510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K211685

B Applicant

Roche Diagnostics

C Proprietary and Established Names

Elecsys Testosterone II

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
CDZ	Class I, reserved	21 CFR 862.1680 - Testosterone Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

Modification of a cleared device to decrease interference to biotin

B Measurand:

Testosterone

C Type of Test:

Quantitative Immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

Immunoassay for the in vitro quantitative determination of testosterone in human serum and plasma.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on the cobas e 601 immunoassay analyzer.

Measurements of testosterone are used in the diagnosis and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism, delayed or precocious puberty, impotence in males and, in females hirsutism (excessive hair) and virilization (masculinization) due to tumors, polycystic ovaries, and adrenogenital syndromes.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Cobas e 601 immunoassay analyzer

IV Device/System Characteristics:

A Device Description:

The Elecsys Testosterone II reagent kit consists of a Reagent Pack (R1, R2, and M (Streptavidin-coated microparticles). The Ready-to-use Rack Pack (kit placed on the analyzer) contains:

· 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)23+ (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.

B Principle of Operation:

The Elecsys Testosterone II immunoassay makes use of a competitive test principle using streptavidin-coated microparticles and electrochemiluminescence detection. Results are

determined using a calibration curve that is generated specifically on each instrument by a 2-point calibration and master curve provided with the reagent bar code.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Elecsys Testosterone II

B Predicate 510(k) Number(s):

k093421

C Comparison with Predicate(s):

Device & Predicate Device(s):	K211685	K093421
Device Trade Name	Elecsys Testosterone II	Elecsys Testosterone II
General Device Characteristic Similarities		
Intended Use/Indications For Use	Measurements of testosterone are used in the diagnosis and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism, delayed or precocious puberty, impotence in males and, in females hirsutism (excessive hair) and virilization (masculinization) due to tumors, polycystic ovaries, and adrenogenital syndromes.	Same
Detection Method	Electrochemiluminescence immunoassay (ECLIA)	Same
Sample Matrix	Human serum, lithium heparin, K2-EDTA and K3-EDTA plasma	Same
Traceability	ID-GC/MS (“Isotope Dilution - Gas	Same

	Chromatography/Mass Spectrometry").	
Assay range	2.50-1500 ng/dL	Same
General Device Characteristic Differences		
Interference from Biotin	This assay has no biotin interference in serum concentrations up to 1200 ng/mL.	< 30 ng/mL

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3; Evaluation of Precision of Quantitative Measurement Procedures, 3rd Edition

CLSI EP17-A2; Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition

CLSI EP07-A3; Interference testing in Clinical Chemistry; Approved Guideline – Third Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision was evaluated on one cobas e 601 analyzer according to CLSI guideline EP05-A3. Two replicates of 2 controls and 5 native human serum samples (2 single samples and 3 sample pools) were tested.

Each control and sample was tested in 2 runs per day over 21 days using 1 reagent lot. Repeatability and intermediate precision were calculated according to CLSI EP05-A3. Assay calibration was performed as specified in the package insert.

Sample	N	Mean (ng/dL)	Repeatability		Intermediate Precision	
			SD (ng/dL)	CV (%)	SD (ng/dL)	CV (%)
Human Serum 1	84	9.33	0.886	9.5	1.37	14.7
Human Serum 2	84	30.3	1.34	4.4	1.81	6.0
Human Serum 3	84	71.7	1.34	1.9	2.33	3.2
Human Serum 4	84	200	2.52	1.3	4.15	2.1
Human Serum 5	84	1347	27.1	2.0	41.2	3.1
Control 1	84	534	9.94	1.9	15.8	3.0
Control 2	84	240	4.59	1.9	7.42	3.1

Lot to lot precision was evaluated on one cobas e 601 analyzer according to CLSI guideline EP05-A3. Five aliquots of each control and human serum samples (2 single samples and 3 sample pools) per run, 1 run per day for 5 days with 3 lots.

Repeatability and intermediate precision were calculated according to CLSI EP05-A3. Assay calibration was performed as specified in the package insert.

Results were similar across three lots. Results from a representative lot are shown below:

Sample	Mean (ng/dL)	Repeatability		Intermediate Precision	
		SD (ng/dL)	CV (%)	SD (ng/dL)	CV (%)
Human Serum 1	10.0	1.1	11.3	1.2	12.2
Human Serum 2	31.4	1.6	5.2	2.2	7.2
Human Serum 3	73.2	1.9	2.6	2.8	3.9
Human Serum 4	207	3.9	1.9	4.9	2.4
Human Serum 5	1380	35.6	2.6	47.4	3.4
Control 1	555	14.0	2.5	21.2	3.8
Control 2	251	6.3	2.5	10.5	4.2

2. Linearity:

For linearity, one reagent lot was tested on one cobas e 601 with one run. One human serum sample with high analyte content above the measuring range was diluted to the lower end of the measuring range with various amounts of human serum sample without analyte content. A similar design was used for each plasma dilution series. The dilution series contained 25 steps for serum and 20 dilution steps for lithium heparin plasma. Samples were assayed in 3-fold determinations (3 sera and 3 plasma samples for one lot). Each dilution series contained samples from approximately 2.0 ng/dL to 1550 ng/dL and covered the claimed measuring range. One representative serum and one representative plasma dilution series are shown below:

