like TSH, TRAbs stimulate both cAMP and the inositol phosphate pathways of the thyroid follicular cell, and thus both stimulates and blocks both thyroid hormone synthesis and the growth of the gland (276). The types of methods developed for TRAb measurements are classified relative to their functional activity, as shown in Table 6. Studies in mice and FRTL-5 cell lines as well as humans, show that a high concentration of human chorionic gonadotropin (hCG) is also a weak TRAb agonist and can stimulate cAMP, iodide transport, and cell growth (49). The marked hCG elevations secondary to choriocarcinoma can in rare cases cause a false positive TRAb result. However, the increase in hCG typically seen with normal pregnancy or in patients treated for a hydatiform mole are usually not high enough to elicit a false positive result.

(ii) Bioassays (TSAb, TBAb/TSBAb and TGI)

Most current bioassays are based on TSH receptor activation of second messenger (cAMP) production from a cell preparation (FRTL-5/ CHO TSH-R) exposed to a serum specimen or IgG preparation (280-282). The recent cloning of the TSH receptor has benefited bioassays by facilitating the development of TSH receptor transfected cell lines (283,284). Although these bioassays are available in several commercial laboratories in the United States and Asia, they are less available in Europe because of regulations that affect the use of genetically altered organisms. Unfortunately, the correlation between TRAb assay results and clinical presentation are still suboptimal. For example, the diagnostic sensitivity for Graves' disease using TRAb bioassays ranges from 62.5 to 81% (276). New approaches employing chimeric assays may be able to target the loci of TRAb epitopes and TSH binding sites and thus provide a better correlation between assay response and clinical outcome (274,277,285-287).

Table 6. TRAB Methods

Antibody	Function	Detection Method
TSAb	Stimulates cAMP production, iodine uptake, thyroglobulin	cell bioassay (FRTL-5/ CHO TSH-R) % stimulation of TSH-induced cAMP synthesis compared to normal pooled serum

TBAb (TSBAb)	Inhibits TSH induces cAMP production, iodine uptake, thyroglobulin synthesis	cell bioassay (same as above) % inhibition of TSH-induced cAMP compared to normal pooled serum
TGI	Stimulates thyroid cell growth	FRTL-5-cells, ^{3}H thymidine uptake/ mitotic arrest assay
TBII	Inhibits ^{125}I TSH binding to receptor	Receptor assay soluble porcine TSH-R or recombinant human TSH-R

TSAb: Thyroid stimulating antibodies
TBAb/TSBAb: TSH receptor blocking antibodies
TGI: Thyroid growth stimulating antibodies
TSH-R: TSH receptor
TBII: Thyroid binding inhibiting immunoglobulins

(iii) Receptor (TBII) Assays

Thyroid binding inhibiting immunoglobulin (TBII) assays are commercially available and are used by many clinical laboratories. These methods quantify the inhibition of the binding of ^{125}I-labeled TSH to either solubilized porcine receptors, or more recently, recombinant human TSH receptors (288-290). This type of method does not distinguish between stimulating and blocking TRAbs. TBII activity is typically quantified against a TRAb-positive serum calibrated against a reference calibrator serum. The most frequently used calibrator serum has been the MRC reference serum, LATS-B. A new World Health Organization (WHO) standard (MRC 90/672) has recently become available. The inherent heterogeneity of TRAb in patient serum and the source of receptors used (porcine versus recombinant human) are likely causes for the wide variability observed between TBII methods, despite the use of the same standard (276,291). Although TBII methods based on recombinant human TSH receptor are now available and may have a higher diagnostic sensitivity for Graves' disease, they do not appear to offer improved specificity or sensitivity for predicting response to anti-thyroid drug (ATD) therapy (290,292).

(iv) TRAb Reference Intervals

Despite the adoption of a new international reference preparation MRC 90/672, TRAb values are still method-dependent and reference intervals vary depending on the selection of the "normal" population used to determine the cut-off level for a positive result. This cut-off is generally defined as two standard deviations from the mean of normal subjects.

