3.0 PRINCIPLE
Immunoenzymometric assay (Type 3)

The essential reagents required for an immunoenzymometric assay include: (1) an antibody that specifically recognizes the antigen, (2) a labeled antibody, and (3) a solid phase on which the antigen can be adsorbed. The labeled antibody is allowed to react with the antigen adsorbed on the solid phase. The antigen-antibody reaction that occurs is then detected by an enzyme label that is covalently attached to the antibody. The application of several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type, and the usual precautions in the collection of venipuncture samples should be followed. The blood sample should be collected in a plain red-top venipuncture tube with or without gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of 5 days. If the specimen(s) can not be assayed within this 5 day period, it shall be stored at -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml of the 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. These controls should be treated as unknowns and values determined in each test procedure performed. Quality control charts should be maintained to follow the performance of the assay. A normal microplate cover for incubation conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer

Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store the buffer at 2-30°C. All products that contain human serum have been found to be non-reactive for Hepatitis B Surface antigen, HIV 1&2 and HCV by manufacturers. The reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

Materials Provided:

A. Thyrotropin Calibrators – 1.0 mlval - Iodosyl-3B

B. Thyrotropin Tracer Reagent – 13 mlval - Iodosyl-3B

C. Light Reaction Wells – 96 wells - Icon

D. Wash Solution Concentrate – 20 ml - Icon

E. Signal Reagent A – 7.0ml/vial - Icon CA

F. Signal Reagent B – 7.0ml/vial - Icon CB

The specimens shall be blood, serum in type, and the usual precautions in the collection of venipuncture samples should be followed. The blood sample should be collected in a plain red-top venipuncture tube with or without gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) can not 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, 0.100ml of the specimen is required.

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
10.0 CALCULATION OF RESULTS

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

1. Record the RLUs obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLUs’s for each duplicate serum reference versus the corresponding TSH concentration in µIU/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration for an unknown, locate the average RLUs for each unknown on the vertical axis of the graph and draw a line intersecting the point on the curve, and read the concentration (in µIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLUs of (25677) of the unknown intersects the calibration curve at 0.1µIU/ml TSH concentration (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.

EXAMPLE 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number Without Wash</th>
<th>Number With Wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.00</td>
<td>2.02</td>
</tr>
<tr>
<td>B</td>
<td>2.05</td>
<td>2.03</td>
</tr>
<tr>
<td>C</td>
<td>2.04</td>
<td>2.08</td>
</tr>
<tr>
<td>D</td>
<td>2.03</td>
<td>2.01</td>
</tr>
<tr>
<td>E</td>
<td>2.06</td>
<td>2.04</td>
</tr>
</tbody>
</table>

The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the G calibrator (greatest light output). This conversion of TSH for an unknown is caused by efficiency of the various instruments that can be used to measure light.

Figure 1

11.0 Q.C. 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 established ranges.

12.0 RISK ANALYSIS

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

12.1 Assay Performance

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) mm from the sides. Pipetters should be positioned to avoid human error.

3. Highly tempered, hemolyzed or grossly contaminated specimen(s) should not be used.

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-depence 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’s IFU may yield inaccurate results.

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, Readers, Washers. The automated instrument used with this device, and to perform routine preventative maintenance.

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.

12.2 Interpretation

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

1. 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 determinations.

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

3. 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.

4. If controls are not 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.

5. Serum TSH concentration is dependent upon a multiplicity of factors: hypothalamic gland function, thyroid gland function, and the responsiveness of pituitary to TRH. Thus, thyrotropin concentration alone is not sufficient to assess clinical status.

6. Serum TSH values may be elevated by pharmacological intervention. Dopamine, carbamazepine, amiodarone, diazepam, and phenytoin have been reported to increase TSH levels.

7. A decrease in thyrotropin values has been reported with the administration of propranolol, mecamylamine, and dopamine (4).

8. Genetic variations or degradation of intact TSH into subunits may affect the binding characteristics of the antibodies and influence the final result. Such samples normally exhibit different results among various assay systems due to the reactivity of the antibodies involved.

9. TSH Values in µIU/ml

<table>
<thead>
<tr>
<th>Sample</th>
<th>TSH Values in µIU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.00</td>
</tr>
<tr>
<td>B</td>
<td>2.00</td>
</tr>
<tr>
<td>C</td>
<td>3.00</td>
</tr>
<tr>
<td>D</td>
<td>4.00</td>
</tr>
<tr>
<td>E</td>
<td>5.00</td>
</tr>
<tr>
<td>F</td>
<td>6.00</td>
</tr>
</tbody>
</table>

It is important to keep in mind that expected values for normal population is dependent upon a multiplicity of factors: genetic, environmental, age, etc. These factors will determine by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the TSH AccuLite™ CLIA method were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 1.2

<table>
<thead>
<tr>
<th>Level Number</th>
<th>X</th>
<th>σ</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>85</td>
<td>2.5</td>
<td>0.05</td>
</tr>
<tr>
<td>2.00</td>
<td>76</td>
<td>3.5</td>
<td>0.06</td>
</tr>
<tr>
<td>3.00</td>
<td>67</td>
<td>4.5</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Within Assay Precision (Values in µIU/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>2.05</td>
<td>0.03</td>
<td>1.5</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>2.06</td>
<td>0.04</td>
<td>2.0</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>2.07</td>
<td>0.05</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Between Assay Precision* (Values in µIU/ml)

The within and between assay precision of the TSH AccuLite™ CLIA method was ascertained by determining the variability of the 0 µIU/ml serum calibration and using the 2σ (95% certainty) statistic to calculate the minimum dose. It was determined to be 0.062 µIU/ml.

Accuracy

The TSH AccuLite™ CLIA assay was compared with a reference Elsa assay. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.01µIU/ml - 4µIU/ml). The total number of such specimens was 181. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data from this is displayed in Table 4.

14.3 Accuracy

The TSH AccuLite™ CLIA 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.

For Orders and Inquiries, please contact:

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Please visit our website to learn more about our other interesting products and services.