



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

I Background Information:

A 510(k) Number

K242505

B Applicant

Roche Diagnostics

C Proprietary and Established Names

Elecsys Cortisol III

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
JFT	Class II	21 CFR 862.1205 - Cortisol (Hydrocortisone And Hydroxycorticosterone) Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Cortisol

C Type of Test:

Quantitative, Enzyme 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 cortisol in human urine. 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 cobas e immunoassay analyzers.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Cobas e 801 analyzer

IV Device/System Characteristics:

A Device Description:

The Elecsys Cortisol III immunoassay reagent working solutions consist of the following:

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-cortisol-Ab-biotin (gray cap), 1 bottle, 21.0 mL:
Biotinylated monoclonal anti-cortisol antibody (mouse) 18 ng/mL; danazol 20 pg/mL;
MES buffer 100 mmol/L, pH 6.0; preservative.

R2 Cortisol-peptide-Ru(bpy) (black cap), 1 bottle, 21.0 mL:
Cortisol derivative (synthetic), labeled with ruthenium complex, 5 ng/mL; danazol 20
pg/mL; MES buffer 100 mmol/L, pH 6.0; preservative.

MES = 2-morpholino-ethane sulfonic acid

B Principle of Operation:

The Elecsys Cortisol III immunoassay makes use of a competition test principle with the following steps for a total duration of 18 minutes:

1st incubation: By incubating the sample (6 μ L) with a cortisol-specific biotinylated monoclonal antibody, immunocomplexes are formed, the amount of which is dependent upon the analyte concentration in the provided sample.

2nd incubation: After addition of streptavidin-coated microparticles and a cortisol-derivative labeled with a ruthenium complex a), the still-vacant sites of the biotinylated antibodies become occupied, with formation of an antibody-hapten complex. The entire 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 II 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 two-point calibration and a master curve provided via the cobas link.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Architect Cortisol Assay

B Predicate 510(k) Number(s):

K062204

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K242505</u>	<u>K062204</u>
Device Trade Name	Elecsys Cortisol III	Architect Cortisol Assay
General Device Characteristic Similarities		
Intended Use/Indications For Use	Immunoassay for the <i>in vitro</i> quantitative determination of cortisol	Same
Measurand	Cortisol	Same
General Device Characteristic Differences		
Sample Type	Urine	Serum, plasma or urine
Reportable Range	0.73 – 18.1 µg/dL (20.0 – 500 nmol/L)	0.8 – 59.8 µg/dL
Detection Method	electrochemiluminescence immunoassay “ECLIA”	chemiluminescent microparticle immunoassay (CMIA)

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition
- CLSI EP09c-Ed3: 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
- CLSI EP06-Ed2: Evaluation of the Linearity of Quantitative Measurement Procedures
- CLSI EP07-Ed3: Interference Testing in Clinical Chemistry.
- CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision

Precision studies were conducted for the Elecsys Cortisol III assay to evaluate repeatability (within-run precision) and intermediate precision (within-laboratory precision) according to CLSI EP05-A3. A precision study was conducted with one reagent lot for the Elecsys Cortisol III assay on one **cobas e 801** analyzer to evaluate repeatability (within-run precision) and intermediate precision (within-laboratory precision) according to CLSI EP05-A3. Four human urine samples (24-hour urine) and two levels of PreciControl Cortisol Urine controls were tested in two replicates per run, two runs per day for 21 days. The results are summarized below.

Sample	Mean (µg/dL)	Repeatability (within-run)		Intermediate Precision (within-lab)	
		SD (µg/dL)	CV (%)	SD (µg/dL)	CV (%)
Human Urine 1	3.22	0.087	2.7	0.12	3.8
Human Urine 2	8.56	0.20	2.3	0.25	2.9
Human Urine 3	10.7	0.21	2.0	0.34	3.2
Human Urine 4	16.4	0.34	2.1	0.53	3.2
Control 1	7.39	0.15	2.0	0.18	2.5
Control 2	13.6	0.33	2.4	0.41	3.0

Reproducibility

A multi-site, multi-lot reproducibility experiment was performed according to CLSI EP05-A3 using four human urine (24 hr urine) samples and two urine control levels. Data were collected at two external sites each using one reagent lot and one external site using three reagent lots.

