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Testosterone II

cobas®

Testosterone

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
05200067 190	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

Intended use

Immunoassay for the in vitro quantitative determination of testosterone 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

References[1,2,3,4,5,6]

The androgen testosterone (17β-hydroxyandrostenone) has a molecular weight of 288 daltons. In men, testosterone is synthesized almost exclusively by the Leydig cells of the testes. The secretion of testosterone is regulated by luteinizing hormone (LH), and is subject to negative feedback via the pituitary and hypothalamus.

Testosterone promotes the development of the secondary sex characteristics in men and serves to maintain the function of the prostate and seminal vesicles.

Most of the circulating testosterone is bound to carrier proteins (SHBG = sex hormone-binding globulin).

In women, small quantities of testosterone are formed in the ovaries. In physiological concentrations, androgens have no specific effects in women. Increased production of testosterone in women can cause virilization (depending on the increase).

The determination of testosterone in women is helpful in the diagnosis of androgenic syndrome (AGS), polycystic ovaries (Stein-Leventhal syndrome) and when an ovarian tumor, adrenal tumor, adrenal hyperplasia or ovarian insufficiency is suspected.

Testosterone is determined in men when reduced testosterone production is suspected, e.g. in hypogonadism, estrogen therapy, chromosome aberrations (as in the Klinefelter's syndrome) and liver cirrhosis.

The Elecsys Testosterone II assay is based on a competitive test principle using a high affinity monoclonal antibody (sheep) specifically directed against testosterone. Endogenous testosterone released from the sample by 2-bromoestradiol competes with the added testosterone derivative labeled with a ruthenium complex[a)] for the binding sites on the biotinylated antibody.

The Elecsys Testosterone II assay shows an improved performance if compared to Isotope Dilution - Gas Chromatography/Mass Spectrometry (ID-GC/MS) reference method in the female concentration range.

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

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample are incubated with a biotinylated monoclonal testosterone-specific antibody. The binding sites of the labeled antibody become occupied by the sample analyte (depending on its concentration).
- 2nd incubation: After addition of streptavidin-coated microparticles and a testosterone derivate labeled with a ruthenium complex, 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.

Reagents - working solutions

The reagent rackpack is labeled as TESTO II.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:

Streptavidin-coated microparticles 0.72 mg/mL, preservative.

R1 Anti-testosterone-Ab~biotin (gray cap), 1 bottle, 10 mL:

Biotinylated monoclonal anti-testosterone antibody (sheep) 40 ng/mL; releasing reagent 2-bromoestradiol; MES buffer 50 mmol/L, pH 6.0; preservative.

R2 Testosterone-peptide~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:

Testosterone derivative, labeled with ruthenium complex 1.5 ng/mL; MES buffer 50 mmol/L, pH 6.0; 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.

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 in a sufficient number and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2- and K_3-EDTA plasma.

Criterion: Recovery within 80-120 % of serum value > 1 ng/mL, recovery of ± 0.2 ng/mL of serum value ≤ 1 ng/mL and slope 0.9-1.1 + intercept 0.05 ng/mL + coefficient of correlation > 0.95.

Stable for 1 week at 2-8 °C, 6 months at -20 °C. Freeze only once.[7]

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.

Testosterone II

Testosterone

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 05202230190, Testosterone II CalSet II, for 4 x 1 mL
- REF 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- 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 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- 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.

MODULAR ANALYTICS E170, **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 via ID-GC/MS ("Isotope Dilution - Gas Chromatography/Mass Spectrometry").[8,9]

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 ng/mL, ng/dL or nmol/L).

Conversion factors:	ng/mL x 3.47 = nmol/L
	ng/mL x 100 = ng/dL
	nmol/L x 0.288 = ng/mL

Limitations - interference

The assay is unaffected by icterus (bilirubin < 513 µmol/L or < 30 mg/dL), hemolysis (Hb < 0.372 mmol/L or < 0.600 g/dL), lipemia (Intralipid < 1000 mg/dL) and biotin (< 123 nmol/L or < 30 ng/mL).

