

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

A. 510(k) Number:

k051733

B. Purpose for Submission:

New Device

C. Measurand:

Cortisol, Salivary

D. Type of Test:

Quantitative Enzyme Immunoassay

E. Applicant:

DRG International, Inc.

F. Proprietary and Established Names:

DRG Salivary Cortisol ELISA KIT

G. Regulatory Information:

1. Regulation section:

21 CFR §862.1205, Enzyme Immunoassay Cortisol, Salivary

2. Classification:

Class II

3. Product code:

NHG

4. Panel:

75 (Chemistry)

H. Intended Use:

1. Intended use(s):

See Indications for Use below.

2. Indication(s) for use:

The DRG Salivary Cortisol ELISA Test is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of active free cortisol (hydrocortisone and hydroxycorticosterone) in saliva. Measurements of cortisol are used in the diagnosis and treatment of disorders of the adrenal gland.

3. Special conditions for use statement(s):

For prescription use.

4. Special instrument requirements:

Calibrated EIA reader adjusted to read at 450 nm, Precision pipettes (100 and 200 µl), Distilled or Deionized water, Timer (60 min. range), Reservoirs (disposable), Test tube or micro-tube rack in a microplate configuration, Linear-linear graph paper or software for data reduction.

I. Device Description:

The DRG Salivary Cortisol ELISA Test consists of Microtiter plate, 8 well snap-off strips, 12 strips, coated with rabbit anti-Cortisol antiserum. Reference Standard Set, 1 ml each, 0.0; 2; 5; 10; 20; 40; 80 ng/ml. Enzyme-Conjugate, 26 ml, Cortisol conjugated to horseradish peroxidase. Substrate Solution –TMB, 25 ml. Stop Solution, and Wash Solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Salimetrics High Sensitivity Salivary Cortisol EIA

KMI/ IBL Cortisol LIA

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

k031348 - Salimetrics

k010790 - KMI/ IBL

3. Comparison with predicate:

The DRG Salivary Cortisol Test is substantially equivalent to the Salimetrics HS Salivary Cortisol EIA (k031348). An additional comparison study was performed versus the KMI Diagnostics, Inc. Cortisol LIA method (k010790).

Comparison table for new device compared to the predicate devices

Item	Predicate Device	Predicate Device	New Device
Device Name	Salimetrics HS Salivary Cortisol EIA (k031348)	KMI/ IBL Cortisol LIA (k010790)	DRG Salivary Cortisol ELISA (k051733)
Analyte	Active Free Cortisol	Active Free Cortisol	Active Free Cortisol
Specimens	Saliva	Saliva and Serum	Saliva
Method	Enzyme immunoassay	Luminescent immunoassay	Enzyme immunoassay
Test Principle	Cortisol in the sample competes with Cortisol-enzyme conjugate for binding sites to antibody bound to a microwell. Unbound components are washed away and bound cortisol-enzyme is measured by a colored reaction with the TMB substrate.	Same except, cortisol-peroxidase is measured by a chemiluminescent reading.	Same as cortisol-peroxidase measured by a colored reaction with the TMB substrate.
Detection	Colorimetric microplate reader	Luminometer	Colorimetric reader
Calculation	Quantitative determination with standard curve	Quantitative determination with standard curve	Quantitative determination with standard curve
Quality Control	Recommended	Recommended	Recommended
Indications for use	Measurements of cortisol are used in the diagnosis and treatment of disorders of the adrenal gland.	Measurements of cortisol are used in the diagnosis and treatment of disorders of the adrenal gland.	Measurements of cortisol are used in the diagnosis and treatment of disorders of the adrenal gland.
Expected Values (Normal Range)	0.094 – 1.551 µg/dL	0.5 – 1.5 µg/dL	0.12 – 1.47 µg/dL
Detection limit	0.007 µg/dL	0.015 µg/dL	0.104 µg/dL

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

None referenced

L. Test Principle:

The DRG Salivary Cortisol ELIA KIT is based on the competitive principle and the microplate separation. An unknown amount of Cortisol present in the sample and a fixed amount of Cortisol conjugated with horse-radish peroxidase compete for the binding sites of rabbit polyclonal Cortisol-antiserum coated onto the wells. After one hour incubation the microplate is washed to stop the competition reaction. After addition of the substrate solution the concentration of Cortisol is inversely proportional to the optical density measured.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The Intra-Assay variation was determined by replicate measurements of 4 saliva samples using DRG ELISA kit. The within assay variation is shown below:

