DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

ROCHE DIAGNOSTICS
KELLI TURNER
REGULATORY AFFAIRS PRINCIPAL
9115 HAGUE ROAD
INDIANAPOLIS IN 46250

April 27, 2016

Re: K152227
Trade/Device Name: Elecsys Cortisol II, Cortisol II CalSet
Regulation Number: 21 CFR 862.1205
Regulation Name: Cortisol (hydrocortisone and hydroxycorticosterone) test system
Regulatory Class: II
Product Code: JFT, JIT
Dated: March 24, 2016
Received: March 25, 2016

Dear Ms. Kelli Turner:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Katherine Serrano -S

For: Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
k152227

Device Name
Elecsys Cortisol II

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*

k152227

Device Name
Cortisol II CalSet

Indications for Use *(Describe)*
Cortisol II CalSet is used for calibrating the quantitative Elecsys Cortisol II assay on the Elecsys and cobas e immunoassay analyzers.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

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510(k) Summary

(k152227)

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

Submitter Name	Roche Diagnostics
Address	9115 Hague Road Indianapolis, IN 46250
Contact	Kelli Turner Phone: (317) 521-4515 FAX: (317) 521-3080 Email: kelli.turner@roche.com
Date Prepared	04/22/2016
Proprietary Name	1) Elecsys Cortisol II 2) Cortisol II CalSet
Common Name	1) Cortisol II immunoassay 2) Cortisol II CalSet
Classification Name	1) Enzyme Immunoassay, Cortisol 2) Secondary, calibrator
Product Codes	1) JFT, 862.1205 2) JIT, 862.1150
Predicate Devices	Elecsys Cortisol (k070788) Elecsys Cortisol CalSet (k070788)
Establishment Registration	Roche Diagnostics GmbH in Mannheim, Germany, is 9610126 Roche Diagnostics GmbH in Penzberg, Germany, is 9610529 Roche Diagnostics in the United States is 1823260

1. DEVICE DESCRIPTION

The Elecsys Cortisol II assay makes use of 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 reagent barcode.

1.1. Reagents

The reagent working solutions include:

- rackpack (kit placed on instrument)
  - Streptavidin coated microparticles,
  - Reagent 1 (Anti-cortisol-Ab~biotin) and
  - Reagent 2 (Cortisol-peptide~$Ru(bpy)^{2+}_{3}$).

1.2. Calibrator

The Cortisol II CalSet is a lyophilized human serum with added cortisol in two concentration ranges.

The CalSet includes:

- Cal 1 (approximately 12.5 nmol/L cortisol in a human serum matrix)
- Cal 2 (approximately 1000 nmol/L cortisol in a human serum matrix)

2. INDICATIONS FOR USE

2.1. Immunoassay

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.

2.2. Calibrator

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

3. TECHNOLOGICAL CHARACTERISTICS

Table 1: Assay Comparison

Assay Comparison		
Feature	**Predicate Device: Elecsys Cortisol (k070788)**	**Candidate Device: Elecsys Cortisol II**
General Assay Features		

Assay Comparison		
Intended Use/ Indications for Use	Immunoassay for the in vitro quantitative determination of cortisol in human serum, plasma, urine and saliva. 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.	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.
Assay Protocol	The Elecsys Cortisol assay makes use of a competition test principle using a polyclonal 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.	The Elecsys Cortisol II assay makes use of 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.
Detection Protocol	Electrochemiluminescent Assay	Electrochemiluminescent Assay.
Applications	18 minute application	18 minute application.
Instrument Platform	Elecsys 1010, Elecsys 2010, MODULAR ANALYTIC E170 and cobas e analyers.	**cobas e** 411
Sample Volume	20 µL	10 µL.
Sample Type	Human serum, plasma, saliva and urine.	Human serum, and plasma.

