510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:
k093421

B. Purpose for Submission:
New device

C. Measurand:
Testosterone

D. Type of Test:
Quantitative, electro-chemiluminescence (ECLIA) assay

E. Applicant:
Roche Diagnostics

F. Proprietary and Established Names:
Elecsys® Testosterone II Immunoassay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
Radioimmunoassay, testosterones and dihydrotestosterone (CDZ)	Class I, reserved	21 CFR 862.1680 Testosterone test system	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   Refer to Indications for use below.

2. 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 Elecsys and cobas e immunoassay analyzers.

   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 androgenital syndromes.

3. Special conditions for use statement(s):
   For Prescription use only

   The sponsor has the following limitations in their labeling:

"In patients receiving therapy with high biotin doses (i.e. > 5mg/day), no sample should be taken until at least 8 hours after the last biotin administration."
"In isolated cases, elevated testosterone levels can be seen in samples from female patients with end stage renal disease (ESRD)."
"In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects have been minimized by suitable test design."
"Implausible elevated testosterone values in women should be verified by an extraction method or a validated LC-MS/MS tandem method."

4. Special instrument requirements:
   Elecsys 2010 analyzer

I. Device Description:

The device is supplied as ready-to-use, three-reagent kit. The Elecsys Testosterone II reagent kit consists of a Reagent Pack (contains R1, R2, and M).
Reagent 1 contains Anti-testosterone-Ab~biotin, Biotinylated monoclonal anti-testosterone antibody, releasing reagent 2-bromoestradiol, MES buffer, and preservative. Reagent 2 contains Testosterone-peptide, Testosterone derivative, MES buffer, and preservative. M contains Streptavidin-coated microparticles.

All human source materials were tested by FDA approved methods and found to be negative for HIV ½, HBsAg, and HCV.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Elecsys Testosterone Assay

2. Predicate 510(k) number(s):

   k964889

3. Comparison with predicate:

Feature	Elecsys Testosterone II Assay (Candidate device)	Elecsys Testosterone Assay (K964889) Predicate Device
Intended Use	Immunoassay for the in vitro quantitative determination of testosterone in human serum and plasma. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.	Immunoassay for the in vitro quantitative determination of testosterone in human serum and plasma. The electrochemiluminescence immunoassay "ECLIA" is intended for use on the Boehringer Mannheim Elecsys 1010 and 2010 immunoassay analyzers.
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
Assay Protocol	Competition principle	Same
Detection Protocol	Electrochemiluminescence immunoassay (ECLIA)	Same
Traceability/ Standardization	ID-GC/MS (Isotope Dilution Gas Chromatography/Mass Spectrometry)	Same
Sample Type	Human serum and plasma	Same
Measuring Range	2.5 – 1500 ng/dL (0.087 – 52.0 nmol/L)	2.0 – 1500 ng/dL (0.069 – 52.0 nmol/L)
Calibrator	Testosterone II CalSet II (Calibrators 1 and 2)	Same
Calibration Interval	Once per reagent lot and 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 specified limits	Same

Controls	PreciControl Universal 1 and 2	Same
Reagent Stability	Unopened at 2-8ºC – up to the expiration date. After opening at 2-8ºC – 12 weeks. Onboard the analyzer – 8 weeks	Unopened at 2-8ºC – up to the expiration date. After opening at 2-8°C – 8 weeks. Onboard the analyzer – 8 weeks

K. Standard/Guidance Document Referenced (if applicable):

- CLSI EP5-A2: Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline Vol 19, No 2.
- CLSI EP6-A: Evaluation of the Linearity of Quantitative Analytical Measurement Procedure: A Statistical Approach; Approved Guideline, Vol 23 No 16.
- CLSI EP17-A: Protocol for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. Vol 24, No 34.

L. Test Principle:

The Elecsys Testosterone II immunoassay is based on a competitive test principle with 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 a master curve provided with the reagent bar code.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Following CLSI EP5-A, the sponsor evaluated the precision using seven human serum (HS) specimens and two controls, at the concentrations listed in the table below. Precision was evaluated in duplicate, 2 runs per day for 21 days (n=84) using Elecsys 2010 analyzer. The results are tabulated below.

Sample	Mean	Within run precision		Between run precision	
		SD	CV (%)	SD	CV (%)
	ng/dL	ng/dL		ng/dL	
HS 1	4.5	0.5	10.2	0.8	18.5
HS 2	9.5	0.4	4.7	0.8	8.4
HS 3	69.1	1.4	2.1	2.2	3.2
HS 4	216	4.2	1.9	6.0	2.8
HS 5	867	22.9	2.6	24.3	2.8

HS 6	1300	15.8	1.2	44.0	3.4
HS 7	1450	22.0	1.5	35.0	2.4
PCU1	630	8.8	1.4	18.2	2.9
PCU2	250	4.7	1.8	9.7	3.7

b. Linearity/assay reportable range:

The sponsor performed linearity studies in accordance with the CLSI EP6-A guideline. Seventeen levels of samples were prepared by diluting a high spiked serum pool sample with a low serum pool sample. The linearity samples were tested in triplicate on the Elecsys 2010 analyzer over the range of 0.5 to 1535.8 ng/dL. The observed values were plotted against the expected values and an appropriate line fitted by standard linear regression resulting in: y = 1.0122x - 0.0299; R=0.9996.

