



DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite E/F, Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

onestep@rapidtest.com

technicalsupport@rapidtest.com

www.rapidtest.com

IVD



See external label



2°C-8°C



Σ=96 tests

REF

#2043-6

SHBG ELISA

Cat. No. 2043-6

INTENDED USE

The SHBG Test is intended to be used for the quantitative determination of sex-hormone-binding globulin (SHBG) in serum.

Clinical background:

Sex-hormone-binding globulin (SHBG) is a β -globulin that specifically binds steroid hormones. The major site of SHBG synthesis is thought to be the hepatocytes. Its production is regulated by androgen/estrogen balance, thyroid hormones, insulin and dietary factors, among others. SHBG is involved in the transport of sex steroids in plasma. Its concentration is a major factor regulating their distribution between protein-bound and free states. Determination of SHBG concentration is mainly of importance in the evaluation of mild disorders of androgen metabolism and it allows identification of women with hirsutism that are likely to respond to estrogen therapy. Testosterone/SHBG –ratios correlate well with both measured and calculated values for free testosterone, and help to discriminate between subjects with excessive androgen activity and normal individuals.

PRINCIPLE OF TEST

A monoclonal antibody specific to SHBG is immobilized on microwell plates, and another monoclonal antibody, also specific to SHBG, is conjugated with horseradish peroxidase (HRP). SHBG from the sample is bound to the plates. After a washing step, HRP conjugate is added. After a second washing step, enzyme substrate is added. The enzymatic reaction is proportional to the amount of SHBG in the sample. Adding stopping solution terminates the reaction. Absorbance is measured on a plate reader.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

1. Multichannel pipette with disposable plastic tips;
100 μ l (assay buffer, conjugate, substrate),
50 μ l (stopping solution), 25 μ l (standards, samples)
2. Lid or sealing tape for microwell plate
3. Reagent troughs
4. Plate shaker
5. Aspiration device
6. Photometer (plate or strip reader), 450 nm

PRECAUTIONS

The SHBG TEST is tended for in vitro use only. The reagents contain the preservative thimerosal. The Control Serum has been prepared from human sera shown to be negative for HBsAg, HIV antibodies and HCV antibody. Nevertheless, such tests are unable to prove the complete absence of viruses, and the Control Serum should therefore be handled taking appropriate precautions.

SPECIMEN COLLECTIONS HANDLING

Serum and heparin plasma can be used. EDTA-plasma may give slightly lower results. No interferences resulting from hemolysis, lipemia or bilirubin has been observed. Specimens may be stored at 2-8°C for brief periods (approximately two days). For longer storage, specimens should be frozen. Frozen specimens should be well mixed after thawing, and before assay. Avoid repeated freezing and thawing.

DILUTION OF SAMPLES

Serum samples with SHBG concentrations greater than 300 nmol/l should be diluted further with the Assay Buffer. Correct the result using an appropriate dilution factor.

CONTENTS OF KIT - STORAGE INSTRUCTIONS

Reagents are sufficient for 96 wells.

Storage and stability: The kit should be stored at 2-8°C. The unopened kit is stable until the expiry date printed on the kit label. The expiry date of each unopened component is printed on the label of the component.

1. Breakpart Wells

96 wells on a plate coated with mouse monoclonal SHBG antibody, packed in a laminate bag.

Ready for use. Stability: After opening the laminate bag, wells are months at 2-8°C.

2. Assay Buffer, 1 x 80 ml

Ready for use. Stability: Once opened, stable for 2 months at 2-8°C.

3. SHBG Standards

Ready for use. Standards A-E 0.5 ml.

Calibrated against human SHBG. The standard values are about 0, 4, 16, 65 and 260 nmol/l.

Stability: Once opened, stable for 2 months at 2-8°C.

4. Control Serum, 0.5 ml

Stability: Once opened, stable for one week at 2-8°C.

5. Enzyme Conjugate (100x conc.), 0.15 ml

Mouse monoclonal SHBG antibody conjugated with horseradish peroxidase.

Dilute with Assay Buffer 1:100. Stability: Once opened, stable for 2 months at 2-8°C.

6. Wash Concentrate, 25 ml (40x conc.)

Before use, dilute in 975 ml distilled water.

Stability: Once diluted, stable for 2 months at 2-8°C.

7. TMB Substrate Solution, 14 ml

Tetramethyl benzidine substrate solution.

Ready for use. Stability: Once opened, stable for 2 months at 2-8°C.

8. Stop Solution, 14 ml

1N HCL. Ready for use. Stability: Once opened, stable for 2 months at 2-8°C.

REAGENT PREPARATION

Preparation of the Enzyme conjugate Solution:

Dilute the Conjugate Concentrate 1:100 with Assay Buffer as follows:

<u>No. of strips</u>	<u>Concentrated Conjugate</u>	<u>Assay Buffer</u>
1	10 µl	1 ml
2	20 µl	2 ml
3	30 µl	3 ml
4	40 µl	4 ml
5	50 µl	5 ml
6	60 µl	6 ml

7	70 µl	7 ml
8	80 µl	8 ml
9	90 µl	9 ml
10	100 µl	10 ml
11	110 µl	11 ml
12	120 µl	12 ml

TEST PROCEDURE

1. Allow all reagents to reach room temperature before use.
Dilute Wash Concentrate (with 1000 ml distilled water).
2. Mark the wells to be used on the plate.
3. Dilute standards, Control Serum and samples 1:20 with Assay Buffer, e.g. Dilute 10 µl standards, controls or samples with 200 µl Assay Buffer.
4. Pipette **100 µl of Assay Buffer** into each well.
5. Pipette **25 µl of diluted standards, control and serum samples** into appropriate wells.
6. Cover the plate and incubate for 30 minutes at room temperature.
7. Aspirate and wash the wells 3 times with 300 µl of washing solution.
8. Pipette 100 µl of **diluted Enzyme Conjugate** into the wells (see Preparation of Reagents).
9. Cover the plate and incubate for 15 minutes at room temperature.
10. Wash the wells as above (3 x 300 µl). At timed intervals add 100 µl of TMB Substrate Solution into each well. Cover the plate and incubate for 12 minutes at room temperature (20-25°C) for 8 minutes at room temperature (26°C and more).
11. Stop the reaction by adding 100 µl of Stop Solution into each well at the same timed intervals as in step 11. Shake the plate gently to mix the solutions.
13. Measure the absorbance at 450 nm using a plate or strip reader at least 5 min after stopping the Substrate reaction.

