Elecsys DHEA-S

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REF		Σ	SYSTEM
07027192190	07027192500	100	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
DHEAS	10068

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 for which the adrenal glands is the sole source in females and the principle source in males. DHEA-S is found in the fetus but declines rapidly in the first year of life. Around 5-7 years of age, DHEA-S production slowly resumes, increases during puberty and reaches a maximum between 20 and 30 years of age. Thereafter DHEA-S levels steadily decline to approximately 10 % of peak levels by the age of 80.[1,2] DHEA-S has a relatively long half-life of 7-10 hours and its concentration is approximately constant over the day.[1]

Measurement of DHEA-S can be useful in the diagnostic work-up of female patients presenting with clinical symptoms of hyperandrogenism.[3] Elevated DHEA-S levels are indicative of an involvement of the adrenal gland. A decrease of DHEA-S and total serum testosterone by more than 50 % upon dexamethasone suppression, is seen as confirmation of hyperandrogenism of the adrenal gland.[4] The most common cause is missense mutations in the 21-hydroxylase gene resulting in a mild or adult-onset or non-classical congenital adrenal hyperplasia (NCCAH). It has been estimated that the incidence of NCCAH is around 1 % in the population of New York.[4] In rare cases the cause is an adrenal tumor; in a study by Carmina et al.,[5] the incidence of an adrenal tumor was 0.2 % (2 out of 950 women with hyperandrogenism). 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 (9 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 II 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 **cobas** link.

Reagents - working solutions

The **cobas e** pack is labeled as DHEAS.

M Streptavidin-coated microparticles, 1 bottle, 6.1 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-DHEA-S-Ab~biotin, 1 bottle, 9.9 mL:
Biotinylated polyclonal anti-DHEA-S antibody (rabbit) 450 ng/mL; phosphate buffer 100 mmol/L, pH 6.8; preservative.

R2 DHEA-S~$Ru(bpy)_3^{2+}$, 1 bottle, 9.9 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 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 dust/fume/gas/mist/vapours/spray.

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.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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 available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **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

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Stability:	
on the analyzers	16 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-heparin, K_2-EDTA and K_3-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + 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.

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 and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators 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 03000095122, DHEA-S CalSet, for 4 x 1.0 mL
- REF 11731416190, PreciControl Universal, for 4 x 3.0 mL
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, 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.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against gravimetrically produced master calibrators consisting of exactly defined DHEA-S concentrations in depleted human serum matrix.

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 **cobas e** pack 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:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack 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 **cobas e** pack, 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.

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 effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 222 µmol/L or ≤ 13 mg/dL
Hemoglobin	≤ 0.35 mmol/L or ≤ 0.56 g/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 287 nmol/L or ≤ 70 ng/mL
Rheumatoid factors	≤ 80 IU/mL

Criterion: For concentrations of 0.2-50 µg/dL the deviation is ≤ ± 5 µg/dL. For concentrations > 50 µg/dL the deviation is ≤ ± 10 %.

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.

Pharmaceutical substances

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

0.005-27.1 µmol/L or 0.2-1000 µg/dL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.005 µmol/L or < 0.2 µg/dL. Values above the measuring

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range are reported as > 27.1 µmol/L or > 1000 µg/dL (or up to 135.7 µmol/L or 5000 µg/dL for 5-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.003 µmol/L (0.1 µg/dL)

Limit of Detection = 0.005 µmol/L (0.2 µg/dL)

Limit of Quantitation = 0.081 µmol/L (3 µg/dL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 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 the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

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 two 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 protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 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	0.012	0.436	0.027	0.160	36.6	0.005	0.174	39.9
HS 2	0.131	4.82	0.015	0.539	11.2	0.018	0.667	13.8
HS 3	8.47	312	0.315	11.6	3.7	0.413	15.2	4.9
HS 4	19.0	699	0.662	24.4	3.5	0.801	29.5	4.2
HS 5	25.5	939	0.912	33.6	3.6	1.29	47.7	5.1
PC[c)] Universal1	5.56	205	0.190	7.01	3.4	0.225	8.29	4.0
PC Universal2	15.4	492	0.426	15.7	3.2	0.619	22.8	4.6

b) HS = human serum

c) PC = PreciControl

Method comparison

A comparison of the Elecsys DHEA-S assay, REF 07027192190 (**cobas e** 801 analyzer; y) with the Elecsys DHEA-S assay, REF 03000087122 (**cobas e** 601 analyzer; x) gave the following correlations (µg/dL):

Number of samples measured: 148

Passing/Bablok[7]	Linear regression
y = 0.986x - 0.715	y = 0.998x - 3.26
τ = 0.971	r = 0.997

The sample concentrations were between 0.252 and 980 µg/dL.

A comparison of the Elecsys DHEA-S assay, REF 07027192190 (**cobas e** 402 analyzer; y) with the Elecsys DHEA-S assay,

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REF 07027192190 (**cobas e** 801 analyzer; x) gave the following correlations (µg/dL):

Number of samples measured: 202

Passing/Bablok[7]	Linear regression
y = 1.016x - 1.37	y = 1.006x +1.90
τ = 0.980	r = 0.998

The sample concentrations were between 2.19 and 944 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

1 Leowattana W. DHEAS as a new diagnostic tool. Clin Chim Acta. 2004;341(1-2):1-15.

2 Enea C, Boisseau N, Diaz V, et al. Biological factors and the determination of androgens in female subjects. J Steroids 2008;73(12):1203-1216.

3 Huang A, Brennan K, Azziz R. Prevalence of Hyperandtrogenemia in the Polycystic Ovary Syndrome by the NIH 1990 Criteria. J Fertil Steril. 2010;93(6):1938-1941.

4 Rachon D. Differential Diagnosis of Hyperandrogenism in Women with Polycystic Ovary Syndrome. J Exp Clin Endocrinol Diabetes 2012;120:205-209.

5 Carmina E, Rosato F, Janni A, et al. Relative Prevalence of Different Androgen Excess Disorders in 950 Women Referred because of Clinical Hyperandrogenism. J Clin Endocrinol Metab 2006;91(1):2-6.

6 Sciarra F, Tosti-Croce C, Toscano V. Androgen-secreting adrenal tumors. Minerva Endocrinol. 1995;20(1):63-8.

7 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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.

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 dialog.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 after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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