

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

A. 510(k) Number:

k062534

B. Purpose for Submission:

New Device

C. Measurand:

17 α -Hydroxyprogesterone

D. Type of Test:

Quantitative

E. Applicant:

DRG International, Inc.

F. Proprietary and Established Names:

Salivary 17 α -OHP Elisa

G. Regulatory Information:

1. Regulation section:

21 CFR 862.1395

2. Classification:

Class I, but meets the limitation to the exemption outlined in 862.9(c)(2)

3. Product code:

JLX

4. Panel:

75 Chemistry

H. Intended Use:

1. Intended use(s):

See indications of use statement below.

2. Indication(s) for use:

An enzyme immunoassay for the quantitative in vitro diagnostic measurement of active free 17-hydroxyprogesterone in saliva. Measurements of 17-hydroxyprogesterone are used as an aid in the diagnosis and treatment of various disorders of the adrenal glands or the ovaries, and as an aid in the diagnosis of late onset of 21-hydroxylase deficiency, a common cause of Congenital Adrenal Hyperplasia. This test is not intended for newborn screening.

3. Special conditions for use statement(s):

For prescription use only. This test is not intended for newborn screening.

4. Special instrument requirements:

A microtiter plate calibrated reader.

I. Device Description:

This DRG Salivary 17 α -OHP Elisa kit contains a 96 well microtiter plate coated with anti-17 α -OHP rabbit antibody, 6 vials of ready-to-use standards (levels 0 to 5) and two vials of controls (1 mL each). The kit also contains 1 vial (26 mL) of enzyme conjugate, one vial of substrate solution, one vial of stop solution and one vial of wash solution. The standards are used to calculate a typical standard curve and their concentrations are 0, 10, 50, 250, 500 and 1000 pg/mL.

J. Substantial Equivalence Information:

1. Predicate device name(s):

BIO-RAD microplate 17-Hydroxyprogesterone Test.

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

k973350

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Methodology	Enzyme Immunoassay	Radio Immunoassay
Calculation	Quantitative determination with standard curve	Same
Indications for Use	Measurements of 17-OHP are used as an aid in the diagnosis and treatment of disorders of the adrenal gland for Congenital Adrenal Hyperplasia	Same

Differences		
Item	Device	Predicate
Matrix	Saliva	Serum
Analyte	Unbound 17-OHP	Total 17-OHP
Quality Control	Included with the device	Not included with the device
Detection limit	3.6 to 1000 pg/mL	2.0 to 208 ng/mL

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

None referenced.

L. Test Principle:

The DRG Salivary 17 α -OHP ELISA kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a polyclonal antibody (rabbit) directed towards an antigenic site on the 17 α -OHP molecule. Endogenous 17 α -OHP of a patient sample competes with a 17 α -OHP - horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of 17 α -OHP in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Eight saliva samples were run in duplicate for 10 days to evaluate inter-assay precision using the DRG Salivary 17-OHP ELISA kit. The mean concentrations, SD and CV are located on the chart below.

Eight saliva samples were run in triplicate from three different DRG Salivary 17-OHP ELISA kits to evaluate inter-lot precision. The mean concentrations, SD and CV are shown in the chart below.

Eight saliva samples were run in 20 replicates using the DRG Salivary 17-OHP ELISA kit to evaluate intra-assay precision. The mean concentrations, SD and CV are shown in the chart below.

Inter-assay	Sample							
N	20	20	20	20	20	20	20	20
Mean (pg/mL)	9.39	15.80	50.68	57.05	101.08	211.8	512.52	800.21
SD (pg/mL)	0.97	1.34	1.71	2.60	7.59	8.30	10.32	38.16
CV (%)	10.37	8.48	3.37	4.57	7.51	3.92	2.01	4.77
Inter-Lot	Sample							
N	9	9	9	9	9	9	9	9
Mean (pg/mL)	10.16	16.95	44.32	52.16	100.38	205.26	515.73	798.12
SD (pg/mL)	1.26	0.84	1.50	2.97	6.14	3.64	19.50	46.82
CV (%)	12.37	4.98	3.38	5.69	6.12	1.77	3.78	5.87
Intra-assay	Sample							
N	20	20	20	20	20	20	20	20
Mean (pg/mL)	11.77	24.19	37.46	63.32	90.25	212.96	447.44	820.43
SD (pg/mL)	0.97	1.93	1.36	4.61	4.47	10.80	17.78	28.96
CV (%)	8.26	7.96	3.64	7.28	4.95	5.07	3.97	3.53

b. *Linearity/assay reportable range:*

The sponsor conducted five separate recovery studies to validate the reportable range of this device (3.6 to 1000 pg/mL).

