Four Nonisotopic Immunoassays of Free Thyroxin Evaluated

Remy Perdrixot,1 Marie-Paule Bounaud,2 Jean-Yves Bounaud,2 and Pierre Jallot1

We evaluated four new nonisotopic immunoassays of free thyroxin (FT4)—Amerlite FT4 (Amerham International), Magic Lite FT4 (Ciba Corning Diagnostics), Stratus FT4 (Dade—Baxter Travenol), and FT4 Enzelsa (Compagnie ORIS Industrie)—by comparison with two FT4 radioimmunoassays: Amerlex and Sclavo. Inter- and intra-assay coefficients of variation were <10% in the working range and there was no significant interaction between the tracer conjugate and albumin. All methods except Enzelsa gave results equivalent to those obtained with the Sclavo chromatographic technique. In a population consisting of 325 euthyroid subjects and 111 hypothyroid and 61 hyperthyroid patients without any treatment, we observed slight overlaps between the hyperthyroid and euthyroid populations and diagnostic sensitivities were −0.95 and 0.80 for hyperthyroidism and hypothyroidism. In specific populations (69 patients with chronic renal failure, 20 patients in intensive care, 17 patients treated with heparin, and 27 pregnant women), results were quantitatively similar to those obtained by the Sclavo assay. We conclude that these nonisotopic methods are a valid alternative to current radioisotopic commercial methods.

Additional Keyphrases: thyroid status · nonthyroidal illness and thyroid hormone assay · effects of albumin, oleic acid · "kit" methods · chromatographic technique compared

The free fraction of thyroxin (FT4) corresponds to the physiologically active form of the hormone. Measurement of FT4 is therefore of value in assessing thyroid function. Direct measurement is difficult, because measurement techniques may modify the dynamic equilibrium between thyroxin and thyroxin-binding protein. Over the last five years, radioimmunoassays (RIA) with labeled analogs have been developed and commercialized. However, in certain disorders, now well known, these methods give erroneous results, caused by artefacts usually related to interactions of the tracer and the T4 transport proteins (1–3). Nonisotopic methods recently being marketed involve conjugated tracers that bind slightly or not at all to the T4 transport proteins.

Here we report our study of four nonisotopic FT4 assays and compare their results with those obtained by two RIA methods, particularly for euthyroid patient populations with specific clinical conditions in which FT4 concentrations are known not to be always correlated with the patient’s euthyroid status.

Materials and Methods

FT4 Assays

The nonisotopic methods we studied were the Amerlite FT4 assay (Amerham International plc, Amersham, Bucks., U.K.), the Magic Lite FT4 (Ciba Corning Diagnostics Corp., Medfield, MA), the Stratus FT4 (Dade—Baxter Travenol, Miami, FL), and the FT4 Enzelsa (Compagnie Oris Industrie, GiffYvette, France). Amerlite, Magic Lite, and Enzelsa are one-step techniques; Stratus is an entirely automated two-step method. Table 1 summarizes the principal methodological characteristics of these kits.

The two RIA methods we used for comparison were our routine tests: the Sclavo two-step chromatographic method (FT4 Sclavo; Sclavo, Sienna, Italy) and the Amerlex analog-based assay (Amerlex M FT4, Amerham International plc). All assays were run exactly according to the manufacturers’ instructions.

Other Assays

Thyrotropin (TSH) concentrations were determined by the Amerlite TSH "ultrasensitive" assay (normal reference interval: 0.15 to 4 milli-int. units/L). Free triiodothyronine (FT3) was measured by a chromatographic method (Sclavo). Thyroxin-binding globulin was quantified with the RIAgnost kit (Hoechst Behring, Frankfurt, F.R.G.), prealbumin (tranthyretnin) and albumin by immunonephelometric assays (Hoechst-Behring).

Stated normal values for these methods, determined from data on 325 euthyroid patients, range from 6.5 to 33.5 mg/L for thyroxin-binding globulin, from 0.15 to 0.5 g/L for prealbumin, and from 37.5 to 50.5 g/L for albumin.