Criterion: Recovery within ± 10 % of initial value (concentration range > 1-15 ng/mL), recovery within ± 15 % of initial value (concentration range > 0.5-1 ng/mL) and recovery of ± 0.075 ng/mL (concentration range of 0.150-0.500 ng/mL).

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 1000 IU/mL.

In vitro tests were performed on 18 commonly used pharmaceuticals. No interference with the assay was found.

Two special drugs were additionally tested. A strong interaction with Nandrolone (INN international nonproprietary name, WHO) was found. Do not use samples from patients under Nandrolone treatment.

In isolated cases, elevated testosterone levels can be seen in samples from female patients with end stage renal disease (ESRD).

Implausible elevated testosterone values in women should be verified by an extraction method or a validated LC-MS/MS tandem method.[5]

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

0.025-15.0 ng/mL or 0.087-52.0 nmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.025 ng/mL or < 0.087 nmol/L. Values above the measuring range are reported as > 15.0 ng/mL or > 52.0 nmol/L.

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Limit of Blank = 0.012 ng/mL or 0.042 nmol/L

Limit of Detection = 0.025 ng/mL or 0.087 nmol/L

Limit of Quantitation = 0.120 ng/mL or 0.416 nmol/L

Testosterone II

cobas®

Testosterone

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

The Limit of Quantitation was determined using the result of functional sensitivity testing.

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 (functional sensitivity) is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %. It has been determined using low concentration testosterone samples.

Dilution

Not necessary due to the broad measuring range.

Expected values

The following tables show the results obtained using the Elecsys Testosterone II assay in a reference population of 95 males (7-18 years) and 100 females (8-18 years), who were in good endocrinological health. Subjects were clinically characterized according to their Tanner Stage. Tanner Stage was characterized according to the method of Marshall and Tanner.[10,11]

Reference values for males (7-18 years) characterized by Tanner Stage

Tanner Stage	N	Median	5-95th percentiles (ng/mL)
1	26	< 0.025	< 0.025
2	18	0.597	< 0.025-4.32
3	15	2.45	0.649-7.78
4	16	3.44	1.80-7.63
5	20	4.46	1.88-8.82

Reference values for females (8-18 years) characterized by Tanner Stage

Tanner Stage	N	Median	5-95th percentiles (ng/mL)
1	37	< 0.025	< 0.025-0.061
2	12	< 0.025	< 0.025-0.104
3	12	0.079	< 0.025-0.237
4	12	0.122	< 0.025-0.268
5	27	0.197	0.046-0.383

The following table shows the results obtained with the Elecsys Testosterone II assay in an apparently healthy group of 214 males and 160 females without intake of contraceptiva and prescription drugs (study number CIM 000669). Blood samples were taken between 6.30 am and 1.00 pm. This clinical study with focus on the Elecsys Testosterone II assay included measurements in parallel with the Elecsys SHBG assay. The results were evaluated for the Elecsys Testosterone II and Elecsys SHBG assays and commonly used parameters derived from different calculation procedures, including albumin as an important parameter involved.[12]

- Free testosterone index (% FTI) or free androgen index (% FAI) as calculated on a molar/molar basis:

  FTI (%) = (testosterone in nmol/L divided by SHBG in nmol/L) x 100
- Free testosterone calculated (FTc) in nmol/L and %
- Bioavailable testosterone calculated (BATc) in nmol/L and %

