

Judit Oldekamp, Cornelia Eichner 2009

Elecsys Testosterone II

Technical Performance of Elecsys Testosterone II

- Current immunoassays for testosterone seem to over-estimate testosterone in the female matrix. The effect is variable and cannot be predicted for any given sample:
 - “Inaccurate calibration or interference by cross-reacting substances is almost certainly the cause of the problem, but for many immunoassays, the exact nature of the interferent is not known”¹.

- A second generation **Elecsys Testosterone II** immunoassay was developed to improve the recovery of testosterone if compared to Isotope Dilution – Gas Chromatography/Mass Spectrometry (ID-GC/MS) reference method. Elecsys Testosterone II uses a new high affinity sheep monoclonal testosterone antibody:
 - Significantly reduced matrix effects and reduced interference by cross-reacting substances.

¹ Kane et al 2007, Measurement of serum Testosterone in women, what should we do?, Clinical Biochem; 44: 5-15

Testosterone – Background II

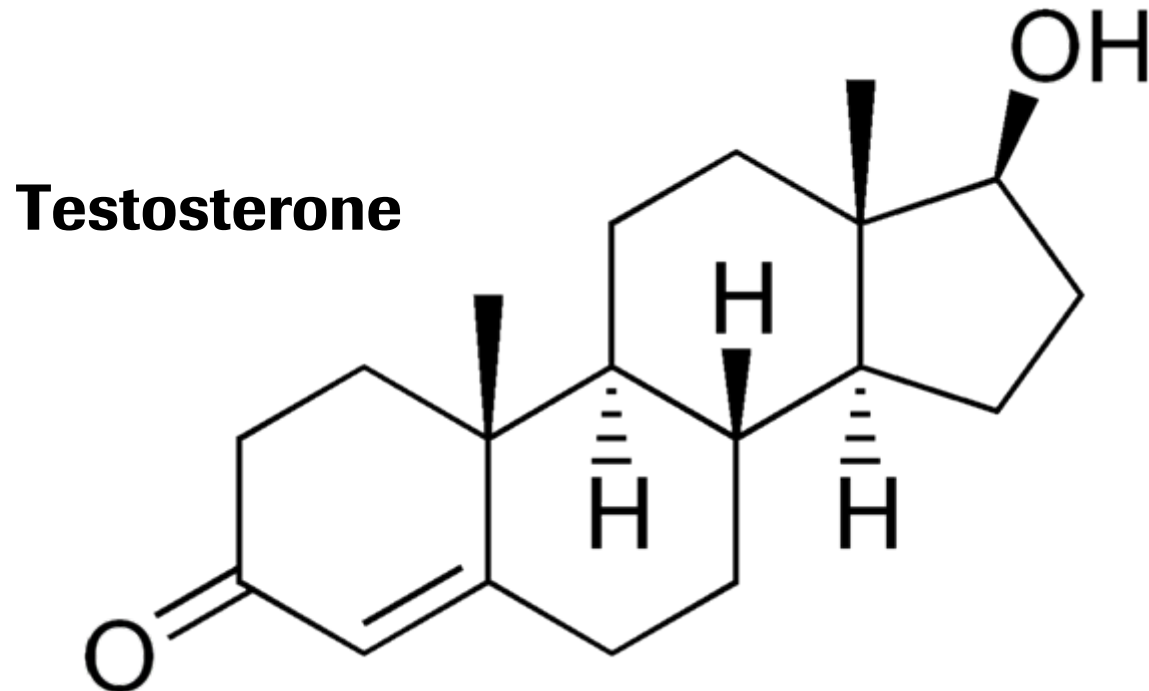


- The new assay shows a significant improved accuracy in female samples. In addition, cross-reactivity to Dehydroepiandrosterone sulphate (DHEAS) is reduced.
- The assay demonstrates a good correlation with liquid chromatography tandem mass spectrometry (LC-MS/MS) and provides excellent imprecision and functional sensitivity data.
- Average value of female testosterone samples (in line with LC-MS/MS) shows approx. 30% negative bias if compared to current Elecsys Testosterone immunoassays .
- On the other hand, there is no significant change in male testosterone values.

Testosterone – General I



- The androgen testosterone (17 β -hydroxyandrost-4-en-3-one) has a molecular weight of 288 Daltons. Most of the circulating testosterone is bound to the carrier proteins (SHBG = sex hormone-binding globulin) and albumin.



Testosterone – General II - men



- 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.
- 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.

Testosterone – General III - women



- In women, small quantities of testosterone are formed in the ovaries from adrenal precursors. In physiological concentrations, androgens appear to be necessary to induce libido in women.
- Increased production of testosterone in women can cause virilization (depending on the increase of the Testosterone level).
- The determination of testosterone in women is helpful in the diagnosis of adrenogenital syndrome (AGS), polycystic ovaries (Stein-Leventhal syndrome) and when an ovarian tumor, adrenal tumor, adrenal hyperplasia or ovarian insufficiency is suspected.

Elecsys Testosterone II – Reagents I



Reagent rackpack *(ready for use)*

- **M** Streptavidin-coated microparticles (transparent cap)
- **R1** biotinylated monoclonal anti-Testosterone antibody (sheep), (gray cap)
- **R2** Testosterone-peptide ~ Ru (black cap). Testosterone derivative, labeled with ruthenium complex

Elecsys Testosterone II – Reagents II



Calibrators *(lyophilisate)*

- **Cal 1** Testosterone calibrator 1 (white cap), 2 bottles (lyophilized) for 1.0 mL
- **Cal 2** Testosterone calibrator 2 (black cap), 2 bottles (lyophilized) for 1.0 mL

PreciControl Universal *(lyophilisate)*

- **PCU 1** Testosterone control, 2 bottles (lyophilized) for each 1.0 mL
- **PCU 2** Testosterone control, 2 bottles (lyophilized) for each 1.0 mL

Reagent rackpack (ready for use)

Calibrators: Dissolve carefully by adding exactly 1.0 mL of distilled water, Mix carefully, avoid formation of foam. Reconstitute for 15 min. Transfer aliquots of the calibrators into empty labeled snap-cap bottles (CalSet Vials). Attach the supplied labels.

- Store immediately at -20°C .

Controls: Dissolve carefully by adding exactly 1.0 mL of distilled water, Mix carefully, avoid formation of foam. Reconstitute for 15 min. Transfer aliquots of the controls into appropriate vials.

- Store immediately at -20°C .

Calibrators and Controls: Perform **only one** calibration or control procedure per aliquot.

- Stability at -20°C : 4 weeks (freeze only once).

Elecsys Testosterone II - Test principle I



Competition principle. Total duration of assay: 18 minutes.

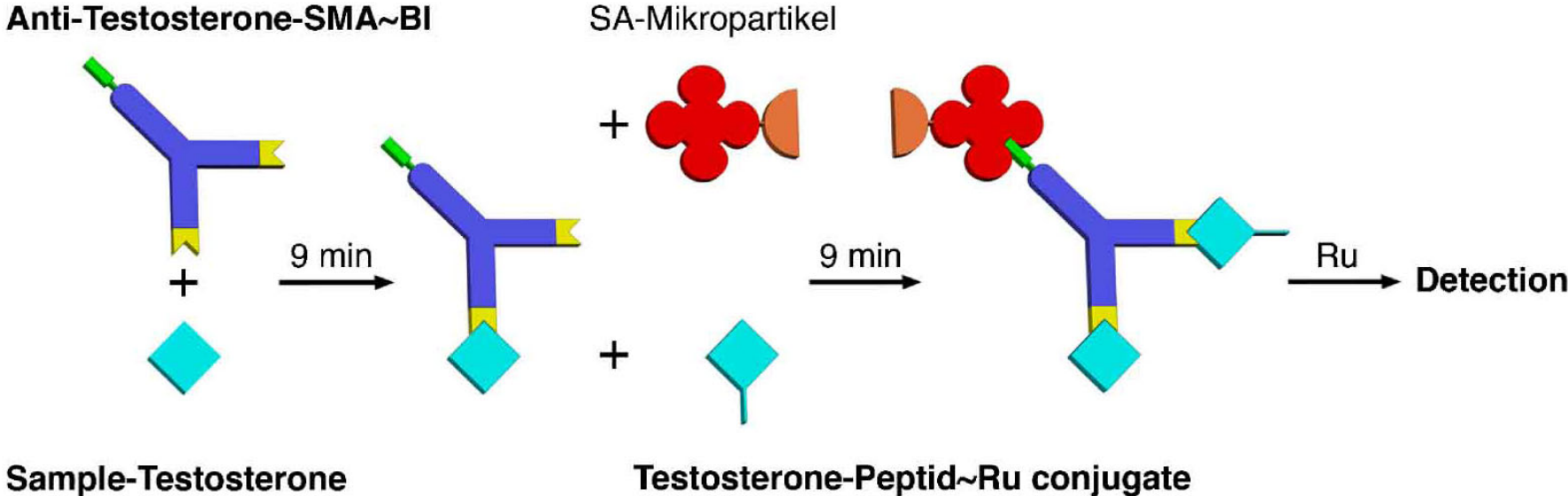
1st incubation: 20 μL of sample are incubated with a biotinylated high affinity monoclonal testosterone-specific antibody (sheep). The binding sites of the labeled antibody become occupied by the sample analyte (depending on its concentration).

2nd incubation: Streptavidin-coated microparticles and a testosterone derivative labeled with a ruthenium complex are added. Still free binding sites are occupied by the labeled conjugate. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.

Measurement: 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. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Elecsys Testosterone II - Test principle II

Competition format



Elecsys Testosterone II – Typical master standard curve

Signal is reciprocally proportional to analyte concentration



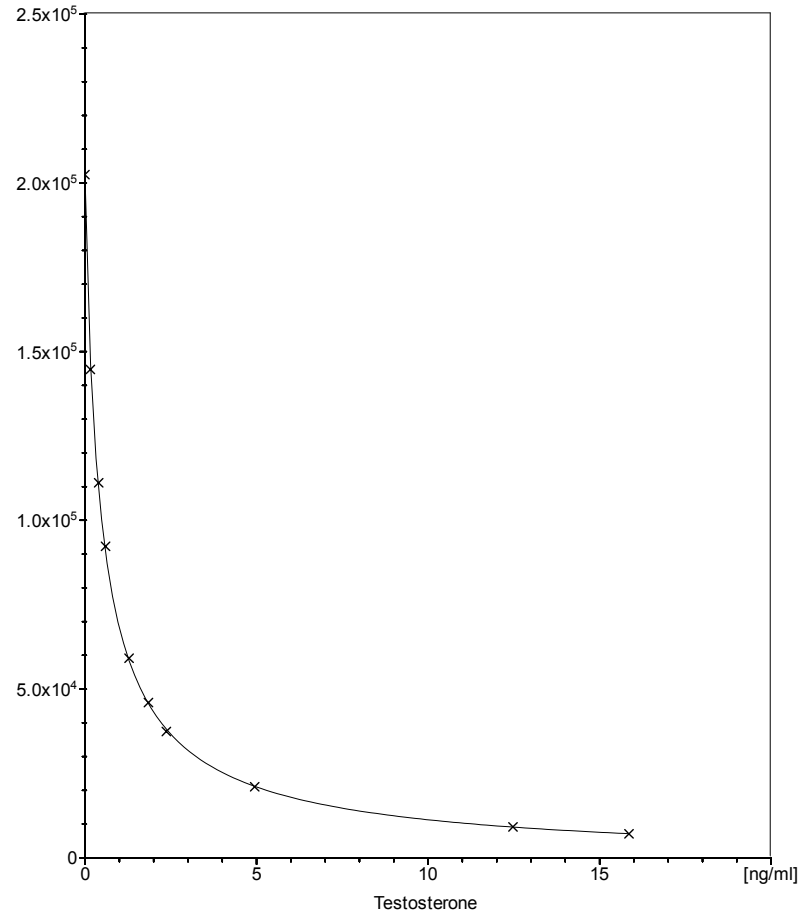
Elecsys Testosterone II Calibration Curve



