

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

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

k142723

**B. Purpose for Submission:**

New device

**C. Measurand:**

Cortisol

**D. Type of Test:**

Quantitative, Chemiluminescent Immunoassay

**E. Applicant:**

Siemens Healthcare Diagnostics Inc.

**F. Proprietary and Established Names:**

ADVIA Centaur® Cortisol (COR) Assay

**G. Regulatory Information:**

1. Regulation section:

21 CFR 862.1250

2. Classification:

Class II

3. Product code:

JFT

4. Panel:

Clinical Chemistry (75)

## H. Intended Use:

1. Intended use(s):

See Indications for use below

2. Indication(s) for use:

The ADVIA Centaur Cortisol assay is for *in vitro* diagnostic use in the quantitative determination of cortisol in serum or urine using the ADVIA Centaur XP system. Measurements of cortisol are used in the diagnosis and treatment of disorders of the adrenal gland.

3. Special conditions for use statement(s):

Prescription use only

4. Special instrument requirements:

ADVIA Centaur XP

## I. Device Description:

The ADVIA Centaur Cortisol assay is a competitive immunoassay using direct chemiluminescent technology. Results are determined using a calibration curve that is generated specifically on each instrument by a 2-point calibration and a master curve with the reagent bar code. The ADVIA Centaur cortisol reagent kit contains the following:

- ADVIA Centaur ReadyPack primary reagent packs containing COR Lite Reagent and Solid Phase. Lite reagent consists of 2.5 mL reagent pack with cortisol labeled with acridinium ester in buffered saline with sodium salicylate, sodium azide, and preservatives. Solid phase reagent consists of 12.5 mL reagent pack with rabbit anti-cortisol antibody bound to monoclonal mouse anti-rabbit IgG antibody covalently coupled to paramagnetic particles in buffered saline with sodium azide and preservatives.
- ADVIA Centaur Calibrator E - required to perform calibration, consists of 2 vials of low calibrator and 2 vials of high calibrator.
- ADVIA Centaur Multi-Diluent 3 - used to dilute high cortisol serum samples only, consists of 5mL of human plasma with sodium azide.

- ADVIA Centaur Cortisol Urine Reconstitution Buffer - used to reconstitute extracted urine samples only, consists of 50 mL of protein buffer solution with sodium azide (0.1%).

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

ADVIA Centaur® Cortisol (COR) Assay

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

k962559

3. Comparison with predicate:

<b>Assay: Similarities and Differences</b>		
Item	Candidate Device: ADVIA Centaur® Cortisol Assay (modified) (Model Number: REF# 10994926 (250T), REF# 10994924 (50T))	Predicate Device: ADVIA Centaur® Cortisol Assay (k962559) (Model Number: REF 04610138 (250T) REF 04610049 (50T))
Intended Use	For the in vitro quantitative determination of cortisol in human serum and urine	Same
Sample type	Serum and urine	Same
Assay Range	Serum: 0.50–75 µg/dL Urine: 0.50–53 µg/dL	Serum: 0.20–75 µg/dL Urine: 0.20–75 µg/dL
Traceability	Internal standards traceable to GCMS	Same
Detection Mechanism	Cortisol labeled with acridinium ester	Same
Capture Antibody	Polyclonal rabbit anti-cortisol antibody	New antibody pool

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

- CLSI Evaluation of the Linearity of Quantitative Measurement Procedures; A Statistical Approach; Approved Guideline-First Edition (EP06-A).

- CLSI Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (EP05-A2).
- CLSI Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition (EP07-A2).
- CLSI Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline- Second Edition (EP17-A2).
- CLSI Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition (EP28-A3c).

#### **L. Test Principle:**

The ADVIA Centaur Cortisol assay is a competitive immunoassay using direct chemiluminescent technology. Cortisol in the patient sample competes with acridinium ester labeled cortisol in the Lite Reagent for binding to polyclonal rabbit anti-cortisol antibody in the Solid Phase. The polyclonal rabbit anti-cortisol antibody is bound to monoclonal mouse anti-rabbit antibody, which is covalently coupled to paramagnetic particles in the Solid Phase. An inverse relationship exists between the amount of cortisol present in the patient sample and the amount of relative light units (RLUs) detected by the instrument.