Assay Comparison		
Reagents	M Streptavidin-coated microparticles: Streptavidin-coated microparticles; preservative. R1 Anti-cortisol-Ab~biotin: Biotinylated polyclonal anti-cortisol antibody (ovine); danazol; MES buffer; preservative. R2 Cortisol-peptide~Ru(bpy): Cortisol derivative (synthetic), labeled with ruthenium complex; danazol; MES buffer; preservative.	M Streptavidin-coated microparticles: Streptavidin-coated microparticles; preservative. R1 Anti-cortisol-Ab~biotin: Biotinylated monoclonal anti-cortisol antibody (ovine); danazol; MES buffer; preservative. R2 Cortisol-peptide~Ru(bpy): Cortisol derivative (synthetic), labeled with ruthenium complex; danazol; MES buffer; preservative.
Calibrator	Elecsys Cortisol CalSet	Elecsys Cortisol II CalSet
Calibration Interval	Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows: · After 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 defined limits	Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows: · 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
Controls	Elecsys PreciControl Universal	Elecsys PreciControl Universal
Traceability / Standardization	standardized against the Enzymun-Test Cortisol method.	Standardized against the IRMM (Institute for Reference Materials and Measurements)/IFCC 451 panel (ID GC/MS, isotope dilution-gas chromatography/mass spectrometry).
Reagent Stability	When stored and handled as directed, reagents are stable until the expiration date. After Opening at 2-8°C – 30 days On the Analyzers – 30 days	Unopened: 2-8°C - Up to the stated expiration date. After Opening at 2-8°C - 12 weeks On the Analyzers – 8 weeks
Measuring Range	0.5 – 1750 nmol/L	3.0- 1750 nmol/L

<table>
<tr><th colspan="3">Assay Comparison</th></tr>
<tr>
<td>Precision</td>
<td>Elecsys 2010/cobas e 411:
Within-run
<table>
<tr><th>Sample.</th><th>Mean (nmol/L)</th><th>SD</th><th>%CV</th></tr>
<tr><td>HS 1</td><td>208</td><td>2.76</td><td>1.3</td></tr>
<tr><td>HS 2</td><td>561</td><td>7.40</td><td>1.3</td></tr>
<tr><td>HS 3</td><td>1268</td><td>14.0</td><td>1.1</td></tr>
<tr><td>PCU* 1</td><td>363</td><td>5.08</td><td>1.4</td></tr>
<tr><td>PCU* 2</td><td>865</td><td>8.54</td><td>1.0</td></tr>
</table>
Total
<table>
<tr><th>Sample.</th><th>Mean (µg/dL)</th><th>SD</th><th>%CV</th></tr>
<tr><td>HS 1</td><td>208</td><td>3.29</td><td>1.6</td></tr>
<tr><td>HS 2</td><td>561</td><td>8.36</td><td>1.5</td></tr>
<tr><td>HS 3</td><td>1268</td><td>19.9</td><td>1.6</td></tr>
<tr><td>PCU* 1</td><td>363</td><td>5.67</td><td>1.6</td></tr>
<tr><td>PCU* 2</td><td>865</td><td>12.5</td><td>1.4</td></tr>
</table>
*PreciControl Universal</td>
<td>cobas e 411:
Serum
Within-run (will be labeled Repeatability)
<table>
<tr><th>Sample</th><th>Mean (nmol/L)</th><th>SD</th><th>CV</th></tr>
<tr><td>HS 1</td><td>3.09</td><td>0.219</td><td>7.1%</td></tr>
<tr><td>HS 2</td><td>35.8</td><td>0.718</td><td>2.0%</td></tr>
<tr><td>HS 3</td><td>283</td><td>7.29</td><td>2.6%</td></tr>
<tr><td>HS 4</td><td>548</td><td>10.4</td><td>1.9%</td></tr>
<tr><td>HS 5</td><td>1592</td><td>29.3</td><td>1.8%</td></tr>
<tr><td>PCU* 1</td><td>308</td><td>4.33</td><td>1.4%</td></tr>
<tr><td>PCU* 2</td><td>719</td><td>10.4</td><td>1.4%</td></tr>
</table>
Total (will be labeled Intermediate precision)
<table>
<tr><th>Sample</th><th>Mean (nmol/L)</th><th>SD</th><th>CV</th></tr>
<tr><td>HS 1</td><td>3.09</td><td>0.392</td><td>12.7%</td></tr>
<tr><td>HS 2</td><td>35.8</td><td>1.36</td><td>3.8%</td></tr>
<tr><td>HS 3</td><td>283</td><td>9.39</td><td>3.3%</td></tr>
<tr><td>HS 4</td><td>548</td><td>17.4</td><td>3.2%</td></tr>
<tr><td>HS 5</td><td>1592</td><td>42.7</td><td>2.7%</td></tr>
<tr><td>PCU* 1</td><td>308</td><td>8.35</td><td>2.7%</td></tr>
<tr><td>PCU* 2</td><td>719</td><td>18.0</td><td>2.5%</td></tr>
</table>
*PreciControl Universal</td>
</tr>
<tr>
<td>Analytical Sensitivity</td>
<td>Limit of Detection = <0.500 nmol/L</td>
<td>Limit of Blank (LoB): = 1.0 nmol/mL
Limit of Detection (LoD): = 1.5 nmol/mL
Limit of Quantitation (LoQ): = 3.0 nmol/mL</td>
</tr>
</table>

