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

A. 510(k) Number:

k083373

B. Purpose for Submission:

New device

C. Measurand:

Free Thyroxine (FT4)

D. Type of Test:

Quantitative, chemiluminescent immunoassay

E. Applicant:

Siemens Healthcare Diagnostics

F. Proprietary and Established Names:

IMMULlTE 2000 Free T4

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CEC	Class II	21 CFR§ 862.1695	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:
   The IMMULITE 2000 Free Thyroxine Assay is for *in vitro* diagnostic use with the IMMULITE 2000 Systems Analyzers - for the quantitative measurement of nonprotein-bound thyroxine (free T4) in serum and heparinized plasma, as an aid in the clinical assessment of thyroid status.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   IMMULITE 2000 Systems Analyzers

I. Device Description:

The IMMULITE 2000 Free T4 is a solid-phase, enzyme-labeled chemiluminescent competitive immunoassay. The assay kit includes bead packs, reagent wedges, and adjustors (calibrators). The solid phase (bead) is coated with monoclonal murine anti-T4 antibody. The liquid phase consists of alkaline phosphatase (bovine calf intestine) conjugated to T4.

Source materials derived from human blood were tested and found nonreactive for syphilis; for antibodies to HIV 1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Siemens Healthcare Diagnostics ADVIA Centaur Free T4 (FrT4) assay

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

   k961510 (ACS Free T4 immunoassay)
   k971418 (ADVIA analyzer – ACS Centaur System)

3. Comparison with predicate:

Characteristics	IMMULITE 2000 Free T4 immunoassay (New Device)	ADVIA Centaur FrT4 Immunoassay (Predicate)
Similarities		
Assay Type	Chemiluminescent immunoassay	Chemiluminescent immunoassay
Analyte	Free T4	Free T4
Calibration	2 point	2 point
Expected Values	Euthyroid: 0.89 – 1.76 ng/dL Hypothyroid: < 0.89 ng/dL Hyperthyroid: > 1.76 ng/dL	Euthyroid: 0.89 – 1.76 ng/dL Hypothyroid: < 0.89 ng/dL Hyperthyroid: > 1.76 ng/dL

Differences		
Sample Type(s)	Serum and plasma	Serum
Solid Phase Reagent	Monoclonal anti-T4 coated bead	Polyclonal anti-T4 bound to paramagnetic particles
Liquid Phase Reagent	Alkaline phosphatase labeled T4	Acridium ester labeled T4
Incubation Time	30 minutes	7.5 minutes
Sample Volume	10 µL	25 µL
Reportable Range	0.3 – 6.0 ng/dL	0.1 – 12.0 ng/dL
Analytical Sensitivity	LoB = 0.05 ng/dL LoD =0.13 ng/dL Functional Sensitivity = 0.25 ng/dL	Minimum Detectable Concentration of 0.1 ng/dL
Precision (Total)	10.2%@ 0.51 ng/dL 6.4% @1.13 ng/dL 3.6% @2.91 ng/dL	6.56%@ 0.47 ng/dL 3.03% @1.08 ng/dL 2.73% @3.09 ng/dL
Endogenous Interference	No significant interference from conjugated/unconjugated bilirubin (up to 20 mg/dL), hemoglobin (up to 634 mg/dL) or triglycerides (up to 1000 mg/dL).	No significant interference from conjugated/unconjugated bilirubin (up to 20 mg/dL), hemoglobin (up to 300 mg/dL) or triglycerides (up to 1000 mg/dL).
Accuracy / Correlation (Serum)	Y =1.06x - 0.001 r =0.981 (vs. ADVIA Centaur FrT4)	Y = 0.99x + 0.02 r = 0.99 (vs. ACS:180 FrT4)

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

- CLSI EP17-A – Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline
- CLSI EP05-2A – Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline- 2nd Edition

