

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

A. 510(k) Number:

K033786

B. Analyte:

Testosterone

C. Type of Test:

Microplate luminescence immunoassay (LIA)

D. Applicant:

Immuno-Biological Laboratories (IBL) - Hamburg

E. Proprietary and Established Names:

IBL Testosterone LIA

F. Regulatory Information:

1. Regulation section:
21 CFR § 862.1150 (calibrator)
21 CFR § 862.1680 (testosterone test system)
21 CFR § 862.1660 (control)
2. Classification:
Class II (calibrator)
Class I (reagent and control)
3. Product Code:
JIT (calibrator), CDZ (testosterone test system), JJX (control)
4. Panel:
Clinical Chemistry

G. Intended Use:

1. Intended use:
The IBL Testosterone Luminescence Immunoassay is intended for the in vitro quantitative measurement of testosterone (a male sex hormone) in oral fluid and serum
2. Indication(s) for use:
The IBL Testosterone Luminescence Immunoassay is intended for the in vitro quantitative measurement of testosterone (a male sex hormone) in oral fluid and serum. Measurement of testosterone is used in the diagnosis and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism, impotence in males and, in females hirsutism (excessive hair) and virilization (masculinization) due to tumors, polycystic ovaries, and adrenogenital syndromes.

3. Special condition for use statement(s):
Prescription Use Only
4. Special instrument Requirements:
Microplate Luminometer System

H. Device Description:

The IBL Testosterone LIA Testosterone Kit contains the following:

- Microtiter Plate coated with rabbit anti-mouse antibody
- Testosterone Antiserum (mouse anti-testosterone)
- Standards A-G (0, 6.4, 16.0, 40.0, 100, 250, 760 pg/mL); Testosterone in buffer with BSA and stabilizers
- Controls Level I and II
- Phosphate buffer with BSA and stabilizers
- Enzyme Conjugate (Alkaline Phosphatase Conjugate with stabilizers)
- Chemiluminescence Reagent AP (acridan based substrate)
- Wash Buffer with Tween and stabilizer
- Adhesive Foil

Oral fluid collection devices are not supplied with the kit, but the sponsor recommends the use of the IBL Saliva Sampling Set. The collection tubes in this set are described as conical shaped polypropylene tubes with a volume of 2.0 mL.

I. Substantial Equivalence Information:

1. Predicate device name(s):
Diagnostic Systems Laboratories, Inc. Testosterone RIA
2. Predicate K number(s):
K854674
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Quantitative Measurement of Testosterone	Same
Calibrators	Multiple Calibrators	Same
Calibration Frequency	Each Run	Same

Differences		
Item	Device	Predicate
Matrices	Oral fluid, Serum	Plasma, Serum
Sample Pretreatment	Serum – dilute X 40 Oral Fluid - none	None
Reportable Range	2 – 500 pg/mL (oral fluid) 76 – 20,000 pg/mL (serum)	Approximately 50 – 25,000 pg/mL (serum, plasma)
Methodology	Luminescent Immunoassay (LIA)	Radioimmunoassay (RIA)

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

None Referenced

K. Test Principle:

The IBL Testosterone Luminescence immunoassay (LIA) is based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labeled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. After addition of the luminescence substrate solution the intensity of the luminescence measured is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

L. Performance Characteristics:

(All performance data was collected using the Berthold microplate system and all samples were collected with the IBL Saliva Sampling Set)

1. Analytical performance:*a. Precision/Reproducibility:*

Oral fluid Intra-assay Precision was assessed by analyzing 12 replicates at five different concentrations. The intra-assay precision of each concentration was then calculated:

Mean Concentration (pg/mL)	4.25	38.37	71.67	195.40	540.88
Standard Deviation	1.13	0.56	1.77	6.34	16.29
CV (%)	26.49	1.47	2.46	3.24	3.01
n	12	12	12	12	12

Oral fluid Inter-assay Precision was assessed by analyzing ten different concentrations in twenty separate runs, with the following results:

Mean Concentration (pg/mL)	5.54	20.75	45.82	53.44	74.67	78.98	236.02	334.87	557.30	196.02
Standard Deviation	1.72	1.44	2.42	2.48	3.38	3.40	8.88	9.72	22.51	11.19
CV (%)	31.1	6.96	5.29	4.63	4.52	4.30	3.76	2.90	4.04	5.71
n	20	20	20	20	20	20	20	20	20	20

Serum* Intra-assay Precision was assessed by analyzing ten replicates at three different concentrations, with the following results:

Mean Concentration (pg/mL)	621.6	3792.0	8890.0
Standard Deviation	47.4	93.3	286.3
CV (%)	7.62	2.46	3.22
n	10	10	10

Serum* Inter-assay Precision was assessed by analyzing four different concentrations in five separate runs, with the following results:

Mean Concentration (pg/mL)	594.40	3532.0	7108.80	6101.60
Standard Deviation	87.69	232.58	471.66	837.65
CV (%)	14.75	6.58	6.63	13.73
N	5	5	5	5

*serum concentrations corrected for X40 dilution factor

b. Linearity/assay reportable range:

Oral fluid – Through linearity and recovery studies the sponsor has demonstrated that the assay is linear from 1.8 to 500 pg/mL.

