**Simplified Reagent Handling**

**Introduction**

Thyroxine (T4) and Triiodothyronine (T3) are thyroid hormones which are essential in the regulation of various metabolic functions. T-Uptake is a measure of the unoccupied binding sites on the thyroid-binding proteins. The Free Thyroxine Index is calculated from the T4 and T-Uptake measurements. These three values provide a useful screening method for assessing thyroid disorders.

**Methodology**

SYNCHRON® Systems T4 Assay is intended for the quantitative determination of total thyroxine concentration in serum while the T-Uptake (TU) Assay is intended for the quantitative determination of thyroxine binding capacity in serum.

Both the T4 and TU assays utilize CEDIA™ technology. No sample pretreatment is required. This methodology relies on the capability of genetically engineered split, inactive β-galactosidase to reassociate and form active enzyme (β-gal) during the assay. One portion of the split enzyme is designated the enzyme acceptor (EA). The other portion, designated the enzyme donor (ED), is covalently linked to thyroxine to yield ED-thyroxine. The amount of active enzyme formed is modulated by the amount of analyte in the sample. The activity of the active enzyme is measured by the rate of hydrolysis of the substrate, o-nitrophenyl-β-d-galactopyranoside (ONPG), which forms a colored product.

T-Uptake is expressed as a percentage and is a measure of the occupancy of the binding sites of the thyroid-binding proteins. During the TU assay, unoccupied thyroid-binding proteins in the sample bind the ED-thyroxine, preventing it from combining with EA. The amount of sites available to bind ED-thyroxine during the assay is inversely proportional to the amount of active enzyme (β-gal) that forms, and thus the percent of T-Uptake.

In the T4 assay, antibody specific to thyroxine is also supplied in the reagent. The thyroxine in the serum is first mixed and incubated with EA and antibody, and has an opportunity to bind to the antibody. ED-thyroxine is then added and will bind to the remaining free antibody. The more thyroxine present in the serum, the less free antibody will be available to bind ED-thyroxine. ED-thyroxine bound to antibody cannot combine with EA to form active β-gal. The amount of thyroxine in the sample is directly proportional to the amount of active β-gal that forms.

* CEDIA is a registered trademark of Beckman Coulter

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**Chemical Reaction Schemes**

**T-Uptake Assay**

**Reaction 1**

\[
\text{ED-Thyroxine} + \text{Thyroxine-Binding Proteins} \rightarrow \text{Thyroxine-Binding Proteins}
\]

**Reaction 2**

\[
\text{Thyroxine-Binding Proteins} + \text{ED} \rightarrow \text{Thyroxine-Binding Proteins}
\]

**Thyroxine Assay**

**Reaction 1**

\[
\text{SAMPLE (Thyroxine)} + \text{REAGENT 1 (EA + Antibody)} \rightarrow \text{R1 MIXTURE}
\]

**Reaction 2**

\[
\text{R1 MIXTURE} + \text{REAGENT 2 (Enzyme-Donor Thyroxine Conjugate)} \rightarrow \text{R1 MIXTURE (Active Enzyme)}
\]
**Performance Characteristics**

### Reference Ranges

These serum values are intended to act as a guide only. Each laboratory should establish its own reference range based on its own population.

<table>
<thead>
<tr>
<th>Test</th>
<th>Conventional Units</th>
<th>S.I. Unit</th>
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<tr>
<td>TU</td>
<td>Female</td>
<td>25.25% to 40.40% uptake</td>
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<td>Male</td>
<td>30.61% to 40.37% uptake</td>
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<td>T4</td>
<td>5 – 12 µg/dL</td>
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### Correlation

Patient correlations spanning the analytical range were performed for both T4 and TU assays. Correlation for T4 was against FPIA (Abbott TDx®); correlation for TU was against RIA.

### Linearity**

T4 linearity was performed by interdiluting a low and high patient specimen to produce three intermediate levels. Four replicates of each sample were run. The method shows excellent linearity from 2 µg/dL to the high calibrator. (The high calibrator is typically about 21 µg/dL.)

### Within-Run Precision**

Typical within-run precision was evaluated by assaying 20 replicates each of hypothyroid and hyperthyroid samples.

### Total Imprecision**

Total imprecision was evaluated by assaying two levels of controls at least twice daily over a one-month period.

### References


*TDx is a trademark of Abbott Laboratories

** These precision, linearity and correlation studies were obtained in limited evaluations and are not intended to represent performance specifications for this reagent.

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**Thyroid**

T4 and T-Uptake (T4/TU)

**Performance Characteristics**

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