

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

A. 510(k) Number:

k152227

B. Purpose for Submission:

Modification of traceability for standardized against the IRMM (Institute for Reference Materials and Measurements)/IFCC-451 panel

C. Measurand:

Cortisol

D. Type of Test:

Quantitative, Enzyme Immunoassay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys Cortisol II

Cortisol II CalSet

G. Regulatory Information:

Product Code	Regulation Name	Classification	Regulation Section	Panel
JFT	Cortisol (hydrocortisone and hydroxycorticosterone) test system	Class II	21 CFR § 862.1205	Chemistry 75
JIT	Calibrator, secondary	Class II	21 CFR§ 862.1150	Chemistry 75

H. Intended Use:

1. Intended use(s):

See Indications for use below.

2. Indication(s) for use:

Immunoassay for the in vitro quantitative determination of cortisol in human serum, and plasma. The determination of cortisol is used for the recognition and treatment of functional disorders of the adrenal gland.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.

Cortisol II CalSet is used for calibrating the quantitative Elecsys Cortisol II assay on the Elecsys and cobas e immunoassay analyzers

3. Special conditions for use statement(s):

Prescription use only

4. Special instrument requirements:

cobas e 411 immunoassay analyzer

I. Device Description:

The Elecsys Cortisol II Immunoassay reagent working solutions contains the following:

- The reagent rack pack labeled as CORT II.
- Streptavidin-coated microparticles and preservative; 1 bottle, 6.5 mL.
- Reagent 1 (Anti-cortisol-Ab~biotin) Biotinylated monoclonal anti-cortisol antibody (ovine), danazol, MES buffer, and preservative; 1 bottle, 10 mL.
- Reagent 2 (Cortisol-peptide~Ru (bpy)²⁺₃) Cortisol derivatives (synthetic), labeled with ruthenium complex, danazol, MES buffer, and preservatives; 1 bottle, 10 mL.

The Cortisol II CalSet is a lyophilized human serum with added cortisol in two concentration range. The two concentration ranges approximately 12.5 nmol/L or 0.45 µg/dL and approximately 1000 nmol/L or 36 µg/dL. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

- CORT II Cal 1: 2 bottles, 1.0 mL
- CORT II Cal 2: 2 bottles, 1.0 mL

J. Substantial Equivalence Information:

1. Predicate device name(s):

Roche Elecsys Cortisol and CalSet

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

k070788

3. Comparison with predicate:

Cortisol Assay

Similarities / Differences		
Item	Candidate Device Elecsys Cortisol II (k152227)	Predicate Device Elecsys Cortisol (k070788)
Intended Use	For the in vitro quantitative determination of cortisol in human serum, and plasma	Same
Test principle	Utilizes a competition test principle using a monoclonal antibody which is specifically directed against cortisol.	Utilizes a competition test principle using a polyclonal antibody which is specifically directed against cortisol.
Detection Method	Electrochemiluminescent Assay	Same
Instrument Platform	Elecsys and cobas e immunoassay analyzers	Same
Sample Type	Human serum and plasma	Human serum, plasma, urine and saliva
Sample volume	10 µL	20 µL
Measuring range	3.0 – 1750 nmol/L	0.5-1750 nmol/L

Cortisol Calibrator

	Candidate Device Cortisol II CalSet (k152227)	Predicate Device Elecsys Cortisol CalSet (k070788)
Intended Use	For calibrating the Cortisol assay on the Elecsys and cobas e immunoassay analyzers	Same
Format	Lyophilized	Same
Traceability	IRMM (Institute for Reference Materials and Measurements)/IFCC-451 panel	Enzymun-Test Cortisol method

	Candidate Device Cortisol II CalSet (k152227)	Predicate Device Elecsys Cortisol CalSet (k070788)
Levels	2	Same
Target Ranges	Cal 1: 12.5 nmol/L Cal 2: 1000 nmol/L	Same
Calibration Intervals	Calibration must be performed once per reagent lot using fresh reagent. Renewed calibration is recommended as follows: <ul style="list-style-type: none"> • After 8 weeks 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 	Same