MP-Kit

04.06.2009 08:54:23
RPZMS000579/TESTO_II/FH0024
Protokoll Id: -Draft- OLDEKAMJ Vers.:5.3.4

E2010

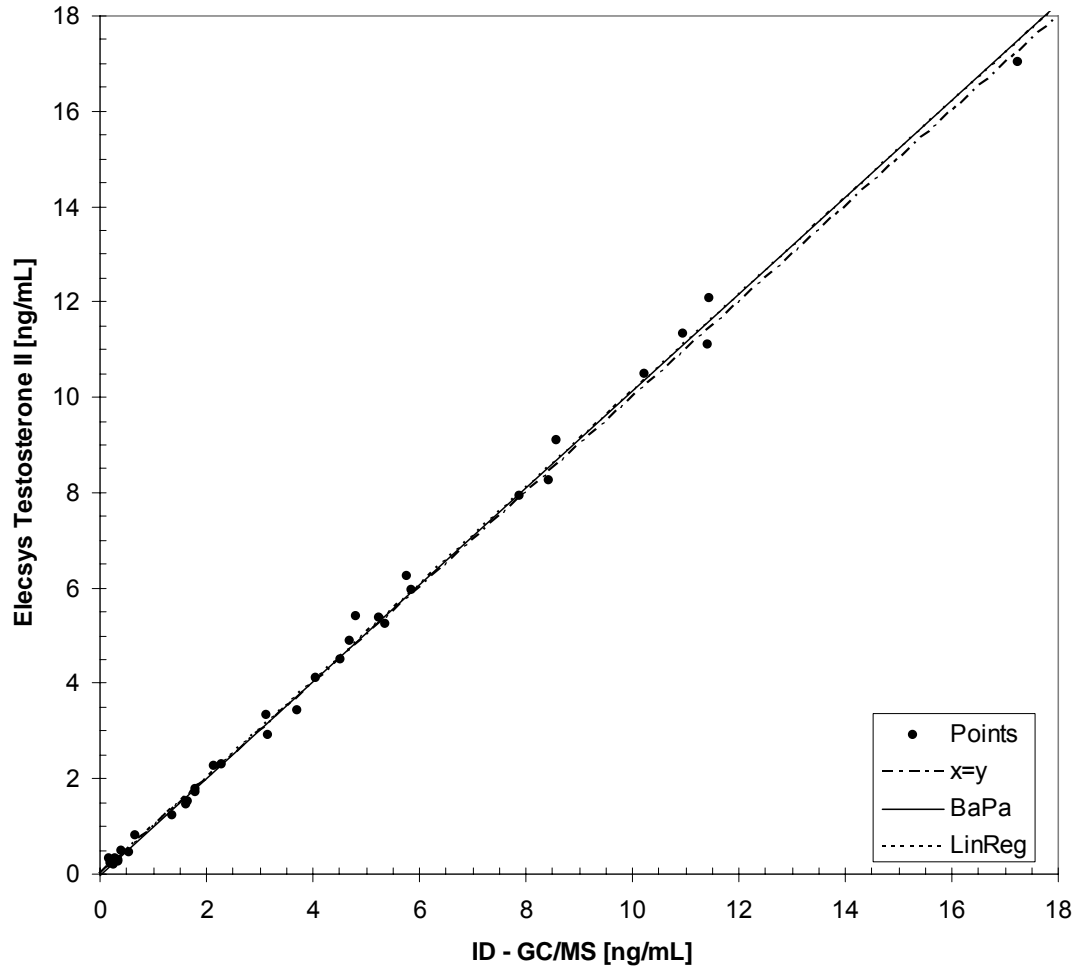


Competitive standard curve

Data source:
Elecsys Testosterone II
Assay development FH0024
MP-Kit

Elecsys Testosterone II – Traceability I

Standardized against ID-GC/MS (female and male samples) - the assay shows a very good correlation to ID-GC/MS



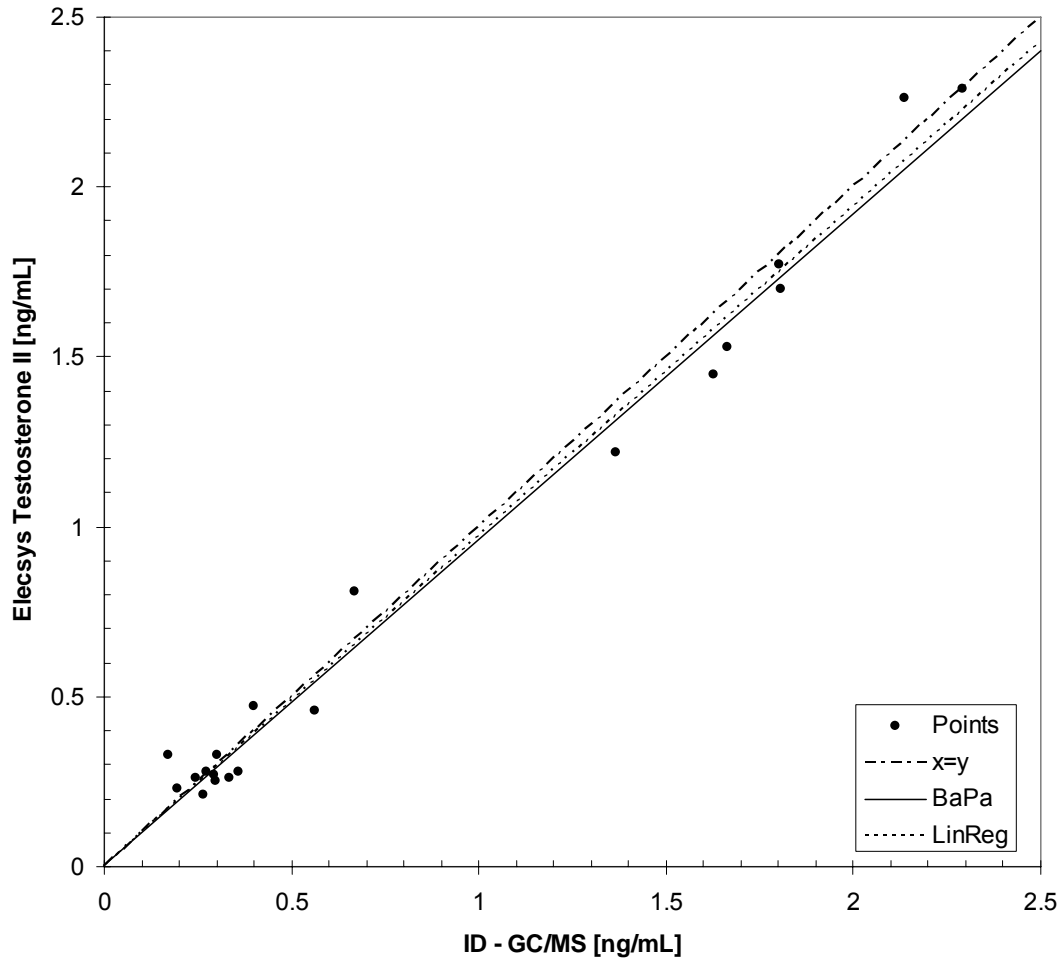
Number of samples measured: 39 total (19 male and 20 female samples)

The sample concentrations were between approx. 0.173 and 17.3 ng/mL.

Data Source:
Elecsys Testosterone II
Ref Stand MP Kit
Elecsys 2010

Elecsys Testosterone II – Traceability II

Standardized against ID-GC/MS (female samples) – the assay shows a very good correlation to ID-GC/MS in female samples



Number of samples measured: 20

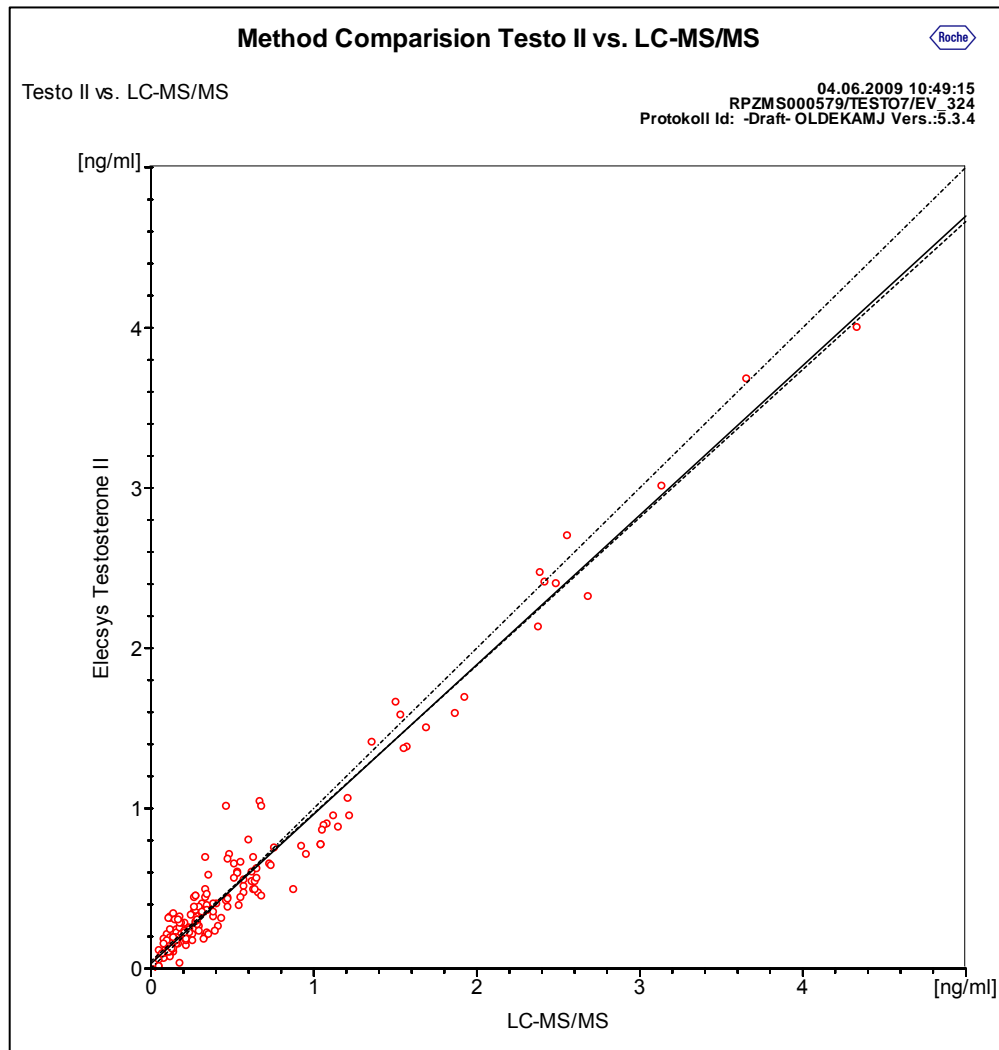
The sample concentrations were between approx. 0.173 and 2.29 ng/mL.