Either serum sample or urine sample (24 hours collection) could be used with the assay. For the urine sample, user can select either the direct urine configuration or the extracted urine configuration. The urinary cortisol extraction procedure utilizes the extraction of free cortisol from the urine sample with methylene chloride before measurement of the sample on the instrument. The extraction procedure is provided in the labeling.

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

All studies were performed on the ADVIA Centaur XP analyzer.

##### 1. Analytical performance:

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

A 20-day precision study was performed according to CLSI EP5-A2. The samples consisted of controls, serum, and urine pools (direct and extracted). Each sample was tested in 2 replicates per run, 2 runs per day for 20 days for a total of 80 replicates. Results from a representative lot are presented below.

Samples	Mean (µg/dL)	Within-run precision		Between-run precision	
		SD (mg/L)	CV (%)	SD (mg/L)	CV (%)
Serum Control 1	2.76	0.10	3.7	0.16	6.0
Serum Control 2	22.28	0.65	2.9	1.00	4.5
Serum Control 3	34.24	1.16	3.4	1.50	4.4
Serum 1	5.69	0.19	3.3	0.30	5.3
Serum 2	50.51	2.10	4.2	2.49	4.9
Direct Urine 1	9.2	0.40	4.3	0.63	6.8
Direct Urine 2	25.6	0.91	3.5	1.73	6.8
Direct Urine 3	50.2	1.99	4.0	4.59	9.1
Extracted Urine 1	9.8	0.33	3.3	0.67	6.8
Extracted Urine 2	26.9	0.99	3.7	1.92	7.2
Extracted Urine 3	40.4	2.12	5.3	3.47	8.6
Extracted Urine 4	51.1	2.97	5.8	4.69	9.2

*b. Linearity/assay reportable range:*

Linearity of the ADVIA Centaur Cortisol assay was assessed according to EP06-A by evaluating equally spaced serum, and urine (direct and extracted) dilutions across the assay claimed measuring range. Nine (9) serum samples and 10 urine samples with cortisol concentrations ranging from approximately 0.5–79.5 µg/dL were tested. Samples were prepared by diluting the high sample (79.5µg/dL) to 87.5%, 75%, 62.5%, 50%, 37.5%, 25% and 12.5% with the low sample (0.5µg/dL) or reconstitution buffer. The samples were assayed in triplicate and the mean of the results was used for the analysis. Expected values were calculated from gravimetric concentration of both high and low samples. Linear regression results:

Serum:  $y=1.057x - 0.051, r^2 = 0.9991$ .

Direct Urine:  $y=1.011x+0.090, r^2 = 0.9975$ .

Extracted Urine:  $y=0.914x +0.017, r^2 = 0.9997$ .

The linearity study results support the sponsor’s claimed measuring range of the following:

Serum: 0.50–75 µg/dL

Urine (direct and extracted): 0.50–53 µg/dL

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

The ADVIA Centaur Cortisol assay is standardized using internal standards manufactured analytically which are traceable to gas chromatography-mass

spectroscopy (GCMS). Assigned values for calibrators are traceable to this standardization.

Controls: Siemens recommends the use of any commercially available quality control materials with at least two levels (low and high).

The ADVIA Centaur Cortisol assay uses the Siemens ADVIA Centaur Calibrator E, which is cleared under k932955. Calibrator E consists of two level calibrators (Low and High), supplied separately. Values of these 2 levels of calibrators were established in 6 runs on 2 reagent lots for a total of 12 runs (using a minimum of 3 ADVIA Centaur instruments) x 6 reps per calibrator for a total of 72 replicates. The calibrator values were calculated as the overall mean of the value assignment runs read off the full standard curve. Level 1 is ~ 3 µg/dL and Level II is ~ 40 µg/dL.

