poses, such criteria should be considered with this fact in mind. Derivation of criteria on the basis of subject-specific intra-individual variation is more complicated, and in practice it suffers from the lack of a sufficient number of individual values. Calculated differences, D(%), are given in Table 3. The values were calculated under the assumption of a long-term analytical precision that represents the present state of the art.

I thank Dr. Haseler (Poliklinik Plauen) for organizing the blood collection of the EH group, and C. Schneider, G. Lochbaum, and P. Junghans (Bezirkskrankenhaus Plauen) for technical assistance.

References


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The SimuTRAC™ FT4/TSH Assay Evaluated as a First-Line Thyroid-Function Test


We evaluated the SimuTRAC FT4 57Co/TSH 125I dual-isotope assay for the simultaneous measurement of free thyroxin (FT4) by radioimmunoassay analog techniques and of thyrotropin (TSH) by immunoradiometry. Inter- and intra-assay CVs were <10% over the entire range tested except for 15.9% at the lowest FT4 concentration. Results obtained by the SimuTRAC assay allowed complete differentiation of 85 hyperthyroid patients and 35 hypothyroid patients from normal subjects. However, such estimations of FT4 or TSH concentrations occasionally were misleading for assessing thyroid status in various clinical conditions. We conclude that the SimuTRAC assay has the same inherent disadvantages possessed by FT4 analog and TSH immunoradiometric assays; however, where results of one of the simultaneous assays may be misleading, the results provided by the other may indicate the underlying pathology without requiring an additional assay.

Additional Keyphrases: free thyroxin · thyrotropin · monoclonal antibodies · immunoradiometric assay · radioimmunoassay · pregnancy · nonthyroidal illness · goiter

Recent developments in thyroid-function testing have allowed measurement of thyrotropin (TSH) by immunoradiometric assay (IRMA) (1). We and many others (2-6) have found TSH measurements by IRMA to be a reliable and convenient screening test for the biochemical assessment of thyroid status, but others have noted that TSH measurements by assays with more sensitive detection limits may be misleading in certain conditions (7-12).

With the development of a dual-isotope assay that allows simultaneous determination of "free" thyroxin (FT4) by radioimmunoassay (RIA) analog techniques and determination of TSH by IRMA, we decided to evaluate the analytical characteristics of this assay, and to compare it with FT4 and TSH assays currently in use in our laboratory for evaluating several clinical conditions.

Materials and Methods

Assays. SimuTRAC™ FT4 57Co/TSH monoclonal antibody 125I kits were supplied by Becton Dickinson, Rutherford, NJ 07070. Standards, supplied in a human serum matrix, contained both TSH (range: 0-155 milli-int. units/L) and FT4 (range: 0-127 pmol/L) and were calibrated by

1 Nonstandard abbreviations: TSH, thyrotropin; FT4 (free) thyroxin; FT3 (free) triiodothyronine; IRMA, immunoradiometric assay.
comparison with the World Health Organization's MRC 80/568 standard for TSH and by equilibrium dialysis, respectively. We followed the assay protocol supplied by the manufacturer, which, briefly, is as follows. Pipet samples (standards or patients' serum) into tubes coated with antibodies to T4 and TSH; then add 35S-labeled T4 analog and 125I-labeled monoclonal antibody to TSH. Mix the contents of the tubes and incubate at 37 °C for 3 h; after washing, drain the tubes and count both kinds of remaining radioactivity simultaneously in a gamma counter. [We used a Berthold LB 2100 (Berthold Laboratories, Wildbad, F.R.G.), with <2% spillover.] Interpolate results with the best-fit logit-log curve; in our hands, the results compared well with those obtained by performing the manual calculations described by the manufacturers.

FT4 and FT3 were measured by RIA analog techniques (Amerlex-M; Amersham, Bucks., U.K.) and TSH by IRMA (Behring RIA-gnost; Behringwerke, Marburg, F.R.G.) in the sera of all patients.

