

Read entire protocol before use.

TESTO-RIA-CT

Bio-Line S.A. - Rue André Fauchille.17 - B-1150 Bruxelles – Belgium

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of human Testosterone (TESTO) in serum.

II. GENERAL INFORMATION

- A. Name : Bio-Line **TESTO-RIA-CT**
B. Catalogue number : **BL-12-CT**: 100 tests
C. Manufactured by : Bio-Line S.A.
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III. CLINICAL BACKGROUND

A. Biological activity

Testosterone is a C-19 steroid hormone (molecular weight: 288 Da) which is produced from androstenedione in the testes, adrenals and ovaries. Testosterone is a precursor along with androstenedione of the estrogen steroids.


B. Clinical applications

- **Clinical significance of testosterone level** : Source of testosterone :
 - Women : Ovary, adrenal cortex, peripheral tissues (by conversion of 50-60% other steroids).
 - Men : Testes > 90%, adrenal cortex, peripheral tissues.
- **Clinical diseases with high level of testosterone** :
 - Women : Hirsutism and virilization, polycystic ovary syndrome, congenital adrenal hyperplasia (with 17OH-PROG), tumors of adrenal and ovarian origin, breast cancer.
 - Men : Disease of the hypothalamic pituitary unit, some malignant testicular tumors, congenital adrenal hyperplasia, prostate cancer.
- **Clinical diseases with low level of testosterone** :
 - Primary or secondary hypogonadism, Klinefelter's syndrome, other chromosomal alteration, hypopituitarism, enzymatic defects, orchidectomy and cryptorchidism, testicular feminization, hepatic cirrhosis, some autoimmune diseases for example : Sjögren's syndrome, systemic lupus.
- **Other domains for measurement of testosterone level** :
 - *In vitro* fertilization : the women with high response to gonadotrophin have a significant increase in testosterone.
 - Parameter of the prepuberty and puberty.
 - Determination of foetal sex in amniotic fluid.
 - Free testosterone is significantly raised in both male and female acne sufferers.
 - Follow up of cancer and in pathological situations; low testosterone syndrome.

IV. PRINCIPLES OF THE METHOD

A fixed amount of ¹²⁵I labelled steroid competes with the steroid to be measured present in the sample or in the calibrator for a fixed amount of antibody sites being immobilized to the wall of a polystyrene tube. Neither extraction nor chromatography are required because of the high specificity of the coated antibodies. After 3 hours incubation at 37°C, an aspiration step terminates the competition reaction. The tubes are then washed with 3 ml of wash solution and aspirated again. A calibration curve is plotted and the testosterone concentrations of the samples are determined by dose interpolation from the calibration curve.

V. REAGENTS PROVIDED

Reagents	100 Test Kit	Colour Code	Reconstitution		
 Tubes coated with anti TESTO	2 x 50	green	Ready for use		
<table border="1" data-bbox="108 600 242 645"> <tr> <td>Ag</td> <td>¹²⁵I</td> </tr> </table> TRACER: ¹²⁵ Iodine labelled TESTO (HPLC grade) in phosphate-citrate buffer with bovine gelatin and azide (<0.1%)	Ag	¹²⁵ I	1 vial 55 ml 180 kBq	red	Ready for use
Ag	¹²⁵ I				
<table border="1" data-bbox="108 790 242 835"> <tr> <td>CAL</td> <td>0</td> </tr> </table> Zero Calibrator in human serum and azide (0.5%)	CAL	0	1 vial 1 ml	yellow	Ready for use
CAL	0				
<table border="1" data-bbox="108 902 242 947"> <tr> <td>CAL</td> <td>N</td> </tr> </table> Calibrators - N = 1 to 5 (see exact values on vial labels) in human serum and azide (0.5%)	CAL	N	5 vials 0.5 ml	yellow	Ready for use
CAL	N				
WASH SOLN CONC Wash solution (TRIS-HCl)	1 vial 10 ml	brown	Dilute 70 x with distilled water (use a magnetic stirrer).		
<table border="1" data-bbox="76 1227 268 1272"> <tr> <td>CONTROL</td> <td>N</td> </tr> </table> Controls - N = 1 or 2 in human serum with thymol	CONTROL	N	2 vials lyophilised	silver	Add 0.5 ml distilled water
CONTROL	N				

Note :Use the zero calibrator for sera dilutions.

