

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

A. 510(k) Number:

k070708

B. Purpose for Submission:

New device

C. Measurand:

Free Thyroxine (FT4)
Total Thyroxine (T4)

D. Type of Test:

Quantitative, Chemiluminescent Immunoassay

E. Applicant:

Olympus Life & Material Science Europa GmbH (Irish branch)

F. Proprietary and Established Names:

Olympus FT4 – Free thyroxine (catalogue no. OSR210102)
Olympus T4 - Total thyroxine (catalogue no. OSR210104)

G. Regulatory Information:

1. Regulation section:

21CFR §862.1695-Free Thyroxine test system

21 CFR §862.1700-Total Thyroxine test system

2. Classification:

Class II

3. Product code:

CEC, CDX

4. Panel:

Chemistry (75)

H. Intended Use:

1. Intended use(s):

See indication for use below

2. Indication(s) for use:

The Olympus FT4 assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of free thyroxine levels in human serum and plasma using the Olympus AU3000i™ Immunoassay System. Measurements obtained by this device are used in the diagnosis and treatment of thyroid disease. For *in vitro* diagnostic use only.

The Olympus T4 assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of total thyroxine levels in human serum and plasma using the Olympus AU3000i™ Immunoassay System. Measurements obtained by this device are used in the diagnosis and treatment of thyroid disease. For *in vitro* diagnostic use only.

3. Special conditions for use statement(s):

Prescription use only

4. Special instrument requirements:

Olympus AU3000i™ Immunoassay System.

I. Device Description:

Olympus FT4 reagent kit contains the following:

1. Reagent 1: Alkaline phosphatase labeled T4 analogue, MES buffer, pH 6.5, with protein stabilizers and preservatives; in a 10 mL bottle
2. Reagent 2: Paramagnetic particles coated with murine monoclonal anti-T4 antibody, HEPES buffer, pH 7.4 with protein stabilizers and preservatives; in a 10 mL bottle
3. Calibrator: FT4 prepared in human matrix with preservatives; in a 1.5 mL bottle
4. Control: FT4 ~1.16 ng/dL prepared in a human matrix with preservatives; in a 2.5 mL bottle

Olympus T4 reagent kit contains the following:

1. Reagent 1: Alkaline phosphatase labeled T4, Tris buffer, pH 7.4, ANS (8-anilino-1-naphthalene sulphonic acid) with protein stabilizers and preservatives; in a 10 mL bottle

2. Reagent 2: Paramagnetic particles coated with rabbit polyclonal anti-mouse antibody and murine monoclonal anti-T4 antibody, Tris buffer, pH 7.4 with protein stabilizers and preservatives; in a 10 mL bottle
3. Calibrator: T4 prepared in human matrix with preservatives; in a 1.5 mL bottle
4. Control: T4 ~9.01 µg/dL prepared in a human matrix with preservatives; in a 2.5 mL bottle

All components derived from human blood have been tested by an FDA approved method and found to be non-reactive for HBsAg, anti-HCV, and anti-HIV-1/HIV-2.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Roche Elecsys® FT4 Assay
Siemens/Bayer Centaur T4 Assay

2. Predicate 510(k) number(s):

k961489, k905532

3. Comparison with predicate:

Similarities and differences for FT4 assay		
Item	Olympus FT4 assay (new device)	Roche Elecsys® FT4 assay (predicate device) k961489
Intended Use	Immunoassay for the quantitative determination of FT4 levels in human serum/plasma.	Same
Standardization/Traceability	Traceable to USP material with values assigned by comparison with a commercially available method	Traceable to Enzymun-Test FT4 method. This in turn was standardized using equilibrium dialysis.
Detection/Operating Principle	Chemiluminescence	Same
Assay Methodology	Competitive immunoassay	Same
Antibody	Monoclonal	Polyclonal
Sample Type	Serum and plasma	Same
Calibrator level(s)	One	Two
Control level(s)	One	Two
Assay range	0.10-7.77 ng/dL	0.023-7.77 ng/dL
Interference	≤ 5% for Bilirubin up to 40 mg/dL ≤ 3% for Hemolysis up to 5 g/dL ≤ 3% for lipemia up to 10 g/L ≤ 5% for Triglyceride up to 10 g/L Biotin: not tested	≤ 10 % for Bilirubin up to 41mg/dL ≤ 10 % for Hemolysis up to 2 g/dL ≤ 10 % for lipemia up to 2 g/L ≤ 10 % for Biotin up to 100 ng/mL Triglyceride: not tested