Assay Comparison						
Analytical Specificity	Compound	Concentration (µg/mL)	% Cross-Reactivity	Cross reactant	concentration tested (µg/mL)	Highest cross-reactivity observed (%)
	Allotetrahydro-Cortisol	0.1	165	11-Deoxycorticosterone	10	0.640
	Corticosterone	10	5.8	11-Deoxycortisol	10	4.90
	Cortisol 21 -sulfate	10	0.04	17-α-Hydroxyprogesterone	10	0.080
	Cortisone	10	0.30	Corticosterone	10	2.48
	11-Deoxycortl-costerone	10	0.69	Cortisone	10	6.58
	11-Deoxycortisol	10	4.1	Dexamethasone	10	Not detectable
	Dexamethasone	10	0.08	Fludrocortisone	10	0.200
	21-deoxycortisol	1	45.4	Prednisone	10	2.23
	6-b-Hydroxycortlsol	1	158	Progesterone	10	0.035
	17-Hydroxy-progesterone	10	1.5	21-Deoxycortisol	1	2.40
	6-a-methyl-prednisolone	0.1	389	Prednisolone	0.1	7.98
	Prednisolone	0.1	171	6-α-Methylprednisolone	0.1	12.0
	Prednisone	10	0.28	Androstendion	10	0.1
	Progesterone	10	0.35	Estradiol	10	0.2
				Estriol	10	Not detectable
				Estron	10	0.8
Linearity	1 to 59.8 µg/dL			3.0 to 1750 nmol/mL		

Assay Comparison		
Limitations	When performed in serum and plasma, the assay is unaffected by: · Bilirubin <60 mg/dL · Hemolysis < 1.9 g/dL · Lipemia < 2700 mg/dL · Biotin< 30 ng/mL · Rheumatoid factors < 1100 IU/mL · In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found. · The risk of interference from potential immunological interactions between test components and rare sera has been minimized by the inclusion of suitable additives. In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. The test contains additives which minimize these effects. · Pregnancy, contraceptives and estrogen therapy give rise to elevated cortisol concentrations. · In samples from patients who have been treated with prednisolone, 6 α Methylprednisolone or prednisone, falsely elevated concentrations of cortisol may be determined. · During metyrapon tests, 11 deoxycortisol levels are elevated. Falsely elevated cortisol values may be deter-mined due to cross reactions (see section on analytical specificity). · Patients suffering from 21 hydroxylase deficiency exhibit elevated 21 deoxycortisol levels and this can also give rise to falsely elevated cortisol results. · The time of sample collection must be taken into account when interpreting results due to the cortisol secre-tion circadian rhythm. Severe stress can also give rise to elevated cortisol levels. For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.	When performed in serum and plasma, the assay is unaffected by: · Bilirubin ≤ 25 mg/dL · Hemolysis ≤ 0.5 g/dL · Lipemia ≤ 1500 mg/dL · Biotin ≤ 30 ng/mL · Rheumatoid factors < 600 IU/mL · IgG ≤ 50 g/L · IgM ≤ 10 g/L · IgA ≤ 10 g/L · In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found. · 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. · Pregnancy, contraceptives and estrogen therapy give rise to elevated cortisol concentrations. · In samples from patients who have been treated with prednisolone, 6 α Methylprednisolone or prednisone, falsely elevated concentrations of cortisol may be determined. · During metyrapon tests, 11 deoxycortisol levels are elevated. Falsely elevated cortisol values may be deter-mined due to cross reactions (see section on analytical specificity). · Patients suffering from 21 hydroxylase deficiency exhibit elevated 21 deoxycortisol levels and this can also give rise to falsely elevated cortisol results. · The time of sample collection must be taken into account when interpreting results due to the cortisol secre-tion circadian rhythm. Severe stress can also give rise to elevated cortisol levels. For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Assay Comparison

