Introduction

The Quantitative Determination of 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 assay methodology that has occurred in the past. The advent of reagents required and the discovery of blocking agents to the T3 binding serum proteins have enabled the development of procedurally simple radioimmunoassay.

This microplate immunoassay methodology provides the technician with rapidity, sensitivity, and less requiring few equipment.

In this method, a sample (Calibrators, Controls, and Patient Specimen) is added to the streptavidin coated microwells of a 96 well microplate followed by tracer (HRP) labeled T3 derivative and biotinylated specific sheep IgG. A competition occurs between the varying amounts of T3 in the sample and fixed amount of T3 analog for a fixed number of binding sites on the antibody.

After the completion of the required incubation period, the antibody bound T3-enzyme conjugate is separated from the unbound T3-enzyme derivative by aspiration or decantation. The activity of the enzyme present on the surface of the well is inversely proportional to the native antigen concentration.

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 between normal values, a majority of the serum samples should be obtained. The blood should be collected in a red-top vial after 72 hours, and the blood cell sample should be clotted for serum or plasma. The clot should be removed by aspiration or decantation.

Materials Provided:
- A. Combi-Cal® T3/T4/TSH Calibrator - 1ml/vial - Ions A-F
- Six (6) vials of serum reference for triiodothyronine at concentrations of 0 (0), 0.5 (B), 1.0 (C), 2.5 (D), 5.0 (E) and 7.5 (F) ng/ml.
- A preservative has been added. Store at 2-8°C.
- For Si units: x 1,536 = nmol/L
- B. tracer: T3-T4 tracer Buffer - 13ml/vial - Ions A-F
- One (1) vial of tracer contains buffer, red dye, preservative, and binding protein inhibitors. Store at 2-8°C.
- C. Signal Reagent A – 7ml/vial - Icon SA
- One (1) vial contains biotinylated anti-triiodothyronine (sheep) reagent in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.
- D. Light Reaction Wells – 96 wells - Icon SB
- H. Signal Reagent B – 7ml/vial - Icon SB
- E. Light Reaction Wells – 96 wells – Icon SC
- 7.5 ng/ml, pipette 0.025ml (25µl) of the specimen and 0.025ml (25µl) of the "0" serum reference into the sample well (this maneuver should be done in a sterile fashion).

Note: Do not use reagents beyond the kit expiration date. Note: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at +4°C.

Product Insert

1.0 INTRODUCTION

Competitive Enzyme Immunoassay – Type 7

Enzyme-antibody conjugate and a serum containing the native antigen, a competitive reaction results between the native antigen and the enzyme-antibody conjugate for a limited number of antibody binding sites. The interaction is illustrated by the following equation:

Enzyme-antigen Conjugate + Antigen-Antibody Complex

AgAbBtn + EnzAgAbBtn

The employment of several serum references of known concentration permits construction of a graph of relative light units. The graph is used to determine the reason for the variations.

1. Pipette capable of delivering 0.050 & 0.100ml (50 & 100µl) volumes with a precision of better than 1.5%.

2. Draw the best-fit curve through the plotted points. 3. Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1 ml of B per two (2) eight well strips (A slight excess of solution is made). Pipette the solutions not used within 36 hours after mixing.

Note: Do not reagents that are contaminated or have bacteria growth.

10.0 CALCULATION OF RESULTS

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

1. Record the RLUs obtained from the printout of the microplate reader.

2. Plot the RLUs for each duplicate serum reference versus the corresponding triiodothyronine concentration in ng/ml on linear graph paper.

3. Draw the best-fit curve through the plotted points.

4. To 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 corresponding concentration (in ng/ml) from the horizontal axis of the graph. The concentration of the unknown may be averaged as indicated (See Figure 1).

Note: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained. Duplicates of the unknown may be averaged as indicated (See Figure 1).

11. Read the Relative Light Units (RLUs) in each well, for minimum 0.5 – 1.0 seconds, using a microplate luminometer.

Software should be ascertained. Duplicates of the unknown may be averaged as indicated (See Figure 1).
9. Multichannel pipettes are recommended for addition of reagents.
10. 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.
11. It is important to calibrate all the equipment e.g. Pipettors, 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.

2. Interpretation
1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are not a substitute 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 procedure have been formulated to eliminate maximal interference; however, potential interference between the serum specimens and test reagents may cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all immunoassays. (Boscato 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 signs.
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 imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Total serum triiodothyronine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration and function, and the binding of triiodothyronine to TBG. Thus, total triiodothyronine concentration alone is not sufficient to assess clinical thyroid status.
8. A decrease in total triiodothyronine values is found with protein-wasting diseases, certain liver diseases and administration of estrogen/steroid sulphate, diphtheria or 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.

11.0 QC PARAMETERS
In order for the assay results to be considered valid the following criteria should be met:
1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS
The MSDS and Risk Analysis Form for this product are 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. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. 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-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.

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 pooled control sera. The number, mean values, standard deviations and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

15.0 REFERENCES