

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

A. 510(k) Number:

k042689

B. Purpose for Submission:

New device

C. Measurand:

Cortisol

D. Type of Test:

Quantitative, Chemiluminescent competitive binding assay

E. Applicant:

Nichols Institute Diagnostics

F. Proprietary and Established Names:

Nichols Advantage Cortisol

G. Regulatory Information:

1. Regulation section:

21 CFR 862.1205, Cortisol (hydrocortisone and hydroxycorticosterone) test system

21 CFR 862.1150, Calibrator

2. Classification:

Class II

3. Product code:

CGR

JIT

4. Panel:

Chemistry (75)

H. Intended Use:

1. Intended use(s):

The Nichols Advantage® Cortisol Assay is intended for use with the Nichols Advantage® Specialty System for the quantitative determination of cortisol concentrations in human serum, EDTA plasma and urine. Measurements of cortisol are used in the diagnosis and treatment of disorders of the adrenal gland.

The Nichols Advantage® Cortisol Assay Calibrators are intended for adjustment of the stored curve for Nichols Advantage Cortisol Assay.

2. Indication(s) for use:

The Nichols Advantage® Cortisol Assay is intended for use with the Nichols Advantage® Specialty System for the quantitative determination of cortisol concentrations in human serum, EDTA plasma and urine. Measurements of cortisol are used in the diagnosis and treatment of disorders of the adrenal gland.

The Nichols Advantage® Cortisol Assay Calibrators are intended for adjustment of the stored curve for the Nichols Advantage Cortisol Assay.

3. Special conditions for use statement(s):

This device is for clinical laboratory use only.

4. Special instrument requirements:

Nichols Advantage® Specialty System

I. Device Description:

The Nichols Advantage Cortisol is composed of the following materials:

One cartridge with the following reagents sufficient for 100 tests:

- One vial containing streptavidin-coated magnetic particles in buffer and preservatives
- One vial containing acridinium ester-labeled rabbit anti-cortisol solution
- One vial containing biotinylated cortisol solution (goat IgG)
- Diluent
- Assay Buffer

- One lot specific Cortisol Master Curve Bar Code Card

Calibrators are required but are sold separately. The two calibrators (with target concentrations are in the dynamic range of 0 µg/dL to 60 µg/dL) are each supplied in four 1.0 mL vials, each containing cortisol in human serum with preservative. The human serum has been tested using FDA approved methods and has been found negative for antibodies to Human Immunodeficiency Virus (HIC I and HIV II) and to Hepatitis C Virus (HCV), as well as for Hepatitis B surface antigen (HBsAg).

J. Substantial Equivalence Information:

1. Predicate device name(s):

Diagnostic Product Corporation Coat-A-Count Cortisol

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

k810891

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for Use	Diagnosis and treatment of disorders of the adrenal gland	Aid in the clinical assessment of adrenal status
Specimens	Serum, EDTA plasma, and urine	Serum, heparinized plasma, and urine
Test Principle	Competitive binding technology	Same

Differences		
Item	Device	Predicate
Binding Technology	Magnetic particles – avidin coated	Antibody coated tubes
Sample Size	12 µL	25 µL
Analytical Sensitivity	≤0.8 µg/dL	0.2 µg/dL

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

NCCLS EP5-A (Evaluation of Precision Performance of Clinical Chemistry Devices)
NCCLS EP7-P (Interference Testing in Clinical Chemistry)
NCCLS EP9-A (Method Comparison)

L. Test Principle:

The Nichols Advantage Cortisol Assay is a one-site chemiluminescence competitive binding assay. Biotinylated-cortisol and the cortisol in the sample compete for a limited amount of chemiluminescent labeled antibody. After an initial incubation period, streptavidin coated magnetic particles are added to the reaction mixture and allowed to incubate, providing separation of biotinylated-cortisol bound to antibody onto the solid phase. Unbound labeled antibody is separated by aspiration of the reaction mixture and subsequent washing. The wells containing the washed magnetic particles are transported to the system luminometer, which injects Triggers 1 and 2, initiating the chemiluminescent reaction. The luminometer quantitates the light and expresses it as relative light unit (RLU). The amount of bound labeled antibody is inversely proportional to the concentration of cortisol in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The within-run and total precision for the Nichols Advantage Cortisol was estimated using the NCCLS EP5-A method (Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline). The data represent one run per day over 21 days for three human serum pools and three commercially available cortisol controls run in duplicate. The study was performed on a single system.

