

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

A. 510(k) Number:

k080167

B. Purpose for Submission:

New Device

C. Measurand:

Free thyroxine (FT4)

D. Type of Test:

Quantitative, Chemiluminescent Immunoassay

E. Applicant:

Siemens Healthcare Diagnostics Inc.

F. Proprietary and Established Names:

ADVIA Centaur FT4 Immunoassay

G. Regulatory Information:

1. Regulation section:
21CFR §862.1695-Free thyroxine test system
2. Classification:
Class II
3. Product code:
CEC
4. Panel:
Chemistry (75)

H. Intended Use:

1. Intended use(s):
The ADVIA Centaur FT4 Immunoassay is for *in vitro* diagnostic use in the quantitative determination of free thyroxine (FT4) in serum or plasma (heparinized or EDTA) using the ADVIA Centaur and ADVIA Centaur XP Systems. Measurements of free thyroxine are used in the diagnosis and treatment of thyroid diseases.
2. Indication(s) for use:
See intended use above.

3. Special conditions for use statement(s):

For prescription use only

The sponsor's stated limitations in the labeling are as follow:

- a. The anticonvulsant drug phenytoin may interfere with total and free T4 levels due to competition for TBG binding sites.
- b. FT4 values may be decreased in patients with non-thyroidal conditions and in patients taking carbamazepine.
- c. Performance of this assay has not been established with neonatal specimens.
- d. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
- e. Thyroid auto antibodies in human serum may interfere and cause falsely elevated FT4 results.

4. Special instrument requirements:

ADVIA Centaur and ADVIA Centaur XP Systems

I. Device Description:

The ADVIA Centaur Free T4 (FT4) Immunoassays are adaptations of the ACS:180, ADVIA Centaur Free T4 (FrT4) Immunoassays. The ADVIA Centaur Free T4 (FT4) Immunoassay consists of two reagents. The Lite Reagent is an acridinium ester labeled T4 derivative. It is diluted to a concentration of about 0.2 ug/mL in sodium barbitol buffer (1.03%) with protein stabilizers, EDTA and sodium azide (<0.1%). The solid phase is a biotinylated polyclonal rabbit anti-T4 antibody (0.525 ug/mL) that is coupled to avidin covalently linked to paramagnetic particles. The particles are diluted in sodium barbitol buffer (1.03%) with protein stabilizers, EDTA and sodium azide (<0.1%).

J. Substantial Equivalence Information:

1. Predicate device name(s):

ACS:180 FrT4 Immunoassay

2. Predicate K number(s):

k961510

3. Comparison with predicate:

Similarities		
Item	New Device ADVIA Centaur FT4 Assay	Predicate ACS:180 FrT4 Assay
Intended Use	For <i>in vitro</i> diagnostic use in the quantitative determination of free thyroxine (FT4) in serum or plasma	For <i>in vitro</i> diagnostic use in the quantitative determination of free thyroxine (FT4) in serum
Assay Method	Competitive immunoassay	Competitive immunoassay
Detection	Chemiluminescence	Chemiluminescence
Assay Range	0.1 – 12.0 ng/dL	0.1 – 12.0 ng/dL
Calibrators	Calibrator A	Calibrator A
Calibration	2 point	2 point
Reagents	Two liquid reagents, ready to use	Two liquid reagents, ready to use
Lite Reagent	Acridium ester labeled T4	Acridium ester labeled T4
Expected Values (ng/dL)	Euthyroid 0.89 – 1.76 Hypothyroid < 0.89 Hyperthyroid > 1.76	Euthyroid 0.89 – 1.76 Hypothyroid < 0.89 Hyperthyroid > 1.76
Standardization	Internal Standard (USP)	Internal Standard (USP)

Differences		
Item	New Device ADVIA Centaur FT4 Assay	Predicate ACS:180 FrT4 Assay
Specimen Type	Human serum or plasma (heparinized or EDTA)	Human serum
Solid Phase Reagent	Biotin-labeled polyclonal anti-T4 bound to avidin paramagnetic particles	Polyclonal anti-T4 bound to paramagnetic particles

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 FT4 immunoassays are competitive immunoassays using direct chemiluminescent technology. FT4 in the patient sample competes with acridinium ester labeled T4 in the Lite Reagent for a limited amount of biotinylated polyclonal rabbit-anti T4 that is coupled to avidin covalently linked to paramagnetic particles. There is an inverse relationship between the concentration of free thyroxine in the sample and relative light units.

