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

A. 510(k) Number:

k181233

B. Purpose for Submission:

Modification of a previously cleared assay (k131244)

C. Measurand:

Free thyroxine (Free T4)

D. Type of Test:

Quantitative, electrochemiluminescent immunoassay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys FT4 III

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1695

2. Classification:

   Class II

3. Product code:

   CEC

4. Panel:

   Chemistry (75)

H. Intended Use:

1. Intended use(s):

   Refer to Indications for Use below

2. *Indication(s) for use:*

   Assay for the in vitro quantitative determination of free thyroxine in human serum and plasma. Measurements obtained by this device are used in the diagnosis and treatment of thyroid diseases.

   The electrochemiluminescence immunoassay "ECLIA" is intended for use on the cobas e 411 immunoassay analyzer.

3. *Special conditions for use stat*ement(s):

   For in vitro diagnostic use only
   For prescription use only

4. Special instrument requirements:

   Roche cobas e411 Analyzer

I. Device Description:

The Elecsys FT4 III assay contains the following components:

M: Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1: Anti T4-Ab~Ru(bpy) (gray cap), 1 bottle, 18 mL: Polyclonal anti T4 antibody (sheep) labeled with ruthenium complex 75 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative.

R2: T4~biotin (black cap), 1 bottle, 18 mL: Biotinylated T4 2.5 ng/mL; free D-biotin 8 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Elecsys FT4 II Assay

2. Predicate 510(k) number:

   k131244

3. Comparison with predicate:

   Elecsys FT4 II assay

Similarities		
Item	New Device Elecsys FT4 III (k181233)	Predicate Elecsys FT4 II (k131244)
Intended Use	Quantitative determination of free thyroxine (FT4) in serum and plasma	Same
Methodology	Quantitative electrochemiluminescence immunoassay	Same
Sample Volume	15 µL	Same
Calibrators	2 levels	Same
Assay Time	18 minutes	Same
Sample type	Serum and plasma (Li-heparin, K2-EDTA, K3-EDTA)	Same
Measuring Range	0.101-7.77 ng/dL	Same
LoB	0.03 ng/dL	Same
LoD	0.05 ng/dL	Same
LoQ	0.101 ng/dL	Same

Differences		
Item	New Device Elecsys FT4 III (k181233)	Predicate Elecsys FT4 II (k131244)
Biotin	D-Biotin concentration in the R2 reagent buffer: 8 ng/mL	D-Biotin concentration in the R2 reagent buffer: 50 ng/mL
Instrument Platform	cobas e 411	Elecsys and cobas e

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

CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods: Approved Guideline, Second Edition

CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A statistical Approach: Approved Guideline

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

L. Test Principle:

This is a competitive assay with an assay time of 18 minutes.
First incubation: 15 µL of sample is combined with a T4 specific antibody labeled with a sulfonyl ruthenium complex.
Second incubation: After addition of biotinylated T4 and streptavidin-coated microparticles, the still free binding sites of the labeled antibody 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 then aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell 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 2 point calibration and a master curve provided via the reagent barcode.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   The repeatability (within-run) and intermediate precision (total) of the Elecsys FT4 III assay were evaluated on one cobas e 411 Immunoassay Analyzer referencing the CLSI EP05-A2 guideline. Five human serum samples and two levels of control material were analyzed. The protocol consisted of testing 2 replicates of each human serum sample and control per run, 2 runs per day for 21 days.

   The results of the precision study were as follows:

Sample	n	Mean (ng/dL)	Repeatability		Intermediate precision	
			SD (ng/dL)	CV (%)	SD (ng/dL)	CV (%)
Human serum 1	84	0.120	0.007	5.7	0.013	10.7
Human serum 2	84	1.05	0.019	1.8	0.037	3.5
Human serum 3	84	1.86	0.032	1.7	0.057	3.0
Human serum 4	84	4.49	0.086	1.9	0.176	3.9
Human serum 5	84	7.15	0.174	2.4	0.423	5.9
PC Universal 1	84	1.23	0.020	1.6	0.037	3.0
PC Universal 2	84	3.19	0.067	2.1	0.108	3.4

b. *Linearity/assay reportable range:*

Linearity of the Elecsys FT4 III assay was assessed using human serum samples on the cobas e 411 immunoassay analyzer according to CLSI EP06-A guideline.
A high analyte human serum sample was diluted with FT4 analyte free human serum to create 8 levels with FT4 concentrations from 0.068 to 8.623 ng/dL. Each sample was measured in triplicate on 3 lots of reagent. Linearity was evaluated using first, second and third polynomial regression analysis based on CLSI EP06-A. The coefficient of the third order polynomial regression was found to be significant; therefore, calculation of the third order model was used to demonstrate linearity. All three reagent lots generated similar results and one representative lot is presented below.

