PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

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

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

B) External Evaluation:

ELECTRA™ Progesterone CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **ELECTRA™ Progesterone** 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) Radwanska, E., Frankenberg, J., and Allen, E., Plasma progesterone levels in normal and abnormal early human pregnancy. *Fertility and Sterility*, 1978; 30, 398-402. (2) Autrere,M.B., and Benson, H., Progesterone: An overview and recent advances, *J. Par. Sci.*, 1976; 65: 783-800. (3) March, C.M., Goebelsmann, U., Nakamura, R.M., and Mishell, D.R. Jr., Roles of estradiol and progesterone in eliciting the midcycle luteinizing hormone and follicle-stimulating hormone surges, *J. Clin. Endo. Metab.*, 1979; 49, 507-513. (4) Ross, G.T., Vande Wiele, R.L., and Frantz, A.G., The Ovaries and the breasts. In: Williams, R.H., ed., *Textbook of Endocrinology*. Saunders Company, Philadelphia; 1981: 355-411. (5) Chattoraj, S.C., Endocrine function. In: Tietz, N.W., ed., *Fundamentals of Clinical Chemistry*. Saunders Company, Philadelphia; 1976: 699-823. (6) Shepard, M.K., and Senturia, Y.D., Comparison of serum progesterone and endometrial biopsy for confirmation of ovulation and evaluation of luteal function. *Fertility and Sterility*, 1977; 28: 541-548. (7) Johansson, E.D.B., and Jonasson, L.-E., Progesterone levels in amniotic fluid and plasma from women: I. Levels during normal pregnancy. *Acta Obstet. Gynec. Scand.*, 1971; 50: 339-343. (8) USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories"" 1984. (9) Tietz, N.W. ed., *Clinical Guide to Laboratory Tests*, 3rd Edition, W.B. Saunders, Co., Philadelphia, 1995: 509-512.

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.

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An ISO 13485 Certified Company

0321/VER-01

electra™ PROG

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

INTENDED USE

ELECTRA™ Progesterone CLIA test is intended for the quantitative determination of Progesterone in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

Progesterone is a C21 steroid which is synthesized from both tissue and circulating cholesterol. Cholesterol is transformed to pregnenolone which is then converted via a combined dehydrogenase and isomerase to progesterone. The principle production sites are the adrenals and ovaries and the placenta during pregnancy. The majority of this steroid is metabolized in the liver to pregnanediol and conjugated as a glucuronide prior to excretion by the kidneys.

Progesterone exhibits a wide variety of end organ effects. The primary role of progesterone is exhibited by the reproductive organs. In males, progesterone is a necessary intermediate for the production of corticosteroids and androgens. In females, progesterone remains relatively constant throughout the follicular phase of the menstrual cycle. The concentration then increases rapidly following ovulation and remains elevated for 4-6 days and decreases to the initial level 24 hours before the onset of menstruation. In pregnancy, placental progesterone production rises steadily to levels of 10 to 20 times those of the luteal phase peak.

Progesterone measurements are thus performed to determine ovulation as well as to characterize luteal phase defects. Monitoring of progesterone therapy and early stage pregnancy evaluations comprise the remaining uses of progesterone assays. The Progesterone CLIA kits are designed for the measurement of total progesterone in human serum.

PRINCIPLE

ELECTRA™ Progesterone Quantitative CLIA assay is for use on **ELECTRA** analyzers. **ELECTRA™ Progesterone 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™ Progesterone CLIA** is based on the principle of competitive binding between progesterone in the test specimen and progesterone-HRP conjugate for a constant amount of rabbit anti- progesterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with progesterone standards & patient samples along with progesterone-HRP Conjugate Reagent and rabbit anti-progesterone reagent at room temperature. During the incubation, a fixed amount of HRP-labeled progesterone competes with the endogenous progesterone in the standard and sample, for a fixed number of binding sites of the specific progesterone antibody. Thus, the amount of progesterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of progesterone in the specimen increases. Unbound progesterone 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 Progesterone in the sample. By reference to a series of Progesterone standards assayed in the same way, the concentration of progesterone 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-Progesterone Reagent. Ready to use.
- Progesterone-HRP Conjugate Concentrate (11X).
- Progesterone-HRP Conjugate Diluent.
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- Progesterone 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**

STORAGE AND STABILITY

1. **ELECTRA™ Progesterone** 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.
- Dilute enzyme conjugate with Conjugate diluent according to the requirement as shown below. Prepare a fresh dilution for each assay.

No. of Strips	0.5	1	2	3	4	5	6	7	8	9	10	11	12
Enzyme Conjugate (µl)	50	100	180	250	320	400	480	550	640	700	760	840	900
Conjugate Diluent (µl)	500	1000	1800	2500	3200	4000	4800	5500	6400	7000	7600	8400	9000

- 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-progesterone reagent into each well, followed by**100 µl** of Working Progesterone-HRP Conjugate reagent into each well. Incubate at room temperature (18-25°C) for **60 mins**. Thoroughly mix for 30 seconds.
3. 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.
4. Add **50** µl of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
5. Cover the **ELECTRA™** microplate and incubate for **10** minutes at room temperature (18-25°C) in dark.
6. 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-Progesterone reagent and **100** µl of diluted Progesterone-HRP conjugate in each well

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Apply plate sealer and incubate for **60** minutes at **18-25°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

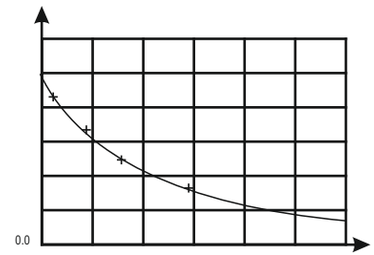
Construct a Standard curve by plotting the mean RLU obtained from each reference standard against its concentration in ng/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding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

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

Progesterone Values (ng/ml)	RLU's
A	2582029
B	1579148
C	759573
D	339954
E	149155
F	60486



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 Progesterone EIA was performed on randomly selected outpatient clinical laboratory samples.

Males:	Adult	0.13 – 0.97 ng/ml
	Prepubertal (children)	0.70 – 0.52 ng/ml
Females:	follicular phase	0.15 – 0.70 ng/ml
	luteal phase	2.00 – 25.0 ng/ml
	post menopausal	0.06 – 1.60 ng/ml
Pregnancy:	1st trimester	10.3 – 44.0 ng/ml
	2nd trimester	19.5 – 82.5 ng/ml
	3rd trimester	65.0 – 229 ng/ml

The minimum detectable concentration of Progesterone by this assay is estimated to be 0.2 ng/ml.

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