1. INTRODUCTION

Intended Use: The Quantitative Determination of Total Triiodothyronine in Human Serum or Plasma sample by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of serum triiodothyronine concentration is generally regarded as a valuable tool in the diagnosis of thyroid dysfunction. This importance has provided the impetus for the significant improvement in methodology that has occurred in the past. The advent of monoclonal antisera and the discovery of blocking agents to the T3 binding serum proteins has contributed significantly to an improvement in assay methodology that has occurred. The enzyme activity on the antibody bound fraction is measured after decantation or aspiration. This method involves the use of labeled T3 derivative and biotin labeled purified anti-T3 specific antibody. The sample is boiled for 5-10 minutes to denature the protein, and the antigen combines with labeled T3 derivative. After cooling, the sample is added to the well, and the enzyme labelled antibody is added. The enzyme activity present on the surface of the well is then measured after adding a substrate solution. The results should be read within 30 minutes of addition of the substrate solution to avoid photobleaching of the chemical following prolonged exposure to light. The enzyme activity in the antibody bound fraction is measured after decantation or aspiration.

2.1 Materials Provided:

A. T3 Calibrators – 1mL vial - Ions A-F
B. Reaction Buffer – 1× Millipore
C. Working Buffer –1× Millipore
D. Working Reagents
- Signal Reagent A – 1 vial
- Signal Reagent B – 1 vial

2.2 Specimen Collection and Preparation

Samples may be refrigerated at 2-8 °C for up to 7 days. Avoid exposure to freezing and thawing. When assayed in duplicate, 0.100 mL (100 µL) of the specimen is required for Total T3.

3.0 REAGENTS

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The enzyme activity present on the surface of the well is then measured after adding a substrate solution. The results should be read within 30 minutes of addition of the substrate solution to avoid photobleaching of the chemical following prolonged exposure to light. The enzyme activity in the antibody bound fraction is measured after decantation or aspiration.

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5.0 PRECAUTIONS

For In Vitro Diagnostic Use

Do not use reagents that are contaminated or have bacterial growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reactivity calibrators and controls to room temperature (20 - 25 °C).

"Test procedure should be performed by a skilled individual or trained professional"

1. Format the microplates with 50 µL each well as follows:
   - Add 0.500 mL (50 µL) of Working Reagent Solution A to the appropriate wells (see Reagent Preparation Section)
   - Add 0.500 mL (50 µL) of Working Reagent Solution B to the appropriate wells (see Reagent Preparation Section)

2. Pipette 0.050 mL (50 µL) of the appropriate serum reactivity calibrator, control or specimen into the assigned wells.

3. Add 0.050 mL (50 µL) of Working Tracer Reagent solution to the appropriate wells (see Reagent Preparation Section)

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

5. Incubate 45 minutes at room temperature.

6. Add 0.350 mL (350 µL) of wash buffer (see Reagent Preparation Section), decant (tap and blot) and aspirate. Repeat four (4) additional times for a total of five (5) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is used, fill each well by depressing the container (avoiding air bubbles) to disperse the wash. Decant the wash and repeat four (4) additional times.

7. Add 0.100 mL (100 µL) of working signal reagent solution to all wells (see Reagent Preparation Section). Always add the reagent to each well at same time in order to minimize reaction time differences between wells.

8. Incubate for five (5) minutes in the dark at room temperature

9. To determine the concentration of T3 for an unknown, locate the curve on the following graph, find the intersecting point on the curve, and read the concentration in ng/mL.

Note: For re-assaying specimens with concentrations greater than 7.5 ng/mL, pipette 0.025mL (25 µL) of the specimen and 0.025mL (25 µL) of the “0” serum reference into the sample well (95 µL total volume) and one (1) µL of a uniform protein concentration). Mix the reagents in the same order to minimize reaction time differences between reagent addition. The results should be read within thirty (30) minutes of addition of the substrate solution to avoid photobleaching of the chemical following prolonged exposure to light. The enzyme activity in the antibody bound fraction is measured after decantation or aspiration.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of triiodothyronine in unknown specimens.

Recorded the RLUs obtained from the printout of the microlute reader as outlined in Example 1.

Plot the RLUs obtained from the printout of the microlute reader as outlined in Example 1.

Draw the best-fit curve through the plotted points.

Determine the concentration of T3 for an unknown, locate the average RLUs for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration in ng/mL from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated in the instruction). For example, the average RLUs (63817) of the unknown interacts the calibration curve at (1.4 ng/mL) T3 concentration (see Figure 1).
Any deviation from Monobind’s IFU may yield inaccurate results.

9. Multichannel pipettes are recommended for addition of reagents.

10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory practices, 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 IVD Directive 98/79/EC for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.1 Interpretation
1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.

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.

3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Bosco LM-Stuart MC: Heterophilic antibodies: a problem for all immunoassays’ Clin.Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.

4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

5. 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.

6. If computer controlled data reduction is used to interpret the results of the test, it is important to note that the predicted values for the calibrators fall within 10% of the assigned concentrations.

7. Total serum triiodothyronine concentration is dependent upon thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of triiodothyronine to TBG. Thus, total triiodothyronine concentration alone is not sufficient to assess clinical status.

8. A decrease in total triiodothyronine values is found with protein-wasting diseases, certain liver diseases and salicylates. A table of interfering drugs and conditions, which affect total triiodothyronine values, has been compiled by the Journal of the American Association of Clinical Chemists.

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for Total T3 SBS™ AccuLite® CLIA method. The mean (X) values standard deviations (e) and expected ranges (s) are presented in Table 1. The total number of samples was 85.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
<th>Concentration</th>
</tr>
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<tbody>
<tr>
<td>I- Triiodothyron</td>
<td>1.0000</td>
<td>-</td>
</tr>
<tr>
<td>I-Thyroxine</td>
<td>&lt; 0.0002</td>
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<td>Sodium Salicylate</td>
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14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision
The within and between assay precision of the Total T3 SBS™ AccuLite® CLIA test system was 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>
<thead>
<tr>
<th>TABLE 2</th>
<th>Within Assay Precision (Values in ng/ml)</th>
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<tbody>
<tr>
<td>Sample</td>
<td>N</td>
</tr>
<tr>
<td>Low</td>
<td>16</td>
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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.

14.4 Specificity
The cross reactivity of the antibodies used to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated using the dose effect curve. An interfering substance to dose of thyroid hormone needed to displace the same amount of tracer.

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15.0 REFERENCES