The linear regression line was $y = 0.982x + 0.009$, $R^2=0.9988$

Summary of the deviation of the third order polynomial regression against the expected linear regression:

Sample	Expected (ng/dL)	Linear regression (ng/dL)	3rd Order (ng/dL)	Absolute Difference (ng/dL)	Relative difference (%)
1	0.068	0.075	0.071	-0.004	-5.91
2	0.134	0.141	0.141	0.000	0.244
3	0.270	0.273	0.282	0.009	3.2
4	0.538	0.538	0.558	0.021	3.83
5	1.080	1.064	1.095	0.027	2.52
6	2.152	2.121	2.113	-0.014	-0.678
7	4.312	4.234	4.055	-0.184	-4.35
8	8.623	8.468	8.623	0.147	1.73

The results of the linearity study and the limit of detection study (below) support the sponsor claims that the measuring range of this assay is 0.101 – 7.7 ng/dL.

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

Traceability:

The Elecsys FT4 III assay is traceable to the Enzymun Test which was standardized using equilibrium dialysis.

d. Detection limit:

The limit of blank (LoB) is the 95th percentile value from 60 measurements of analyte-free samples over several independent series (60 measurements /lot, 3 lots of regents). The LoB corresponds to the concentration below which analyte-free samples are found with a probability of 95% and was determined to be 0.03 ng/dL (highest of the 3 lots).

The limit of detection (LoD) is determined based on the LoB and the standard deviation of low concentration samples. The LoD corresponds to the lowest analyte concentration which can be detected (value above the LoB with a probability of 95%). Five level of low samples were measured in two replicates per sample per run for 6 runs (one run per day, over multiple days), using 3 lots of reagents. LoD was determined to be 0.05 ng/dL (highest of the 3 lots).

The limit of quantitation (LoQ) was determined by measuring five low serum samples, in five replicates per run, one run per day, over 5 days, using 3 lots of reagents. LoQ is defined as the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 % and was determined to be 0.101 ng/dL (highest of the 3 lots).

The claimed measuring range of this assay is: 0.101 – 7.7 ng/dL.

e. Analytical specificity:

1. Endogenous substances

The effect of endogenous substances was evaluated on the e 411 Analyzer using pooled human serum samples spiked with L-Thyroxine. For each potential interferent, three serum samples containing low, mid, and high concentrations of FT4 were analyzed. For all substances tested, no significant interference was defined as recovery ± 10% of initial value. The potential interferents and the highest concentration tested which did not cause significant interference are listed below:

Potential interferent	Highest concentration at which no interference was observed
Bilirubin	66 mg/dL
Hemoglobin	1.0 g/dL
Lipemia (Intralipid)	2000 mg/dL
Rheumatoid Factors	1500 IU/mL
IgG	7.0 g/dL
IgA	1.6 g/dL
IgM	1.0 g/dL

2. Structurally similar substances

The effect of structurally similar substances was determined using human serum samples spiked with potential cross-reactant compounds and analyzed in duplicate on the e 411 Analyzer. For all substances tested, no significant interference was defined as recovery ± 10% of initial value. The following cross-reactivities were found at FT4 concentrations of approximately 0.974 ng/dL and 2.66 ng/dL:

Cross-reactant	Concentration tested (ng/dL)	Cross-reactivity %
L-T3	50000	0.005
D-T3	50000	0.002
rT3	190000	0.007
3-iodo-L-tyrosine	10000000	0.000
3,5-diiodo-L-tyrosine	10000000	0.000
3,3',5-triiodothyroacetic acid	100000	0.000
3,3',5,5'-tetraiodothyroacetic acid	100000	0.001

3. Common drugs

The effect of common therapeutic drugs and thyroid drugs was evaluated on the e 411 Analyzer using human serum pools spiked with L-Thyroxine. For each drug, two serum samples containing a low and high concentration of FT4 were analyzed. For all substances tested, no significant interference was defined as recovery ± 10% of initial value. The drugs and the highest concentration tested which did not cause significant interference are listed below.