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

CLSI EP05-A2: Evaluation of Precision of Quantitative measurements Procedure; Approved Guideline

CLSI EP09-A: Evaluation of Linearity of Quantitative measurements procedure: a Statistical Approach; Approved Guideline

CLSI EP09-A3: Measurement Procedure Comparison, and Bias Estimation using Patient Samples; Approved Guideline

CLSI EP17-A2: Evaluation of Detection Capability for Clinical laboratory Measurement procedures; Approved Guideline

L. Test Principle:

The Elecsys Cortisol II assay is a competition test principle using a monoclonal antibody which is specifically directed against cortisol. Endogenous cortisol which has been liberated from binding proteins with danazol competes with exogenous cortisol derivative in the test which has been labeled with ruthenium complex for the binding sites on the biotinylated antibody. 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision of the Elecsys Cortisol II assay for serum was evaluated according to CLSI EP5-A2 guideline using one cobas e 411 immunoassay analyzer, one lot of Elecsys Cortisol II Immunoassay reagent, 5 human sera and two controls. The protocol consisted of testing 2 runs per day in duplicate for 21 days (n=84). The following precision results were obtained:

Sample	Mean nmol/L (µg/dL)	Repeatability (Within-run)		Intermediate precision (Within-Laboratory)	
		SD nmol/L (µg/dL)	CV %	SD nmol/L (µg/dL)	CV %
Human Serum 1	3.09 (0.112)	0.219 (0.008)	7.1	0.392 (0.014)	12.7
Human Serum 2	35.8 (1.30)	0.718 (0.026)	2.0	1.36 (0.049)	3.8
Human Serum 3	283 (10.3)	7.29 (0.264)	2.6	9.39 (0.340)	3.3
Human Serum 4	548 (19.9)	10.4 (0.377)	1.9	17.4 (0.631)	3.2
Human Serum 5	1592 (57.7)	29.3 (1.06)	1.8	42.7 (1.55)	2.7
PreciControl Universal 1	308 (11.2)	4.33 (0.157)	1.4	8.35 (0.303)	2.7
PreciControl Universal 2	719 (26.1)	10.4 (0.377)	1.4	18.0 (0.653)	2.5

b. *Linearity/assay reportable range:*

i.) Linearity of serum using the Elecsys Cortisol II assay was evaluated following CLSI guideline EP6-A on the cobas e 411 immunoassay analyzer. A high serum pooled cortisol sample was diluted with Diluent Universal (protein matrix). A total of 19 levels of cortisol concentrations were tested. The cortisol concentrations range from 1.66-1898 nmol/L. Samples were assayed in triplicate within a single run.

Statistical evaluation using linear regression showed that the assay is linear from 2.854 -1898 nmol/L, yielding a linear regression result of $y = 1.014x - 1.41$ with a correlation coefficient of $R^2 = 0.9995$

ii.) Linearity of Lithium Heparin plasma using the Elecsys Cortisol II assay was evaluated following CLSI guidelines EP6-A on the cobas e 411 immunoassay. A high plasma pooled cortisol sample was diluted with Diluent Universal (protein matrix). A total of 19 concentrations were tested. The cortisol concentrations range from 1.71 – 1959 nmol/L. Samples were assayed in triplicate within a single run.

Statistical evaluation using linear regression showed that the assay is linear from 2.94– 1959 nmol/L, yielding a linear regression result of $y = 1.004x - 1.75$ with a correlation coefficient of $R^2 = 0.9982$.

The results of the linearity study support the sponsors claim that the assay is linear throughout the claimed measuring range of 3 to 1750 nmol/L.

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

Traceability/Standardization

The Elecsys Cortisol II assay has been standardized against the reference materials (IRMM/IFCC-451 panel) which were value assigned by the reference measurement procedure (ID-GC/MS (isotope dilution-gas chromatography/mass spectrometry). Ghent University’s ID-GC/MS is a recognized reference method procedure used to assign the reference materials.