L. Test Principle:

The patient sample and the reagent are incubated together with the coated bead for 30 minutes. During this time, free T4 in the sample competes with enzyme conjugated T4 in the buffer for a limited number of antibody binding sites on the bead. Unbound patient sample and enzyme conjugate are then removed by centrifugal washes. Finally, the chemiluminescent substrate is added to the reaction tube containing the bead and the signal is generated in proportion to the bound enzyme.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was evaluated in accordance with CLSI document EP5-A2. 6 serum pools and one control sample were processed in duplicate over the course of 20 days, two runs per day, for a total of 40 runs and 80 replicates using the IMMULITE 2000 analyzer. The precision data are summarized as follows:

			Within-Run		Total	
Material/ Sample #	# Rep	Mean (ng/dL)	SD (ng/dL)	CV (%)	SD (ng/dL)	CV (%)
Pool 04	80	0.39	0.037	9.5	0.052	13.3
Pool 05	80	0.51	0.040	7.8	0.052	10.2
Pool 06	80	0.85	0.038	4.5	0.060	7.1
Control	80	1.13	0.067	5.9	0.072	6.4
Pool 07	80	1.49	0.072	4.8	0.090	6.0
Pool 08	80	2.91	0.103	3.5	0.104	3.6
Pool 09	80	4.82	0.144	3.0	0.172	3.6

b. Linearity/assay reportable range:

Linearity was evaluated by assaying free T4 calibrators A through F (ranging from 0 to 6.4 ng/dL) as unknowns. Calibrators are mixed to create intermediate values for a total of 10 different concentrations. Each sample was measured in replicates of 5 using the IMMULITE 2000 analyzer. The percent recovery across all samples was calculated and the % recovery ranged from 91% to 119%.

In addition, the expected values (X) were plot against the observed values (Y) and a line fit was plotted. The linear regression is calculated as $Y= 0.9725X + 0.0617$. $R^2= 0.995$.

The data provided support the sponsor's claim for a reportable range of 0.3 ng/dL – 6 ng/dL.

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

The IMMULITE 2000 Free T4 assay is traceable to an internal material.

Stability:

Stability testing included real-time testing of the assay kit in addition to stress testing designed to simulate storage/stress conditions. Stress testing studies support real-time stability claims and the conditions tested were 3 day storage

at 37 °C, 7 day storage at room temperature (15-30 °C), and 3 freeze/thaw cycles (freeze -30 °C to -5 °C, thaw at 2-8 °C).

The IMMULITE 2000 Free T4 assay kit is stable for 330 days (unopened) when stored at 2-8 °C. On-board the IMMULITE 2000 analyzer, the reagent is stable after opening for 92 days. The calibrators are stable at 2-8 °C for 30 days or at -20°C for 6 months (aliquoted).

d. Detection limit:

The limit of the blank (LoB) and limit of detection (LoD) were determined in accordance with the guidelines of CLSI document EP17-A using the IMMULITE 2000 analyzer. A zero calibrator was assayed 60 times on 3 instruments 60 using 3 lots to determine the LoB. Three low serum pools and zero calibrator were assayed twice a day for 20 days on 2 instruments, using 3 lots to determine the LoD. Functional sensitivity of the assay was determined by plotting the results of the precision studies (coefficient of variance (CV) vs. mean Free T4 concentration) and reporting the Free T4 concentration with a CV of 20%. The results were as follows:

LoB = 0.05 ng/dL (non parametric)
LoD = 0.13 ng/dL (parametric)
Functional Sensitivity = 0.25 ng/dL

The assay has a reportable range is 0.3 ng/dL – 6 ng/dL.

e. Analytical specificity:

i. Interference from endogenous substances:

Potential interfering substances (unconjugated and conjugated bilirubin, triglycerides, hemoglobin) were spiked into aliquots of patient samples. Unspiked aliquots served as the control. Samples were selected so that free T4 levels spanned the range of the assay, and interferents were tested at a range of concentrations using the IMMULITE 2000 analyzer. The sponsor states that no significant interference occurs if the percent recovery is between 85 – 115% across samples. No significant interference was observed for the following levels of interferents:

Triglycerides: up to 1000 mg/dL
Conjugated and unconjugated bilirubin: up to 20 mg/dL,
Hemoglobin: up to 634 mg/dL.