Similarities and differences for T4 assay		
Item	Olympus T4 assay (new device)	Siemens/Bayer Centaur T4 assay (predicate device) k905532
Intended Use	Immunoassay for the quantitative determination of T4 levels in human serum/plasma.	Same
Standardization/Traceability	Traceable to USP material with values assigned by comparison with the IDMS method	Traceable to USP material
Detection/Operating Principle	Chemiluminescence	Same
Assay Methodology	Competitive immunoassay	Same
Antibody	Monoclonal and Polyclonal	Polyclonal only
Sample Type	Serum and plasma	Serum only
Calibrator level(s)	One	Two
Control level(s)	One	Two
Assay range	0.30-23.3 µg/dL	0.30-30.0 µg/dL
Interference	≤ 5% for Bilirubin up to 20 mg/dL ≤ 5% for Hemolysis up to 5 g/dL ≤ 10% for lipemia up to 10 g/L	NS for Bilirubin up to 20 mg/dL NS for Hemolysis up to 0.5 g/dL NS for Triglyceride up to 1 g/L NS=Not significant

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

CLSI - EP05-A2 - *Evaluation of Precision Performance of Clinical Chemistry Devices*
 CLSI - EP07-A2 - *Interference Testing in Clinical Chemistry*
 CLSI - EP09-A2 - *Method Comparison and Bias Estimation Using Patient Samples*
 CLSI - C28-A2 - *How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline Second edition*

L. Test Principle:

The Olympus FT4 assay is a one-step paramagnetic particle enzyme immunoassay. It is based on the competitive principle and used to quantitate FT4 in serum/plasma. The Olympus FT4 assay reagent and sample are added to the assay cuvette in the following sequence:

1. Samples are incubated first with T4 analogue conjugated with alkaline phosphatase and a monoclonal anti-T4 antibody bound to magnetic particles. Unbound FT4 from the sample and the conjugated T4 analogue compete to form an antibody-antigen complex. Washing steps remove the unbound material.
2. The chemiluminescent substrate is added to the assay cuvette and reacts with the bound alkaline phosphatase (ALP). Light generated by the reaction is measured by the luminometer. The light emission is inversely proportional to the quantity of FT4 in the sample.

- Results are calculated from a pre-defined calibration curve. The Olympus AU3000i system automatically calculates the FT4 concentration of each sample in pmol/L or ng/dL.

The Olympus T4 assay is a one-step paramagnetic particle enzyme immunoassay. It is based on the competitive principle and used to quantitate FT4 in serum/plasma. The Olympus T4 assay reagent and sample are added to the assay cuvette in the following sequence:

- Samples are incubated with T4 conjugated with alkaline phosphatase and a monoclonal anti-T4 antibody bound to magnetic particles. T4 from the sample and the conjugated T4 compete to form an antibody-antigen complex. Washing steps remove the unbound material.
- The chemiluminescent substrate is added to the assay cuvette and reacts with the bound alkaline phosphatase (ALP). Light generated by the reaction is measured by the luminometer. The light emission is inversely proportional to the quantity of T4 in the sample.
- Results are calculated from a pre-defined calibration curve. The Olympus AU3000i system automatically calculates the T4 concentration of each sample in nmol/L or µg/dL.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

i.) For the FT4 test system: A precision study was conducted using pooled human sera according to CLSI protocol EP5-A. Samples were run twice a day in duplicate for 20 days (n=80) on the Olympus AU 3000i analyzer. The precision results are shown below:

Sample	Within run			Total	
	Mean [ng/dL]	SD [ng/dL]	CV [%]	SD [ng/dL]	CV [%]
1	0.564	0.008	1.4	0.014	2.5
2	0.929	0.005	0.6	0.013	1.4
3	2.91	0.036	1.2	0.081	2.8

ii.) For the T4 test system: A precision study was conducted using pooled human sera according to CLSI protocol EP5-A. Samples were run twice a day in duplicate for 20 days (n=80) on the Olympus AU 3000i analyzer. The precision results are shown below:

