

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

**A. 510(k) Number:**

K153301

**B. Purpose for Submission:**

New Device

**C. Measurand:**

Digoxin

**D. Type of Test:**

Quantitative enzyme immunoassay

**E. Applicant:**

Roche Diagnostics

**F. Proprietary and Established Names:**

Elecsys Digoxin Immunoassay

Elecsys PreciControl Cardiac II

**G. Regulatory Information:**

1. Regulation section:

21 CFR 862.3320

21 CFR 862.1160

2. Classification:

Class II, Class I

3. Product code:

KXT

JJY

4. Panel:

Toxicology

Clinical Chemistry

**H. Intended Use:**

1. Intended use(s):

See indications for use below

2. Indication(s) for use:

**Elecsys Digoxin Immunoassay**

Immunoassay for the in vitro quantitative determination of digoxin in human serum and plasma. Measurements are used in the diagnosis and treatment of digoxin overdose and in monitoring levels of digoxin to ensure proper therapy. The electrochemiluminescence immunoassay “ECLIA” is intended for use on Elecsys and cobas e immunoassay analyzers.

**Elecsys PreciControl Cardiac II**

PreciControl Cardiac II is used for quality control of specified immunoassays on the Elecsys and cobas e immunoassay analyzers.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

This submission demonstrates performance on the cobas e 411 immunoassay analyzer.

**I. Device Description:**

The assay consists of three liquid components supplied as working solutions:

- Streptavidin-coated microparticles, 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- Ruthenylated Anti-digoxin-antibody, 1 bottle, 10 mL: Monoclonal anti-digoxin antibody (mouse) labeled with ruthenium complex 15 µg/L; phosphate buffer 100 mmol/L, pH 7.0; preservative.

- Biotinylated digoxin-derivative, 1 bottle, 10 mL: Biotinylated digoxigenin 1.06 ng/mL; biotin 15 µg/L; phosphate buffer 100 mmol/L, pH 7.0; preservative.

The Elecsys PreciControl Cardiac II controls are sold separately. These are lyophilized control serum based on human serum, and consist of two concentration ranges. The two available concentration ranges (PC CARDII 1 and PC CARDII 2) are each supplied as 2 bottles; each bottle contains 2mL of control serum.

The Digoxin CalSet is sold separately. The CalSet calibrator is bovine serum matrix with added digoxin with two concentration ranges. The two available concentration ranges (DIGO Cal1 and DIGO Cal2) are each supplied as two bottles; each bottle contains 1.5 mL of calibrator.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
Elecsys Digoxin Immunoassay
2. Predicate 510(k) number(s):  
K973112
3. Comparison with predicate:

<b>Similarities</b>		
<b>Item</b>	<b>Predicate – Elecsys Digoxin Immunoassay (K973112)</b>	<b>Candidate – Elecsys Digoxin Immunoassay (K153301)</b>
Intended Use/Indications for Use	Immunoassay for the in vitro quantitative determination of digoxin in human serum and plasma.	SAME
Assay Protocol	Competitive Immunoassay	SAME
Detection Protocol	Electrochemiluminescent Assay	SAME
Applications	18 minute application	SAME
Calibrator	Elecsys Digoxin CalSet	SAME
Calibration Interval	Calibration must be performed once per reagent lot using fresh reagent Renewed calibration is recommended: -after 1 month (28 days) when using the same reagent lot -after 7 days (when using the same reagent kit on the	SAME

<b>Similarities</b>		
<b>Item</b>	<b>Predicate – Elecsys Digoxin Immunoassay (K973112)</b>	<b>Candidate – Elecsys Digoxin Immunoassay (K153301)</b>
	analyzer) -as required: e.g. quality control findings outside the specified limits	
Traceability/Standardization	(USP) digoxin reference material into analyte free human serum.	SAME
Storage and Stability	Store at 2-8 °C. Do not freeze. Store upright. Stability: -unopened at 2-8 °C: up to the stated expiration date -after opening at 2-8 °C: 12 weeks -on the analyzers: 8 weeks	SAME
Hook Effect	not applicable	SAME

