# Radioassay

## Evaluation of New $T_3$ Uptake and Total $T_4$ Tests

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***The $T_3U$ and $T_4$ tests from Ames Co. were compared with two reference tests in their ability to classify populations of patients with regard to thyroid function. The CLINIRIA $T_3U$ is the first all-liquid $T_3$ uptake test that lends itself to automation. In specificity and reproducibility, it was comparable to existing methods. The CLINIRIA $T_4$ and the reference method were equal in misclassification of patients but were different in the range of normal values. Both tests were of equal value when compared with already established thyroid function test procedures. Any advantage over previously introduced tests may come from considerations of cost-effectiveness and ease of automation and thus depends on specific laboratory circumstances.***

Many methods to determine the degree of saturation of serum thyroxine-binding sites are presently in use. Recently, Ames Co. introduced an additional technique called the CLINIRIA $T_3$ Uptake ($T_3U$) test kit, which is the first totally liquid competitive protein-binding test introduced with the claim that it is precise, rapid, and stable for more than 20 weeks and can be readily automated. Concurrently, a compatible $T_4$ radioimmunoassay (CLINIRIA $T_4$) is available, which, when performed with the $T_3U$, facilitates economizing through the use of common tubes, pipets, and counting equipment.

The combination of $T_4$ radioimmunoassay and $T_3U$ permits calculation of the free thyroxine index ($FT_4I$), which is considered at present to be the most reliable and widely used single test for thyroid function screening (*1–3*).

We have evaluated the diagnostic reliability of these new tests in patients with different thyroid function states and varying blood levels of thyroid-binding globulin.

### Patients and Methods

Sera were obtained from 35 hypothyroid patients (9 men), 100 euthyroid patients (40 men) with or without thyroid replacement therapy, 31 euthyroid patients who were either pregnant or taking oral contraceptives, and 35 hyperthyroid patients (11 men). The 100 normal subjects were further subdivided into "healthy" normals (n = 55) and "conditional" normals (n = 45). Conditional normals were euthyroid on thyroid replacement therapy or were patients with severe illness or taking drugs not known to affect thyroid function, such as sedatives and cardiac glycosides. Clinical assessment of thyroid status was supported in all hypothyroid patients by increased levels of serum thyroid-stimulating hormone and low levels of serum $T_4$ using an RIA from the Mayo Medical Laboratories ("reference $TT_4$"). The clinical diagnosis of hyperthyroidism was supported by elevated levels of serum $T_4$ supplemented, when indicated, by additional tests of thyroid function, such as I-131 uptake, $T_3$ suppression, or thyroid-releasing hormone stimulation tests. Table 1 gives information about the population studied.

After the thyroid status of patients was classified by clinical evaluation, reference $TT_4$, and supporting thyroid function tests, the normal ranges for the Ames $T_4$ and $T_3U$ were determined at the 95% confidence level and were compared with results from the reference methods. The reference method for $T_3U$ was a commercial

**TABLE 1. Descriptive Statistics by Group**

| Group | N | Mean Age (yr) | S.D. | Range (yr) |
|---|---|---|---|---|
| Normal healthy | 55 | 39.8 | 13.3 | 22-71 |
| Normal conditional | 45 | 55.2 | 11.5 | 20-73 |
| Normal | | | | |
| Males | 40 | 44.3 | 14.8 | 22-73 |
| Females | 60 | 48.3 | 14.4 | 20-72 |
| Therapy with oral contraceptive | 20 | 39.3 | 14.0 | 24-68 |
| Pregnant | 11 | 24.6 | 3.5 | 20-31 |
| Hypothyroid | 35 | 50.8 | 16.4 | 19-80 |
| Hyperthyroid | 35 | 46.3 | 18.6 | 16-82 |

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**TABLE 2. CLINIRIA $T_3U$ and $T_4$: Precision Studies for Ames Sera A, B, and C**

| | | Intra-Assay | | | | Interassay | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ames Serum | Replicates | Mean | 1 S.D. | CV (%)* | Replicates | Mean | 1 S.D. | CV (%)* |
| $T_3U$ | A | 60 | 30.75 | 0.611 | 1.99 | 60 | 30.75 | 0.272 | 0.88 |
| | B | 60 | 34.52 | 0.580 | 1.68 | 60 | 34.52 | 0.492 | 1.43 |
| | C | 60 | 39.26 | 0.552 | 1.41 | 60 | 39.26 | 0.738 | 1.88 |
| $T_4$ | A | 60 | 2.43 | 0.194 | 7.98 | 60 | 2.43 | 0.257 | 10.57 |
| | B | 60 | 8.47 | 0.504 | 5.95 | 60 | 8.47 | 0.431 | 5.08 |
| | C | 60 | 17.19 | 0.590 | 3.43 | 60 | 17.19 | 0.876 | 5.10 |

*Coefficient of variation.

test (Squibb), and the reference method for $TT_4$ was the procedure performed at the Mayo laboratories.

The Ames $T_3U$ utilizes the competitive protein-binding principle. The Ames $T_4$ is an RIA based on the use of a specific rabbit $T_4$ antiserum. The thyroid-binding-globulin blocking agent ANS (8-anilino-1-naphthalene sulfonic acid) is used. The detailed procedures are described in the manufacturer's literature or are available from us on request.

