Elecsys Androstenedione

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REF		Σ	SYSTEM
07679831190	07679831500	100	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 1530
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 304

Intended use

Immunoassay for the in vitro quantitative determination of androstenedione in human serum and plasma. The determination of androstenedione is used as an aid in diagnosis and differential diagnosis of androgens related endocrine function in conjunction with other clinical and laboratory findings.

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

Summary

Androstenedione (ASD) is a 19-carbon androgenic steroid hormone produced by the adrenal glands and the gonads. Steroid hormones represent a class of signaling molecules that help to control a variety of processes, including metabolism, inflammation, immune functions, salt and water balance and the development of sexual characteristics. Steroid hormones are fat-soluble, therefore, they can pass through the lipid membrane into the circulation. They are subsequently delivered to their site of action before exerting their cellular effects by binding to specific steroid receptors. They can be grouped into glucocorticoids, mineralocorticoids, androgens, estrogens and progestogens, based on the receptors to which they bind.[1] The production of androstenedione by the adrenal glands is governed by the adrenocorticotropic hormone (ACTH), whereas production of gonadal androstenedione is controlled by gonadotropins. Androstenedione is formed as an intermediate step in the biochemical pathway for production of the sex hormones testosterone, estrone and estradiol. Therefore, it is the common precursor for male and female sex hormones.[2]

Measurement of androstenedione levels is useful in the evaluation of adrenal gland function, androgen production, ovarian/testicular function, as well in the diagnosis and monitoring of patients who have suspected cortisol-related enzyme deficiencies resulting in hyperandrogenism. As per the most recent guidelines androstenedione is indicated for the investigation of hyperandrogenism in women suspected of having polycystic ovary syndrome (PCOS), if total or free testosterone is not elevated.[3]

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: By incubating the sample (10 µL) with an androstenedione-specific biotinylated antibody and an ASD conjugate labeled with a ruthenium complex[a)], immunocomplexes are formed. The still-vacant sites of the biotinylated antibodies become occupied, with formation of an antibody-hapten complex, the amount of which is dependent upon the analyte concentration in the sample.
- 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 ASD.

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

R1 Anti-androstenedione-Ab~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated monoclonal anti-androstenedione antibody (recombinant, sheep) 10 ng/mL; phosphate buffer 50 mmol/L, pH 6.5; preservative.

R2 Androstenedione-peptide~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
Androstenedione coupled to a synthetic peptide labeled with ruthenium complex 3 ng/mL; phosphate buffer 50 mmol/L, pH 6.5; 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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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, USA: 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 automatically from the respective reagent barcodes.

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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	8 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.

Serum separation tubes containing polyester-based polymer gels are not suitable.[4]

Li-heparin, K_2-EDTA and K_3-EDTA plasma as well as plasma separation tubes. Plasma separation tubes containing polyester-based polymer gels are not suitable.[4]

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, 6 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, 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 07679866190, CalSet Androstenedione, for 4 x 1.0 mL
- REF 08740062190, PreciControl Maternal Care, for 6 x 3.0 mL
- REF 08740062160, PreciControl Maternal Care, for 6 x 3.0 mL (for USA)
- REF 05192943190, Diluent Universal 2, 2 x 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **cobas e** 411 analyzer:

- 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 materials for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution
- REF 11298500160, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution (for USA)

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 prior to use and the reading in of the test-specific parameters via the reagent barcode take place automatically. No manual input is necessary. 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: The Elecsys Androstenedione assay has been standardized by isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS). The method is traceable to the certified reference material NMIA M955 from the National Measurement Institute of Australia.

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:

- 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 Maternal Care.

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.

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

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Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L, ng/mL or in µg/L with **cobas e** 601 and **cobas e** 602 analyzers).

Conversion factors: nmol/L x 0.286 = ng/mL (µg/L)

ng/mL x 3.497 = nmol/L

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	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1200 mg/dL
Biotin	≤ 14326 nmol/L or ≤ 3500 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
IgG	≤ 7.0 g/dL
IgA	≤ 1.6 g/dL
IgM	≤ 1.0 g/dL
Human serum albumin	≤ 5.0 g/dL

Criterion: Recovery ≤ 0.06 ng/mL if initial value with samples < 0.60 ng/mL or within ± 10 % of initial value with samples > 0.60 ng/mL.