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

- Distilled water
- Pipettes for delivery of: 50 µl and 500 µl (the use of accurate pipettes with disposable plastic tips is recommended)
- Vortex mixer
- Magnetic stirrer
- Incubator at 37°C
- 5 ml automatic syringe (Cornwall type) for washing
- Aspiration system (optional)
- Any gamma counter capable of measuring ¹²⁵I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

- A. Controls:** Reconstitute the controls with 0.5 ml distilled water.
- B. Working Wash solution:** Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash Solution (70x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, controls are stable for 7 days at 2-8°C. For longer storage periods, aliquots should be made and kept at -20°C for maximum 3 months. Avoid subsequent freeze-thaw cycles.
- Freshly prepared Working Wash solution should be used on the same day.

- After its first use, tracer is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

IX. SPECIMEN COLLECTION AND PREPARATION

- Serum samples must be kept at 2-8°C.
- If the test is not run within 24 hrs, storage at -20°C is recommended.
- Avoid subsequent freeze-thaw cycles.

X. PROCEDURE

A. Handling notes

Do not use the kit or components beyond expiry date. Do not mix materials from different kit lots. Bring all the reagents to room temperature prior to use. Thoroughly mix all reagents and samples by gentle agitation or swirling. Use a clean disposable pipette tip for addition of each different reagent and sample in order to avoid cross-contamination. High precision pipettes or automated pipetting equipment will improve the precision. Respect the incubation times. Prepare a calibration curve for each run, do not use data from previous runs.

B. Procedure

- Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes
- Briefly vortex calibrators, controls and samples and dispense 50µl of each into the respective tubes.
- Dispense 500 µl of ¹²⁵Iodine labelled TESTO into each tube, including the uncoated tubes for total counts.
- Shake the tube rack gently by hand to liberate any trapped air bubbles.
- Incubate for 3 hours at 37°C.
- Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
- Wash tubes with 3 ml Working Wash solution (except total counts) and aspirate (or decant). Avoid foaming during the addition of the Working Wash solution.
- Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
- Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

- Calculate the mean of duplicate determinations.
- Calculate the bound radioactivity as a percentage of the binding determined at the zero calibrator point (0) according to the following formula :

$$B/B_0(\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

- Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the (B/B₀(%)) values for each calibrator point as a function of the TESTO concentration of each calibrator point. Reject obvious outliers.
- Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
- By interpolation of the sample (B/B₀ (%)) values, determine the TESTO concentrations of the samples from the calibration curve.
- For each assay, the percentage of total tracer bound in the absence of unlabelled TESTO (B₀/T) must be checked.

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

TESTO	cpm	B/Bo (%)
Total count	56034	
Calibrator ng/ml	0.00	100.0
	0.11 ng/ml	70.5
	0.48 ng/ml	46.7
	1.55	27.3
	3495	12.3
ng/ml	1678	5.9
	5.40 ng/ml	
	16.40 ng/ml	

XIII. PERFORMANCE AND LIMITATIONS

A. Detection limit

Twenty zero calibrators were assayed along with a set of other calibrators.

The detection limit, defined as the apparent concentration two standard deviations below the average counts at zero binding, was 0.05 ng/ml.

B. Specificity

The percentage of cross-reaction estimated by comparison of the concentration yielding a 50% inhibition are respectively:

Compound	Cross-Reactivity (%)
DiHydroTestosterone	0.31
Androstenedione	0.28
17-β-Estradiol	0.004
17-OH-Progesterone	0.004
Progesterone	0.01
DHEA	0.0006
DHEA-sulphate	0.0002
Cortisol	< 0.0001
Danazol	0.001
Ethinylestradiol	0.0004
Ethisterone	0.003
Cyproterone acetate	< 0.0001
Dihydroprogesterone	0.004
Mesterolone	0.39
19 Nortestosterone	1.8

Note: this table shows the cross-reactivity for the anti TESTO

C. Precision

INTRA-ASSAY PRECISION

INTER-ASSAY PRECISION

Serum	N	<X> ± SD (ng/ml)	CV (%)	Serum	N	<X> ± SD (ng/ml)	CV (%)
A	10	0.69 ± 0.03	4.6	A	20	0.55 ± 0.03	6.2
B	10	4.35 ± 0.14	3.3	B	20	3.51 ± 0.17	4.8
C	9	9.82 ± 0.44	4.4				

SD: Standard Deviation; CV: Coefficient of variation

D. Accuracy

DILUTION TEST

Sample	Dilution	Theoretical Concent. (ng/ml)	Measured Concent. (ng/ml)
A	1/1	-	8.72
	1/2	4.36	4.08
	1/4	2.18	2.00
	1/8	1.09	1.08
	1/16	0.55	0.56
	1/32	0.27	0.23
B	1/1	-	6.98
	1/2	3.49	3.33
	1/4	1.75	1.59
	1/8	0.87	0.81
	1/16	0.44	0.42
	1/32	0.22	0.18

Samples were diluted with zero calibrator.