Sample	Mean (ng/mL)	Within-Run		Total Precision	
		SD	%CV	SD	%CV
Pool 1	9.0	0.4	4.4	1.0	11.1
Pool 2	13.7	0.5	3.6	1.0	7.3
Pool 3	35.2	1.3	3.7	3.0	8.5
Control 1	2.3	0.2	8.7	0.4	17.4
Control 2	17.0	0.7	4.1	1.2	7.1
Control 3	28.9	1.1	3.8	2.5	8.7

The within-run and total precision for the urinary cortisol extraction method was estimated using the NCCLS EP5-A method. The data represent one run per day over 21 days for three urine samples assayed in duplicate. The study was performed on a single instrument.

Sample	Mean (µg/dL)	Within-Run		Total Precision	
		SD	%CV	SD	%CV
Sample A	2.2	0.1	4.5	0.3	13.6
Sample B	11.2	0.4	3.6	2.0	17.9
Sample C	25.4	0.8	3.1	3.3	13.0

b. Linearity/assay reportable range:

Parallelism

Samples with varying concentrations of cortisol were diluted on-board with sample diluent and assayed in duplicate. Results demonstrate good linearity across the range of the assay.

Sample	Dilution	Observed (µg/dL)	% Recovery
A	Undiluted	15.2	97
	1:10	14.8	
B	Undiluted	21.4	94
	1:10	20.2	
C	Undiluted	28.4	104
	1:10	29.6	
D	Undiluted	39.0	107
	1:10	41.8	
E	Undiluted	48.6	99
	1:10	48.2	
F	Undiluted	57.7	102
	1:10	58.8	

Urine samples were extracted following the normal protocol. The reconstituted extracts were diluted with Nichols Advantage Cortisol Sample Diluent and assayed in duplicate.

Sample	Dilution	Observed (µg/dL)	% Recovery
A	Undiluted	46.0	
	1:2	21.3	93
	1:4	11.2	97
	1:8	5.4	93
B	Undiluted	36.7	
	1:2	19.4	105
	1:4	9.7	105
	1:8	4.1	89
C	Undiluted	36.4	
	1:2	18.6	103
	1:4	10.0	110
	1:8	4.9	107

Recovery

Three sets of a high and a low/normal sample were mixed in 1:2, 1:1, and 2:1 ratios and assayed in duplicate. The recoveries were determined from the undiluted results. The results demonstrate good recovery of cortisol in patients' samples (97-109% recovery).

A high and low urine sample was extracted and assayed in duplicate. In addition, the reconstituted extracts from the high and low urine samples were mixed in 2:1, 1:1, and 1:2 ratios and assayed in duplicate. The results demonstrate good recovery (88-107%).

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

The cortisol standards are prepared analytically after UV spectrophotometric calibration. Calibrator A and Calibrator B make up two points on the Master Curve. New calibrator values are obtained after testing against a previous batch or lot of released standards and calibrators, and also against primary standards. Generally, at least 30 assays are run to obtain calibrator values. The mean value, their precision, and their target value must be within specification before they are released for distribution. The assigned values are lot-specific.

The standards/calibrators are stable until the expiration date on the vials when stored at 2-8°C. The calibrators were heat spiked for up to 8 days at 37°C (equivalent to 12 months of extrapolated stability), and up to 4 days at 45°C (equivalent to 12 months of extrapolated stability). The heat spiked standards were compared to an identical set of standards stored at -20°C (control set). After the heat spike, the control set and the test set were run in parallel. The heat spiked standards were used to estimate the concentration of controls and QC pools. The heat stressed standards yielded an average RLU recovery of 108% recovery at 37°C and 105% RLU recovery at 45°C. The controls and QC pools were within acceptable limits, indicating the standards maintained their full potency after 8 days or 4 days of heat stress testing.

d. Detection limit:

The analytical sensitivity ($\leq 0.8 \mu\text{g/dL}$) of the assay is defined as the smallest single value which can be distinguished from zero with 95% confidence.

e. Analytical specificity:

The specificity of the cortisol antibody was measured against several naturally occurring steroids and glucocorticoid drugs that may be present in patient samples. Several levels of each cross reactant were spiked into the Sample Diluent, and the response was determined in $\mu\text{g/dL}$. The greatest observed cross reactivities were 56% for prednisolone, 23% for 6- α -

methylprednisolone, 20% for prednisone, 13% for 11-deoxycortisol, and 7% each for corticosterone and 21-deoxy-cortisol. The greatest observed cross reactivities for the other compounds ranged from undetectable to 1.6%.