Pharmaceutical substances

In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

A strong interaction with Exemestane (INN international nonproprietary name, WHO) was found. Do not use samples from patients under Exemestane treatment.

In addition, the following special drugs were tested. No interference with the assay was found.

Special drugs

Drug	Concentration tested mg/L
ACTH	0.150
Clomiphene	40.7
Follicle stimulating hormone (FSH)	0.077
Flutamide	450
Metformin	1800

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

0.525-34.9 nmol/L or 0.150-10.0 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.525 nmol/L or < 0.150 ng/mL. Values above the measuring range are reported as > 34.9 nmol/L or > 10.0 ng/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.262 nmol/L (0.0750 ng/mL)

Limit of Detection = 0.525 nmol/L (0.150 ng/mL)

Limit of Quantitation = 1.05 nmol/L (0.30 ng/mL)

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 defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable error of ≤ 30 %.

Dilution

Samples with androstenedione concentrations above the measuring range can be diluted with Diluent Universal 2 or a suitable human serum with a low analyte concentration. The recommended dilution is 1:10. The concentration of the diluted sample must be > 4.19 nmol/L (> 1.20 ng/mL).

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

Expected values were determined at 2 trial sites. Samples used in the study were derived from clinical cohorts/sample collections with defined inclusion/exclusion criteria.

Test subjects	N	5th percentile ng/mL	Median ng/mL	95th percentile ng/mL
Apparently healthy women	84	0.490	0.825	1.31

Test subjects	N	2.5th percentile ng/mL	Median ng/mL	97.5th percentile ng/mL
Postmenopausal women	140	0.187	0.451	1.07
Polycystic ovary syndrome (PCOS)	125	0.645	1.54	3.47
Apparently healthy men	138	0.280	0.640	1.52
Apparently healthy children	140	< 0.150	< 0.150	0.519

Test subjects	N	5th percentile nmol/L	Median nmol/L	95th percentile nmol/L
Apparently Healthy women	84	1.71	2.89	4.58

Test subjects	N	2.5th percentile nmol/L	Median nmol/L	97.5th percentile nmol/L
Postmenopausal women	140	0.654	1.58	3.74
Polycystic ovary syndrome (PCOS)	125	2.26	5.39	12.1
Apparently healthy men	138	0.979	2.24	5.32
Apparently healthy children	140	< 0.525	< 0.525	1.81

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

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Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). 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	1.61	0.056	3.4	0.073	4.6
Human serum 2	3.38	0.080	2.4	0.115	3.4
Human serum 3	11.9	0.406	3.4	0.507	4.3
Human serum 4	20.2	0.657	3.3	0.734	3.6
Human serum 5	31.5	1.03	3.3	1.33	4.2
PreciControl MC[b)] 1	1.76	0.056	3.1	0.080	4.5
PreciControl MC 2	10.5	0.283	2.7	0.357	3.4
PreciControl MC 3	27.6	0.878	3.2	1.17	4.2

b) MC = Maternal Care

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.461	0.016	3.4	0.021	4.6
Human serum 2	0.966	0.023	2.4	0.033	3.4
Human serum 3	3.39	0.116	3.4	0.145	4.3
Human serum 4	5.77	0.188	3.3	0.210	3.6
Human serum 5	9.00	0.294	3.3	0.380	4.2
PreciControl MC 1	0.504	0.016	3.1	0.023	4.5
PreciControl MC 2	3.00	0.081	2.7	0.102	3.4
PreciControl MC 3	7.89	0.251	3.2	0.335	4.2

