

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

**A. 510(k) Number:**

k083844

**B. Purpose for Submission:**

New device

**C. Measurand:**

Thyroid-stimulating hormone (TSH, thyrotropin)

**D. Type of Test:**

Quantitative chemiluminescent immunoassay

**E. Applicant:**

Siemens Healthcare Diagnostics Inc.

**F. Proprietary and Established Names:**

ADVIA Centaur TSH3-Ultra (TSH3-UL)

**G. Regulatory Information:**

1. Regulation section:

21 CFR § 862.1690 Thyroid Stimulating Hormone Test System

2. Classification:

Class II

3. Product code:

JLW

4. Panel:

75, Clinical Chemistry

**H. Intended Use:**

1. Intended use(s):

See indication(s) for use below:

2. Indication(s) for use:

For in vitro diagnostic use in the quantitative determination of thyroid-stimulating hormone (TSH, thyrotropin) in serum and plasma using the ADVIA Centaur XP system. A thyroid stimulating hormone test system is a device intended to measure thyroid stimulating hormone, also known as thyrotrophin and thyrotrophic hormone, in serum and plasma. Measurements of thyroid stimulating hormone produced by the anterior pituitary are used in the diagnosis of thyroid or pituitary disorders.

3. Special conditions for use statement(s):

For prescription use

4. Special instrument requirements:

The performance of the assay was established using the Advia Centaur XP System.

**I. Device Description:**

ADVIA Centaur TSH3-Ultra (TSH3-UL) kit consists of the following reagents:

**Ultra-Lite Reagent-** bovine serum albumin (BSA) conjugated to monoclonal anti-TSH (~0.3 µg/mL) labeled with acridinium ester in HEPES buffered saline, mouse IgG, BSA, goat serum, surfactant, and preservatives

**Solid Phase Reagent** - anti-fluorescein monoclonal mouse antibody covalently linked to paramagnetic particles (PMP) (~85 µg/mL) in HEPES buffered saline, BSA, goat serum, surfactant, and preservative

**Ancillary Well Reagent** - FITC conjugated to monoclonal anti-TSH (~3 µg/mL) in HEPES buffered saline, mouse IgG, BSA, goat serum, surfactant, and preservative.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

ADVIA Centaur TSH-3 Assay

2. Predicate K number(s):

k951796

3. Comparison with predicate:

Both the Centaur TSH3-Ultra and the predicate thyroid stimulating hormone (TSH) assay have a similar intended use and both are chemiluminescent immunoassays. The new device and the predicate use different reagents and the antibodies differ. The new device is intended for use in serum and plasma and the predicate is intended for serum only. The assay range of the new device is 0.008 – 150 µIU/mL and the predicate assay range is 0.01 -150 µIU/mL.

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

Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline- Second Edition (CLSI EP5-A2)

Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (CLSI EP9-A2)

**L. Test Principle:**

The ADVIA Centaur TSH3-Ultra assay employs anti-FITC monoclonal antibody covalently bound to paramagnetic particles, an FITC-labeled anti-TSH capture monoclonal antibody, and a tracer consisting of a proprietary acridinium ester and an anti-TSH mAb antibody conjugated to bovine serum albumin (BSA) for chemiluminescent detection.

The system automatically performs the following actions:

- dispenses 100 µL of sample into a cuvette
- dispenses 50 µL of Ancillary Reagent and 50 µL of Lite Reagent and incubates for 2.75 minutes at 37°C
- dispenses 200 µL of Solid Phase and incubates for 5.5 minutes at 37°C
- separates, aspirates, and washes the cuvettes with Wash 1
- dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction
- reports results according to the selected option, as described in the system operating instructions or in the online help system.

A direct relationship exists between the amount of TSH present in the patient sample and the amount of relative light units (RLUs) detected by the system.