Twenty five (25) repeated measurements were performed for each sample appearing combination of sample (concentration level, site and lot) with five repetitions per day for five days on a **cobas e 801** analyzer. Testing was completed at all three sites for a total N=125. Results are summarized in the table below.

Sample	N	Mean (µg/dL)	Reproducibility	
			SD (µg/dL)	CV (%)
Human Urine 1	125	7.58	0.16	2.1
Human Urine 2	125	11.67	0.26	2.2
Human Urine 3	125	15.04	0.32	2.1
Human Urine 4	125	17.58	0.44	2.5
Control 1	125	7.54	0.17	2.3
Control 2	125	13.63	0.33	2.4

2. Linearity:

Linearity was evaluated using three spiked high analyte human urine samples diluted with low analyte human urine sample on the cobas e 801 analyzer according to CLSI EP06-Ed2. The dilution series contained a minimum of 9 concentrations ranging from approximately 0.22 to 193.58 µg/dL (6.1 to 534 nmol/L) to cover the measuring range. Samples were assayed in a 4-fold determination within a single run. Linear regression results representative of the assay linearity is as follows:

	Linear Regression
Range	6.26 – 534 nmol/L
Intercept	0
Slope	0.960
Correlation Coefficient (Pearson's r)	0.999
R ²	0.995

The results support the claimed measuring interval of 0.73-18.1 µg/dL (20-500 nmol/L).

3. Analytical Specificity/Interference:

Interference

Interfering substances (endogenous and exogenous) were tested according to CLSI EP07 using 3 human urine samples (24 hour urine) containing low, mid, and high concentrations (~20, ~200, and ~450 nmol/L) of cortisol, with the exception of hydrochlorothiazide (HCTZ), Lisinopril, and Metformin which were tested using two human urine samples (24-hour urine) containing approximately 25 nmol/L and 375 nmol/L. Each potential interfering substance was spiked into the urine samples with one aliquot left unspiked to serve as the control. Samples were tested in replicates of five. The results are summarized below.

Exogenous Substances

Interferent	Highest Concentration of substance tested which demonstrated no significant interference
Bilirubin	$\leq 1130 \mu\text{mol/L}$ or $\leq 66 \text{ mg/dL}$
Hemoglobin	$\leq 0.621 \text{ mmol/L}$ or $\leq 1000 \text{ mg/dL}$
Intralipid	$\leq 2000 \text{ mg/dL}$
Biotin	$\leq 204658 \text{ nmol/L}$ or $\leq 50000 \text{ ng/mL}$
Rheumatoid factors	$\leq 1200 \text{ IU/mL}$
IgG	$\leq 7.0 \text{ g/dL}$

Exogenous Substances

Interferent	Highest Concentration of substance tested which demonstrated no significant interference (mg/dL)
Amlodipine	0.008
Betamethasone	0.0345
Beclomethasone	0.000631
Budenoside	0.00063
Canrenone	0.075
Dexamethasone	1.20
Fludrocortisone	0.120
Fluticasone propionate	0.0003
HCT (hydrochlorothiazide)	4.77
Lisinopril	27
Losartan potassium	0.092

Metformin	850
Metoprolol	0.150
Mometasone	0.000045
Prednisone	0.010
Spirolactone	0.0555
Triamterene	0.059
Valsartan	1.17
Verapamil	0.160
Triamcinolone	0.003
Atorvastatin	0.075
Danazol	0.030
Diclofenac	2.40
β -Sitosterol	1.00

The sponsor has included the following limitations in the labeling:

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.