Labeled Performance Characteristics

Method Comparison (LC/MS)

n = 208	**Passing/Bablok**	**Deming regression**
Min = 10.9 nmol/L		
Max = 1599 nmol/L		
Slope	1.022	1.055
Intercept	2.92	-6.10
Tau/r	0.930	0.993

Method Comparison (Cortisol II (y) versus Cortisol (x))

n = 536	**Passing/Bablok**	**Deming regression**
Min = 1.54 nmol/L		
Max = 1680 nmol/L		
Slope	0.758	0.806
Intercept	10.20	-10.31
Tau/r	0.8725	0.9676

Reference range serum/plasma

Subject cohort	**Sample size**	**Min**	**Max**	**Mean**	**Median**	**2.5th percen-tile**	**5th percen-tile**	**95th percen-tile**	**97.5th percen-tile**
	N	**nmol/L**							
6 – 10 am	296	23.7	835	316	303	133	166	507	537
LCL – UCL*	-	-	-	-	-	70.1 - 161	133 - 183	488 - 537	511 - 584
4 – 8 pm	300	19.1	544	172	161	68.2	73.8	291	327
LCL – UCL*	-	-	-	-	-	46 - 72	68 - 87	269 - 327	301 - 401

Table 2: CalSet Comparison

Characteristic	Predicate device: Elecsys Cortisol CalSet (k070788)	Candidate device: Cortisol II CalSet
Intended Use	Cortisol CalSet is used for calibrating the quantitative Elecsys Cortisol assay on the 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.
Analyte	Cortisol (synthetic)	Same
Matrix	Human serum matrix	Same
Levels	Two	Same
Target Ranges	Cal 1: 12.5 nmol/L Cal 2: 1000 nmol/L	Same.
Format	Lyophilized	Same
Traceability	standardized against the Enzymun-Test Cortisol method.	The Elecsys Cortisol II assay has been standardized against the IRMM(Institute for Reference Materials and Measurements)/IFCC-451 panel (ID-GC/MS, isotope dilution- gas chromatography/mass spectrometry)
Handling	Carefully dissolve the contents of one bottle by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 15 minutes to reconstitute. Mix carefully, avoiding foam formation. Transfer aliquots of the reconstituted calibrators into empty labeled snap-cap bottles (CalSet Vials). Attach the supplied labels to the additional bottles. Store the aliquots immediately at -20 °C. Perform only one calibration procedure per aliquot.	Same.

4. NON-CLINICAL PERFORMANCE EVALUATION

Non-clinical performance evaluations for the Elecsys Cortisol II executed with the study briefly summarized.

4.1. Precision (Human Serum)

Precision of the Elecsys Cortisol II assay was evaluated on one cobas e 411 Immunoassay Analyzer according to CLSI EP5-A2 guideline. One reagent lot was evaluated.

The protocol consisted of testing 2 replicates each of the controls (PC Universal) and 5 human serum samples (HS) per run, 2 runs per day for 21 days. The samples were run in randomized order on the analyzer. Samples used were native, diluted and spiked human serum pools.