#### **M. Performance Characteristics (if/when applicable):**

##### **1. Analytical performance:**

##### ***a. Precision/Reproducibility:***

CLSI protocol EP5-A2 was used as a guideline to study precision using 2 Centaur XP analyzers for 20 days with 2 runs per day. Four controls and six patient serum pools were assayed in duplicate in two runs for 20 days on at least two ADVIA Centaur systems using two reagent lots and two calibrator lots ( $n \geq 80$  for each sample). Precision also was evaluated for patient plasma pools. Five plasma pools were assayed in duplicate in two runs per day for 20 days on one ADVIA Centaur XP System using two reagent lots and two calibrator lots ( $n = 80$  for each sample). The Serum and Plasma Pools were prepared using commercially available serum and plasma units. Different lots of serum and plasma units were used in the preparation of each pool. Serum Pool 1 and Plasma Pool 1 were not spiked with stock TSH concentrate. Serum Pools 2 to 6 and Plasma Pools 2 to 5 were spiked with stock TSH concentrate. The results of the study are presented in the table below.

<b>Sample</b>	<b>Mean (µIU/mL)</b>	<b>Within-run %CV</b>	<b>Run-to-Run %CV</b>	<b>Total %CV</b>
Control 1	0.026	4.69	3.62	6.64
Control 2	0.383	1.97	4.13	5.13
Control 3	4.17	1.95	4.28	5.58
Control 4	16.6	2.26	3.99	5.18
Serum Pool 1	1.03	2.85	2.35	4.45
Serum Pool 2	5.41	2.37	0.88	3.64
Serum Pool 3	10.7	1.70	1.20	3.77
Serum Pool 4	32.0	1.93	1.87	4.37
Serum Pool 5	56.7	1.44	1.59	4.29
Serum Pool 6	132.8	1.82	1.41	5.17

Sample	Mean ( $\mu$ IU/mL)	Within-run %CV	Run-to-Run %CV	Total %CV
Plasma Pool 1	0.659	2.56	0.70	3.55
Plasma Pool 2	5.00	1.87	1.77	3.18
Plasma Pool 3	34.2	2.27	1.88	4.01
Plasma Pool 4	78.5	2.51	1.50	4.90
Plasma Pool 5	129.2	2.93	3.20	5.51

**b. Linearity/assay reportable range:**

The claimed measuring range is 0.008 mIU/L – 150 mIU/L. The sponsor performed two recovery studies to demonstrate the claimed measuring range.

**Study #1**

Six patient samples in the range of 0.296–146.24  $\mu$ IU/mL (mIU/L) of TSH were diluted 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64 with Multi-Diluent 1 and assayed. The mean recoveries ranged from 93.6–105.9%. All these levels for each set of samples were run in triplicate on the Centaur XP system and one reagent lot.

	Sample 1			Sample2		
Dilution	Observed	Expected	%Recovered	Observed	Expected	%Recovered
	0.296			1.674		
<b>1:2</b>	0.139	0.148	94.1	0.769	0.837	91.8
<b>1:4</b>	0.071	0.074	95.8	0.383	0.419	91.6
<b>1:8</b>	0.036	0.037	96.6	0.199	0.209	95.1
<b>1:16</b>	0.016	0.018	88.4	0.102	0.105	97.2
<b>1:32</b>	0.009	0.009	96.0	0.052	0.052	99.9
<b>1:64</b>	0.004	0.005	91.0	0.028	0.026	106.1
<b>MEAN</b>			<b>93.6</b>			<b>97.0</b>

	Sample 3			Sample 4		
Dilution	Observed	Expected	%Recovered	Observed	Expected	%Recovered
	11.72			72.26		
<b>1:2</b>	5.97	5.86	101.8	35.32	36.13	97.8
<b>1:4</b>	3.05	2.93	104.1	18.09	18.06	100.2
<b>1:8</b>	1.55	1.47	105.5	8.99	9.03	99.5
<b>1:16</b>	0.79	0.73	107.7	4.46	4.52	98.8
<b>1:32</b>	0.40	0.37	108.1	2.32	2.26	102.6
<b>1:64</b>	0.20	0.18	108.3	1.12	1.13	98.8
<b>MEAN</b>			<b>105.9</b>			<b>99.6</b>

