Evolution of TSH Assays: A Third Generation Viewpoint

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First-, second- and third-generation assays are defined in terms of functional sensitivity, i.e. the lowest TSH concentration at which the interassay CV is less than 20%.[1] For each subsequent generation of TSH assays, the functional sensitivity limit shifts to lower concentrations by one order of magnitude (see Table 1).

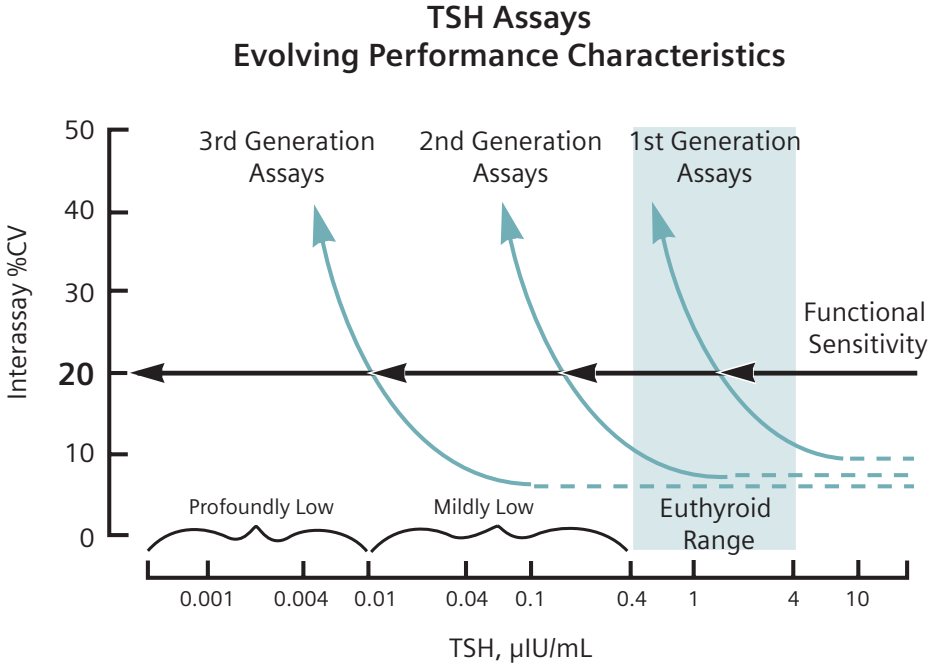
Table 1. Functional sensitivities for three generations of TSH assays.

Generation	Functional Sensitivity*
First	1 – 2 µIU/mL
Second	0.1 – 0.2 µIU/mL
Third	0.01 – 0.02 µIU/mL

*<20% interassay precision within these ranges.

Precision profiles for three generations of TSH assays are depicted in Figure 1. The functional sensitivity limit of first-generation assays (1 to 2 µIU/mL) occurs at approximately the middle of the euthyroid range for TSH concentrations. Clearly, these assays cannot distinguish between normal and suppressed TSH levels. In contrast, second-generation assays allow quantitation of TSH in the low normal and subnormal ranges, down to 0.1 µIU/mL; and third-generation assays extend the range another tenfold, down to 0.01 µIU/mL. In addition, third-generation assays have far superior precision in the subnormal TSH range (0.1 to 0.4 µIU/mL) compared to second-generation assays.

Figure 1. Interassay precision profiles for three generations of TSH assays. (Adapted from Spencer, et al.[1])



The functional range of third-generation TSH assays spans more than four orders of magnitude of TSH concentrations, from 0.01 to approximately 100 µIU/mL. Accordingly, they can distinguish between the profoundly low basal TSH levels of thyrotoxicosis (below 0.01 µIU/mL) and mildly subnormal values (0.01 to 0.4 µIU/mL), while also providing precise and accurate TSH results throughout the euthyroid (0.4 to 4 µIU/mL) and hypothyroid (over 4 µIU/mL) ranges. This broad functional range and the markedly improved precision constitute the most relevant distinguishing features of the third-generation TSH assays.