Repeatability and Intermediate precision were calculated according to EP5-A2.

4.2. Limit of Blank

For the analytical sensitivity studies, 2 **cobas e** 411 analyzers and 2 lots of reagents were used. The Limit of Blank (LoB) was determined using native human serum pools and Universal Diluent. A total of n = 60 LoB measurements were made (5 blank samples, 1 replicate, 2 runs per day on 2 instruments over 3 days). The LoB was calculated according to CLSI EP17-A2 (non-parametric approach).

4.3. Limit of Detection

For the analytical sensitivity studies, 2 **cobas e** 411 analyzer and 2 lots of reagents were used. The Limit of Detection (LoD) was determined using 5 low-level human serum samples (diluted). A total of n = 60 LoD measurements were made (5 samples, 2 runs per day on 2 instruments over 3 days). The LoD was calculated according to CLSI EP17-A2.

4.4. Limit of Quantitation

The Limit of Quantitation (LoQ) was determined using a set of nine human serum samples, three reagent lots on two **cobas e** 411 analyzer. The Limit of Quantitation (LoQ) was determined in accordance with CLSI Guideline EP17-A2. Each sample was analyzed in replicates of 2, two runs per day over 3 days.

4.5. Linearity

4.5.1. Serum

The linearity results were obtained with serum samples on the **cobas e** 411 Immunoassay analyzer. A high analyte serum sample (pooled human serum sample, spiked) was diluted with Diluent Universal (protein matrix). 19 concentrations (thereof 17 dilutions) throughout the

measuring range were prepared. Samples were assayed in 3-fold determination within a single run.

The linearity data were analyzed with regards to linear, quadratic and cubic polynomials according to CLSI EP6-A. In a first step, a linearity check was performed with a first order (linear) regression and then with higher order models (quadratic and cubic).

4.5.2. Plasma

Linearity of the Elecsys Cortisol II assay was assessed on the cobas e 411 Immunoassay Analyzer according to CLSI EP6-A.

A high analyte plasma sample (pooled human plasma sample, spiked) was diluted with Diluent Universal (protein matrix). 19 concentrations (thereof 17 dilutions) throughout the measuring range were prepared. Samples were assayed in 3-fold determination within a single run.

The linearity data were analyzed with regards to linear, quadratic and cubic polynomials according to CLSI EP6-A. In a first step, a linearity check was performed with a first order (linear) regression and then with higher order models (quadratic and cubic).

4.6. Analytical Specificity

4.6.1. Serum/Plasma

The specificity of the Elecsys Cortisol II assay was determined using two human serum sample pools (diluted as well as spiked) spiked with potential cross-reactant compounds. The analyte concentration of the samples was at approx. 60 and 300 µg/L Cortisol (conversion factor: µg/L x 2.7586 = nmol/L). The spiked and non-spiked samples were tested in duplicates on the **cobas e** 411 Immunoassay Analyzer. Cross-reactivity results will be reported in the method sheet.

4.7. Endogenous Interferences

The effect on quantitation of analyte in the presence of endogenous interfering substances using the Elecsys Cortisol II assay was determined on the cobas e 411 Immunoassay analyzer using human serum samples (single samples as well as pools, native, diluted and spiked).

For each interfering substance three human serum samples containing low, mid and high concentrations of Cortisol were tested. The recovery for each sample was calculated by comparison to the reference sample. Predefined acceptance criterion was met. The claims included in the method sheet were set to the specification concentration.

4.8. HASA Effect

The effect of the presence of human anti-sheep antibodies (HASA) on the Elecsys Cortisol II assay was assessed on the cobas e 411 analyzer.

As no native human sample with this type of interference was available the effect of human anti-sheep antibodies was investigated in a model system using donkey anti-sheep polyclonal antibodies (DASA).

Two serum samples with cortisol concentrations of 173 respectively 789 nmol/L were divided into two aliquots each. One aliquot was spiked with the 20µg/mL DASA and was then diluted with the unspiked aliqout of the serum sample in 10% increments.

