New Simple Kit Method for Radioimmunoassay of Serum Triiodothyronine

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Our experience with a new kit used for measuring serum triiodothyronine (T_3) is reviewed. The method's advantages include its simplicity, rapidity, relatively long shelf-life, the fact that it does not require expensive and elaborate laboratory equipment, and the small amount of radioactivity involved. The method has good specificity for T_3 and its reproducibility is satisfactory. The results with this method compare favorably with those obtained by previously published assays.

Measurement of triiodothyronine (T_3) in blood and other biologic fluids is receiving increasing attention. Even though the role of T_3 is not yet completely clear, there is evidence to suggest that T_3 may be the only active form of the thyroid hormone at the cellular level and perhaps one of the most important factors responsible for the clinical expression of the functional status of the thyroid *(1)*. Measurement of T_3 has been of considerable help in characterizing a new clinical syndrome: T_3 hyperthyroidism *(2, 3)*. With a new method for the measurement of serum T_3 now commercially available, the assay can be performed in approximately 4 hr in any laboratory equipped with a well gamma counter.

At present, measurement of T_3 is primarily for research purposes. However, from the clinical point of view, the test is specifically indicated for the patient who presents with a clinical picture of hyperthyroidism but who has results within the normal limits on the usual thyroid function tests. The reported incidence of T_3 hyperthyroidism has fluctuated from 5% of all cases of hyperthyroidism in an area representative of the iodide intake in the United States *(4)* to 10% in an area of previous iodide deficiency *(5)*.

Another indication for serum T_3 measurement would be as a test for the integrity of the hypophysis-thyroid axis. Larsen *(6)* has demonstrated that the administration of thyroid-stimulating hormone (TSH) to euthyroid patients evokes a marked increase in serum T_3 levels—105% above basal levels in 8 hr—while the increase in serum thyroxine (T_4) levels is only 41% above basal levels in the same amount of time. Such an increase, at least to that extent, would not be expected to occur in patients with primary hypothyroidism. In spite of the fact that the need for measurement of serum T_3 for clinical reasons may arise infrequently, the fact that the radioisotope (^{125}I) used in the test has a long half-life (60 days) permits the kits to remain usable after storage for 60 days.

We have evaluated a new commercially available kit (RIA-Mat circulating T_3 I125 kit, Mallinckrodt, Inc., St. Louis, Mo.) for the determination of serum T_3.

Materials and Methods

As in all radioimmunoassays, the test is based on the antigen-antibody reaction. The kit consists of 100 reaction vials (containing 250 μg of magnesium 8-anilino-1-naphthalene sulfonate and less than 0.2 μCi of ^{125}I-T_3 in 1 ml of barbital buffer); 1.5-ml vials of five T_3 standards (0, 0.5, 1.0, 2.0, and 6.0 ng/ml); 2.5-ml vials of T_3 antiserum (goat; dilution, 1:150,000); one 100-ml bottle of barbital buffer; and anion exchange resin strips, 50 per bottle. According to information supplied by the manufacturer, the reaction vials, standards, and antiserum should be stored at -10° to -20° C; the buffer and the resin strips may be stored at refrigerator temperature. Once the T_3 standards have been thawed for use, they can be stored at refrigerator temperature and used up to the expiration date stated on the label. The

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antiserum cannot be refrozen after the initial thawing but, if kept at refrigerator temperature, it will remain usable for 3 weeks afterward.

The required number of reaction vials are thawed and counted for 1 min in a well gamma counter ("precount"); they can be counted before thawing. Then 100 μl of each standard is added to a set of reaction vials, and 100 μl of each serum is added to the other reaction vials. A Hamilton syringe or Eppendorf-type pipet is recommended. We have used precision pipets produced by Medical Laboratory Automation, Inc. (Mount Vernon, N.Y.) with good results, with the following precautions: (A) the solution is drawn up and expelled at least once before dispensing; (B) the plastic tip is discarded after each addition; (C) the presence of a meniscus in the plastic tip is not allowed and, if one forms, a different tip is used; and (D) the tip is wiped before delivery. The next step is addition of 100 μl of T_3 antiserum to all reaction vials followed by gentle mixing by agitation or rotation for 1 min; vortex mixing is not recommended. We use a slow-speed rotator supplied by Mallinckrodt (12—14 rpm).

All the vials are then incubated for 1 hr in a water bath at 37° C; on completion of incubation, 1.0 ml of barbital buffer is added to each vial, followed by a resin strip. These strips should be carefully removed from the storage flask with forceps without being allowed to dry or fragment before insertion into the vials. This step is followed by slow rotation again for 1 hr, after which the resin strips are removed with forceps and discarded after careful wiping and draining into the vial.