D.11 Guideline: TSH Receptor Antibody (TRAb) Tests used by Clinical Labs

Clinical laboratory TRAb assays are either:

- Receptor or TSH binding inhibition tests (TBII) do not measure stimulatory activity directly, but detect factors in the specimen that block the binding of labeled TSH to an in-vitro TSH receptor preparation. This type of test is commonly used in clinical laboratories.
- TSH receptor bioassays (TSAb) use cultured cells (FRTL-5 cells, or CHO cells transfected with human TSH receptor) to detect thyroid stimulating immunoglobulins (TSAb) that either stimulate cAMP or iodide uptake. These tests are not routinely available in all countries.
- In general, there is a poor correlation between TSAb and TBII results (60-75%). TSAb assays are claimed to be positive in 80-100% of untreated Graves' patients while TBII assays are positive in 70 to 90% of these patients. Neither test has adequate specificity or sensitivity for predicting remission from Graves'

hyperthyroidism.
- Normal hCG as well as abnormal hCG in choriocarcinoma are known to interact with the TSH receptor which could lead to false positive results. This might be observed in rare cases of choriocarcinoma but not in normal pregnancy or treated hydatiform mole in which the level of hCG is not high enough to cause a false positive.

(v) Clinical Uses of TRAb Measurement

The clinical use of TRAb measurements for the diagnosis and follow-up of AITD remains a matter of controversy and is geographically sensitive. The differential diagnosis of hyperthyroidism can be resolved in most patients without resorting to TRAb testing. Nevertheless, the presence of TRAb may distinguish Graves' disease from factitious thyrotoxicosis and other manifestations of hyperthyroidism such as subacute or post-partum thyroiditis and toxic nodular goiter. TRAb measurements have also been proposed as a means for predicting the course of Graves' disease. A declining TRAb level is often seen in hyperthyroid patients in clinical remission after treatment with antithyroid drugs (ATD). After ATD withdrawal, very high levels of TRAb correlate quite well with prompt relapse, but this situation involves very few patients. Conversely, a significant number of patients with a negative TRAb will relapse. A meta-analysis of the relationship between TRAb levels and the risk of relapse has shown that 25% of patients are misclassified by TRAb assays (258). This suggests that after ATD therapy, a follow-up of the patients is necessary whatever the TRAb level at the time of ATD withdrawal and that TRAb measurement is not cost effective for this purpose.

D.12 Guidelines: Clinical Uses of TRAb Measurement:

- To investigate the etiology of hyperthyroidism when the diagnosis is not clinically evident.
- TRAb measurement should be an option for all patients with Graves' disease both at the time of diagnosis and when the physician needs to correlate the TRAb values to a treatment protocol.
- To evaluate patients suspected of "euthyroid Graves' opthalmopathy". Although a negative TRAb measurement does not necessarily exclude this condition.
- For pregnant women with a past or present history of Graves' disease. Note: Pregnant women who are euthyroid after receiving prior antithyroid drug treatment for Graves' disease have a negligible risk for fetal or neonatal hyperthyroidism.
- Euthyroid pregnant women (+/- L-T4 treatment) who have had prior radioiodine treatment for Graves' disease should have TRAb measured both in early pregnancy, when an elevated value is a risk factor for fetal hyperthyroidism (2-10%), and during the third trimester of pregnancy to evaluate the risk of neonatal hyperthyroidism.
- Pregnant women who are receiving antithyroid drugs (ATD) for Graves' disease should have TRAb measured in the third trimester of pregnancy. A high TBII value should prompt a clinical and biochemical evaluation of the neonate for hyperthyroidism, both at birth (cord blood) and at 4 – 7 days after the effects of transplacental passage of ATD have disappeared.
- Although TSAb assays have a theoretical advantage, some believe that TBII tests, which detect both stimulating (TSAb) and rare case, of blocking (TBAb/TSBAb) antibodies, can be equally useful.
- The assessment of the risk of fetal and neonatal thyroid dysfunction necessitates the detection of either blocking or stimulating TRAb when mothers have no intact thyroid following previous therapy for Graves' hyperthyroidism.
- To identify neonates with transient hypothyroidism due to TSH receptor blocking antibodies.

There is general agreement that TRAb measurements can be used to predict fetal and/or neonatal thyroid dysfunction in pregnant women with a previous or present history of AITD (9,246). High levels of TRAb in the mother during the third trimester of pregnancy suggest a risk of thyroid dysfunction in the offspring (9,275). Two to 10% of pregnant women with hight levels of TRAb deliver newborns with hyperthyroidism (9). The risk for neonatal hyperthyroidism is negligible following successful treatment of hyperthyroidism with antithyroid drugs, but can develop after radioiodine treatment if TRAb levels remain elevated (9). Euthyroid pregnant women (+/- L-T4 treatment) who have had prior radioiodine therapy for Graves' disease should have TRAb measured both in early pregnancy, when an elevated value is a significant risk factor for fetal hyperthyroidism, and during the third trimester, to evaluate for the risk of neonatal hyperthyroidism (9). Pregnant women who take antithyroid drugs