The data support the sponsor's claim that the measuring range of this device is 2.5 to 1500 ng/dL.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Calibrators used with this device have been previously cleared in k003411. Control materials have been previously cleared in k090541. The sponsor provided the stability study protocols for reagent stability. Based on the real-time stability studies, the reagent kit is stable up to 12 weeks. The on-board stability for open vials is 56 days.

d. Limit of Detection:

The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) were determined in accordance with CLSI EP17-A document. LoB were conducted using a blank sample tested five times on 2 Elecsys 2010 analyzers over 3 days using 2 runs, and 2 reagent lots (N=60). For LoD, five serum samples were selected and diluted to obtain lower concentrations. The samples were run over 3 days with 2 runs per day using 2 reagent lots (N=60). For LoQ, 7 serum samples were tested once per run, 2 runs per day for over 5 days (N=70). LoB was determined to be 1.2 ng/dL and LoD was determined to be 2.5 ng/dL. The LoQ was calculated as the lowest concentration for which the CV is less than 20%. The results demonstrated that the LoQ is 12.0 ng/dL

The claimed measuring range of the device is 2.5 to 1500 ng/dL.

e. Analytical specificity:

Interference studies were performed using four different levels of testosterone samples spiked with 5 endogenous substances, using the same protocol as follows: One aliquot of each serum sample was spiked with the interfering substance; another aliquot was spiked with the same volume of the solvent (control sample). The interfering pool was then diluted into the dilution pool in 10% increments, generating a total of 11 different concentrations of interference substances. Results of the spiked samples were compared with the control samples and % recovery was calculated. 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). Based on the data, the sponsor claims no significant interference for the substances and concentrations listed below:

Icterus (bilirubin up to 30 mg/dL),
Hemolysis (Hemoglobin up to 600 mg/dL),
Lipemia (Intralipid up to 1000 mg/dL),
Biotin: up to 30 ng/mL,
Rheumatoid Factor: up to 1000 IU/mL.

In addition, common pharmaceutical compounds were spiked into native human serum samples and tested with Testosterone II assay. Two serum samples pools containing approximately 60 ng/dL and 500 ng/dL testosterone were spiked with potential interferents (multiples of maximal daily doses). The reference sample (control) without interferent was spiked with the respective amount of solvent. Based on the sponsor's definition of non-significant interference (greater than ± 10% of control value), the sponsor claims no interference for the compounds and concentrations listed in the table below:

Compound	Concentration
Acetylcystein	150 mg/L
Ampicillin	1000 mg/L
Ascorbic acid	300 mg/L
Ca-Dobesilate	200 mg/L
Cyclosporine	5 mg/L
Cefoxitin	2500 mg/L
Heparin	5000 U
Levodopa	20 mg/L
Methyldopa	20 mg/L
Metronidazole	200 mg/L
Phenylbutazone	400 mg/L
Doxycyclin	50 mg/L
Acetylsalicylic Acid	1000 mg/L

Rifampicin	60 mg/L
Acetaminophen	200 mg/L
Ibuprofen	50 mg/L
Theophylline	100 mg/L
Heparin Clexane	5000 U
Dexamethasone	20 mg/L

Nandrolone was tested and was considered as interference substance; therefore, the sponsor has the following limitations in the labeling:

"Do not use samples from patients under Nandrolone treatment"

Cross-reactivity study was performed using native human serum samples spiked with potential cross-reactant compounds. The spiked and non-spiked samples were tested in duplicates on the Elecsys 2010 analyzer. Results are summarized below:

Substances	Concentration tested (ng/mL)	Cross-reactivity (%)
Androstendione	100	≤ 2.5
Cortisol	1000	≤ 0.01
Cortisone	2000	≤ 0.001
Danazol	1000	≤ 0.5
Dexamethasone	2000	≤ 0.001
DHEA	1000	≤ 0.016
DHEA-S	50000	≤ 0.003
D-5-Androstene-3β,17β-diol	1000	≤ 0.29
Estradiol	1000	≤ 0.16
Estrone	1000	≤ 0.004
Ethisterone	1000	≤ 2.40
Norgestrel	1000	≤ 0.91
Testosterone propionate	100	≤ 2.46
5-α-Androstane-3β,17β-diol	1000	≤ 2.11
5-α-Dihydro-testosterone	500	≤ 0.86
11-β-Hydroxy-testosterone	100	≤ 18.0
11-Keto-testosterone	1000	≤ 3.22
Prednisone	1000	≤ 0.001
Prednisolone	1000	≤ 0.002
Progesterone	1000	≤ 0.001

f. Assay cut-off:
Not Applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

Method comparisons between the candidate device and the predicate device were performed according to the CLSI EP9-A guideline. A total of 387 serum samples (239 males and 148 females) ranging from 2.5-1400 ng/dL were used in the study. The candidate device results on the Elecsys 2010 (Y) were compared to the corresponding predicate results on the Elecsys 2010 (X). Deming regression analysis resulted in: Y = 0.989X – 2.87; R = 0.992.