Drug	Highest concentration at which no interference was observed
Acetaminophen	200 µg/mL
Acetylcysteine	1660 µg/mL
Acetylsalicylic Acid	300 µg/mL
Amiodarone	200 µg/mL
Ampicillin-Na	1000 µg/mL
Ascorbic acid	300 µg/mL
Carbimazole	6.0 µg/mL
Cefoxitin	2500 µg/mL
Cyclosporine	5 µg/mL
Doxycycline	50 µg/mL
Fluocortolone	100 µg/mL
Heparin	5000 U
Hydrocortisone	200 µg/mL
Ibuprofen	50 µg/mL

Drug	Highest concentration at which no interference was observed
Iodide	0.2 µg/mL
Levodopa	20 µg/mL
Methyldopa	20 µg/mL
Metronidazole	200 µg/mL
Octreotide	0.3 µg/mL
Perchlorate	2000 µg/mL
Phenylbutazone	100 µg/mL
Prednisolone	100 µg/mL
Propranolol	240 µg/mL
Propylthiouracil	300 µg/mL
Rifampicin	60 µg/mL
Theophylline	100 µg/mL
Thiamazole	80 µg/mL

Thyroid drugs Furosemide and Levothyroxine caused elevated Free T4 findings at the daily therapeutic dosage level. This is indicated in the labeling.

The sponsor has the following limitation statement in the labeling:

Any influence that might affect the binding behavior of the binding proteins can alter the result of the FT4 tests (e.g. drugs, NTIs (Non Thyroid Illness)), patients suffering from FDH (Familial Dysalbuminemic Hyperthyroxinemia).

The test cannot be used in patients receiving treatment with lipid-lowering agents containing D-T4. If the thyroid function is to be checked in such patients, the therapy should first be discontinued for 4-6 weeks to allow the physiological state to become re-established.
Autoantibodies to thyroid hormones can interfere with the assay.

4. Biotin interference:

Biotin interference was tested up to 1200 ng/mL in serum samples at low, medium and high concentrations of FT4. Interference was defined as a difference >±10% of the control sample values. The results are summarized in the tables below:

% Bias for samples containing various concentrations of Biotin					
Sample ng/dL	Biotin concentrations (ng/mL)				
	52	65	78	91	104
0.96	1.2	2.1	3.6	5.7	8.5
1.66	-1.8	-1.4	1.3	3.0	7.3
2.61	-0.5	0.6	2.3	4.7	7.5

% Bias for samples containing various concentrations of Biotin					
Sample ng/dL	Biotin concentrations (ng/mL)				
	117	150	300	600	1200
0.96	10.9	17.6	57.1	256	*
1.66	7.6	16.1	53.7	291	*
2.61	9.7	15.9	56.5	*	*
* Values above the measuring range. % Bias cannot be calculated.					

The labeling states:
Specimens with biotin concentrations up to 104 ng/mL demonstrated ≤ 10% bias in results. Biotin concentrations greater than 104 ng/mL can lead to falsely increased fT4 results. Some studies have shown that serum concentrations of biotin can reach 355 ng/mL within the first hour after biotin ingestion for subjects consuming supplements of 20 mg biotin per day and up to 1160 ng/mL for subjects after a single dose of 300 mg biotin.
Do not test samples from patients who take biotin.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed with 141 human serum samples (138 native, 3 spiked) with concentrations ranging from 0.18 to 7.14 ng/dL. The comparison of the Elecsys FT4 III assay (y) with the predicate device, Elecsys FT4 II assay (x), produced the following results:

Linear regression	y = 1.02x + 0.047	r = 0.999
Passing-Bablok	y = 1.03x + 0.014	r=0.957

b. Matrix comparison:

A total of 53 serum/plasma pairs per sample material were tested in singleton with one reagent lot on one cobas e 411 immunoassay analyzer. Serum concentrations ranged from 0.155 – 7.72 ng/dL.

The Passing-Bablok regression results are the following:
Serum/Li-Heparin: y=1.03x-0.017, r=0.999
Serum/K2-EDTA: y= 1.01x-0.10, r=0.999
Serum /K3 EDTA: y=1.03x-0.035, r=0.999

The results of the study support the sponsor claims that K2-EDTA, K3-EDTA, and lithium heparin plasma are acceptable anti-coagulants for the FT4 III 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:

   Euthyroid: 0.93 1.7 ng/dL

   These values correspond to the 2.5^{th} and 97.5^{th} percentile of results from a total of 801 healthy test subjects studied.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

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