liver or spleen (13). In our study, we established four calibration curves with four ferritin standards from liver; the reactivity of the standards is not the same, giving very different results for patients' serum samples. Consequently, it is necessary to establish the normal range for each method. Generally, there is a good correlation among the different methods ($r > 0.98$), but absolute values can vary greatly (14). To standardize the results, all liver ferritin standards should be calibrated vs the National Institute for Biological Standards and Controls reference standard (15).

In conclusion: the method described here is simple, fast, and presents the same precision and accuracy as the preceding (1) two-step method. The non-significant batch to batch variation (due to coating of tubes) does not affect the long-term reproducibility of the assay. We recommend use of the National Institute for Biological Standards and Controls reference standard to establish the standard curve. Furthermore, this assay procedure is less expensive than ferritin commercial kits.

References

Identification of Hyperthyroid Patients by Means of a Sensitive Assay for Thyrotropin

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We evaluated a new assay (TSH3 MAIAclone) for thyrotropin (TSH) with improved sensitivity, testing a series of hospital inpatients with increased free thyroxin indices (thyroxin concentration x triiodothyronine uptake on resin). This assay involves use of three monoclonal antibodies and an antibody–magnetic particle conjugate that rapidly and completely separates bound and free tracer in a magnetic field. The assay turnaround time is 3 h. By the TSH3-MAIAclone assay, 65% of these patients with an increased free thyroxin index were identified on the basis of a TSH value ≥0.50 milli-int. unit/L. In contrast, another commercially available assay for TSH detected suppressed TSH concentrations in less than 5% of these patients. We conclude that the TSH3 MAIAclone assay markedly improves our ability to discriminate hyperthyroidism from euthyroidism.

Additional Keyphrases: thyroid status • immunoradiometry by use of magnetizable solid phase • cutoff value

The regulation of pituitary secretion of thyrotropin (TSH)

1 Nonstandard abbreviations: TSH, thyrotropin (thyroid-stimulating hormone); T4, thyroxin; T3, triiodothyronine; FTI, free thyroxin index; TRH, thyroliberin (thyrotropin-releasing hormone); ECS, Environmental Chemical Specialties.
independent determinants on the TSH molecule, and a unique antibody–magnetic particle conjugate permits rapid, complete separation of bound from free tracer in a magnetic field. One can detect 0.25 milli-int. unit of TSH per liter (3, 4). For comparison, we also evaluated another commercially available assay for TSH.

Materials and Methods

We studied 65 patients attending the Hospital of the University of Pennsylvania between November 1983 and June 1984, who had free thyroxin indices (FTI) exceeding 11.8. The thyroid-function tests were repeated on more than one occasion, to confirm the increased FTI. We also measured triiodothyronine (T3) in these patients: 46% had an increased T3 as measured by RIA (>2.00 μg/L), the rest having T3 concentrations within the normal interval. In general, the population under study had significant medical problems that necessitated hospitalization, multi-drug therapy, and in some cases surgery. Complicating medical problems were cardiopulmonary disease, malignancy, diabetes, and neurologic disorders. Medications taken by the patients included drugs known to influence the thyroid–pituitary axis, the binding of T4 to serum proteins, and thyroxin metabolism—e.g., L-thyroxin, propylthiouracil, phenytoin, furosemide, and amiodarone (I, 5).

The euthyroid group comprised 18 healthy individuals who were participating in a study of thyroliberin (TRH) stimulation testing. None had a history of thyroid disease, and none was using any medications known to affect the thyroid or thyroid-function tests. Each euthyroid control had TSH values determined just before and 30, 60, and 180 min after administration of 400 μg of TRH as an intravenous bolus. All of these individuals had basal values for FTI within the normal interval (3 ± SD = 7.0 ± 1.85, normal 3.8–11.8) and T3 by RIA in the normal interval (0.85 ± 0.29, normal 0.60–2.00 μg/L), and all responded appropriately to TRH stimulation with respect to TSH secretion.

Blood samples from each participant were collected into evacuated specimen-collection tubes (Vacutainer Tubes, Becton Dickinson) and allowed to clot. The serum, obtained by centrifugation, was stored at 4°C until assayed for TSH, T3, and T4 resin uptake, and T3 by RIA with commercially available methods (Corning Medical, Medfield, MA). Specimens for TSH determination were stored at −20°C for no longer than three months before measurement.

The TSH3-MAIAclone assay (Serono Diagnostics, Braintree, MA) was provided by the manufacturer and has been described elsewhere (3, 4). Briefly: an aliquot of serum or standard is incubated for 2 h with anti-TSH reagent (a mixture of two radiiodinated monoclonal antibodies to TSH), then mixed with anti-TSH–magnetic particle conjugate. An additional 5-min incubation is then followed by separation and washing. We counted radioactivity for 1 min in a Micromed System 4/200 automated gamma counter equipped with a Hewlett-Packard HP200 microprocessor for data reduction. Standard curves were run for each assay, from TSH standards provided by Serono (0.25, 0.5, 1, 5, 10, and 30 milli-int. units/L). The 0.5 milli-int. unit/L standard was diluted with an equal volume of zero standard to produce the 0.25 milli-int. unit/L standard. Patients’ specimens and controls were at first run in quadruplicate, but later in duplicate. Any serum containing TSH greater than 50 milli-int. units/L was diluted and reassayed.