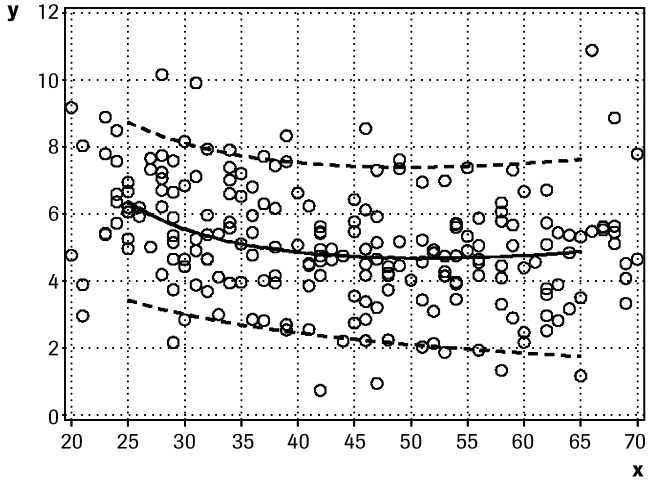
FTc and BATc were calculated by means of individual concentrations for total testosterone, SHBG, and albumin and via the association constant of albumin to testosterone. A detailed description of the calculation procedure is available on request. Refer also to the homepage of www.issam.ch/freetesto.htm.

The following results were obtained:

Testosterone

Test subjects	Percentiles				
	N	Median	5-95th	Median	5-95th
		ng/mL		nmol/L	
Males 20-49 years	136	5.36	2.49-8.36	18.6	8.64-29.0
Males ≥ 50 years	78	4.76	1.93-7.40	16.5	6.68-25.7
Females 20-49 years	89	0.271	0.084-0.481	0.941	0.290-1.67
Females ≥ 50 years	71	0.162	0.029-0.408	0.563	0.101-1.42

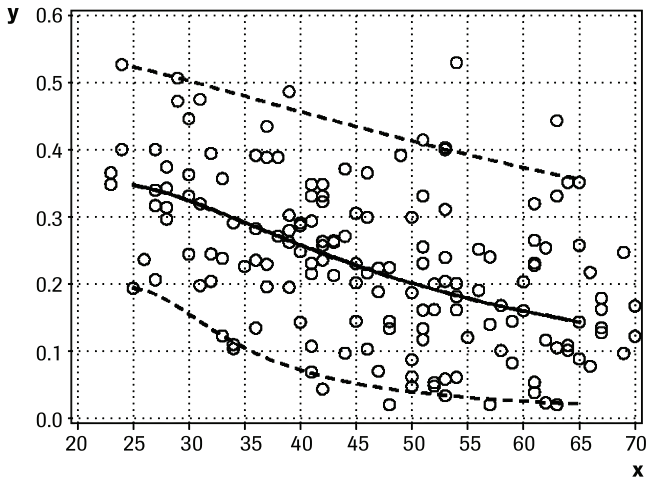
Distribution of testosterone values in the apparently healthy male group based on age (n = 214). Solid line: 50 % percentile, upper line: 95 % percentile, lower line: 5 % percentile.



x: Age (years)

y: Testosterone (ng/mL) - male group

Distribution of testosterone values in the apparently healthy female group based on age (n = 160). Solid line: 50 % percentile, upper line: 95 % percentile, lower line: 5 % percentile.



x: Age (years)

y: Testosterone (ng/mL) - female group

Testosterone II

Testosterone

SHBG

Test subjects	N	Median	5-95th percentiles
		nmol/L	
Males 20-49 years	136	33.5	16.5-55.9
Males ≥ 50 years	78	40.8	19.3-76.4
Females 20-49 years	89	64.3	24.6-122
Females ≥ 50 years	71	57.4	17.3-125

Free testosterone index or free androgen index

Test subjects	N	Median	5-95th percentiles
		FTI or FAI (%)	
Males 20-49 years	136	57.2	35.0-92.6
Males ≥ 50 years	78	38.2	24.3-72.1
Females 20-49 years	89	1.53	0.297-5.62
Females ≥ 50 years	71	1.15	0.187-3.63