An additional method comparison study using 123 pediatric samples (69 males age 7-18 years old and 54 females' age 8-18 years old) was performed. Samples ranging from 2.74 to 1048.2 ng/dL. Deming regression analysis resulted in: Y= 1.06X – 2.79; R= 0.93.

b. *Matrix comparison:*

The sponsor performed a matrix comparison study using 45 unaltered paired serum, K2-EDTA plasma, K3-EDTA plasma and Lithium Heparin plasma samples. Samples ranging from 10 to 1460 ng/dL were analyzed on the Elecsys 2010 analyzer. The plasma results (Y) were compared to the corresponding serum results (X) and the Passing/Bablok regression analysis results are summarized in the table below:

Anticoagulant	Regression Analysis
Lithium Heparin	Y=1.0008 - 0.0001; R = 0.997
K2-EDTA	Y=0.998 + 0.0005; R = 0.998
K3-EDTA	Y=0.993 + 0.0022; R = 0.998

The sponsor claimed that K2-EDTA, K3-EDTA and Lithium heparin are acceptable anticoagulants.

3. Clinical studies:

a. *Clinical Sensitivity:*

Not Applicable

b. *Clinical specificity:*

Not Applicable

c. *Other clinical supportive data (when a. and b. are not applicable):*

Not Applicable

4. Clinical cut-off**:**
Not Applicable

5. Expected values/Reference range:

A reference range study was performed using healthy adult (214) males and (160) females without intake of contraceptives and prescription drugs. Results are summarized below:

		Percentiles		
Test subjects	N	Median	5-95th	2.5-97.5th
		ng/dL		
Males 20-49 years	136	536	249-836	218-906
Males ≥ 50 years	78	476	193-740	132-892
Females 20-49 years	89	27.1	8.4-48.1	5.0-52.2
Females ≥ 50 years	71	16.2	2.9-40.8	< 2.5-46.1

In addition, a reference range study using pediatric population (95 males and 100 females) under 18 years old, who were in good endocrinological health was performed. Subjects were clinically characterized according to their age as well as Tanner Stage. Tanner Stage was characterized according to the method of Marshall and Tanner.[a,b] Results are summarized below:

Reference values for males under the age of 18 characterized by age

Age	N	Range, ng/dL	
		Min	Max
7	3	< 2.5	< 2.5
8	6	< 2.5	< 2.5
9	8	< 2.5	< 2.5
10	2	< 2.5	< 2.5
11	13	< 2.5	236.6
12	6	29.40	278.4
13	8	< 2.5	432
14	9	40.1	778.4
15	23	78.7	762.6
16	14	237.5	1048.2
17	1	505.5	505.5
18	2	557.1	685.1

Reference values for females under the age of 18 characterized by age

Age	N	Range, ng/dL	
		Min	Max
7	0	—	—
8	11	< 2.5	6.14
9	16	< 2.5	7.49
10	7	< 2.5	5.45
11	13	< 2.5	17.06
12	10	< 2.5	26.23
13	7	< 2.5	23.66
14	4	11.21	28.82
15	21	7.64	39.77
16	8	< 2.5	29.4
17	0	—	—
18	3	15.33	31.13

Reference values for males under the age of 18

			Percentiles	
Tanner Stage	N	Median	5-95th	2.5-97.5th
			ng/dL	
1	26	< 2.5	< 2.5	< 2.5-4.2
2	18	59.7	< 2.5-432	< 2.5-432
3	15	245	64.9-778	64.9-778
4	16	344	180-763	180-763
5	20	446	188-882	138-1050

Reference values for females under the age of 18

			Percentiles	
Tanner Stage	N	Median	5-95th	2.5-97.5th
			ng/dL	
1	37	< 2.5	< 2.5-6.12	< 2.5-7.5
2	12	< 2.5	< 2.5-10.4	< 2.5-10.4
3	12	7.9	< 2.5-23.7	< 2.5-23.7
4	12	12.2	< 2.5-26.8	< 2.5-26.8
5	27	19.7	4.6-38.3	4.5-39.8

Sponsor state that each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

References:

a. Marshall, W.A., Tanner, J.M. Variations in the pattern of pubertal changes in boys. Arch. Dis. Childh. 1970;45: 13-23.
b. Marshall, W.A., Tanner, J.M. Variations in the pattern of pubertal changes in girls. Arch. Dis. Childh. 1969;44: 291-303.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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