TESTOSTERONE
Product Code: 3725-300

SUMMARY AND EXPLANATION OF THE TEST

Testosterone, (17β)-Hydroxy-4-androstene-3-one-1, a C19 steroid, is the most potent naturally secreted androgen. In normal postpuberal males, testosterone is secreted primarily by the testes with only a small amount derived from peripheral conversion of 4-Androstene-3,17-dione (AOD). In adult women, it has been estimated that over 50% of serum testosterone is secreted by peripheral conversion of ASD secreted by the adrenal and ovary, with the remainder from direct secretion of testosterone by these glands.

The male, testosterone is mainly synthesized in the interstitial Leydig cells and the testis, and is regulated by the interstitial cell stimulating hormone (ICSH), or luteinizing hormone (LH) of the anterior pituitary (the female equivalent is FSH). Testosterone is responsible for the development of secondary sex characteristics, such as the accessory sex organs, the prostate, seminal vesicles and the growth of pubic and auxiliary hair. Testosterone measurements have been very helpful in evaluating hypogonadal states. Increased testosterone levels in males can be found in complete androgen resistance (testicular feminization). Common causes of decreased testosterone levels in males include: hypogonadism, orchidectomy, estrogen therapy, Klinefelter’s syndrome, hypothalamic, and hepatic cirrhosis.

In the female, testosterone levels are normally found to be much lower than those encountered in the healthy male. Testosterone in the female comes from three sources. It is secreted in small quantities by both the adrenal glands and the ovaries, and in healthy women 50-60% of the daily testosterone production arises from peripheral metabolism of prohormone, chiefly androstenedione. Common causes of increased serum testosterone levels in females include polycystic ovaries (Stein-Leventhal syndrome), ovarian tumors, adrenal androgen hyperplasia. Virilization in women is associated with the administration of androgens and endogenous overproduction of testosterone. There appears to be a correlation between serum testosterone levels and the degree of virilization in women, although approximately 25% of women with varying degrees of virilism have serum testosterone levels that fall within the female reference range.

PRINCIPLE

Competitive Enzyme Immunoassay (TYPE T)

The essential reagents required for a enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the followed equation:

\[
\frac{k_a}{k_a} = \frac{Ag + Ab}{Ag + Ab} \cdot \frac{AgAb + EAgAb}{AgAb + EAgAb}
\]

where:

- \( Ag \) = Monospecific Immobilized Antibody (Constant Quantity)
- \( Ag + Ab \) = Enzyme-antigen Conjugate (Variable Quantity)
- \( EAgAb \) = Enzyme-antigen Conjugate (Antibody Complex)
- \( Ab \) = Rate Constant of Association
- \( k_a \) = Rate Constant of Dissociation
- \( k_a + k_a \) = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after deceleration or aspiration.

\[
Ag + Ab = \text{immobilized complex}
\]

\[
\text{Streptavidin} = \text{Streptavidin} + \text{immobilized complex}
\]

\[
\text{Streptavidin} + \text{Immobilized complex} = \text{sandwich complex bound to the solid surface}
\]

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

REAGENTS MATERIALS PROVIDED:

A. Human Serum References -- 1ml/vial - Icons A-G

B. Testosterone Enzyme Reagent – 1.0 ml/vial

C. Testosterone Biotin Reagent --6.0 ml - Icon

D. Testosterone Enzyme Coated Plate -- 96 wells –Icon

E. Streptavidin Coated Plate -- 96 wells –Icon

F. Wash Solution -- 20ml - Icon

G. Substrate A --7ml/vial - Icon

H. Substrate B -- 7ml/vial - Icon

Note: Do not use the working substrate if it looks blue.

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 23°C).

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-6°C.

2. Pipette 0.010 ml (10µL) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.050 ml (50µL) of the working Testosterone Enzyme Reagent to all wells (see Reagent Preparation Section).

4. Swirl the microwell gently for 20-30 seconds to mix.

5. Add 0.050 ml (50µL) of Testosterone Biotin Reagent to all wells.

6. Swirl the microwell gently for 20-30 seconds to mix.

7. Cover and incubate for 60 minutes at room temperature.

8. Discard the contents of the microwhite by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

9. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat once (2) additional times for a total of three (3) washes.

10. Add 10.0 ml (100µL) of working substrate solution to all wells (see Reagent Preparation Section). Incubate (tap and blot) aspirate. Add 0.050ml (50µL) of stop solution to each well and gently mix for 15-20 seconds.

11. Incubate at room temperature for fifteen (15) minutes.

12. Add 0.050ml (50µL) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

Note: Dilute the samples suspected of concentrations higher than 12 ng/mL 1:5 and 1:10 with Testosterone '0' ng/mL calibrator or female patient sera with a known low value for testosterone.

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. If the individual laboratory should set any mandatory performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variation.

Note: Enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the followed equation:
CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Testosterone in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding Testosterone concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of Testosterone for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated).

In the following example, the average absorbance (1.764) intersects the dose response curve at (0.57 ng/ml) Testosterone concentration (See Figure 1).

EXAMPLE 1

<table>
<thead>
<tr>
<th>ID</th>
<th>Sample</th>
<th>Number</th>
<th>Well</th>
<th>Abs (A)</th>
<th>Abs (B)</th>
<th>Mean</th>
<th>(1/S.D.(A))</th>
<th>(1/S.D.(B))</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal A</td>
<td>A1</td>
<td>2.780</td>
<td>2.787</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal B</td>
<td>C1</td>
<td>2.576</td>
<td>2.611</td>
<td>0.100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal C</td>
<td>D1</td>
<td>2.646</td>
<td>1.789</td>
<td>F1</td>
<td>1.965</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal D</td>
<td>G1</td>
<td>1.391</td>
<td>1.392</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal E</td>
<td>A2</td>
<td>0.780</td>
<td>0.788</td>
<td>2.500</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal F</td>
<td>C2</td>
<td>0.530</td>
<td>0.538</td>
<td>D2</td>
<td>0.547</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal G</td>
<td>E2</td>
<td>0.301</td>
<td>0.308</td>
<td>H2</td>
<td>0.455</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctrl 1</td>
<td>G2</td>
<td>1.040</td>
<td>0.760</td>
<td>12.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>A3</td>
<td>1.751</td>
<td>1.764</td>
<td>0.570</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Q.C. PARAMETERS

In order for the assay results to be considered valid, the following criteria should be met:

1. The absorbance (OD) of calibrator 0 ng/ml should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

LIMITATIONS OF PROCEDURE

A. Assay Performance

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
4. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time-deviation during reaction.
5. Plate readers measure vertically. Do not touch the bottom of the wells.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.

B. Interpretation

1. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a “normal” adult population, the expected ranges for the Testosterone AccuBind™ ELISA Test System are detailed in Table 1.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>1.0000</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.0009</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>0.0178</td>
</tr>
<tr>
<td>Cortisol</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortisol</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Progesterone</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>17α-OH Progesterone</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DHEA sulfate</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Estradiol</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Estrone</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of “normal” persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

PERFORMANCE CHARACTERISTICS

A. Precision

The within and between assay precision of the Testosterone AccuBind™ Microplate EIA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>10</td>
<td>1.47</td>
<td>0.09</td>
<td>6.3%</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>4.90</td>
<td>0.29</td>
<td>5.9%</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>8.99</td>
<td>0.54</td>
<td>6.0%</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate over a ten day period.

REFERENCES

4. Leshansky, G, et. al., J Clin Endocrinol Metab, 58, 674 (1991)