Serum – Through linearity and recovery studies the sponsor has demonstrated that the assay is linear from 76 to 20,000 pg/mL.

c. Traceability (controls, calibrators, or method):

The sponsor states that the preparation of calibrators is traceable to a GC-MS method and to an in-house reference standard.

The sponsor states that prior to release, the kit controls are assayed repeatedly and compared to previously released controls and a

quality control panel consisting of ten samples which span the range of the standard curve.

d. Detection limit:

Oral fluid – the analytical sensitivity for oral fluid was defined as the concentration corresponding to two standard deviations from the signal at zero concentration (standard A) and was calculated to be 1.8 pg/mL

Serum – the analytical sensitivity was similarly calculated using steroid-free serum diluted X 40 with standard A. When corrected for the dilution factor, the analytical sensitivity was calculated to be 76 pg/mL (7.6 ng/dL)

e. Analytical specificity:

The following compounds were tested as possible interferents in serum:

Potential Interferent	Approximate Concentration Testosterone Tested (pg/mL)	Concentration of Interferent Below Which No Significant Effect Was Observed*
Bilirubin	23, 106, 261	0.5 mg/mL
Hemoglobin	20, 91, 255	4 mg/mL
Triolein	17, 95, 252	30 mg/mL
Bovine Serum Albumin	3	1%

* defined as +/- 15%

The following compounds were tested as possible interferents in oral fluid:

Potential Interferent	Approximate Concentration Testosterone Tested (pg/mL)	Concentration of Interferent Below Which No Effect Observed
Whole Blood	25, 116	0.1%
Thimerosal	<2.5, 59, 67, 143	0.1%
Sodium Azide	<2.5, 19, 63, 132	0.1%

f. Assay cut-off:

N/A

2. Comparison studies:

a. Method comparison with predicate device:

Oral fluid – a method comparison study was performed between the IBL method and an RIA method modified for use with oral fluid. Sixty-nine specimens ranging from 3.7 to 122.5 pg/mL by the RIA

method were run on both systems. Linear regression produced the following results:

$$\text{IBL} = 0.91 \times \text{RIA} + 5.5 \text{ and } r = 0.93 \text{ for } n = 69$$

The modified RIA method used in the study above was described in Granger et al, Horm Behav. 1999 Feb;35(1):18-27. This article also investigated the relationship between saliva and serum free testosterone concentrations. For males the relationship was [serum free] = 0.212 [saliva] + 1.731 and for females [serum free] = 0.095 [saliva] + 0.574.

Serum – a method comparison study was performed between the IBL method and a predicate RIA method. Per the manufacturer's instructions, the serum samples were diluted X 40 prior to analysis using the IBL method. Seventy-one specimens ranging from 251 to 5819 pg/mL by the RIA method were run on both systems. Linear regression produced the following results:

$$\text{IBL} = 0.75 \times \text{RIA} + 261 \text{ and } r = 0.97 \text{ for } n = 71$$

b. Matrix comparison:

N/A – oral fluid and serum matrices are not claimed to be equivalent by the sponsor.

3. Clinical studies:

a. Clinical sensitivity:

N/A

b. Clinical specificity:

N/A

c. Other clinical supportive data (when a and b are not applicable):

4. Clinical cut-off:

N/A

5. Expected values/Reference range:

Oral fluid – an in-house reference range study was performed using apparently healthy males and females, with the following results.

Age (y)	Oral fluid Testosterone (pg/mL)					
	FEMALES			MALES		
	Median	Range (5 - 95 %)	N	Median	Range (5 - 95 %)	N
20 – 29	19.0	5.5 – 49.0	40	78.8	41.4 – 142.5	55
30 – 39	17.3	5.2 – 49.0	39	58.8	31.8 – 100.4	35
40 – 49	13.8	4.5 – 49.0	47	54.4	30.1 – 97.8	48
50 – 59	13.2	3.6 – 49.0	53	54.8	30.0 – 92.0	64
60 – 69	15.8	2.9 – 38.8	33	42.9	23.2 – 86.9	63

Serum – The sponsor states that a normal range for this method using serum as the matrix has not been established. Citing the scientific literature, the sponsor states the following: “Published ranges for testosterone concentrations in serum of “normal” males and females tend to vary slightly depending on the method used for assessment. It has been observed that adult men can fall within 836 – 20017 pg/mL (2.9 – 69.4 nmol/L) and adult women can range within 29 – 5451 pg/mL (0.1 – 18.9) nmol/L for typical commercially available immunoassays. Using GC-MS as a reference range, adult men fall within the range of 2365 – 16613 pg/mL (8.2 – 57.6 nmol/L) while adult women can range between 173 – 2076 pg/mL (0.6 – 7.2 nmol/L)”.

M. Conclusion:

Based upon the information provided for the file, I recommend that IBL Testosterone LIA be found substantially equivalent to the predicate device.