The recovery for each sample was calculated by comparison to the unspiked reference sample.

4.9. Exogenous Interferences- Drugs

The effect on quantitation of analyte in the presence of drugs was determined by comparing values obtained from samples spiked with 16 pharmaceutical compounds spiked into two human serum samples (native, diluted and spiked human serum pools as well as single samples) and tested on the **cobas e** 411 Immunoassay Analyzer. The analyte concentrations of the samples were approximately 170 and 750 nmol/L. The drug concentrations tested are in accordance with the recommendation (if available) given in the CLSI guideline EP7-A2. The two serum samples were divided into aliquots and spiked with the potential interferents. The reference sample without interferent was spiked with the respective amount of solvent only.

The Cortisol II concentration of the spiked aliquots was determined in 3-fold determination and compared to the Cortisol concentration determined for the reference aliquot (also in 3-fold determination) on one cobas e 411 Immunoassay Analyzer.

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4.10. Exogenous Interferences- Anticoagulants

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 (native, diluted and spiked human serum pools as well as single samples) drawn into Serum, Li-Heparin, K2-EDTA-, K3-EDTA-plasma primary tubes and Li-Heparin Plasma Gel Separation Tubes. A minimum of 48 serum/plasma pairs per sample material were tested in duplicate with one reagent lot on one **cobas e** 411 Immunoassay Analyzer. Potential effects are assessed by regression analysis.

4.11. Method Comparison

To show correlation, the following two method comparisons were performed:

Method Comparison 1: Elecsys Cortisol II vs. LC-MS (Reference Method)

Method Comparison 2: Elecsys Cortisol II vs. Cortisol I (k070788)

4.11.1. Method Comparison 1: Elecsys Cortisol II vs. LC-MS (Reference Method)

A total of 208 human serum samples (all native single donors) were measured in singleton covering the entire measuring range. The study was performed on the cobas e 411 (Y) and the LC-MS method (X) at UZ Gent, Lab voor Klinische Biologie (Gent, Belgium).

4.11.2. Method Comparison 2: Elecsys Cortisol II vs. Cortisol I (k070788)

The second method comparison shows the correlation of the Elecsys Cortisol II to the current Elecsys Cortisol assay. The expected differences due the improved specificity of the Elecsys Cortisol II assay are shown. The study was performed on the cobas e 411 (Y and X) analyzer at UZ Gent, LMU Großhadern, Uni Klinik Leipzig and Labor Limbach, respectively. A total of 536 human serum samples (all native single donors) were measured.

4.12. Reagent Stability

To test reagent stability, three studies were executed with two studies completed.

4.12.1. Study 1. Reagent stability onboard (8 weeks)

Reagent onboard stability for the Elecsys Cortisol II assay was tested on one cobas e 411 Immunoassay Analyzer.

A fresh reagent rackpack was placed on the analyzer and calibrated. Reference values for the samples tested were determined. After measurement the rackpack was removed from the analyzer and kept at 20°C + 3°C (on-board condition). On day 8, 36 and day 64, the same samples were measured with this reagent kit using the calibration curves established on day 1, 29 and 57, respectively.

Samples tested in duplicate include five human serum (HS) samples and two controls. Native, diluted and spiked human serum samples were used.

4.12.2. Study 2. Reagent stability after first opening at 2-8°C (84 days)

Reagent stability after first opening for the Elecsys Cortisol II assay was tested on one cobas e 411 Immunoassay Analyzer.

A fresh reagent rackpack was placed on the analyzer and calibrated. Reference values for the samples tested were determined. After measurement the kit was removed from the analyzer and kept at 2-8 °C for 29 days, 57 days and 92 days. After 29 days, 57 days and 92 days the kit was placed on the analyzer again, calibrated and the test samples were determined.

Samples tested in duplicate include five human serum (HS) samples and two controls. Native, diluted and spiked human serum samples were used.