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean nmol/L	SD nmol/L	CV %	SD nmol/L	CV %
Human serum 1	1.56	0.045	2.9	0.070	4.5
Human serum 2	3.23	0.059	1.8	0.122	3.8
Human serum 3	11.2	0.262	2.4	0.413	3.7
Human serum 4	18.8	0.346	1.8	0.703	3.7
Human serum 5	32.0	0.682	2.1	1.24	3.9
PreciControl MC 1	1.70	0.038	2.3	0.080	4.7
PreciControl MC 2	9.97	0.196	2.0	0.381	3.8
PreciControl MC 3	25.8	0.769	3.0	1.19	4.6

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.446	0.013	2.9	0.020	4.5
Human serum 2	0.923	0.017	1.8	0.035	3.8
Human serum 3	3.19	0.075	2.4	0.118	3.7
Human serum 4	5.39	0.099	1.8	0.201	3.7
Human serum 5	9.15	0.195	2.1	0.355	3.9
PreciControl MC 1	0.487	0.011	2.3	0.023	4.7
PreciControl MC 2	2.85	0.056	2.0	0.109	3.8
PreciControl MC 3	7.37	0.220	3.0	0.339	4.6

Method comparison

A comparison of the Elecsys Androstenedione assay (y) with ID-LC-MS/MS (x) gave the following correlations (nmol/L):

Number of samples measured: 332

Passing/Bablok[5]	Linear regression
y = 1.04x + 0.004	y = 1.03x + 0.025
τ = 0.922	r = 0.996

The sample concentrations (ID-LC-MS/MS concentrations) were between 0.49 and 35.3 nmol/L (0.14 and 10.1 ng/mL).

Analytical specificity

For the Elecsys Androstenedione assay, the following cross-reactivities (CR; in %) were found at the respective additive concentration (AC; in ng/mL), tested with androstenedione concentration of approximately 1.4 ng/mL (4.9 nmol/L):

	CR (%)	**AC (ng/mL)**
Androsterone	0.019	10000
Aldosterone	n.d.[c)]	10000
Cortisol	n.d.	10000
Dihydrotestosterone (DHT)	0.275	2500
Dihydroepiandrosterone (DHEA)	0.394	1600
Estriol	n.d.	10000
16-Epiestriol	0.001	10000
Estradiol	n.d.	10000
Estriol-3-glucuronide	n.d.	10000
Estriol-16-glucuronide	n.d.	10000
Estrone	0.030	10000
17-OH-Progesterone	0.021	10000
Progesterone	0.011	10000
Testosterone	0.383	1000
Dexamethasone	n.d.	10000
Prednisolone	n.d.	10000
Fludrocortisone	n.d.	10000
Spironolactone	0.013	10000
Cyproterone acetate	n.d.	10000
Finasteride	0.001	10000
Ethinyl estradiol	n.d.	10000
Cholesterol	n.d.	10000
Dehydroepiandrosterone-3-sulfate (DHEA-S)	n.d.	10000
Prednisone	n.d.	10000
Pregnenolone	n.d.	10000

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	CR (%)	AC (ng/mL)
Norethindrone	0.002	10000
Corticosterone	n.d.	10000
11-Keto-testosterone	0.001	10000
Adrenosterone	0.221	2800
4-Androstene-11β-ol-3,17-dione	0.141	4400
Cortisone	0.001	10000
Fulvestrant	n.d.	10000
Progestin (Medroxyprogesterone acetate)	0.001	10000
Canrenone	0.046	10000

c) n. d. = not detectable

References

1 Luu-The, V. Assessment of steroidogenesis and steroidogenic enzyme functions. J Steroid Biochem Mol Biol 2013;137:176-182.

2 Devlin TM. Textbook of Biochemistry: with Clinical Correlations (7th ed.). Hoboken, NJ: John Wiley & Sons. 2010: p. 432.

3 Teede HJ, Misso ML, Costello MF, et al. International PN Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2018;33(9):1602-1618.

4 Hepburn S, Wright MJ, Boyder C, et al. Sex steroid hormone stability in serum tubes with and without separator gels. Clin Chem Lab Med (CCLM) 54.9 2016: 1451-1459.

5 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 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 for reconstitution
GTIN	Global Trade Item Number

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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