Analytical Evaluation

Three pools of serum at three analyte concentrations were measured 10 times within the same series to establish intra-assay reproducibility. These pools corresponded to hypothyroid (I), euthyroid (II), and hyperthyroid (III) samples. Interassay reproducibility was estimated by systematically including pools I, II, and III in each series. Pool II was assayed at the beginning and end of each analytical run, to determine assay drift. The effects of albumin and non-esterified fatty acids on the different methods were studied by analyzing a pool of sera from normal subjects after supplementing it with (per liter) 15, 30, and 45 g of albumin (Sigma), and 0.125, 0.5, 1, 10, and 20 mmol of oleic acid sodium salt (Sigma). Samples from each such serum specimen were measured five times.

We also studied two serum samples from euthyroid patients known to have autoantibodies to thyroxin. These
autoantibodies were detected by measuring the percentage of radioactive T₄ analog precipitated by polyethylene glycol (200 g/L), as described by Allan et al. (4).

Clinical Evaluation

The following patient populations, defined on the basis of clinical and biological criteria, were included in the study:

- Euthyroid population: 325 patients with no clinical sign of thyroid dysfunction and a normal biological assessment, including assays of FT₃, ultrasensitive assay of TSH, and Sclavo FT₄ assay. None of these patients had nonthyroidal illnesses (NTI).
- Hyperthyroid population: 111 patients with clear signs of hyperthyroidism, undetectable TSH, increased concentrations of FT₃ and FT₄, and an absent TSH response to thyroliberin.
- Hypothyroid population: 61 patients whose TSH concentrations exceeded the basal value and who over-responded to thyroliberin with clinically moderate or clear hypothyroidism. None of these patients was receiving any treatment of any kind.
- Particular patient populations: serious NTI represented by chronic hemodialyzed renal failure (n = 69); 20 patients in the acute intensive-care unit whose blood was sampled before any treatment; 17 patients being treated with heparin but with no serious organic disease; and 27 women in the third trimester of pregnancy, a time when alteration in transport proteins is known to occur (2). None of these 133 patients showed any sign of thyroid dysfunction on clinical examination and their values for FT₃ and TSH were within the normal reference intervals.

Data Analysis

To assess the performance of each assay in the euthyroid, hypothyroid, and hyperthyroid groups we used cumulative frequency data analysis. We plotted the three FT₄ distributions on the same graph. As shown by the theoretical example given in Figure 1, such a representation:

- makes graphic the overlap between the euthyroid and hypothyroid populations and between the euthyroid and hyperthyroid populations;
- defines the interval containing data for 95% of the euthyroid subjects (the reference interval); its limits, a and b, are the abscissas of points 0.975 and 0.025 taken from the FT₄ distribution of the euthyroid population;
- allows one to determine the measurement sensitivity (i.e., the proportion of subjects correctly classified) in the diagnosis of hyperthyroidism or hypothyroidism, according to points a and b. For the diagnosis of hypothyroidism, sensitivity is given by the value on the ordinate of the hypothyroid curve at which the abscissa is a. For the diagnosis of hyperthyroidism, sensitivity is given by 1 minus the value on the ordinate of the hyperthyroid curve point at which the abscissa is b.

In the "particular" populations, we compared the kits by counting the number of subjects with an FT₄ concentration below or above the reference interval.

Results and Discussion

Reproducibilities: Inter-assay and intra-assay CVs obtained with the non-isotopic method were about the same as for the RIAs techniques (Table 2). The Enzelsa method gave lower values for pools I and II in the interassay comparison.

Intra-assay drift: A significant drift was observed only with Stratus FT₄ assay (Table 2). This is not a very satisfactory result for a completely automated technique and should be investigated further.

Albumin-dependence of FT₄ results: Increasing the albumin concentration did not affect results for FT₄ with the Amerlite, Magic Lite, and Stratus methods (Figure 2). Values dropped slightly with the Enzelsa method according to the law for equilibrium between thyroxin and its transport proteins. As expected, an increase of as much as 50% occurred with Amerlex and can be explained by the analog binding to the albumin (5, 6).