	Sample 5			Sample 6		
Dilution	Observed	Expected	%Recovered	Observed	Expected	%Recovered
	109.39			146.24		
1:2	53.05	54.70	97.0	70.40	73.12	96.3
1:4	26.31	27.35	96.2	35.92	36.56	98.3
1:8	13.17	13.67	96.3	17.67	18.28	96.6
1:16	6.54	6.84	95.7	9.36	9.14	102.5
1:32	3.31	3.42	96.9	4.77	4.57	104.4
1:64	1.71	1.71	99.8	2.37	2.29	103.6
MEAN			97.0			100.3

#### Study #2

The linearity study was performed using eleven samples, 9 equally spaced combinations of a low serum sample and a high serum sample covering the range of 0.006 to 160 mIU/L of the assay. All these levels for each set of samples were run in quadruplicate on the Centaur XP system and one reagent kit; the mean sample recovery ranged 98.1- 105.0% demonstrating recovery over the claimed assay range.

% Dilution	Expected mIU/L	Observed mIU/L	% Recovery
100 %	160.030		
90%	146.830	144.027	98.1
80%	132.980	128.024	96.3
70%	113.640	112.021	98.6
60%	97.760	96.018	98.2
50%	81.950	80.015	97.6
40%	62.790	64.012	101.9
30%	48.560	48.009	98.9
20%	30.850	32.006	103.7
10%	15.240	16.003	105.0
0%	0.006		

The standardization of the TSH3-Ultra method is traceable to the WHO International Standard for Human TSH (IRP 81/565). A comparison over the full range of the assay gave the following correlation:

ADVIA Centaur TSH3-Ultra = 1.103 (WHO) - 0.111 mIU/L;  $r=1.000$

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.

Shelf life stability experiments showed that the reagent is stable for 24 months at 2-8°C

**d. Detection limit:**

The Limit of Blank (LoB) for the TSH3-Ultra assay was determined as recommended in CLSI guideline EP17-A. A TSH3-Ultra negative equine base pool was used as a blank and was analyzed on two ADVIA Centaur XP instruments over 12 days. The sample was tested twice daily using two different reagent pilot lots with 2 replicate determinations per run and the daily analyses were separated by at least 2 hours. Each instrument was calibrated on day one only. The LoB was calculated to be 0.001  $\mu$ IU/mL.

**Limit of Detection (LOD)** - Four low human sample pools with mean values ranging from 0.0027 to 0.0111 mIU/L were used to determine the LoD. Human serum pools were prepared by pooling samples with low TSH concentration at 0.0056, 0.0072, 0.009 and 0.0097 mIU/L. The human pools were analyzed with 2 different reagent lots, on 1 ADVIA Centaur XP system over 10 days with 2 replicates/sample/run for a total of 40 replicates/sample. The LoD was determined nonparametrically since the requirement of normality was not met for these pools for all combinations of reagent lot and system. The LoD was calculated to be 0.006  $\mu$ IU/mL.

**Functional Sensitivity** - Six levels of serum based pools were used to determine functional sensitivity. Human serum pools were prepared by pooling samples with low TSH concentration at 0.0023, 0.0042, 0.0056, 0.0072, 0.009 and 0.0097 mIU/L. Six human serum pools each were assayed on one Centaur XP system using two reagent pilot lots, twice per day for 20 days. For functional sensitivity, the total imprecision results generated at each of six pool levels for each reagent lot and system were fitted with an appropriate nonlinear function using the nonlinear regression program. The functional sensitivity was determined to be 0.008  $\mu$ IU/mL, which is the lowest analyte concentration at which the total imprecision for the assay does not exceed 20%. The results of the study are presented below.

