

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

A. 510(k) Number:

k121951

B. Purpose for Submission:

New device

C. Measurand:

Free Thyroxine (FT4)

D. Type of Test:

Quantitative chemiluminescent immunoassay

E. Applicant:

DiaSorin Inc.

F. Proprietary and Established Names:

LIAISON® FT4
LIAISON® Control Thyroid 1
LIAISON® Control Thyroid 2
LIAISON® Control Thyroid 3

G. Regulatory Information:

Product	Classification	Regulation	Panel
CEC	Class II	21 CFR 862.1695 Free Thyroxine Test System	Clinical Chemistry(75)
JJX	Class I, reserved	21 CFR 862.1660 Quality Control Material	Clinical Chemistry(75)

H. Intended Use:

1. Intended use(s):

The DiaSorin LIAISON® FT4 assay is an *in vitro* chemiluminescent immunoassay (CLIA) intended for the quantitative determination of free thyroxine (FT4) in human serum using the LIAISON® Analyzer. It is intended

for use as an aid in the clinical assessment of thyroid status.

The DiaSorin LIAISON® Control Thyroid 1, LIAISON® Control Thyroid 2 and LIAISON® Control Thyroid 3 are intended for use as assayed quality control samples to monitor the accuracy and precision of the DiaSorin LIAISON® FT4 assay.

2. Indication(s) for use:

See intended use in H.1. above

3. Special conditions for use statement(s):

For *in vitro* diagnostic use only
For prescription use only

4. Special instrument requirements:

For use on the LIAISON® Analyzer

I. Device Description:

1) FT4 Reagent Integral contains:

- a) Magnetic particles - coated with T4 protein polyclonal antibody (rabbit), BSA, phosphate buffer and 0.09% sodium azide; 2.3 mL
- b) Conjugate – Anti-T4 monoclonal antibody (mouse) labeled with isoluminal derivative, in Tris buffer BSA, surfactant and 0.09% sodium azide; 23.0 mL
- c) Calibrator 1, low – containing human serum spiked with T4 antigen and 0.09% sodium azide; 1.0 mL
- d) Calibrator 2, high – containing human serum spiked with T4 antigen and 0.09% sodium azide; 1.0 mL

2) Thyroid Control 1, Control 2 and Control 3

- a) Three separate sets each consisting of one concentration;
low, medium or high .
- b) 4 vials each level per set; lyophilized
- c) Contains FT4 hormone in human serum, stabilizers and preservatives

FT4 reagent and controls contain human source material and have been tested using FDA-approved methods and found negative for the presence of HBsAg, antibody to HIV-1/2 and HCV.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Roche Elecsys® FT4 Test System,
Roche Elecsys PreciControl Universal

2. Predicate k number(s):

k961489
k090541

3. Comparison with predicate:

Similarities and Differences		
Characteristic	New Device LIAISON® FT4	Predicate Device Roche Elecsys® FT4 (k961489)
Intended Use	The DiaSorin LIAISON® FT4 Assay is a chemiluminescent immunoassay (CLIA) intended for the quantitative determination of free thyroxine (FT4) in human serum.	Same
Assay method	Chemiluminescent Immunoassay	Electrochemiluminescent Immunoassay
Measurand	Free thyroxine	Same
Measuring range	0.29 – 7.7 ng/dL	0.023 – 7.7 ng/dL
Reference range	0.69-1.59 ng/dL	0.93-1.70 ng/dL
Sample Size	50 µL	15 µL
Calibrators	Included – 2 levels	Not included – 2 levels

Similarities and Differences		
Characteristic	New Device DiaSorin Control Thyroid 1, 2 and 3 Control set	Predicate Device controls (K090541)
Intended Use	Intended for used as assayed quality control samples to monitor the accuracy and precision of the FT4 assay.	Same
Form	Lyophilized	Same
Levels	3 levels	2 levels

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

CLSI EP05-A2 Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline Second edition

CLSI EP6-A Evaluation of the Linearity of Quantitative Analytical Methods; Approved Guideline

CLSI EP07-A2 Interference Testing in Clinical Chemistry; Approved Guideline-Second edition

CLSI EP09-A2-IR Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline Second edition

CLSI EP17-A Protocols for Demonstration, Verification, and Evaluation of Limits of Detection and Quantitation; Approved Guideline

CLSI C28-A3 How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline Second edition

