Measurement of Serum T_4 by RIA-Mat T_4 Kit

Kazuyuki YAMAUCHI, Shunmatsu WANIBE, Yukihiro IMAI, Terunori MITSUMA, Akio NAGASAKA and Noriyuki NIHEI
1st Department of Internal Medicine, School of Medicine, Nagoya University

Fundamental and clinical studies on T_4 radioimmunoassay kit (RIA-Mat T_4 kit) developed by the Daiichi Radioisotope Labolatory were performed to estimate clinical usefullness of this kit.

Methods: The reaction mixture which contained 1 m*l* of T_4-^{125}I barbital buffer, 600 ug of 8-anilino-1-naphthalene sulfonate, 0.1 m*l* of T_4 antiserum and 10 u*l* of T_4 standard serum or unknown serum was incubated at 37°C for 30 min. After a resin strip was added, each vial was incubated for 30 min on rotator to separate free and bound T_4.

Results: 1) Fundamental study; T_4 antiserum showed a crossreaction of 1.3% with Triac, 1.0% with T_3 and 0.05% with DIT. In the 1st incubation, B T% was not affected by change of incubation time from 15 to 150 min, or by change of temperature at 23°C or 37°C. B/T% was decreased by the prolongation of 2nd incubation time. 30 min second incubation gave the best standard curve in the range of 0.3 ug/d*l* to 40 ug/d*k* of T_4. The recovery of added T_4 in the system was 92.4 to 98.9%. Intra and inter assay reproducibilities were satisfactory. 2) Clinical study; Serum T_4 concentration in 42 normal subjects was 7.5 ± 1.5 (M$\pm$SD)ug/d*l* and normal range was calculated as 4.5$\sim$10.5 ug/d*l*.

T_4 values by RIA-Mat T_4 kit correlated well to those by Res-0-Mat T_4 kit or T_4 RIA kit.

Summary: RIA-Mat T_4 kit was relatively simple, rapid and had an reasonable recovery and reproducibility and corresponded well to clinical thyroid state, even though using small sample volume as 10 u*l*.

Measurement of a New Radioimmunoassay for Serum L-Thyroxine (T_4) in Patients with Various Thyroid Diseases and in Normal Subjects

Sadao SHIMOYAMA, San-E ISHIKAWA and Akiko KAJIHARA
First Department of Internal Medicine, School of Medicine, Gunma University, Maebashi, Japan

Several methods have been reported for the determination of l-thyroxine (T_4) which usually need the extraction procedure of iodothyronines from the serum. The serum T_4 was measured in 53 normal subjects and 67 patients with various thyroid disorders using a newly developed radioimmunoassay with a specific antiserum to the T_4 (RIA-MAT T_4 kit, Dai-ichi Radioisotope Lab.) to examine the accuracy and reliability in comparison to the T_4-I determination (Oxford-T_4) which have been previously used. The principle of this procedure is as follows: the serum T_4 bound with carrier proteins is liberated from TBG and TBPA by the administration of ANS (magnesium 8-anilino-1-naphthalene sulfonate) and veronal, and the liberated free-T_4 is measured by the competitive binding with ^{125}I T_4. The antibody bound- and free-T_4 are separated simply by employing the resin strip. In this system, the mean recovery of the T_4 added to serum was 95.6% and the mean coefficient of inter- and intraassay variations in 5 determinations were 9.5% and 4.9%, respectively, indicating that this system bears less variation than previous methods. In normal subjects, the range of RIA-T_4 concentration was 4.7–11.5 μg/d*l* in men and 3.4–10.5 μg/d*l* in women, respectively. The measured values of RIA-T_4 reflected satisfactorily on various clinical thyroid states, and the serum T_4 concentrations determined by RIA-MAT T_4 kits are well-correlated not only

with Oxford-T_4 but also with T_3-RSU values. We conclude that RIA-MAT T_4 kit is a simple, rapid and reliable method compared to previously used CPBA or Oxford method and will be able to be applied for the clinical use.

TRH-T Radioimmunoassay

S. WANIBE, K. YAMAUCHI, Y. IMAI, T. MITSUMA, A. NAGASAKA and N. NIHEI
First Department of Internal Medicine, Nagoya University, School of Medicine

We reported that synthetic TRH-tartrate (TRH-T) released TRH in human and rats as TRH and that synthetic TRH-T had immunoreactivity to TRH antibody as TRH. TRH-T radioimmunoassay was developed with using TRH-T as antigen to produce TRH-T antibody.

Method:

1. Antibody; TRH-T-BDB-BSA conjugate was injected to rabbits every four weeks and TRH-T antibody was harvested a week after each booster.
2. ^{125}-I-TRH-T; ^{125}-I-TRH-T was made by Hunter Greenwood's method and purified through Sephadex G-10 colomn.

Results:

TRH-T antibody was made in a rabbit after second booster. It had the titer of 1: 6000. This antibody had immunoreactivity as TRH and had few immunoreactivity to twenty four TRH analogues and hormones. The practical method of TRH-T radioimmunoassay was performed as; Samples or standards 0.1 m*l*, ^{125}-I-TRH-T 0.1 m*l* TRH-T antibody 0.05 m*l* and 0.01 M PO_4 buffer with 0.15 M NaCl, 1% BSA and 0.1 M EDTA 0.35 m*l* were mixtured and incubated at 4°C for 24 hrs. After first incubation, separation of bound and free was performed with second antibody or polyetheyleneglycol. With this method, significant dose response curve of TRH-T was observed from 2 pg/m*l* to 25 ng/m*l* of TRH-T.

The measurement of human plasma immunoreactive TRH-T was performed as; The plasma was separated at 1°C as soon as after sampling, extracted by ethanol and dried up. Plasma without TRH and extractant by ethanol was 92.7%. The plasma TRH levels in normal male were from undetectable range (less than 2 pg/m*l*) to 150 pg/m*l* and in female were from undetectable range to 100 pg/m*l*.

Conclusion:

We established a suitable radioimmunoassay system for human plasma TRH with using TRH tartrate antiserum.