Enzyme Immunoassay for the Quantitative Determination of Testosterone Concentration in Human Serum

Catalog Number: 11150
FOR RESEARCH USE ONLY

Store at 2 to 8°C.

INTRODUCTION

Testosterone is the most important androgen secreted into the blood. In males, testosterone is secreted primarily by the Leydig cells of the testes; in females, ca. 50% of circulating testosterone is derived from peripheral conversion of androstenedione, ca. 25% from the ovary and ca. 25% from the adrenal glands.

Testosterone is responsible for the development of secondary male sex characteristics and its measurements are helpful in evaluating the hypogonadal states.

In women, high levels of testosterone are generally found in hirsutism and virilization, polycystic ovaries, ovarian tumors, adrenal tumors and adrenal hyperplasia.

In men, high levels of testosterone are associated to the hypothalamic pituitary unit diseases, testicular tumors, congenital adrenal hyperplasia and prostate cancer.

PRINCIPLE OF THE TEST

The Testosterone EIA is based on the principle of competitive binding between Testosterone in the test specimen and Testosterone-HRP conjugate for a constant amount of rabbit anti-Testosterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 10 µl of Testosterone standards, controls, testing samples, 100 µl Testosterone-HRP conjugate reagent and 50 µl rabbit anti-Testosterone reagent at 37°C for 90 minutes. During the incubation, a fixed amount of HRP-labeled Testosterone competes with the endogenous Testosterone in the standard, sample, or quality control serum for a fixed number of binding sites of the specific Testosterone antibody. Thus, the amount of Testosterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of Testosterone in the specimen increases. Unbound Testosterone peroxidase conjugate is then removed and the wells washed. Next, a solution of TMB Reagent is then added and incubated at room temperature for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 1N HCl, and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled Testosterone in the sample. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The Testosterone concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve.

REAGENTS

Materials provided with the kit:

- Goat Anti-Rabbit IgG-coated microtiter wells, 96 wells
- Testosterone Reference Standards: 0, 0.1, 0.5, 2.0, 6.0 and 18.0 ng/ml. Liquids, 0.5 ml each, ready to use.
- Rabbit Anti-Testosterone Reagent (pink color), 7 ml
- Testosterone-HRP Conjugate Reagent (blue color), 12 ml
- Testosterone Control 1, Liquid, 0.5 ml, Ready to use.
- Testosterone Control 2, Liquid, 0.5 ml, Ready to use.
- TMB Reagent (One-Step) 11 ml.
- Stop Solution (1N HCl), 11 ml.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 O.D. at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.
2. Samples with expected testosterone concentrations over 18 ng/ml may be quantitated by dilution with diluent available from your vendor.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 10 µl of standards, specimens and controls into appropriate wells.
3. Dispense 100 µl of Testosterone-HRP Conjugate Reagent into each well.
4. Dispense 50 µl of rabbit anti-Testosterone reagent to each well.
5. ***Thoroughly mix for 30 seconds. It is very important to mix them completely.***
6. Incubate at **37°C** for **90** minutes.
7. Rinse and flick the microwells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
9. Incubate at room temperature (18-25°C) for 20 minutes.

10. Stop the reaction by adding 100µl of Stop Solution to each well.
11. Gently mix 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely***.
12. Read absorbance at 450 nm with a microtiter well reader ***within 15 minutes***.

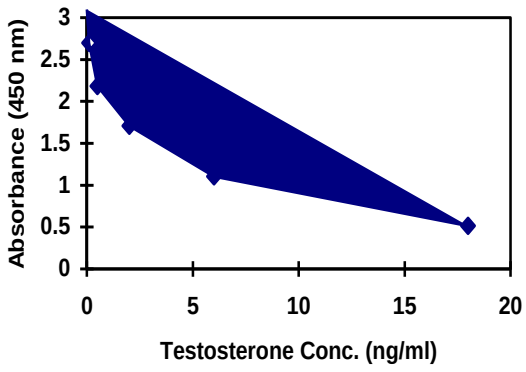
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on a ***linear-linear graph paper***, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of Testosterone in ng/ml from the standard curve.
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against Testosterone concentrations shown in the X axis. **Note:** This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

Testosterone (ng/ml)	Absorbance (450 nm)
0	3.096
0.1	2.700
0.5	2.185
2.0	1.709
6.0	1.105
18.0	0.516



PERFORMANCE CHARACTERISTICS

1. Sensitivity

The minimum detectable concentration of the Testosterone ELISA assay as measured by 2 SD from the mean of a zero standard is estimated to be 0.05 ng/ml.

2. Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of four different serum samples in one assay. Within-assay variability is shown below:

Samples	1	2	3	4
# Replicates	24	24	24	24
Mean Testosterone (ng/ml)	0.44	3.7	5.1	12.7
S.D.	0.03	0.4	0.4	0.6
C.V. (%)	6.4	10.0	8.3	5.0

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of six different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Samples	1	2	3	4
# Replicates	20	20	20	20
Mean Testosterone (ng/ml)	0.45	3.4	5.0	13.3
S.D.	0.02	0.3	0.2	0.5
C.V. (%)	4.4	8.4	4.4	3.7

3. Recovery Study

Various serum samples of known Testosterone levels were combined and assayed in duplicate. The mean recovery was 95.3%.

PAIR NO.	EXPECTED [Testosterone] (ng/ml)	OBSERVED [Testosterone] (ng/ml)	% RECOVERY
1	8.7	9.2	105.9
2	9.3	9.6	103.6
3	6.3	5.2	83.2
4	5.0	5.0	99.9
5	2.6	3.3	127.5
6	2.4	2.3	97.5
7	0.66	0.46	70.4
8	0.61	0.46	74.6

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