The final count ("postcount") is determined for each vial and the calculation is as follows:

$$\frac{\text{Postcount (cpm)}}{\text{Precount (cpm)}} \times 100 = \%\ ^{125}\text{I-}T_3 \text{ bound.}$$

A standard curve is plotted on two-cycle semilog paper with percentage bound on the ordinate and T_3 standard concentrations on the abscissa. Values for the unknowns are read from the standard curve.

In this test, ANS (magnesium 8-anilino-1-naphthalene sulfonate) is used to displace T_3 from thyroxine-binding globulin (TBG), so that all T_3 in the serum can be free to bind to the antibody. The anion exchange resin strip used in the final step has a high capacity to bind T_3 that is not bound to the antibody.

We studied a total of 135 patients: 11 patients with hyperthyroidism (8 had Graves' disease, 2 had nodular goiter, and 1 had Hashimoto's thyroiditis with Graves' disease); 23 patients with hypothyroidism (6 had Hashimoto's thyroiditis, 3 had had ^{131}I treatment, 4 had had thyroidectomy, and 10 had myxedema of unknown cause); 90 normal patients (27 were healthy, without disease, 50 were well with nonthyroid disease, and 13 were ill with nonthyroid disease); and 11 patients with thyroid disease but considered to be euthyroid (7 had adenomatous goiter, 1 had Hashimoto's thyroiditis, 1 had Graves' disease, 1 had had ^{131}I treatment, and 1 had a solitary nodule).

All blood samples were drawn while the patient was fasting, and the serum was either processed immediately or kept frozen for later processing. Surks, et al *(7)* have shown that there is no change in T_3 concentration after repeated freezing and thawing, the serum remaining at room temperature for 24 hr or frozen at – 20° C for periods as long as 2 months.

The effect of changing antiserum concentrations was tested by running a standard curve in triplicate with 0.05, 0.1, and 0.2 ml of the antiserum. The effect of the duration of incubation also was studied with standards at 0 and 2.0 ng/ml. The effect of temperature was assessed by running standard curves with incubation at room temperature (22° C), in a 37° C water bath, and in a refrigerator (4° C).

Reproducibility was tested by determining the coefficient of variation for 14 measurements on different days from a serum pool (interassay variation). Intra-assay variation was determined from five simultaneous determinations in samples taken from a serum pool.

The test for specificity was done by comparing the reactivities of L-T_3, D-T_4, L-T_4, and diiodotyrosine (DIT) with the antibody; concentrations ranging from 10 ng/dl to 1,000 μg/dl were used.

All chemicals were reagent grade (Sigma Chemical Co., St. Louis, Mo.).

Results

The intra-assay coefficient of variation was 6.2% and appears to be satisfactory and compares well with values obtained by others *(7, 8)* using the traditional techniques. The interassay coefficient of variation was larger, 16.1%, but this does not constitute a problem for clinical interpretation of the results.

Regarding specificity of the supplied antiserum, of greatest importance is L-T_4, which is present in the blood in microgram amounts, and D-T_4, which may be present in the blood in much higher levels in patients who take a thyroxine preparation (Choloxin) for hypercholesteremia. Cross-reactivity was found to be 2.5% with L-T_4 and 0.4% with D-T_4 (Fig. 1).

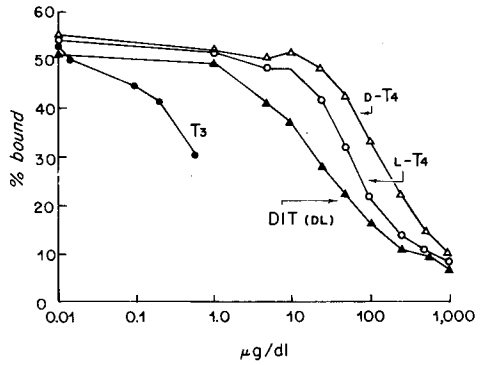

FIG. 1. Specificity of antibody used in assay. D- and L-T_3 were not distinguished by the antibody. Cross-reactivity with L-T_4 was about 2.5% and about 0.4% with free D-T_4, which is acceptable.

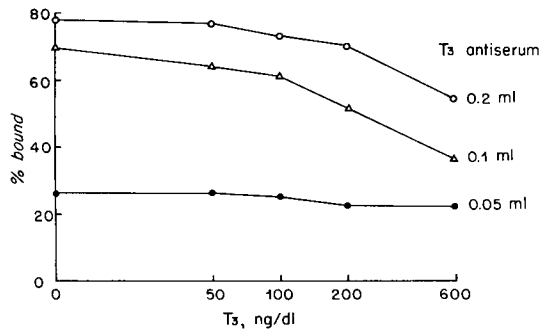

FIG. 2. Effect of different volumes of supplied antiserum on standard curve. The best standard curve is obtained by using the recommended 0.1 ml of antiserum.