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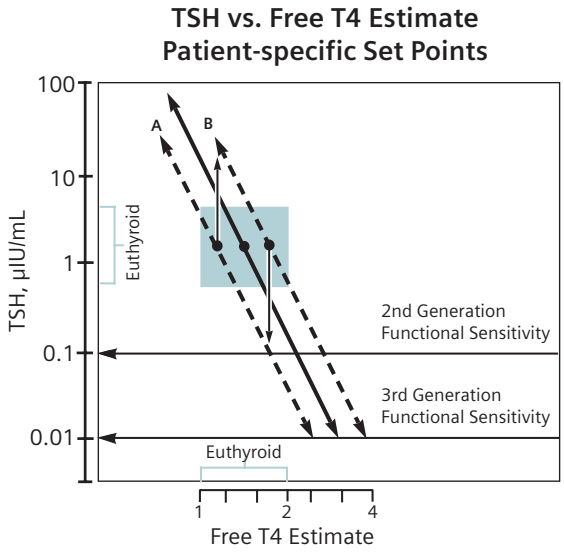
Thyroid hormone homeostasis is regulated by a feedback inhibition mechanism which operates along the hypothalamic-pituitary axis. The hypothalamus secretes thyrotropin-releasing hormone (TRH, protirelin) which stimulates the pituitary to secrete TSH. TSH, in turn, stimulates the thyroid gland to produce and secrete thyroid hormones (T4 and T3) into the circulation. According to a now familiar negative feedback mechanism, once adequate levels of T4 and T3 have been achieved, further production of TSH is suppressed. There is, therefore, an inverse relationship between TSH and thyroid hormone levels. Since it is the free hormones which exert physiological activity, this inverse relationship actually holds between TSH and the free thyroid hormones.

TSH and Thyrometabolic Status

The TSH/T4 interaction was quantified in an early study of primary hypothyroid patients who were given gradually increasing doses of T4.[3] Plotting matched TSH and total T4 results for each patient revealed a log-linear TSH/T4 relationship in which a twofold change in the T4 concentration caused a 100-fold change in TSH concentration.

A study employed results of a third-generation TSH assay and free T4 estimates. A plot of matched TSH/free T4 results for ambulatory patients (with and without thyroid disease, but with intact hypothalamic-pituitary function) yielded a log-linear relationship similar to that reported earlier.[4] Furthermore, the characteristic log-linear relationship was found to be continuous down to 0.01 µIU/mL.

Figure 2. Log-linear relationship between TSH and free T4 estimate.[3,4]



The central line in Figure 2 depicts this log-linear relationship in an average subject and demonstrates the extreme sensitivity of TSH to slight changes in free T4 concentrations. This TSH/free T4 interaction explains why TSH is the most sensitive marker for detecting subclinical thyroid disease—much better than estimates of the thyroid hormones themselves. In developing hypothyroidism, TSH becomes abnormally elevated well before either free T4 or free T3 descends below the euthyroid range. Similarly, in developing Graves' hyperthyroidism, TSH decreases to abnormally low levels well before either free T4 or free T3 ascends above the euthyroid range. TSH thus serves as an exquisitely sensitive probe of thyrometabolic status.[1]

Uses of Sensitive TSH Assays

- Ruling out thyroid dysfunction in patients with an intact hypothalamic-pituitary axis
- Monitoring and normalizing TSH levels
- Replacing (most) TRH-stimulated TSH testing
- Optimizing suppressive therapy

Individual Specific TSH/ Free T4 Set Points

Another important characteristic of the TSH/free T4 interaction is reflected in the log-linear lines for patients A and B in Figure 2. Note that these lines are displaced from each other and from the line for the average subject. The three lines traverse the area delineated by euthyroid reference ranges for TSH and free T4 at different points along the free T4 axis. Individual specific set points are located on the segment of the lines contained within the euthyroid area. For example, in patient A, a low normal free T4 value is required for complete normalization of TSH. This free T4 value would, however, be inadequate in patient B, whose TSH level would remain abnormally elevated at this free T4 concentration. Similarly, the ideal free T4 concentration for patient B would be excessive for patient A, and would result in an abnormally low TSH level in this patient.

