2.0 SUMMARY AND EXPLANATION OF THE TEST

Thyroxine Binding Globulin (TBG), a 54 kD liver glycoprotein, is the principal carrier protein for T4 and T3 in circulation. Electrophoretic analyses indicate that T4 is bound, in decreasing order, to TBG, to a T4 binding prealbumin (TBPA) and to albumin. The ratio of TBG to total protein is a major determinant of overall binding capacity. The interaction between T4 and its binding proteins conforms to a reversible Michaelis-Menten equilibrium in which the majority of the hormone is bound and a very small portion (~0.05%) is free. T3 is not bound by TBPA and is bound by TBG less firmly than is T4. As a consequence, proportion of free T3 is normally 8-10 times greater than T4. Only free (T3/T4) hormones are available to the tissues, therefore the metabolic state of the patient will correlate more closely with the free than with the total concentration of the hormones.

The diagnostic accuracy of the total hormone measurements would be equal to the free hormone if all the patients had similar binding protein concentrations. Unfortunately, serum TBG abnormalities that distort the total-free relationship are commonly encountered in clinical practice. Additionally, the presence of antibodies to TBG may, in some patients render total hormone measurements unreliable. Considerable confusion still exists regarding the validity of free hormone testing. There is controversy regarding the clinical utility of free hormone testing in conditions associated with binding protein abnormalities of pregnancy and non-thyroidal illness. Methods that are sensitive to albumin concentrations, the effect of certain drugs, high free fatty acid and levels of hormones binding inhibitors are considered more desirable. However, the techniques for physically separating the exceedingly small amounts of free hormones from the dominant protein bound moiety are technically demanding, inconvenient and expensive for a routine clinical laboratory. Such methods that employ equilibrium dialysis, ultrafiltration and gel-filtration are typically used by researchers. In routine analysis the clinical laboratories rely on direct measurements of free and total hormones and their binding proteins, mainly TBG.

Based on their serum concentrations, familial TBG variants are encountered in clinical practice. Additionally, the presence of ally binding protein concentrations. Unfortunately, serum TBG would be equal to the free hormone if all the patients had similar

2.1 INTRODUCTION

Thyroxine Binding Globulin (TBG) is a minor liver glycoprotein. The enzyme activity in the antibody-bound fraction is inversely proportional to the antibody concentration. A simultaneous reaction between the biotin attached to the microtiter plate and the enzyme-antigen conjugate results in the formation of an insoluble complex that is measured with an automated microtiter plate luminometer.

The employment of several serum references of known TBG levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with TBG concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):

The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the following equation:

\[ E_{\text{Ag}} + \text{Ag + Ab}_{\text{Ag}} \rightleftharpoons \text{AgAb}_{\text{Ag}} + E_{\text{Ag}} \text{Ab}_{\text{Ag}} \]

where:

- \( E_{\text{Ag}} \) = Native Antigen (Variable Quantity)
- \( \text{Ag} \) = Enzyme-antigen Conjugate (Constant Quantity)
- \( \text{Ab}_{\text{Ag}} \) = Antigen-antibody Complex
- \( \text{AgAb}_{\text{Ag}} \) = Enzyme-antigen Conjugate - Antibody Complex
- \( K_d = \text{Rate Constant of Association} \)
- \( K_a = \text{Rate Constant of Dissociation} \)
- \( K = K_a / K_d = \text{Equilibrium Constant} \)

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effect is the separation of the antigen bound fraction after decapitation or aspiration.

3.0 REAGENTS

Materials Provided:

- TBG Calibrators – 0.5 mIU/l - Icons A-F
- TBG tracer – 1 mIU/l - Icon E
- TBG Antibody (Variable Quantity)
- TBG diluent (1:20)
- TBG standard and TBG calibrator concentrations.
- To use, dilute as outlined in Example 1.
- One (1) vial containing Streptavidin (1:100,000 dilution) in buffer, dye, and preservatives. Store at -20°C.
- 1. Light Reaction Wells – 96 wells
- 2. Working Signal Solution 8-90°C
- 3. Wash Buffer
- 4. Wash Buffer Concentrate – 20.0ml/l - Icon B
- 5. Signal Solution – 20.0ml/l - Icon C
- 6. Wash Solution Concentrate – 20.0ml/l - Icon D
- 7. Wash Solution Concentrate – 20.0ml/l - Icon E

Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the international reference material (IS 88/638).

2.8 STORAGE AND STABILITY

- The calibrators and controls are stable for 60 days when stored at 2-8°C.
- The kit and component stability are identified on the label.
- The kit components are stable for 60 days when stored at 2-8°C.

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

4.0 REAGENT PREPARATION

4.1 Required But Not Provided:

- Pipette capable of delivering 0.010 and 0.050 ml (10 & 50 µl) volumes with a precision of better than 1.5%.
- Multi-channel dispensers (for delivering 0.10 and 0.50 ml (100 & 500 µl) volumes with a precision of better than 1.5%.
- Microplate washers or a squeeze bottle (optional).
- Microplate washer or a pipet (optional).
- Absorbent Paper for blotting the microwell.
- Plastic wrap or microplate covers for incubation steps.
- Vacuum aspirator (optional) for wash steps.
- Quality control materials.