Three saliva samples containing different amounts of analyte were serially diluted with zero standard and assayed with the DRG Elisa. The percentage recovery was calculated by comparing the expected and measured values for saliva 17-OHP.

Sample	Dilutions Factor	Measured Conc. 17 OHP (pg / ml)	Expected Conc 17 OHP (pg / ml)	Recovery (%)
1 SLV	undil	96.63	96.63	
	1:2	47.82	48.32	99.0
	1:4	22.96	24.16	95.0
	1:8	11.66	12.08	96.5
	1:16	5.95	6.04	98.5
	1:32	2.60	3.02	86.1
2 SLV	undil	39.31	39.31	
	1:2	20.32	19.66	103.4
	1:4	10.56	9.83	107.4
	1:8	4.28	4.91	87.2
3 SLV (spiked)	undil	194.88	194.88	
	1:2	97.06	97.44	99.6
	1:4	47.38	48.72	97.2
	1:8	23.19	24.36	95.2
	1:16	12.98	12.18	106.6
	1:32	5.57	6.09	91.5
	1:64	3.44	3.04	113.2

Recovery of the DRG ELISA was determined by adding increasing amounts of the analyte to three different saliva samples containing different amounts of endogenous analyte. Each sample was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recovery was calculated by comparing the expected and measured values for saliva 17-OHP.

Sample	Endogenous 17OHP pg/ml	Added 17OHP pg/ml	Measured 17OHP pg/ml	Expected 17OHP pg/ml	Recovery (%)
Saliva 1	824.11	0	824.11	824.11	100.0
		500.00	>1000	1324.11	75.5
		250.00	>1000	1074.11	93.1
		125.00	984.92	949.11	103.8
		25.00	831.65	849.11	97.9
		5.00	821.86	829.11	99.1
Saliva 2	282.83	0	282.83	282.83	100.0
		500.00	773.64	782.83	98.8
		250.00	583.64	532.83	109.5
		125.00	400.61	407.83	98.2
		25.00	333.71	307.83	108.4
		5.00	303.89	287.83	105.6
Saliva 3	150.09	0	150.09	150.09	100.0
		500.00	595.02	650.09	91.5
		250.00	361.33	400.09	90.3
		125.00	249.18	275.09	90.6
		25.00	166.72	175.09	95.2
		5.00	161.79	155.09	104.3

Two saliva samples were spiked with 5000 and 100,000 pg/mL 17-OHP and were serially diluted with zero standard and assayed with the DRG ELISA. The percentage recovery was calculated by comparing the expected and measured values for the analyte.

Sample	Dilution	Measured Conc. 17OHP pg/ml	Expected Conc 17OHP pg/ml	Recovery (%)
Saliva1	1 : 1	>1000	100000.00	
	1 : 10	>1000	10000.00	
	1 : 50	>1000	2000.00	
	1:60	>1000	1666.67	
	1:70	>1000	1428.57	
	1:80	>1000	1250.00	
	1:90	990.19	1111.11	89.1
	1:100	903.18	1000.00	90.3
	1:110	830.74	909.09	91.4
	1:120	771.45	833.33	92.6
	1:130	713.54	769.23	92.8
	1:140	653.95	714.29	91.6
	1:150	617.66	666.67	92.6
	1:300	299.60	333.33	89.9
	1:600	147.01	166.67	88.2
1:1000	92.89	100.00	92.9	
Saliva2	1:1	>1000	5000.00	
	1:2	>1000	2500.00	
	1:4	>1000	1250.00	
	1:5	939.71	1000.00	94.0
	1:6	825.98	833.33	99.1
	1:8	575.74	625.00	92.1
	1:10	469.95	500.00	94.0
	1:20	237.84	250.00	95.1

To evaluate the lower end of the DRG ELISA assay, recovery was assessed by adding increasing amounts of analyte to three different saliva samples containing different amounts of endogenous analyte. The samples were assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples.