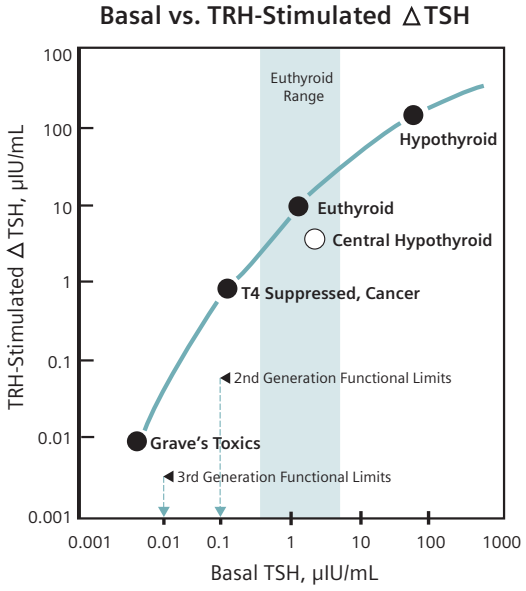
If the objective of a treatment regimen is to maintain the patient in an optimally euthyroid state, this would be best achieved by normalizing not just thyroid hormone levels but TSH levels as well, in terms of individual specific set points. Normalizing TSH is expected to decrease possible cardiac, hepatic and bone effects resulting from iatrogenic subclinical hyperthyroidism associated with prolonged overreplacement of thyroid hormones. The sensitive TSH assays (second- and third-generation) are effective tools for guiding clinicians toward realizing this increasingly — but not yet universally — accepted treatment objective.

A potentially confounding characteristic of the TSH/free T4 interaction must be considered. A minimum of 6 weeks is required to achieve steady-state TSH levels after changes induced by a thyroid hormone replacement regimen. TSH levels obtained before achieving steady state may be clinically misleading, and thyroid hormone levels are, therefore, a more reliable indicator of thyroid status during this transition period.

Subnormal and Suppressed TSH

The historic "gold standard" for determining the degree to which a patient may be suppressed — either as a result of hyperthyroidism or because of suppressive therapy — has been the TRH-stimulation test. Typically, the test involves determining TSH basally and again 15 to 30 minutes after an intravenous bolus of TRH. The relationship between basal and TRH-stimulated TSH values has been studied with second-generation assays;[5] more recently with a third-generation; and, for TSH values below 0.01 µIU/mL, with a fourth-generation assay.[2] This latter study included a large cohort of ambulatory subjects representing a variety of thyroid conditions. The mean values for basal TSH plotted against matching TRH-stimulated TSH values observed for hyperthyroid (Graves' toxicosis), T4-suppressed thyroid cancer, euthyroid and hypothyroid patients are depicted in Figure 3. The TRH/TSH interaction is defined by the curve connecting these population means; this curve represents a continuum of values from which the means were calculated. At the profoundly low basal TSH levels (below 0.01 µIU/mL) observed for the Graves' toxics, the corresponding stimulated TSH values are also extremely low (mean: 0.007 µIU/mL), with 89% of these patients having no detectable response (detection limit: 0.002 µIU/mL). Then, as basal TSH levels increase, so does the corresponding TRH-stimulated response over the whole expanse of basal TSH concentrations, from 0.01 to over 100 µIU/mL.