<b>Differences</b>		
<b>Item</b>	<b>Predicate</b>	<b>Device</b>
Sample Type	Serum, Li-, Na-, NH <sub>4</sub> <sup>+</sup> - heparin, K <sub>3</sub> -EDTA, sodium citrate, and sodium fluoride/potassium oxalate plasma.	Serum, Li-Heparin, K <sub>2</sub> - and K <sub>3</sub> -EDTA plasma. Li-Heparin plasma tubes containing separating gel
Controls	A suitable commercially available control	Elecsys PreciControl Cardiac II or other suitable control material
Measuring Range	0.150 (LDL)-5.00 ng/mL	0.4 ng/mL (LoQ) – 5.00 ng/mL
LoB	Not Reported	0.15 ng/mL
LoD	Not Reported	0.2 ng/mL
LoQ	Not Reported	0.4 ng/mL
Lower Detection Limit	0.150 ng/mL	Not Reported

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

CLSI EP5-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition

CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition

CLSI EP6-A Evaluation of the Linearity of Quantitative Measurement Procedures: A

Statistical Approach; Approved Guideline

**L. Test Principle:**

The Elecsys Digoxin assay employs a competitive test principle using a monoclonal antibody specifically directed against digoxin. Digoxin in the sample competes with the added digoxin derivative labeled with biotin for the binding sites on the ruthenylated antibody-complex. The total duration of the assay is 18 minutes.

By incubating the sample (10 µL) with a digoxin specific ruthenium-labeled antibody, an immunocomplex is formed, the amount of which is dependent upon the analyte concentration in the sample. After addition of streptavidin-coated microparticles and a digoxin derivative labeled with biotin, the still-vacant sites of the ruthenium labeled 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. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

**M. Performance Characteristics (if/when applicable):**

All performance characteristics were established on the cobas e 411 analyzer.

1. Analytical performance:

*a. Precision/Reproducibility:*

**Precision**

Repeatability and intermediate precision of the Elecsys Digoxin assay were evaluated on one cobas e 411 analyzer. One reagent lot was evaluated.

A seven sample panel consisting of five pooled human serum samples (HS) spiked with digoxin and two controls (PCC II = PreciControl Cardiac II, Level 1 and 2) were measured. The protocol consisted of testing 2 replicates of each control and human serum sample per run, divided into 2 runs per day for 21 operating days. The samples were run in randomized order.

		Repeatability		Intermediate Precision		
Sample	Mean [ng/mL]	SD [ng/mL]	CV [%]	SD [ng/mL]	CV [%]	n
Human Serum 1	0.565	0.019	3.4	0.036	6.4	84
Human Serum 2	1.09	0.027	2.5	0.063	5.8	84
Human Serum 3	1.85	0.039	2.1	0.083	4.5	84
Human Serum 4	2.38	0.055	2.3	0.092	3.8	84
Human Serum 5	4.67	0.119	2.5	0.299	6.4	84
PCC II 1	1.20	0.035	2.9	0.051	4.3	84
PCC II 2	2.74	0.102	3.7	0.111	4.1	84

### Reproducibility

Reproducibility of the Elecsys Digoxin assay across multiple reagent lots was evaluated on three cobas e 411 analyzers according to the 3 x 5 x 5 design described in CLSI EP5-A3. The protocol consisted of testing 5 aliquots for each of two control solutions (PeciControl Cardiac II, ) and five serum sample pools spiked with digoxin over 5 days on three instruments with three reagent lots, with 1 run per aliquot per day, for a total of 225 measurements per sample.

Sample material	n	Mean [ng/mL]	SD [ng/mL]	CV [%]
HS 01	225	0.442	0.074	16.65
HS 02	225	0.810	0.051	6.28
HS 03	225	1.07	0.063	5.85
HS 04	225	2.30	0.144	6.27
HS 05	225	4.46	0.274	6.13
Control 01	225	1.21	0.060	4.97
Control 02	225	2.71	0.139	5.13

#### b. Linearity/assay reportable range:

Linearity of the Elecsys Digoxin assay was assessed on the cobas e 411 analyzer according to CLSI EP6-A.

A high analyte serum sample pool spiked with digoxin was diluted with digoxin free human serum. Fifteen concentrations throughout the measuring range were prepared. Samples were measured in triplicate within a single run.

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

(quadratic and cubic). Results of this study support the claimed measuring range of 0.4 to 5.0 ng/mL.

A dilution study was performed to determine the recommended dilution factor for samples. The dilution study for Elecsys Digoxin assay was performed on two cobas e 411 analyzers using four human serum samples with Digoxin concentrations above the measuring range. Samples were diluted 1:2 manually and automatically by the instrument. Results support the recommended dilution factor of 1:2 yielding a diluted sample concentration of 2.5 ng/mL or lower.

