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SEX HORMONE BINDING GLOBULIN (SHBG) ELISA

EU: CE IVD **CAN:** IVD **USA:** For Research Use Only. Not for Use in Diagnostic Procedures

REF: CAN-SHBG-4010 Version 8.1 (COMB)
Effective: October 17, 2023

INTENDED USE

For the quantitative determination of Sex Hormone Binding Globulin by an enzyme immunoassay in human serum.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for SHBG is immobilized onto the microplate and another monoclonal antibody specific for a different region of SHBG is conjugated to horse radish peroxidase (HRP). SHBG from the sample and standards are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of SHBG in the sample.

A set of standards is used to plot a standard curve from which the amount of SHBG in patient samples and controls can be directly read.

CLINICAL APPLICATIONS

Sex hormone binding globulin (SHBG) is a glycoprotein composed of 373 amino acid residues and three carbohydrate side chains. SHBG has been known by many other names including Testosterone-estradiol Binding Globulin (TeBG), Sex steroid Binding Protein (sBP) and Sex Steroid Binding Globulin (SSBG). One of the main properties of SHBG is its high affinity for steroids, especially the C18, C19 and 17α-hydroxyl groups. The binding of steroids to SHBG is temperature and pH dependent. The three steroids that have a high avidity for SHBG are Dihydrotestosterone, Testosterone and Estradiol. Very small amounts of these steroids are free in biological fluid; the majority are bound to SHBG and albumin. These two fractions, that is, free and bound exist in a state of dynamic equilibrium. When the level of SHBG concentration changes, a remarkable change occurs in both albumin-bound hormone and also in the free fraction.

Throughout life SHBG increases until the eighties in both sexes. During the menstrual cycle SHBG does not seem to vary appreciably, however, according to some authors the concentration of SHBG is elevated in the luteal phase. During pregnancy the level of SHBG rises rapidly until about the 30th week of gestation.

Many agents or conditions that affect SHBG levels include:

- Estrogens and thyroid hormone cause an increase in the concentration of plasma SHBG levels.
- Androgens, growth hormone (GH) (in vivo), prolactin (in vivo), insulin (in vitro), obesity and high lipids cause a decrease in the concentration of plasma SHBG levels.

Clinical Trends:

- Increased SHBG levels occur in hypogonodal men, hyperthyroidism, alcoholic liver disease, primary biliary cirrhoses (women), anorexia nervosa (women).
- Decreased SHBG levels occur in myxedema, hyperprolactinaemia (women), acromegaly growth hormone therapy, obesity and congenital adrenal hyperplasia.

PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of SHBG in human serum. The kit is not calibrated for the determination of SHBG in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only assay buffer may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.
6. Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

Dilute patient serum samples 1:10 in assay buffer before use. Example: To 90 µL of assay buffer add 10 µL of serum sample.

*Do not dilute the standards and controls, they are ready for use.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 10, 20, 50, 90, 150 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 13)

REAGENTS PROVIDED

1. Mouse Anti-SHBG Antibody-Coated Break-Apart Well Microplate — Ready To Use
Contents: One 96-well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

2. Mouse Anti-SHBG Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate — Requires Preparation X50
Contents: Anti-SHBG monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 0.4 mL/vial
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µL of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 300 µL of HRP in 15 mL of assay buffer. Discard any that is left over.

3. SHBG Calibrators — Ready to Use
Contents: Six vials containing SHBG in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of SHBG. Calibrated against World Health Organization (WHO) 1st IS 95/560.
* Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Calibrator	Concentration	Volume
Calibrator A	0 nmol/L	0.4 mL/vial
Calibrator B	3.3 nmol/L	0.4 mL/vial
Calibrator C	12.5 nmol/L	0.4 mL/vial
Calibrator D	55 nmol/L	0.4 mL/vial
Calibrator E	160 nmol/L	0.4 mL/vial
Calibrator F	295 nmol/L	0.4 mL/vial

Storage: Refrigerate at 2–8°C.
Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. Controls — Ready to Use
Contents: Two vials containing SHBG in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of SHBG. Refer to vial labels for the acceptable range.
Volume: 0.4 mL/vial
Storage: Refrigerate at 2–8°C
Stability: 12 months in unopened vials or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate — Requires Preparation X10
Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water

6. Assay Buffer — Ready to Use
Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
Volume: 55 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

7. TMB Substrate — Ready To Use
Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume: 16 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

8. Stopping Solution — Ready To Use
Contents: One bottle containing 1M sulfuric acid.
Volume: 6 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

ASSAY PROCEDURE

Specimen Pretreatment:
Dilute 1:10 With Assay Buffer Before Use.