L. Test Principle:

The LIAISON® FT4 assay's method for quantitative determination of FT4 is based on the Solid Phase Antigen Linked Technique (SPALT) principle. A T4-protein conjugate is coated on the magnetic particles (solid phase), and a monoclonal anti-T4 antibody is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the incubation, FT4 competes with the T4-protein-conjugate for the binding sites on the labeled antibody. After incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminolantibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is inversely proportional to the FT4 concentration present in calibrators, patient samples or controls. All assay steps and incubations are performed automatically by the LIAISON® Analyzer.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
 - a. *Precision/Reproducibility:*

The precision performance of the DiaSorin LIAISON FT4 assay was evaluated by a twenty day reproducibility/precision study performed at DiaSorin Inc. and 1 external site according to CLSI EP05-A2 using a panel of 4 frozen serum samples that contained 2 samples each at FT4 concentrations of medium (1.21 and 1.91 ng/dL) and high (5.67 and 5.84 ng/dL). All samples were tested on one lot of reagent, in two replicates per run, 2 runs per day for 20 days for a total of 80 replicate results per sample. The LIAISON Control Thyroid Level 1, 2 and 3 (Kit Control) were also tested in the study.

The 20 day results for a typical site are summarized below.

Sample ID	Mean (ng/dL)	Between Run precision		Total precision	
		SD	%CV	SD	%CV
KC 1	1.02	0.02	1.8%	0.08	7.9%
KC 2	3.18	0.07	2.3%	0.22	6.9%
KC 3	6.59	0.33	4.9%	0.46	7.0%
Sample 3	1.19	0.03	2.7%	0.09	8.0%
Sample 4	1.90	0.05	2.4%	0.14	7.5%
Sample 5	5.56	0.17	3.1%	0.40	7.1%
Sample 6	5.72	0.17	2.9%	0.40	7.1%

The sponsor performed an additional 5 day study at DiaSorin and 1 external site to support the precision performance of this assay at FT4 concentrations that approximate the medical decision points.. Samples were assayed once per day in replicates of 12 for a total of 60 replicate results per sample.

The 5 day results for a typical site for one lot are summarized below.

Sample ID	Mean (ng/dL)	Between Run precision		Total precision	
		SD	%CV	SD	%CV
1	0.851	0.08	9.7%	0.08	9.5%
2	1.57	0.14	9.0%	0.13	8.6%
3	3.25	0.24	7.4%	0.24	7.5%

b. *Linearity/assay reportable range:*

The sponsor provided a linearity study with one run using one lot of reagents with each sample tested in replicates of 4. Eleven diluted samples with FT4 concentrations evenly distributed were prepared by diluting a high FT4 sample. This yielded linearity samples with FT4 levels that spanned a range from 0.12 to 7.7 ng/dL. The sponsor calculated linear regressions from mean observed values versus expected values using an unweighted regression model and observed maximum % difference from observed values versus linear fit of 10%.

The fitted linear model regression is:

$$\text{Measured FT4} = 1.025 (\text{Expected FT4}) + 0.084, R = 0.998.$$

Based on this linearity study and the limits of detection study (below), the sponsor claimed that the measuring range of this device is 0.29 – 7.7 ng/dL.

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

Traceability:

Currently there is no reference method for the FT4 assay. The LIAISON FT4 assay kit calibrators are traceable to concentrations of internal primary reference standards of a synthetic T4 antigen in T4-stripped human serum prepared by symmetric dialysis.

Stability:

LIAISON FT4 assay kits are stable for two weeks, when stored either on board at 12-19°C or at refrigerated conditions of 2-8°C. LIAISON FT4 assay kits are stable for at least 12 month (shelf life) when stored at 2-8°C. The stability study protocol and acceptance criteria were reviewed and found to be adequate.

Value assignment of the calibrators and controls:

The kit calibrators (level 1 and 2) are manufactured by diluting a stock solution of T4 with a human serum based matrix. The kit calibrators are tested with a specific reagent lot against the master standards (10 points) to assess the FT4 concentration. The target ranges of the calibrators are:

Calibrator 1 is between 0.45 - 0.55 ng/dL

Calibrator 2 is between 5.0 - 7.0 ng/dL.

Controls for FT4:

The controls (level 1 and 2 and 3) are prepared from pooled human serum with added constituents of human serum, pure chemicals and therapeutic drugs. The controls are tested on 3 LIAISON analyzers, using two reagent lots for a minimum of 3 days to yield a minimum of 60 valid test results. FT4 mean values and standard deviations are calculated from the results. The target ranges are established using the mean values \pm 3SD. The target values

of the controls are:
Control 1 is targeted to 1 ng/dL
Control 2 is targeted to 3 ng/dL
Control 3 is targeted to 6 ng/dL.

d. Detection limit:

The sponsor provided a detection limits study to determine the LoB, LoD, and LoQ for the FT4 assay.