Data Source:
Elecsys Testosterone II
Ref Stand MP Kit
Elecsys 2010

Elecsys Testosterone II – Comparison to LC-MS/MS I



High correlation to the LC-MS/MS method (female samples from clinical routine, ARUP, USA)



Anzahl Wertepaare: 171

———— P/B	$y = 0.0297 + 0.9333 \cdot x$ (Kendalls tau)= 0.7752)
----- LinRegYX	$y = 0.0446 + 0.9232 \cdot x$ (Pearsons r)= 0.9808)
..... W.Halb	$y = x$

Data Source:
Elecsys Testosterone II
Ev_324
Research Kit

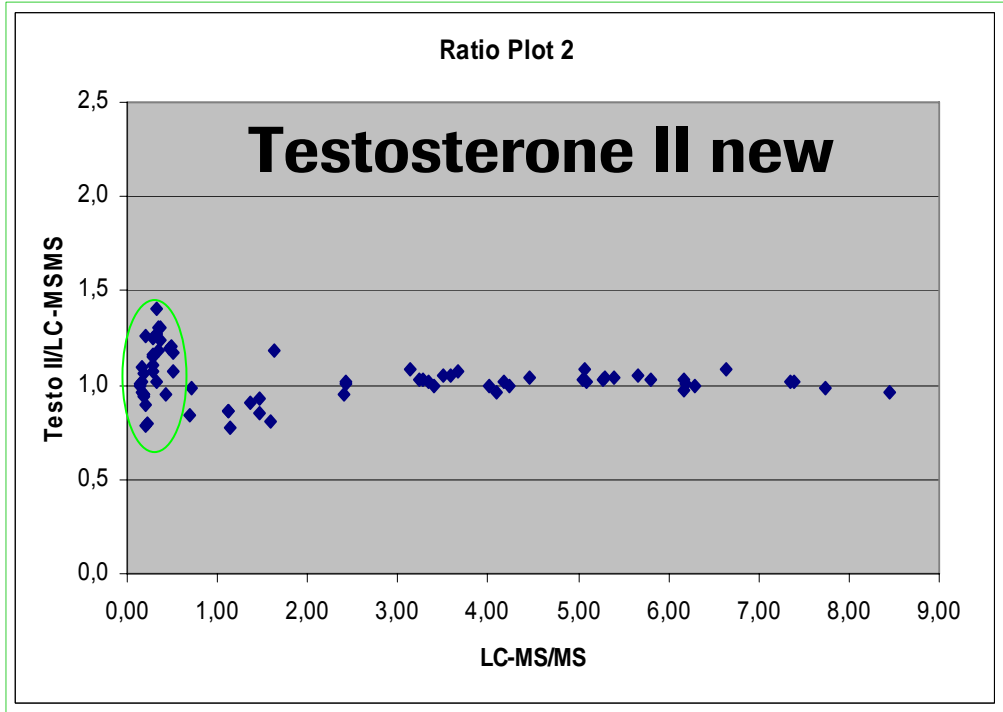
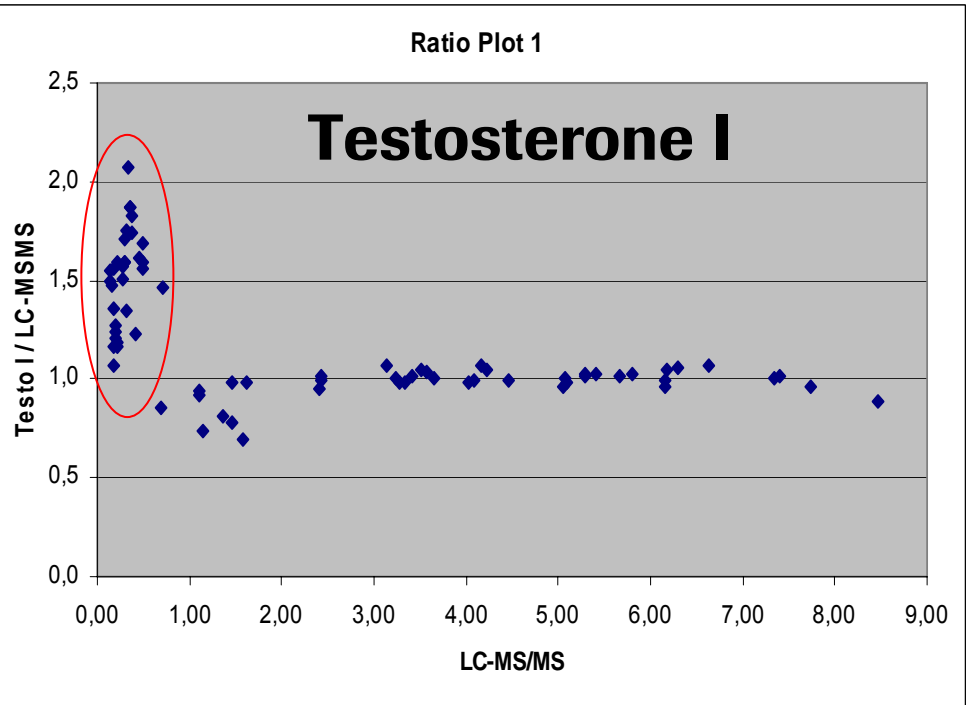
Elecsys Testosterone II – Comparison to LC-MS/MS II



Ratio plots confirm the improved results in accuracy of female samples with Testosterone II if compared to LC-MS/MS

Ratio plots 1 and 2: Comparison of Elecsys Testosterone I and Testosterone II with LC-MS/MS (Roche internal method) using UK-NEQAS female and male ring trial samples (UK NEQAS Distr. 334-343, 345-348; 33 females and 40 male samples; no spiked serum samples).

The x-axis (in ng/mL) represents the testosterone concentration measured by LC-MS/MS; the y-axis represents the immunoassay testosterone concentration divided by the LC-MS/MS testosterone concentration. Ratio of 1 means: the two methods gave the same result.