Patients. The thyroid status of all patients was determined on clinical grounds and on the basis of FT4 and FT3 (Amersham) and TSH (Behring) concentrations. The study group consisted of 85 newly diagnosed patients with hyperthyroidism, 25 patients with overt or mild hypothyroidism (13), 10 patients with subclinical hypothyroidism [i.e., patients who had no signs or symptoms of hypothyroidism and normal FT4 and FT3 results by Amersham tests, but increased TSH concentrations by Behring test (13)], 35 women with normal pregnancies, 37 patients from the intensive-care unit with severe nonthyroidal illnesses (14), one patient each with T3 intoxicosis (15) and selective pituitary resistance to thyroid hormones (16), two patients with antibodies to T4 (17), and six selected euthyroid patients with nodular goiter and normal FT4 and FT3 (Amersham) but suppressed TSH (Behring) concentrations. The control group comprised 112 healthy adult volunteers. Table 1 gives details of the age and sex of the study group.

**Statistical treatment.** We used the paired and unpaired Student's *t*-test and the Mann–Whitney U-test, where appropriate, to detect statistical differences between groups; Spearman’s rank-correlation test was used to determine correlation between groups. Reference ranges for FT4 and TSH were determined by calculating the central 95% of values in the control group.

**Results**

Table 2 shows the intra-assay CVs for the SimulTRAC FT4/TSH kit, calculated by performing at least 12 replicates at each concentration. Except for a CV of 15.9% at the lowest FT4 concentration, the CVs were <10% for both FT4 and TSH over the range tested. Inter assay CVs, calculated from duplicate analyses of Lymphochek control sera (Bio-Rad Laboratories, Richmond, CA) in five assays, were also <10% for both FT4 and TSH (Table 2).

The detection limit, defined as the concentrations obtained at mean + 2 SD (for TSH) and mean – 2 SD (for FT4) counts/min for 15 replicates of the zero standard was 0.06 milli-int. unit/L and 0.81 pmol/L for TSH and FT4, respectively, in the SimulTRAC assay. The linear regression equation for the comparison of results for FT4 obtained by the SimulTRAC (y) and the Amersham (x) methods was: y = 1.01x – 0.23 (r = 0.91, *P* < 0.0001, n = 263). The equation obtained for the comparison of results of TSH obtained by the SimulTRAC (y) and the Behring (x') methods was: y = 0.89x' – 0.63 (r = 0.89, *P* < 0.0001, n = 188). TSH results were significantly lower (*P* < 0.0001) in the control group when measured by the SimulTRAC assay than by the Behring assay, but there was no significant difference between FT4 concentrations determined by the SimulTRAC and Amersham methods. Table 1 gives the reference ranges and results for FT4 and TSH by all assays.

In terms of the results obtained by the SimulTRAC assay only, the FT4 and TSH concentrations in the various clinical conditions were as follows (see Figure 1).

**Hyperthyroidism:** All patients had increased FT4 and suppressed TSH concentrations (with regard to the refer-

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<table>
<thead>
<tr>
<th>Table 1. FT4 and TSH Concentrations (Mean ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FT4, pmol/L</strong></td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>----</td>
</tr>
<tr>
<td>Normal reference interval</td>
</tr>
<tr>
<td>Controls</td>
</tr>
<tr>
<td>Hyperthyroid</td>
</tr>
<tr>
<td>Overt hypothyroid</td>
</tr>
<tr>
<td>Subclinical hypothyroid</td>
</tr>
<tr>
<td>Nonthyroidal illness</td>
</tr>
<tr>
<td>Pregnancy</td>
</tr>
</tbody>
</table>

*Significantly different from values for control group: *P* < 0.0001, *P* < 0.0005, *P* < 0.005.

*Undetectable.

*Concentrations > 50 milli-int. units/L assigned a value of 50 milli-int. units/L.

F = number of females in group.

<table>
<thead>
<tr>
<th>Table 2. Intra- and Interassay CVs (%) for SimulTRAC Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FT4, pmol/L</strong></td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>16</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>TSH, milli-int. units/L</td>
</tr>
<tr>
<td>0.25</td>
</tr>
<tr>
<td>16</td>
</tr>
</tbody>
</table>

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ence ranges established for the SimulTRAC assay).

Hypothyroidism: TSH concentrations were increased in all patients with hypothyroidism, but FT₄ was within the reference range in three patients with overt or mild hypothyroidism and in all 10 patients with subclinical hypothyroidism.

Nonthyroidal illnesses: Fifteen patients had low FT₄ concentrations, one patient had undetectable TSH concentrations, and four had increased TSH concentrations. However, only three patients had abnormal concentrations of both FT₄ and TSH: two had low FT₄ and increased TSH concentrations, and one had low concentrations of both FT₄ and TSH.