TSH3-Ultra Conc.	Total %CV	Predicted Total %CV	Lower 97.5 <sup>th</sup> Percentile Prediction Limit	Upper 97.5 <sup>th</sup> Percentile Prediction Limit
0.0028	41.6	41.7617	36.9184	46.6049
0.0046	32.3	31.5018	27.4816	35.5219
0.0055	27.6	27.4148	23.4718	31.3578
0.0059	24.8	25.7858	21.8498	29.7218
<b>0.0076</b>		<b>19.9970</b>	16.0617	23.9323
0.0080	18.8	18.8784	14.9501	22.8067
0.0081	17.6	18.6123	14.6862	22.5383
0.0085	17.3	17.6000	13.6848	21.5151
0.0090	20.2	16.4492	12.5493	20.3492
<b>0.0092</b>		16.0235	12.1292	<b>19.9178</b>
0.0095	13.0	15.4210	11.5334	19.3086
0.0109	13.5	13.1561	9.2250	17.0871

TSH3-Ultra Conc.	Total %CV	Predicted Total %CV	Lower 97.5 <sup>th</sup> Percentile Prediction Limit	Upper 97.5 <sup>th</sup> Percentile Prediction Limit
0.0125	12.1	11.5914	7.2685	15.9143
0.0126	10.9	11.5276	7.1622	15.8930

Hook effect: High TSH levels can cause a paradoxical decrease in the RLUs (high dose hook effect). In this assay, TSH levels as high as 3000 mIU/L will assay greater than 150 mIU/L. Hook Effect: A concentrated sample of purified TSH was measured both neat and on dilution within the measuring range of the TSH-3 Ultra assay. There was no high dose effect observed at TSH concentrations up to 3,000 mIU/mL. This assay uses a two-step design that significantly reduces the risk of a high dose hook effect.

**e. Analytical specificity:**

The purpose of specificity study is to determine if other glycoproteins with alpha sub-units similar to that of TSH cross-react with TSH antibodies in the TSH3-Ultra assay. The following substances were tested: Human Chorionic Gonadotropin (hCG), Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Specificity of the ADVIA Centaur-Ultra assay was evaluated by adding the purified hormones, hCG at 200,000 mIU/mL, FSH at 1500 mIU/mL and LH at 600 mIU/mL into three serum samples. Each test sample was compared to a matched unspiked control using two reagent lots.

The following substances were tested in human serum samples: Human Chorionic Gonadotropin (hCG -Scripps 2286001) at 200,000 mIU/mL, Follicle Stimulating Hormone (FSH- Scripps 2187401) at 1500 mIU/mL and Luteinizing Hormone (LH- SCRIPPS 2270102) at 600 mIU/mL. Three serum samples with TSH concentrations of 0.95, 1.93 and 3.66 uIU/mL were spiked with the above hormones at the target levels. The resulting samples were assayed for TSH along with the matched unspiked controls on one Centaur XP system using two lots of reagents. The % cross-reactivity was *less than 5%* for all three hormones.

**Interfering Substances:**

Interference by endogenous substances was evaluated in serum sample according to the guidelines of CLSI Document EP7-A2. The following substances were tested: conjugated and unconjugated Bilirubin, hemoglobin (lysed RBC) and triglycerides. Each sample was spiked with an interferant to the maximum and half maximum levels. Each test sample was compared to a matched unspiked control. The percent change of TSH recovery was calculated as % Recovery = (Observed spiked sample dose/ Observed unspiked sample dose) x 100

Two serum samples with TSH concentrations of 1.10 uIU/mL and 6.40 uIU/mL, respectively, were spiked with the above interferents at the target and half target levels. The resulting samples were assayed for TSH along with the matched unspiked controls. The recoveries ranged from 96.3% to 100.9%