Sample		Within run		Total	
	Mean [µg/dL]	SD [µg/dL]	CV [%]	SD [µg/dL]	CV [%]
1	2.304	0.03	1.1	0.096	4.2
2	8.059	0.11	1.3	0.304	3.8
3	13.125	0.21	1.6	0.5544	4.1

b. Linearity/assay reportable range:

A recovery study was conducted to assess the measuring range of the FT4 assay. Twelve pooled samples prepared from a commercial human serum matrix that has been spiked with USP material were used. The samples tested ranged from 0.00 to 15.50 ng/dL. The recovery of each sample was measured using the Olympus FT4 assay on the Olympus AU3000i analyzer and ranged from 99.3% to 101.4%. The sponsor's acceptance criterion was $\leq 10\%$ deviations from the mean recovery.

The data provided in the linearity study, the detection limit study below and the method comparison study below support the sponsor's claim that this test has a reportable range of 0.10 - 7.77 ng/dL.

A recovery study was also conducted to assess the measuring range of the T4 assay. Five patient samples ranging in concentration from 19.31 to 24.91 µg/dL were serially diluted with a zero, stripped serum. The recovery of each sample was measured using the Olympus T4 assay on the Olympus AU3000i analyzer and ranged from 95.4% to 108.5 %. The sponsor's acceptance criterion was $\leq 10\%$ deviations from the mean recovery.

The data provided in the linearity study, detection limit study below and method comparison study below support the sponsor's claim that this test has a reportable range of 0.30 - 23.3 µg/dL.

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

The Olympus FT4 calibrator is a liquid human serum matrix and is traceable to USP material with values assigned by comparison with a commercially available device.

The Olympus T4 calibrator is a liquid human serum matrix and is traceable to USP material with values assigned by IDMS (Isotope dilution mass spectrometry).

The FT4 and T4 calibration materials are prepared by creating a master calibrator from the reference material in which the value assignment is verified using an in house protocol. The shelf-life of the calibrator materials is at least 12 months based upon accelerated studies with ongoing real time stability studies. Once open, the calibrator is stable at 2-8 °C for 28 days.

The FT4 and T4 control materials are liquid human based matrix and the target concentration range is set at the euthyroid range. Value assignments are determined

on multiple instruments. The shelf-life of the control materials is at least 12 months with ongoing real time stability studies. Once open, the calibrator is stable at 2-8 °C for 28 days.

d. Detection limit:

A detection limit study was performed to assess the limit of blank (LoB) and the limit of detection (LoD) for the Olympus FT4 assay. LoB is defined as the lowest detectable level of FT4 that can be distinguished from zero. LoD was conducted by assaying 20 replicates of an analyte free sample and 5 replicates of a low non-zero sample in a single run for the FT4 assay. The calculated LoB is 0.015 ng/dL and the calculated LoD for FT4 assay is 0.023 ng/dL. The sponsor chose 0.1 ng/dL as the limit of detection.

A detection limit study was performed to assess the limit of blank and the limit of detection for the Olympus T4 assay. LoB is defined as the lowest detectable level of T4 that can be distinguished from zero. LoD was conducted by running 20 replicates of an analyte free sample and 5 replicates of a low non-zero sample in a single run for the T4 assay. The calculated LoB is 0.052 µg/dL and the calculated LoD is 0.069 µg/dL. The sponsor chose 0.3 µg/dL as the limit of detection.

e. Analytical specificity:

i.) FT4 assay:

The cross-reactivity study was carried out by adding known amounts of potential cross reactants to a low and a high FT4 serum and measuring the FT4 concentrations on the Olympus AU 3000i analyzer. The analytical specificity study was measured by comparing the FT4 recovery in the samples with cross reactant added versus no cross reactant added. The analytical specificity results are shown below:

Cross-reactant	Concentration tested (µg/L)	% cross-reactivity
Diiodothyronine	5000	<0.001
Tetraiodothyroacetic Acid	1000	0.004
Triiodothyroacetic Acid	1000	0.003
Triiodothyropropionic Acid	10000	<0.001
Diiodothyrosine	10000	<0.001
L-Triiodothyronine	1000	0.002
Monoiodotyrosine	10000	<0.001
Reverse T3	1000	<0.001

Interfering substances studies were evaluated based on CLSI EP7-A guideline. Results of studies conducted to evaluate the susceptibility of the FT4 assay to various potential interferents were shown as follows:

Icterus: Interference ≤ 5% for up to 40 mg/dL bilirubin.