(ATD) for Graves' disease should have TRAb measured in the third trimester. High TRAb levels in such patients should prompt a thorough clinical and biochemical evaluation of the neonate for hyperthyroidism, both at birth (cord blood) and at 4 – 7 days, after the effects of the transplacental passage of ATD have disappeared (293). It is worth noting that the TBII receptor assays are often used for this purpose since they detect both stimulating (TSAb) and in rare cases, blocking antibodies (TBAb/TSBAb) which cause transient hypothyroidism in 1:180,000 of newborns (294). It is also advisable to test for both stimulating and inhibiting antibodies because the expression of thyroid dysfunction may be different in the mother and the infant (247).

D.13 Recommendations for Improvements to Thyroid Antibody Tests

- Current thyroid autoantibody assays should be submitted to a comparative study of their analytical and clinical performance.
- A comparative study of the antigen preparations currently in use would allow the identification of the most suitable method(s) for clinical thyroid autoantibody testing.
- The characteristics of the antigen preparations used in the test should be stated for all thyroid autoantibody assays.
- Reference preparations of antigens should be made available.

The role of TRAb in thyroid associated opthalmopathy (TAO) is uncertain (295). TAO appears to be exacerbated by radioiodine therapy (296). Furthermore, TRAb and other thyroid antibody levels increase significantly after radioiodine therapy (297-299). This suggests that TRAb measurements prior to radioiodine therapy may be useful to predict the risk of TAO but as yet there are no prospective studies to document this fact.

D.14 Guidelines for Manufacturers Developing Thyroid Antibody Tests

- Absolute or "gold standard" methods remain a target for the future.
- Methods used to produce the antigen reagents, together with differences in assay design and the experimental conditions in which they are performed, affect the antigen-antibody interactions that are used to produce the quantified result. Such information should be included in the kit package insert.
- The specificity of the secondary standards should be selected relative to the interaction between the autoantibodies present in patient sera and their specific antigens.
- Immunometric assays for TPOAb and TgAb should be checked for hook problems before release by measuring a minimum of 20 specimens with antibody concentrations above 1,000 µg/L kIU/L (IU/ml) and a minimum of 20 specimens with values above 10,000 µg/L kIU/L (IU/ml).
- TgAb methods should be checked for interferences by high antigen (Tg) concentrations by assessing the effect of spiking TgAb-positive sera with serum Tg > 10,000 µg/L (ng/ml) and >100,000 µg/L (ng/ml).
-

(h) Future Directions

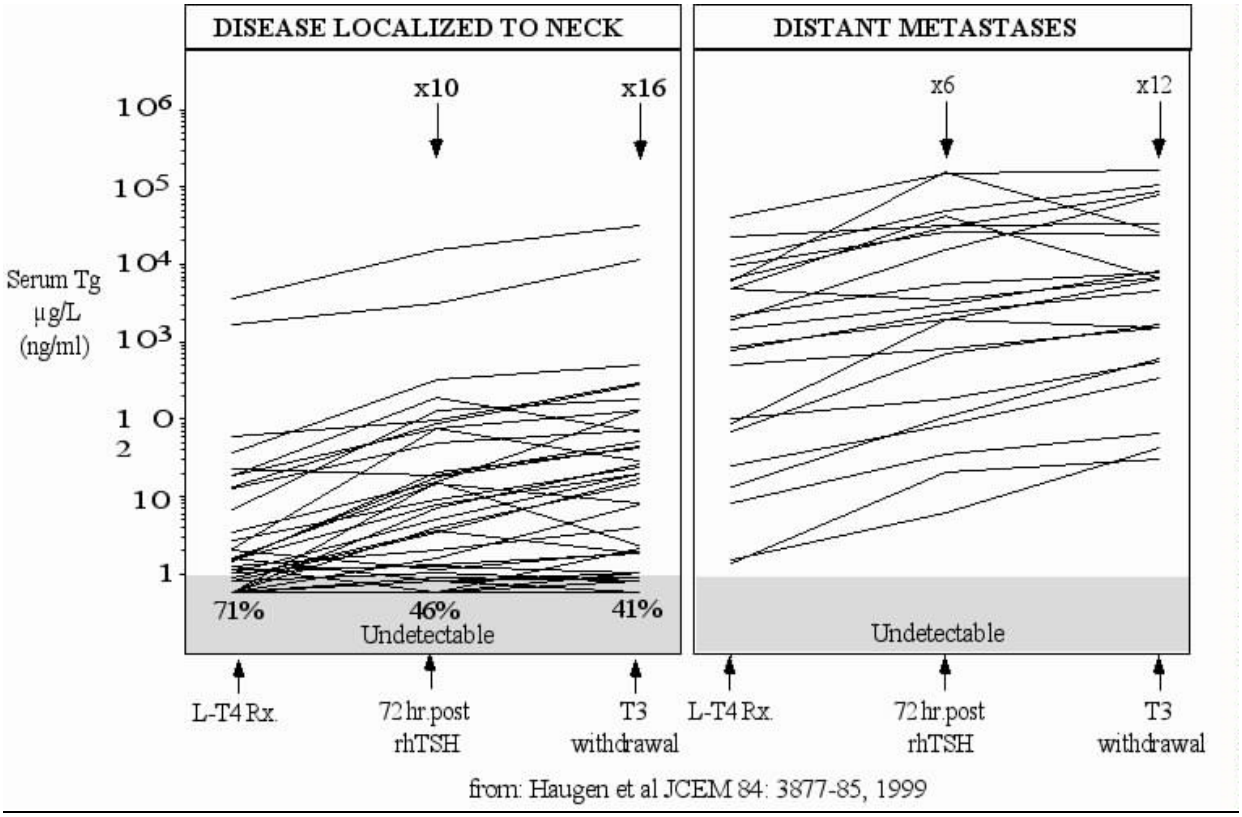
It is important that a well-structured comparative study of the commercially available thyroid autoantibody assays be performed. This would provide irrefutable evidence that differences exist in the performance of current assay methods (289). It would also help to convince clinical laboratory scientists to avoid using assays that have poor clinical performance and encourage manufacturers to improve their products or drop them from the market.