Biotin Interference

To evaluate the candidate device's susceptibility to biotin interference, biotin was spiked into urine samples containing different concentrations of cortisol (26, 200, and 365 ng/mL). Samples were assay in multiple replicates. The results are summarized below.

Sample (nmol/L)	Biotin concentration (ng/mL)				
	10,000	20,000	30,000	40,000	50,000
25.9	+0.3 nmol/L	-0.1 nmol/L	+0.7 nmol/L	+0.9 nmol/L	+2 nmol/L
206	+1%	+2%	+5%	+5%	+6%
365	+1%	+2%	+5%	+4%	+5%

Sample (nmol/L)	Biotin concentration (ng/mL)				
	60,000	70,000	80,000	90,000	100,000
25.9	+2 nmol/L	+3 nmol/L	+6 nmol/L	+10 nmol/L	+60 nmol/L
206	+11%	+12%	+17%	+23%	+88%
365	+6%	+11%	+17%	+35%	+99%

The sponsor has included the following limitations in the labeling:

Specimens with biotin concentrations up to 50,000 ng/mL demonstrated ≤ 4.2 nmol/L bias (absolute deviation) for samples with initial value 10.0-42 nmol/L and ≤ 10 % bias for samples with initial value > 42 -500 nmol/L in cortisol results on the **cobas e 801** analyzer. Biotin concentration greater than 50,000 ng/mL can lead to falsely elevated cortisol results on the **cobas e 801** analyzer.

The highest urine concentrations of biotin reported in literature for subjects consuming supplements of 20 mg per day vary with one study reporting up to 31,700 ng/mL in 24 hours (*Goodrum et al, 2023*) and another study reporting up to 3120 ng/mL in 24 hours (*Zempleni & Mock, 1999*). Patients may take supplements as high as 300 mg per day, however, the urinary concentrations resulting from higher-dose biotin supplements have not been established in the literature.

At concentrations corresponding to the daily therapeutic dose, the special drugs prednisolone and hydrocortisone caused elevated concentrations of cortisol. For the special drug 6-methylprednisolone, no interference was observed for concentrations ≤ 0.157 mg/dL

Cross-Reactivity Study

A cross-reactivity study was conducted according to CLSI EP07 to evaluate the potential cross-reacting compounds using human urine (24-hour urine) with a low analyte level (~ 0.6 $\mu\text{g/dL}$). One aliquot of urine sample was spiked with potential cross-reactants at the specified concentrations below and the other aliquot was left unspiked to serve as the control. Samples were measured in the presence and absence of the potential cross-reactants and cross-reactivity was calculated with one lot of reagent on the cobas e 801 analyzer. Cross reactivity was calculated according to the formula:

$$\% \text{ Cross-reactivity} = ((\text{Mean analyte concentration of spiked sample} - \text{mean analyte concentration of unspiked sample}) / \text{Spiked concentration of cross-reactant}) \times 100\%$$

The following cross-reactivities were found at the respective cross-reactant concentration tested with a cortisol concentration of approximately 17 nmol/L (0.6 $\mu\text{g/dL}$).

Cross-Reactant	Concentration tested µg/dL	Cross reactivity %
11-Deoxy-Corticosterone	100	0.174
11-Deoxy-Cortisol	50	24.3
17a-Hydroxyprogesterone	1000	0.412
21-Deoxy-Cortisol	100	2.33
Corticosterone	750	0.368
Cortisone	500	1.49
Androstendione	100	n.d.
DHEAS	1000	n.d.
DHEA	1000	n.d.
Progesterone	1000	0.00930
Testosterone	1000	n.d.
Estradiol	1000	n.d.
Estriol	1000	n.d.
Estrone	1000	n.d.
Aldosterone	1000	n.d.
Pregnenolone	1000	n.d.
6 alpha-OH-Cortisol	100	0.0417
6 beta-OH-Cortisol	100	0.0173
Cortisol-21 glucuronide	1000	n.d.
Allotetrahydrocortisol	10	n.d.
17-alpha-Hydroxypregnenolone	1000	0.0698
11-beta-Hydroxyprogesterone	1000	0.0301
Pregnanetriol	1000	11.3
Cortisol-21-sulfate	1000	n.d.
beta-Cortol	1000	n.d.
beta-Cortolone	1000	n.d.
Pregnanediol	1000	n.d.
Tetrahydrocortisol	10	n.d.