Sample	Endogenous 17-OH-P pg/ml	Added 17-OH-P pg/ml	Measured Conc. SLV 17-OH-P pg/ml	Expected conc pg/ml	Recovery (%)
1	6.16	0.00	6.16		
		25.00	30.64	31.16	98.3
		125.00	133.10	131.16	101.5
		250.00	257.05	256.16	100.3
		500.00	540.11	506.16	106.7
2	17.24	0.00	17.24		
		25.00	42.13	42.24	99.7
		125.00	141.57	142.24	99.5
		250.00	271.54	267.24	101.6
		500.00	522.08	517.34	100.9
3	45.86	0.00	45.86		
		25.00	69.32	70.86	97.8
		125.00	178.50	170.86	104.5
		250.00	281.27	295.86	95.1
		500.00	531.52	545.86	97.4

An additional study used three spiked saliva samples containing different amounts of analyte that were serially diluted with zero standard and assayed to determine upper end detectability. The percentage recovery was calculated by comparing the expected and measured 17-OHP values. . The results support the sponsor linearity upper range of 1000 pg/mL.

Spiked saliva Sample	Dilution	Measured Conc. 17-OH-P pg/ml	Expected Conc 17-OH-P pg/ml	Recovery (%)
Sample 1	undil	>1000	1200.00	
	1:1.1	>1000	1090.91	
	1:1.2	945.46	1000.00	94.5
	1:1.3	892.03	923.08	96.6
	1:1.4	867.25	857.14	101.2
	1:1.5	840.91	800.00	105.1
	1:1.8	700.92	666.67	105.1
	1:2	581.18	600.00	96.9
	1:4	310.32	300.00	103.4
	1:8	148.81	150.00	99.2
	1:10	122.27	120.00	101.9
sample2	undil	>1000	1050.00	
	1:1.1	975.68	954.55	102.2
	1:1.2	868.47	875.00	99.3
	1:1.3	796.47	807.69	98.6
	1:1.4	776.87	750.00	103.6
	1:1.5	703.79	700.00	100.5
	1:1.8	580.40	583.33	99.5
	1:2	519.81	525.00	99.0
	1:4	269.38	262.50	102.6
	1:8	122.80	131.25	93.6
	1:10	98.34	105.00	93.7
sample 3	undil	816.64		
	1:1.1	696.17	742.40	93.8
	1:1.2	688.63	680.53	101.2
	1:1.3	600.93	628.18	95.7
	1:1.4	559.15	583.31	95.9
	1:1.5	563.63	544.43	103.5
	1:1.8	427.88	453.69	94.3
	1:2	403.43	408.34	98.8
	1:4	196.07	204.17	96.0
	1:8	96.71	102.08	94.7
1:10	83.72	81.66	102.5	

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

The DRG Salivary 17 α -OHP ELISA test device is traceable to commercially available purified 17-OHP.

The sponsor conducted real time closed stability on two lots for 16 months at storage of 2 and 8 °C. The results support a stability of 16 months and real time stability studies are ongoing.

The sponsor conducted real time open stability of the DRG Salivary 17 α OHP ELISA test. Two control samples were assayed on day 0, 3 weeks, 6 weeks and 12 weeks in duplicate. The results support a stability of 6 weeks when stored at 4 °C.

Three lots of DRG ELISA test that were stored at 37 °C for ten days were used to assay 5 saliva samples. The results were compared with the values for the 5 samples obtained by the corresponding lots that were stored at 4 °C. The results support the stress stability conditions of 37 °C for ten days.

d. Detection limit:

The sponsor conducted a limit of the blank (LoB) study in which the zero standard was assayed 20 times to assess the lowest level detectable by the assay. The sponsor calculated the LoB by subtracting 2 standard deviations from the mean of the 20 replicates of the zero standard. The LoB was 2.513 pg/mL.