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the anti-SHBG-HRP conjugate and wash buffer.
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 20 µL of each calibrator, control and diluted specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 200 µL of assay buffer into each well. (We recommend using a multichannel pipette.)
5. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
6. Wash the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette 150 µL of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
8. Incubate on a plate shaker (approximately 200 rpm) for 15 minutes at room temperature.
9. Wash the wells again in the same manner as step 6.
10. Pipette 150 µl of TMB substrate into each well at timed intervals.
11. Incubate on a plate shaker for 10–15 minutes at room temperature (or until calibrator F attains dark blue colour for desired OD).
12. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 10.
13. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the “0” calibrator from the mean absorbance values of the calibrators, controls and serum samples.
4. Draw a calibrator curve on log-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
5. Read the values of the unknowns directly off the calibrator curve.
6. If a sample reads more than 295 nmol/L, then dilute it with assay buffer at a dilution of no more than 1:10 (from original 1:10 dilution). The result obtained should be multiplied by the dilution factor.

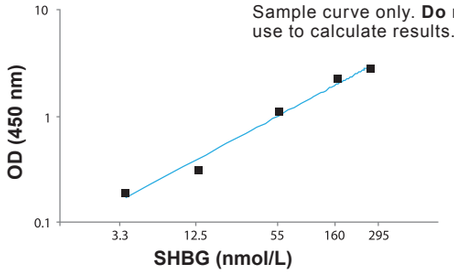
TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	OD 1	OD 2	Mean OD	Value (nmol/L)
A	0.088	0.086	0.087	0
B	0.185	0.189	0.187	3.3
C	0.309	0.311	0.310	12.5
D	1.090	1.075	1.083	55
E	2.263	2.250	2.257	160
F	2.772	2.738	2.755	295
Unknown	0.365	0.387	0.376	16.8

TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the DBC SHBG ELISA kit is **0.1 nmol/L**.

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the SHBG ELISA kit was determined by measuring the apparent SHBG value of samples spiked with high levels of Thyroxine Binding Globulin (TBG):

Substance	Concentration Range (mg/L)	Apparent SHBG Value (nmol/L)
Thyroxine Binding Globulin (TBG)	10–500	Not Detected

INTRA-ASSAY PRECISION

Four samples were assayed ten times each on the same calibrator curve. The results (in nmol/L) are tabulated below:

Sample	Mean	SD	CV %
1	4.5	0.39	8.6
2	16	0.68	4.3
3	57	1.70	3.0
4	158	8.4	5.3

INTER-ASSAY PRECISION

Four samples were assayed ten times over a period of four weeks. The results (in nmol/L) are tabulated below:

Sample	Mean	SD	CV %
1	3.8	0.44	11.6
2	19	1.60	8.4
3	63	5.50	8.7
4	194	14.0	7.2

RECOVERY

Spiked samples were prepared by adding defined amounts of SHBG to three patient serum samples. The results (in nmol/L) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1 Unspiked	39	-	-
+ 6.5	42	45.5	92.3
+ 28.5	67	67.5	99.3
+ 165	208	204	102.0
2 Unspiked	61	-	-
+ 6.5	63	67.5	93.3
+ 28.5	91	89.5	101.7
+ 165	224	226	99.1
3 Unspiked	157	-	-
+ 6.5	170	163.5	104.0
+ 28.5	210	185.5	113.2
+ 165	307	322.0	95.3

LINEARITY

Three patient serum samples were diluted with assay buffer. The results (in nmol/L) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1	58	-	-
1:2	29	29	100.3
1:5	12.4	11.6	106.9
1:10	5.6	5.8	96.6
2	85	-	-
1:2	42.5	42.5	100.0
1:5	19.9	17	117.1
1:10	9.2	8.5	108.2
3	120	-	-
1:2	61.2	60	102.0
1:5	24	24	100.0
1:10	12.2	12	101.7

HIGH DOSE HOOK EFFECT

The SHBG ELISA kit did not experience a high dose hook effect when it was tested up to a SHBG concentration of 10,000 nmol/L.

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Mean (nmol/L)	Range (nmol/L)
Males	104	31	7–70
Females	44	50	15–120

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SYMBOLS

CE European Comformity
IVD In vitro diagnostic device
Consult instructions for use
Contains sufficient for <n> tests
Storage Temperature
Legal Manufacturer
Use by
REF Catalogue Number
EC REP Authorized representative
LOT Lot number
X # Dilute 1: # Before use