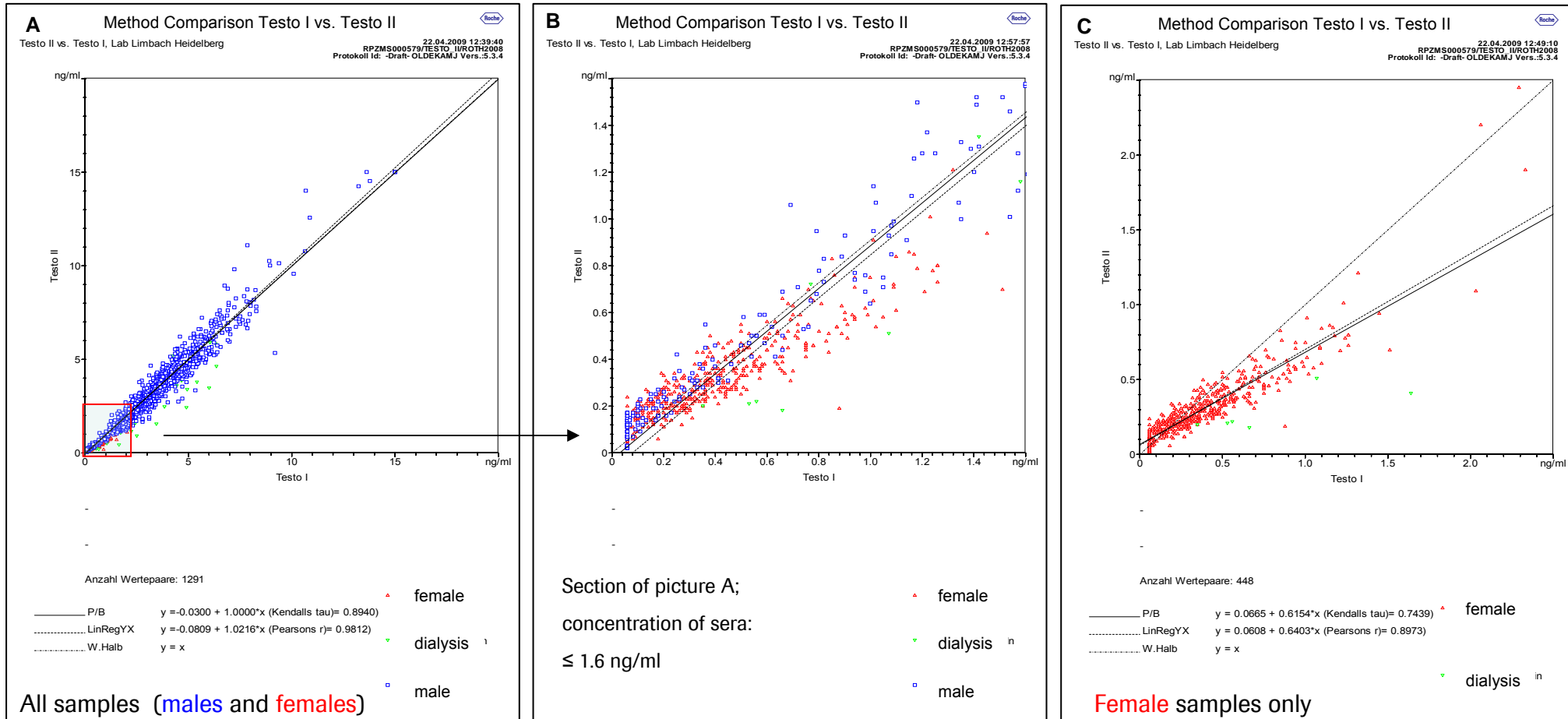


Elecsys Testosterone II – Method Comparison II



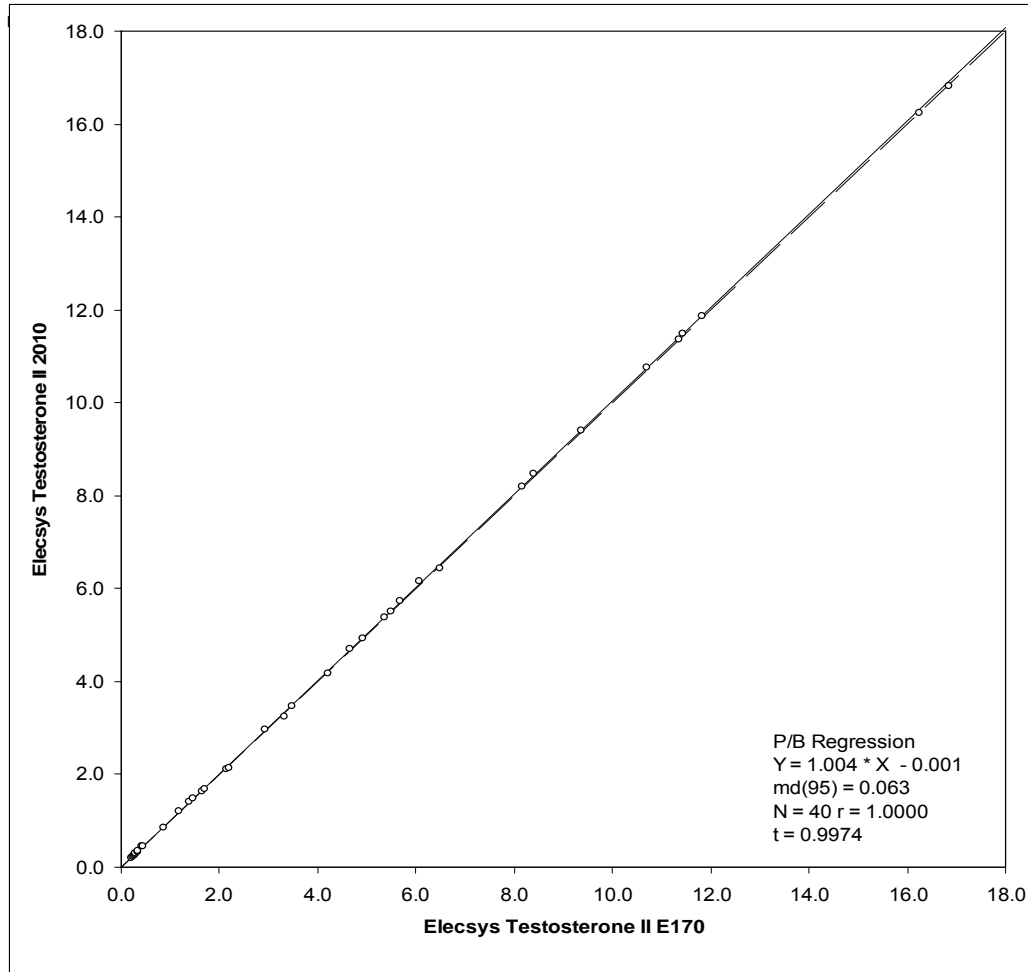
Comparison of routine samples with Elecsys Testosterone I and II

An approx. 30% negative bias in female samples can be expected with Testo II compared to Testo I, male samples are highly similar with both methods



Elecsys Testosterone II- Method Comparison I

Method comparison E170 vs. Elecsys 2010 - shows an excellent correlation between both platforms



Data source:

Elecsys Testosterone II
Standardization D031/09
P03 kit

Elecsys Testosterone II

Multicenter Evaluation Data – very good repeatability and intermediate precision over the whole measuring range on Elecsys 2010 cobas e 411



Elecsys 2010 and cobas e 411 analyzers								
			Repeatability ^a			Intermediate Precision ^b		
Sample	Mean		SD		CV	SD		CV
	ng/mL	nmol/L	ng/mL	nmol/L	%	ng/mL	nmol/L	%
Human Serum 1	0.095	0.330	0.004	0.014	4.7	0.008	0.028	8.4
Human Serum 2	0.691	2.40	0.014	0.048	2.1	0.022	0.076	3.2
Human Serum 3	2.16	7.50	0.042	0.146	1.9	0.060	0.208	2.8
Human Serum 4	8.67	30.1	0.229	0.795	2.6	0.243	0.843	2.8
Human Serum 5	13.0	45.1	0.158	0.548	1.2	0.440	1.53	3.4
PreciControl U1	6.30	21.9	0.088	0.305	1.4	0.182	0.632	2.9
PreciControl U2	2.65	9.20	0.047	0.163	1.8	0.097	0.337	3.7

Data source:
 Elecsys Testosterone II
 MCE study
 MP kit
 CLSI EP5-A2

a) Repeatability = within-run precision
 b) Intermediate precision = total precision

Elecsys Testosterone II – Precision II



Multicenter Evaluation Data - very good repeatability and intermediate precision over the whole measuring range on Modular Analytics E170 and cobas e 601

MODULAR ANALYTICS E170 and cobas e 601 analyzers								
		Repeatability			Intermediate precision			
Sample	Mean		SD		CV	SD		CV
	ng/mL	nmol/L	ng/mL	nmol/L	%	ng/mL	nmol/L	%
Human Serum 1	0.091	0.316	0.014	0.049	14.8	0.017	0.059	18.1
Human Serum 2	0.696	2.42	0.029	0.097	4.1	0.030	0.104	4.4
Human Serum 3	2.13	7.39	0.059	0.017	2.8	0.067	0.232	3.2
Human Serum 4	8.79	2.53	0.236	0.833	2.7	0.292	1.01	3.3
Human Serum 5	13.1	45.8	0.281	0.081	2.1	0.331	1.15	2.5
PreciControl U1	6.08	21.1	0.179	0.625	2.9	0.190	0.659	3.1
PreciControl U2	2.56	8.88	0.066	0.229	2.6	0.093	0.323	3.6

Data source:
 Elecsys Testosterone II
 MCE study
 MP kit Precision
 CLSI EP5-A2.

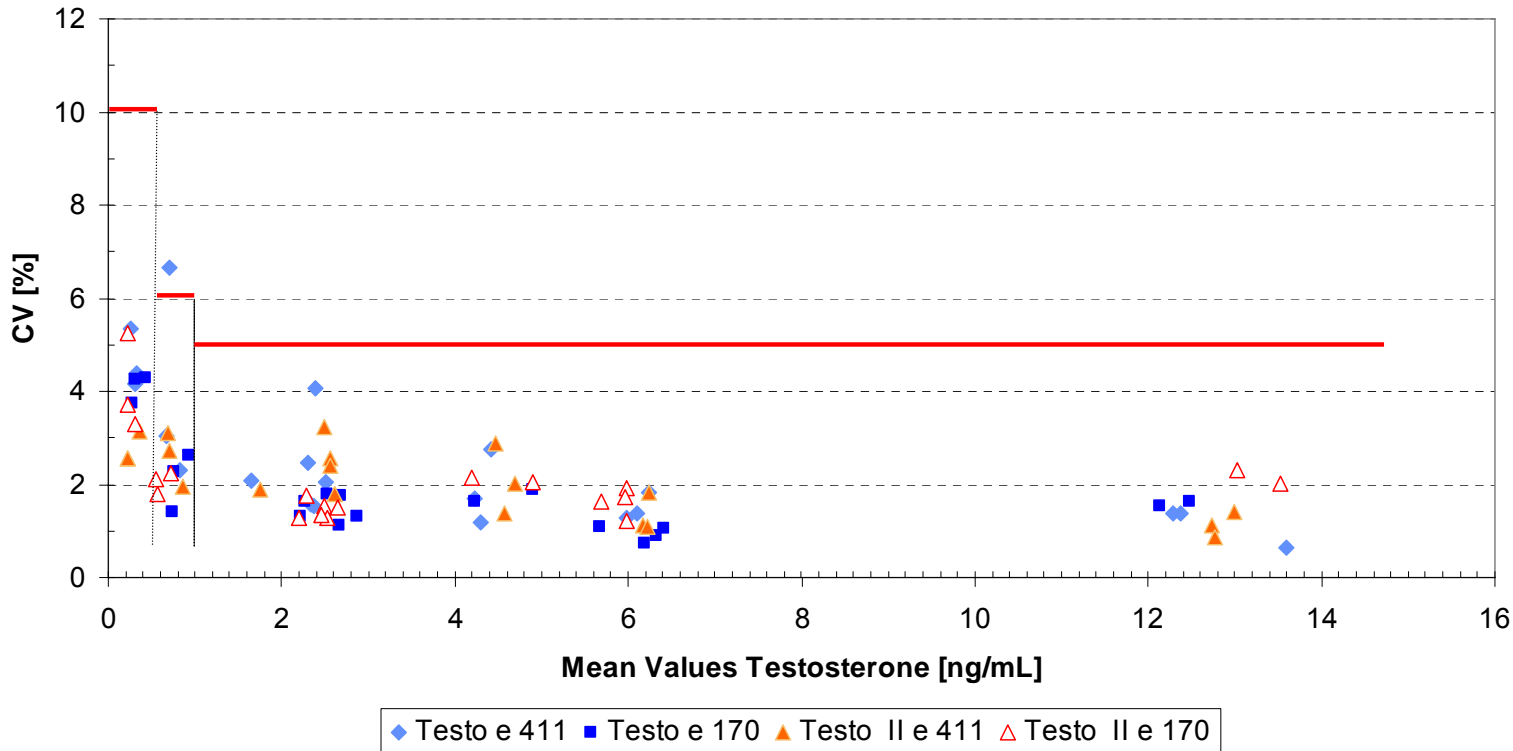
- a) Repeatability = within-run precision
- b) Intermediate precision = total precision

Elecsys Testosterone II - Precision III

Multicenter Evaluation Data – very good repeatability over the whole measuring range - comparable to Testosterone I or even better



Repeatability Data (n=21)
MCE Elecsys Testosterone II

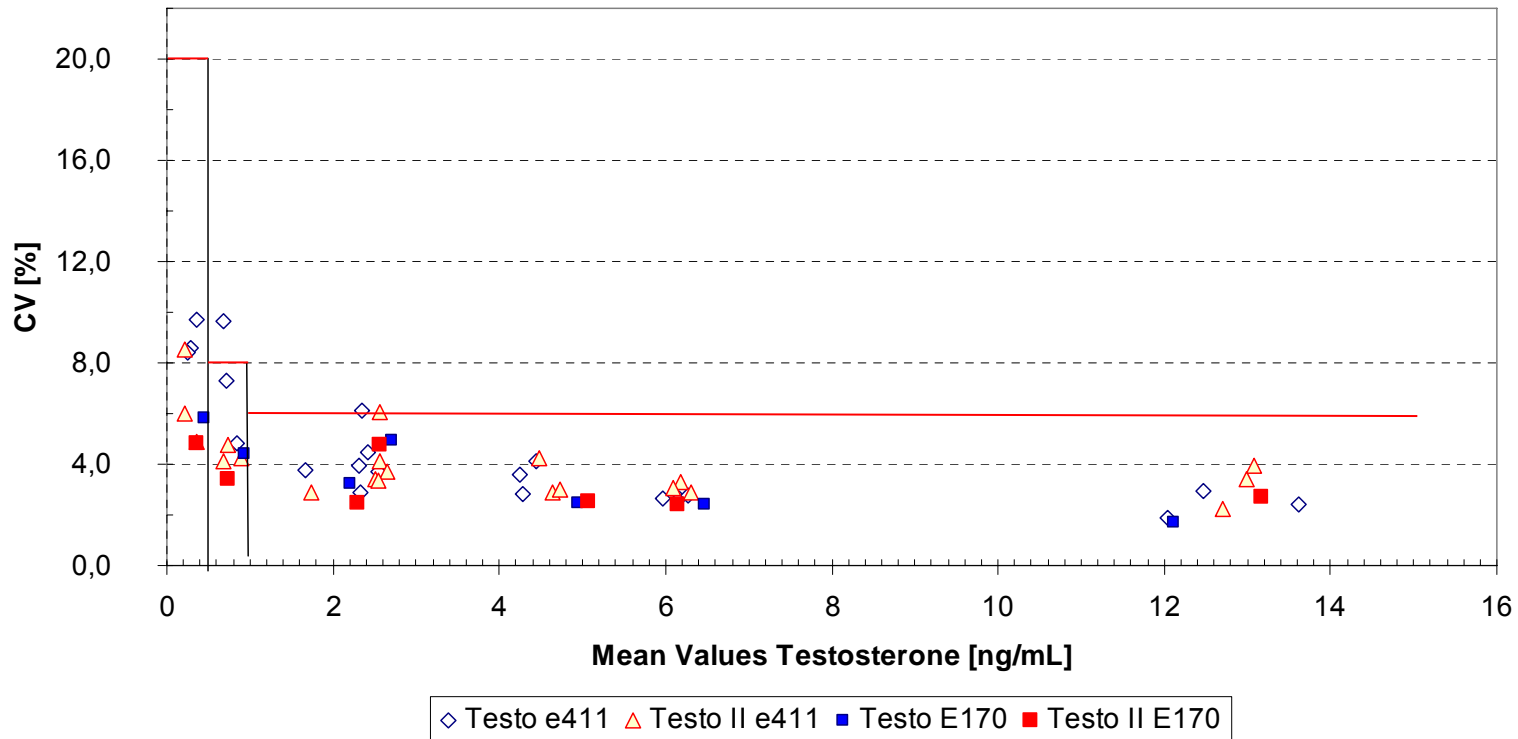


Elecsys Testosterone II – Precision IV



Multicenter Evaluation Data – very good intermediate over the whole measuring range - comparable to Testosterone I or even better