ii. Cross-reactivity:

Concentrations of potentially cross-reacting substances were spiked into a zero analyte sample. Percent cross-reactivity was calculated using the

following equation:

(zero analyte sample dose recovery) / (amount of compound added) x 100 = % cross-reactivity

The results were as follows:

IMMULITE 2000 Cross-Reactivity			
Material	Amount Added (ng/dL)	Apparent Free T4 (ng/dL)	% Cross-reactivity
L-T3	100000	1.15	Not Detectable
3,5-Diiodo-L-thyronine	10000	Not Detectable	Not Detectable
3-Monoiodo-L-tyrosine	100000	Not Detectable	Not Detectable
3,5-Diiodo-L-tyrosine	100000	Not Detectable	Not Detectable
D-T4	1000	Not Detectable	Not Detectable
5,5-Diphenylhydantoin (phenytoin)	1000000	Not Detectable	Not Detectable
5,5-Diphenylhydantoin (phenytoin)	4000000	0.70	Not Detectable
3,3',5,5'-Tetraiodothyroacetic Acid	2000	Not Detectable	Not Detectable
Salicylic Acid	50	0.63	1.26%
Albumin	4.0×10^9	Not Detectable	Not Detectable

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed using 282 human samples (13 of which were spiked) ranging from 0.3 ng/dL to 5.2 ng/dL on the IMMULITE 2000 analyzer using Free T4 assay (new device) and ADVIA Centaur FT4 assay (predicate). The linear regression correlation is summarized as follows:

IMMULITE 2000 FREE T4 *vs.* ADVIA Centaur FT4				
Regression Method	Sample Size	Coefficient Correlation	Slope (95% CI)	Intercept (95% CI)
Linear regression	282	0.981	1.06 (1.03, 1.08)	-0.001 (-0.04, 0.04)

b. *Matrix comparison:*
A matrix comparison study was performed using 49 paired serum (plain red-top and serum separator tubes) and heparinized plasma samples. 9 samples were spiked to cover the hard-to-find ranges. Testing was performed using the IMMULITE 2000 analyzer. The linear regression correlations are summarized as follows:

Red-Top Serum (X) vs. Heparinized Plasma (Y), Range: (0.81 ng/dL – 5.02 ng/dL)

Regression Method	N	Coefficient of Correlation	Slope (95% CI)	Intercept (95% CI)	%Bias (95% CI)
Linear	49	0.99	1.05 (1.00, 1.09)	-0.02 (-0.10 , 0.07)	2.9% (1.1%,4.6%)

Red-Top Serum (X) vs. SST (Y), Range: (0.80 ng/dL – 4.96 ng/dL)

Regression Method	N	Coefficient of Correlation	Slope (95% CI)	Intercept (95% CI)	%Bias (95% CI)
Linear	49	1.00	1.04 (1.01, 1.06)	-0.04 (-0.09, 0.001)	0.3% (-1.3%, 1.8%)

Samples assayed for the red-top serum ranged from 0.77 ng/dL to 4.97 ng/dL.

In the labeling, the sponsor recommends serum and heparinized plasma as the samples to use.

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:

Expected values generated by the predicate device (ADVIA FT4 assay) using 283 samples were verified by using 158 apparently healthy subjects with the IMMULITE 2000 FT4 assay (candidate device). Using the 158 apparently healthy subjects analyzed using the non parametric percentile (2.5 and 97.5) the range obtained was 0.88 to 1.87. Based on the closeness of the method comparison between the predicate and candidate devices, the sponsor claimed that the expected values are transferable to the candidate device. Method comparison regression equation: y=1.06X – 0.0001, coefficient correlation = 0.981, (see section M.2.a above).

Expected results presented in the labeling are as follows:

Clinical Condition	FT4 Range (ng/dL)	FT4 Range (pmol/L)
Euthyroid	0.89 -1.76	11.5 -22.7
Hypothyroid	less than 0.89	less than 11.5
Hyperthyroid	greater than 1.76	greater than 22.7

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.