Effect of oleic acid on FT₄ results: We observed qualitatively identical results with all the methods, except for

---

*Table 1. Features of the Six Kits Compared*

<table>
<thead>
<tr>
<th>Kit</th>
<th>Method</th>
<th>Tracer</th>
<th>Extraction step</th>
<th>Signal detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sclavo</td>
<td>RIA two-step</td>
<td>¹²⁵I-T₄</td>
<td>Chromatography</td>
<td>Gamma counter</td>
</tr>
<tr>
<td>Amerlex</td>
<td>RIA one-step</td>
<td>¹²⁵I-T₄O³</td>
<td>None</td>
<td>Gamma counter</td>
</tr>
<tr>
<td>Amerlite</td>
<td>LEIA one-step</td>
<td>T₄-HRPε</td>
<td>None</td>
<td>Enhanced luminescence</td>
</tr>
<tr>
<td>Magic Lite</td>
<td>LIA one-step</td>
<td>AE-IgG-T₄γ</td>
<td>None</td>
<td>Chemiluminescence</td>
</tr>
<tr>
<td>Stratus</td>
<td>EIA two-step</td>
<td>PhA-T₄ stereotype</td>
<td>None</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>Enzelsa</td>
<td>EIA one-step</td>
<td>Anti-T₄ Ab¹</td>
<td>None</td>
<td>Spectrophotometry</td>
</tr>
</tbody>
</table>

¹LEIA, luminescent enzyme immunoassay; LEIA, luminescent immunoassay; EIA, enzyme immunoassay.
²¹²⁵I-labeled T₄ derivative.
³Horseradish peroxidase-labeled T₄ derivative.
⁴Acridinium-ester-labeled T₄-immunoglobulin conjugate.
⁵Alkaline phosphatase-labeled T₄.
¹Monoclonal anti-T₄ antibody-peroxidase conjugate.

---

![Fig. 1. Diagnostic values of FT₄ in hypothyroid (○), euthyroid (□), and hyperthyroid (■) populations demonstrated for a hypothetical set of subjects](image_url)
Amerlex (Figure 3): FT4 values were unaffected by oleic acid concentrations up to 1 mmol/L. Higher concentrations caused a more or less marked increase as a result of competition of oleic acid for thyroxin binding sites. The decrease in values observed with Amerlex resulted from changes in the free/bound analog concentration ratio (3).

Interference of T4 autoantibodies: The results were higher with Amerlite, Magic Lite, and Enzelsa, all of which are one-step techniques (Table 3). The Stratus (two-step) technique gave normal values. We found the same difference with the RIA methods: FT4 values were very high with the one-step Amerlex method but normal with the two-step Scavo technique. The falsely high values obtained with the one-step methods (Amerlex, Amerlite, and Magic Lite) resulted from autoantibodies to thyroxin cross-reacting with

### Table 2. Analytical Properties of the Assays

<table>
<thead>
<tr>
<th>Reproducibilities (CV), %</th>
<th>Within run (n = 10)</th>
<th>Between run (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I  II  III</td>
<td>I  II  III</td>
</tr>
<tr>
<td>Sclavo</td>
<td>8  5  3.5</td>
<td>12 10  7</td>
</tr>
<tr>
<td>Amerlex</td>
<td>7  5  3.5</td>
<td>15 11  8</td>
</tr>
<tr>
<td>Amerlite</td>
<td>6  4  2.5</td>
<td>12  9  7</td>
</tr>
<tr>
<td>Magic Lite</td>
<td>6  4  2.5</td>
<td>12  7  4</td>
</tr>
<tr>
<td>Stratus</td>
<td>6.5 4  3</td>
<td>11 10  8</td>
</tr>
<tr>
<td>Enzelsa</td>
<td>6  3  2.5</td>
<td>8  5  8</td>
</tr>
</tbody>
</table>

Intra-assay drift, %

- Sclavo: +6.5
- Amerlex: +2.5
- Amerlite: +1.3
- Magic Lite: +1.9
- Stratus: +20.6
- Enzelsa: +0.4

*  I, II, and III were pooled sera from euthyroid, hypothyroid, and hyperthyroid subjects, respectively. Their average concentrations in pmol/L were 5, 12, and 30 by Sclavo; 8, 16.5, and 28 by Amerlex; 7, 16, and 24 by Amerlite; 10, 20, and 30 by Magic Lite; 10.5, 18.5, and 32 by Stratus; and 8, 11, and 16 by Enzelsa.