The ADVIA Centaur FT4 assay utilizes a factory set master curve generated by the FT4 master curve standards. The master curve values are contained on the master curve card provided with each kit. The barcode reader or keyboard is used to enter the master curve values on the system. The 2 calibrators in the kit are assayed when the lot is first used or after expiration of the calibrator interval. If the calibration run is valid as determined by prearranged parameters, the values are stored and used to “normalize” test values to the master curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

An internal precision protocol, using serum and control samples, based on a modification of CLSI EP5-2A, was used: A precision study was done with 27 runs over 16 days with three reagent lots on four ADVIA Centaur systems, 1-4 runs per day, n=3 per run for a total of n = 81 for each sample. Coefficient of Variation was calculated as shown below:

Specimen	Level (ng/dL)	Within-run		Total	
		SD	CV (%)	SD	CV (%)
Serum 1	0.37	0.0300	8.11	0.0414	11.2
Control 1	0.71	0.0233	3.28	0.0344	4.85
Control 2	1.31	0.0449	3.43	0.0521	3.98
Control 3	3.35	0.0891	2.66	0.1363	4.07
Serum 2	5.32	0.1569	2.95	0.2139	4.02
Serum 3	9.68	0.4327	4.47	0.6321	6.53

An additional study using low level patient samples pool was assayed as part of the study. This sample was tested over 4 days, 20 replicates per day on 1 system. The precision of this low sample was comparable to the low samples in the other studies. The results are presented in the table below;

Specimen	Level (ng/dL)	Within-run		Total	
		SD	CV (%)	SD	CV (%)
Serum Pool	0.206	0.0214	10.4	0.0214	10.4

b. *Linearity/assay reportable range:*

Twelve samples prepared in a commercial human serum matrix containing USP material at various concentrations were tested. The FT4 concentrations of the samples tested ranged from 0 to 12.50 ng/dL (Expected Values). The recovery of each sample was measured using 5 replicates at each level.

Expected ng/dL	Mean % Recovery
0	none
0.0825	107
0.165	97

0.33	102
0.872	107
1.92	101
3.4	106
5.81	99.6
7.48	94.7
9.16	93
10.8	99.3
12.5	99.8

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

The ADVIA Centaur FT4 assay is traceable to an internal standard manufactured using U.S.P. (United States Pharmacopeia) material. Assigned values of calibrators and ranges of Ligand Plus controls are traceable to this standardization.

On-board stability experiments showed that the reagent is stable for 28 days at 2-8°C, and the sponsor requires that it is calibrated every 7 days.

The calibrator and control materials were cleared in previous submissions as follows:

Calibrator A (k970539), Ligand Plus Control (k030452).

d. *Detection limit:*

Sponsor determined the Limit of Blank (LoB) and the Limit of Detection (LoD) for the ADVIA Centaur FT4 by assaying a zero sample and a low sample (pool of patient samples) in the following manner:

Eighty replicates of each sample were tested in 4 runs over 4 days, with 20 replicates per run. The results of the study are shown below.

Product	Number of observations	Mean (ng/dL)	Total SD (ng/dL)	LoB (ng/dL)	LoD (ng/dL)
Blank	80	0.0323	0.0258	0.08	0.12
Low sample #1	80	0.206	0.0214		
Low sample #2	80	0.405	0.0268		

LoB = Mean of Blank + (1.645 x SD of Blank)
LoB = 0.0323 + (1.645 x 0.0258) = 0.075 ng/dL

LoD = Mean of Blank + (1.645 x SD of Blank) + (1.645 x pooled SD of Low samples)
LoD = 0.0323 + (1.645 x 0.0258) + (1.645 x 0.0242) = 0.12 ng/dL

e. *Analytical specificity:*

i.) Cross-reactivity studies:

The cross-reactivity of the ADVIA Centaur FT4 assay with a substance has been expressed as the ratio of:

- The amount of T4 required to displace 50% of the maximally bound labeled T4 from the anti-T4 antibody, and
- The amount of the cross-reactant to give the same 50% displacement -