	Sample 1	Sample 2	Sample 3	Sample 4
Mean (ng/mL)	3.21 ng/mL	19.09 ng/mL	32.51 ng/mL	1.19 ng/mL
SD	0.188	1.085	1.806	0.083
CV (%)	5.85	5.68	5.55	6.96
n =	20	20	20	20

The Inter-Assay (between-run) variation was determined by quadruplicate measurements of commercial control samples in three different day runs. The inter-assay variation is shown below:

Mean	24.29 ng/mL	40.85 ng/mL
SD	1.81 ng/mL	2.38 ng/mL
CV (%)	7.47	5.82
n =	12	12

The Inter-Lot (between-lot) variation was determined by duplicate measurements of five saliva samples in three different kit lots. The between run variability is shown below:

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean	2.17 ng/mL	14.01 ng/mL	22.85 ng/mL	1.74 ng/mL	2.03 ng/mL
SD	0.12 ng/mL	1.17 ng/mL	1.44 ng/mL	0.13 ng/mL	0.15 ng/mL
CV (%)	5.50	8.32	6.29	7.56	7.43
n =	6	6	6	6	6

b. Linearity/assay reportable range:

Three samples (saliva) containing different amounts of analyte were serially diluted 1:16 with zero standard and assayed with the DRG ELISA. The percentage recovery was calculated by comparing the expected and measured values for the SLV cortisol. An assay linearity of 1.04 – 77 ng/mL has been identified as the usable range. Samples above this range must be diluted and re-run.

		Sample 1	Sample 2	Sample 3
Concentration	ng/mL	33.13	80.0	24.32
Average Recovery %		107	99.1	97.5
Range of	from	101.1	97.8	90.8
Recovery %	To	114.1	99.6	104.4

The Linearity study has been expanded to include additional higher samples to yield an upper detection level. As can be seen in the Table below the test is linear through to the highest Calibrator. Previous data demonstrated the lowest level of detectability.

Upper Linearity Study

In order to identify the upper level of detectability, three (3) native saliva samples, containing different amounts of analyte, were spiked with purified cortisol to obtain a starting level (undiluted) around and above the highest Calibrator. The spiked saliva were serially diluted with zero standard and assayed to determine upper end of detectability with the DRG ELISA. The percentage recovery was calculated by comparing the expected and measured values for SLV cortisol.

Percentage recovery is calculated as follows:

$$\frac{\text{Measured values}}{\text{Expected values}} \times 100$$

Spiked saliva Sample	Dilution	Measured OD mean of duplicate (450 nm)	Measured Conc. Cortisol Saliva ng/mL	Expected Conc Cortisol Saliva ng/mL	Recovery (%)
Sample 1	undil	0.197	33.13	33.13	
	1 : 2	0.320	17.04	16.57	102.9
	1 : 4	0.518	8.37	8.28	101.1
	1 : 8	0.744	4.43	4.14	107.0
	1:16	1.016	2.14	2.07	103.4
	1:32	1.227	1.12	1.04	108.2
	1:64	1.380	0.59	0.52	114.1
	1:128	1.486	0.29	0.26	112.3
Sample 2	undil	0.066	80.00		
	1 : 2	0.084	80.00		
	1 : 4	0.111	73.36		
	1 : 8	0.183	36.47	36.68	99.4
	1:16	0.305	18.22	18.34	99.3
	1:32	0.490	9.13	9.17	99.6
	1:64	0.740	4.48	4.58	97.8
	sample3	undil	0.247	24.32	24.32
1 : 2		0.394	12.68	12.16	104.3
1 : 4		0.612	6.35	6.08	104.4
1 : 8		0.917	2.81	3.04	92.4
1:16		1.149	1.45	1.52	95.4
1:32		1.348	0.69	0.76	90.8

	Sample 1	Sample 2	Sample 3
Concentration ng/mL	33.13	80.00	23.23
Average % Recovery	107.0	99.1	97.5
Range of % Recovery from	101.1	97.8	92.4
% Recovery to	114.0	99.6	104.4

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

The calibrators are buffer based (artificial saliva matrix). The calibrators were prepared by appropriate dilution from the maximum standard (Smax.: 80 ng/mL). The Cortisol for the Calibrators was purchased from a commercially available source and is weighed in to make the 80 ng/mL.

