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

A. 510(k) Number:

k131244

B. Purpose for Submission:

Modification of a previously cleared assay (k961489)

C. Measurand:

Free thyroxine (Free T4)

D. Type of Test:

Quantitative, electrochemiluminescent immunoassay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys FT4 II Assay

Elecsys FT4 II CalSet

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1695

   21 CFR 862.1150

2. Classification:

   Both Class II

3. Product code:

   CEC
   JIT

4. Panel:

   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   Refer to Indications for Use below

2. Indication(s) for use:

   The Elecsys FT4 II assay is 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 Elecsys and cobas e immunoassay analyzers

   The Elecsys FT4 II CalSet is used for calibrating the quantitative Elecsys FT4 II assay on the Elecsys and cobas e immunoassay analyzers.

3. Special conditions for use statement(s):

   For in vitro diagnostic use only
   For prescription use only

4. Special instrument requirements:

   Roche Cobas e411 Analyzer only

I. Device Description:

The 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; phosphate buffer 100 mmol/L, pH 7.0; preservative.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Elecsys FT4 Assay, Elecsys FT4 CalSet

2. Predicate 510(k) number:

   k961489

3. Comparison with predicate:

   Elecsys FT4 II assay

Similarities		
Item	New Device (reagent)	Predicate
Intended Use	Quantitative determination of free thyroxine (FT4) in serum and plasma	Same
Methodology	Quantitative electrochemiluminescence immunoassay	Same
Reference Range	Euthyroid: 0.93‑1.7 ng/dL	Same
Sample Volume	15 µL	Same
Calibrators	2 levels	Same
Assay Time	18 minutes	Same

Differences		
Item	New Device (reagent)	Predicate
Measuring Range	0.1 – 7.77 ng/mL	0.02 – 7.77 ng/mL
Acceptable Sample Matrices	Serum Plasma anticoagulated with K^2 EDTA, K^3 EDTA, and Lithium Heparin	Serum Plasma anticoagulated with K^3 EDTA, Lithium Heparin, Sodium Heparin, NH4+ Heparin, Sodium Citrate, Sodium Fluoride/Potassium Oxalate
Traceability/Standardization	Standardized against the Elecsys FT4 method. The Elecsys FT4 assay is traceable to the Enzymun-Test which was standardized using equilibrium dialysis.	Standardized against the Enzymun-Test FT4 method, which was standardized using equilibrium dialysis.

Elecsys FT4 II CalSet

Similarities		
Item	New Device (calibrators)	Predicate
Intended Use	Intended for the calibration of Roche Free T4 Immunoassay	Same
Calibrator concentrations (exact calibrator concentration is lot-specific)	Cal 1: Approximately 0.78 ng/dL Cal 2: Approximately 3.5 ng/dL	Same
Opened stability at 2 – 8° C	12 weeks	Same

Differences		
Item	New Device (calibrators)	Predicate
Name	Elecsys FT4 II CalSet	Elecsys FT4 CalSet

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods: Approved Guideline, Second Edition

CLSI EP 6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A statistical Approach: Approved Guideline

CLSI EP 17-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 II assay were evaluated on one cobas e 411 Immunoassay Analyzer referencing the CLSI EP5-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, two (2) runs per day for 21 days.

Results were as follows:

Sample	Mean (ng/dL)	Repeatability		Intermediate Precision		n
		SD (ng/dL)	CV (%)	SD (ng/dL)	CV (%)	
Control Level 1	1.22	0.011	0.9	0.022	1.8	84
Control Level 2	3.11	0.032	1.0	0.089	2.9	84
Human Serum 1	0.14	0.006	4.0	0.011	7.6	84
Human Serum 2	1.03	0.013	1.3	0.023	2.3	84
Human Serum 3	1.90	0.024	1.3	0.040	2.1	84
Human Serum 4	4.93	0.082	1.7	0.163	3.3	84
Human Serum 5	7.09	0.127	1.8	0.319	4.5	84

b. Linearity/assay reportable range:

Linearity of the Elecsys FT4 II assay was assessed on the cobas e 411 Immunoassay Analyzer referencing CLSI EP6-A.

Three high analyte serum sample pools (spiked with L-Thyroxine) were diluted with FT4 depleted human serum to create three linearity sets. For each linearity set, 13

concentrations (11 dilutions) throughout the measuring range were prepared. All samples were assayed in triplicate within a single run. All three linearity sets generated similar results and one representative set is presented below. Linearity was evaluated using first, second and third polynomial regression analysis based on CLSI EP6-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 from 0.095 to 8.20 ng/dL and a results summary is shown in the table below.

The linear regression line was y = 0.9752x + 0.0622

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

No. of sample	Expected in ng/dL	Linear regression in ng/dL	3rd Order in ng/dL	Absolute difference in ng/dL	Relative difference in %
1	0	0.052	0.044	-0.008	NA
2	0.095	0.144	0.143	-0.001	-0.6
3	0.142	0.190	0.192	0.002	1.2
4	0.213	0.259	0.266	0.007	2.6
5	0.320	0.363	0.375	0.013	3.5
6	0.480	0.518	0.537	0.020	3.8
7	0.720	0.751	0.777	0.026	3.5
8	1.08	1.10	1.13	0.029	2.6
9	1.62	1.62	1.64	0.018	1.1
10	2.43	2.41	2.39	-0.022	-0.9
11	3.64	3.59	3.48	-0.104	-2.9
12	5.47	5.35	5.19	-0.170	-3.2
13	8.20	8.01	8.22	0.210	2.6

Based on this linearity study and the limit of detection study (below), the claimed measuring range of this assay is 0.1 – 7.7 ng/dL.