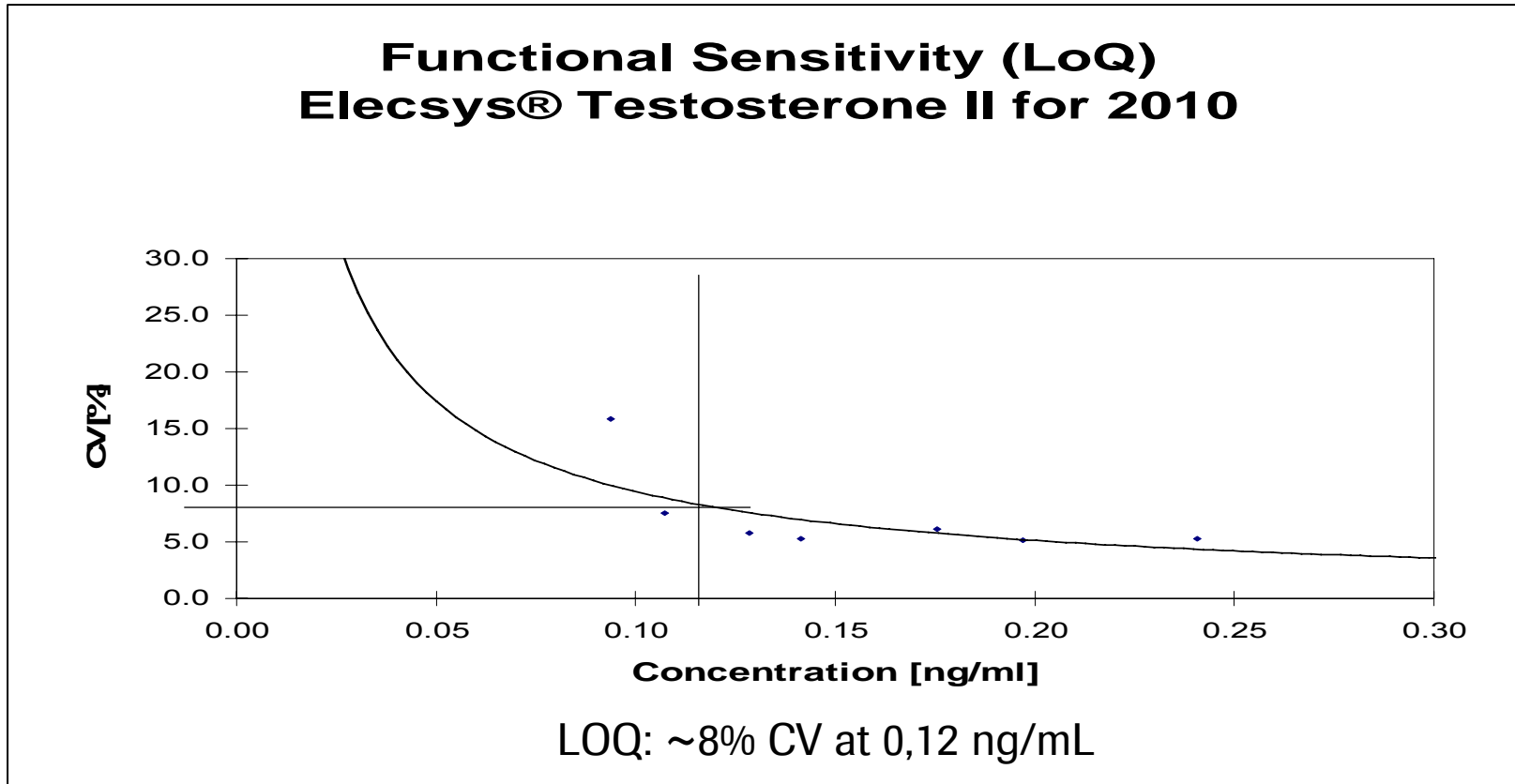
Within - Laboratory ("Total") Precision Data
(MCE Elecsys® Testosterone II, 21 Days, 2 Runs per Day, 2 Replicates)



Elecsys Testosterone II – Limit of Quantitation



Application Report Data – Limit of Quantitation (LoQ or Functional Sensitivity)
– very good precision in the lower range on 2010/ cobas e 411 and E170/cobas e 601



LoB: 0.012 ng/ml; LoD: 0.025

Elecsys Testosterone II – Cross-Reactivity

Accuracy of results not compromised by cross-reactive substances



	Concentration (ng/mL)	Cross reactivity %	Known physiological highest concentration
Androstendione	100	≤ 2.5	10 ng/mL
Danazol	1000	≤ 0.5	1 ug/mL
DHEA-S	50000	≤ 0.003	20 ug/mL
D-5-Androstene-3β,17β-diol	1000	≤ 0.29	unknown
Estradiol	1000	≤ 0.16	30 ng/mL
Ethisterone	1000	≤ 2.40	unknown
Norgestrel	1000	≤ 0.91	2 ng/mL
Testosterone propionate	100	≤ 2.46	unknown
5-α-Androstane-3β,17β-diol	1000	≤ 2.11	unknown
5-α-Dihydrotestosterone	500	≤ 0.87	1,5 ng/mL
11-β-Hydroxytestosterone	100	≤ 18.0	unknown
11-keto-Testosterone	1000	≤ 3.22	1 ng/mL
Prednisone	1000	n.d.	1 ug/mL
Prednisolone	1000	≤ 0.002	1 ug/mL
Progesterone	1000	n.d.	40 ng/mL
Cortisol	1000	≤ 0.01	550 ng/mL
Cortisone	2000	n.d.	550 ng/mL
Dexamethasone	2000	n.d.	unknown
Estrone	1000	≤ 0.004	500 pg/mL
DHEA	1000	≤ 0.016	15 ng/mL

Data source:
Application report
data
MP kit



Elecsys Testosterone – Expected Values I

in package insert lot 154749-01, expiry date 07/2010

Results obtained with the Elecsys Testosterone II assay in an apparently healthy group of 214 males and 190 females without intake of contraceptiva and prescription drugs (MCE study number CIM 000669)

		Percentiles					
	N	Median	5-95 th	2.5-97.5 th	Median	5-95 th	2.5-97.5 th
		ng/mL			nmol/L		
Males (20-50y)	137	5.36	2.50-8.36	2.18-9.05	18.6	8.69-29.0	7.58-31.4
Males (> 50y)	77	4.76	1.93-7.44	1.32-8.99	16.5	6.68-25.8	4.57-31.2
Females (21-50y)	105	0.248	0.058-0.476	0.035-0.513	0.860	0.200-1.65	0.120-1.78
Females (> 50y)	85	0.170	0.026-0.432	< 0.025-0.520	0.590	0.090-1.50	< 0.087-1.79

Elecsys Testosterone – Expected Values Ila - **update**



Testosterone panel in combination with SHBG Data, package insert will be updated with these new data with next Testosterone lot.

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 (MCE study number CIM 000669)

Test subjects	N	Percentiles					
		Median	5-95th	2.5-97th	Median	5-95th	2.5-97th
		ng/mL			nmol/L		
Males 20-49 years	136	5.36	2.49-8.36	2.18-9.06	18.6	8.64-29.0	7.57-31.4
Males ≥ 50 years	78	4.76	1.93-7.40	1.32-8.92	16.5	6.68-25.7	4.59-31.0
Females 20-49 years	89	0.271	0.084-0.481	0.050-0.522	0.941	0.290-1.67	0.173-1.81
Females ≥ 50 years	71	0.162	0.029-0.408	< 0.025-0.461	0.563	0.101-1.42	0.070-1.60

Elecsys Testosterone – Expected Values IIb - **update**



Testosterone panel - SHBG Data

Results were evaluated with focus on Testosterone II assay included measurements in parallel with the Elecsys SHBG assay (MCE study number CIM 000669).

SHBG	N	Percentiles		
		Median	5-95th	2.5-97.5th
		nmol/mL		
Males 20-49 years	136	33.5	16.5-55.9	15.4-63.8
Males ≥ 50 years	78	40.8	19.3-76.4	14.2-78.0
Females 20-49 years	89	64.3	24.6-122	19.1-145
Females ≥ 50 years	71	57.4	17.3-125	14.4-136

Elecsys Testosterone – Expected Values IIC - **update**



Testosterone panel – Free Testosterone index (FTI) of free androgen index (FAI)

*Results were evaluated with focus on Testosterone II assay included measurements in parallel with the Elecsys SHBG assay (MCE study number CIM 000669) and commonly used parameters derived from different calculation procedures, including albumin as an important parameter involved.**

Test subjects	N	Percentiles		
		Median	5-95th	2.5-97.5th
		FTI or FAI (%)		
Males 20-49 years	136	57.2	35.0-92.6	24.0-104
Males ≥ 50 years	78	38.2	24.3-72.1	21.6-77.1
Females 20-49 years	89	1.53	0.297-5.62	0.178-7.07
Females ≥ 50 years	71	1.15	0.187-3.63	0.104-4.95

* Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666-3672.

Elecsys Testosterone – Expected Values IId - **update**



Testosterone panel – Free Testosterone, calculated

*Results were evaluated with focus on Testosterone II assay included measurements in parallel with the Elecsys SHBG assay (MCE study number CIM 000669) and commonly used parameters derived from different calculation procedures, including albumin as an important parameter involved.**

		Percentiles					
Test subjects	N	Median	5-95th	2.5-97.5th	Median	5-95th	2.5-97.5th
		FTc (nmol/L)			FTc (%)		
Males 20-49 years	136	0.379	0.198-0.619	0.174-0.672	2.10	1.53-2.88	1.47-2.97
Males ≥ 50 years	78	0.304	0.163-0.473	0.129-0.567	1.91	1.23-2.59	1.16-2.81
Females 20-49 years	89	0.011	0.003-0.33	0.001-0.034	1.19	0.701-2.19	0.601-2.36
Females ≥ 50 years	71	0.008	0.001-0.020	0.001-0.022	1.26	0.685-2.64	0.632-2.80

* Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666-3672.