Shelf-Life and Open Vial Stability testing protocols and acceptance criteria for the reagents were described and found to be adequate.

*d. Detection limit:*

The determinations of the Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) were performed according to CLSI guideline EP17-A2.

Limit of Blank (LoB) was determined as the 95th percentile of measurement of 6 blank samples with 5 replicates per day over 3 days (n=90). The LoB for the serum matrix of the ADVIA Centaur Cortisol assay is 0.06 µg/dL. The LoB values for direct and extracted urine are 0.19 µg/dL and 0.18 µg/dL.

The Limit of Detection (LoD) is the smallest amount that the assay can reliably detect to determine presence or absence of an analyte. The LoD was determined using five low cortisol serum samples tested over 3 days, 5 replicates per day (n=75). The LoD for the ADVIA Centaur Cortisol assay is 0.14 µg/dL for serum, 0.45 µg/dL for direct and 0.44 µg/dL for extracted urine.

The Limit of Quantitation (LoQ) was determined from the precision profile. Sponsor defined the LoQ as the concentration of the analyte having an observed within laboratory CV of 20% and not less than the LoD. Six samples with GCMS assigned doses were tested over 3 days, in 5 replicates (n=60). The LoQ for the ADVIA Centaur Cortisol assay is 0.31 µg/dL for serum, and 0.48 µg/dL for direct urine and 0.44 µg/dL for extracted urine.

The measuring ranges of the ADVIA Cortisol assay are: Serum: 0.50–75 µg/dL, Urine (direct and extracted): 0.50–53 µg/dL

e. *Analytical specificity:*

Interference Study:

Interfering substances were tested using serum and direct urine sample pools. The extracted urine samples were not tested in these studies because methylene chloride used in extraction steps removes potential interferents and direct urine is representative of urine matrix. Each potential interfering substance was tested at least 2 different concentrations and was spiked into 2 sample pools at 5 µg/dL and 30 µg/dL of cortisol. The same sample pools with no interferents were used as controls. All samples were run in triplicate on an ADVIA Centaur XP with one reagent lot. Sponsor defined non-significant interference as ≤10% difference between the spiked and unspiked samples. Results are summarized below.

Serum:

Tested substance	Concentration with ≤10% interference
Bilirubin - conjugated	20 mg/dL
Bilirubin - unconjugated	20 mg/dL
Hemoglobin	500 mg/dL
Intralipid (Triglycerides)	1000 mg/dL

Urine:

Tested substance	Concentration with ≤10% interference
Boric acid	10 g/dL
Creatinine	56.6 mg/dL
Glucose	36 mg/dL
HSA	60 mg/dL
NaCl	5844 mg/dL(1000 mmol/L)
Urea	2102 mg/dL(350 mmol/L)

Cross-Reactivity Study:

The sponsor evaluated cross-reactivity by spiking each compound into hum serum specimens with Cortisol levels of approximately 6 µg/dL. The spiked and unspiked samples were tested in triplicate. The cross-reactivity results are summarized in the table below:

Tested substance	Concentration Tested	% Cross Reactivity
Aldosterone	1,000 µg/dL	0.4%
Allotetrahydrocortisol	100 µg/dL	11.9%
Androstenedione	1,000 µg/dL	0.2%
Canrenone	1,000 µg/dL	0.2%
Corticosterone	1,000 µg/dL	2.6%
Cortisone	100 µg/dL	11.5%
Dehydrocorticosterone	1,000 µg/dL	2.7%
Dexamethasone	1,000 µg/dL	0.5%
Prednisolone	50 µg/dL	92%
Prednisone	100 µg/dL	10.7%
Pregnanetriol	1,000 µg/dL	0.0%
Pregnenolone	1,000 µg/dL	0.1%
Progesterone	1,000 µg/dL	0.5%
Spirolactone	1,000 µg/dL	≤0.1%
Testosterone	1,000 µg/dL	0.3 %
Tetrahydro-11-deoxycortisol	1,000 µg/dL	≤0.7%
Tetrahydrocortisol	1,000 µg/dL	≤1.1%
Tetrahydrocortisone	1,000 µg/dL	≤0.5%
α Cortol	1,000 µg/dL	≤0.6%
α Cortolone	1,000 µg/dL	0.1%
β Cortol	1,000 µg/dL	0.1%
β Cortolone	1,000 µg/dL	0. %
11 β hydroxyandrosterone	1,000 µg/dL	0.0%
11 β hydroxyetiocholanone	1,000 µg/dL	0.0%
11 β hydroxyprogesterone	1,000 µg/dL	1.0
11-deoxycorticosterone	1,000 µg/dL	0.9%
11-deoxycortisol	100 µg/dL	18.3%
11-keto-androsterone	1,000 µg/dL	0.1%
11-keto-etiochalanonlone	1,000 µg/dL	0%
17α hydroxyprogesterone	1,000 µg/dL	1.4%
17α hydroxypregnenolone	1,000 µg/dL	0.1%
20 α dihydrocortisol	1,000 µg/dL	2.5%
20 α -dihydrocortisone	1,000 µg/dL	0.5%
20 β dihydrocortisol	1,000 µg/dL	2.5%
20 β dihydrocortisone	1,000 µg/dL	0.3%
21 deoxycortisol	100 µg/dL	10.3%
6 β hydroxycortisol	1,000 µg/dL	2.3%
6α-methyl-prednisolone	100 µg/dL	23.1%

The following limitations are provided in the labeling:

- Circulating cortisol results from patients receiving Prednisolone or Prednisone (which is converted to Prednisolone in vivo) therapy may be falsely elevated. Exercise caution with cortisol determinations for patients undergoing therapy with these and structurally related synthetic corticosteroids.
- 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.
- The performance of the ADVIA Centaur systems Cortisol assay has not been established with neonatal specimens.

*f. Assay cut-off:*

Not applicable

2. Comparison studies:

*a. Method comparison with predicate device:*

Method comparison testing between the candidate device and the predicate device was performed in duplicate over 2 days and involved 243 serum samples, 98 direct urine samples and 111 extracted urine samples. In order to obtain a hard-to-find sample range, 5% of the serum samples and 9% of the extracted urine samples were altered. All the direct urine samples were native urine samples. Data were analyzed using one replicate of the results from the candidate device and weighted Deming regressions are summarized below:

Sample Category	N	Range	Regression Equation
Serum	243	0.53–67.42 µg/dL	$y=1.00x + 0.07 \mu\text{g/dL}$ (r) = 0.996
Direct Urine	98	2.64–47.00 µg/dL	$y=1.11x + 0.68 \mu\text{g/dL}$ (r) = 0.969
Extracted Urine	111	1.13–50.69 µg/dL	$y = 0.86 x + 0.38 \mu\text{g/dL}$ (r) = 0.991

*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:

A reference interval study was performed according to CLSI EP28-A3c using the ADVIA Centaur COR assay on 252 serum samples from apparently healthy male and female individuals. Based on a central 95% interval, the following reference intervals were established:

N=127; AM Serum (7–9 AM); Reference Interval: 5.27–22.45 (µg/dL)

N=125; PM Serum (3–9 PM); Reference Interval: 3.44–16.76 (µg/dL)

Reference intervals for 24 hour direct urine and extracted urine, previously established with the predicate device (ADVIA Centaur Cortisol assay), were verified using 20 apparently healthy subjects according to the CLSI EP28-A3c criteria of transferability. Sponsor's reference range for the direct urine and extracted urine are as follows:

Direct Urine: N=105; 20.9–292.3 µg/24 hr

Extracted Urine: N = 105; 9.5–136.2 µg/24 hr

Sponsor stated the following in the labeling: “As with all diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.

**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.