## Results

*Precision of Assays and Variability of the Standard Curve*: Interassay and intra-assay variabilities were within the accepted range for both tests (Table 2). While the precision was essentially constant for Ames $T_3U$, it varied with the concentration in Ames $T_4$. The ranges of values obtained from normal subjects (healthy and conditional) are given in Table 3. The ranges for the Ames $T_3U$ and $T_4$ were similar to the reference $T_3U$ and $T_4$ tests. The ranges of the more reliable free $T_4$ index, however, were more clearly different with regard to Ames versus reference tests. There was no significant difference between the two $T_4$ values from healthy and conditional normals. Thus, the two populations were grouped together for the definition of the normal range.

Using thus-defined normal ranges, we examined patient groups who had known abnormal thyroid function and tried to examine misclassifications of these groups (Table 4).

**TABLE 3. 95% Confidence Limits Estimated Nonparametrically from Data on 100 Normal Subjects (Conditional Plus Healthy)**

| | 2.5 percentile | 97.5 percentile |
|---|---|---|
| Reference $T_3U$ | 22.95 | 34.90 |
| Ames $T_3U$ | 25.40 | 34.65 |
| Reference $T_4$ | 3.95 | 11.50 |
| Ames $T_4$ | 4.80 | 13.60 |
| Reference $FT_4I$ | 113.66 | 344.48 |
| Ames $FT_4I$ | 150.90 | 389.98 |

**TABLE 4. Classification Results Based on Univariate Limits**

| | | Predicted thyroid state based on normals* (conditional plus healthy) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Euthyroid | | | Hypothyroid | | | Hyperthyroid | | |
| Category | | $T_3U$ | $T_4$ | $FT_4I$ | $T_3U$ | $T_4$ | $FT_4I$ | $T_3U$ | $T_4$ | $FT_4I$ |
| Normals (healthy + conditional) | Mayo | 96 | 96 | 95 | 2 | 2 | 2 | 2 | 2 | 3 |
| (n=100) | Ames | 95 | 95 | 95 | 2 | 2 | 2 | 3 | 3 | 3 |
| Normal healthy | Mayo | 53 | 53 | 52 | 1 | 2 | 2 | 1 | 0 | 1 |
| (n=55) | Ames | 54 | 53 | 53 | 0 | 1 | 1 | 1 | 1 | 1 |
| Normal conditional | Mayo | 43 | 43 | 43 | 1 | 0 | 0 | 1 | 2 | 2 |
| (n=45) | Ames | 41 | 42 | 42 | 2 | 1 | 1 | 2 | 2 | 2 |
| Hypothyroid | Mayo | 21 | 2 | 0 | 14 | 33 | 35 | 0 | 0 | 0 |
| (n=35) | Ames | 10 | 1 | 0 | 25 | 34 | 35 | 0 | 0 | 0 |
| Hyperthyroid | Mayo | 8 | 1 | 0 | 0 | 0 | 0 | 26 | 34 | 35 |
| (n=35) | Ames | 6 | 1 | 0 | 0 | 0 | 0 | 29 | 34 | 35 |
| High thyroid-binding globulin | Mayo | 13 | 13 | 18 | 7 | 0 | 0 | 0 | 7 | 2 |
| (n=20) | Ames | 6 | 14 | 18 | 14 | 0 | 0 | 0 | 6 | 2 |
| Pregnant | Mayo | 3 | 9 | 10 | 8 | 0 | 0 | 0 | 2 | 1 |
| (n=11) | Ames | 2 | 7 | 11 | 9 | 0 | 0 | 0 | 4 | 0 |

*95% confidence intervals were estimated nonparametrically for each of the three variables ($T_3U$, $T_4$, and $FT_4I$) for Ames and Mayo (see Table 1).

*CLINIRIA $T_3U$*: The $T_3U$, when used as the only test, misclassified a smaller number of hypothyroid patients relative to the reference method (ten versus 21). About an equal number of hyperthyroid patients were misclassified by the two tests (six versus eight). The reference method misclassified a smaller number of patients with high thyroid-binding globulins, as well as pregnant patients, compared with the $T_3U$ reference method (seven versus 14 and eight versus nine).

*CLINIRIA $T_4$*: The $T_4$ and the reference $TT_4$ methods were equal in misclassification of patients from different groups (normal, hypothyroid, hyperthyroid, high thyroid-binding globulins, and pregnancy).

*$FT_4I$*: $FT_4I$ was calculated as the product of $T_4$ ($\mu$g/dl) $\times$ $T_3U$ (%). For all practical purposes, there was no difference between the two methods with respect to misclassification of patients in different groups.

## Conclusions

The results of the CLINIRIA $T_3U$, CLINIRIA $T_4$, and reference methods of estimating thyroid function are comparable (as regards misclassification of patient groups). Normal values for $T_4$ and $FT_4I$, however, are different from the reference method, so that before the test can be used, a normal range has to be established. The technical advantages of the Ames assay—economy, stability, and ease of automation—appear to be worthwhile.

The CLINIRIA $T_3U$ test, as a single thyroid function test, was superior to the reference method in classifying the patient groups but, as expected, was inadequate as a thyroid function test when used alone.

## References


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