Stability

The shelf-life, accelerated, and open-vial stability testing protocols for the Cortisol II CalSet calibrators and the acceptance criteria were described and found to be acceptable.

Cortisol II CalSet calibrator are stable until expiration date (3 months) when stored at -20 °C. The Cortisol CalSet calibrator open vial on the analyzer at 20 – 25 °C is stable for 4 hours and should only be used once.

Value Assignment of Calibrators

For each Cortisol II CalSet manufactured, the calibrators are run in duplicate on at least three (3) cobas e 411 analyzers and at least three (3) Modular Analytics E170/cobas e 601/cobas e 602 analyzers with all Cortisol II reagent lots available. The assigned value of each calibrator is defined as the mean value obtained over at least six (6) runs on at least three (3) analyzers of the respective calibrator. The exact lot-specific calibrator values are encoded on the calibrator bottle label as well as printed on the calibrator barcode sheet provided or electronically available.

Target values for Cortisol II CalSet

Level	Target Value (nmol/L)	Target Range (nmol/L)
Calibrator 1	10	5-15
Calibrator 2	1000	900-1100

d. *Detection limit:*

The limit of Blank (LoB) of the Elecsys Cortisol II assay on the cobas e 411 Immunoassay analyzer was determined according to CLSI EP17-A2. Two reagent lots, two cobas e 411 instruments, five blank samples, one replicate per sample per run, two runs per day for three days for a total of 60 determinations were performed. The zero-level (blank) samples used were prepared by diluting the native human serum pool with the Universal Diluent. The LoB was defined as the 95th percentile of measurements of blank samples. The linear interpolation between the 57th and 58th replicate yields a LoB of the following:

Reagent Lot	LoB (nmol/L)
1	0.5270
2	0.6645

The LoB was determined to be = 1.0 nmol/L

The limit of Detection (LoD) of the Elecsys Cortisol II assay on the cobas e 411 Immunoassay analyzer was determined according to CLSI EP17-A2. LoD was defined as the lowest amount of cortisol in a sample that can be detected with 95% probability. Two reagent lots, two cobas e 411 instruments, five low cortisol level samples, one replicate per sample per run, two runs per day, for 3 days for a total of 60 replicates per sample per reagent lot were performed. A low-level sample set of 5 human serum samples (diluted human serum pools) of known cortisol concentration was evaluated for each reagent lot, with the results obtained as follows.

LoD = LoB + 1.653 x SD total (of low cortisol samples)

Reagent Lot	LoD
1	1.1227
2	1.2846

LoD was determined to be = 1.5 nmol/L

The limit of Quantitation (LoQ) of the Elecsys Cortisol II assay on the cobas e 411 Immunoassay analyzer was determined according to CLSI EP17-A2. Three reagent lots, two cobas e 411 instruments, nine low level serum samples, two replicate per sample per run, two runs per day for three days were performed. A low-level sample set of 9 human serum samples (diluted human serum pools) of known cortisol concentration was evaluated for each reagent lot. For each reagent lot, the sample with the lowest cortisol concentration that meets the specifications for precision is taken as the LoQ, with the results obtained as follows. The LoQ is set to 3.0 nmol/L with a precision of $\leq 20\%$ CV.

Reagent Lot	LoQ (nmol/L)
1	1.94
2	2.85
3	2.99

The Cortisol II assay has a measuring range of 3.0 to 1750 nmol/L (0.109 to 63.4 µg/dL).

e. Analytical specificity:

Interference Study Protocol:

An interference study was performed following the CLSI Guideline EP7-A2 guideline using human serum samples with cortisol concentrations 24.2, 476, and 1314 nmol/L. Interference substances were spiked into the serum samples and the results were measured and compared between the spiked and unspiked samples.

The interference from the following compounds was observed to be ≤ 10% (non-significant interference) at the levels indicated.