4.12.3. Study 3: A real-time stability study is ongoing to support shelf-life stability claim.

In the ongoing real-time stability study, the Elecsys Cortisol II assay material is stored at 2-8°C. The stored assay reagents are tested at time point T=0 and at specified intervals over the shelf life of the device up to the planned shelf life plus one month. Testing will be performed using

PreciControl Universal, Level 1 and 2 (lyophylized, stored at -20°C) and human serum samples (stored at -80°C).

Data for the time-points at 0, 13, 16 and 19 months for MP lot and at 0, 7, 16 and 19 months for P2 and P3 lot tested in duplicate will be available. The average on-test recovery value will be calculated as percent recovery compared to the reference value (Assigned value for PreciControl Universal, Level 1 and 2).

4.13. Sample Stability

To test sample stability, three studies were completed. Because these studies are not analyzer dependent, and the assay is a global product currently available rest of world (ROW), the studies were executed on the cobas e 601. Study results can be applied to the cobas e 411 since sample stability is independent of the analyzer. For each study, the samples used were all single donors (native, spiked and diluted) and the following acceptance criteria was applied.

4.13.1. Study 1. Sample stability at 2-8°C

Twelve human serum, Li-Heparin, K2-EDTA, and K3-EDTA plasma samples were aliquoted and measured fresh (reference value) and after storage at 2-8°C for 4 days.

Measurements were performed with three-fold determination on a cobas e 601 Immunoassay Analyzer and recovery was calculated as percent of the reference value.

The human serum samples used were native, spiked and diluted single donors.

4.13.2. Study 2. Sample stability at Room Temperature (15-25°C)

Twelve human serum, Li-Heparin, K2-EDTA, and K3-EDTA plasma samples were aliquoted and measured fresh (reference value) and after storage at 20-25°C for 24 hours.

Measurements were performed with three-fold determination on a cobas e 601 Immunoassay

Analyzer and recovery was calculated as percent of the reference value.

The human serum samples used were native, spiked and diluted single donors.

4.13.3. Study 3. Sample stability -15 to -25°C

Twelve human serum, Li-Heparin, K2-EDTA, and K3-EDTA plasma samples were aliquoted and measured fresh (reference value) and after storage of 12 months at -15 to -25°C.

Measurements were performed with three-fold determination on a cobas e 601 Immunoassay Analyzer and recovery was calculated as percent of the reference value.

The human serum samples used were native, spiked and diluted single donors.

4.14. Calibration Stability

To test calibration stability, two studies were completed, including:

- Study 1. Lot calibration stability
- Study 2. On-board calibration stability

4.14.1. Study 1

Calibration of an Elecsys Cortisol II reagent lot is recommended every 8 weeks. During that time period fresh reagent kits of the same lot can be used without calibration using the calibration curve of the Day 1 reagent kit.

Elecsys Cortisol II was calibrated with a fresh reagent kit on Day 1 using a cobas e 411 Immunoassay Analyzer. After 29 days, 57 days and 92 days a new reagent kit of the same lot was used and recovery of samples was determined using the calibration curve of day 1.

Five human serum (HS) samples and two control samples were tested; each sample was tested with two-fold determination. The human serum samples used were native, diluted and spiked.

4.14.2. Study 2

Reagent Calibration Stability for the Elecsys Cortisol II assay was tested on one cobas e 411 Immunoassay Analyzer.

A fresh Reagent Rack-Pack was placed on the analyzer and calibrated. All samples were measured on day 1. On day 8 the same samples were measured with the same reagent kit kept at 20°C + 3°C (on-board condition) using the calibration curves established on day 1.

Five human serum (HS) samples and two control samples were tested; each sample was tested with two-fold determination. The human serum samples used were native, diluted and spiked.

4.15. Calibrator studies

The Elecsys Cortisol II CalSet was evaluated for value assignment, reconstitution and stability.

