



Free TESTOSTERONE-RIA-CT

Radioimmunoassay for the Quantitative Determination of Free Testosterone in Human Serum

BL-39-CT

IN VITRO DIAGNOSTIC USE



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1. INTENDED USE :

For IN VITRO determination of Free Testosterone (FT) levels in hirsutism and hypogonadism.

Free testosterone diffuses through cell membranes and binds to specific receptor proteins (androgen receptors); the Testosterone-receptor complexes act as transcriptional modulators on cis-regulatory regions of many genes.

Excess of Androgens in women causes hirsutism and signs of virilization; Testosterone level in serum has to be determined before and after ovarian and adrenal stimulation and suppression to identify the source of excessive hormone production.

Primary and secondary hypogonadism in men result in clinical hypoandrogenization, correlated with the degree of gonadal failure in Testosterone production. The determination of serum Testosterone together with that of LH allows the correct assessment of those conditions.

The diagnosis of true anorchia also requires to discriminate this condition from cryptorchidism. Under prolonged hCG stimulation, Testosterone levels remain very low in true anorchia while cryptorchid testes can respond to stimulation.

Androgen resistance syndromes, due to X linked androgen receptor gene deficiencies, are made of various ambiguity. Whatever the severity of the phenotypical abnormalities, serum Testosterone is systematically high in regards to elevated LH serum levels in these conditions.

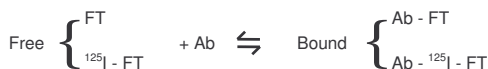
Testosterone assays include total testosterone (direct, extraction, coated tubes) and free testosterone determinations.

Total Testosterone in plasma includes free Testosterone and Testosterone bound to SHBG, albumin, CBG. The mean percentage of each in normal men is 2.7, 32, 65 and <0.1 respectively.

Solvents break the protein binding in extraction assays whereas blocking agents release Testosterone from proteins in direct assays. The advantage of a free testosterone assay is that free testosterone concentrations are in equilibrium with testosterone bound to receptors in the organs.

2. PRINCIPLE OF THE METHOD :

The Free Testosterone (FT) CT RIA obeys the law of mass action according to the following equation :




Since the concentrations of ¹²⁵I - FT and coated antibodies are constant, the advancing state of the equation depends on the concentration of FT. The amount of ¹²⁵I - FT bound to the coated tube is inversely proportional to the concentration of FT in the sample.

Following the incubation, the tube is aspirated to remove excess unbound labelled T. Patient sample concentrations are read from a calibration curve.

3. MATERIAL PROVIDED AND STORAGE :

Stored at 2 - 8°C, the material can be used up to the expiration date printed on each label.

- 3.1.  2 x 50 Polypropylene tubes (12 x 75 mm) coated with anti-Testosterone polyclonal antibodies. Systematically allow the coated tubes to reach room temperature before opening the bag. Store the unused tubes in the provided minigrip bag at 2-8°C.. Do not forget to reseal the bag.
- 3.2.

Ag	125I
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 yellow, 105 ml
1 bottle of 125I-labelled TESTOSTERONE in phosphate buffer with gelatine as stabilizer, a preservative (THIOMEROSAL < 0.05 %) and a yellow dye. Each bottle contains less than 185 Kbc (5 µCi)
- 3.3.

CAL	N
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 1 ml in each vial - N=0 to 5
6 vials of FREE TESTOSTERONE in human serum containing preservative (NaN3< 0.1 %). The concentrations are printed on the labels.

3.4.

Lyophilized - N=1 or 2

2 vials of human serum containing preservative (NaN₃ < 0.1 %). The control sera are to be assayed along with the patient samples. The ranges for the control sera are printed on the vial labels.

CONTROL	N
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Before use , reconstitute the content of the controls with 1 ml of distilled water. Mix gently to avoid foaming. Wait at least 15 minutes after solubilization before dispensing. After reconstitution, the solutions are stable for 3 weeks at 2-8°C or for a longer period if stored at -20°C.

4. MATERIAL REQUIRED BUT NOT PROVIDED :

- bench surfaces, protected by absorbent paper to reduce the effects of radioactive spillage.
- waste disposal containers, appropriately labelled and suitable for solid or liquid radioactive materials.
- manual or automated precision micropipettes for dispensing samples or reagents without cross-contamination.
- absorbent paper.
- vacuum pump, connected through a trap, for aspiration.
- Reciprocating or orbital shaker (max. 350 rpm).
- a gamma scintillation counter
- appropriate graph paper for plotting the results.

5. METHODOLOGY

5.1. Collection and handling of blood samples :

The blood sample can be collected into a dry tube.

After separation from the red blood cells, serum samples can be assayed immediately, within 24 hours if stored at 2 - 8°C, or later, after a period of up to several months if stored at -20°C. Repeatedly freezing and thawing must be avoided.

5.2. Assay procedure :

Reagents stored at 2°- 8° C. must be brought at room temperature prior to use. Do not mix reagents of different lots. Label the tubes for T (« Total Counts » do not use coated tubes) calibrators, samples and controls. Calibrators and controls should be mixed before use by inverting or swirling rather than vortexing.