Free testosterone, calculated

Test subjects	N	Percentiles			
		Median	5-95th percentiles	Median	5-95th percentiles
		FTc (nmol/L)		FTc (%)	
Males 20-49 years	136	0.379	0.198-0.619	2.10	1.53-2.88
Males ≥ 50 years	78	0.304	0.163-0.473	1.91	1.23-2.59
Females 20-49 years	89	0.011	0.003-0.033	1.19	0.701-2.19
Females ≥ 50 years	71	0.008	0.001-0.020	1.26	0.685-2.64

Bioavailable testosterone, calculated

Test subjects	N	Percentiles			
		Median	5-95th percentiles	Median	5-95th percentiles
		BATc (nmol/L)		BATc (%)	
Males 20-49 years	136	9.10	4.36-14.3	49.8	35.0-66.3
Males ≥ 50 years	78	6.63	3.59-11.0	42.1	27.5-60.7
Females 20-49 years	89	0.246	0.059-0.756	25.7	15.3-47.7
Females ≥ 50 years	71	0.168	0.030-0.430	28.0	15.1-55.2

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, samples and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers					
			Repeatability		
Sample	Mean		SD		CV
	ng/mL	nmol/L	ng/mL	nmol/L	%
Human serum 1	0.095	0.330	0.004	0.014	4.7
Human serum 2	0.691	2.40	0.014	0.048	2.1
Human serum 3	2.16	7.50	0.042	0.146	1.9
Human serum 4	8.67	30.1	0.229	0.795	2.6
Human serum 5	13.0	45.1	0.158	0.548	1.2
PreciControl U[b)]1	6.30	21.9	0.088	0.305	1.4
PreciControl U2	2.65	9.20	0.047	0.163	1.8

b) U = Universal

Elecsys 2010 and **cobas e** 411 analyzers					
			Intermediate precision		
Sample	Mean		SD		CV
	ng/mL	nmol/L	ng/mL	nmol/L	%
Human serum 1	0.095	0.330	0.008	0.028	8.4
Human serum 2	0.691	2.40	0.022	0.076	3.2
Human serum 3	2.16	7.50	0.060	0.208	2.8
Human serum 4	8.67	30.1	0.243	0.843	2.8
Human serum 5	13.0	45.1	0.440	1.53	3.4
PreciControl U1	6.30	21.9	0.182	0.632	2.9
PreciControl U2	2.65	9.20	0.097	0.337	3.7

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
			Repeatability		
Sample	Mean		SD		CV
	ng/mL	nmol/L	ng/mL	nmol/L	%
Human serum 1	0.091	0.316	0.014	0.049	14.8
Human serum 2	0.696	2.42	0.029	0.097	4.1
Human serum 3	2.13	7.39	0.059	0.205	2.8
Human serum 4	8.79	30.5	0.236	0.833	2.7
Human serum 5	13.1	45.8	0.281	0.975	2.1
PreciControl U1	6.08	21.1	0.179	0.625	2.9
PreciControl U2	2.56	8.88	0.067	0.229	2.6

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
			Intermediate precision		
Sample	Mean		SD		CV
	ng/mL	nmol/L	ng/mL	nmol/L	%
Human serum 1	0.091	0.316	0.017	0.059	18.1
Human serum 2	0.696	2.42	0.030	0.104	4.4
Human serum 3	2.13	7.39	0.067	0.232	3.2
Human serum 4	8.79	30.5	0.292	1.01	3.3
Human serum 5	13.1	45.8	0.331	1.15	2.5
PreciControl U1	6.08	21.1	0.190	0.659	3.1
PreciControl U2	2.56	8.88	0.093	0.323	3.6