Note: n.d. = not detectable

The sponsor has included the following limitations in the labeling:

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

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

4. Assay Reportable Range:

0.73-18 ug/dL (20-500 nmol/L)

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

Traceability

The Elecsys Cortisol III assay has been standardized by an isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) based method. The method is traceable to the certified standard reference material SRM 921a Cortisol from the National Institute of Standards and Technology (NIST).

On-Board Stability

A stability study was conducted to ensure that the reagent performed consistently throughout the claimed in use/on board stability. All protocols and results were reviewed and found to be acceptable. The study results support the claims of 16 weeks.

6. Detection Limit:

The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) were determined according to CLSI guideline EP17-A2.

Limit of Blank

For determination of LoB, five analyte-free urine samples (24-hour urine) were measured using 3 reagent lots over \geq three days in 6 runs with 2-fold determination per run for a total of 60 replicates per reagent lot on one **cobas e 801** analyzer. LoB was calculated according to the parametric function as described in CLSI EP17-A2.

Limit of Detection

For determination of LoD, three reagent lots were evaluated on one **cobas e 801** analyzer. Five low-level human urine samples (24-hour urine) were measured on one instrument over \geq three days in 6 runs with a two-fold determination per run. LoD was calculated according to CLSI EP17-A2.

Limit of Quantitation

Determination of the LoQ of the Elecsys Cortisol III was performed using the **cobas e 801** analyzer. A low-level sample set (ten 24-hour urine samples) of known measurand concentration was tested in two replicates (aliquots) on one instrument per run, for six runs over \geq 3 days. LoQ was defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable error of \leq 30%.

LoB, LoD and LoQ as reported in the labeling are shown below.

Limits of Detection	Cortisol Concentration
LoB	4.00 nmol/L (0.145 µg/dL)
LoD	7.50 nmol/L (0.272 µg/dL)
LoQ	10.0 nmol/L (0.363 µg/dL)

7. Assay Cut-Off:

Not Applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study of the Elecsys Cortisol III assay on the **cobas e 801** analyzer (candidate device) with the predicate device was performed according to CLSI EP09-A3. Urine samples were measured with the Elecsys Cortisol III on the **cobas e 801** analyzer and with the Architect Cortisol on Abbott Architect i2000 using one lot of each assay. 125 native urine samples (24-hour urine) that spanned the measuring range were tested with one run per sample. The Passing Bablok regression analysis results between the candidate device (dependent variable, y-axis) and the predicate device (x-axis, comparator) are shown below:

N	Concentration Range (µg/dL)*	Slope	Intercept	Correlation coefficient (r)
125	0.837 – 17.7	1.051	-0.810	0.933

*As measured by the candidate device

2. Matrix Comparison:

Not Applicable.

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:

The US reference range study was performed using 144 apparently healthy subjects. The reference range was computed and reported as the 2.5th and 97.5th percentiles for pooled data from all study sites and with all sites combined. The 2.5th and 97.5th percentiles and corresponding intervals were computed using a nonparametric method as described in CLSI EP28-A3c.

App. healthy males and females, no relevant medication	N	Median	2.5th percentile (95% CI)	97.5th Percentile (95% CI)
24-hour urine, µg per 24 hours	144	26.4	8.98	86.28
24-hour urine, nmol per 24 hours	144	72.8	24.8	238

The reference range for Elecsys Cortisol III in 24 hr urine will be listed 24.8 – 238 nmol/24 h in the “Expected values” section of the labeling. Sponsor stated the following in the labeling: “Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.”

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.