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SHBG

cobas®

Human sex hormone-binding globulin

REF	Σ	SYSTEM
03052001 190	100	Elecsys 2010 MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

Intended use

Immunoassay for the in vitro quantitative determination of sex hormone-binding globulin in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Sex hormone-binding globulin (SHBG) is the blood transport protein for testosterone and estradiol. It is a large glycoprotein with a molecular weight of about 95 kD, and exists as a homodimer composed of two identical subunits. Each subunit contains two disulfide bridges.[1]

Planar C^{18} and C^{19} steroids with a 17α-hydroxyl group bind particularly well,[2,3] whereas C^{19} 17-ketosteroids such as dehydroepiandrosterone (DHEA) and androstendione do not bind so easily. SHBG has a high binding affinity to dihydrotestosterone (DHT), medium affinity to testosterone and estradiol, and only a low affinity to estrone, DHEA, androstendione, and estriol.

SHBG binds reversibly to sexual steroids. Albumin, which exists in far higher concentrations than SHBG, also binds sexual steroids – although with a clearly lower binding affinity (e.g. about 100 times lower for testosterone).

SHBG has a half-life of about 7 days and is produced mainly by the liver. Its synthesis and secretion are regulated by estrogen.[4,5] SHBG serum concentrations depend on the extent, duration, and the kind of estrogen applied, and how regulation takes place. Androgens and gestagens with androgenic residual action have the opposite effect.

In the serum SHBG mainly takes over the transportation of steroids and the reduction/regulation of the effect of androgen.[6,7] Decreased SHBG serum levels are associated with conditions where elevated androgen levels are present or where the effect of androgen on its target organs is excessive. This explains the gender-related differences seen between men and women, especially during puberty.

Measurement of SHBG can be an important indicator of an excessive/chronic androgenic action where androgen levels are normal, but where clinical symptoms would seem to indicate androgen in excess. SHBG is a useful supplementary parameter in the determination of androgen where a relatively high concentration of free androgen (e.g. testosterone) is suspected.[8]

By calculating the free androgen index (FAI), also called free testosterone index (FTI), from the ratio of total testosterone (TT) to SHBG [% FAI or FTI = (TT/SHBG) * 100], it is possible to calculate the approximate amount of free testosterone (FTc), as there is a direct correlation between FAI and FT. By additionally taking the non-specifically albumin-bound testosterone into account, it is possible to calculate the bioavailable testosterone (BATc), which is the sum of free testosterone and the albumin-bound testosterone fraction, calculated via the association constant to albumin. Only free testosterone is biologically active, and it best indicates the clinical situation of the patient. Free and bioavailable testosterone are also referred to as non-SHBG-bound testosterone and can be obtained by precipitation of the SHBG-bound-testosterone with ammonium sulfate, and by equilibrium dialysis.[9]

Elevated SHBG levels can be seen in elderly men, and are often found in patients with hyperthyroidism and cirrhosis of the liver. SHBG levels also increase when oral contraceptives or antiepileptic drugs are taken. Pregnant women have markedly higher SHBG serum concentrations due to their increased estrogen production. Decreased SHBG concentrations are often seen with hypothyroidism, polycystic ovarian syndrome (PCOS), obesity, hirsutism, elevated androgen levels, alopecia, and acromegaly.

The Elecsys SHBG assay employs two monoclonal antibodies specifically directed against human SHBG.

Cross-reactivity with α_1-fetoprotein (AFP), corticosteroid binding globulin (CBG), DHT, estradiol, fibrinogen, human immunoglobulin-A (IgA), human immunoglobulin G (IgG), plasminogen, thyroxine binding globulin (TBG), testosterone, thyroglobulin (Tg), transferrin, and thyrotropin (TSH) is negligible.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample, a biotinylated monoclonal SHBG-specific antibody, and a monoclonal SHBG-specific antibody labeled with a ruthenium complex[a)] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as SHBG.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-SHBG-Ab~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated monoclonal anti-SHBG antibody (mouse) 1.25 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 Anti-SHBG-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL:
Monoclonal anti-SHBG antibody (mouse) labeled with ruthenium complex 1.25 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines.

