Immunoenzymatic Quantification of Low Concentrations of Thyrotropin

Hain-Yu Lee, A. Eugene Pekary, Viera P. Smith, Julia Sladk, and Jerome M. Herschman

We evaluated an immunoenzymatic assay (Abbott HTSH EIA) for thyrotropin (TSH) as a tool for detecting hyperthyroidism and for monitoring thyroid hormone suppressive therapy in patients with nodular goiter, thyroid carcinoma, and hypopituitarism. We also tested with thyroliberin (TRH), to determine the correlation between peak and basal TSH in suppressed patients. For comparison, we used a nonequilibrium radioimmunoassay optimized for maximum sensitivity (J Clin Endocrinol Metab 1975;41:876). Hyperthyroid patients with values for either or both triiodothyronine and thyroxin above the normal reference interval had Abbott assay values = 0.2 milli-int. unit/L, clearly below the Abbott assay normal range, as determined in 116 euthyroid subjects. We detected one-third of the suppressed patients (0.3 milli-int. unit/L) with RIA, 69% with the Abbott assay (TSH ≥ 0.04 milli-int. unit/L). Only 20% of patients with undetectable basal TSH values in the Abbott assay responded to TRH with a detectable peak TSH value; the peak TSH value after TRH was proportional to the basal TSH value. A single basal TSH measurement by the Abbott HTSH EIA should be adequate for monitoring the degree of thyroidal suppression in thyroid-hormone-treated patients.

Highly sensitive assays of thyrotropin (TSH, thyroid-stimulating hormone) that can measure TSH accurately in the range 0.1 to 1 milli-int. unit/L have been proposed as a primary screening assay for thyroid diseases and as a replacement for expensive testing for responses to thyroliberin (TRH) stimulation in thyroid-hormone-suppressed patients (1). Their utility for these purposes will depend on how well they can discriminate between low-normal values and suppressed TSH values, which can result from various pathological and therapeutic causes. Recent reports indicate that disorders of the hypothalamic–pituitary–thyroid axis can be identified by using recently developed assays for TSH that can detect concentrations below the normal reference range (2). We have utilized a representative state-of-the-art TSH assay (Abbott immunoenzymatic assay) and an "in-house" radioimmunoassay optimized for maximum sensitivity (3) to determine whether subnormal concentrations of TSH in serum can be detected reliably and used in screening for thyroid disorders and in therapeutic management.

Subjects and Methods

We studied the following subjects, dividing them into groups according to the results of measuring the concentrations of TSH, T4, and T3 in their serum by previously described "in-house" (WVA) radioimmunoassay methods (3, 4): (a) euthyroid with normal serum TSH, T4, and T3 (n = 116); (b) hypothyroid with TSH >15 milli-int. units/L (n = 50); (c) hyperthyroid with both T3 and T4 above the normal range (n = 67); (d) nodular goiter treated with thyroid hormone to suppress the concentration of TSH in serum (this group was not preselected on the basis of the measured serum TSH value; n = 44); (e) other patients receiving thyroid hormone therapy, selected because they had suppressed serum TSH concentration by our in-house assay (n = 33); (f) patients with thyroid carcinoma suppressed with thyroid hormone (n = 25); (g) hypopituitary patients being treated by thyroid hormone replacement (n = 30); and (h) patients receiving a 500-μg bolus injection of TRH to evaluate their thyroid function, mainly for diagnosis of equivocal hyperthyroidism (n = 40).

Abbott HTSH EIA. The TSH diagnostic test kit (Abbott HTSH EIA) was provided by Abbott Laboratories, North Chicago, IL. A detailed description of the assay reagents and methods, as used in the present study, has been published (5). To improve precision in the low-dose region, we used highly accurate positive-displacement manual pipetting devices in place of the siphon-type dispensing bottles supplied by the manufacturer for adding the o-phenylenediamine and 0.5 mol/L sulfuric acid solutions.

In-house "research" radioimmunoassay. We used this previously reported radioimmunoassay (3) (WVA TSH RIA), optimized for maximum sensitivity (minimum detectable dose: 0.3 milli-int. unit/L), for classifying subjects and for comparing absolute assay values.

Statistical analysis. We used Student’s paired t-test, the Pearson correlation, and regression analysis with the aid of "Statview 512+" (BrainPower, Calabases, CA 91302), a statistical program for the Apple Macintosh computer.

