Elecsys DHEA-S

cobas®

REF		Σ	SYSTEM
03000087122	03000087501	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 740
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 142

Intended use

Immunoassay for the in vitro quantitative determination of dehydroepiandrosterone sulfate (DHEA-S) in human serum and plasma.

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

Summary

DHEA-S is a steroid hormone which is produced from the precursor cholesterol in the zona reticularis and broad fascia of the adrenal cortex.[1] The determination of elevated DHEA-S values is an important aid in the diagnosis of hirsutism and virilism.[2,3] In addition to a differential diagnosis of hirsutism and virilism further indications for this parameter are all forms of androgenisation, hyperprolactinemia, polycystic ovarian syndrome, and the exclusion of an androgen producing tumor of the adrenal cortex.[2] DHEA-S exhibits only a weak androgenic activity but can be metabolized to more active androgens such as androstenedione and testosterone, which can indirectly cause hirsutism and virilism.[2,4]

From 7 years of age onwards, an increase in DHEA-S levels is observed which then gradually after the age of 30 begins to fall again.[5] Only elevated DHEA-S concentrations are of clinical importance; other factors which can be responsible for DHEA-S excess production are genetic enzyme defects of the adrenal cortex (adrenogenital syndrome),[6] hyperplasia of the adrenal cortex as well as androgen producing tumors.[2]

The rate of secretion of DHEA-S into the blood stream is only slightly more than the rate observed for DHEA. As a consequence of the DHEA-S half-life of approximately 1 day, the DHEA-S level is however about a thousand fold greater.[7] DHEA-S is relatively strongly bound to albumin, only a small portion is non-protein bound, and none appears to be bound to sex hormone-binding globulin (SHBG).[8] Due to its high concentration and low inter- and intra-day variability, DHEA-S is an excellent indicator of adrenal cortex androgen production.[7,9]

Together with testosterone, DHEA-S assays represent the assay of choice for initial screening tests to determine whether androgen values are elevated in hirsutism. Approximately 84 % of the women suffering from hirsutism exhibit elevated androgen levels.[10] The main purpose of this is to exclude the presence of androgen producing tumors (from the adrenal cortex or the ovaries). Tumor relevant values in women are those values exceeding 700 µg/dL DHEA-S.[6]

The Elecsys DHEA-S assay makes use of a competition test principle using a polyclonal antibody (rabbit) specifically directed against DHEA-S. Endogenous DHEA-S in the sample competes with added DHEA-S derivative labeled with a ruthenium complex[a)] for the binding sites on the biotinylated antibody.

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

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: By incubating the sample (15 µL) with a DHEA-S-specific biotinylated antibody, an immunocomplex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 2nd incubation: After addition of streptavidin-coated microparticles and a DHEA-S derivative labeled with a ruthenium complex, the still-vacant sites of the biotinylated antibodies become occupied, with formation of an antibody-hapten complex. The entire 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.

Reagents - working solutions

The reagent rackpack is labeled as DHEA-S.

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

R1 Anti-DHEA-S-Ab~biotin (gray cap), 1 bottle, 9 mL:
Biotinylated polyclonal anti-DHEA-S antibody (rabbit) 450 ng/mL; phosphate buffer 100 mmol/L, pH 6.8; preservative.

R2 DHEA-S~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
DHEA-S derivative (synthetic) labeled with ruthenium complex 0.32 ng/mL; phosphate buffer 100 mmol/L, pH 6.8; preservative.

Precautions and warnings

For in vitro diagnostic use for healthcare 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.

Disposal:

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

Hazardous components:

- 2-methyl-2H-isothiazol-3-one hydrochloride

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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	8 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.

Li-, Na-, NH_4^+-heparin, K_3-EDTA, sodium citrate, potassium oxalate and sodium fluoride 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, 14 days at 2-8 °C, 12 months at -20 °C (± 5 °C). Freeze only once.[11,12]

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 03000095122, DHEA-S CalSet, for 4 x 1.0 mL
- REF 11731416160, PreciControl Universal, for 4 x 3.0 mL
- 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 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- 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.

cobas e 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

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 gravimetrically produced master calibrators consisting of exactly defined DHEA-S concentrations in depleted human serum matrix.

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).

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

Renewed calibration is recommended as follows:

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

Quality control

Use PreciControl Universal or other suitable controls for routine quality control procedures.

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.

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

Elecsys DHEA-S

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Calculation

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

Conversion factors: µmol/L x 36.846 = µg/dL

µg/dL x 0.02714 = µmol/L

µg/dL x 0.01 = µg/mL

Limitations - interference

The assay is unaffected by icterus (bilirubin < 222 µmol/L or < 13 mg/dL), hemolysis (Hb < 0.35 mmol/L or < 0.56 g/dL), lipemia (Intralipid < 2000 mg/dL) and biotin (< 123 nmol/L or < 30 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 80 IU/mL.

In vitro tests were performed on 17 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

0.003-27.0 µmol/L or 0.100-1000 µg/dL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.003 µmol/L or < 0.100 µg/dL. Values above the measuring range are reported as > 27.0 µmol/L or > 1000 µg/dL (or up to 135 µmol/L or 5000 µg/dL for 5-fold diluted samples).

Linearity range: 1.09-27.0 µmol/L or 40-1000 µg/dL

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: 0.003 µmol/L (0.100 µg/dL)

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

Dilution

Samples with DHEA-S concentrations above the measuring range can be diluted using human samples with a low analyte concentration. The recommended dilution is 1:5. The concentration of the diluted sample must be > 1.22 µmol/L (> 45 µg/dL).