Elecsys Testosterone – Expected Values IIe - **update**



Testosterone panel – Bioavailable Testosterone, calculated

*Results were evaluated with focus on Testosterone II assay included measurements in parallel with the Elecsys SHBG assay (MCE study number CIM 000669) and commonly used parameters derived from different calculation procedures, including albumin as an important parameter involved.**

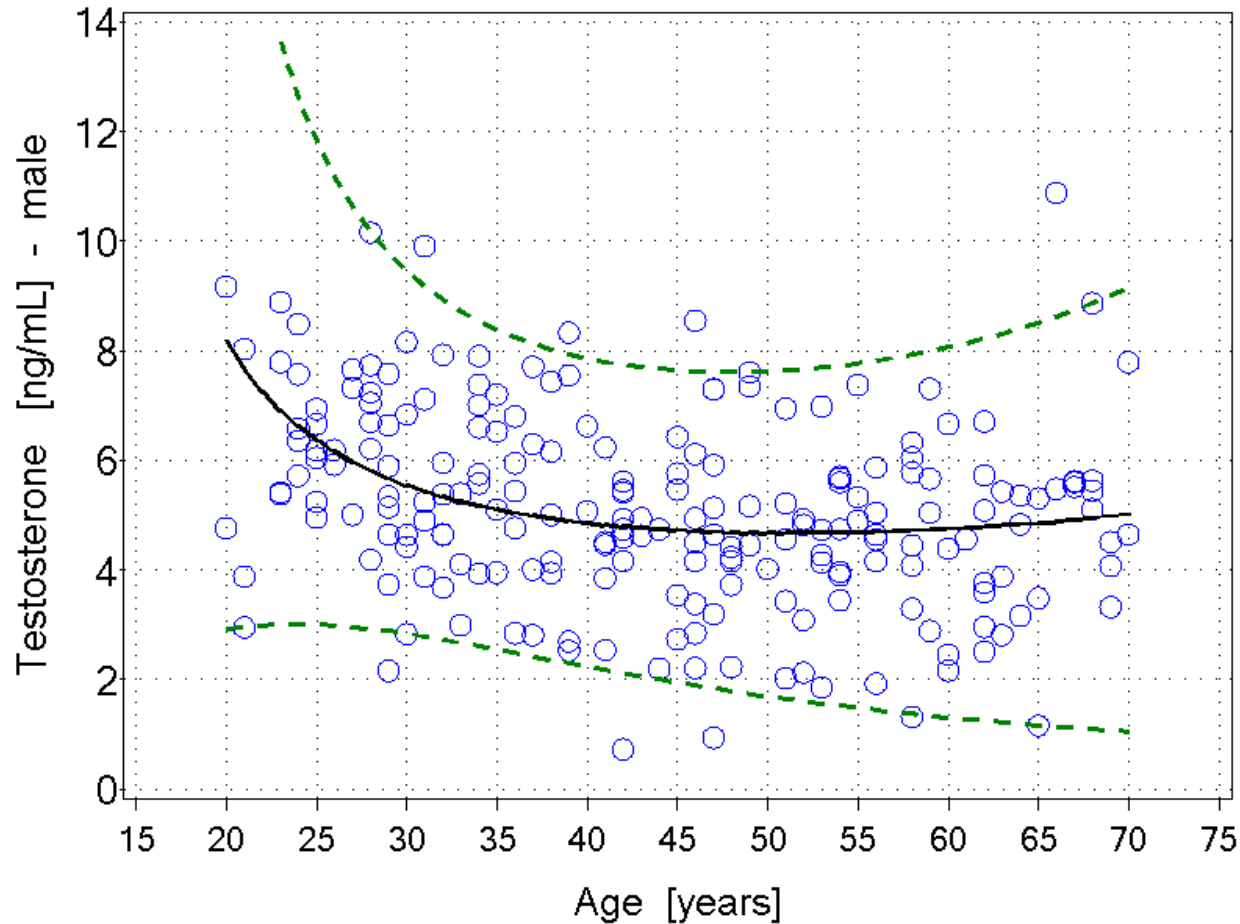
		Percentiles					
Test subjects	N	Median	5-95th	2.5-97.5th	Median	5-95th	2.5-97.5th
		BATc (nmol/L)			BATc (%)		
Males 20-49 years	136	9.10	4.36-14.3	3.68-15.3	49.8	35.0-66.3	31.7-68.2
Males ≥ 50 years	78	6.63	3.59-11.0	3.07-12.6	42.1	27.5-60.7	24.2-63.7
Females 20-49 years	89	0.246	0.059-0.756	0.033-0.774	25.7	15.3-47.7	13.0-56.1
Females ≥ 50 years	71	0.168	0.030-0.430	<0.025-0.460	28.0	15.1-55.2	14.3-59.8

* Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666-3672.

Elecsys Testosterone II – Expected values IIe - **update**



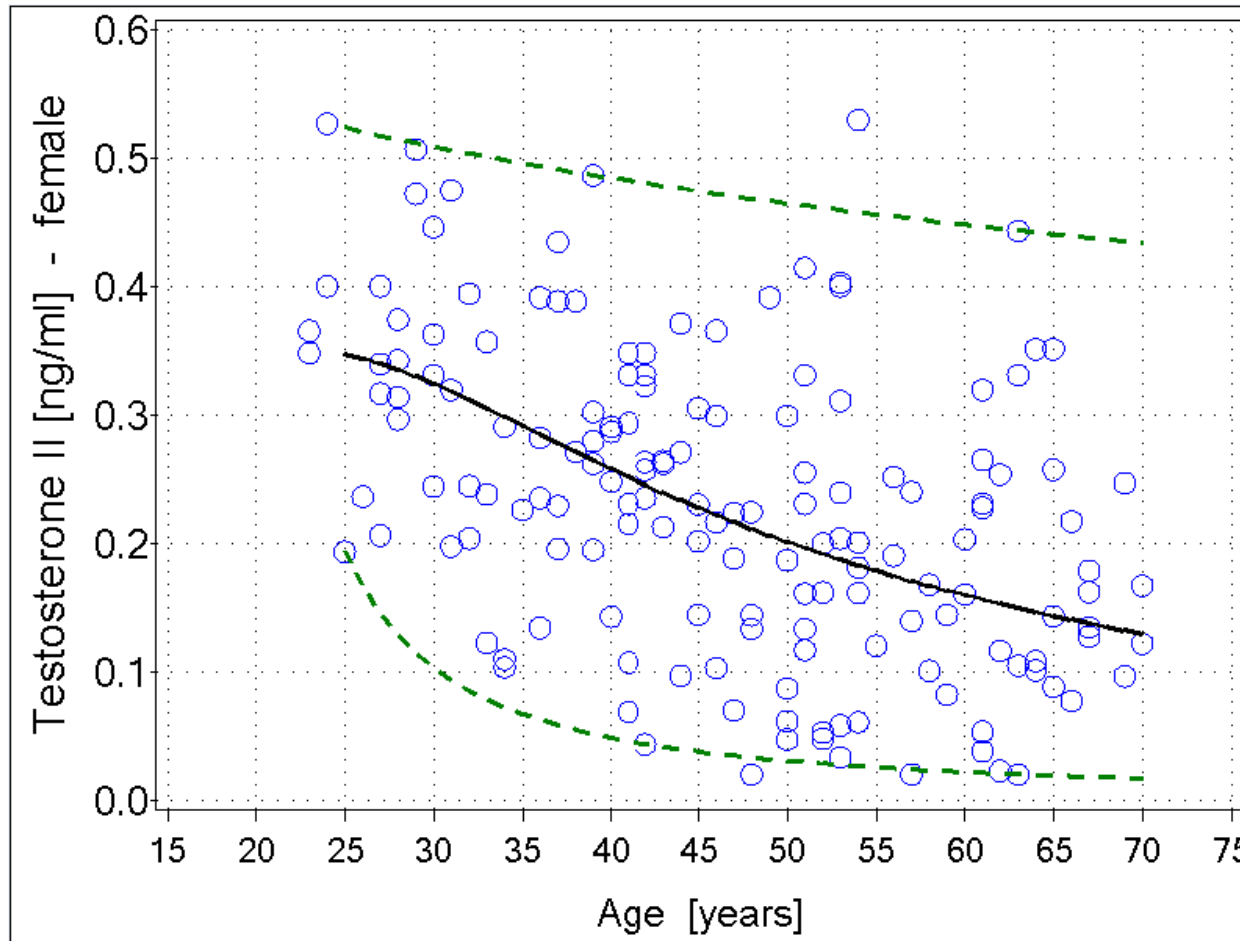
Distribution of testosterone values in the apparently healthy male control group dependent on age, n = 214. Solid line: 50% percentile, upper line: 97.5% percentile, lower line 2.5% percentile



Elecsys Testosterone II – Expected values If - **update**

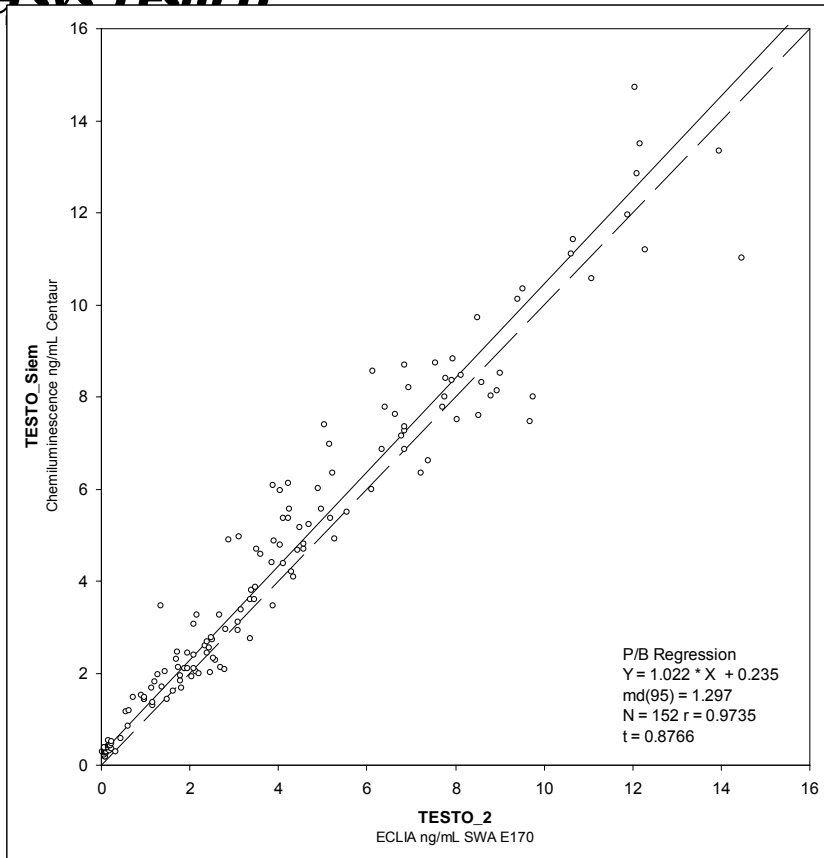


Distribution of testosterone values in the apparently healthy female control group dependent on age, n = 160. Solid line: 50% percentile, upper line: 97.5% percentile, lower line 2.5% percentile

