1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Sex Hormone Binding Globulin (SHBG) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric.

2.0 SUMMARY AND EXPLANATION OF THE TEST

In order for the sex-steroids testosterone, 5α-dihydrotestosterone, and 17β-estradiol to reach the tissues, a transport molecule known as the Sex Hormone Binding Globulin (SHBG) is used. No more than 10% of these steroids are actually in plasma unbound and, therefore, biologically active. This protein’s steroid binding capacity is highly dependent on the temperature and pH of its environment.1,2,3 Originating in the liver, this glycoprotein is a 93.4 kDa homodimer. Overall, SHBG is responsible for the balance of steroid levels and in certain instances has an influence on the effects these steroids have on their targets. The serum levels of this globulin have been shown to vary drastically between individuals, even if considered to be in a “normal” state of health.2,3,4

Physiological status changes due to hormonal, metabolic, and nutritional factors are reflected in the concentration of SHBG in serum. SHBG is also decreased by the presence of a steroid, whereas a rise in androgens inhibits SHBG production. Aging and conditions like polycystic ovarian syndrome demonstrate this effect.2,3,4 SHBG levels also correlate strongly with conditions like hyperthyroidism, insulin resistance, central adiposity, and dyslipidemia. More importantly, low levels of SHBG shows an increased risk for diabetes and cardiovascular disease.2

SHBG is also used to calculate the Free Androgen Index (FAI). The calculation method has been applied frequently to the determination of Free Testosterone (FT) levels. In the past, analog methods and dialysis have been used to obtain testosterone levels, but each has a very striking downfall: analog methods tend to give values substantially lower than the actual clinical status and dialysis is a laborious technique. This calculated method uses SHBG, total testosterone (T) and albumin concentrations to calculate the free Testosterone in human serum.5,6

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3): The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-SHBG antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labelled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$\text{Enz}_{\text{Ab}} + \text{Ag}_{\text{SHBG}} + \text{Ab}_{\text{SHBG}} \rightarrow \text{Enz}_{\text{Ab}} + \text{Ag}_{\text{SHBG}} + \text{Ab}_{\text{SHBG}}$$

Immobilized complex = sandwich complex bound to the well surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decapitation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing 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.

4.0 REAGENTS

Materials Provided:

A. SHBG Calibrators – 1ml/vial - Icons A

B. SHBG Enzyme Reagent – 12ml/vial - Icons C

C. SHBG Diluent – 60ml/vial – Icons D

D. Streptavidin Plate – 96 wells – Icon E

E. Wash Solution Concentrate – 20ml/vial – Icon F

F. Substrate A – 0.250ml (250µl) of 0.05mM 3,3’5,5’-Tetramethylbenzidine (TMB) in acetate buffer.

G. Substrate B – 0.250ml – Icon G

H. Stop Solution – 8ml/vial - Icon H

I. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.

Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

1. Pipette capable of delivering 0.025 & 0.050ml (25 & 50µl) volumes with a precision of better than 1.5%.

2. Dispensers (for repetitive deliveries of 0.100 & 0.300ml (100 & 300µl) volumes with a precision of better than 1.5%.

3. Microplate washers or a squeeze bottle (optional).

4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.

5. Absorptometer Paper for blotting the microplate wells.

6. Plastic wrap or microplate sealing strips.

7. Vacuum aspirator (optional) for wash steps.

8. Timer.


5.0 PRECAUTIONS

Don't use for the any imaging diagnosis.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum and the usual precautions in the collection of venipuncture samples should be observed. For further details refer to the established normal values, a fasting morning sample serum should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anticoagulants. All blood to clot for samples. 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) cannot be assayed within this time, the sample(s) should be stored at -20°C for up to 30 days. Avoid use of contaminated containers. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated ranges for monitoring assay performance. These controls should include knowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents.

Preparation of an acceptable quality control procedure should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or deterioration 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 diluted buffer at 2-30°C for up to 60 days.