Using linear regression analysis, we compared results from the TSH3-MAIAclone assay with data obtained by another commercially available assay for TSH, based on polyclonal anti-TSH antibodies (Immophase; Corning Medi-
cal), performed according to the manufacturer’s instructions.

"Serono Low" and "Sero-test" controls were obtained from Serono Diagnostics. Environmental Chemical Specialties (ECS) controls were purchased from Bio-Rad Corp., Anaheim, CA.

Results

By using log-log data reduction, we found that a linear relationship between bound/total radioactivity vs TSH concentration was maintained over the clinically relevant range of 0.25 to 50 milli-int. units/L. Standard curves and controls were run on eight separate occasions over a 30-day period to evaluate the precision and reproducibility of individual points for the controls run in quadruplicate. The inter-assay imprecision (mean, SD, and CV) was: Serono Low, 0.89 ± 0.10 milli-int. unit/L, 11.7%; Sero-test, 4.54 ± 0.31 milli-int. units/L, 4.9%; ECS 2, 11.0 ± 0.24 milli-int. units/L, 2.2%; and ECS 3, 30.2 ± 1.10 milli-int. units/L, 3.6%.

We determined the cross reactivity of the antibodies with follitropin, lutein, and chorionicadotrophin by assaying TSH in sera from clinically euthyroid individuals having known increased concentrations of these hormones and normal values for the FTI. Cross reactivity was <0.1% for all of these hormones, confirming the specificity claimed for the antibodies by the manufacturer.

For 18 healthy men and nonpregnant women with normal FTI values, the mean TSH (± SD) was 1.32 ± 0.32 milli-int. unit/L, consistent with results of a previous study of 46 normal individuals at another center with the TSH3 MAIAclone assay (4). Consequently, we defined the lower limit of normal as 0.5 milli-int. unit/L, as suggested by Cobb et al. (4).

We compared the performance of the TSH3-MAIAclone assay with that of commercially available Corning Immophase TSH assay, using linear regression analysis of TSH values obtained by the two methods from 15 normal, euthyroid controls just before and 30, 60, and 180 min after TRH administration. The correlation between the two methods was excellent for these euthyroid individuals (y = 0.88x + 1.21, r = 0.96). However, for patients with above-normal FTIs and presumably suppressed TSH concentrations, the Immophase assay identified only three of 65 (4.6%) as being hyperthyroid on the basis of a TSH concentration below the lower limit of normal of 1.5 milli-int. unit/L. The TSH3-MAIAclone assay yielded an abnormally low TSH concentration (<0.5 milli-int. unit/L) in 42/65 (65%) of these patients (Figure 1). Of these 42 patients, 25 had increased T3 values.

Of the 23 patients who had TSH values within the normal range (0.5–5.6 milli-int. units/L) with the TSH3-MAIAclone assay in spite of increased FTI values, 17 had normal values for T3. Five patients had T3 values within 0.11 μg/L. Most of the patient population with increased FTIs and normal TSH concentration were hospitalized with critical cardiopulmonary or neurologic disorders, or both. Review of available charts indicated that most of these patients were not consid-
ered clinically hyperthyroid on follow-up examination. This is in marked contrast to the patients who presented with increased FTIs and decreased TSH concentrations. Chart review revealed that this group consisted of patients with newly diagnosed, clinically significant hyperthyroidism or patients with known thyroid disease.

Drugs with known effects on T4 binding to serum proteins or T4 metabolism—including furosemide, phenytoin, and amiodarone—had little apparent interaction with the assay, because 21 (81%) of the 26 patients on these and other
medications had appropriately low serum TSH concentrations in conjunction with an increased FTI.

Discussion

Radioimmunoassays for T₄, T₃, and TSH have greatly simplified and improved the diagnosis of thyroid disorders. However, diagnosis of mild primary hyperthyroidism is still difficult. Routinely used TSH assays are not sufficiently sensitive to discriminate depressed TSH values from those found in euthyroid individuals (6). The TSH₂-MAIAclone assay reported here is sensitive to at least 0.25 milli-int. unit/L, with an acceptable CV, allowing the identification of primary hyperthyroidism (3, 4, 7). Linearity of the assay is maintained to 50 milli-int. units/L, covering the clinically significant range. Additionally, the TSH₂ MAIAclone assay correlates highly significantly with results by the Immophase TSH assay for TSH values in the moderately above-normal range, and the assay requires only 3 h.

Of particular interest is the performance of the TSH₂ MAIAclone assay with the sera of the 65 inpatients with increased FTI values. Most of these patients were on multidrug regimens and were hospitalized with severe illness. The TSH₂-MAIAclone assay identified two-thirds of these patients as being hyperthyroid by detecting suppressed TSH concentrations; by comparison the TSH Immophase assay identified less than 5% by this criterion.

Although two-thirds of the patients we investigated had suppressed TSH concentrations as measured with the TSH₂-MAIAclone assay, a third of them had TSH values within the normal range. T₃ as measured by RIA in most of the latter patients (22 of 23) were either normal or within 0.11 μg/L of the upper limit of normal. Normal T₃ concentrations associated with increased FTIs and normal TSH concentrations have been described in patients with severe systemic illness. This is thought to be the result of impaired peripheral conversion of T₄ to T₃ (8). Hence it is likely that some of the apparent discrepancies between TSH values and FTIs are a consequence of the complex and severe nature of the disease processes (predominantly cardiopulmonary and neurologic) in this subgroup of patients.

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References