

## D<sub>2</sub>. Measurement C (In Vitro Assay, Thyroid Hormone)

### Radioimmunoassay of Thyroxine

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T-4 RIA Diagnostic Kit was supplied by Dina-bott Lab. for the quantitative measurement of total circulating serum thyroxine. The evaluation of T-4 RIA Kit has been studied. To the 50  $\mu$ l of the unknown or standard was added 0.5N Sodium Trichloroacetate for inhibition of T-4 binding to thyroxine-binding proteins. Separation of bound and free I-125 T-4 was carried out with resin sponge. Standard curve was obtained from plotting %sponge uptake in T-4 concentrations of 0, 5, 10, 15, and 20  $\mu$ g/100ml. The standard curve consisted with dilutions of hyperthyroid serum with the T-4-free serum. Recoveries of T-4 from normal serum ranged from 92 to 124%. The mean recovery was 103.9%. Similar recoveries were obtained when T-4 was added to hypothyroid sera. For normal sera, the withinassay variation and the betweenassay was 3.5% and 6.2%, respectively.

The mean normal T-4 was 9.1  $\mu$ g/100ml with a range of 4.6 to 13.6  $\mu$ g/100ml. The mean serum T-4 levels in chronic thyroiditis, hypothyroid, pregnant, and renal failure were 6.5, 2.5, 14.2, 4.8  $\mu$ g/100ml respectively. The T-4 levels in hyperthyroid were more than 14.0  $\mu$ g/100ml. When in 17 sera from patients with various thyroid states, serum T-4 values were measured with two methods; T-4 RIA and T-4 CPBA, there was excellent agreement between these two techniques, the correlation coefficient being 0.95. The primary advantages of the T-4 RIA would appear to be one of efficiency in that preextraction of the serum is not necessary and only small volumes of serum are required.

These results suggested that T-4 RIA Kit is useful in the routine measurement of thyroxine.

### Study on the Radioimmunoassay of Serum Thyroxine

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The concentration of thyroxine in the blood is one of the indexes which indicates most clearly

the functioning conditions of the thyroid.

Competitive Protein Binding Analysis has been

widely used as a means of measuring this concentration. Whereas, a new method of measuring thyroxine in the blood has recently been developed using radioimmunoassay.

Basic studies were made with T<sub>4</sub> RIA Kit Dainabot which was introduced from Laboratories.

The following is a report on the results obtained with regard to the correlations among the time and temperature of incubation, frequency of washing with the resin sponges after incubation, and the standard curve and Compu-Curve (provided with the Kit), and among recurring rate, normal and abnormal bounds of thyroxine concentration in the blood and the correlation with values measured by CPBA.

As for the incubation time, study was made for 30', 45' 60', 75', 90', and 120'.

The measured values became stable in 60 minutes. Among temperatures of incubation in ice, refrigerator and in room, favorable result was observed in ice and refrigerator and no difference was found between them.

However, the measured values were more stable with ice than with refrigerator. In the study on frequency of washing with the resin sponge, it was

revealed that decontamination factor became equilibrated after washing made 2 times or more if the whole liquid has been absorbed away.

As regards, the standard curve and Compu-Curve the Kit was favorable as represented by  $r=0.837$  under the given condition and also favorable in recurring rate. Distribution of thyroxine values in the blood was 6.0–10.3 ng/100 ml in euthyroid subjects (6 males and 6 females). Its mean value and standard deviation were  $8.58 \text{ ng/ml} \pm 0.61$ . In comparison with CPBA the Kit was favorable as shown by  $r=0.973$ .

As compared with CPBA, in the case of T<sub>4</sub> RIA Kit, the required quantity of the serum was so small as 0.05 ml and no extraction of thyroxine from the blood was required because of the existence of Extractant Solution.

With this Kit, measurement can be made with many samples at one time at a specified temperature and the measured values are not affected by difference in temperature.

Measurement of radioactivity requires only 30 seconds. Another merit is the small variations that occur on the standard curve by use of Compu-Curve.

### On the Use of T<sub>4</sub> RIA Test Kit

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Recently developed T<sub>4</sub> RIA test kit was technically and clinically evaluated. Elongation of incubation time (60 minutes indicated) up to 90 minutes had little significant effect on T<sub>4</sub> value when patients' sera were measured with standard solution under the same condition. Elevation of incubation temperature decreased steepness of

slope of the standard curves, especially in the higher T<sub>4</sub> region. Indicated temperature at 0°C gave the best result. The intra- and interassay reproducibility proved to be satisfactory. The mean C.V. were 5.0 and 5.2% respectively. Recovery test, performed by adding 0 to 20 µg/dl of T<sub>4</sub> to the serum of a hypothyroid patient, also gave the satisfactory