Perform the assay in duplicate. Calibrators, controls and samples must be assayed at the same time.

1. Calibrator curve :

Pipette 50 µl of each calibrator into the corresponding tubes.

2. Samples and control sera :

Pipette 50 µl of each sample or control serum into the corresponding tubes.

3. Add 1 ml of ¹²⁵I - TESTOSTERONE analog tracer to each tube and mix with a vortex.

4. Incubate 2 hours and 30 minutes at room temperature on a reciprocating shaker (max. 160 rpm).

5. Carefully aspirate or decant the solution of all tubes. (do not wash)

6. Count the radioactivity fixed in each tube for at least 60 seconds

5.3. Data processing :

Determine the mean count rate for each set of duplicate tubes.

Calculate the ratio B/B0 as follows :

$$B/B0 \% = [\text{Cal or Smp cpm} / B0 (\text{Cal } 0) \text{ cpm}] \times 100$$

Draw the calibrator curve on semilogarithmic paper by plotting the ratio B/B0 % (linear scale) obtained for each calibrator versus its respective concentration expressed in pg/ml (logarithmic scale). FREE TESTOSTERONE concentrations in samples can be read directly from the calibrator curve.

If a computer is used to calculate the results, the data can be fitted to the appropriate equation : smoothed spline.

5.4. Example of a typical assay :

	Contents (pg/ml)	cpm 1st duplicate	cpm 2nd duplicate	Mean count rate	B/Bo (%)	Free Testosterone (pg/ml)
Total counts	-	33231	33031	33131	-	-
Cal 0	0	12866	12140	12503	100	-
Cal 1	0.25	10966	10395	10681	85.4	-
Cal 2	1	7624	7981	7803	62.4	-
Cal 3	5	5204	5335	5270	42	-
Cal 4	20	3371	3553	3252	26.9	-
Cal 5	65	1943	1970	1956	15.6	-
C 1 low	1.6 – 2.7	10731	10175	10453	55.6	1.75
C 2 high	16 – 29	5489	5332	5411	26	21.3
Sample 1		11455	11521	11488	91.9	0.16
Sample 2		9458	9362	9410	75.3	0.45
Sample 3		6996	6864	6880	55	1.8

Example of a typical assay, do not use for calculations

6. PERFORMANCE CHARACTERISTICS :

6.1. Specificity

Steroid	% Cross-reactivity
Testosterone	100
5 α DHT	0.006
androstenedione	0.02
β estradiol	0.0003
DHEA-S	0.000001
Androsterone, Corticosterone, 11 DOC, estriol, estrone, progesterone, DHEA	N.D.

6.2. Minimum detectable concentration of FREE TESTOSTERONE :

The minimum detectable concentration has been assayed at 0.13 pg/ml and corresponds to the concentration given by two standard deviations below the mean cpm of 20 replicate determinations of the zero calibrator.

6.3. Reproducibility :

	Mean value (pg/ml)	Within assay variation (% CV) 10 replicates	Between assay variation (% CV) 5 Separate assays in duplicate
Pool 1	0.8	11.4	8.32
Pool 2	9.74	5.7	2.3
Pool 3	28.6	9.3	0.03

7. LIMITATION OF THE PROCEDURE

- The results obtained from this or any other diagnostic kit should be used and interpreted only in the context of an overall clinical picture.
- Do not use lipemic, haemolyzed, icteric or turbid specimens.
- Do not use plasma samples

8. EXPECTED VALUES

It is recommended that each laboratory establishes its own reference values.

	FREE TESTOSTERONE Mean (pg/ml)	Range (pg/ml)
Males (N=71)	25	8.9 - 42.5
Females (N = 68)	1	0.02 - 3.09

9. WARNING AND PRECAUTIONS

For IN VITRO DIAGNOSTIC use only

CAUTION : Radioactive material

This radioactive material may be received, acquired, possessed and used only by authorized persons in clinical or hospital laboratories and only for in vitro clinical or laboratory tests, not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to State and local regulations.

WARNING : Sodium azide

Some components contain sodium azide as preservative agent ($\text{NaN}_3 < 0.1\%$). Dispose of the reagents by flushing with large amount of water through the plumbing system.

WARNING : Potentially infectious material

Handle all components (and all patient samples) as if capable of transmitting viral diseases such as hepatitis B and C and the acquired immunodeficiency syndrome (AIDS).

Source material derived from human body fluids or organs and used in the preparation of this kit were tested and found negative for HBsAg and anti-HCV by immunoassay. However, no known test can guarantee that such material does not contain the causative agent of viral hepatitis.

Likewise, all human materials used in the preparation of this kit were screened for the presence of antibodies against HIV-1 and -2 by enzyme-immunoassay and were found negative. However, absence of this antibody cannot guarantee the absence of the viral agent responsible for the acquired immunodeficiency syndrome.

10. BIBLIOGRAPHY

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