Sample	Substance	Amount spiked (mg/dL)	Unspiked Dose (uIU/mL)	Spike Dose (uIU/mL)	% Recovery
1	Triglyceride	500	1.10	1.10	100
		1000	1.09	1.05	96.3
	HB	50	1.14	1.13	99.1
		100	1.12	1.15	102.7
	Bilirubin Unconj	20	1.13	1.12	99.1
		40	1.13	1.10	97.3
	Bilirubin conj	20	1.10	1.14	103.6
		40	1.12	1.13	100.9
2	Triglyceride	500	6.44	6.36	98.8
		1000	6.27	6.27	100
	HB	50	6.48	6.52	100.6
		100	6.59	6.51	98.8
	Bilirubin Unconj	20	6.55	6.61	100.9
		40	6.35	6.37	100.3
	Bilirubin conj	20	6.42	6.40	99.7
		40	6.44	6.30	97.8

**Heterophile Interference Study:** Interference by HAMA Type 1 and HAMA Type 2 pools as well as 19 HAMA positive samples ranging from 62.5 to 215 ng/ml were evaluated using four reagent lots on one ADVIA Centaur XP system. The recoveries of HAMA Type 2 for four TSH3-Ultra reagents ranged from 1.18 to 1.28 uIU/mL. The recoveries of HAMA Type 1 for four TSH3-Ultra reagents ranged from 3.50 to 6.71 uIU/mL. Assay of a wide range of HAMA positive samples showed that there was no significant difference in the TSH values between the four TSH3-Ultra reagents and the current TSH3 reagent. The results demonstrate that TSH3-Ultra reagents like the current TSH reagent are not affected by interference of heterophile antibodies.

- f. *Assay cut-off:*  
Not applicable.
2. Comparison studies:
  - a. *Method comparison with predicate device:*

The new TSH3-Ultra assay was compared to the predicate device by testing 241 clinical samples consisting of stored (previously frozen) and fresh serum samples. The samples ranged from 0.009 to 148.2 mIU/L were performed on the Advia Centaur XP system. This protocol was run in accordance with CLSI document EP09-A2. Samples were procured from commercial vendors (retrospective study). In-house donor samples were collected from apparently



healthy donors without known thyroid conditions. Summary of method comparison correlation:  $y = 0.99x + 0.09$ ,  $r = 0.998$

*b. Matrix comparison:*

Matched heparinized plasma, EDTA plasma and serum samples were collected from patients in the range of 0.008 to 150 mIU/L TSH. Correlation was performed on 92 pairs of serum and lithium heparin plasma samples and 93 pairs of serum and EDTA plasma samples on the Centaur XP system. Some of the paired samples were spiked with a TSH stock to achieve concentrations covering the analytical assay range. The following results support the use of lithium heparin and EDTA plasma samples with the Centaur TSH3-Ultra assay.

**Summary of Serum/Plasma Correlation:**

Sample	n	Linear Regression	Correlation coefficient
Serum vs Li-heparin	92	$0.97 x + 0.10$	0.995
Serum vs EDTA	93	$0.95 x + 0.11$	0.997

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 National Academy of Clinical Biochemistry established the following TSH reference intervals <sup>[1]</sup>:

**TSH Reference Ranges**

Age	TSH Ranges mIU/L
14 months	0.4-7.0
5 years	0.4-6.0
14 years	0.4-5.0
Adult	0.4-4.0

<sup>[1]</sup> LABORATORY MEDICINE PRACTICE GUIDELINES, op. cit.

**The sponsor determined their own reference ranges for adults, adolescents and pediatric populations.** To determine the reference range for the ADVIA Centaur TSH3-Ultra Assay, aliquots of each patient sample were run on each of two ADVIA Centaur XP systems. Two lots of TSH3-Ultra reagents and one lot each of FT3 and FT4 reagents were used for all samples, and one lot each of aTPO and aTG reagents were used for the adult ( $\geq 18$  years old) age group only.

**Adult Reference Interval:** Two hundred and twenty nine adult serum specimens were obtained from apparently healthy adult blood bank donors and tested for aTPO, aTG, FT4, FT3 and TSH. Seventy samples were considered abnormal because the FT3, FT4, aTPO, aTG values were outside the ranges given in their corresponding current Centaur Instructions for Use.