NOTES ON TECHNIQUE

1. Protect the plates from draught, strong light or direct sunlight during the test procedure.
2. Careful aspiration of the washing solution is essential for good assay precision.
3. Since timing of the incubation steps is important to performance of the assay, pipette the samples and the conjugate without interruption. Pipetting of samples should not exceed 10 minutes to avoid assay drift. If more than one plate is used in the same run, it is recommended to include a standard curve on each plate.
4. Adding of the TMB Substrate Solution starts a kinetic reaction that is terminated by dispensing the Stopping Solution. Keep the incubation times for each well the same by adding reagents at timed intervals.
5. Protected from light, absorbance values are stable for 60 minutes.
6. Plate readers measure absorbance vertically. Do not touch the bottoms of the wells.

TEST PROCEDURE – SUMMARY

	Standards 0 – 300 nmol/l (1:20)	Control Serum (1:20)	Samples (1:20)
Mark the strips			
Pipette Assay Buffer (µl)	100	100	100
Pipette diluted standards, Control Serum and samples (µl)	25	25	25
Incubate for 30 minutes at room temperature – Wash 3 times			
Pipette diluted Enzyme Conjugate (µl)	100	100	100
Incubate for 15 minutes at room temperature – Wash 3 times			
Pipette TMB Substrate Solution (µl)	100	100	100

Pipette Stopping Solution (μl)

Cover and incubate for 12 minutes at room temperature (20-25°C)		
100	100	100
Mix		
Measure absorbance at 450 nm		

RESULTS

Calculation of results

1. Calculate the mean absorbance for each duplicate.
2. Subtract the absorbance value of the zero standard from the mean absorbance values of standards, control and samples.
3. Draw the standard curve on log-log graph paper by plotting absorbance values of standards against appropriate SHBG concentrations.
4. Read off the SHBG concentrations for the control and the samples.

Quality Control

It is recommended that internal controls be used in every assay. Control results should be within established ranges.

EXPECTED VALUES

Serum samples from apparently healthy women and men were assayed using the SHBG ELISA TEST, with the following results:

	Number of Samples	SHBG nmol/l	
		mean	range
Men	102	43	15 – 100
Women	44	62	15 - 120

Each laboratory should determine its own reference range.

PERFORMANCE CHARACTERISTICS

1. Detection limit

On the basis of results of 16 replicate determinations of the zero standard, the minimum SHBG concentration detectable by the method is 0.2 nmol. The detection limit is defined as the value deviating by 2 SD from that of the zero standard.

2. Precision

Analysing three patient sera of different SHBG concentrations established Intra- and inter-assay precisions. The results are shown in Table 1 and 2.

Table 1. Intra-assay precision

Patient	Number of Replicates	Mean nmol/l	SD nmol/l	CV %
1	16	4.5	0.39	8.6
2	16	16	0.68	4.3
3	16	57	1.7	3.0
4	16	158	8.4	5.3

Table 2. Inter-assay precision

Patient	Number of Replicates	Mean nmol/l	SD nmol/l	CV %
1	16	3.8	0.44	11.6
2	16	19	1.6	8.4
3	16	63	5.5	8.7
4	16	194	14	7.2

3. Recovery

A known amount of SHBG was added to three patient sera and the quantities recovered were measured. The results are shown in Table 3.

Table 3. Recovery

Sample	Endogenous SHBG nmol/l	Added SHBG nmol/l	Expected SHBG nmol/l	Observed SHBG nmol/l	Recovery %
1	39	6.5	45.5	42	92
1	39	28.5	67.5	67	99
1	39	165	204	208	102
2	61	6.5	67.5	63	93
2	61	28.5	89.5	91	102
2	61	165	226	224	99
3	157	6.5	163.5	170	104
3	157	28.5	185.5	210	113
3	157	165	322	307	95

4. Linearity (Dilution test)

Four patient samples were diluted with Assay Buffer to 1:2, 1:5 and 1:10. SHBG values were assayed, and the results were corrected using dilution factors. Recovery results of these dilution tests are shown in Table 4.

Table 4. Dilution of samples

Sample	Undiluted SHBG nmol/l	Recovery %		
		1:2	1:5	1:10
1	58	100	107	97
2	85	100	117	108
3	120	102	100	102
4	185	89	95	96

5. Specificity

Specificity of the SHBG ELISA TEST was studied by measuring apparent SHBG response caused by high levels of TBG (Thyroxine Binding Globulin) and CBG (Cortisol Binding Globulin). No cross-reactions were found when testing up to 500 mg/l of TBG and 500 mg/l of CBG.

6. High-dose hook effect

The assay was tested for a hook effect up to a SHBG concentration of 10000 nmol/l. No hook effect was observed.

LITERATURE

- Moore, J.W. and Bulbrook R.D. (1988) *The epidemiology and function of sex hormone binding globulin*. IN: Oxford Reviews of Reproductive Biology, 10: 180-236.
- Selby, C. (1980) *Sex hormone binding globulin: origin, function and clinical significance*. Ann Clin Biochem 27: 532-541



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