Results

The minimum detectable dose (0.04 milli-int. unit/L) was calculated from the average of 2 SD at zero dose of TSH (0.00133 A405) divided by the average slope of the standard curve at zero dose (0.0362 A405/milli-int. unit of TSH per liter). Table 1 summarizes the within- and between- assay CVs; Table 2 summarizes the TSH values for the various groups. The TSH values for 116 euthyroid subjects as measured with the Abbott EIA significantly exceeded the corresponding results obtained with the WVA assay, as assessed by Student’s paired t-test. The TSH values in the euthyroid group fit a log-normal rather than an untransformed normal distribution. If we use the WVA TSH assay minimum detectable dose of <0.3 milli-int. unit/L for both the VA and the Abbott assay, then all of the 116 euthyroid samples were detectable with the WVA assay and 93% were

<table>
<thead>
<tr>
<th>Table 1. Coefficients of Variation for the Two Assays</th>
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<tr>
<td><strong>Abbott EIA</strong></td>
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<tr>
<td><strong>TSH concn, milli-int. unit/L</strong></td>
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<tr>
<td><strong>Within assay</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>9</td>
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* n = 12; † n = 4; ‡ n = 5; § n = 10.
detected with the Abbott assay. The Abbott data for the hypothyroid patients were not significantly different from corresponding measurements with the WVA TSH assay. Results obtained for 64 hyperthyroid samples by the Abbott kit (all with undetectable TSH in the WVA TSH assay, <0.3 milli-int. unit/L) showed that TSH was undetectable in 40 (<0.04 milli-int. unit/L) and was 0.09 (SD 0.05) for the other 24 samples. All of the hyperthyroid values were <0.3 milli-int. unit/L in the Abbott assay. Half of the 44 nodular-goiter patients on suppressive therapy (group d) had TSH >0.3 milli-int. unit/L in the WVA assay; detectable values were found in 64% of the thyroid carcinoma patients receiving thyroid hormone suppressive therapy (group f) and in 60% of the hypopituitary patients (group g). In the Abbott assay the proportion of detectable TSH values were: hyperthyroid (group c), 41%; nodular goiter (group d), 75%; suppressed group (group e), 85%; suppressed thyroid carcinoma (group f), 68%; and hypopituitary (group g), 76%.

Figure 2 shows the correlation between basal and peak TSH response, as measured in the Abbott assay, to a 500-μg bolus injection of TRH in 40 patients whose basal TSH value was <0.3 milli-int. unit/L (according to the WVA assay). The corresponding correlation of log (basal TSH) vs log (peak TSH) is given in the right panel of Figure 2. The n value for this latter correlation is smaller because several basal TSH values were also undetectable in the Abbott assay and could not be represented appropriately on a logarithmic scale. A highly significant correlation was obtained with both the linear and logarithmic transformations of the data, suggesting that the Abbott assay can be used adequately to monitor the degree of thyroid hormone suppression in the hyperthyroid range without the need for TRH testing. For example, 15 basal TSH samples were undetectable in the Abbott assay (<0.04 milli-int. unit/L). Of these, only three showed a detectable response to TRH. Of the remaining 25 samples, with TSH values >0.04 milli-int. unit/L, 22 had a corresponding peak TSH value >0.3 milli-int. unit/L. The regression slopes in Figure 2 (right panel) are not statistically different (6) from the regression slopes calculated for hypothyroid subjects (7). Many undetectable values are superimposed at the origin of Figure 2 (left panel) or are not representable on the log scale of Figure 2 (right panel).

### Table 2. Concentration of Thyrotropin in Serum of Seven Groups of Patients as Measured by Both Methods: Mean ± SD TSH Conc., milli-int. units/L (and 95% range)*

