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

A. 510(k) Number:

k052649

B. Purpose for Submission:

Premarket Notification 510(k) of intention to manufacture and market the DRG Salivary Testosterone Elisa Kit.

C. Measurand:

Testosterone

D. Type of Test:

Enzyme Immunoassay

E. Applicant:

DRG International, Inc.

F. Proprietary and Established Names:

DGR Salivary Testosterone Elisa Kit

G. Regulatory Information:

1. Regulation section:

    21 CFR §862.1680 Testosterone Test System

2. Classification:

    Class 1 (reserved)

3. Product code:

    CDZ

4. Panel:

    75 (Chemistry)

H. Intended Use:

1. Intended use(s):

    See Indications for use below.

2. Indication(s) for use:

    An Enzyme Immunoassay for the *in vitro diagnostic* quantitative measurement of free active testosterone in saliva. Measurement of testosterone is used in the diagnosis and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism, delayed or precocious puberty, impotence in males and, in females hirsutism (excessive hair) and virilization (masculinization) due to tumors, polycystic ovaries, and adrenogenital syndromes.

3. Special conditions for use statement(s):

    For Professional use only. For in-vitro diagnostic use only

4. Special instrument requirements:

    Calibrated EIA reader adjusted to read at 450nm.

I. Device Description:

The DRG Salivary Testosterone Elisa Kit consists of the following:

1. Microtiter plate, 8 well snap-off strips, 12 strips, coated with (mouse) anti-Testosterone antiserum.
2. Reference Standard Set, 1 ml each, 0.0; 10; 50; 100; 500; 1000; 5000 pg/ml.
3. Enzyme-Conjugate, 26 ml, Testosterone conjugated to horseradish peroxidase, ready to use.
4. Substrate Solution - TMB, 25 ml, ready to use.
5. Stop Solution, 0.5M H_2SO_4, 14 ml, ready to use.
6. Wash Solution, 30 ml, Concentrate for 1200 ml.

J. Substantial Equivalence Information:

1. Predicate device name(s):

    IMMUNO BIOLOGICAL LABORATORIES, IBL Testosterone LIA

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

    k033786

3. Comparison with predicate:

Similarities		
Item	**Predicate Device**	**New Device**
Device Name	IBL Testosterone LIA	DRG SLV Testosterone ELISA
Analyte	Free active Testosterone	Same
Specimen	Serum or Saliva	Saliva
Method	Luminescence Immunoassay	Enzyme Immunoassay
Test Principle	Competitive Immunoassay. Competition is between a labeled and non-labeled antigen for a fixed number of antibody binding sites. The amount of labeled analyte bound to the antibody is inversely proportional to the concentration of the analyte present in the sample.	Same
Detection	Luminescence detection	Colorimetric detection
Calculation	Quantitative determination with standard curve	Same
Quality Control	2 Controls at different levels	Recommended separate external controls
Indications for Use	Measurements of testosterone are used in the diagnosis and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism, delayed or precocious puberty, impotence in males and , in females hirsutism (excessive hair) and virilization (masculinization) due to tumors, polycystic ovaries, and adrenogenital syndromes.	Same
Detection Limit	1.757 pg/mL	1.857 pg/mL

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

Haeckel, R., R.F. Walker and D. Colic (1989): Reference ranges for mixed saliva collected from literature. J. Clin. Chem. Clin. Biochem. 27, 249-252

L. Test Principle:

The DRG Salivary Testosterone ELISA Kit is based on the competition principle and the microplate separation. An unknown amount of free testosterone present in the sample and a fixed amount of testosterone conjugated with horseradish peroxidase compete for the binding sites of mouse monoclonal testosterone 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 testosterone is inversely proportional to the optical density measured.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

      INTRA-ASSAY PRECISION
      The intra-assay (within-run) variation of the DRG SLV Testosterone ELISA was determined by repeated measurements of four saliva samples.