4.15.1. Value assignment

Value assignment testing was conducted and passed pre-defined acceptance criteria. The target values for the two levels of the Cortisol II CalSet kit are chosen to obtain the best fit with the Master Calibration Curve, together with the Rodbard curve parameters encoded in the reagent barcode. For each Elecsys Cortisol II CalSet lot manufactured, the calibrators are run in duplicate on at least three (3) **cobas e** 411 analyzers and at least three (3) **cobas e** 601/**cobas e** 602/MODULAR ANALYTICS E170 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.

Measurement values for PreciControl Universal (Levels 1 & 2), a multi-analyte control recommended for use to monitor accuracy and precision of specified analytes, are read from the calibration curves generated. The pre-defined acceptance criteria for PreciControl Universal have to be met to release the Assigned Values for Cortisol II CalSet.

4.15.2. Reconstitution

Reconstitution time for the lyophilized Cortisol II CalSet was tested. Two sets of Cortisol II CalSet were reconstituted, one for 15 minutes and the other for 30 minutes. Signal recovery after 30 minutes reconstitution was compared to the signal value after 15 minutes.

Cortisol II CalSet was evaluated in duplicate on the cobas e 411 analyzer.

4.15.3. Stability

Three studies were performed in order to verify the stability claims for the Cortisol II CalSet. Stability studies after reconstitution and an accelerated stability study were completed on the Elecsys 2010.

4.15.3.1. Study 1- Stability at -20ºC (after reconstitution)

The on-test and reference materials were tested in duplicate on the Elecsys 2010. The on-test material was reconstituted and stored in closed vials for 3 months at -20°C. The on-test signal recovery was calculated as percent of the reference value.

4.15.3.2. Study 2- Onboard stability at 20-25ºC after reconstitution

The on-test and reference materials were tested in duplicate on the Elecsys 2010. The on-test material was reconstituted and stored in open vials for 4 hours at 20-25°C. The on-test signal recovery was calculated as percent of the reference value.

4.15.3.3. Real-time Stability

Since there was no change in formulation and target values to the Cortisol II CalSet the real-time stability data (18 months at 2-8°C) of the current Cortisol CalSet is applicable.

In the real-time stability study, the Cortisol CalSet on-test material was stored at 2 - 8°C. The CalSets were tested at specified intervals over the shelf life of the device up to the planned shelf life plus one month.

Data for the time-points after manufacturing, 7 (Lot 1), 6 (Lot 2 & 3), 14 (Lot 1), 13 (Lot 2 & 3), and 19 months were tested in duplicate. The stability of the CalSet was assessed via accurate recovery of PreciControl Universal.

4.15.3.4. Reconstitution study

Reconstitution time for the lyophilized Cortisol II CalSet was tested. Two sets of Cortisol II CalSet were reconstituted, one for 15 minutes and the other for 30 minutes. Signal recovery after

30 minutes reconstitution was compared to the signal value after 15 minutes. Cortisol II CalSet was evaluated in duplicate on the cobas e 411 analyzer.

5. CLINICAL PERFORMANCE EVALUATION

Clinical samples were collected at three sites in the United States in order to establish the reference range values for the Elecsys Cortisol II assay. Reference ranges for self-reported healthy males and females were determined using the median value, the 5^{th} -95^{th} and the 2.5^{th} - 97.5^{th} percentiles (nmol/L) as lower and upper limit of normal, respectively. The evaluation was done at one site in St Louis, Missouri with one reagent lot (MP) using one **cobas e** 411 analyzer. Samples were all native human serum samples measured in singleton, over 2 runs within 10 days.

6. ADDITIONAL INFORMATION

Two additional products are used with the Elecsys Cortisol II assay; Cortisol CalCheck and the PreciControl Universal. There has been no change to the manufacturing process, value assignment process, product stability or specifications of these products.

The Elecsys Cortisol CalCheck labeling has been updated to incorporate the Cortisol II information and has been included for review.

7. CONCLUSIONS

The information provided in this Premarket Notification [510(k)] will support a determination of substantial equivalence for the Elecsys Cortisol II, and Elecsys Cortisol II CalSet. The data supports a safe, effective device which performs as well as or better than the predicate devices.