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

Traceability:

This assay has been standardized against the Elecsys FT4 method cleared under k961489. The Elecsys FT4 assay is traceable to the Enzymun‑Test which was standardized using equilibrium dialysis.

Calibrator Stability:

When stored unopened at 2 - 8ºC the shelf life for the calibrators is 18 months. After opening, the calibrators are stable for 12 weeks when stored at 2 – 8° C. Onboard the cobas e 411, the calibrators are stable for up to 5 hours at 20° C. The stability protocols and acceptance criteria were reviewed and found to be adequate.

Value assignment for the calibrators:

The Elecsys FT4 II Calset is value assigned based on an internal value assignment procedure using the master calibrators as the reference value. The kit calibrator (CalSet) values are assigned by reading their respective concentrations from the master calibration curve. The assigned value for each level of the CalSet is the median over a minimum of 6 determinations. Kit calibrator (CalSet) values are assigned for each instrument platform. Examples of the target value for calibrator 1 and 2 are shown in the table below.

Level	Target Value
Calibrator 1	0.78 ng/dL
Calibrator 2	3.5 ng/dL

d. Detection limit:

The Limit of Blank, Limit of Detection and Limit of Quantitation were evaluated referencing CLSI EP17 – A2.

The Limit of Blank (LoB) is the 95th percentile value from 60 measurements of analyte‑free samples over several independent series. 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.

The Limit of Detection (LoD) is determined based on the Limit of Blank 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%) and was determined to be 0.05 ng/dL.

The Limit of Quantitation (functional sensitivity) is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 % and was determined to be 0.1 ng/dL. Values measured as less than 0.1 ng/dL will be reported as < 0.1 ng/dL.

The FT4 II assay has a measuring range of 0.1 to 7.77 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	41 mg/dL
hemoglobin	1.0 g/dL
Lipemia (intralipid)	2000 mg/dL
biotin	20 ng/mL
albumin	6.3 g/dL
IgG	7 g/dL
IgA	1.6 g/dL
IgM	1 g/dL
Rheumatoid Factor	1200 IU/mL

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	≤ 0.001
rT3	190000	≤ 0.003
3-iodo-L-tyrosine	10000000	≤ 0.000

Cross-reactant	Concentration tested (ng/dL)	Cross-reactivity %
3,5-diiodo-L-tyrosine	10000000	≤ 0.000
3,3′,5-triiodothyroacetic acid	100000	≤ 0.0002
3,3′,5,5′-tetraiodothyroacetic acid	100000	≤ 0.001

3. Common drugs

The effect of 29 common 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	150 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
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

Drug	Highest concentration at which no interference was observed
Propranolol	240 µg/mL
Propylthiouracil	300 µg/mL
Rifampicin	60 µg/mL
Theophylline	100 µg/mL
Thiamazole	80 µg/mL

In in vitro studies the drugs Furosemide and Levothyroxine caused elevated Free T4 findings at the daily therapeutic dosage level. In rare cases, interference due to extremely high titers of antibodies to analyte‑specific antibodies, streptavidin or ruthenium can occur. Cautions regarding Furosemide, Levothyroxine, analyte ‑ specific antibodies, streptavidin and ruthenium are stated in the labeling.

The sponsor also has the following limitation in their 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) or increased TBG in pregnancy."

"Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration."

"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."

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed with 170 human serum samples with concentrations ranging from 0.161 to 7.05 ng/dL. Eleven samples were spiked to produce high concentrations and four samples were diluted to produce low concentrations. The comparison of the Elecsys FT4 II assay (y) with the predicate device, Elecsys FT4 assay (x), produced the following correlation:

Linear regression $y = 1.02x - 0.075$ $r = 0.996$

b. Matrix comparison:

A matrix comparison study was performed using 72 samples collected into serum and K_2-EDTA, K_3-EDTA, and Li Heparin anticoagulant tubes. Serum concentrations ranged from 0.35 – 7.29 ng/dL. Testing was performed using the cobas e 411 Analyzer. The linear regressions results were as follows:

Serum vs. K_2-EDTA

slope	1.02
intercept	-0.02
r^2	0.999

Serum vs. K_3-EDTA

slope	1.00
intercept	0.00
r^2	0.998

Serum vs. Lithium Heparin

slope	1.01
intercept	-0.01
r^2	0.998

The sponsor claims that K_2-EDTA, K_3-EDTA, and Lithium heparin plasma are acceptable anti-coagulants for the FT4 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

The reference range was established according to the CLSI C28-A3 guideline *Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory;* based on the capability of transferring the predicate device's reference range to the candidate device's reference range because of the comparability of the two testing systems. Sponsor followed the transferability guidance and verification procedure of the CLSI and has confirmed that the established reference range from the predicate device is transferable to the candidate device using 60 apparently healthy subjects (30 males and 30 females) with a normal TSH value. The reference range was previously established in k961489 for the predicate device by conducting a reference range study using 801apparently healthy subjects.

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

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.