Safety data sheet available for professional user on request.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

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All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	7 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel, or lithium heparin plasma.

Do not use EDTA plasma.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 3 days at 2-8 °C, 1 month at -20 °C. Freeze only once.[10]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 03052028190, SHBG CalSet, for 4 x 1 mL
- REF 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- REF 03609987190, Diluent MultiAssay, 2 x 16 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against the 1st International Standard for SHBG from the National Institute for Biological Standards and Control (NIBSC) code 95/560.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Universal.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample either in nmol/L, µg/mL or mg/L (selectable).

Conversion factors: nmol/L x 0.095 = µg/mL (mg/L)

µg/mL (mg/L) x 10.53 = nmol/L

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Limitations - interference

The assay is unaffected by icterus (bilirubin < 1026 µmol/L or < 60 mg/dL), hemolysis (Hb < 1.8 mmol/L or < 2.9 g/dL), lipemia (Intralipid < 2700 mg/dL) and biotin (< 246 nmol/L or < 60 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1160 IU/mL.

There is no high-dose hook effect at SHBG concentrations up to 1000 nmol/L.

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.350-200 nmol/L (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.350 nmol/L. Values above the measuring range are reported as > 200 nmol/L (or up to 2000 nmol/L for 10-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: 0.350 nmol/L

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution

Samples with SHBG concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:10 (either automatically by the MODULAR ANALYTICS E170, Elecsys 2010 or **cobas e** analyzers or manually). The concentration of the diluted sample must be > 20 nmol/L.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

Expected values

The following table shows the results obtained from a group of 415 males and 343 females using the Elecsys SHBG assay. All subjects were apparently healthy, non-obese (BMI, body mass index ≤ 30), non-pregnant adults without intake of any contraceptive or relevant prescription drugs (study number CIM 000669). Blood samples (fasting) were taken between 6.30 am and 2.00 pm. This clinical study focusing on the Elecsys Testosterone II assay included measurements in parallel using the Elecsys SHBG assay. Please refer to the Elecsys Testosterone II package insert for SHBG values in combination with testosterone.

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		SHBG (nmol/L)	
	N	Median	5-95th percentiles
Males (20-49 years)	241	33.2	18.3-54.1
Males (≥ 50 years)	174	40.6	20.6-76.7
Females (20-49 years)	166	67.8	32.4-128
Females (≥ 50 years)	177	62.4	27.1-128

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean nmol/L	SD nmol/L	CV %	SD nmol/L	CV %
Human serum 1	14.1	0.30	2.1	0.39	2.7
Human serum 2	44.2	1.05	2.4	1.24	2.8
Human serum 3	204	5.61	2.7	11.4	5.6
PreciControl U[b)]1	34.9	0.76	2.2	0.92	2.6
PreciControl U2	19.0	0.41	2.2	0.52	2.7

b) U = Universal

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean nmol/L	SD nmol/L	CV %	Mean nmol/L	SD nmol/L	CV %
Human serum 1	14.9	0.17	1.1	13.7	0.24	1.8
Human serum 2	45.7	0.60	1.3	42.0	0.89	2.1
Human serum 3	219	3.76	1.7	189	7.58	4.0
PreciControl U1	35.3	0.46	1.3	33.1	0.63	1.9
PreciControl U2	19.2	0.21	1.1	18.1	0.42	2.3

Method comparison

A comparison of the Elecsys SHBG assay (y) with a commercially available SHBG test (x) using clinical samples gave the following correlations:

Number of samples measured: 109

Passing/Bablok[11]	Linear regression
y = 1.17x - 3.26	y = 1.15x - 1.82
τ = 0.909	r = 0.981

The sample concentrations were between approx. 11.2 and 155 nmol/L.

Analytical specificity

For the monoclonal antibodies used, non-detectable cross-reactivities were found for the following substances:

AFP, CBG, DHT, estradiol, fibrinogen, human IgA, human IgG, plasminogen, TBG, testosterone, Tg, transferrin, and TSH.

References

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing

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