Free TESTO-RIA-CT

KIRI19000

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History

Summary of change:

Previous Version: 190711/1	Current Version: 200122-1
	
LOT	Version
Room temperature	Room temperature (18-25°C)
	Text added: **3. MATERIAL PROVIDED AND STORAGE** : CAL N and CONTROL N **(See exact values on vial labels)**
	Text added: **5.2 Assay Procedure** Each tube can only be used once
5.2 Assay Procedure: 5. Carefully aspirate or decant (before to decant, add 2 ml of washing solution to each tube) the solution of all tubes. (Except total counts tubes). 6.Add 2 ml of washing solution to each tube. Aspirate or decant carefully.	**5.2 Assay Procedure:** 5. Carefully aspirate the solution of all tubes. (Except total counts tubes). 6. Add 2 ml of washing solution to each tube. Aspirate carefully. (Except total count tubes). 7.Add 2 ml of washing solution to each tube. Aspirate carefully. (Except total count tubes).
5.3. Data processing : 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.	**5.3. Data processing :** Draw the calibrator curve 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.
	Text added: **9. WARNING AND PRECAUTIONS** For more information, see Material Safety Data Sheet (MSDS)

Free TESTOSTERONE-RIA-CT

Radioimmunoassay for the Determination of Free Testosterone in Human Serum

KIRI19000

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

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1. INTENDED USE :

For determination of Free Testosterone (FT) levels in human serum. For research use only, not for use in diagnostic procedures.

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.

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 :

$$\text{Free} \begin{cases} \text{FT} \\ {}^{125}\text{I - FT} \end{cases} + \text{Ab} \rightleftharpoons \text{Bound} \begin{cases} \text{Ab - FT} \\ \text{Ab - } {}^{125}\text{I - FT} \end{cases}$$

Since the concentrations of ^{125}I - FT and coated antibodies are constant, the advancing state of the equation depends on the concentration of FT. The amount of ^{125}I - 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.

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 48 Polystyrene tubes (12 x 75 mm) coated with anti-Testosterone polyclonal antibodies.
Systematically allow the coated tubes to reach room temperature (18-25°C) before use.

3.2. **Ag** | **125I** — yellow, 42 ml
1 bottle of 125I-labelled FREE TESTOSTERONE analogue in protein based buffer containing < 0.1 % NaN3 as preservative.
Each bottle contains less than 185 Kbq (5 µCi)

3.3. **CAL** | **N** — 0.5 ml in each vial - N=0 to 6
7 vials of FREE TESTOSTERONE in human serum containing preservative (NaN3< 0.1 %).
The concentrations are printed on the labels.
(See exact values on vial labels)

3.4. **CONTROL** | **N** — 2 vials, lyophilized - N=1 or 2
2 vials of human plasma containing preservative (Thymol
The controls are to be assayed along with the samples.
The ranges for the controls are printed on the vial labels.
Before use, reconstitute the content of the controls with 0.5 ml of distilled water
After reconstitution, the controls should be aliquoted and kept at –20°C for maximum 3 months.
(See exact values on vial labels)

3.5. **WASH** | **SOLN** | **CONC** — 70 x concentrated, 10 ml
1 bottle concentrated buffered solution containing sodium azide (NaN_3 < 0.1 %). Poor the solution in 700 ml of distilled water.
The reconstituted washing solution is stable for 2 weeks at 2-8°C if covered with adhesive film to avoid contamination.

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.
- water bath.
- 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 (18-25°C)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.

Each tube can only be used once

1. Calibrator curve :

   Pipette 50 µl of each calibrator into the corresponding tubes.

2. Samples and controls :

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

3. Add 400 µl of ^{125}I - TESTOSTERONE analogue tracer to each tube. Vortex and cover.

4. Incubate 2 hours at 37 ± 2°C.

5. Carefully aspirate the solution of all tubes. (Except total counts tubes).

6. Add 2 ml of washing solution to each tube. Aspirate carefully. (Except total count tubes).

7. Add 2 ml of washing solution to each tube. Aspirate carefully. (Except total count tubes).

8. 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 :

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

Draw the calibrator curve 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 Testoste-rone (pg/ml)
Total counts	-	52039	51647	51843	-	-
Cal 0	0	25839	25961	25900	100	-
Cal 1	0.3	21086	21170	21128	81.6	-
Cal 2	1	16509	16203	16356	63.2	-
Cal 3	3	12437	12428	12433	48	-
Cal 4	10	8317	7916	8117	31.3	-
Cal 5	30	5017	4711	4864	18.8	-
Cal 6	90	2611	2528	2570	9.9	
C 1 low	1.5 – 2.9	13461	13557	13508	52.2	2.3
C 2 high	15 – 29	5736	5002	5369	20.8	21
Sample 1		17478	16742	16975	65.5	0.86
Sample 2		7538	7423	7481	28.9	11.5
Sample 3		4681	4645	4663	24.3	33

Example of a typical assay, do not use for calculations

6. PERFORMANCE CHARACTERISTICS :

6.1. Specificity

Steroid	% Cross-reactivity
Testosterone	100
5α DHT	0.006
andostenedione	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 LOB (Limit of Blank) was calculated by measuring the blank several times and was calculated as the mean - 1.65 standard deviations of the distribution of these values. The LOB was calculated to be 0.08 pg/ml.

The LOD (limit of detection) was calculated as the LOB – 1.65 standard deviations of a low concentration sample tested in 10 different runs. The LOD was calculated to be 0.40 pg/ml.

6.3. Reproducibility :

	Within assay variation		Between assay variation	
	Mean value (pg/ml)	10 replicates (% CV)	Mean value (pg/ml)	7 Separate assays in duplicate (% CV)
Pool 1	29,86	9,3	0,73	19,5
Pool 2	8,17	5,7	10,89	7,3
Pool 3	0,63	11,5	33,94	9,1

7. LIMITATION OF THE PROCEDURE

7.1. The results obtained from this or any other kit should be used and interpreted only in the context of an overall clinical picture.

7.2. Do not use lipemic, haemolyzed, icteric or turbid specimens.

7.3. Do not use plasma samples

8. TYPICAL DATA

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

Age group	Males			Females		
	Number of subjects	Median pg/ml	Absolute Range pg/ml	Number of subjects	Median pg/ml	Absolute Range pg/ml
<15 years	49	0,3	ND - 1,8	45	0,3	ND -2,7
15-39 years	154	13,3	5,4 - 40,0	145	2,3	ND- 4,6
40-59 years	97	11,8	3,6-25,7	77	1,5	ND- 4,0
>60 years	87	9,7	1,5-28,8	92	1,4	ND -5,0

9. WARNING AND PRECAUTIONS

For Research use only, not for use in diagnostic procedures.

CAUTION : Radioactive material

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.

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

For more information, see Material Safety Data Sheet (MSDS)

WARNING : Sodium azide

Some components contain sodium azide as preservative agent (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 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.

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