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

**Traceability**

This method is standardized to United States Pharmacopoeia (USP) digoxin reference material

**Stability**

Protocols and acceptance criteria for PreciControl Cardiac II control solution stability were reviewed and found acceptable to support stability of reconstituted control serum for up to 3 months at -20°C, 3 days at 2-8°C, on-board the analyzer at 20-25°C. Controls should not be re-frozen after thawing. Protocols and acceptance criteria for stability of lyophilized control were reviewed and found acceptable to support stability of lyophilized control serum stored between 2-8°C for up to 18 months; real-time stability studies are ongoing.

d. *Detection limit:*

**Limit of Blank (LoB):**

The Limit of Blank (LoB) was defined as the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte were tested. The distribution of values for five analyte-free human serum samples was determined with three reagent lots on two **cobas e 411** analyzers with two runs per day over a period of three days.

The sample was measured in one-fold determination in each run. In summary, 30 measuring points were collected per instrument for a total of 60 measured values per reagent lot. Results support the LoB of 0.15 ng/mL.

**Limit of Detection (LoD)**

The distribution of values for five low level human serum samples was determined using three reagent lots on two **cobas e 411** analyzers with two runs per day over a period of three days. Each sample was measured once per run. In summary, 30 measuring points were collected per instrument for a total of 60 measured values per reagent lot. The Limit of Detection (LoD) was defined as  $LoB + 1.653 \times SD_{total}$  where  $SD_{total}$  refers to the total standard deviation of low analyte samples. Results support the LoD of 0.2 ng/mL.

### **Limit of Quantitation (LoQ)**

The Limit of Quantitation (LoQ) was defined as the lowest amount of analyte that can be quantitatively determined with the stated accuracy. The distribution of values for  $\geq 4$  low level samples each diluted to concentrations which covered the range between LoB to 2x LoQ was determined with three reagent lots on a **cobas e 411** analyzer with six runs distributed over three days. Each sample was measured in two replicates per run with a total of twelve replicates per sample per reagent lot.

A set of ten human serum samples (sample pools spiked with Digoxin) with known concentrations in the specified LoQ-area (approx. from LoB to 2x LoQ) was evaluated for each reagent lot. Target values for the Low Level Sample Set were determined using mass spectrometry during Reference Standardization. Results support the LoQ of 0.4 ng/mL.

*e. Analytical specificity:*

### **Cross Reactivity**

A cross reactivity study was performed to determine the specificity of the Elecsys Digoxin assay with both co-analytes and cross-reactant compounds. A dilution series was prepared and assayed for each co-analyte and cross reactant with Digoxin concentration of 0.5 and 2.0 ng/mL.

<b>Co-Analyte</b>	<b>Cross-reactivity [%]</b>
$\beta$ -Methyldigoxin	87.9 %
$\alpha$ -Acetyldigoxin	77.9 %
$\beta$ -Acetyldigoxin	84.4 %
Lanatoside C	65.2 %
Deslanoside	85.6 %
Digoxigenin-bis-digitoxoside	108.1 %
Digoxigenin-mono-digitoxoside	141.1%

Substance	Concentration tested [ng/mL]	Cross Reactivity
<b>Digoxin Metabolites</b>		
Digoxigenin	6	< 50 %
Dihydrodigoxin	1000	< 20 %
<b>Other substances used as drugs &amp; their metabolites</b>		
Digitoxin	250	< 2 %
Digitoxigenin	250	< 5 %
Ouabain	5000	< 0.1 %
k-Strophanthin	1250	< 0.2 %
<b>Steroids</b>		
Cortisol	5000	< 0.01 %
Prednisone	5000	< 0.01 %
Dexamethasone	5000	< 0.01 %
D-Aldosterone	5000	< 0.01 %
Progesterone	5000	< 0.05 %
Estradiol	5000	< 0.01 %
DHEA	5000	< 0.01 %
Testosterone	5000	< 0.15 %
<b>Others</b>		
Oleandrin	5000	< 0.01 %
Furosemide	5000	< 0.01 %
Sulthiame	5000	< 0.01 %
Quinidine (free base)	5000	< 0.01 %

### Human Anti-Mouse Antibodies (HAMA)

The effect of the presence of human anti-mouse antibodies in patient samples on the Elecsys Digoxin assay was assessed on the cobas e 411 analyzer. A high HAMA and HAMA-free serum pool were each divided into two aliquots and spiked with analyte to yield two different digoxin concentrations: 0.843 and 2.37 ng/mL. Each high HAMA serum pool was diluted in 11 steps with the corresponding HAMA-free serum pool containing the same digoxin concentration. Each dilution was analyzed in 3-fold determination. The sponsor defined significant interference as recovery of the HAMA serum sample beyond  $\pm 10\%$  of the corresponding HAMA-free sample. No significant interference was observed at all tested concentrations.