The functional sensitivity was calculated by measuring two saliva samples 20 times and determining the concentration that gives a CV of $\leq 20\%$. The sponsor reports a functional sensitivity of 3.6 pg/mL

e. Analytical specificity:

Cross reactivity of the antiserum used for the ELISA kit was evaluated for various compounds. The percent cross-reactivity is expressed as a ratio of 17-OHP concentration. Equations and % cross-reactivity are below:

$$\text{Cross reactivity} = \frac{\text{Concentration of 17-OHP at 50\% B/B0}}{\text{Concentration of cross-reactant giving 50\% B/B0}} \times 100$$

Steroid	% Cross reaction
17- α -OH Progesterone	100%
Estriol	< 0.01
Estradiol 17 β	< 0.01

Steroid	% Cross reaction
Testosterone	< 0.01
Dihydrotestosterone	< 0.01
DOC	0.05%
11-Desoxycortisol	1.40%
Progesterone	1.20%
DHEA	<0.01
DHEA-S	< 0.001
Cortisol	< 0.01
Corticosterone	< 0.05
Aldosterone	< 0.01
Androstendion	< 0.01
Dehydroepiandrosten sulfate	< 0.01
Prednisone	< 0.01

Sodium azide was studied for its interference with the DRG SLV 17-OHP ELISA on two saliva samples after adding varying concentrations of sodium azide. The sponsor states that concentrations that are greater than 0.02% sodium azide significantly interfere with the test (+/- 15% of the expected results). The sponsor has added the following note to their package insert: “*Note: Samples containing sodium azide should not be used in the assay.*”

The influence of longer and shorter incubation times on the results of SLV 17-OH-P ELISA was determined by measuring three samples using the incubation times (15 minutes) as recommended in the instructions for users (IFU), 20% longer incubation (18 minutes), and 20% shorter incubation (12 minutes) times. The incubation time did not interfere with the expected test results.

The influence of blood contamination in saliva was examined by assaying two saliva samples that were enriched with different concentrations of whole blood, and run in the DRG SLV 17-OHP ELISA. The results demonstrate that blood contamination at or above 0.08% do interfere with test results (result exceeded +/-15% of expected values). Since this level may sometimes be difficult to visualize, the sponsor has placed the following two limitations in the package insert.

- 1) The patient should not eat, drink, chew gum or brush teeth for 30 minutes before sampling. Otherwise rinse mouth thoroughly with cold water 5 min prior to sample collection. Do not collect samples when oral diseases, inflammation, or lesions exist (blood contamination).
- 2) Blood contamination of more than 0.08% in saliva samples will affect results, and usually can be seen by the eye. Therefore, samples containing any visible blood should not be used.

The sponsor does not specifically recommend a saliva collection tube for collection of sample since such devices are commercially available. However, to assess the possible influence of a cotton swab on 17-OHP concentrations, six saliva samples were collected in Salivette tubes (collection with cotton swab) and compared with those collected in Sali tubes (collection through a straw) using DRG SLV 17 -OHP kit. Samples collected in Salivette tubes (cotton swab) revealed artificially high values. This interference is described in the package insert and users are instructed not to use cotton swabs for sampling.

The influence of increased or decreased pipetting volumes on the results of the DRG ELISA was determined by measuring three control samples using the pipetting volumes as prescribed in the instructions for users (IFU) (25 µL), 20% increased volumes (20 µL), and 20% decreased volumes (20 µL). The sample volumes tested do not interfere with the test results (all results were within +/- 15% recovery of the expected results).

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

The sponsor compared the DRG Salivary 17-OHP ELISA test to a well-validated reference LC-MS/MS method for measuring 17-OHP in saliva. Seventy-six non spiked saliva samples from adult normal and CAH-Patients were assayed in parallel with the DRG Salivary ELISA test and the LC-MS/MS reference method. The LC-MS/MS and DRG ELISA values ranged from 10 to 1090 pg/mL and 21 to 945 respectively. The resulting linear equation was $y=1.004x + 3.609$ with a correlation constant of 0.982.

b. Matrix comparison:

Not applicable as this assay is only for saliva.

