Elecsys SHBG

cobas®

REF		Σ	SYSTEM
03052001160	03052001501	100	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

For use in the USA only

System Information

For **cobas e** 411 analyzer: test number 750
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 146

Intended use

Immunoassay for the in vitro quantitative determination of sex hormone-binding globulin in human serum and plasma. This assay is intended for use as an aid in the diagnosis of androgen disorders.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **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 2 identical subunits. Each subunit contains 2 disulfide bridges.[1]
Planar C^{18} and C^{19} steriods 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 steriods 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]
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 2 monoclonal antibodies specifically directed against human SHBG.
Cross-reactivity with α_1-fetoprotein (AFP), corticosteroid binding globulin (CGB), 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 or e-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 for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.
Prevention:	
P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.
Response:	
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

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Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: 1-800-428-2336

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.

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 5 days at 20-25 °C, 7 days at 2-8 °C, 12 months at -20 °C (± 5 °C). Freeze only once.[9]

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.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 03052028190, SHBG CalSet, for 4 x 1.0 mL
- REF 11731416160, PreciControl Universal, for 4 x 3.0 mL
- REF 03609987190, Diluent MultiAssay, 2 x 16 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **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, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **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, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Additional material for all analyzers:

- REF 11298500160, ISE Cleaning solution/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 approximately 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:

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

- 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.

If necessary, repeat the measurement of the samples concerned.

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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

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.

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

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

0.800-200 nmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.800 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

Limit of Blank, Limit of Detection and Limit of Quantitation
Limit of Blank = 0.500 nmol/L
Limit of Detection = 0.800 nmol/L
Limit of Quantitation = 2.00 nmol/L

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of 30 %. It has been determined using low concentration SHBG samples.
Please note: When reporting values < 2.00 nmol/L, the client report should be annotated with the following information. "Values < 2.00 nmol/L are not reliable as the total allowable error is > 30 %."

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 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 software automatically takes the dilution into account when calculating the sample concentration.

Expected values

The following tables show the results obtained using the Elecsys SHBG and Elecsys Testosterone assays in a reference population of 214 males and 160 females, who were classified as apparently healthy. The subjects health status of the apparently healthy group was confirmed by a standard clinical chemistry and hematology profile and a brief medical examination.

		SHBG (nmol/L)		**Testosterone (nmol/L)**	
	N	Median	5th-95th percentile	Median	5th-95th percentile
Males 20-49 yrs	136	33.5	16.5-55.9	18.6	8.64-29.0
Males ≥ 50 yrs	78	40.8	19.3-76.4	16.5	6.68-25.7
Females 21-49 yrs	89	64.3	24.6-122	0.941	0.290-1.67
Females ≥ 50 yrs	71	57.4	17.3-125	0.563	0.101-1.42

The free testosterone index (FTI), or free androgen index (FAI), was obtained as follows:
% FTI or FAI = ((Testosterone (nmol/L) ÷ SHBG (nmol/L)) x 100

	Free Testosterone Index (FTI)/Free Androgen Index (FAI), (nmol/L)[b)]		
	N	Median	5th-95th percentile
Males 20-49 yrs	136	57.2	35.0-92.6
Males ≥ 50 yrs	78	38.2	24.3-72.1
Females 21-49 yrs	89	1.53	0.297-5.62
Females ≥ 50 yrs	71	1.15	0.187-3.63

b) The 5th and 95th percentiles were calculated from the distribution of individual FTIs.

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:

cobas e 411 analyzer					
		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[c)]1	34.9	0.76	2.2	0.92	2.6
PreciControl U2	19.0	0.41	2.2	0.52	2.7

c) U = Universal

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

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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 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[10]	Linear regression
y = 1.17x - 3.26	y = 1.15x - 1.82
τ = 0.909	r = 0.981

The sample concentrations were between 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 user guide or operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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