If the endogenous DHEA-S concentration is negligible, multiply the result by the dilution factor or calculate using the following equation:

C = c + 4 (c - D)

C = true DHEA-S concentration of the sample

c = measured DHEA-S concentration

D = DHEA-S concentration in the diluent (human sample)

Expected values

Extended studies with the Elecsys DHEA-S assay conducted in 2 clinical centers in Germany covering a total of 519 samples from female individuals, a total of 489 samples from male individuals and a total of 269 samples from children gave the following values for the age groups listed below (study protocols No.: C00P032 and C01P005 - status 05/01 to 11/01):

Age (years)	N	50th percentile		5-95th percentile	
		µmol/L	µg/dL	µmol/L	µg/dL
Females:					
10-14	73	3.34	123	0.92-7.60	33.9-280
15-19	55	4.26	157	1.77-9.99	65.1-368
20-24	36	6.46	238	4.02-11.0	148-407
25-34	64	4.96	183	2.68-9.23	98.8-340
35-44*	85	4.38	161	1.65-9.15	60.9-337
45-54*	89	3.28	121	0.96-6.95	35.4-256
55-64	59	2.08	76.7	0.51-5.56	18.9-205
65-74	29	1.75	64.4	0.26-6.68	9.40-246
≥ 75	29	1.65	60.9	0.33-4.18	12.0-154
Males:					
10-14	74	2.74	101	0.66-6.70	24.4-247
15-19	67	7.57	279	1.91-13.4	70.2-492
20-24	28	9.58	353	5.73-13.4	211-492
25-34	60	7.68	283	4.34-12.2	160-449
35-44	70	6.00	221	2.41-11.6	88.9-427
45-54	45	5.94	219	1.20-8.98	44.3-331
55-64	69	3.75	138	1.40-8.01	51.7-295
65-74	55	2.45	90.2	0.91-6.76	33.6-249
≥ 75	21	1.53	56.2	0.44-3.34	16.2-123
Children:					
< 1 week	37	7.60	280	2.93-16.5	108-607
1-4 weeks	25	3.91	144	0.86-11.7	31.6-431
1-12 months	69	0.59	21.6	0.09-3.35	3.4-124
1-4 years	59	0.14	5.0	0.01-0.53	0.47-19.4
5-9 years	79	0.63	23.1	0.08-2.31	2.8-85.2

* Effects of the menopause on the results obtained for the women of the corresponding age groups were tested and found to be negligible.

DHEA-S values of newborns are strongly influenced by maternal hormonal exchange via placenta.

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 analyzers								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	µmol/L	µg/dL	µmol/L	µg/dL	%	µmol/L	µg/dL	%
HS[b] 1	3.18	117	0.09	3.28	2.8	0.11	4.16	3.6
HS 2	10.7	395	0.26	9.46	2.4	0.50	18.4	4.7
HS 3	26.7	984	0.46	17.0	1.7	0.63	23.3	2.4
PC U[c]1	4.15	153	0.09	3.33	2.2	0.11	3.99	2.6

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cobas e 411 analyzers								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	µmol/L	µg/dL	µmol/L	µg/dL	%	µmol/L	µg/dL	%
PC U2	3.34	123	0.09	3.41	2.8	0.10	3.83	3.1

b) HS = human serum

c) PC U = PreciControl Universal

cobas e 601 and **cobas e** 602 analyzers					
			Repeatability		
Sample	Mean		SD		CV
	µmol/L	µg/dL	µmol/L	µg/dL	%
HS 1	2.60	96.0	0.08	3.03	3.2
HS 2	10.9	402	0.29	10.5	2.6
HS 3	21.3	784	0.49	18.0	2.3
PC U1	5.81	214	0.10	3.60	1.7
PC U2	14.1	519	0.21	7.71	1.5

cobas e 601 and **cobas e** 602 analyzers					
			Intermediate precision		
Sample	Mean		SD		CV
	µmol/L	µg/dL	µmol/L	µg/dL	%
HS 1	2.53	93.2	0.06	2.29	2.5
HS 2	10.7	395	0.29	10.6	2.7
HS 3	20.4	753	0.48	17.7	2.4
PC U1	5.69	210	0.14	4.99	2.4
PC U2	13.6	501	0.29	10.8	2.2

Method comparison

A comparison of the Elecsys DHEA-S assay (y) with a commercially available DHEA-S test (x) using clinical samples gave the following correlations (µg/dL):

Number of samples measured: 603

Passing/Bablok[13]	Linear regression
y = 1.06x - 4.78	y = 0.94x + 14.0
τ = 0.865	r = 0.952

The sample concentrations were between 0.33 and 19.8 µmol/L (12 and 730 µg/dL).

Analytical specificity

For the Elecsys DHEA-S assay, the following cross-reactivities were found:

Substance	**Cross-reactivity %**	**Additive concentration µg/dL**
Androstenedione	10.8	1000
DHEA	8.90	1000
Androsterone	2.10	2000
Testosterone	2.55	2000
Aldosterone	0.320	5000
Androsterone-sulfate	1.10	5000
DHEA-glucuronide	2.08	5000
Estradiol	n. d.[d)]	5000
Estriol	n. d.	5000
Estrone	0.740	5000
Estrone-3-sulfate	0.500	5000
Progesterone	1.32	5000
5-α-Dihydrotestosterone	1.12	5000
19-Hydroxyandrostendione	1.66	5000
Cortisol	0.060	10000

d) n. d. = not detectable

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

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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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