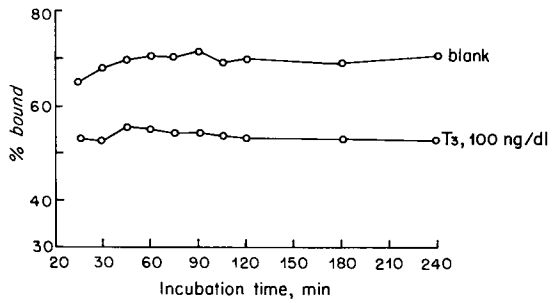

FIG. 3. Effect of duration of incubation on antibody-antigen binding. With incubation times less than 60 min, less was bound and the amount bound was time dependent. There was little change in binding with incubation times more than 60 min.

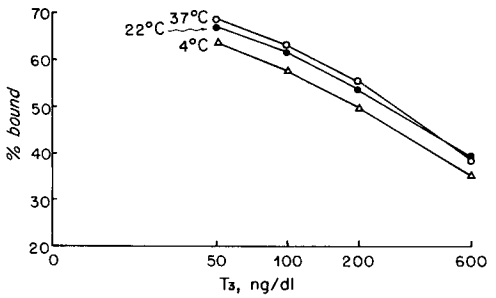

FIG. 4. Effect of incubation temperature. Binding was maximal at 37° C and decreased with decreasing temperature, but the difference was not great. Incubation time was 1 hr.

The amount of antiserum used should be 100 μl, as suggested by the supplier. With increasing amounts of antiserum, binding increases and the standard curve becomes flat and not accurate for T_3 values lower than 100 ng/dl (Fig. 2).

When standard curves were prepared with incubation time varying between 15 and 240 min, there was a slight increase in binding up to 1 hr and then a fairly stable line between 1 hr and 4 hr (Fig. 3). In this assay, the incubation time would be critical only if it were shorter than 45 min.

Binding during a 1-hr incubation decreased with decreasing incubation temperature (Fig. 4). The recommended temperature is 37° C but it appears from this curve that the assay probably could be performed at room temperature.

The serum T_3 values found in the different groups of patients are given in Table 1.

Discussion

Our values for serum T_3 compare favorably with those obtained by others (not using a kit method) with the exception of those of Gharib, et al *(9)*

TABLE 1. Serum T_3 Values

Category	No.	T_3 (ng/dl) Mean	s.d.	Range
Hypothyroid				
Hashimoto's thyroiditis	6	141.2	66.3	86–262
^{131}I treatment	3	65.7	35.3	30–101
Thyroidectomy	4	61.2	17.6	45–83
Myxedema, no known cause	10	74.5	69.7	0–237
Total	23	88.5	64.8	0–262
No thyroid disease				
Normal, no disease	27	121.2	25.6	78–178
Nonthyroid disease, well	50	115.3	45.1	27–272
Nonthyroid disease, ill	13	124.5	47.5	90–270
Total	90	118.5	42.1	27–272
Hyperthyroid				
Graves' disease	8	357.2	162.1	163–>600
Toxic nodular goiter	2	268.0	56.6	228–308
Toxicosis	1	96.0	...	...
Total	11	317.3	159.3	96–>600

TABLE 2. Summary of Serum T_3 Values Obtained by Radioimmunoassay

Study	T_3, mean ± s.d. (ng/dl) Hypothyroid	Normal	Hyperthyroid
Larsen *(6)*	39 ± 21	110 ± 25	546 ± 442
Surks, et al *(7)*	44 ± 26	146 ± 24	665 ± 289
Gharib, et al *(9)*	103 ± 43	218 ± 55	760 ± 289
Chopra, et al *(10)*	100	100 – 170	100 – 1,300
Mitsuma, et al *(11)*	62 ± 9	138 ± 23	494 ± 265
Lieblich and Utiger *(8)*	99 ± 24	145 ± 25	429 ± 146
Guansing, et al *(12)*	40 – 160	40 – 290	80 – 1,100
Sterling and Milch *(13)*	47 ± 39	189 ± 30	838 ± 398
Present study	88.5 ± 64.8	118.5 ± 42.1	317.3 ± 159.3

which are probably the highest so far reported (Table 2) *(6—13)*. Lower values have been reported in a later publication *(14)*. So far, the exact values of circulating T_3 in serum have not been established, and the possible technical reasons for the discrepancy among the reported values have been discussed by Gharib, et al *(14)* and Sterling and Milch *(13)*. It is clear that our values discriminate much better between hyperthyroid and normal than between hypothyroid and normal. This may have a physiologic reason *(3)*.

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