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 concentration integrates three major factors: (1) the mass of differentiated thyroid tissue present; (2) any inflammation or injury to thyroid tissue which causes the release of Tg; and (3) 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. Patients with preoperative serum Tg levels within the normal range may have tumors that do not secrete abnormal amounts of Tg. Changes in serum Tg post-operatively represent changes in tumor mass, provided that a constant TSH level is maintained with L-T4 therapy.

Figure 8. Serum Tg Responses to rhTSH & T3 Withdrawal



A serum Tg measured during TSH stimulation [endogenous TSH or recombinant human TSH (rhTSH)] is more sensitive for detecting DTC than a basal Tg measurement made during L-T4 treatment (Figure 8) (300). 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 (301). Poorly differentiated tumors that do not concentrate iodine may display a blunted response to TSH stimulation (302).

(a) 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 (303). 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 offers 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 (199). 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 methods are also compromised by differences in standardardization and specificity and generally show poor sensitivity, sub-optimal between-run precision and high dose "hook" effects (199).

(i) Standardization

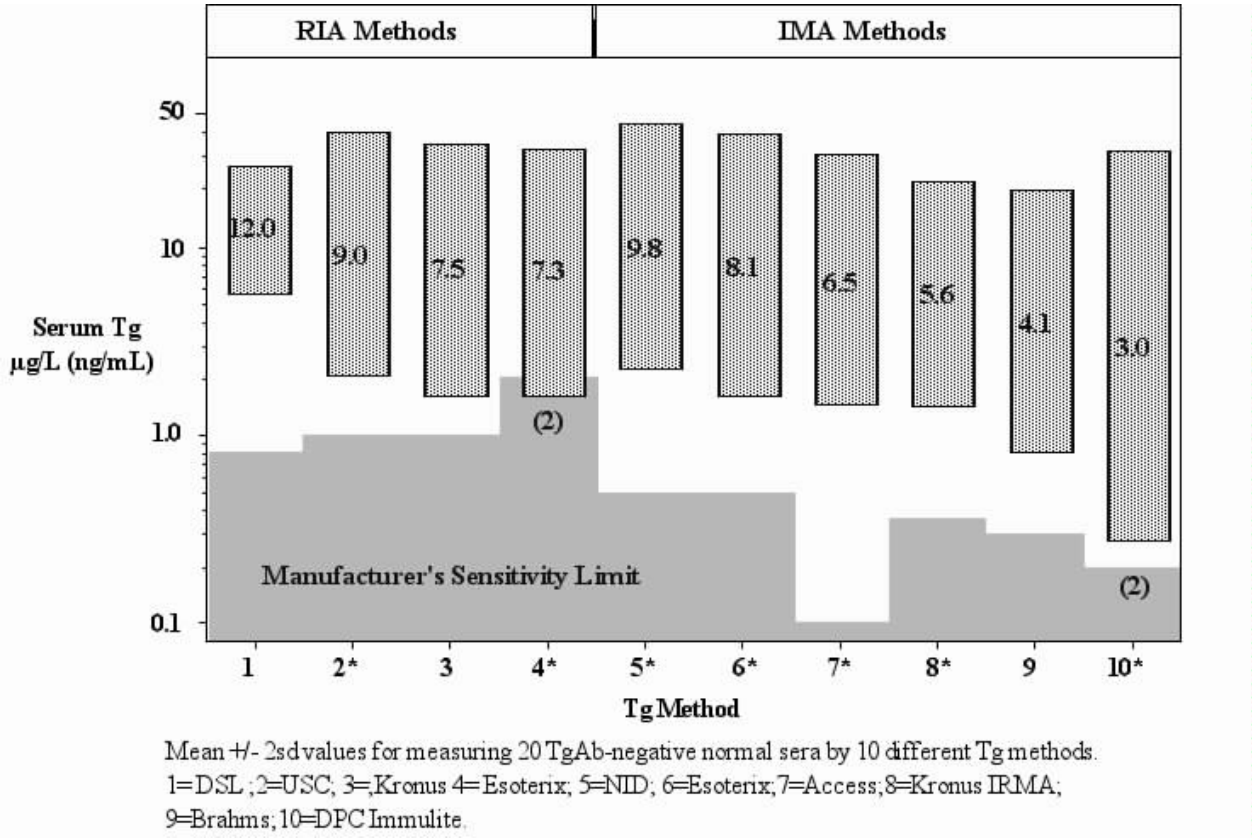
Serum Tg concentrations measured by either RIA or IMA methods, vary widely (199,304). A recent collaborative effort sponsored by the Community Bureau of Reference of the Commission of the European Communities has developed a new international Tg reference preparation, CRM-457 (291,305). This material can be obtained from Dr. Christos Profilis, BCR, Rue de la Loi 200, B 1049 Brussels, Belgium.

E.1 Guideline 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 the Tg method to allow re-baselining of DTC patients.

Figure 9. Different Tg Methods used for Measuring TgAb-Negative Normal Sera



Mean +/- 2sd values for measuring 20 TgAb-negative normal sera by 10 different Tg methods.
1=DSL ;2=USC; 3=;Kronus 4=Esoterix; 5=NID; 6=Esoterix;7=Access;8=Kronus IRMA;
9=Brahms;10=DPC Immulite.

The widespread adoption of this standard was projected to reduce, but not eliminate the significant method-to-method variability that exists with this method. It was hoped that standardization worldwide 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 appreciably decreased between-method variability as much as expected. Currently, serum Tg values measured by methods that use CRM-457 standards can differ by three-fold (Figure 9). These method-to-method differences which are greater than the goal for maximum imprecision needed for monitoring an individual (Table 5) precludes the use of different Tg methods for monitoring thyroid cancer patients.