Figure 3. Relationship between basal and TRH-stimulated TSH observed in 500 ambulatory patients with an intact hypothalamic-pituitary axis (310 euthyroid, 106 T4-suppressed thyroid cancer, 17 Graves' toxicosis, 61 primary hypothyroid) and 6 patients with untreated central (secondary/ tertiary) hypothyroidism. (Based on data published by Spencer et al.[2])



The proportionality between basal and stimulated TSH confirms previous conclusions that a subject's thyrometabolic status can be determined directly from basal TSH values alone. Furthermore, this study demonstrates that the TRH/TSH interaction is a continuum which extends down to the profoundly low basal TSH values associated with frank hyperthyroidism. Therefore, basal TSH provides essentially the same information as TRH-stimulated TSH throughout the whole range of basal TSH concentrations accessible with third-generation TSH assays. Appropriately sensitive TSH assays can thus circumvent the historic reliance on TRH-stimulation testing in subjects with intact hypothalamic-pituitary axes for assessing degrees of suppression.

Patients with central hypothyroidism, i.e. hypothalamic and/or pituitary disorders, are an exception. The reduction in physiological but not immunological potency of circulating TSH found in many of these patients can confound interpretation of TSH results. Such patients may exhibit inappropriately normal TSH levels in conjunction with low free T4 values.7 TRH-stimulated TSH values in these patients tend to be significantly blunted, and stimulation testing may, therefore, continue to be a useful adjunct for the differential diagnosis of central hypothyroidism.1

Third-Generation TSH Assay for Optimizing Suppressive Therapy

The desired degree of suppression is determined by the specifics of both disease and patient. In general, patients with differentiated thyroid cancer are treated aggressively, due to the risk of metastases. Suppression is pushed to the limit of clinical tolerance, as close as possible to the degree of suppression associated with hyperthyroidism, i.e. to TSH values below 0.01 µIU/mL. However, for surgically cured cancer patients and patients with benign nodular or diffuse goiter, the treatment regimen is far less aggressive: TSH levels may be maintained between 0.01 and 0.4 µIU/mL depending on the disease, the patient and the judgment of the attending clinician. Therapy should be carefully balanced to achieve the desired degree of suppression while avoiding the potentially deleterious effects of iatrogenic hyperthyroidism on cardiac and liver function, as well as accelerated osteoporosis. In this context, third-generation TSH results provide accurate and precise guidelines for optimizing suppressive therapy.

Utility of TSH Determinations among Hospitalized Patients

Severe nonthyroidal illness (NTI) and acute drug therapy, such as with dopamine or glucocorticoids, may cause suppression of TSH to values below 0.1 µIU/mL.[8,9] Not surprisingly, therefore, thyroid disease was confirmed in only 24% of a hospitalized patient population with TSH results below the functional limit (0.1 µIU/mL) of second-generation TSH assays. The high incidence of misleading results rendered the second-generation TSH assay virtually useless when applied to this population. However, as stated earlier, frank hyperthyroidism is associated with TSH values below 0.01 µIU/mL,[2] and this suggests that the specificity of TSH determinations could be improved by using a third-generation assay with a limit of 0.01 µIU/mL for the diagnosis of hyperthyroidism among hospitalized patients.

This approach was evaluated using a group of 91 hospitalized patients (54 with hyperthyroidism, and 37 with NTI).[9] The results of this evaluation are summarized in Table 2. Note that all 54 hyperthyroid patients had TSH values below 0.1 µIU/mL; hence a second-generation assay might even have had 100% sensitivity for identifying hyperthyroid patients. However, 18 out of the 37 NTI patients also had TSH values below 0.1 µIU/mL; hence the results, if classified according to a second-generation cutoff of 0.1 µIU/mL, would have reflected a specificity of only 49%. This high incidence of results falling below the normal range, in the absence of thyroid disease, is excessive. In contrast, third-generation results, evaluated in terms of the limit of 0.01 µIU/mL, would have had a very respectable specificity of 86% while maintaining a sensitivity of 96%. (Two hyper-thyroid patients with TSH results between 0.01 and 0.02 µIU/mL caused the sensitivity to fall short of 100%.) Therefore, the most effective laboratory test for the differential diagnosis of hyperthyroidism among hospitalized patients is a third-generation TSH assay with a 0.01 µIU/mL or better functional limit; TSH values below this limit strongly suggest hyperthyroidism.

