B) External Evaluation:

Qualisa™ Testosterone ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Qualisa™ Testosterone** ELISA has demonstrated 95% correlation with the reference method.

*Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics Pvt. Ltd.).

IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

BIBLIOGRAPHY

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4. Heinonen, P.K., Androgen production by epithelial ovarian tumours in post-menopausal women. Maturitas, 1991; 13: 117-117-122.
5. Tietz, N.W. ed., Clinical Guide to Laboratory Tests, 3rd Edition, W.B. Saunders, Co., Philadelphia, 1995: 578-580.
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SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	LOT Batch Number / Lot Number
Manufacturer	IVD *In vitro* Diagnostic Medical Device	This side up	Σ Contains sufficient for <n> tests
Use by	REF Catalogue Number	Do not reuse	

Manufactured by:

Zephyr Biomedicals

A Division of Tulip Diagnostics (P) Ltd.

M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.

Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

1120/VER-01

Qualisa™ Microwell Enzyme Immunoassay | TESTO

Testosterone

Enzyme linked Immunosorbent Assay (ELISA) for the Quantitative determination of Testosterone in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Qualisa™ Testosterone Competitive ELISA test is intended for the quantitative determination of Testosterone in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

Testosterone (17β-hydroxyandrost-4-ene-3-one) is a C19 steroid with an unsaturated bond between C-4 and C-5, a ketone group in C-3 and a hydroxyl group in the β position at C-17. This steroid hormone has a molecular weight of 288.4.

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. Low levels of testosterone can be found in patients with the following diseases: Hypopituitarism, Klinefelter's syndrome, Testicular feminization, Orchidectomy and Cryptorchidism, enzymatic defects and some autoimmune diseases. The Testosterone EIA kits are designed for the measurement of total Testosterone in human serum.

PRINCIPLE

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 testosterone standards & patient samples along with testosterone-HRP Conjugate Reagent and rabbit anti-testosterone reagent at 37°C. During the incubation, a fixed amount of HRP-labeled testosterone competes with the endogenous testosterone in the standard and sample, 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, resulting in the development of blue color. The reaction is stopped after specified time with stop solution and absorbance is determined for each well using an ELISA reader. 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 run concurrently with the standards can be calculated from the standard curve.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with Goat Anti-Rabbit IgG.
- Rabbit Anti-Testosterone Reagent. Ready to use
- Testosterone-HRP Conjugate Reagent.
- TMB Substrate. Ready to use
- Stop Solution. Ready to use
- Testosterone Standard set of 6 standards labeled as A to F in liquid form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).

Materials required but not provided

- Precision pipettes: 10µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. **Qualisa™ Testosterone** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the dessicant has changed from blue to white at the time of opening the

pouch, another coated microwells pouch should be used.
3. Diluted Wash Buffer is stable upto one week when stored at 2-8°C.

SPECIMEN COLLECTION

1. Collect Blood specimen by venipuncture according to standard procedure.
2. Serum only should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

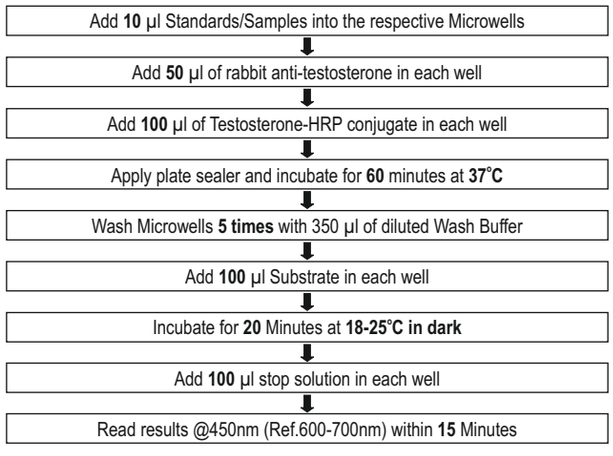
1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens and standards should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **10 µl** of standards and serums into the appropriate wells.
2. Dispense **50 µl** of rabbit anti-testosterone reagent into each well and mix thoroughly for 30 seconds, followed by **100 µl** of testosterone-HRP Conjugate into each well and mix thoroughly for 30 seconds.
3. Incubate at 37°C for **60 mins**.
4. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
5. Dispense **100 µl** of TMB substrate into each well. Incubate at room temperature (18-25°C), in the dark, for **20 minutes**.
5. Stop the reaction by adding **100 µl** of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
6. Read the optical density at 450/630 nm with a microtiter plate reader within 15 minutes.



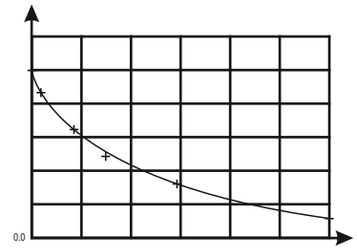
CALCULATION OF RESULTS

Construct a standard curve by plotting the absorbance obtained from each reference standard against its concentration in ng/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of testosterone in ng/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve

Results of a typical standard run with optical density reading at 450nm (ref 600 – 700nm) shown in the Y axis against testosterone concentrations shown in the X axis.

Testosterone (ng/ml)	Absorbance (450nm)
A	1.854
B	1.490
C	1.159
D	0.807
E	0.553
F	0.414



This Standard curve is for the purpose of illustration only, and should not be used to calculate samples. Each user should obtain his or her own standard curve and data.

Expected ranges of values

Each laboratory should establish its own normal range based on the patient population. The Testosterone EIA was performed on randomly selected outpatient clinical laboratory samples. The results of these determinations are as follows:

Males:

prepubertal (late)	0.1 – 0.2 ng/ml
Adult	3.0 – 10.0 ng/ml

Females:

prepubertal (late)	0.1 – 0.2 ng/ml
follicular phase	0.2 – 0.8 ng/ml
luteal phase	0.2 – 0.8 ng/ml
post menopausal	0.08 – 0.35 ng/ml

The minimum detectable concentration of Testosterone by this assay is estimated to be 0.05 ng/ml.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. In an internal Study **Qualisa™ Testosterone** was evaluated against commercially available licensed kit with 90 random clinical samples and **Qualisa™ Testosterone** has demonstrated 95% clinical correlation with the commercially available licensed kit.
2. Precision: **Qualisa™ Testosterone** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with Qualisa™ Testosterone	Coefficient of Variable (CV)
Level 1	10	0.783	6.49
Level 2	10	12.16	5.98
Level 3	10	27.10	4.05