**  Mean percentage increase in measured FT4 (pool II) from beginning to end of 12 consecutive assay runs.

---

### Table 3. Interference from Autoantibody to T4

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured FT4, pmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sclavo</td>
<td>15</td>
<td>7–15</td>
</tr>
<tr>
<td>Amerlex</td>
<td>52</td>
<td>9.5–23</td>
</tr>
<tr>
<td>Amerlite</td>
<td>95</td>
<td>9.5–18</td>
</tr>
<tr>
<td>Magic Lite</td>
<td>35</td>
<td>11–21.5</td>
</tr>
<tr>
<td>Stratus</td>
<td>21</td>
<td>11–24</td>
</tr>
<tr>
<td>Enzelsa</td>
<td>&gt;51</td>
<td>8–17</td>
</tr>
</tbody>
</table>

*The percentage of [125I]T4 precipitated with polyethylene glycol was 30% for patient 1 and 60% for patient 2, but only 3% for control subjects.*

---

**Fig. 2.** Effect of albumin on FT4 concentration measured in a serum from an euthyroid subject.

A: Amerlex; ML: Magic Lite; ST: Stratus; AT: Amerlite; SC: Sclavo; EN: Enzelsa

---

**Fig. 3.** Effect of oleic acid on FT4 concentration measured in a serum from an euthyroid subject.

Ax, Amerlex; ML, Magic Lite; ST, Stratus; AT, Amerlite; SC, Sclavo; EN, Enzelsa.

---

Thirty to fifty percent of the hemodialyzed patients gave low values with the Amerlite, Stratus, Magic Lite, and Amerlex methods. A moderate decrease (15% of these patients were in the hypothyroid zone) was also seen with the

---

**CLINICAL CHEMISTRY, Vol. 35, No. 1, 1989 117**
Fig. 4. Values for FT₄ in the hypothyroid (V), euthyroid reference (□), and hyperthyroid (○) populations.
Methods for data analysis are given in the text.
Sclavo method. There is no clear explanation for this decrease in values, but it is probably related to the disorders from which the patients were suffering and to the multiple therapies prescribed for them (8,9). No decrease in FT4 was observed with the Enzelsa technique.

The patients receiving heparin all showed increased values with the four non-isotopic methods, owing to the increase in the concentration of nonesterified fatty acids, which displace T4 from its binding sites on the transport proteins. These results are close to those obtained with the Sclavo RIA method and correspond to the findings reported in the literature (3). Only the Amerlex method gave different results (two of 17 were in the hypothyroid range). The experiment in supplementation with oleic acid illustrates the particular behavior of the analog assay.

The four kits—Amerlite, Stratus, Magic Lite, and Enzelsa—gave similar qualitative results for the patients in acute intensive care. Respectively 10%, 30%, 36%, and 15% of the patients were classified as hyperthyroid. The few high values can be explained in part by the increase in concentrations of nonesterified fatty acids that happens in all intense stress situations (3).

For pregnant women, lower values were obtained with all the methods. The Enzelsa technique and the Sclavo method showed the least variations in relative values in comparison with the euthyroid subjects.