. 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 sponsor conducted a normal range study that was further supported by published literature. The following several studies measured saliva 10-OHP in children, adults, women and men that were both normal and afflicted with CAH.

A study was conducted to determine the normal range. Salvia samples from 129 normal healthy children (ages 6 to 12) were collected. The values ranged from 3.0 to 32.9 pg/mL. Saliva samples were collected from healthy women with regular cycles and assayed using the DRG Salivary kit. The women (ages 21 to 50) were either in follicular (n=124) or luteal phase (n=128) and the values ranged from 8.2 to 41.1 pg/mL and 28.1 to 84.8 pg/mL respectively. Saliva samples from 132 healthy men (ages 21 to 70) had values ranging from 10.6 to 54.8 pg/mL. The sponsor has placed the following chart in their labeling:

	Age group	N	Mean	S.D.	Range 5-95%
Children	6-12 yrs	129	16.9 pg/mL	9.5	3.0-32.9 pg/mL
Women	21-50 yrs	Follicular phase: n = 124	22.0 pg/mL	11.1	8.2-41.1 pg/mL
	21 - 50 yrs.	Luteal phase: n = 128	51.2 pg/mL	17.3	28.1-84.8 pg/mL
Men	21 – 50 yrs	N = 132	24.9 pg/mL	12.6	10.6-54.8 pg/mL

The sponsor’s normal reference range study above produced similar results to the following results from the literature.

<i>Zerah et al., 1987</i>				
	Age group	Salivary	Serum pg/mL	Ratio sal/ser
Women	Follicular phase: 18 – 42 yrs	17 - 67 pg/mL	381-1925	4.5 – 3.4 %
	Luteal phase: 19 – 38 yrs	47 – 106 pg/mL	1012-3212	4.6- 3.3 %
Men	25 - 46 yrs	24 – 109 pg/mL	636-3496	3.8-3.1%

Zerah, M., Pang, S., and New, M Morning Salivary 17-Hydroxyprogesterone Is a Useful Screening Test for Nonclassical 21-Hydroxylase Deficiency, *J. Clinical Endo. And Metabolism*, Vol. 65, No.2, 1987.

The following serum/saliva normal and abnormal ranges from a matrix method comparison studies were obtained in a sponsor study.

1.) Healthy individuals

40 Children 6 - 12 years adipose

	DRG SLV-Saliva	DRG EIA-Serum
Mean	15.2 pg/ml	0.62 ng/ml
SD	8.9 pg/ml	0.41 ng/ml
Median	13.3 pg/ml	0.55 ng/ml
5% - 95% Percentile	4 - 31 pg/ml	0.08 – 1.32 ng/ml

40 Adults healthy

	DRG SLV-Saliva	DRG EIA-Serum
Mean	24.0 pg/ml	1.2 ng/ml
SD	19.5 pg/ml	1.1 ng/ml
Median	17.6 pg/ml	0.8 ng/ml
5% - 95% Percentile	7 - 59 pg/ml	0.36 – 3.06 ng/ml

2.) Individuals with CAH

57 Children 6 - 12 years afflicted with CAH

	DRG SLV-Saliva	DRG EIA-Serum
Mean	199.8 pg/mL	11.8 ng/mL
SD	172.4 pg/mL	9.5 ng/mL
Median	129.3 pg/mL	8.9 ng/mL
5% - 95% Percentile	28.6 – 526.5 pg/mL	2.2 – 30.2 ng/mL

21 Children 6 - 12 years afflicted with CAH

	DRG SLV-Saliva	DSL RIA Serum
Mean	452.5 pg/ml	32.03 ng/ml
SD	376.0 pg/ml	22.29 ng/ml
Median	344.0 pg/ml	25.45 ng/ml
5% - 95% Percentile	64 - 1390 pg/ml	9.9 – 67.7 ng/ml

39 Adults and Adolescent 12-25 years afflicted with CAH

	DRG SLV-Saliva	DSL RIA Serum
Mean	998.7 pg/ml	61.2 ng/ml
SD	954.0 pg/ml	54.6 ng/ml
Median	782.0 pg/ml	42.6 ng/ml
5% - 95% Percentile	72 - 3083 pg/ml	6.8 - 162 ng/ml

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.