Measurement	Saliva 1		Saliva 2		Saliva 3		Saliva 4	
	OD_{450}	**Conc. pg/ml**	OD_{450}	**Conc. pg/ml**	OD_{450}	**Conc. pg/ml**	OD_{450}	**Conc. pg/ml**
1	1.789	**2.94**	1.665	**13.82**	1.438	**44.07**	1.539	**6.75**
2	1.785	**3.22**	1.683	**11.99**	1.429	**45.58**	1.561	**5.19**
3	1.816	**1.18**	1.700	**10.35**	1.430	**45.41**	1.542	**9.50**
4	1.778	**3.73**	1.711	**9.32**	1.471	**38.76**	1.579	**4.95**
5	1.791	**2.80**	1.654	**14.97**	1.462	**40.17**	1.540	**9.41**
6	1.802	**2.05**	1.641	**16.37**	1.455	**41.29**	1.560	**6.50**
7	1.789	**2.94**	1.690	**11.31**	1.472	**38.60**	1.538	**9.22**
8	1.795	**2.52**	1.658	**14.55**	1.449	**42.26**	1.557	**8.50**
9	1.769	**4.41**	1.647	**15.72**	1.431	**45.28**	1.562	**5.59**
10	1.791	**2.80**	1.689	**11.40**	1.479	**37.52**	1.563	**6.67**
11	1.785	**3.22**	1.662	**14.13**	1.438	**44.07**	1.574	**8.41**
12	1.815	**1.24**	1.665	**13.82**	1.446	**42.75**	1.550	**5.11**
13	1.786	**3.15**	1.691	**11.21**	1.471	**38.76**	1.156	**8.68**
14	1.792	**2.73**	1.670	**13.30**	1.450	**42.10**	1.202	**5.92**
15	1.775	**3.95**	1.667	**13.61**	1.435	**44.57**	1.159	**7.52**
16	1.772	**4.18**	1.681	**12.19**	1.428	**45.74**	1.156	**7.18**
17	1.771	**4.26**	1.668	**13.51**	1.440	**43.73**	1.144	**5.84**
18	1.801	**2.12**	1.673	**13.00**	1.456	**41.13**	1.173	**5.92**
19	1.810	**1.54**	1.682	**12.09**	1.467	**39.38**	1.161	**7.61**
20	1.794	**2.59**	1.681	**12.19**	1.440	**43.73**	1.202	**6.67**

	Saliva 1	Saliva 2	Saliva 3	Saliva 4
Mean (pg/ml)	2.88	12.94	42.25	7.06
SD	0.946	1.787	2.655	1.485
CV (%)	32.87	13.81	6.28	21.04
n =	20	20	20	20

The functional sensitivity of the assay is 7.1 pg/mL. This assumes that the lowest concentration having a CV% of approximately 20% is considered the functional sensitivity.

INTER ASSAY PRECISION: LOT TO LOT

The inter-assay (between-run) variation was determined by triplicate measurements of five saliva samples in three different kit lots. Results reported in the table below:

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean (pg/ml)	64.5	352.89	517.65	44.00	116.54
SD (pg/ml)	3.77	13.44	15.01	1.53	5.00
CV (%)	5.85	3.81	2.90	3.47	4.29
n =	9	9	9	9	9

b. *Linearity/assay reportable range:*

Two saliva samples containing different amounts of analyte were serially diluted with zero standard and assayed using DRG SLV testosterone ELISA. Percentage recovery was calculated by comparing the expected and observed values for SLV testosterone.

Sample	Dilution	OD 450 nm	Observed conc. pg/ml	Expected Conc. pg/ml	Recovery %
1(spiked)	undiluted	0.113	>5000	9000	
	1:2	0.139	4410.07	4500.00	98.00
	1:4	0.247	2285.54	2250.00	101.58
	1:8	0.435	1108.65	1125.00	98.55
	1:16	0.670	582.77	562.50	103.60
	1:32	1.025	265.73	281.30	94.46
	1:64	1.350	132.78	140.60	94.44
	1:128	1.622	67.89	70.30	96.57

Sample	Dilution	OD 450 nm	Observed conc. pg/ml	Expected Conc. pg/ml	Recovery %
2 (spiked)	undiluted	0.457	1033.58	1033.58	
	1:2	0.730	505.48	516.79	97.81
	1:4	1.030	262.95	258.40	101.76
	1:8	1.385	122.69	129.20	94.96
	1:16	1.660	60.89	64.60	94.26
	1:32	1.855	30.84	32.30	95.48
	1:64	1.985	15.46	16.15	95.73
	1:128	2.125	2.78	8.07	34.45

	Sample 1	Sample 2
Concentration pg/ml	9000.00	1033.58
Average % recovery	98.20	96.70
Range of from	94.4	94.3
% recovery to	103.6	101.7
Accepted from	85%	85%
recovery to	115%	115%

The upper end detectability of SLV testosterone is **4410 pg/m**L. These results demonstrate that the test system is performing equivalent to original.