Potential Interferent	Highest Concentration of substance tested which demonstrated no significant interference
Biotin	30 ng/mL
Hemoglobin	500 mg/dL
Intralipid (Lipemia)	1500 mg/dL
Bilirubin	25 mg/dL
Rheumatoid Factor (RF)	600 IU/mL
IgG	50 g/L
IgM	10 g/L
IgA	10 g/L

The package insert contains the following limitations:

“Pregnancy, contraceptives and estrogen therapy give rise to elevated cortisol concentrations.”

“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.”

Exogenous Drug Interference:

16 pharmaceutical compounds were spiked into two human serum samples with cortisol concentrations of approximately 170 and 750 nmol/L. The two sample pools were divided into aliquots and spiked with the potential interferents. The cortisol

concentration of the spiked aliquots was run in triplicate and compared to the cortisol concentration of the reference aliquot also run in triplicate on one cobas e 411 Immunoassay analyzers.

The interference from the following was observed to be $\leq 10\%$ (non-significant interference) at the levels indicated.

The below compounds at the indicated concentrations do not cause significant interference with the assay.

Drug	Highest Concentration of substance tested which demonstrated no significant interference
Acetylcystein	1663 mg/L
Ampicillin-Na	1000 mg/L
Ascorbic acid	300 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
Doxycycline	50 mg/L
Acetylsalicylic acid	1000 mg/L
Rifampicin	60 mg/L
Acetaminophen	200 mg/L
Ibuprofen	500 mg/L
Theophylline	100 mg/L

The effects of the presence of human anti-sheep antibodies (HASA) on the Elecsys Cortisol II assay was conducted using the cobas e 411 analyzer and two serum samples with cortisol concentrations of 172 and 789 nmol/L. Both samples were divided into two aliquots each. One aliquot was spiked with 20 $\mu\text{g}/\text{mL}$ of donkey anti-sheep polyclonal antibodies (DASA) and then diluted with the unspiked aliquot of the serum in 10% increments. The recovery for each sample was calculated by comparison to the unspiked reference samples.

No effect of DASA up to 20 $\mu\text{g}/\text{mL}$ on the Cortisol result has been observed.

Cross reactivity Study Protocol:

The specificity of the Elecsys Cortisol assay was determined using two human serum sample pools (diluted and spiked) with potential cross-reactant compounds. The analyte concentration of the samples was approximately 60 and 300 $\mu\text{g}/\text{L}$ of cortisol.

The spiked and non-spiked samples were tested in duplicate on the cobas e 411 Immunoassay analyzer. The percentage of cross reactivity was calculated using the following formula:

$$\text{Percent cross reactivity} = \frac{\text{analyte concentration}}{\text{Concentration of cross-reactant spiked}} \times 100$$

Cross reactant tested	Concentration Tested (µg/mL)	Highest cross-reactivity observed (%)
Cortisone	10	6.58
11-Deoxy-cortisol	10	4.90
Corticosterone	10	2.48
Progesterone	10	0.035
17-α-OH-Progesterone	10	0.080
21-Deoxy-cortisol	1	2.40
Fludrocortisone	10	0.200
6-α-MethylPrednisolone	0.1	12.0
Prednisone	10	2.23
Prednisolone	0.1	7.98
Dexamethasone	10	Not detectable
11-Deoxycorticosterone	10	0.640
Androstendione	10	0.1
Estradiol	10	0.2
Estriol	10	Not detectable
Estrone	10	0.8

The package insert states the following in the Limitation-interference section:

“In samples from patients who have been treated with prednisolone, 6-α-Methylprednisolone or prednisone, falsely elevated cortisol values may be determined due to cross reactions. “

“During metyrapon tests, 11-deoxycortisol levels are elevated. Falsely elevated cortisol values may be determined due to cross reactions. “

“Patients suffering from 21-hydroxylase deficiency exhibit elevated 21-deoxycortisol levels and this can also give rise to falsely elevated cortisol results.”

