Chemiluminescence assay

**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 second trimester pregnancy evaluations comprise the remaining uses of progesterone assays. The Progesterone CLIA kits are designed for the measurement of total progesterone in human serum.

**PERFORMANCE CHARACTERISTICS**

A) Internal Evaluation:

1. Accuracy: 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:

<table>
<thead>
<tr>
<th>Controls</th>
<th>No. of testings</th>
<th>Mean Control values with ELECTRA Progesterone</th>
<th>Coefficient of Variation (CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>10</td>
<td>0.783</td>
<td>6.49</td>
</tr>
<tr>
<td>Level 2</td>
<td>10</td>
<td>12.16</td>
<td>5.98</td>
</tr>
<tr>
<td>Level 3</td>
<td>10</td>
<td>27.10</td>
<td>4.05</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Level</th>
<th>No of Testings</th>
<th>Mean Control value with ELECTRA Progesterone</th>
<th>Coefficient of Variation (CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>10</td>
<td>0.783</td>
<td>6.49</td>
</tr>
<tr>
<td>Level 2</td>
<td>10</td>
<td>12.16</td>
<td>5.98</td>
</tr>
<tr>
<td>Level 3</td>
<td>10</td>
<td>27.10</td>
<td>4.05</td>
</tr>
</tbody>
</table>

**BIBLIOGRAPHY**


**SYMBOL KEYS**

- Temperature Limitation
- Contact Instructions for use
- Date of Manufacture
- Catalogue Number
- Test side up
- Do not reuse
- Contains sufficient for 40 tests

**MATERIALS & COMPONENTS**

- 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.
- Rabbit Anti-Progesterone Reagent. Ready to use.
- Proc. 382

**INTENDED USE**

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

**MANUFACTURER**

Zephyr Biomedicals

**Certified Company**

An ISO 13485

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Catalogue Number</th>
<th>BIBLIOGRAPHY</th>
</tr>
</thead>
</table>

**REFERENCES**

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Use protective clothing and wear gloves when handling samples.
5. Do not mix components of one kit with another.
6. Do not heat inactivate before use.
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 allow liquid from one well to mix with other wells.
11. Always use new tip for each specimen and reagent.
12. Do not let the strips dry in between the steps.
13. Do not pipelease any material by mouth.

PRECAUTIONS

1. Collect blood specimen by venipuncture according to the standard procedure.
2. Diluted Wash Buffer is stable up to one week when stored at 2-8°C.
3. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be sealed to protect from moisture. If the colour of the dessicant has changed from blue to white, the kit should not be used.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
7. Specimen should be free from particulate matter and microbial contamination.
8. Specimen should be free from particulate matter and microbial contamination.
9. Dilute Wash Buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
10. Dilute Wash Buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
11. 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.
12. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
13. Avoid grossly hemolytic, lipemic or turbid samples.
14. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be sealed to protect from moisture. If the colour of the dessicant has changed from blue to white, the kit should not be used.
15. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
16. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
17. 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.
18. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
19. Avoid grossly hemolytic, lipemic or turbid samples.
20. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.

SPECIMEN COLLECTION

1. Add 50 µl of anti-Progesterone reagent and 100 µl of diluted Progesterone-HRP conjugate in each well.
2. Apply plate sealer and incubate for 60 minutes at 18-25°C.
3. Wash Microwells 5 times with 350 µl of diluted Wash Buffer.
4. Add 50 µl Substrate in each well.
5. Incubate for 10 Minutes at 18-25°C in dark.
6. Read results on ELECTRA™ Analyzer exactly at the end of 10 Minutes.

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 25 µl of Standards & Serums into the appropriate wells.
2. Add 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.

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.