Interference testing using hemoglobin, triglycerides, bilirubin, and albumin was performed up to the concentrations recommended in the NCCLS guideline EP7-P (Interference Testing in Clinical Chemistry). Each substance was spiked into a serum pool in which baseline cortisol was determined. The spiked pool was then assayed in duplicate and the recovery of cortisol was determined. Results indicate no significant interference from hemoglobin up to 5000 mg/dL, triglycerides up to 3000 mg/dL, bilirubin up to 20 mg/dL, or albumin up to 6 g/dL. Recoveries ranged from 85% to 111%.

Interference testing with albumin and creatinine was performed by spiking these substances into a 24-hour urine sample. Results indicate that albumin up to 3000 mg and creatinine up to 20 g have no significant effect in the assay. Recoveries ranged between 98% and 105%.

Extraction efficiency was determined by spiking known quantities of cortisol into three urine samples and measuring the recovery of the spiked cortisol. Specifically, cortisol standard was spiked in quadruplicate into a urine sample, extracted and assayed in duplicate. Samples 1, 2, and 3 resulted in recoveries of 99%, 95%, and 106%, respectively, each representing the average of 8 replicates.

f. Assay cut-off:

The functional sensitivity, the concentration with a coefficient of variation (CV) not to exceed 20%, is ≤ 2.0 $\mu\text{g/dL}$ in serum or plasma.

2. Comparison studies:

a. Method comparison with predicate device:

The Nichols Advantage Cortisol Assay was compared to a commercially available cortisol RIA method. One hundred and fifty (150) serum samples were assayed by both methods. The range of values observed with the commercial RIA method was 1.9-68.2 $\mu\text{g/dL}$; and the range of values observed with the Nichols Advantage Cortisol method was 2.8-49.5 $\mu\text{g/dL}$. Deming regression analysis yielded an equation of $y = 0.70x + 2.5$ (95% confidence intervals of the slope and intercept were 0.67 to 0.73 and 1.9 to 3.2, respectively). Pearson's correlation coefficient of the paired data was $r = 0.97$ (95% confidence interval was 0.96 to 0.98).

b. Matrix comparison/Sample Tube Types comparison:

To evaluate similarities and differences between different specimen types, blood was collected from 30 normal volunteers into Becton Dickinson brand Vacutainer™ blood collection tubes : plain red top, heparinized, EDTA, and serum separator (SST™) Vacutainer™ tubes. Data analysis indicates good agreement between the plain red top, SST™, and EDTA plasma tubes. On average, heparinized plasma will yield results that are 7-15% lower than serum (SST™ and plain) or EDTA plasma.

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:

Serum samples were obtained between 8 a.m. and 10 a.m. from fifty-eight (58) healthy fasting adults (24 males and 34 females) age 18-66 years. To evaluate diurnal variation the 4-6 p.m. sera were obtained from the same cohort. Each individual had a normal serum chemistry panel and complete blood count, were prescription medication free, and had normal blood pressure. Serum cortisol results were normally distributed after log transformation and the 95% confidence intervals were determined.

8 -10 a.m. 6.5 – 26 µg/dL

4 - 6 p.m. 3.5 – 15 µg/dL

Twenty-four hour urine was collected from 81 (41 females and 40 males) apparently healthy normal adults 18 to 78 years of age. Serum creatinine and BUN were normal in all individuals. None of the females were pregnant, taking birth control pills, or on estrogen treatment and none were on medications known to affect adrenal function. After square root transformation of the data, the 95%

confidence interval for normal 24-hour urinary cortisol results was as follows:
8 – 77 µg/24 hours.

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.