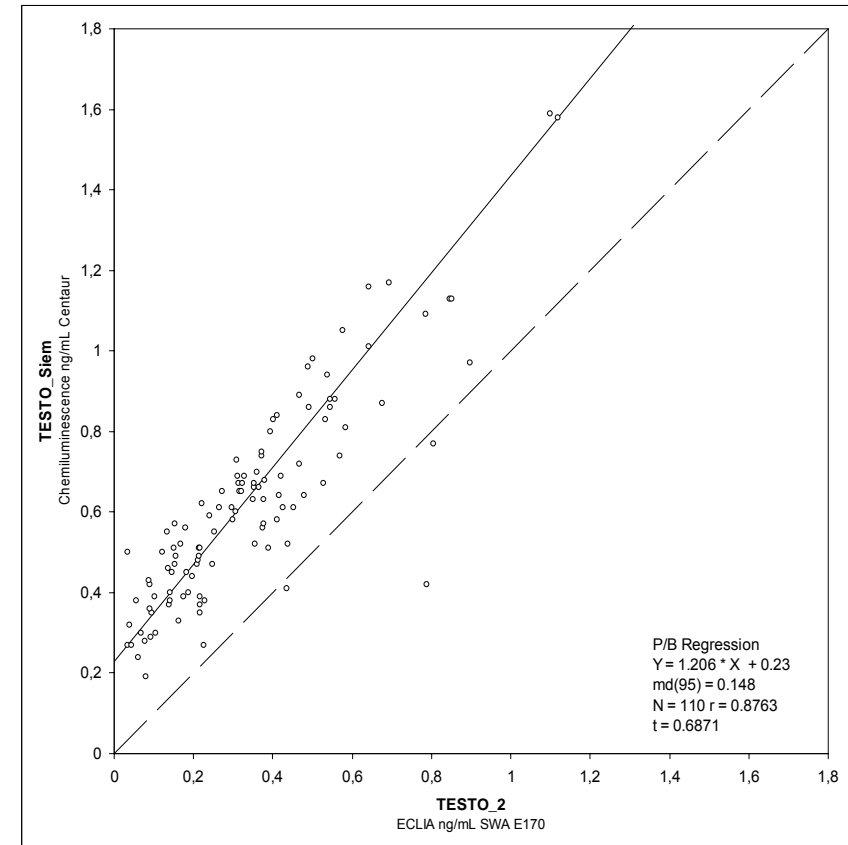


Elecsys Testosterone II – Competitor Comparison

Comparison of Elecsys Testosterone with Bayer Centaur – good correlation in male samples, in female samples a positive bias of around 20% with an intercept of 0.23 can be expected with Bayer Centaur when comparing to Elecsys Testosterone II



Males

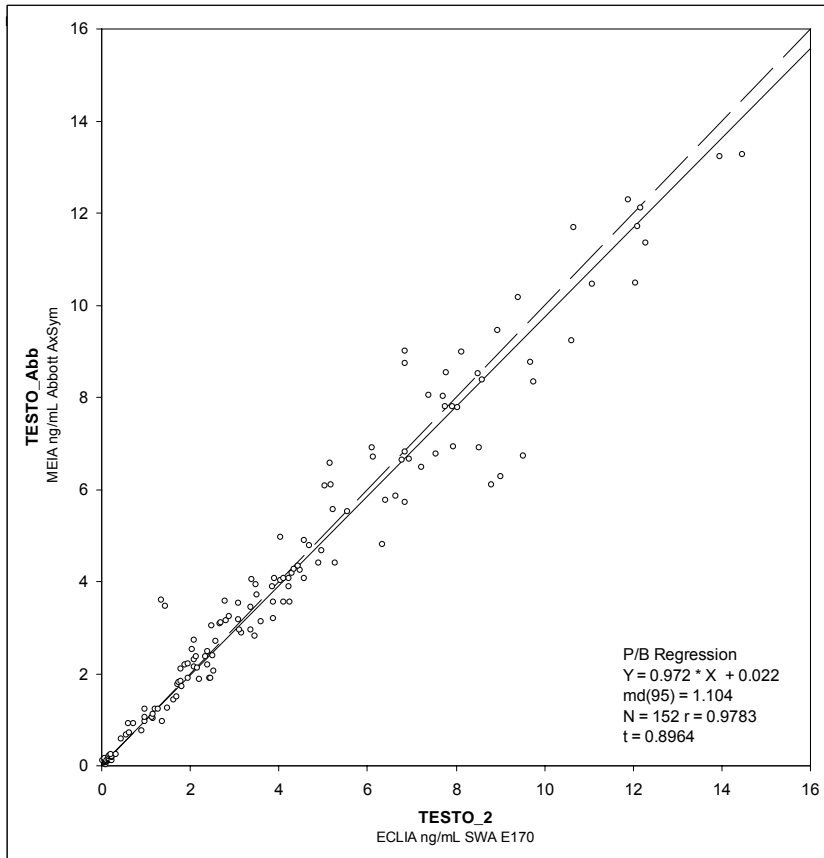


Females

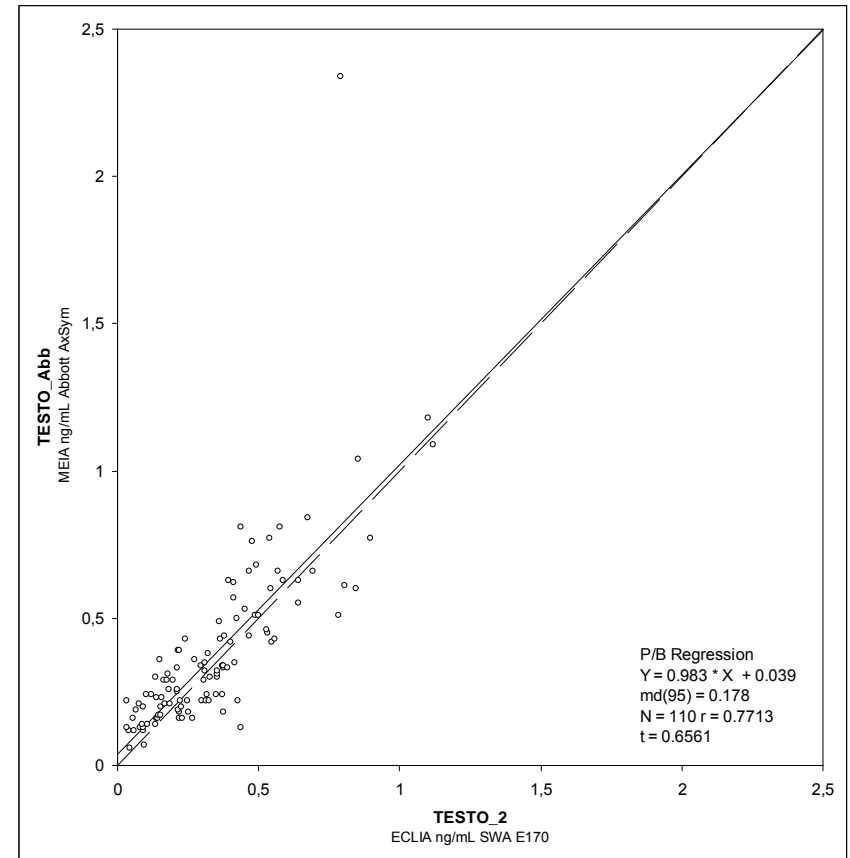
Elecsys Testosterone II – Competitor Comparison



Comparison of Elecsys Testosterone with ABBOTT AxSym - good correlation in male samples and female samples



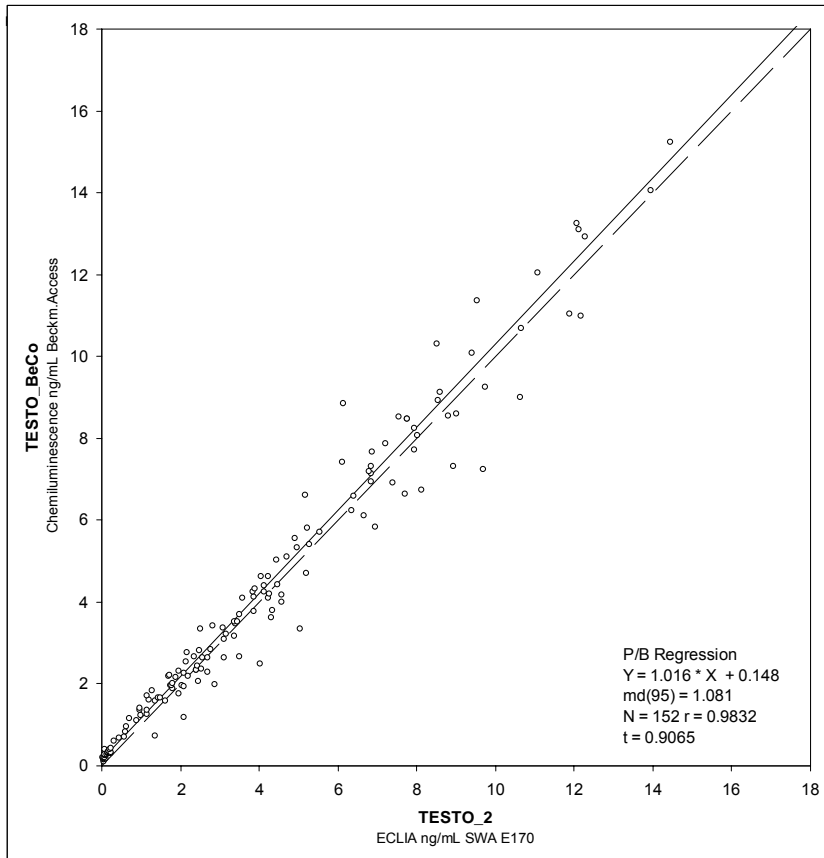
Males



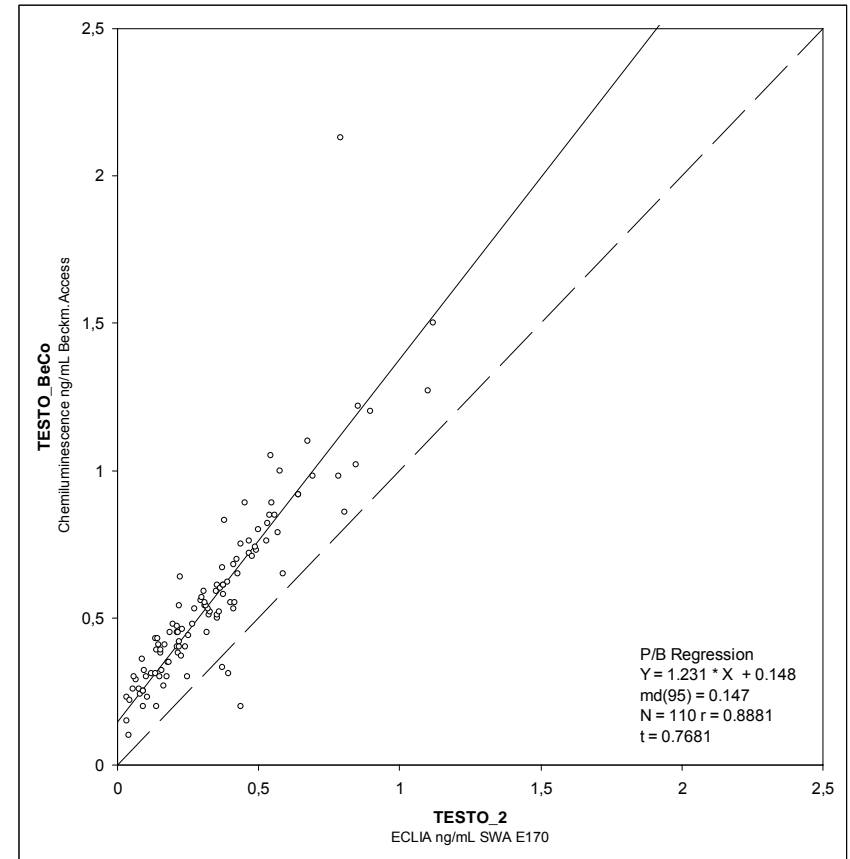
Females

Elecsys Testosterone II – Competitor Comparison

Comparison of Elecsys Testosterone with Beckman Access - good correlation in male samples, in female samples a positive bias of around 23% with an intercept of 0.15 can be expected with Beckman Access when comparing to Elecsys Testosterone II