The non-isotopic techniques gave similar results, except for the Enzelsa technique, in the pregnant women and the dialyzed patients. This was somewhat foreseeable, given the lack of sensitivity of this last method in the detection of hypothyroidism and hyperthyroidism. However, whatever method is used to measure free T4, certain categories of patients cannot be satisfactorily classified according to their thyroid status with only this one test. Ultrasensitive TSH is undoubtedly more suited for this purpose in the specific populations we studied.
We conclude that conjugated tracers gave, overall, good results in the four methods studied, results similar to those obtained with the Scavo two-step chromatographic technique. They are better than those observed with the one-step analog-based methods, which are still widely used despite their disadvantages. These non-isotopic methods are thus a valid alternative to the isotopic two-step methods currently available.

We acknowledge Professors J. C. Bigorgne and V. Rohmer and Drs. J. F. Subra, C. Fanello, and J. L. Bourrier for helpful collaboration in the clinical part of this work; and all the manufacturers, for supplying their kits and reagents and for their invaluable technical advice.

References


Measurement of Cyclosporine Concentrations in Whole Blood: HPLC and Radioimmunoassay with a Specific Monoclonal Antibody and 3H- or 125I-Labeled Ligand Compared

Bryan A. Wolf,1 Michael C. Deft,2 John W. Koenig,3 M. Wayne Flye,3 John W. Turk,1 and Mitchell G. Scott1

We compared cyclosporine concentrations in whole blood as measured by HPLC and by RIA with a monoclonal antibody specific for cyclosporine with 3H- or 125I-labeled cyclosporine ligand. The 3H-RIA kit slightly underestimated cyclosporine concentrations (>600 μg/L) in comparison with HPLC. Over a wide range of concentrations, cyclosporine measured with the 125I-RIA kit correlated well with HPLC (slope = 0.99, n = 301, r = 0.98), observed for samples from recipients of kidney, heart, or liver allografts (respective slopes: 1.01, 0.93, and 1.00). The 125I-RIA standard curve was linear to 1000 μg of cyclosporine per liter. Inter- and intra-assay CVs for 125I-RIA measurements of cyclosporine were ≤7%. Evidently, the 125I-RIA kit involving a monoclonal antibody specific for cyclosporine is equivalent to the HPLC assay and can replace it for therapeutic drug monitoring of cyclosporine therapy.

Additional Keyphrases: intermethod comparison · monitoring therapy · organ transplants · "kit" methods

Cyclosporine, a cyclic undecapeptide with strong immunosuppressive properties (1), has been widely used in human transplants of solid organs or bone marrow during the last decade (2). Cyclosporine concentrations in blood are monitored because of the narrow therapeutic range for immunosuppression, the substantial inter- and intra-patient variability in cyclosporine pharmacokinetics, and the frequency of nephrotoxicity during cyclosporine therapy (1–5).

Measurement of cyclosporine concentrations has been complicated by several issues, recently reviewed by the Task Force on Cyclosporin Monitoring (3). HPLC measurement of cyclosporine in whole blood has been recommended because of the analytical specificity of HPLC (3). Many clinical laboratories nonetheless utilize RIA for cyclosporine measurements because of its technical simplicity and its adaptability for use with large numbers of samples. RIA measurements of cyclosporine have been confounded, however, by the nonspecificity of the antisera, which cross-react to various extents with cyclosporine metabolites (7). Because both the immunosuppressive and nephrotoxic properties of cyclosporine metabolites are incompletely characterized and because there is considerable individual variation in cyclosporine metabolism, the clinical utility of these pan-specific RIAs has been questioned (7). To address this problem, Sandoz Ltd., the manufacturer of the drug, has recently developed a monoclonal antibody specific for parent cyclosporine (6, 7), and has incorporated the specific monoclonal antibody into an RIA kit in which [3H]cyclosporine is used as ligand (8). A comparison of cyclosporine concentrations determined with this kit and by HPLC showed that results correlated well, so this specific RIA was proposed to replace HPLC for therapeutic monitoring of cyclosporine concentrations (8). Recently, a technically simpler RIA has been

1 Division of Laboratory Medicine, Departments of Pathology and Internal Medicine, Box 8118, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110.
2 Department of Laboratories, Barnes Hospital, St. Louis, MO 63110.
3 Department of Surgery, Washington University School of Medicine.

Received July 11, 1988; accepted September 6, 1988.