### Endogenous Interference

The potential interfering effect of endogenous substances on the Elecsys Digoxin assay was determined on the cobas e 411 analyzer using human serum samples spiked with digoxin. For each interfering substance 3 serum samples containing low, mid and high concentrations of digoxin were tested.

One aliquot of each serum sample was spiked with the potentially interfering substance; another aliquot was spiked with the same volume of isotonic NaCl solution

(dilution pool). The interfering pool was then diluted into the dilution pool in 10% increments. The recovery for each sample was calculated by comparison to the reference (unspiked) sample.

The sponsor defined significant interference as recovery of the digoxin sample spiked with potential interferent beyond  $\pm 0.08$  ng/mL at concentrations less than 0.8 ng/mL, beyond  $\pm 10\%$  at concentrations between 0.8 and 4.0 ng/mL, and beyond  $\pm 12\%$  at concentrations above 4.0 ng/mL when compared to the corresponding un-spiked reference sample. No significant interference was observed for any tested endogenous substance at the levels indicated in the table below:

<b>Compound</b>	<b>Highest concentration with no significant interference</b>
Lipemia	1500 mg/dL
Biotin	100 ng/mL
Bilirubin	66 mg/dL
Hemoglobin	1000 mg/dL
Rheumatic Factor	1630 IU/mL
human Serumalbumin	11.2 g/dL
human IgG	8.0 g/dL

### **Exogenous Interference**

To test for interference from exogenous substances, seventeen common pharmaceutical compounds were spiked into two human serum samples and tested with the Elecsys Digoxin assay. The analyte concentrations of the samples were approximately 0.6 and 2.4 ng/mL. In addition, 17 cardiac drug compounds were assessed using the same serum sample pools and tested with the Elecsys Digoxin assay.

For testing, two serum sample pools were divided into aliquots and spiked with the potential interferents. The interferent concentrations were determined based on recommendations in the CLSI guideline EP7-A2. The reference samples without interferent were spiked with solvent.

The digoxin concentration of the spiked samples was determined in triplicate and compared to the digoxin concentration determined for the reference samples (also measured in triplicate). These studies were conducted on a cobas e 411 analyzer.

**Common Pharmaceutical Compounds:**

<b>Drug</b>	<b>Drug concentration [mg/L]</b>	<b>Recovery in <math>\approx 0.6</math> ng/mL Digoxin [%]</b>	<b>Recovery in <math>\approx 2.4</math> ng/mL Digoxin [%]</b>
Acetylcysteine	1660	105.3	98.7
Ampicillin-Na	1000	95.6	92.4
Ascorbic acid	300	102.1	94.5
Cyclosporine	5	98.6	91.1
Cefoxitin	2500	111.7	106.3
Heparin	5000 U	99.8	97.5
Levodopa	20	97.7	96.2
Methyldopa	20	96.3	97.5
Metronidazole	200	104.7	96.6
Phenylbutazone	400	105.5	92.7
Doxycycline	50	98.4	97.5
Acetylsalicylic Acid	1000	102.4	97.1
Rifampicin	60	99.1	99.2
Acetaminophen	200	105.8	95.5
Ibuprofen	500	101.1	95.5
Theophylline	100	102.8	94.3

**Cardiac Drugs**

<b>Drug</b>	<b>Drug concentration [mg/L]</b>	<b>Recovery in <math>\approx 0.6</math> ng/mL Digoxin [%]</b>	<b>Recovery in <math>\approx 2.4</math> ng/mL Digoxin [%]</b>
Carvedilol	37.5	100.2	100.9
Clopidogrel	75	101.1	100.0
Epinephrine	0.5	107.7	99.6
Insulin	1.6	102.9	100.0
Lidocaine	80	98.3	102.2
Lisinopril	10	102.9	97.8
Methylprednisolone	7.5	98.0	109.0
Metoprolol	150	96.6	101.9
Nifedipine	30	103.3	106.0
Phenprocoumon	3	107.2	104.8
Propafenone	300	103.1	107.1
Reteplase	33.3	102.9	102.7
Simvastatin	30	98.3	105.0
Tolbutamide	1500	101.3	107.2
Torasemide	15	102.9	101.8
Verapamil	240	102.9	98.7
Fluindione	20	102.2	100.4

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

A method comparison was performed using the Elecsys Digoxin assay (K973112) as the predicate device.