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed to compare the Elecsys Cortisol II assay (candidate device) to the current Elecsys Cortisol assay (predicate device). A total of 536 human serum samples (all native single donors) with cortisol values ranged between 10.55-1735 nmol/L were assayed in singlcate on the cobas e 411. The regression analysis results are summarized below:

N	536	
Range (nmol/L)	9.21-1680	
	Passing/Bablok	Deming
Slope	0.758	0.806
intercept (nmol/L)	10.2	-10.31
Correlation coefficient (r)		0.9676

The slope showed a significant bias and shift between the candidate device and the predicate device, however this shift is expected because the purpose of the device modification was to standardize against the IRMM (Institute for Reference Materials and Measurements)/IFCC-451 panel to both improve the specificity (cross-reactivity) and the accuracy of the assay. Therefore, test results from the candidate device do not, and are not expected to, directly correlate with test results from the predicate device. Laboratories that use the Roche Elecsys Cortisol assay should be aware of the significantly different performance of the modified assay.

An additional method comparison study was conducted to evaluate the accuracy between the candidate device and a recognized reference measurement procedure, University of Ghent's ID- GC-MS method (RMP). The method comparison against the RMP was the basis of the substantial equivalency determination.

A total of 208 human serum samples (all native single donors) were measured in order to cover the entire measuring range. Cortisol values ranged between 10.9–1599 nmol/L for the LC-MS and between 10.6 – 1711 nmol/L for the Elecsys Cortisol II assay.

N	208	
Range (nmol/L)	10.9 - 1599	
	Passing/Bablok	Deming Regression
Slope	1.022 (1.002,1.044)*	1.055 (1.026-1.084)*
intercept (nmol/L)	2.92 (-1.81, 8.82)*	-6.10 (-15.8/3.63)*
Correlation coefficient (r)		0.993
*Lower/Upper Confidence (95%)		

b. *Matrix comparison:*

A matrix comparison study using different tube type was performed to demonstrate equivalency of the specimen types. The effect on quantitation of analyte in the presence of anticoagulants with the Elecsys Cortisol II Immunoassay was determined by comparing values obtained from samples drawn into serum, Li-heparin plasma (with and without gel), K2-and K3-EDTA plasma primary tubes.

Specimen Type	n	Slope	Range (nmol/L)	Intercept	Correlation Coefficient (r)
Serum / Li-Heparin Plasma	48	0.987	5.22 - 1729	-0.418	0.987
Serum / Li-Heparin (Gel) Plasma	48	0.991	5.22 - 1729	-1.02	0.973
Serum / K2-EDTA Plasma	48	0.985	5.22 - 1729	-0.564	0.980
Serum / K3-EDTA Plasma	58	0.966	4.49 - 1729	-0.882	0.966

Sponsor claimed that Lithium heparin plasma, K2 EDTA plasma, and K3 EDTA plasma are acceptable sample type for the Cortisol II assay.

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:

In the reference range studies with the Elecsys Cortisol II assay, the following values were determined using samples from 300 self-reported healthy individuals, aged 21 years or older. Exclusion criteria were: pregnancy, lactation, use of oral contraceptives and medication with cortisone/cortisol. No statistical difference was observed between males and females.

Cortisol in serum:

5th-95th percentile:

Morning hours 6-10 a.m.: 166-507 nmol/L (6.02-18.4 µg/dL), n = 296*

Afternoon hours 4-8 p.m.: 73.8-291 nmol/L (2.68-10.5 µg/dL), n = 300

2.5th-97.5th percentile:

Morning hours 6-10 a.m.: 133-537 nmol/L (4.82-19.5 µg/dL), n = 296*

Afternoon hours 4-8 p.m.: 68.2-327 nmol/L (2.47-11.9 µg/dL), n = 300

* Four samples were excluded from data analysis because samples were drawn outside the time of 6-10 am.

The sponsor states in the package insert each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

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.