2. **Monitoring of Androgen Suppressing Drugs:**
   Testosterone measurements may be utilized in women for the adjustment of androgen suppressing drugs and their dosages.
3. **Pregnancy:**
   Testosterone concentrations are relatively consistent during the pregnancy.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

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

Controls	No. of testings	Mean Control values with ELECTRA™ Testosterone	Coefficient of Variation (CV)
Level 1	10	1.17	7.58
Level 2	10	3.71	5.95
Level 3	10	8.92	3.38

B) External Evaluation:

Electra™ Testosterone CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Electra Testosterone** CLIA has demonstrated 95% correlation with the reference method.
*Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics (P) 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 RLU values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the RLU 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 RLU values.

BIBLIOGRAPHY

(1) Chen, A., Bookstein, J.J., Meldrum, D.R., Diagnosis of a testosterone-secreting adrenal adenoma by selective venous catheterization. *Fertil. Steril.*, 1991; 55: 1202-1203. (2) Granoff, A.B. and Abraham, G.E., Peripheral and adrenal venous levels of steroids in a patient with virilizing adrenal adenoma. *Obstet. Gynecol.*, 1979; 53:111-115. (3) Bricaire, C., Raynaud, A., Benotmane, A., et al., Selective venous catheterization in the evaluation of hyperandrogenism. *J. Endocrinol Invest.,* 1991; 14: 949-956. (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. (6) USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories"" 1984.

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.

An ISO 13485 Certified Company

0321/VER-01

electra™ TESTO

Chemiluminescence Assay for Quantitative Determination of Testosterone in Human Serum.
FOR IN VITRO DIAGNOSTIC USE ONLY
Store at 2°C to 8°C

INTENDED USE

ELECTRA™ Testosterone CLIA competitive 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

ELECTRA™ Testosterone Quantitative CLIA assay is for use on **ELECTRA** analyzers. **ELECTRA™ Testosterone CLIA** works on the principle of chemiluminescence wherein light is produced by a chemical reaction from a substance as it returns from an electronically excited state to the ground state. When catalysed by HRP, the oxidation of luminol by hydrogen peroxide produces an electronically excited form of 3-aminophthalate which on relaxation emits light with maximum intensity at λ=425nm.
The **ELECTRA™ Testosterone CLIA** 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 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. A solution of chemiluminescent substrate is then added and Luminescence is measured in RLU. The intensity of the emitting light is directly proportional to the amount of enzyme present and is inversely related to the amount of unlabeled Testosterone in the sample. By reference to a series of Testosterone standards assayed in the same way, the concentration of Testosterone in the unknown sample is quantified.

MATERIALS & 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.
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- 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: 10-100µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- **ELECTRA™ Analyzer**

electra™ Chemiluminescence assay
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STORAGE AND STABILITY

1. **ELECTRA™ Testosterone** kit is stable at 2-8°C up to the 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.
4. Working Substrate (A+B) must be used immediately.

SPECIMEN COLLECTION

1. Collect blood specimen by venipuncture according to the standard procedure.
2. Only serum 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.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

REAGENT PREPARATION

- 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.
- Dilute Wash Buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
- Prepare a Working Substrate by Mixing Substrate A and Substrate B in equal volume (1:1 ratio) before addition to the micro-wells.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Substrate-A µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450
Substrate-B µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **25** µl of Standards & Serums into the appropriate wells.
2. Dispense **50** µl of rabbit anti-testosterone reagent into each well, followed by **100** µl of testosterone-HRP Conjugate into each well.
3. Incubate at 37°C for **60 mins**. Thoroughly mix for 30 seconds.
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 the absorbent paper or paper towels to remove all residual water droplets.
5. Add **50** µl of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
6. Cover the **ELECTRA™** microplate and incubate for **10** minutes at room temperature (18-25°C) in dark.
7. Read the **ELECTRA™** micro-plate exactly at **10** minutes in **ELECTRA™ Analyzer.** If **ELECTRA™** micro-plate is not read between 10-15 minutes the test results should be considered as invalid.

Add **25** µl Standards/Samples into the respective Microwells

⬇

Add **50** µl of anti-Testosterone reagent and **100** µl of Testosterone-HRP conjugate in each well

⬇

Apply plate sealer and incubate for **60** minutes at **37°C**

⬇

Wash Microwells **5 times** with 350 µl **of** diluted Wash Buffer

⬇

Add **50** µl Substrate in each well

⬇

Incubate for **10** Minutes at **18-25°C in dark**

⬇

Read results on **ELECTRA ™** Analyzer exactly at the end of **10** Minutes

CALCULATION OF RESULTS

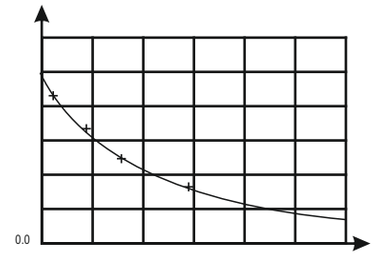
Construct a Standard curve by plotting the mean RLU obtained from each reference standard against its concentration in ng/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding 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 RLU's shown in the Y axis against Testosterone concentrations in the X axis.

Suggest: Use 4-Parameter Standard curve to calculate sample values.

Testosterone Values (ng/ml)	RLU's
A	4935398
B	4505360
C	3339844
D	1671095
E	624290
F	136117



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 VALUES AND SENSITIVITY

Each laboratory should establish its own normal range based on the patient population. The Testosterone Assay 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 the Testosterone CLIA assay as measured by 2 SD from the mean of a zero standard is estimated to be 0.05 ng/ml.

CLINICAL APPLICATION

In Males:

In men, the determination of testosterone is used as an indicator for the function of the testes: low hormone levels are found in cases with Klinefelter's syndrome, cryptorchism or anorchia. Males with testosterone deficiency often present with a number of symptoms such as decreased libido, as well as decreased muscle strength, gynecomastia and infertility.

In Females:

1. **Virilizing Disorders:**
   Testosterone measurements are frequently utilized in the evaluation of virilizing disorders. Testosterone concentrations >2.0 ng/ml may indicate androgen secreting ovarian or adrenal neoplasms.

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