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Read entire protocol before use.

TESTO-RIA-CT

I. INTENDED USE

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

II. GENERAL INFORMATION

A. Proprietary name : DIAsource TESTO-RIA-CT Kit

B. Catalog number : KIP1709 : 96 tests

C. Manufactured by : DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

For technical assistance or ordering information contact :
Tel : +32 (0)10 84.99.11 Fax : +32 (0)10 84.99.91

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 170H-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 respons 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 syndrom.

IV. PRINCIPLES OF THE METHOD

A fixed amount of ^{125}I 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	96 Test Kit	Colour Code	Reconstitution
Tubes coated with anti TESTO	2 x 48	green	**Ready** for use
Ag | **^{125}I** TRACER: ^{125}Iodine labelled TESTO (HPLC grade) in phosphate-citrate buffer with bovine gelatin and azide (<0.1%)	1 vial 55 ml 180 kBq	red	**Ready** for use
CAL | **0** Zero Calibrator in human serum and azide (0.5%)	1 vial 1 ml	yellow	**Ready** for use
CAL | **N** Calibrators - N = 1 to 5 (see exact values on vial labels) in human serum and azide (0.5%)	5 vials 0.5 ml	yellow	**Ready** for use
WASH | **SOLN** | **CONC** Wash solution (TRIS-HCl)	1 vial 10 ml	brown	**Dilute** 70 x with distilled water (use a magnetic stirrer).
CONTROL | **N** Controls - N = 1 or 2 in human serum with thymol	2 vials lyophilised	silver	**Add** 0.5 ml distilled water

Note : Use the zero calibrator for sera dilutions.

VI. SUPPLIES NOT PROVIDED

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

1. Distilled water
2. Pipettes for delivery of: 50 µl and 500 µl (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Incubator at 37°C
6. 5 ml automatic syringe (Cornwall type) for washing
7. Aspiration system (optional)
8. Any gamma counter capable of measuring ^{125}I 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

1. Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes
2. Briefly vortex calibrators, controls and samples and dispense 50µl of each into the respective tubes.
3. Dispense 500 µl of ^{125}Iodine labelled TESTO into each tube, including the uncoated tubes for total counts.
4. Shake the tube rack gently by hand to liberate any trapped air bubbles.
5. Incubate for 3 hours at 37°C.
6. 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.
7. 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.
8. Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
9. Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

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

$$B/B0\,(\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

3. 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.
4. 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.
5. By interpolation of the sample (B/B0 (%)) values, determine the TESTO concentrations of the samples from the calibration curve.
6. 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	0.00 ng/dl	28383	100.0
	11.0 ng/dl	20016	70.5
	48.0 ng/dl	13255	46.7
	155.0 ng/dl	7756	27.3
	540.0 ng/dl	3495	12.3
	1640.0 ng/dl	1678	5.9

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 5.0 ng/dl.

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

Serum	N	<X> ± SD (ng/dl)	CV (%)
A	10	69.0 ± 3.0	4.6
B	10	435.0 ± 14.0	3.3
C	9	982.0 ± 44.0	4.4

INTER-ASSAY PRECISION

Serum	N	<X> ± SD (ng/dl)	CV (%)
A	20	55.0 ± 3.0	6.2
B	20	351.0 ± 17.0	4.8

SD: Standard Deviation; CV: Coefficient of variation

D. Accuracy

DILUTION TEST

Sample	Dilution	Theoretical Concent. (ng/dl)	Measured Concent. (ng/dl)
A	1/1	-	872
	1/2	436	408
	1/4	218	200
	1/8	109	108
	1/16	55	56
	1/32	27	23
B	1/1	-	698
	1/2	349	333
	1/4	175	159
	1/8	87	81
	1/16	44	42
	1/32	22	18

Samples were diluted with zero calibrator.

RECOVERY TEST

Sample	added TESTO (ng/dl)	Recovered TESTO (ng/dl)	Recovered (%)
1	22	19	86.4%
	46	51	110.9%
	136	150	110.3%
	328	309	94.2%
	980	1220	124.5%

Conversion factor :

From ng/dl to nmol/L : x 0.035
From nmol/L to ng/dl : x 28.8

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 post-menopausal status or premature ovarian failure.

Identification	Range (*) (ng/dl)	Median	n
Females (determined in UK)			
. Premenopausal	ND - 77	30	66
. Postmenopausal	ND – 58	20	26
Males	267 – 1012	531	77

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

XVI. PRECAUTIONS AND WARNINGS

Safety

For *in vitro* diagnostic use only.

This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations.

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

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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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