Hemolysis: Interference ≤ 3% for up to 5 g/L hemolysate

Lipemia: Interference ≤ 3% for up to 10 g/L Intralipid®

Triglyceride: Interference $\leq 5\%$ for up to 10g/L Triglyceride

ii.) T4 assay:

The cross-reactivity study was carried out by adding known amounts of potential cross reactants to a low and a high FT4 serum and measuring the FT4 concentrations on the Olympus AU 3000i analyzer. The analytical specificity study was measured by comparing the FT4 recovery in the samples with cross reactant added versus no cross reactant added. The analytical specificity results are shown below:

Cross-reactant	Concentration tested (µg/L)	% cross-reactivity
Diiodothyronine	5000	<0.2
Tetraiodothyroacetic Acid	25	68
Triiodothyroacetic Acid	250	6
Triiodothyropropionic Acid	50	6
Diiodothyrosine	5000	<0.2
L-Thyroxine	40	108
D-Thyroxine	50	72
L-Triiodothyronine	1000	6
Monoiodotyrosine	5000	<0.2
Reverse T3	50	6

Interfering substances studies were evaluated based on CLSI EP7-A guideline. Results of studies conducted to evaluate the susceptibility of the T4 assay to various potential interferents were shown as follows:

Icterus: Interference $\leq 5\%$ for up to 20 mg/dL bilirubin.

Hemolysis: Interference $\leq 5\%$ for up to 5 g/L hemolysate

Lipemia: Interference $\leq 10\%$ for up to 10 g/L Intralipid®

As limitations, the applicant stated the followings in the package insert:

As with all tests containing monoclonal antibodies, some samples from patients who have been treated with monoclonal antibodies or have received them for diagnostic purposes could give erroneous findings. Human anti-mouse antibodies (HAMA), heterophilic antibodies, and rheumatoid factors in human serum can react with the immunoglobulins included in the assay components causing interference and an anomalous result. Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference. These reagents have been formulated to minimize the risk of interference; however, potential interactions between rare sera and test components can occur.

f. Assay cut-off:

Not Applicable

2. Comparison studies:

a. Method comparison with predicate device:

108 patient serum samples were used to compare the Olympus FT4 assay on the Olympus AU3000i analyzer against Roche Elecsys FT4 assay based on the CLSI protocol EP9-A. Results of regression by Passing-Bablok analysis were as follows:

N	Range of sample concentrations [ng/dL]	Intercept [ng/L]	Slope	Correlation Coefficient
108	0.14-5.30	0.093	0.932	0.984

122 patient serum samples were used to compare the Olympus T4 assay on the Olympus AU3000i analyzer against Bayer Centaur T4 assay based on the CLSI protocol EP9-A. Results of regression by Passing-Bablok analysis were as follows:

N	Range of sample concentrations [µg/dL]	Intercept [µg/dL]	Slope	Correlation Coefficient
122	0.46-22.79	3.44	1.023	0.9291

b. Matrix comparison:

A matrix comparison study was conducted using 87 paired sera and Lithium heparinized plasma samples. Testing was performed using the Olympus FT4 assay on the Olympus AU 3000i analyzer. Serum samples range tested was 0.22-5.11 ng/dL. The Deming regression correlation is as follows: $Y = 0.972X + 0.176$, $r=0.9995$.

(Y = plasma values, X = serum values)

A matrix comparison study was also conducted using 87 paired sera and Lithium heparinized plasma samples. Testing was performed using the Olympus T4 assay on the Olympus AU 3000i analyzer. Serum samples range tested was 0.33-17.26 µg/dL. The Deming regression correlation is as follows: $Y = 1.015X - 0.394$, $r=0.9983$.

(Y = plasma values, X = serum values)

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:

A reference range study was performed based on the CLSI C28-A2 guideline using 355 apparently euthyroid subjects (equal proportions of male and female). Serum samples were run using the Olympus FT4 assay and the Olympus T4 assay on the Olympus AU3000i analyzer. The reference ranges were calculated based on all the values. The sponsor claimed that the reference interval for the FT4 assay is 0.85- 1.59 ng/dL and T4 assay is 3.97- 9.95 µg/dL.

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.