Males

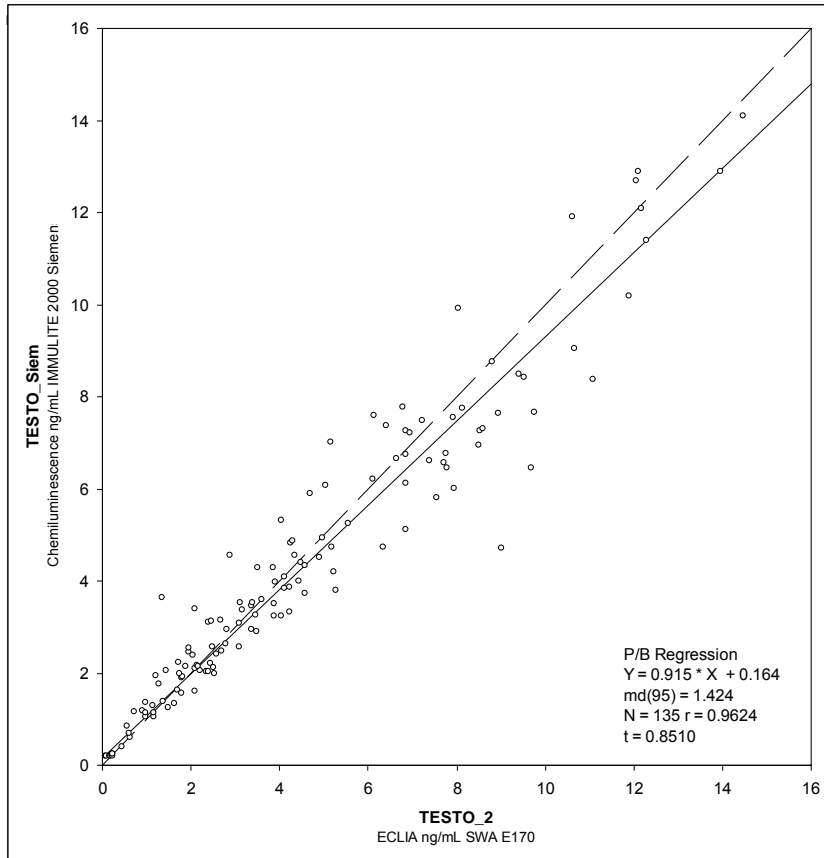


Females

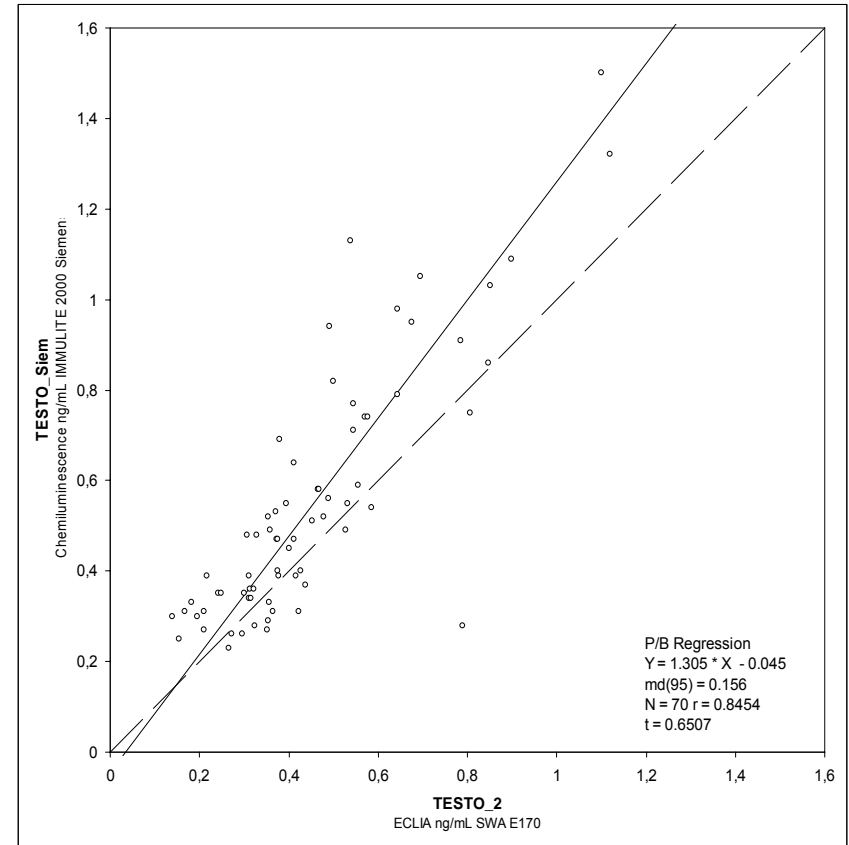
Elecsys Testosterone II – Competitor Comparison



Comparison of Elecsys Testosterone with Siemens Immulite 2000 - good correlation in male samples, in female samples a positive bias of around 30% with an intercept of - 0.05 can be expected with Siemens Immulite when comparing to Elecsys Testo II



Males

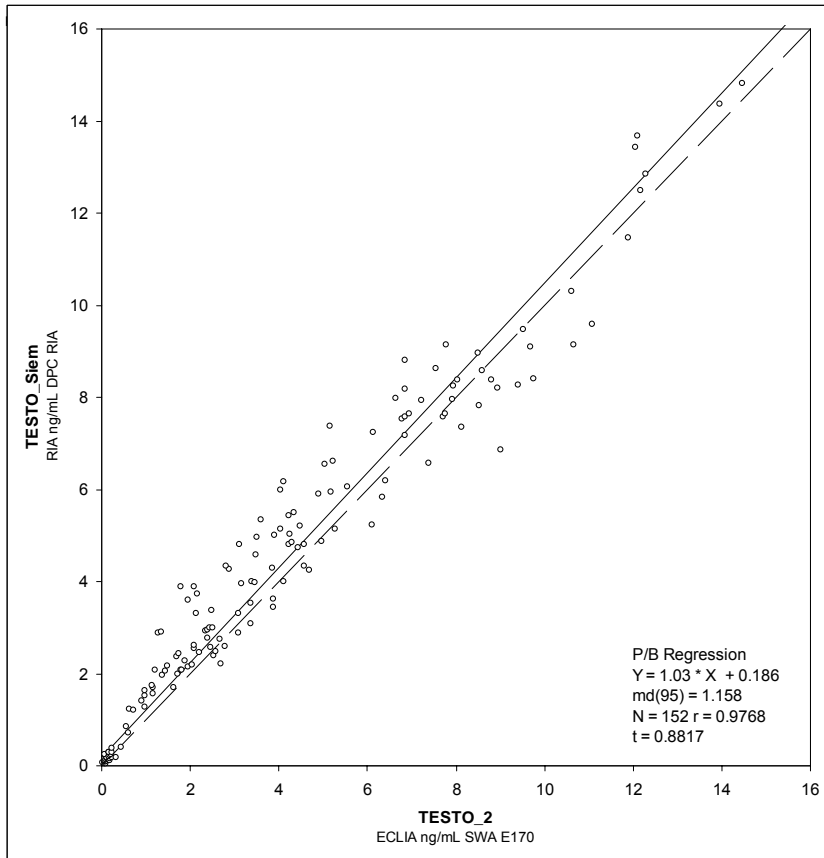


Females

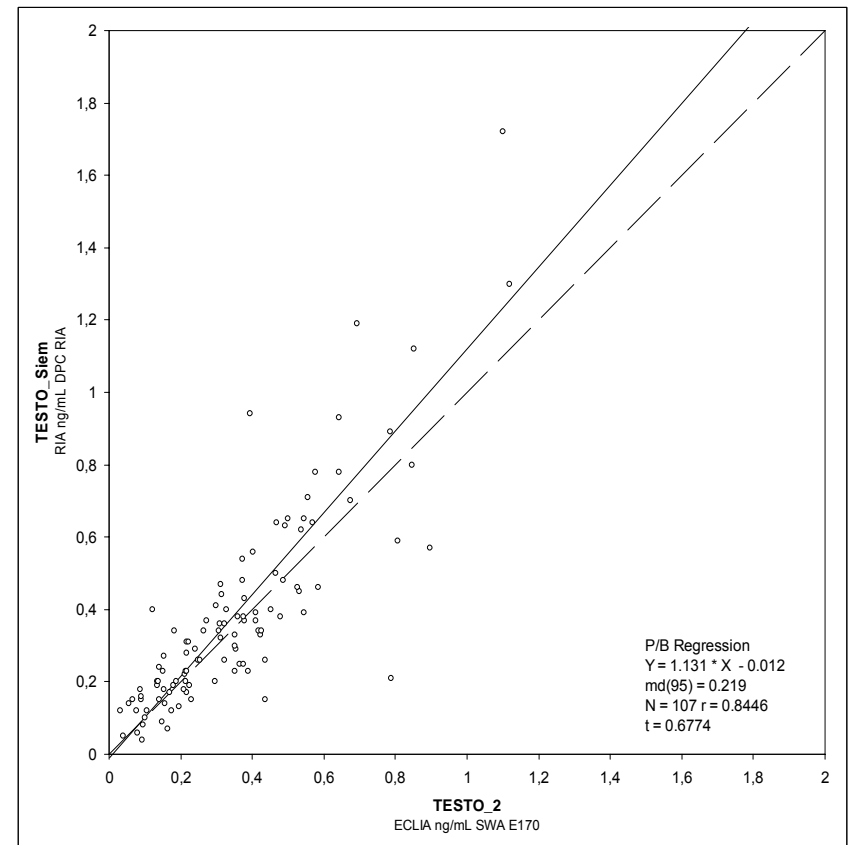
Elecsys Testosterone II – Competitor Comparison



Comparison of Elecsys Testosterone with DPC RIA - good correlation in male samples, in female samples a fairly good correlation can be expected with DPC RIA when comparing to Elecsys Testo II



Males



Females

Elecsys Testosterone II - Key Performance Overview



Total Duration	18 Minutes
Assay Principle	Competitive
Sample Volume	20 µL
LOB	≤ 0.012 ng/mL
LOD	≤ 0.025 ng/mL
LOQ	≤ 0.12 ng/ml
Measuring range	0.025 (= LOD) – 15 ng/mL
Traceability	ID-GC/MS targeted female and male testosterone samples
Total precision	CV% 2.2 – 8.5% (0.22-13.18 ng/ml), MCE CIM000669
Sample material	Serum and plasma (Li-heparin; K2- and K3-EDTA plasma)
Reagent stability	Unopened at 2 – 8°C up to the stated expiration date
Onboard stability	8 weeks on all analyzers
Dilution	Generally not necessary due to the broad measuring range
Expected values	Please refer to the page 20 - 22 for the details.