The employment of several serum references of known unsaturated thyroid hormone binding capacity permits construction of a graph of light and concentration. From comparison to the dose response curve, an unknown specimen’s absorbance can be correlated with thyroid hormone binding capacity.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (Type 5)

The required components for assessing the binding capacity of human serum are enzyme-T3 conjugate, thyroxine, binding protein (P), and immobilized thyroid hormone antibody (Ab). Upon mixing the enzyme-conjugate and thyroxine with the specimen, a binding reaction occurs between the patient’s binding proteins and the added thyroxine but not with the enzyme conjugate. This interaction is represented by:

\[ T4 + P \rightarrow T4-P(1) \]

\[ T4 = Thryoxine \]
\[ P = Specific binding proteins \]

The added thyroxine (T4) not consumed in reaction 1 then competes with the enzyme-T3 conjugate for a limited number of insolubilized binding sites. The interaction is illustrated by the following equation:

\[ \Delta T4 = T4 + A pw + \frac{A pw}{T4-P} = \frac{E_{T3-A-pw}}{T4} \]

\[ \Delta T4 = T4 unreacted in reaction (1) \]
\[ T4 = Thryoxine \]
\[ a = Antibody Constant \]
\[ \Delta T4 = T4 unreacted in reaction (1) \]
\[ T4-Apw = Immobilized Antibody Constant \]
\[ \Delta T4 = T4 unreacted in reaction (1) \]
\[ T4-Abw = Thyroxine-Antibody Complex \]
\[ \Delta T4-Abw = Enzyme-Thyroxine Conjugate - Antibody Complex \]

Since the metabolic processes are regulated entirely by the concentration of the free thyroid hormones, which are inversely related to the levels of the binding proteins, an assessment of the binding capacity of human serum was developed in 1957 by deMars et al. The T3-Uptake (T3U) test used to detect specimen of whole blood. After an incubation period, the mixture was centrifuged and the red cells washed. The radioactivity uptake of the red cells was inversely related to the binding capacity of the serum. Although this method had severe limitations, it proved to be a valuable diagnostic tool.

Further technical improvements in the assay methodology of the T3-uptake test resulted as various separation agents such as coated charcoal (5), ion-exchange resins (6), denatured albumin (7), silicates (8), antibodies and organic polymers were employed in place of the red cells. This microparticle chemiluminescence immunoassay methodology provides the technician with optimum sensitivity while requiring fewer technical manipulations. In this method, serum reference, patient specimen, or control is first added to a microparticle well. Enzyme-T3 conjugate and thyroxine (T4) are added, and then the reactions are mixed. The endogenous binding proteins of the sample react with the microparticle well but not with the enzyme conjugate. This leads to a higher binding of the enzyme conjugate to the antibody combining sites, reactive for triiodothyronine and thyroxine, immobilized on the well as the binding capacity of the specimen increases. After the completion of the required incubation period, the antibody bound enzyme conjugate is separated from the unbound enzyme conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce light.

The required components for assessing the binding capacity of human serum are enzyme-T3 conjugate, thyroxine, binding protein (P), and immobilized thyroid hormone antibody (Ab). The antibody bound enzyme conjugate is separated from the unbound enzyme conjugate by decantation or aspiration. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce light.

The specimens shall be blood, serum or plasma in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate results, the serum (or plasma) is handled as potentially hazardous and capable of transmitting disease. Patients the sale for use in the detection of disease. Diabetic patients are recommended to store at temperatures of -20°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required. The specimens shall be blood, serum or plasma in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate results, the serum (or plasma) is handled as potentially hazardous and capable of transmitting disease.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimens should be avoided.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-decay during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Accurate and precise pipetting, as well as following the exact protocol steps should be performed.
9. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
10. It is important to calibrate all the equipment (e.g., pipettes, washers) and the automated instruments used with this device, and to perform routine preventative maintenance.
11. Risk Analysis: as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12. Interpretation
12.1 Measurements and interpretation of results must be performed by a skilled individual or trained professional.
12.2 Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
12.3 For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
12.4 If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
12.5 If computer-controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
12.6 The T3-Uptake Test is dependent upon a multiplicity of factors: thyroid gland and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of the thyroid hormones to TBG. Thus, the T3-Uptake Test alone is not sufficient to assess clinical status.
12.7 The free thyroxine index (FTI), which is the product of the T3-Uptake Ratio and the total thyroxine concentration, has gained wide clinical acceptance as a more accurate assessment of thyroid status (9). The FTI value compensates for any condition or drug, such as pregnancy or estrogen, which alters the TBG and the T4 levels but does not change the thyrometabolic status. A table of interfering drugs and conditions, which affect the T3-Uptake Test, has been compiled by the Journal of the American Association of Clinical Chemists (10).

13.0 EXPECTED RANGES OF VALUES
A study of an euthyroid adult population (85 specimens) was undertaken to determine expected values for the T3-Uptake and are presented in Table 1.

### TABLE 1

<table>
<thead>
<tr>
<th>Thyroid Status</th>
<th>% T3 Uptake</th>
<th>T-Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>25 - 35</td>
<td>0.83-1.17</td>
</tr>
<tr>
<td>Hypothyroid or TBG</td>
<td>&lt; 25</td>
<td>&lt; 0.83</td>
</tr>
<tr>
<td>Hypothyroid or TBG</td>
<td>&gt; 35</td>
<td>&gt; 1.17</td>
</tr>
</tbody>
</table>

14.0 PERFORMANCE CHARACTERISTICS

#### 14.1 Precision
The within and between assay precision of the T-Uptake AccuLite™ CLIA method were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>10</td>
<td>26.1</td>
<td>0.28</td>
<td>1.1%</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>35.5</td>
<td>1.22</td>
<td>3.4%</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>45.6</td>
<td>0.70</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate over a ten day period.

#### 14.2 Accuracy (Method Comparison)
The T-Uptake AccuLite™ CLIA assay was compared with a T3 Uptake enzyme immunoassay method. Biological specimens from hypothyroid, euthyroid and hyperthyroid and pregnancy populations were used (The values ranged from 15% - 45%). The total number of such specimens was 105. The least square regression equation and the correlation coefficient were computed for this T-Uptake Chemi in comparison with the reference method. The data obtained is displayed in Table 4.

### Table 4

<table>
<thead>
<tr>
<th>Method Reference</th>
<th>Mean (x)</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.8</td>
<td>30.2</td>
<td>y = 1.06+0.576(x)</td>
<td>0.987</td>
</tr>
</tbody>
</table>

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

15.0 REFERENCES


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