AgAbC.W. + EnzAgAbC.W.

enzyme-antigen conjugate and a serum containing the native conjugate and native antigen. Upon mixing immobilized antibody, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity, determined by reaction with a substrate that generates light, in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different antigen-antibody pairs and store at 2-8°C.

After equilibrium is attained, the antibody-bound fraction is measured by reaction with a suitable substrate that generates light. In the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different antigen-antibody pairs and store at 2-8°C.

4.0 REAGENTS

Materials Provided:

A. T4 Calibrators – 1.0 ml/vial - Icons A-F
Six (6) vials of serum reference for thyroxine at concentrations of 0.0 (B), 5.0 (C), 10.0 (D), 15.0 (E) and 25.0 (F) µg/dl. Store at 2-8°C. A preservative has been added. For 51 units: µg x 12.9 = nmol/L.

B. One (1) vial of thyroxine-antibody conjugate in a shielded protein bound (PB) test to the theoretically sophisticated radioimmunassay (2).

This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-antigen conjugate is added, and then the reactants are mixed. A competition reaction results between the enzyme conjugate and the antigen conjugate for the antibody sites immobilized on the well.

After the completion of the required incubation period, the antibody bound enzyme-thyroxine conjugate is separated from the unbound enzyme-antibody conjugate by aspiration or decantation. The activity of the enzyme present on the unbound enzyme-antibody conjugate can indicate unnoticed change in experimental conditions or limitations that may affect the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 90 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, with each run, the entire set of controls should be run to determine the reason for the variations.

8.0 REAGENT PREPARATION:

1. Working T4 Tracer Solution
Dilute the T4-Tracer 1:11 with Total T3/T4 tracer buffer in a suitable container. For example, dilute 160µl of conjugate with 1,550µl of buffer for 16 wells (A slight excess of solution is made). This reagent should be used within twenty-four hours for maximum performance of the assay. Store at 2-8°C.

2. Enzyme-antigen Conjugate

(a) Preparing the T4-antigen conjugate

1. Prepare a 1:10,000 dilution in OptiPlate Buffer of the T4 conjugate. This is the antibody-antigen conjugate.

2. Prepare a 1:10 dilution of the T4-antigen conjugate in OptiPlate Buffer. This is the antigen-antibody conjugate.

3. Prepare a 1:10 dilution of the T4-antigen conjugate in OptiPlate Buffer. This is the antigen-antibody conjugate.

3. Enzyme-antigen Conjugate

(a) Preparing the T4-antigen conjugate

1. Prepare a 1:10,000 dilution in OptiPlate Buffer of the T4 conjugate. This is the antibody-antigen conjugate.

2. Prepare a 1:10 dilution of the T4-antigen conjugate in OptiPlate Buffer. This is the antigen-antibody conjugate.

3. Prepare a 1:10 dilution of the T4-antigen conjugate in OptiPlate Buffer. This is the antigen-antibody conjugate.

4. Enzyme-antigen Conjugate

(a) Preparing the T4-antigen conjugate

1. Prepare a 1:10,000 dilution in OptiPlate Buffer of the T4 conjugate. This is the antibody-antigen conjugate.

2. Prepare a 1:10 dilution of the T4-antigen conjugate in OptiPlate Buffer. This is the antigen-antibody conjugate.

3. Prepare a 1:10 dilution of the T4-antigen conjugate in OptiPlate Buffer. This is the antigen-antibody conjugate.

5. Enzyme-antigen Conjugate

(a) Preparing the T4-antigen conjugate

1. Prepare a 1:10,000 dilution in OptiPlate Buffer of the T4 conjugate. This is the antibody-antigen conjugate.

2. Prepare a 1:10 dilution of the T4-antigen conjugate in OptiPlate Buffer. This is the antigen-antibody conjugate.

3. Prepare a 1:10 dilution of the T4-antigen conjugate in OptiPlate Buffer. This is the antigen-antibody conjugate.
4. If more than one plate is used, it is recommended to repeat the assay.

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-deviation 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 time and temperature requirements prescribed are essential. Any deviation from Monobind Inc.'s IFU may yield inaccurate results.

9. Patient Specimens with T4 concentrations greater than 25 µg/dl can be diluted by pipetting 12.5µl of the specimen and 1.5µl of the 0.0 serum reference to the well to dilute. This maintains a uniform protein concentration. Multiply the readout value by 2 to obtain the thyroxine concentration.

10. Applicable national standards and regulations, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

11. It is important to keep in mind that establishment of a range of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analyst using the method in their laboratory.

14.0 PERFORMANCE CHARACTERISTICS

The within and between assay precision of the T4 AccuLite™ CLIA were determined by analyses on three different levels of pools. 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>C.V.</th>
<th>T</th>
<th>S</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>16</td>
<td>3.7</td>
<td>0.25</td>
<td>6.8%</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Normal</td>
<td>16</td>
<td>8.6</td>
<td>0.31</td>
<td>3.6%</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>High</td>
<td>16</td>
<td>14.5</td>
<td>0.82</td>
<td>5.7%</td>
<td>5%</td>
<td>5%</td>
</tr>
</tbody>
</table>

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

14.2 Sensitivity

The T4 AccuLite™ CLIA procedure has a sensitivity of 80 pg/ml. This is equivalent to a sample containing a concentration of 0.318 pg/dl.

The sensitivity was ascertained by determining the variability of the 0 pg/dl serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The T4 AccuLite™ CLIA method was compared with a microplate Elisa method. Biological samples from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.5 µg/dl – 28 µg/dl). The total number of such specimens was 120. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data are displayed in Table 4.

TABLE 4

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean (x)</th>
<th>Regression Coefficient</th>
<th>Correlation Coefficient</th>
<th>Method Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Least Square</td>
<td>3.87</td>
<td>y = 0.18+0.965x</td>
<td>0.965</td>
<td>8.25</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 method least square equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity (Specificity) of the thyroxine antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between the dose of interfering substance to dose of thyroxine needed to displace the same amount of tracer.

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