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

The standards prepared for the SLV Testosterone kits are buffer based (artificial saliva matrix). The range of calibrators were prepared by appropriate dilution from the maximum standard (Smax: 5000 pg/mL). The testosterone for the standards is purchased from a commercially available source, and is weighed in to make the 5000 pg/mL.

The reference values (calibrators/controls) were established using (Gas chromatography-mass spectrophotometry) GC-MS methods, as per the guidelines for quality assurance in medical laboratories, Instand E.V. Germany (L.D. Dikkesche. Et al. 1988: De toepassing kwaliteitcontroleprogramma's voor progesterone-, cortisol-, testosterone- en oestradiolbe[alingen in serums. Tijdschr NVKC 13: 148-155).

WHO standard is not available.

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

Real time stability, Accelerated stability, and Saliva sample stability were validated for this assay.

d. *Detection limit:*

The analytical sensitivity of SLV Testosterone ELISA was calculated from the mean minus 2SD of 20 replicate analyses of the zero standard.

Mean	1.718
SD	0.018
2 x SD	0.035
Mean – 2SD	1.683 **corresponds to 1.857 pg/ml**
N	20

e. *Analytical specificity:*

Cross reactivity was tested with the following compounds whose chemical structure could potentially cause interference with the SLV Testosterone 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 below.

The cross-reactivity is defined as:

Concentration of testosterone at 50% B/BO   x 100
Concentration of cross-reactant giving 50% B/BO

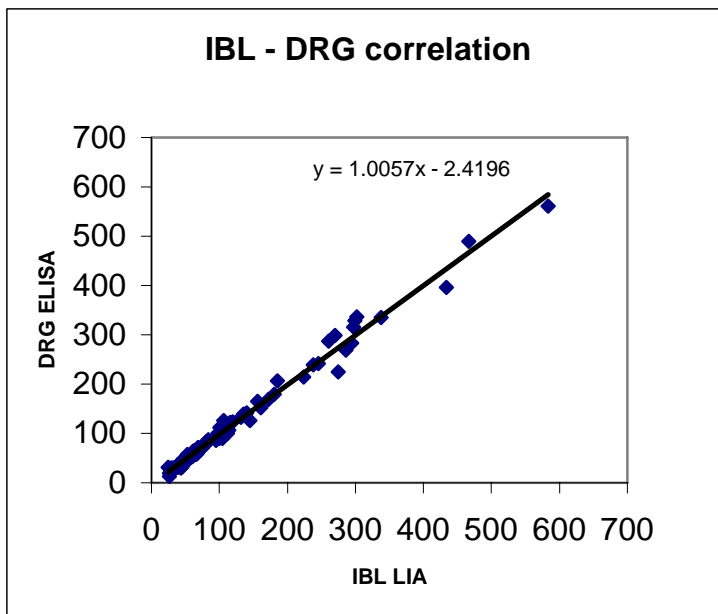| Steroid	% Cross reaction
Testosterone	100%
5α-Dihydrotestosterone	0.80%
Androstenedione	0.90%
11β-hydroxysterone	3.30%
17α-methyltestosterone	0.10%
19-Nortestosterone	3.30%
Epitestosterone	0.10%
Estradiol	0.10%
Progesterone	< 0, 10%
Cortisol	< 0, 10%
Estrone	< 0, 10%
Danazol	< 0, 10%

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

COMPARISON TO IBL LIA

Concentration of testosterone in 81 saliva samples collected from 40 - 65 year old men and women using DRG SLV testosterone kit. The results were compared with those obtained from IBL-LIA method.

Correlation Coefficient = 0.99328

IBL - DRG correlation

$y = 1.0057x - 2.4196$

Number of XY Pairs = 81
Pearson r = 0.9933
95% confidence interval = 0.9895 to 0.9957

R squared = **0.9866**

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):

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

In order to determine the normal range of SLV Testosterone, saliva samples from 187 adult male and 188 adult female apparently healthy subjects, age 21 to 75 years, were collected in the morning and analyzed using the DRG SLV Testosterone ELISA kit. The following range was calculated from this study.

Age Group Years	Men ♂			Women ♀		
	Range (5-95%)	Median	n	Range (5-95%)	Median	n
21-30	47.2-136.2	92.8	42	7.9-50.4	20.8	40
31-40	46.8-106.8	73.6	37	<7.0-44.8	17.1	40
41-50	36.5-82.7	58.8	34	<7.0-39.4	18.3	38
51-60	19.2-89.0	44.5	36	<7.0-29.8	19.2	38
61-75	12.2-68.6	38.9	38	<7.0-29.3	16.0	32

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.