The reference intervals for each combination of Advia Centaur XP system and reagent lot are given below:

Reagent Lot	System	Lower Reference Limit	Upper Reference Limit
114080	AF26108B	0.549	4.796
	AT26108E	0.572	4.603
114081	AF26108B	0.570	4.631
	AT26108E	0.561	4.515

**Adult Reference Interval:** The adult reference interval determined for ADVIA TSH3-Ultra assay is **0.550 to 4.783 mIU/L**. The reference intervals were established by calculating the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles of the distribution of values obtained with the assay

**Adolescent Reference Interval:** One hundred and eighty three ‘leftover’ adolescent (12 years of age to less than 18 years of age) serum specimens were obtained from routine laboratory testing and tested for FT4, FT3 and TSH. Nine samples were considered abnormal because the FT3 and FT4 values were outside their respective reference intervals given in the current reference literature.

All adolescent serum specimens that met the following criteria were included in the study: If the FT3 values were between 2.6 and 4.8 pg/mL,<sup>[1]</sup> and if the FT4 values were between 0.6 and 1.4 ng/dL.<sup>[2]</sup>

<sup>[1]</sup> Tietz, Clinical Guide to Laboratory Tests, 1995, 4<sup>th</sup> edition, p. 1076.

<sup>[2]</sup> “NACB – Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease”, p. 8.

The adolescent reference intervals for each combination of Advia Centaur XP system and reagent lot are given below:

Reagent Lot	System	Lower Reference Limit	Upper Reference Limit
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114080	AF26108B	0.53	4.53
	AT26108E	0.50	4.48
114081	AF26108B	0.52	4.98
	AT26108E	0.51	4.39

**Adolescent Reference Interval:** The adolescent reference interval for the ADVIA TSH-3 Ultra is **0.505 to 4.942 mIU/L**. The reference intervals were established by calculating the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles of the distribution of values obtained with the assay

**Pediatric Reference Interval:** One hundred and thirty seven ‘leftover’ pediatric (2 years of age to less than 12 years of age) serum samples were obtained from routine laboratory testing and tested for FT4, FT3 and TSH. Eleven samples were considered abnormal because the FT3 and FT4 values were outside the range given in the current reference literature. These intervals are given below.

All pediatric serum specimens that met the following criteria were included in the study: If the FT3 values were between 2.6 and 4.8 pg/mL,<sup>[1]</sup> and If the FT4 values were between 0.8 and 1.7 ng/dL.<sup>[2]</sup>

<sup>[1]</sup> Tietz, Clinical Guide to Laboratory Tests, 1995, 4<sup>th</sup> edition, p. 1076.

<sup>[2]</sup> “NACB – Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease”, p. 8.

The pediatric reference intervals for each combination of Advia Centaur XP system and reagent lot are given below:

Reagent Lot	System	Lower Reference Limit	Upper Reference Limit
114080	AF26108B	0.66	6.31
	AT26108E	0.66	5.62
114081	AF26108B	0.67	5.68
	AT26108E	0.63	5.59

**Pediatric Reference Interval:** The pediatric reference interval determined for ADVIA Centaur TSH3-Ultra assay is **0.636 to 6.267 mIU/L**. The reference intervals were established by calculating the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles of the distribution of values obtained with the assay.

**Conclusion:** The pediatric, adolescent and adult TSH3-Ultra reference intervals are similar to those cited in the National Academy of Clinical Biochemistry (NACB) reference publication:

Age	NACB TSH Ranges mIU/L	TSH3-Ultra Reference Ranges
5 years	0.4-6.0	

Age	NACB TSH Ranges mIU/L	TSH3-Ultra Reference Ranges
14 years	0.4-5.0	
Adult	0.4-4.0	
2 years to less than 12 years		0.636 – 6.267
12 years to less than 18 years		0.505 – 4.942
≥18 years		0.550 – 4.806

**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.