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

\[
   k_{a} = \frac{k_{b}}{k_{d}} = \frac{[A]^{n}[B]^{m}}{[C]^{p}[D]^{q}}
\]

\( k_{a} \) – Enzyme-labeled Biotinylated Monoclonal Antibody (Excess Quantity)
\( k_{b} \) – Enzyme-labeled Biotinylated Monoclonal Antibody (Excess Quantity)
\( k_{d} \) – Rate Constant of Dissociation
\( k_{e} \) – Rate Constant of Association

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated by:

\[
   k_{f} = \frac{k_{g}}{k_{h}} = \frac{[E]^{r}}{[F]^{s}}
\]

\( k_{f} \) – Rate of Formation of Complex
\( k_{g} \) – Rate Constant of Dissociation
\( k_{h} \) – Rate Constant of Association

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different sera references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

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.27,28 Originating in the liver, this glycoprotein is a 93 kDa homodimer. Only SHBG is responsible for the majority 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. Rises in oestrogens occur with an increase in SHBG, whereas a rise in androgens inhibits SHBG production. Aging and conditions like polycystic ovarian syndrome demonstrate the actual effect.2,3 SHBG levels also correlates strongly with conditions like polyscystic ovarian syndrome demonstrate this effect.2,3,5 SHBG levels also correlates strongly with conditions like polyscystic ovarian syndrome demonstrate this effect.2,3,5 SHBG levels also correlates strongly with conditions like hyperthyroidism, insulin resistance, central adiposity, and conditions like polyscystic ovarian syndrome.5,6,7

SHBG antibody.

Several different sera 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 - Icos A-F
       Six (6) vials of human serum reference calibrators at concentrations of 0.0 (10), 25 (B), 50 (D), 100 (E) and 250 (F) nmol/L. A preservative has been added. Store at 2-8°C.<br>(The Calibration are normalized against WHO’s 27 IS 08/2666 for SHBG)

B. SHBG Enzyme Reagent – 2ml/vial - Icon
       Contains biotinylated monoclonal antibody (Varied Quantity) and biotinylated anti-human SHBG presented in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

C. SHBG Diluent – 60ml/vial – Icon

   One (1) vial of a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

D. Streptavidin Plate – 96 wells – Icon

   One (1) 96-well microplates coated with streptavidin and packaged in an aluminum bag with a dry agent. Store at 2-8°C.

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

   Wash Solution Concentrate is intended for use in the enzyme-linked immunosorbent assay (ELISA) technique. Store at 2-8°C.

F. Substrate A – 7ml/vial - Icon

   Substrate A is composed of tetramethylbenzidine (TMB) in acetate buffer. Store at 2-8°C.

G. Substrate B – 7ml/vial - Icon

   Substrate B contains hydrogen peroxide (H₂O₂) in acetate buffer. Store at 2-8°C.

H. Stop Solution – 8ml/vial - Icon

   Stop Solution contains a strong acid (1N HCl). Store at 2-8°C.

1. Product Instructions.

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

   Note 2: Do not use reagents that are contaminated or have expired. The reagents are stable for sixty (60) days when stored at 2-8°C.

2. Working Substrate Solution – Stable for several days to store at 2-8°C. Kit and component stability are identified on the label.

3. Place the kit in a refrigerator and allow it to reach room temperature. The kit is stable at room temperature for five (5) minutes.

4. Add 0.050ml (50µl) of stop solution to each well and gently mix.

5. Incubate 30 minutes at room temperature.

6. Discard the contents of the microplate by decantation or aspiration. Rinse, tap and blot the plate dry with absorbent paper.

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

2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control and patient specimen to be assayed.

   *Patient sample dilution required see 8.0 Reagent Preparation.

3. Add 0.100ml (100µl) of the SHBG Enzyme Reagent to each well.

   It is very important to dispense all reagents close to the bottom of the well.

4. Spin the microplate gently for 20-30 seconds to mix and for a total of (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instructions for proper usage. If a squeeze bottle is employed, fill well each well by depressing the container (avoiding air bubbles) to displace the wash. Decant the wash and repeat two (2) additional times.

5. Add 0.100ml (100µl) of working substrate solution to all wells (see Reagent Preparation). Allow substrate to react in both (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instructions for proper usage. If a squeeze bottle is employed, fill well each well by depressing the container (avoiding air bubbles) to displace the wash. Decant the wash and repeat two (2) additional times.

6. Add 0.050ml (50µl) of stop solution to each well and gently mix.

Note 3: Do not use the printed chart to determine the results.

7. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The average should be read within thirty (30) minutes of adding the stop solution.

10.0 CALCULATION OF RESULTS

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

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.1.

2. Plot the absorbance for each duplicate serum reference versus the corresponding SHBG concentration in nmol/L (for example: 0.100µl of a reference calibrator, control and patient specimen to be assayed in duplicate).

3. Create a computer data reduction software designed for ELISA assays. This allows the computer to utilize the validated software for the test.

4. To determine the concentration of SHBG for an unknown, locate the average absorbance of the duplicates for each unknown specimen. The average should be read within thirty (30) minutes of adding the stop solution.

Note: Computer data reduction software designed for ELISA assays may not be used for this test. If such software is utilized, the validation of the software should be ascertained.

Note: Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have bacteria growth.
1.10 QC PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:
1. The absorbance (OD) of calibrator 'A' should be \(< 0.05\).
2. The absorbance (OD) of calibrator 'F' should be \(> 1.3\).
3. Four out of six quality control pools should be within the established ranges.

1.20 RISK ANALYSIS

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

1.2.1 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 determinations.

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. Heterophile antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. "Heterophilic antibodies: a problem for all immunoassays" Clin.Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be used in conjunction 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 incompletely interpreted, Monobind shall have no liability.

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.