The reference values (calibrators/controls) were established using (Gas chromatography-mass spectrophotometry) methods as per the guidelines

for quality assurance in medical laboratories, Instand E.V. Germany (L.D. Dikkesche. Et al. 1988: De toepassing van gaschromatografie-massaspectrometrie als referentiemethode in kwaliteitcontroleprogramma's voor progesterone-, cortisol-, testosterone- en oestradiolbepalingen in serums. Tijdschr NVKC 13: 148-155).

WHO standard is not available.

The functional quality of the kit lots were tested using the Lyphocheck controls from BioRad, but controls are not included in the kit. These controls are commercially available and can be purchased by the customers.

d. Detection limit:

The analytical sensitivity of the DRG ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Zero Standard (S₀).

Standard curve:

Standard	Conc. ng/mL	OD ₄₅₀ mean of duplicate
S0	0	2.05
S1	2,0	1.24
S2	5,0	0.85
S3	10,0	0.55
S4	20,0	0.33
S5	40,0	0.20
S6	80	0.11

Controls:

	Conc. ng/ml	Acc. Range.
Lyphocheck 142/10	21.518	15.5 –36.3
Lyphocheck 143/10	41.437	22.1 – 51.6

Replicate	OD ₄₅₀ of S ₀
1	1.940
2	1.984
3	1.945
4	1.901
5	1.866
6	1.816
7	1.850
8	1.795
9	1.963
10	1.989
11	1.999
12	1.950
13	1.975
14	1.991
15	1.927
16	1.927
17	2.066
18	2.078
19	1.818
20	1.600

Mean =	1.919
SD =	0.108
2xSD =	0.216
Mean - 2xSD	1.703 = 0.537 ng/mL
N =	20.00

e. Analytical specificity:

Cross-reactivity was tested with the following compounds whose chemical structure could potentially cause interference with the SLV cortisol ELISA. The specificity of the antiserum used for the ELISA was evaluated by determination of the cross-reactivity at 50% displacement of various compounds listed in the table.

The cross reactivity is defined as:

$$\frac{\text{Concentration of cortisol at 50\% B/BO}}{\text{Concentration of cross-reactant giving 50\% B/BO}} \times 100$$

Steroid	% Cross Reaction
Cortisol	100%
Corticosterone	29%
Cortisone	3.00%
11-Deoxycortisol	< 1,00%

17-OH Progesterone	< 0,50%
Prednisone	< 0, 10%
Progesterone	< 0, 10%
Dexamethasone	< 0, 10%
Desoxycorticosterone	< 0, 10%
Dehydroepiandrosterone sulfate	< 0, 10%
Estradiol	< 0, 10%
Estriol	< 0, 10%
Estrone	< 0, 10%
Testosterone	< 0, 10%

f. Assay cut-off: Not Applicable for this type of device.

2. Comparison studies:

a. Method comparison with predicate device:

Two studies were performed to evaluate the performance of the SLV Cortisol Saliva ELISA versus two commercially available saliva Cortisol kits.

One study evaluated saliva samples from 41 male and female subjects between ages 40 – 70 years. The samples were run in duplicate on the DRG test and another commercially available LIA method to determine the concentration of Cortisol in the samples. An overall correlation of 0.9876 and a regression formula of $y = 0.9726x + 0.095$ was obtained versus this method. The samples ranged in concentration from 3.10 to 13.01 ng/mL.

A second study was performed using 30 saliva samples collected from men and women ages 40 – 65 years and run in duplicate on DRG and another commercially available EIA test. A correlation of 0.95742 with a regression formula of $y = 0.9812x + 0.1515$ was observed. The samples ranged in concentration from < 0.5 to 11.86 ng/mL.

b. Matrix comparison:

Not applicable since this assay is for use with saliva samples only.

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):

4. Clinical cut-off:

Not applicable for this type of device

5. Expected values/Reference range:

109 saliva samples from apparently health adult male and female subjects, ranging in age from 20 to 80 years were collected in the morning and analyzed using the DRG SLV Cortisol ELISA kit. The salivary cortisol concentration did not show any significant differences based on age. Hence the normal range was calculated for the entire group. The normal range of salivary cortisol analyzed using DRG SLV Cortisol ELISA Test.

Adults: 0.12 – 1.47 µg/dL or 1.2 – 14.7 ng/mL (AM collection)

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.