(ii) Sensitivity

Some Tg methods are too insensitive to detect the lower euthyroid reference interval (~ 3.0 μg/L (ng/mL) when iodine intake is adequate). Such methods have suboptimal sensitivity for monitoring DTC patients for recurrence. As with TSH, the functional sensitivity (20% CV low range between-run precision) determines the minimum assay detection limit [Section 3C(b)]. The protocol used to determine Tg assay functional sensitivity is the same as described for TSH (Guideline C.3) with the three stipulations (Guideline E.3).

E.2 Guidelines 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.

(a) *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.
(b) *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 the laboratory report:

- **FOR IMA METHODS*:***

IMA methods may give inappropriately lower or underestimated serum Tg concentrations 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 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 in determining the presence of residual thyroid tissue or tumor.

(iii) 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 E.3).

The within-run precision for immunoassay methods is as expected better than between-run precision. This is because measurements made within a single run are not subject to the variability introduced by using different batches of reagents and different instrument calibrations. The longer the interval between runs the greater the variability and the worse the between-run precision. Low-range precision estimates that are typically used to determine functional sensitivity (Guidelines C.3 and E.3) may be unrealistic if a non-human matrix is used instead of TgAb-free serum. It is important to establish functional sensitivity and the between-run precision of the method from data spanning a 6 to 12 month period, since this is a typical clinical interval used for monitoring DTC patients. Performance criteria established using this time-span can be used to validate the

reliability of the method for long-term monitoring of individual patients, in whom the suggested goal for maximum imprecision is <5% (Table 5). In contrast, within-run precision may be the more relevant parameter when assessing the serum Tg response to rhTSH stimulation (300). In this setting, a basal and rhTSH-stimulated specimen are drawn 3 to 5 days apart and usually measured in the same run (Figure 8) (300,301). Between-run imprecision for an individual patient can be overcome by measuring the archived, stored sample in the same run as the current specimen (11).