<table>
<thead>
<tr>
<th>n</th>
<th>Abbott</th>
<th>WVA</th>
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<tr>
<td>(a) Ethyroid (116)</td>
<td>1.82 (0.5–6.7)</td>
<td>1.19 (0.4–5.8)</td>
</tr>
<tr>
<td>(b) Hypothyroid (50)</td>
<td>57.0 (7.91–242)</td>
<td>50.5 (15.3–239)</td>
</tr>
<tr>
<td>(c) Hyperthyroid (64)</td>
<td>ND in 40, 0.09 ± 0.05 in 24</td>
<td>ND in 67</td>
</tr>
<tr>
<td>(d) Nodular goiter (44)</td>
<td>ND in 12, 0.60 ± 0.66 in 32</td>
<td>ND in 22, 1.0 ± 0.8 in 22</td>
</tr>
<tr>
<td>(e) Suppressed (33)</td>
<td>ND in 5, 0.34 ± 0.39 in 28</td>
<td>ND in 33</td>
</tr>
<tr>
<td>(f) Thyroid carcinoma (25)</td>
<td>ND in 9, 0.54 ± 0.60 in 16</td>
<td>ND in 15, 0.98 ± 0.94 in 10</td>
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<tr>
<td>(g) Hypopituitary (30)</td>
<td>ND in 12, 0.61 ± 0.54 in 18</td>
<td>ND in 19, 0.88 ± 0.52 in 11</td>
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*Based on mean ±2 SD of logarithmically transformed data.
ND, not detectable (<0.04 milli-int. unit/L in EIA, <0.3 in WVA).

**Discussion**

The Abbott immunoenzymatic assay is representative of a new generation of TSH assays with minimum detectable doses below 0.1 milli-int. unit/L. Because the lower end of the normal range is about 0.3 milli-int. unit/L (5, 8, 9), the subnormal range of serum TSH values is now accessible for quantification with the latest generation of TSH assays, which utilize immunoenzymatic (5), fluorescence (10), or chemiluminescence (9) technologies. If TSH concentrations were measurable in patients with nodular goiter and thyroid carcinoma who are on suppressive thyroid-hormone therapy, then the therapeutic response could, in principle, be monitored by use of the serum TSH value alone. Primary screening for hyperthyroidism could also be carried out if the normal range for euthyroid subjects did not overlap that for hyperthyroid patients.

The data presented in Figure 1 suggest that this goal has been largely attained with the Abbott immunoenzymatic assay with 0.5 milli-int. unit/L used as the low end of the normal range. For the suppressed patients as a combined group (groups d–f together), the proportion of detectable TSH values was 75% and for the hypopituitary patients it was 76% with the Abbott assay. The corresponding proportions of TSH values detectable with the WVA assay were 40% and 56%, respectively. TRH testing in thyroid-hormone-suppressed patients appears to be unnecessary, according to Figure 2, because the TSH response to TRH remains proportional to the basal TSH value (7) throughout the range corresponding to that for the thyroid-hormone-suppressed group (2, 11). This proportionality may ultimately be extendable to the range below 0.04 milli-int. unit/L with the availability of still-more-sensitive assays for human thyrotropin.

![Fig. 1. Scatter plots for TSH results obtained with the Abbott immunoenzymatic assay](image-url)
Several authors have discussed aspects of the use of highly sensitive TSH assays as a primary screen for hyperthyroidism and for the monitoring of the adequacy of thyroid hormone suppression in thyroid cancer and nodular goiter patients (12, 13). Major points that have been raised include: (a) T₄ to T₃ conversion is autoregulated to maintain serum T₃ concentrations within the normal range (14); thus a compensatory fall in peripheral T₃ production rate from T₄ tends to decrease the suppressive effects of exogenous T₃ suppressive therapy. (b) The kinetics of pituitary-thyroid re-equilibration after changes in the level of thyroid hormone replacement therapy is slow. During treatment for thyrotoxicosis, thyroid hormone concentrations may decline rapidly, while TSH concentrations may remain suppressed for weeks. (c) Uncommon disorders such as thyroid hormone resistance or TSH-dependent hyperthyroidism will be misdiagnosed unless thyroid hormone concentrations are also measured (13). (d) There may be significant stimulation of the thyroid gland even when TSH is below the normal range. When serum thyroglobulin was used as an index of TSH-responsive thyroid gland stimulation, 76% of patients presenting with a subnormal basal value for TSH exhibited significant suppression of thyroglobulin one week after a 3.0-mg oral T₄ load (12).

Evidence showing that complete TSH suppression makes a significant difference in the recurrence rate of thyroid cancer and nodular goiter is difficult to obtain. Moreover, it is not yet clear whether a fully suppressive dose of T₄ can be selected that does not result in mild hyperthyroidism. Maintenance of such a dose, if it is achievable, will require careful monitoring with a highly sensitive TSH assay.

We are grateful to B. J. Green and Abbott Laboratories for supplying kits free of charge for this evaluation. This work was supported by Veterans Administration Medical Research Funds.