Pregnancy: Eight pregnant patients, all in the third trimester of pregnancy, had low FT₄ concentrations but their TSH concentrations were within the reference range.

Nodular goiter: TSH concentrations were undetectable in all six euthyroid patients with nodular goiter, but FT₄ concentrations were all within the reference range.

Table 3 summarizes the results of thyroid-function tests in the remainder of the patients. When FT₄ concentrations were increased or decreased in the SimulTRAC assay, parallel changes were seen in the FT₄ Amersham assay with regard to its established reference range. Findings were similar when the SimulTRAC TSH and Behring TSH assays were compared.

**Discussion**

Analytical performance of the dual-isotope SimulTRAC assay for the measurement of FT₄ and TSH compares favorably with that published for the FT₄ Amersham (18) and TSH Behring (19) assays, respectively. Correlation coefficients for both FT₄ and TSH concentrations obtained by the SimulTRAC method with results obtained by the Amersham and Behring assays were each about 0.9; the comparisons included both very high and very low concentrations of analyte. TSH concentrations were substantially lower when measured by the SimulTRAC assay than by the Behring assay. We are not sure why this was, because the TSH standards in both assays are calibrated against the WHO 80/558 standard. The reference range of measurements by the SimulTRAC method was markedly different from that of the Behring assay, for which the reference range is similar to that of other IRMAs (5). The accuracy of

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**Table 3. Comparison of Thyroid-Function Test Results for Four Women**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Age, y</th>
<th>FT₄, pmol/L</th>
<th>TSH, mIU-int. units/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SimulTRAC</td>
<td>Amersham</td>
</tr>
<tr>
<td>Pituitary resistance to thyroid hormones</td>
<td>54</td>
<td>32.6</td>
<td>35.6</td>
</tr>
<tr>
<td>Antibodies to T₄</td>
<td>43</td>
<td>&gt;127</td>
<td>&gt;120</td>
</tr>
<tr>
<td>Antibodies to T₃</td>
<td>22</td>
<td>33.8</td>
<td>46.8</td>
</tr>
<tr>
<td>T₃ toxicois</td>
<td>25</td>
<td>16.0</td>
<td>17.3</td>
</tr>
</tbody>
</table>

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the TSH SimuTRAC assay thus needs further study in detail.

Nonetheless, this finding does not detract from the overall diagnostic utility of the SimuTRAC assay if results are interpreted only after comparison with the reference range established for the assay. Precision and accuracy of the SimuTRAC FT₄ assay were acceptable, and the reference range was similar to that of the Amersham assay.

When the reference ranges established for the respective assays were taken into account, FT₄ concentrations measured by the SimuTRAC and Amersham assays, and TSH by the SimuTRAC and Behring assays, yielded similar clinical information. With reference to the SimuTRAC assay, FT₄ concentrations were significantly lower in nonthyroidal illnesses and pregnancy than in the control group; falsely increased in the two patients with antibodies to T₄, within the reference range in the single patient with T₃ toxicosis; and often not low in hypothyroidism. TSH SimuTRAC concentrations were suppressed in the euthyroid patients with multinodular goiter, but were not suppressed in the single patient with pituitary resistance to thyroid hormones. These findings were similar to those noted in the Amersham FT₄ and Behring TSH assays, respectively, and suggest that criticisms of FT₄ analog assays [14, 15, 17, 20, 21] and TSH IRMAs [11, 22] apply equally to the SimuTRAC assay.

Results obtained by the SimuTRAC assay allowed complete differentiation of hyperthyroid and hypothyroid patients from the control group, as would TSH measurement by any IRMA (2–6). Similarly, our finding of significantly higher TSH SimuTRAC concentration in the ill patients than in the control group has also been reported previously [14].

We conclude that the SimuTRAC FT₄ and TSH dual assay provides the same information as separate FT₄ analog and TSH IRMAs, with the same drawbacks as these separate assays. The obvious advantage of the SimuTRAC assay is that where measurement of either FT₄ or TSH alone may be misleading, the answer provided by the other may provide a clue to the underlying pathology. Thus on consideration of cost per test, saving in manpower, and clinical information generated, the SimuTRAC dual-isotope assay has a definite place in assessment of thyroid status.

References