Table 2. Sensitivity and specificity for hyperthyroidism: second- versus third-generation TSH assays. (Based on data cited by Spencer, et al.[9])

	3rd Gen. TSH (0.01 µIU/mL cutoff)	2nd Gen. TSH (0.1 µIU/mL cutoff)
Sensitivity	96%	100%
Specificity	86%	49%

Conclusion

Clinical Applications: Second- or Third-Generation TSH Assays

Sensitive TSH assays (either second- or third-generation) can be used effectively to exclude or diagnose suspected thyroid disease in ambulatory patients with an intact hypothalamic-pituitary axis. A normal TSH result virtually excludes thyroid hormone excess or deficiency and does not require follow-up with additional tests. An elevated TSH result provides very strong evidence for hypo-thyroidism and a low TSH result provides strong evidence for hyperthyroidism. The American Thyroid Association has recommended using both a sensitive TSH assay and a free thyroxine estimate in a complementary manner to confirm thyroid abnormalities.

A second- or third-generation TSH assay may also be used to monitor the effect of replacement therapy, particularly when the objective of such therapy is to normalize TSH.

Clinical Applications Requiring a Third-Generation TSH Assay

Distinguishing between mildly subnormal TSH values (ranging from 0.01 to 0.4 µIU/mL) and profoundly low TSH values (below 0.01 µIU/mL) requires a third-generation TSH assay. Such an assay allows subclassification of hyperthyroid patients according to the degree of suppression and optimization of suppressive therapy without having to rely on TRH-stimulation testing. Diagnosing hyperthyroidism in hospitalized patients also requires an assay with a functional limit of at least 0.01 µIU/mL to differentiate effectively between hyperthyroidism and nonthyroidal illnesses.

System	ADVIA Centaur® CP	ADVIA Centaur® XP	IMMULITE® 1000/ 2000 XPi/2500	Dimension® RxL Max™/Dimension® Xpand® Plus	Dimension Vista™	Dimension EXL™ with LM
TSH	✓	✓	✓	✓		
Third Generation TSH	✓	✓	✓		✓	✓

References:

1. Spencer CA. Thyroid profiling for the 1990s: free T4 estimate or sensitive TSH measurement. J Clin Immunoassay 1989;12:82-9.
2. Spencer CA, Schwarzbein D, Guttler RB, LoPresti JS, Nicoloff JT. Thyrotropin (TSH)-releasing hormone stimulation test responses employing third and fourth generation TSH assays. J Clin Endocrinol Metab 1993;76:494-8.
3. Wehmann RE, Nisula BC. Radioimmunoassay of human thyrotropin: analytical and clinical developments. CRC Crit Rev Clin Lab Sci 1984;20:243-83.
4. Nicoloff JT, Spencer CA. The use and misuse of the sensitive thyrotropin assays. J Clin Endocrinol Metab 1990;71:553-8.
5. Klee G, Hay ID. Assessment of sensitive thyrotropin assays for an expanded role in thyroid function testing: proposed criteria for analytical performance and clinical utility. J Clin Endocrinol Metab 1987;64:461-71.
6. Hay ID, Klee G. Linking medical needs and performance goals: clinical and laboratory perspectives on thyroid disease. Clin Chem 1993;39:1519-24.
7. Faglia G, Bitensky L, Pinchera A, Ferrari C, Paracchi A, Beck-Peccoz, et al. Thyrotropin secretion in patients with central hypothyroidism: evidence for reduced biological activity of immunoreactive thyrotropin. J Clin Endocrinol Metab 1979;48:989-98.
8. Spencer CA. Clinical utility and cost-effectiveness of sensitive thyrotropin assays in ambulatory and hospitalized patients. Mayo Clin Proc 1988;63:1214-22.
9. Spencer CA, LoPresti JS, Patel A, Guttler RB, Eigen A, Shen D, et al. Applications of a new chemiluminometric thyrotropin assay to subnormal measurement. J Clin Endocrinol Metab 1990;70:453-60.

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