Serum

Dilution Step	Theoretical Concentration (ng/dL)
1	2.4
2	4.90
3	9.70
4	19.4
5	38.8
6	77.6
7	155
8	233
9	310
10	388
11	466
12	543
13	621
14	698
15	776

Dilution Step	Theoretical Concentration (ng/dL)
16	854
17	931
18	1010
19	1090
20	1160
21	1240
22	1320
23	1400
24	1470
25	1550

Plasma

Dilution Step	Theoretical Concentration (ng/dL)
1	1.7
2	3.4
3	6.8
4	13.5
5	27.0
6	54.0
7	108
8	216
9	324
10	432
11	540
12	648
13	757
14	865
15	973
16	1080
17	1190
18	1300
19	1400
20	1620

The linearity data were analyzed with regards to linear, quadratic and cubic polynomials. In a first step, a linearity check was performed with a first order (linear) regression and then with higher order models (quadratic and cubic).

The averaged linear regression for the 3 serum dilution series was as follows:
Y= 1.006x-0.042; R = 0.9991

The averaged linear regression for the 3 plasma dilution series was as follows:
Y= 1.005x-0.030; R = 0.9974

3. Analytical Specificity/Interference:

Endogenous Interference

The effect on quantitation of analyte in the presence of endogenous interfering substances was determined for testosterone concentrations and a dilution set of the added interfering substances. One reagent lot was tested on 3 samples of each interfering substance. One aliquot of each testosterone sample was spiked with the interfering endogenous substance and used as "interference pool". Another aliquot of the sample was spiked with the same volume of the solvent of the interfering endogenous substance (without interfering substance) and used as the related "dilution pool". A series of 11 dilution steps were prepared by mixing the interference pools and the related dilution pools in 10 % increments. The recovery (absolute deviation or % recovery) was calculated for each sample compared to the expected value.

Definitions of interference were as follows:

Non- significant interference was defined as recovery of ±10% (for concentration range of >100 ng/dL), ±15% (for concentration range of >50 to 100 ng/dL), and ±7.5 ng/dL (for concentration range of 15 to 50 ng/dL).

The sponsor claims no significant interference at the following concentrations:

Interferent	Highest Concentration tested without interference
Hemoglobin	600 mg/dL
Intralipid	800 mg/dL
Bilirubin	30 mg/dL
Rheumatoid factor	1000 IU/mL

Biotin

One aliquot of each testosterone sample was spiked with biotin up to 3600 ng/mL and used as "interference pool". Another aliquot of the sample was spiked with the same volume of the solvent of the interfering endogenous substance (without interfering substance) and used as the related "dilution pool". A series of 11 dilution steps were prepared by mixing the interference pools and the related dilution pools in 10 % increments. The recovery (absolute deviation or % recovery) was calculated for each sample compared to the expected value.

The acceptance criteria were the same as described above. The data supports the labeling claim of no significant interference observed with biotin up to 1200 ng/mL with this device.

Common Drug Interferences

The effect on quantitation of analyte in the presence of drugs was determined by comparing values obtained from samples spiked with 17 common pharmaceutical compounds with the reference sample (unspiked). All samples used were native human serum pools. Samples

(with testosterone concentrations near 5 ng/dL and near 500 ng/dL) were divided into aliquots and spiked with the common drug interferents. The reference sample without drug was spiked with the respective amount of solvent. The definition of significant interference was set to less than or equal to 10 percent bias compared to the reference sample.

Drug	Highest Drug Concentration tested without interference
Acetyl cysteine	150 mg/L
Acetylsalicylic Acid	30 mg/L
Ampicillin-Na	75 mg/L
Ascorbic acid	52.5 mg/L
Cefoxitin	750 mg/L
Doxycycline	18.0 mg/L
Heparin	3300 IU/L
Levodopa	7.5 mg/L
Methyldopa	22.5 mg/L
Metronidazole	123 mg/L
Rifampicin	48 mg/L
Acetaminophen	156 mg/L
Cyclosporine	1.8 mg/L
Ibuprofen	219 mg/L
Theophylline	60 mg/L
Phenylbutazone	107 mg/L
Itraconazole	30 mg/L
Rifampicin	48 mg/L

Special Drug Interferences

Two additional drugs were tested using the same protocol as above.

Drug	Drug Concentration Tested
Testosterone undecanoate	32 mg/L
Nandrolone	11.4 mg/L

Significant interference was observed for Testosterone undecanoate and Nandrolone at the concentrations tested above. The labeling for the device includes the following statements:

"A strong interaction with Nandrolone (INN international nonproprietary name, WHO) was found. Do not use samples from patients under Nandrolone treatment. Testosterone undecanoate (INN international nonproprietary name, WHO) is metabolized to testosterone after administration. The Elecsys Testosterone II assay does not differentiate between endogenous testosterone and exogenous testosterone resulting from metabolized testosterone under testosterone supplementation therapy."