Cross Reactant	Levels Tested (mg/mL)	% Cross Reactivity
L-Triiodothyronine	0.0001, 0.0005, 0.001, 0.005, 0.010	<0.02%
Diiodotyrosine	0.001, 0.005, 0.010, 0.050, 0.100, 1.00	<0.02%
Monoiodotyrosine	0.001, 0.005, 0.010, 0.050, 0.100, 1.00	<0.02%
3,5-Diiodo-L-thyronine	0.0001, 0.001, 0.005, 0.010, 0.050, 0.100	<0.02%
Reverse Triiodothyronine (rT3)	0.0001, 0.0005, 0.001, 0.005, 0.015	<0.02%

ii.) Endogenous substances:

The effect of albumin (up to 4.9 g/dL), hemoglobin (up to 300 mg/dL), lipemic (up to 1000 mg/dL of triglyceride) and icteric (up to 20 mg/dL of free and conjugated bilirubin) was evaluated by testing patient samples with two levels of FT4 (0.91 ng/dL and 1.40 ng/dL) and comparing the results to the Predicate Device on the ACS:180 and ADVIA Centaur. All the studies met the sponsor's internal acceptance criteria and no significant interference was seen with the above tested compounds at the levels tested. Less than 10% interferences are considered to be not significant by the sponsor.

iii.) Drug interferences:

Known amounts of propylthiouracil (4.0 mg/dL), methimazole (0.40 mg/dL), phenylbutazone (15.0 mg/dL), phenytoin (4.00 mg/dL), sodium salicylate (50.0 mg/dL) and aspirin (50.0 mg/dL) were added to serum samples containing 0.91 to 6.10 ng/dL of FT4 and tested in the ADVIA Centaur FT4 assay. The percent change to the dose by the addition of the substance was determined. Phenylbutazone was found to result in an average % change of 6.38%, while all others resulted in changes below 0.2 and 4%. All results passed the sponsor's internal acceptance criteria of ≤10% (average % change) as an acceptable level of interference.

f. *Assay cut-off:*

Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

Correlation studies were performed with the ADVIA Centaur FT4 assay using the predicate ACS:180 FrT4 immunoassay as the comparative method. The correlation studies were done over 10 days using 237 patient serum samples.

The sample distribution is described below.

Sample Type (natural samples)	Number Tested Sample Range (~ 0.14 – 11.14 ng/dL)
Normal	132 (~ 0.70 – 2.12 ng/dL)
Hypothyroid*	80 (0.14 – 2.04 ng/dL) (5 samples below 0.20 ng/dL)
Hyperthyroid*	71 (~ 0.99 – 11.14 ng/dL)
Total	283

*Internally classified based on vendor's TSH Values - not clinically confirmed

Samples ranged from FT4 concentrations of 0.14 ng/dL to 11.14 ng/dL. Data were plotted as single values, and the regression analysis yielded the following:

$$y = 0.973x + 0.016 \text{ with an } r^2 \text{ value of } 0.995.$$

b. *Matrix comparison:*

The serum/plasma equivalence study was carried out using lithium heparin and K₂EDTA plasma samples and their corresponding serum samples. Samples were collected from self-described healthy volunteers. 91 matched heparinized plasma samples were compared with serum with T4 values ranging from 0.17-11.4 ng/dL. Samples include natural samples, and spiked in order to cover the hard to find sample range. Mean % bias was found to be 4.33 %. Linear regression of the data yielded the following values:
 $y = 0.979x + 0.058$, $n=91$, $r=0.997$.

In another study, 73 matched K₂EDTA plasma samples were compared with serum with T4 values ranging from 0.17-11.4 ng/dL. Samples include natural samples and spiked samples in order to cover the hard to find sample range. Mean % bias was found to be -2.25 %. Linear regression of the data yielded the following values:
 $y = 0.967x + 0.007$, $n=73$, $r=0.998$.

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:
The expected results for the ACS:180® FrT4 assay were previously established. Data was obtained on serum samples from 388 apparently healthy individuals. Based on this population, the following reference ranges were established:

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

The sponsor claims that these expected values are transferrable to the ADVA Centaur FT4 assay by analyzing 283 samples in the range of 0.14 to 11.1 ng/dL in the method comparison study above and obtaining a linear regression equation of $Y = 0.979X + 0.058$, $n=91$, $r=0.997$ with a mean bias of 4.33%.

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.