SUMMARY AND EXPLANATION OF THE TEST

TBG (Thyroxine Binding Globulin) a 54 kD liver glycoprotein is the principal binding 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. By virtue of its size and abundance in serum, TBG is by far the major determinant of overall binding capacity. The interaction between T4 and its binding proteins conforms to a reversible binding 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 tissues 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 antibodies to thyroid hormones, 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. 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 decreases TBG levels.

The specimen shall be blood; serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to establish normal values, a homogenous serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow blood to clot for 4 hours and aspirate or centrifuge the serum. Refrigerate the specimen to separate the serum from the cells. Samples may be refrigerated at 2-8°C for a maximum period of 48 hours. If the specimen cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C or below for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.020ml of the specimen is required.

REAGENT PREPARATION:

Provided:

A. TBG Calibrators – 0.5 ml/ul - Icons A-F

B. TBG Enzyme Reagent – 5.5 ml/ul

Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the international reference material (IS 68/638).

SPECIMEN COLLECTION AND PREPARATION:

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REAGENT PREPARATION:

A. Wash Buffer – 7ml/vial - Icon SB

B. Stop Solution – 8ml/vial - Icon

For In Vitro Diagnostic Use

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed changes in the reagents or the instrument state. Frequent quality control reagents should be used to determine the reason for the variations.

CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of TBG in unknown specimens.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of TBG for an unknown, locate the average absorbance of the duplicates 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). (See Figure 1).

EXAMPLE 1

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction.

Q.C. PARAMETERS
In order for the assay results to be considered valid the following criteria should be met:
1. The absorbance (OD) of calibrator F should be > 1.3.
2. Four out of six quality control pools should be within the established ranges.

EXPECTED RANGES OF VALUES
Based on a study of an apparent normal population and established references a normal range for AccuBind™ TBG EIA Microplate Test System was established as mentioned below.

Normal Range

<table>
<thead>
<tr>
<th>Substance</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipids</td>
<td>ND</td>
</tr>
<tr>
<td>Lipoproteins</td>
<td>ND</td>
</tr>
<tr>
<td>Human IgG</td>
<td>1.10 - 2.86</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0.0 - 1.0 mg/dl</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.0 - 2.0 mmol/L</td>
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REFERENCES

For information on other Monobind products please visit the website or contact techsupp@monobind.com.

Monobind offers several instruments, including the Impulse 2 Luminometer CLIA Plate Reader designed hand-in-hand with our products and capable of 2-point calibration. Visit our website for more information.

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Monobind's immunoassay products are designed to work in both manual and automated lab environments. AccuBind™ and AccuLite™ are compatible with any open-ended instrumentation, including chemistry analyzers, microscope readers and microplate washers. There may or may not be an application developed for your particular instrument, please visit the instrument section of our website, or contact techsupp@monobind.com.

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Table C.

<table>
<thead>
<tr>
<th>Code</th>
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<tbody>
<tr>
<td>Cal A</td>
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<td>1.06</td>
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<tr>
<td>Cal B</td>
<td>11.8</td>
<td>1.10</td>
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<tr>
<td>Cal C</td>
<td>19.6</td>
<td>1.60</td>
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</table>

Within Assay Precision (Values in µg/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
<th>Level</th>
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<th>20</th>
<th>40</th>
<th>80</th>
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<tbody>
<tr>
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</table>

*As measured in ten experiments in duplicate.