2. Add 0.100ml (100µl) of 0.05mM 3,3’5,5’-Tetramethylbenzidine (TMB) in acetate buffer. Store diluted buffer at 2-30°C for up to 60 days.

3. Add 0.100ml (100µl) of 0.05mM 3,3’5,5’-Tetramethylbenzidine (TMB) in acetate buffer. Store diluted buffer at 2-30°C for up to 60 days.

3. Add 0.100ml (100µl) of 0.05mM 3,3’5,5’-Tetramethylbenzidine (TMB) in acetate buffer. Store diluted buffer at 2-30°C for up to 60 days.

4. To determine the concentration of SHBG for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting points and read the concentration (in nmol/L) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the absorbance for 0.050ml of 0.05mM 3,3’5,5’-Tetramethylbenzidine (TMB) is read at 420.60nm SHBG concentration (See Figure 1).

Note: Data computation software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.
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. Pipettes, 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 V/Sl Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. Measurements and interpretation of results must 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 data.

3. The reagents for AccuBind® ELISA procedures 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 immunosassays (Boscato LM, Stuart MC. "Heterophilic antibodies: a problem for all immunosassays" 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 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 predictive values for the calibrators fall within 10% of the assigned concentrations.

7. Serum SHBG concentration is dependent upon a multiplicity of factors: including if the patient is sensitized, how many times the patient has been exposed to a specific allergen etc. Total SHBG concentration alone is not sufficient to assess the clinical status. All the clinical findings especially specific allergy testing should be taken into consideration while determining the clinical status of the patient.

8. Since all atopic reactions are not SHBG mediated, all relevant clinical information should be taken into consideration before making any determination for patients who may be in the normal range.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" population the expected ranges for the SHBG AccuBind® ELISA Test System are detailed in Table 1.

13.0.1 Expected Values for the SHBG AccuBind® ELISA test system

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Monobind</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>8.96</td>
<td>4.6</td>
</tr>
<tr>
<td>High</td>
<td>160.39</td>
<td>184.02</td>
</tr>
<tr>
<td>Mean</td>
<td>56.09</td>
<td>49.79</td>
</tr>
<tr>
<td>Intercept</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>6.3037</td>
<td></td>
</tr>
<tr>
<td>Corr (R²)</td>
<td>0.9299</td>
<td></td>
</tr>
</tbody>
</table>

Only slight amounts of bias between this method and the reference method were indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The specificity of the SHBG AccuBind® ELISA test system, to closely related immunoglobulins was evaluated by adding those at twice the physiological concentrations to a serum matrix.

15.0 REFERENCES


---

**Figure 1: SHBG ELISA**

![SHBG ELISA Graph](image-url)

**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Monobind</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>8.96</td>
<td>4.6</td>
</tr>
<tr>
<td>High</td>
<td>160.39</td>
<td>184.02</td>
</tr>
<tr>
<td>Mean</td>
<td>56.09</td>
<td>49.79</td>
</tr>
<tr>
<td>Intercept</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>6.3037</td>
<td></td>
</tr>
<tr>
<td>Corr (R²)</td>
<td>0.9299</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Level 1</td>
<td>24</td>
<td>16.331</td>
<td>0.764</td>
<td>4.7</td>
</tr>
<tr>
<td>Control Level 2</td>
<td>24</td>
<td>49.516</td>
<td>2.128</td>
<td>4.3</td>
</tr>
<tr>
<td>Control Level 3</td>
<td>24</td>
<td>83.955</td>
<td>4.799</td>
<td>5.7</td>
</tr>
</tbody>
</table>

**TABLE 3**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Monobind</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>8.96</td>
<td>4.6</td>
</tr>
<tr>
<td>High</td>
<td>160.39</td>
<td>184.02</td>
</tr>
<tr>
<td>Mean</td>
<td>56.09</td>
<td>49.79</td>
</tr>
<tr>
<td>Intercept</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>6.3037</td>
<td></td>
</tr>
<tr>
<td>Corr (R²)</td>
<td>0.9299</td>
<td></td>
</tr>
</tbody>
</table>