2.0 SUMMARY AND EXPLANATION OF THE TEST

Thyroxine Binding Globulin (TBG), a 54 kD liver glycoprotein, is the principal transport 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. Such an order is of little help in a clinical setting for T4, TBG is by far the major determinant of overall binding capacity. The interaction between T4 and its binding proteins conforms to a reversible 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 against this protein in some patients renders 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 reliable measurements. However, the techniques for physically separating the exceedingly small amounts of free hormones from the dominant protein bound moiety are too 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 divided into four major categories: excess, normal, partial deficiency and complete absence. The studies show that estrogens (pregnancy and oral contraceptives), acute intermittent porphyria and chronic liver disease increase TBG concentrations, while androgenic and anabolic steroids, large doses of glucocorticoids and nephrosis decrease TBG levels.

In this method, TBG calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated polyclonal antibody (highly specific for TBG) and enzyme labeled TBG are added in sequence, and the reactants mixed. Reaction between the TBG antibodies, enzyme labeled TBG and native TBG forms a complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the excess enzyme conjugate is separated from the bound fraction via a wash step. The competition reaction on the surface of the well is quantified by reaction with a suitable signal reagent to produce light.

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 antigen of interest, 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:

\[
\frac{\text{K}_{\text{a}}}{\text{K}_{\text{d}}} = \frac{\text{K}_{\text{a}}}{\text{K}_{\text{d}}} = \text{Equilibrium Constant}
\]

AbBtn = Biotinylated Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
EnzAgBtn = Enzyme-antigen Conjugate (Constant Quantity)
AgBtn = Antigen-Antibody Complex
EnzAgBtn = Enzyme-Antigen-Conjugate Antibody Complex
K_a = Rate Constant of Association
K_d = Rate Constant of Dissociation
K = K_a / K_d = 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 antibody bound fraction after decapitation or aspiration.

After completion of the required incubation period, the excess antibody and enzyme conjugate is separated from the bound fraction via a wash step. The activity of the enzyme present on the surface of the well is then quenched.

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Thyroxine Binding Globulin (TBG) concentration in Human Serum, Plasma or Whole Blood by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 REAGENT PREPARATION

8.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of TBG in unknown samples. Repeat the RU obtained from the printout of the luminescence as outlined in Example 1.

3.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20 ± 2°C).

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

2. Pipette 0.010ml (10 µl) of the corresponding serum reference control, calibrator or specimen into the assigned wells.

3. Add 0.050ml (50 µl) of the TBG Tracer Reagent to each well. Mix gently to ensure complete mixing. It is very important to dispense all reagents close to the bottom of the well coated. Stir gently.

4. Add 0.300ml (300 µl) of the TBG Biotin Reagent to each well. Mix gently. Allow the reaction to continue for 3-4 hours at room temperature.

5. Discard the contents of the microwell by decantation or aspiration. If decamping, tap and plate the dry with absorbent paper.

6. Incubate 30 minutes at room temperature.

7. Add 0.350ml (350 µl) of wash buffer (see Reagent Preparation Section) to each well. Decamp (tap and blot) and aspirate. Repeat four 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 employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Do not shake the wash and repeat four (4) additional times.

8. Add 0.350ml (350 µl) of wash buffer (see Reagent Preparation Section) to each well. Decamp (tap and blot) and aspirate. Repeat four 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 employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Do not shake the wash and repeat four (4) additional times.

9. Add 0.100ml (100 µl) of working Signal Reagent A to all wells (see Reagent Preparation Section).

10. Read the RLU’s (Relative Light Units) in each well in a microplate luminometer for at least 0.2 seconds/well. The result can be read within 30 minutes of adding the signal solution.
12.1 Assay Performance

Following criteria should be met:

- Prepared with each assay.
- The data presented in Example 1 and Figure 1 is for illustration purposes.

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.

12.2 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 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 immunossays. (Boscato, LM, Stuart, MC. "Heterophilic antibodies: a problem for all immunossays" Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, pathologic examination, and all other clinical findings.

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.

### TABLE 1

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>ND</td>
</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>
</tbody>
</table>

14.5 Linearity & Hook Effect

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

15.0 REFERENCES


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