E.3 Recommended Protocol for Functional Sensitivity & Between –Run Precision

Functional sensitivity and between-run precision should be established using the same protocol as for TSH (Guideline C.3) 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 for functional sensitivity determination) should have a target serum Tg value that is 30 to 50 % higher than the expected functional sensitivity limit (FS).
  [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 limit suggested by manufacturer.
- The test period used to assess between-run precision should be at least 6 months. This is representative of the clinical interval used for assessing DTC patients and thus more clinically relevant than the 6-8 week interval recommended for TSH (Guideline C.3).

E.4 Guidelines for Manufacturers and Laboratories

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

- Assays should be standardized against the CRM-457 reference preparation. Assays not standardized against CRM-457 should provide a correction factor.
- The reference range for TgAb-negative normal euthyroid subjects should be cited. Note: All normal subjects have detectable serum Tg concentrations when appropriately sensitive Tg assays are used.
- The matrix used to dilute the standards should be checked for bias (Guideline E.1).
- Functional sensitivity and within and between-run precisions should be established using Guideline E.3.
- TgAb interference should be assessed by checking for RIA:IMA discordance in TgAb-positive sera [TgAb levels 100 to >1000 kIU/L (IU/ml)].
- Exogenous Tg recovery studies should not be used to detect TgAb interference (Guideline E.6).
- Do not report serum Tg values for TgAb-positive specimens unless the method can be shown to give appropriate values for TgAb-positive DTC patients with disease.

(iv) 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 metastatic disease (306). 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)) (199). Manufacturers of IMA methods try 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.
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.

E.5 Guidelines for Laboratories and Manufacturers: 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 a discrepancy in the two results.
- All assays (two-step or one-step) should be checked for a hook effect before manufacturer release. A check for a hook effect should be made by measuring serial 10-fold dilutions on a minimum of 20 different TgAb-negative specimens with serum Tg concentrations above 10,000 µg/L (ng/ml) and a minimum of 20 different TgAb-negative specimens with serum Tg values above 100,000 µg/L. A sufficient number of dilutions should be made until parallelism is demonstrated.

(v) Thyroglobulin Autoantibody (TgAb) Interference

Thyroglobulin autoantibodies (TgAb) are detected in a higher percentage of DTC patients than the general population (~20 versus ~10 %, respectively) (268). Serial serum TgAb measurements may be an independent prognostic indicator of the efficacy of treatment or the recurrence of DTC in TgAb-positive patients (268-270,307). Any TgAb present in a serum specimen has the potential to interfere with any Tg method (308). Because TgAb is heterogeneous, neither the measured TgAb concentration nor an exogenous Tg recovery test can be used to reliably predict whether the TgAb in a specimen will cause interference (268,309,310).

E.6 TgAb Interference and Recovery Tests

- Serum Tg recovery tests do not reliably detect TgAb and should be discouraged. Previous studies have shown that low recoveries sometime 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).

Non-competitive immunometric assay (IMA) methods appear to be more prone to TgAb interference than RIA methods, as determined by the finding of undetectable Tg values in normal euthyroid subjects with an isolated TgAb abnormality. It appears that IMAs fail to quantify the Tg complexed with TgAb in some specimens, and this can result in an underestimation of the specimen's total Tg concentration. In contrast, RIA methods which seem able to quantify both the free and TgAb-bound Tg in the specimen, typically produce higher values than IMA methods, when TgAb is present in a specimen (268,301). Since even low levels of TgAb have the potential to interfere with a Tg measurement, it is essential to perform a sensitive TgAb immunoassay measurement on every specimen sent for Tg analysis. The TgAb test should be made in the laboratory performing the Tg testing because of the variability in sensitivity and specificity of current TgAb tests.

E.7 Recommendation for Laboratories: Importance of Quantitative TgAb Testing

The TgAb concentration should be measured in <u>ALL patient</u> serum prior to Tg analysis:

- Since the sensitivity and specificity of TgAb methods varies so widely, specimens <u>should not be pre-screened for TgAb by the originating laboratory</u> but should be measured by the laboratory performing the Tg analysis unless the originating laboratory performs both tests.
- Serum Tg recovery tests do not reliably detect TgAb and should not be used (Guideline E.6).