A total of 168 human serum samples (all single donors, native and spiked) were measured in singlicate. Digoxin values ranged from 0.413 to 4.78 ng/mL. The study was performed on the cobas e 411 analyzer over 3 runs. Regression analysis for all samples comparing performance on the predicate device Elecsys Digoxin assay (X) and the new device Elecsys Digoxin assay (Y) are as follows:

	N	Sample Concentration Range	Slope (95% CI)	Y intercept (95% CI)	Correlation coefficient
<b>Passing/Bablok Regression Analysis</b>	168	0.413 – 4.78 ng/mL	1.03 (1.017 – 1.047)	0.001 ng/mL (-0.018 to 0.017 ng/mL)	t=0.960
<b>Linear Regression</b>			1.04 (1.03 – 1.05)	-0.009 ng/mL (-0.027 to 0.009 ng/mL)	r = 0.998

b. *Matrix comparison:*

**Anticoagulants**

The effect on quantitation of analyte in the presence of anticoagulants with the Elecsys Digoxin assay was determined by comparing values obtained from native samples spiked with digoxin (single donors) drawn into Serum, Li-Heparin, K<sub>2</sub>-EDTA-, and K<sub>3</sub>-EDTA-plasma primary tubes, and Li-Heparin Plasma Separation Tubes.

Either 65 or 66 serum/plasma pairs per sample material were tested in duplicate with one reagent lot on a cobas e 411 analyzer. Potential effects were assessed by Passing/Bablok regression analysis.

Anticoagulant	Slope	Intercept	Correlation coefficient (r)
K2-EDTA	0.980	0.00595	0.999
K3-EDTA	0.981	0.00753	0.999
Li-Heparin	0.993	0.005	0.999
Li-Heparin Plasma with Gel Separator	0.992	-0.008	0.999

No significant interference was observed.

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:

The sponsor included the following information and references in the labeling:

The recommended therapeutic range for digoxin is 0.6-1.2 ng/mL (0.77-1.5 nmol/L) (ESC Guideline 2008<sup>10</sup>) or even 0.5-1.0 ng/mL (0.64-1.3 nmol/L).<sup>20</sup> Particularly the upper end of the therapeutic range is controversial and concentrations up to 2.0 ng/mL (2.6 nmol/L) may still be applied.<sup>6,7</sup> Concentrations above 2.0 ng/mL are generally considered toxic.<sup>21</sup> Some overlap of toxic and non-toxic values has been reported.<sup>22</sup> Therefore, clinical diagnosis should be based on clinical and laboratory data.

Each laboratory should establish an acceptable reporting format and identify procedures for the reporting of abnormal results. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

References:

6. Lindenbaum J, Mellow MH, Blackstone MO, et al. Variation in Biologic Availability of Digoxin from Four Preparations. *New Engl J Med* 1971;285:1344-1347.
7. Lindenbaum J, Butler VP Jr., Murphy JE, et al. Correlation of Digoxin- Tablet Dissolution Rate with Biological Availability. *Lancet* 1973;1:1215-1217.
10. Keys PW, Stafford RW. In: Taylor WJ, Finn AL, eds. *Individualizing Drug Therapy: Practical Applications of Drug Monitoring*. New York, Gross, Townsend, Frank, Inc; 1981;vol 3:1-21.
20. Terra SG, Washam JB, Dunham GD, et al. Therapeutic Range of Digoxin's Efficacy in Heart Failure: What Is The Evidence? *Pharmacotherapy* 1999;19(10):1123-1126.
21. Matzuk MM, Shlomchik M, Shaw LM. *Therapeutic Drug Monitoring* 1991;13:215-219.
22. Beller GA, Smith TW, Abelmann WH, et al. Digitalis Intoxication: A Prospective Clinical Study with Serum Level Correlations. *New Engl J Med* 1971;284:989-997.

**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.