Analytical Specificity/Cross-Reactivity

The analytical specificity of the Elecsys Testosterone II assay was determined with one reagent lot on one cobas e 601 analyzer using a human serum matrix with one testosterone level 5 ng/dL). The sample aliquots were spiked with potential cross-reactants and measured in the presence or absence of the potential cross-reactants and cross reactivity was calculated using the following equation:

%Cross-reactivity = ((mean analyte conc.of spiked sample −mean analyte conc.of unspiked sample)/ spiked concentration of cross−reactant) x 100%

Cross Reactant	Concentration	% X-reactivity
DHEA-S	50000 ng/mL	0.003
Androstenedione	100 ng/mL	3.15
Danazol	1000 ng/mL	0.504
Estradiol	5000 ng/mL	0.211
Ethisterone	300 ng/mL	3.57
19-Norethisterone	40 ng/mL	5.51
Norgestrel	1000 ng/mL	0.539
Δ5-Androstene-*3β17β*-diol	1000 ng/mL	0.289
Testosterone propionate	100 ng/mL	0.718
5α-Androstane-*3β17β*-diol	500 ng/mL	2.15
5α-Dihydrotestosterone	500 ng/mL	1.3
11β-OH-Testosterone	50 ng/mL	20.6
11keto-Testosterone	200 ng/mL	4.87
Prednisone	5000 ng/mL	n.d.
Prednisolone	5000 ng/mL	n.d.
Progesterone	5000 ng/mL	0.009
Cortisol	5000 ng/mL	n.d.
Cortisone	5000 ng/mL	n.d.
Dexamethasone	2000 ng/mL	n.d.
Estrone	5000 ng/mL	n.d.
DHEA	5000 ng/mL	0.014

n.d. = not detectable; cross-reactivity (%) is ≤ 0.001

4. Assay Reportable Range:

The sponsor claims a range of 12 -1500 ng/dL.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

The device is traceable to the ID-GC/MS “Isotope Dilution-Gas Chromatography/ Mass Spectrometry”.

6. Detection Limit:

Limit of Blank (LoB)

For determination of LoB five analyte free samples were measured in two-fold determinations in 6 runs, distributed over 3 days, with 2 different lots on one cobas e 601 analyzer. In total 60 measured values of analyte free samples were obtained per lot.

The LoB claim in the labeling is 1.5 ng/dL.

Limit of Detection (LoD)

For determination of LoD five samples with low-analyte concentration (from LoB up to approx. 4 times the LoB, including 2 native single samples and 3 diluted single samples) were measured in 2-fold determination in 6 runs, distributed over ≥ 3 days, with 2 different lots on one cobas e 601 analyzer. In total 60 measured values of samples with low analyte concentrations were obtained per lot. Data analysis was based on determination of the 60 measured values of the 5 low analyte samples as follows: LoD = LoB + 1.653 x SD total (of low analyte samples).

The LoD claim in the labeling is 2.5 ng/dL.

Limit of Quantitation (LoQ)

For the determination of LoQ, 2 lots were evaluated, each with 9 human serum (native single samples) covering the range between 9.8-21.5 ng/dL. Each sample was measured in 2 replicates one run per day over 6 days on one cobas e 601 analyzer. Assay calibration was performed as specified in the package insert. Analyte-low native samples or sample pools were used for the determination of LoQ. The LoQ is set as the lowest concentration of analyte that can be reproducibly measured with a total error of ≤ 20 %.

The LoQ claim in the labeling is 12.0 ng/dL.

7. Assay Cut-Off:

Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

Serum samples were measured internally using both the current and updated reagent formulations. A total of 168 samples, including 5 spiked samples, that span the measuring range were tested with 1 run per sample.

The Passing-Bablok regression analysis performed yielded the following equation:
Y=0.948x + 0.023 R= 0.998
The 95% confidence intervals for the slope were 0.940-0.957 and the 95% confidence intervals for the intercept were 0.023-1.44.

2. Matrix Comparison:

The effect on quantitation of analyte in the presence of anticoagulants on the Elecsys Testosterone II assay were determined. Values obtained from serum samples were compared to Li-Heparin, K2-EDTA and K3-EDTA plasma. A total of 57 serum/plasma pairs were tested in one run per matrix type on one cobas e 601 analyzer. Data was assessed by Passing-Bablok regression analysis.

The following results were obtained:

Serum compared to Lithium Heparin Plasma: y=1.003x -0.744; R = 0.999

Serum compared to K_2- Heparin Plasma: y=1.029x -6.03; R = 0.998

Serum compared to K_2- Heparin Plasma: y=1.018x +0.77; R = 0.997

C Clinical Studies:

1. Clinical Sensitivity:

Not Applicable

2. Clinical Specificity:

Not Applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not Applicable

D Clinical Cut-Off:

Not Applicable

E Expected Values/Reference Range:

Established in k093421

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.