from the unbound enzyme-triiodothyronine 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 color.

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

2.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 5): The required components for measuring the binding capacity of human serum are enzyme-T3 conjugate, thyroxine, binding protein (P), and immobilized thyroxine antibody (Ab). Upon addition of enzyme conjugate and thyroxine with the specimen, a binding reaction results between the patient's binding proteins and the added thyroxine but not with the enzyme conjugate. This interaction is illustrated below:

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

T4 = Thyroxine added (constant quantity)

P = Specific binding proteins (varying quantity)

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

\[ \text{EnzT3 + T4 + Abc}_w \rightarrow k_a \text{EnzT3Ab}_w + k_b \text{EnzT3Ab}_w \] (2)

\[ \text{Abc}_w = \text{Immobilized Antibody (Constant Quantity)} \]

\[ \text{T4} = \text{Unreacted thyroxine in reaction 1 (Variable Quantity)} \]

EnzT3 = Enzyme-antigen Conjugate (Constant Quantity)

T4AbC.W. = Immobilized Antibody (Constant Quantity)

4.1 Required But Not Provided:

1. Pipette capable of delivering 25µl volumes with a precision of better than 1.5%.
2. Pipette and tips for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200µl) and (200-1000µl) dispenser(s) for conjugate and substrate additions.
4. Microplate washers or squeegee bottle (optional).
5. Microplate washer or squeegee bottle, 450ml and 620ml wavelength absorbance capability.
6. Test tube(s) for dilution of enzyme conjugate and substrate A and B.
7. Absorbent paper for blotting the microplate wells.
8. Plastic wrap or microplate cover for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
11. Quality control materials.

Note 1: Do not use reagents beyond the expiration date.

Note 2: Do not use reagents that are contaminated or have reduced absorbance capability.

Note 3: Avoid extended exposure to heat and light.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1 and 2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human sera have been treated in plasma to inactivate any potential infectious and capable of transmitting disease. Good laboratory procedures for handling and production of plasma are found in the “Guideline for Clinical Use of Human Blood and Blood Components” in the CDC and National Institute of Health, “Infectious Disease of Transfusion”, 2nd Edition, 1988, HHS Publication No.

(CDC) 88-5839.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

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 comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red top tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot at room temperature for 1-2 hours. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°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, 0.05ml of the diluted specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. Controls should be treated as unknowns and values determined in every test procedure performed. Quality control reference has been maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate a need for an unchanged condition in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION:

1. Working Reagent A - T3U-Enzyme Reagent Solution

Dilute the T3U-enzyme reagent 1:11 with T3 Uptake conjugate buffer to the specified concentration. For example: dilute 160µl of T3-U conjugate buffer with 16ml 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.

General Formula:

Amount of Buffer required = Number of wells * 0.1 ml

Quantity of T3-Enzyme necessary = # of wells * 0.01 ml i.e., 9.6 µl (1ml) T3-U conjugate buffer 16 x 0.01 = 0.16 ml (160µl) for T3 enzyme conjugate.

2. Wash Buffer

Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at 2-8°C for up to 60 days.

3. Substrate Solution

Pour the contents of the amber vial labeled Solution ‘A’ into the clear vial labeled Solution ‘B’. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2-8°C.

Note: Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have reduced absorbance capability.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).

**Test Procedure should be performed by a skilled individual or trained professional**

1. Format the microplates’ wells for each serum reference, control and patient specimen to be assayed in duplicate. Place any unused microtiff strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or patient specimen into the assigned wells.

3. Add 0.100 ml (100µl) of Working Reagent A, T3U-enzyme solution to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate 60 minutes at temperature for up to 60 days.

6. Discard the contents of the microplate gently for 20-30 seconds to mix and cover.

7. Incubate at room temperature for 15 minutes.

8. Do not use reagents that are contaminated or have reduced absorbance capability.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).

**Test Procedure should be performed by a skilled individual or trained professional**

1. Format the microplates’ wells for each serum reference, control and patient specimen to be assayed in duplicate. Place any unused microtiff strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or patient specimen into the assigned wells. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2-8°C.

Note: Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have reduced absorbance capability.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).

**Test Procedure should be performed by a skilled individual or trained professional**

1. Format the microplates’ wells for each serum reference, control and patient specimen to be assayed in duplicate. Place any unused microtiff strips back into the aluminum bag, seal and store at 2-8°C.
10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the unsaturated thyroid binding capacity in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot for each duplicate serum reference versus the corresponding %T3-Uptake (%) on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with the best-fit curve.
4. To determine the %T3-Uptake for an unknown, locate the absorbance (OD) of calibrator A should be > 1.3.
5. Four out of six quality control pools should be within the established ranges.
6. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
7. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
8. The substances in the substrate solution initiate a kinetic reaction, which is terminated by the addition of the stop solution.
9. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
10. Plate reader measures vertically. Do not touch the bottom of the wells.
11. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
12. Use components from the same lot. No intermixing of reagents from different lots.
13. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind’s IFU may yield inaccurate results.
14. All applicable national standards, regulations and laws, including, but not limited, to laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
15. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
16. Risk Analysis- as required by CE Mark IV Directive 98/79/EC for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid, the following requirements from different batches:
1. The absorbance (OD) of calibrator A should be > 1.3.
2. Four out of six quality control pools should be within the established ranges.
3. High lipemic, hemolyzed or grossly contaminated specimens should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate reader measures vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different lots.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind’s IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited, to laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind inc.

12.1 Assay Performance
1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. High lipemic, hemolyzed or grossly contaminated specimens should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate reader measures vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different lots.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind’s IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited, to laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IV Directive 98/79/EC for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com

13.0 EXPECTED RANGES OF VALUES

A study of 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>% T-Uptake</th>
<th>T-Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>&gt; 25</td>
<td>&lt; 0.83</td>
</tr>
<tr>
<td>Hypothyroid or TBG excess binding</td>
<td>&gt;25</td>
<td>&lt;0.83</td>
</tr>
<tr>
<td>Hyperthyroid or TBG saturation</td>
<td>&gt;35</td>
<td>&gt;1.17</td>
</tr>
</tbody>
</table>

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity 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 with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

To determine precision of the T-Uptake AccuBind™ test system, analyses were performed on three different levels of pool control sera. The number (N), mean (X), standard deviation (σ), and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

### Table 2

Within Assay Precision (Values in %U )

<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>24</td>
<td>28.7</td>
<td>0.39</td>
<td>1.37%</td>
</tr>
<tr>
<td>Normal</td>
<td>24</td>
<td>37.8</td>
<td>0.51</td>
<td>1.36%</td>
</tr>
<tr>
<td>High</td>
<td>24</td>
<td>45.4</td>
<td>0.63</td>
<td>1.45%</td>
</tr>
</tbody>
</table>

### Table 3

Between Assay Precision (Values in %U )

<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>28.4</td>
<td>0.45</td>
<td>1.6%</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>37.1</td>
<td>0.65</td>
<td>1.8%</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>45.1</td>
<td>0.55</td>
<td>1.15%</td>
</tr>
</tbody>
</table>

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

14.2 Accuracy (Method Comparison)

The T-Uptake AccuBind™ test method was compared with a T3 Uptake radioassay method. Biological specimens from hypothyroid, euthyroid and hyperthyroid and pregnancy populations were used. The data obtained is displayed in Table 4.

### Table 4

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean (x)</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3-Uptake AccuBind™</td>
<td>29.3</td>
<td>y = 1.56+0.956(x)</td>
<td>0.972</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