5.0 PRECAUTIONS

- All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and Antibodies to Hepatitis C Virus.

Note 1:

- The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the international reference material (IS 88/638).

Note 2:

- The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the international reference material (IS 88/638).

6.0 SPECIMEN COLLECTION AND PREPARATION

- The specimens shall be blood, serum or heparinised plasma in tubes with the following characteristics:
- One (1) vial containing Streptavidin (1:100,000 dilution) in buffer, dye, and preservatives. Store at -20°C.
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12.0 RISK ANALYSIS

1. The RLU of calibrator F should be > 50,000. In order for the assay results to be considered valid the Q.C. PARAMETERS should not.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The RLU of calibrator F should be > 50,000.
2. Four out of six quality control Polos should be within the established ranges.

12.0 RISK ANALYSIS

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. 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-variation 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.

13.0 EXPECTED RANGES OF VALUES

Based on a study of an apparent normal population and established references, a normal range for TBG AccuLite® CLIA Test System was established, as mentioned below.

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 procedure to selected substances was evaluated by adding the interfering substance to a pooled serum matrix at various concentrations; the cross-reactivity was calculated by deriving a ratio between interfering substance to dose of TBG needed to produce the same RLU.
9. 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.
10. 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.

11. 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.

1.2 Interpretation

1. Measurements and interpretation of results should 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 AccuLite® CLIA procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimen 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. (Boscato, LM, Stuart, MC, "Heterophile 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, pathology, laboratory 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 imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the TBG AccuLite® CLIA Test System 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 2 and Table 3.

As measured in ten experiments in duplicate.

14.2 Sensitivity

The TBG AccuLite® CLIA Test System procedure has a sensitivity of 1.0 µg/ml (lowest calibration). The sensitivity was ascertained by determining the variability of the 0.1 µg/ml calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The TBG AccuLite® CLIA test system was compared against a reference method. Biological specimens (n=167) from population (symptomatic and asymptomatic) were used. The values ranged from 0 – 97µg/ml. The correlation is presented in Table 4.

14.4 Linearity & Hook Effect

The test will not be affected by TBG concentrations up to 3400 µg/ml in serum or plasma.

15.0 REFERENCES


Revision: 3 Date: 2013-APR-18 DCO: 0839 Product Code: 3575-300

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
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<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>4.3</td>
<td>0.16</td>
<td>3.6%</td>
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<tr>
<td>Level 2</td>
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<td>11.8</td>
<td>1.10</td>
<td>9.3%</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
<td>19.6</td>
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<table>
<thead>
<tr>
<th>Method</th>
<th>Least Squares Regression Analysis</th>
<th>Correlation Coefficient</th>
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<tbody>
<tr>
<td>Monobind (y)</td>
<td>15.2767 y = 1.0997+1.0192(x) 99.1% Reference (x)</td>
<td>15.3709</td>
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</table>

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
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<tbody>
<tr>
<td>Bilirubin</td>
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</tr>
<tr>
<td>Lipids</td>
<td>ND</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>ND</td>
</tr>
<tr>
<td>Human IgG</td>
<td>ND</td>
</tr>
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</table>

TABLE 1

\[
\text{Mean (x)} = 15.3709, \text{C.V.} = 9.0% \\
\text{Regression Analysis} = 15.2767 y = 1.0997+1.0192(x) 99.1%
\]

TABLE 2

Within Assay Precision (Values in µg/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>4.3</td>
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<td>20</td>
<td>19.6</td>
<td>1.60</td>
<td>8.2%</td>
</tr>
</tbody>
</table>

| Level 1 | 10 | 4.6 | 0.31 | 6.7% |
| Level 2 | 10 | 12.1 | 1.09 | 9.0% |
| Level 3 | 10 | 21.1 | 1.01 | 4.8% |

*As measured in ten experiments in duplicate.

1. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
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3. 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.

13.0 EXPECTED RANGES OF VALUES

Based on a study of an apparent normal population and established references, a normal range for TBG AccuLite® CLIA Test System was established, as mentioned below.

11.0 O.C.Q. PARAMETERS

<table>
<thead>
<tr>
<th>Sample</th>
<th>RLU</th>
<th>Mean (x)</th>
<th>C.V.</th>
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<tbody>
<tr>
<td>Cal A</td>
<td>34717</td>
<td>100000</td>
<td>1</td>
</tr>
<tr>
<td>B1</td>
<td>105283</td>
<td>40661</td>
<td>16</td>
</tr>
<tr>
<td>Cal B</td>
<td>77485</td>
<td>67687</td>
<td>4</td>
</tr>
<tr>
<td>D1</td>
<td>76248</td>
<td>60151</td>
<td>8</td>
</tr>
<tr>
<td>Cal C</td>
<td>F1</td>
<td>58436</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>38983</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1</td>
<td>41429</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal D</td>
<td>A2</td>
<td>24998</td>
<td>23933</td>
</tr>
<tr>
<td>B2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cal E</td>
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<td></td>
</tr>
<tr>
<td>D2</td>
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<tr>
<td>B3</td>
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</table>

Note: Computer data reduction software designed for CLIA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.