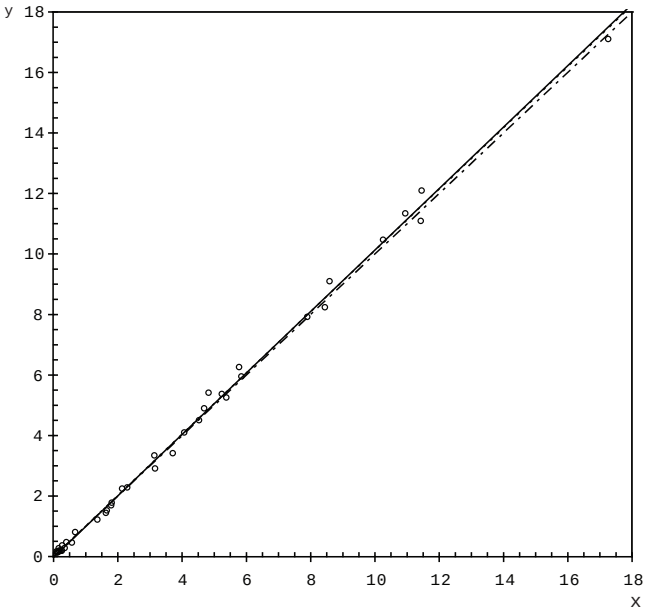
Testosterone II

Testosterone

Method comparison

a) A method comparison of the Elecsys Testosterone II assay (y) with the ID-GC/MS method (x) using 39 serum samples gave the following correlations (ng/mL):

Samples from males and females (n = 39):



x: ID-GC/MS (ng/mL)

y: Elecsys Testosterone II assay (ng/mL)

∘	Points	– - – -	x = y
—	Passing/Bablok	- - - -	Linear regression

Passing/Bablok[13]	Linear regression
y = 1.02x - 0.027	y = 1.01x - 0.003
τ = 0.928	r = 0.999

The sample concentrations were between 0.173 and 17.3 ng/mL (0.600 and 60.0 nmol/L).

Samples from females (n = 20):

Passing/Bablok[13]	Linear regression
y = 0.959x + 0.005	y = 0.969x + 0.007
τ = 0.780	r = 0.992

The sample concentrations were between 0.173 and 2.29 ng/mL (0.600 and 7.95 nmol/L).

b) A comparison of the Elecsys Testosterone II assay (y) with the Elecsys Testosterone assay (x) using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 239 males, 149 females

Results from external multicenter study (study number CIM 000669).

Samples from males (n = 239):

Passing/Bablok[13]	Linear regression
y = 0.977x + 0.032	y = 0.957x + 0.155
τ = 0.870	r = 0.985

The sample concentrations were between 0.063 and 14.0 ng/mL (0.219 and 48.5 nmol/L).

Samples from females (n = 149):

Passing/Bablok[13]	Linear regression
y = 0.715x + 0.023	y = 0.957x - 0.061
τ = 0.697	r = 0.972

The sample concentrations were between 0.023 and 9.26 ng/mL (0.080 and 32.1 nmol/L) with two highly elevated samples of 4.16 ng/mL (14.44 nmol/L) and 9.26 ng/mL (32.1 nmol/L), respectively.

Analytical specificity

For the antibody derivative used, the following cross-reactivities were found (in %):

	Concentration (ng/mL)	Cross-reactivity (%)
Androstendione	100	≤ 2.50
Cortisol	1000	≤ 0.01
Cortisone	2000	n.d.[c)]
Danazol	1000	≤ 0.500
Dexamethasone	2000	n.d.
DHEA	1000	≤ 0.016
DHEA-S	50000	≤ 0.003
D-5-Androstene-3β,17β-diol	1000	≤ 0.290
Estradiol	1000	≤ 0.160
Estrone	1000	≤ 0.004
Ethisterone	1000	≤ 2.40
Norgestrel	1000	≤ 0.910
Testosterone propionate	100	≤ 2.46
5-α-Androstane-3β,17β-diol	1000	≤ 2.11
5-α-Dihydro-testosterone	500	≤ 0.860
11-β-Hydroxy-testosterone	100	≤ 18.0
11-Keto-testosterone	1000	≤ 3.22
19-Norethisterone	40	≤ 6.00
Prednisone	1000	n.d.
Prednisolone	1000	≤ 0.002
Progesterone	1000	n.d.

c) n.d. = not detectable

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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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