References
The accuracy of three commercial kit methods and one liquid-chromatographic (HPLC) method for determining 25-hydroxyvitamin D₃ in serum was evaluated by using isotope dilution–mass spectrometry as a reference technique. The kit methods were based on radioimmunoassay and radioreceptor assay. Serum samples were analyzed from male and female volunteers, some of whom had been exposed to ultraviolet B light or given vitamin D₃ orally. Results obtained with the three commercial kits were less accurate than those by HPLC. The agreement between the HPLC method and the comparison method was relatively good: *r* = 0.99; slope = 1.23; intercept = −2.9 μg/L. We conclude that methods based on HPLC should be preferred in routine clinical work and that the accuracy of the three commercial kits tested is not sufficient for their intended use.

**Additional Keyphrases:** isotope dilution–mass spectrometry · radioimmunoassay · radioreceptor assay · chromatography · reversed-phase

Determination of the concentration of 25-hydroxyvitamin D₃ in serum to evaluate vitamin D status in various clinical disorders has increased, with “high-performance” liquid-chromatographic (HPLC) methods especially coming into wider use. These methods involve one or more purification steps before quantification by measuring ultraviolet absorption. Analytical procedures involving two HPLC steps have also been used (1,2).

Quite recently, commercial kits for assay of 25-hydroxyvitamin D₃ by radioimmunoassay or protein-binding procedures have been introduced that are much easier to use than the HPLC methods.

We have now compared results obtained by three different kits with those obtained by isotope dilution–mass spectrometry (ID-MS) (3), the latter highly accurate method being generally considered to be a suitable reference method (for a review, see ref. 4). For comparison, we have also included results obtained with a method based on one reversed-phase and one straight-phase HPLC method. The accuracy of different HPLC methods for determining 25-hydroxyvitamin D₃ has been evaluated in a previous report from this laboratory (2).

**Materials and Methods**

**Materials**

We used HPLC equipment from Waters Associates, Milford, MA (Model 440 ultraviolet detector; Model 600 pump; Model U 6 K injector). The 4.6 × 250 mm columns were packed with Nucleosil C₁₈ (5-μm particles) or Zorbax-Sil (3 μm).

For gas chromatography–mass spectrometry we used an LKB 9000 instrument (LKB, Bromma, Sweden) equipped with a 1.5% SE-30 column (Chromosorb W, 80–100 mesh, 2 mm × 1.4 m).

We evaluated the following kits: 25-Hydroxyvitamin D₃ ³H (Amersham International, Amersham, Bucks, U.K.); 25-Hydroxyvitamin D (25-OH-D) by Radioimmunoassay (Immuno Nuclear Corp, Stillwater, MN; INC); and 25-OH Vitamin D-BP-50 (Institut National Des Radioelements, Fleurus, Belgium; IRE).

For standards we used: [26,27-³H]-25-hydroxyvitamin D₃ (175 kCi/mol; Amersham); [26-³H]–25-hydroxyvitamin D₃, synthesized as described previously (3), and unlabeled 25-hydroxyvitamin D₃, a gift from Drs. Babcock and Campbell (The Upjohn Co., Kalamazoo, MI).

"HPLC"-grade methanol, acetonitrile, hexane, and isopropanol were obtained from Rathburn Chemicals Ltd, Scotland. The scintillation liquid was Lumage® (Lumac/3M, Schaesberg, The Netherlands). Sep-Pak C₁₈ cartridges were from Waters Associates.

**Samples**

Human serum was sampled from male and female volunteers, some of whom had been exposed to ultraviolet B light (light with a spectrum optimal for vitamin D synthesis) or given vitamin D₃ orally. The concentrations of 25-hydroxyvitamin D₃ in serum from these subjects (pre- and post-treatment) ranged from 11 to 60 μg/L as measured by the mass-spectrometric method.

**Methods**

**Kit procedures:** The Amersham kit involves a simple purification step with Sep-Pak C₁₈ cartridges before a competitive protein-binding step. The INC kit involves an acetonitrile extraction step before the radioimmunoassay. The kit from IRE involves an extraction step (cyclohexane/ethylacetate 1:1 by vol) prior to a competitive protein-binding step.

**Liquid chromatography.** The HPLC method involves protein precipitation and extraction with Sep-Pak C₁₈ car-

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