LoB Test Protocol

One blank sample was tested on 2 LIAISON[®] Analyzers with 2 reagent lots and 2 technicians over 4 runs (three replicates/sample/run) yielding 60 results.

LoD Test Protocol

Four samples in the range of the LoB to 4 times the LoB were tested on 2 LIAISON[®] Analyzers with 2 reagent lots and 2 technicians over 4 runs (three replicates/ sample/run) yielding a minimum of 192 results.

LoQ Test protocol

Eight samples with FT4 concentrations ranging from the LoB to 4XLoB were tested using two analyzers by two technicians using 3 reagent lots over several days generating 40 results per sample. LoQ was defined as inter-assay precision of < 20% CV. The sponsor claimed the following detection limits:

LoB = 0.06 ng/dL
LoD = 0.13 ng/dL
LoQ = 0.29 ng/dL.

The Liaison FT4 assay has a measuring range of 0.29 to 7.7 ng/dL.

e. Analytical specificity:

i. Interference from endogenous substances.

Interference testing was performed to determine whether the presence of endogenous substances such as hemoglobin, triglycerides, rheumatoid factor, conjugated and unconjugated bilirubin may interfere with assay results. Two human serum samples were used with FT4 concentrations of 1.0-1.3 ng/dL and 2.5 – 3.4 ng/dL. Each sample level was spiked with a single high concentration of interferent. The spiked results were used to calculate the % recovery (measured concentration compared to FT4 concentration with zero interferent). Non-significant interference is defined as within $\pm 10\%$ recovery for the tested analyte concentrations when compared against the non-spiked sample. The non-significant interference substances with the highest

concentration tested are summarized in the table below.

Substance	Highest Tested Concentration
Hemoglobin	1000 mg/dL
Triglycerides	3000 mg/dL
Bilirubin (conj)	20 mg/dL
Bilirubin (unconj)	20 mg/dL
Rheumatoid Factor	54 IU/mL
Albumin	6 g/dL

ii. Interference from cross-reactive substances.

The sponsor assessed the following substances for cross-reactivity according to their internal protocol: D-T4, 3-iodo-L-Tyrosine, 3,5-diiodo-L-Tyrosine, and 3,3',5,5'-tetra-iodothyroacetic acid by triplicate measurement of spiked samples in T4-free zero sample and evaluating displacement versus a T4-spiked sample. Results are summarized in the table below.

Cross reactant	Highest Tested Concentration	% cross-reactivity
D-T4	150 ng/dL	100%
3-iodo-L-tyrosine	1400 ng/dL	0%
3,5-diiodo-L-tyrosine	1500 ng/dL	0%
3,3',5,5'-tetra-iodothyroacetic acid	11 ng/dL	0%

iii. Interference from common drugs

Ten human serum samples divided into two aliquots were used with FT4 concentrations ranging from 0.878 to 1.75 ng/dL. One aliquot was not spiked with any drugs and was used as the reference sample. The remaining samples were spiked with the respective amount of drug. Then the concentration of FT4 was determined using two kit lots compared to the reference aliquot. FT4 recovery was within $\pm 10\%$ for the tested analyte concentrations. Non-significant interference is defined as within $\pm 10\%$ recovery for the tested analyte concentrations when compared against the reference sample. Non-significant interference was found at therapeutic concentration of any of the following drugs: salicylic acid, acetylsalicylic acid, phenylbutazone, diphenylhydantoin, amiodarone, 8-anilino-1-naphthalene sulfonic acid ammonium salt, furosemide, iopanic acid, and DL-propranolol.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

A method comparison study was performed with 212 human serum samples with concentrations ranging from 0.29 – 6.99 ng/dL tested by the DiaSorin FT4 assay (expressed as ng/dL) and the predicate device. Sixteen samples were engineered by spiking in human FT4 to achieve hard-to-find high FT4 concentrations. Ten samples were diluted to obtain samples with low FT4 concentrations. Eleven samples with FT4 concentrations outside the measuring range were excluded from data analysis. Samples were tested in duplicate on the candidate device and the first replicate result was analyzed by linear regression.

Data were analyzed by least squares linear regression with 95% CI indicated in parentheses:

Slope = 1.07 (1.03 to 1.09), intercept = - 0.12 (-0.17 to -0.08), r = 0.9633

b. *Matrix comparison:*

Not applicable

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:

To determine the reference range for this assay, a reference range study was performed according to CLSI Approved Guideline C28-A using 130 samples from apparently healthy adults. The reference range is determined to be 0.69-1.59 ng/dL based on the central 95% observed values. The sponsor recommends the

user to consider these limits as guidelines only. Each laboratory should establish its own reference range.

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.