RECOVERY TEST

Sample	added TESTO (ng/ml)	Recovered TESTO (ng/ml)	Recovered (%)
1	0.22	0.19	86.4%
	0.46	0.51	110.9%
	1.36	1.5	110.3%
	3.28	3.09	94.2%
	9.8	12.2	124.5%

Conversion factor :

From ng/ml to nmol/L : x 3.47

From nmol/L to ng/ml : x 0.288

The concentrations of the calibrators are determined with the ID-GC/MS reference method.

E. Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 30 minutes after the calibrator has been added to coated tubes.

TIME DELAY

Serum B/T values	0'	10'	20'	30'
C 1	27.9	28	28.6	28
C 2	11.9	11.5	11.9	11.4

XIV. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises.

XV. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

Premenopausal women were with normal luteal phase (Progesterone > 30 nmol/l), not on clomid and with no evidence of irregular cycle. Postmenopausal women (age : 28 to 61) had FSH > 30 IU/l and most of these patients were routine assessment of confirming recent postmenopausal status or premature ovarian failure.

Identification	Range (*) (ng/ml)	Mean	SD	n
Females (determined in UK)	0.11 - 0.79	0.39	0.17	100
	< 0.06 - 0.50	0.22	0.13	60
Males	1.34 - 6.25	3.89	1.46	71

(*) The range is based on 2.5 % and 97.5 % percentiles

XVI. PRECAUTIONS AND WARNINGS

Safety

For *in vitro* diagnostic use only.

This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area, away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HbsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

XVII. BIBLIOGRAPHY

1. J.L. ANDREYKO et al. (1986)
Role of Serum Androgens and Sex Hormone Binding Globulin Capacity in the Evaluation of Hirsutism in Women.
Clin. Biochem, Vol. 19, 58-61.
2. A. BIZZARO et al.
Influence of Testosterone Therapy on Clinical and Immunological features of Autoimmune Diseases Associated with Klinefelter's Syndrome
J. Clin. Endocrin. Metab. Vol. 64, N°1: 32-36
3. M. CARRABBA et al (1985)
Abnormalities of Sex hormones in Men with Systemic Lupus Erythematosus.
Clin. Rheumatology, N° 4: 422-425.
4. P. HILL et al. (1985)
Plasma Testosterone and Breast Cancer.
Eur.J Cancer Clin. Oncol, Vol. 21, N°10, pp. 1265-1266
5. CG. MAHLCK et al (1986)
Testosterone, SHBG and Albumin in Patients with ovarian carcinoma.
Acta Obstet. Gynecol. Scand. 65: 533-S38.
6. D.M.D PERERA et al (1987)
Amniotic Fluid Testosterone and testosterone Glucuronide Levels in the determination of Foetal Sex.
J. Steroid Biochem., Vol. 26,N°2,pp.273-277.
7. J. TRACHTENBERG (1987)
Experimental Treatment of Prostatic Cancer by intermittent Hormonal Therapy.
J. Urology, Vol. 137, pp. 785-788.
8. R. MARUYAMA et al. (1987)
Sex-Steroid-Binding Plasma Protein (SBP), Testosterone, Oestradiol and DHEA in Prepuberty and Puberty.
Acta Endocrinologica, 114: 60-67
9. P. HOLDUNIA, C. WALTER et al (1992)
A clinical evaluation of a direct radioimmunoassay of testosterone.
Clin. Chim. Acta, 214:31-43.

XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS µl	CALIBRATORS µl	SAMPLE (S) CONTROLS µl
Calibrators (0 to 5) Samples, Controls Tracer	- - 500	50 - 500	- 50 500
Incubation	3 hours at 37°C		
/Separation Working Wash solution Separation	-	Aspirate (or decant) 3.0 ml Aspirate (or decant)	
Counting	Count tubes for 60 seconds		

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