- Even low levels of TgAb can interfere with a serum Tg measurement and cause either falsely low or even undetectable values when using IMA methods. The presence of TgAb may produce an overestimated serum Tg value when using some competitive immunoassay (RIA) methods.
- Serial serum TgAb concentrations provide useful prognostic information on changes in tumor growth.

Many consider that the underestimation of serum Tg typical of IMA measurements in TgAb-positive sera is the most serious type of interference encountered, since an underestimation of a serum Tg value has the potential to mask metastatic disease. When serum containing TgAb are measured by both an RIA and IMA method, an RIA:IMA discordance (Tg ≥2 μg/L (ng/mL) by RIA: Tg undetectable by IMA) is frequently observed. This discordance appears to be a characteristic of TgAb interference with one or both types of methods. Since the current rhTSH-stimulated threshold for a positive Tg response is 2 μg/L (ng/mL), this magnitude of method-related discordance has the potential to influence a physician's clinical decision- (300). Some RIA methods claim to produce clinically valid serum Tg results for TgAb-positive patients (268,311). However, RIA methods may either over- or underestimate serum Tg concentrations, which is also problematic, since this may prompt over-treatment and cause other complications. (267). The influence of TgAb on different RIA methods is usually quite variable and relates to the assay design, the specificity of the Tg polyclonal antibody reagent and the quality of the ^{125}I-Tg tracer. In the United States, there is currently a trend for laboratories to restrict the use of IMA methods to TgAb-negative patients while retaining older RIA methods for TgAb-positive patients.

(b) Tg mRNA Testing

In the future, thyroglobulin messenger RNA (Tg mRNA) testing may be used to facilitate the therapeutic decision-making for DTC, especially when patients have detectable TgAb in their blood. 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 (312-314). 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 (315). 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 (316).

A number of groups have now developed quantitative RT-PCR methods to detect Tg mRNA transcripts in blood (317-319). These studies generally find detectable Tg mRNA in all normal subjects but with a poor correlation with serum Tg as measured by immunoasay (317,318). 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 (317,319). 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 (314). Further studies are needed to determine whether the detectable Tg mRNA levels reported for athyreotic patients without known metastases reflect clinically occult disease or an assay artifact.

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 Tg mRNA measurements will be reserved for high-risk patients where serum Tg has been shown to be diagnostically unreliable. Tg mRNA determinations would be particularly useful for evaluating TgAb-positive patients with rising TgAb concentrations.

(c) Serum Tg Reference Values

(i) Normal Euthyroid Subjects

Serum Tg concentrations are log-normally distributed in normal euthyroid individuals. Values tend to be

slightly higher in women, but gender-related reference ranges are unnecessary (320). Cigarette smoking is a factor associated with goiter and higher serum Tg values (321). Tg reference ranges are geographically sensitive, since serum Tg is influenced by iodine availability and intake (322). Subject selection for the normal cohort for Tg reference range evaluation should have the following exclusion criteria:

- Goiter
- 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
- Cigarette smoking

E.8 Guidelines for Establishing the Reference Interval for a Tg Method

(c) Laboratories should establish their Tg normal reference interval independent of the manufacturer. The serum Tg reference range should be obtained 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 serum thyroid autoantibodies (TgAb and/or TPOAb).

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

(ii) Serum Tg Values after Surgery

The Tg reference interval cited on laboratory reports does not apply to patients who have had recent thyroid surgery! In the first few weeks after surgery, the serum Tg concentration will relate to the completeness of the surgery, the leakage of Tg from the surgical margins and most importantly whether thyroid hormone has been given to prevent a 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 patients are receive thyroid hormone to prevent TSH from rising (Feldt Rasmussen). 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. 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 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 8, because TSH usually stimulates serum Tg more than five-fold, a TSH-stimulated serum Tg is more sensitive for detecting disease confined to the neck, than serum Tg levels measured during TSH suppression (300,301). 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 (302).

(d) Clinical Uses of Serum Tg Measurement

The serum Tg concentration reflects thyroid mass, thyroid injury and TSH receptor stimulation (323). It follows that an elevated serum Tg is a non-specific finding associated with virtually any thyroid pathology.

(i) Non-Neoplastic Conditions

Serum Tg is elevated in patients with goiter and 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 hypothyrodism (324,325).

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) (326). One recent study proposed the use of serum Tg measurement as a parameter to reflect the iodine status in a given population (322).

E.9 Guidelines: 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 (Guideline 2.5).

(ii) 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) (323). 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.

• 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. 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 >3 weeks following FNA.)

E.10 Guidelines: Clinical Utility of Serum Tg Measurements in DTC 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 guideline: 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.
- The magnitude of serum Tg stimulation in response to rhTSH is approximately half that seen following thyroid hormone withdrawal (~8 versus ~16-fold stimulation above basal, rhTSH vs. withdrawal, respectively).

• Serum Tg 1-2 months after Thyroid Surgery

Following thyroid surgery, serum Tg concentrations fall rapidly with a half-life of ~3-4 days. 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 whose serum TSH is maintained below 0.1 mU/L.

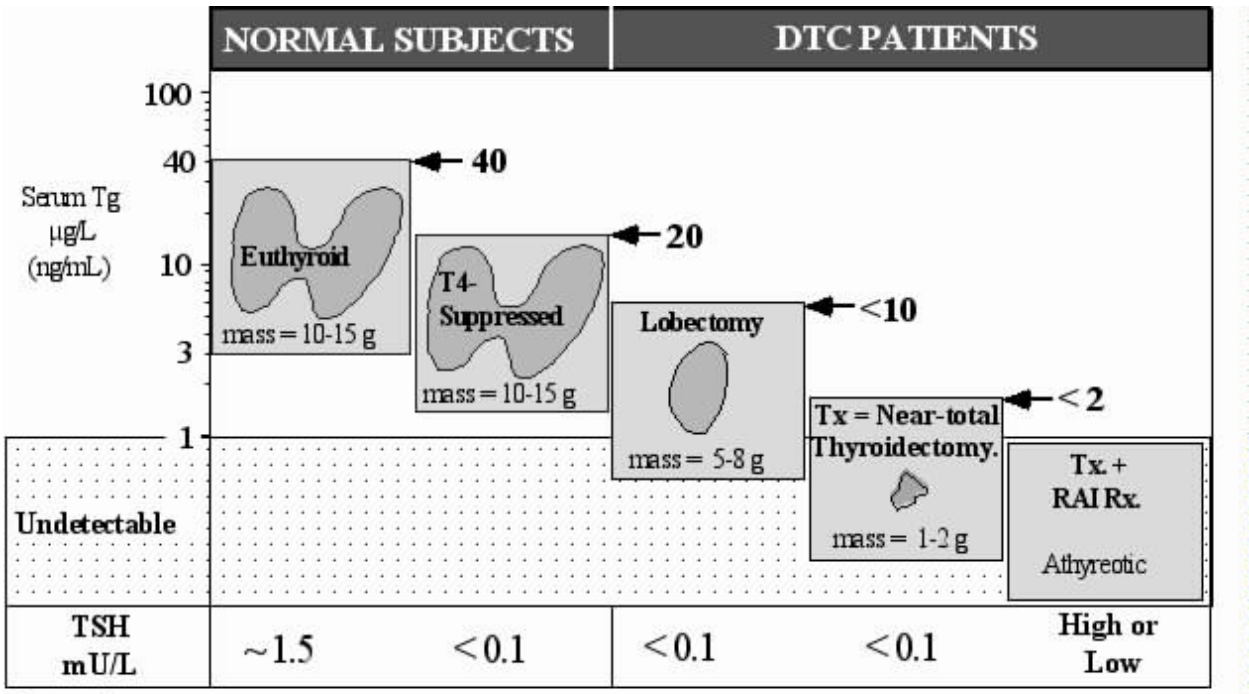
• Long-term Monitoring during L-T4 Suppression Rx.

When TSH 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 have 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 (323). 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 9.

• 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 (300,301). Typically, TSH stimulation of normal thyroid remnants or a well-differentiated tumor produces on average a 10-fold increase in serum Tg above basal (TSH-suppressed) levels, in TgAb-negative patients (Figure 8). The serum Tg response to an endogenous TSH rise is typically greater than for rhTSH (300). Moreover, poorly differentiated tumors, display a blunted (< 3-fold) increase in serum Tg in response to TSH stimulation (302). 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.

Figure 10. Serum Tg Responses to Changes in Thyroid Mass and TSH Status



Assumptions:

- The Tg assay normal reference range = 3-40 µg/L (ng/mL)
- The mass of normal thyroid tissue =10-15 grams
- One gram of normal thyroid tissue secretes ~1µg/L (ng/mL) Tg into the circulation @normal TSH
- One gram of normal thyroid tissue secretes ~0.5µg/L (ng/mL) Tg when TSH is < 0.1 mU/L