ichroma™ Progesterone

INTENDED USE
ichroma™ Progesterone is a fluorescence immunoassay for the quantitative determination of progesterone in human serum or plasma. ichroma™ Progesterone is used as an aid in the screening of determination of the cause of infertility, track ovulation, diagnose an ectopic or failing pregnancy, monitor the health of a pregnancy. For in vitro diagnostic use only.

INTRODUCTION
Progesterone also known as P4 (pregn-4-ene-3,20-dione) is a C-21 steroid hormone involved in the female menstrual cycle, pregnancy (support gestation) and embryogenesis of humans and other species. Progesterone belongs to a class of hormones called progestogens, and is the major naturally occurring human progestogen.

In mammals, progesterone, like all other steroid hormones, is synthesized from pregnenolone, which in turn is derived from cholesterol. Progesterone is essential for the regulation of normal female reproductive functions. The major physiological actions of progesterone are: a) in the uterus and ovary: induction of ovulation, facilitation of implantation, and maintenance of early pregnancy; b) in the mammary gland: lobular-alveolar development in preparation for milk secretion; c) in the brain: neurobehavioural expression associated with sexual responsiveness and d) in the bone: prevention of bone loss. During the follicular phase of the cycle, progesterone levels remain low. Following the LH surge and ovulation, luteal cells in the ruptured follicle produce progesterone in response to LH. During this, the luteal phase, progesterone rises rapidly to a maximum of 10-20 ng/mL at day 5-7 following ovulation. During the luteal phase, progesterone transforms the estrogen-primed endometrium from a proliferative to a secretory state. If pregnancy does not occur, progesterone levels decrease during the last four days of the cycle due to the regression of the corpus luteum.

If conception occurs, the levels of progesterone are maintained at mid-luteal levels by the corpus luteum until about week six. At that time the placenta becomes the main source of progesterone and levels rise from approximately 10-50 ng/mL in the first trimester to approximately 50-280 ng/mL in the third trimester. ichroma™ Progesterone measures quantitatively progesterone concentration in human serum and plasma.

<Reference range of Progesterone in human blood\textsuperscript{12}>

<table>
<thead>
<tr>
<th>Type</th>
<th>ng/ml</th>
<th>nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[SI : 1 ng/ml = 3.18 nmol/L]</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular phase</td>
<td>0.2 – 1.5</td>
<td>0.6 – 4.8</td>
</tr>
<tr>
<td>Ovulatory phase</td>
<td>0.8 – 3.0</td>
<td>2.5 – 9.5</td>
</tr>
<tr>
<td>Luteal phase</td>
<td>1.7 – 27.0</td>
<td>5.4 – 85.9</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>0.1 – 0.8</td>
<td>0.3 – 2.5</td>
</tr>
<tr>
<td>1\textsuperscript{st} Trimester</td>
<td>9.0 – 47.0</td>
<td>28.6 – 149.5</td>
</tr>
<tr>
<td>2\textsuperscript{nd} Trimester</td>
<td>17.0 – 146.0</td>
<td>54.1 – 464.8</td>
</tr>
<tr>
<td>3\textsuperscript{rd} Trimester</td>
<td>55.0 – 255.0</td>
<td>174.9 – 810.9</td>
</tr>
</tbody>
</table>

* It is recommended that each laboratory establish its own reference range.

PRINCIPLE
ichroma™ Progesterone uses a competitive immunoassay using direct fluorescence technology, such that the fluorescence labeled anti-progesterone antibody in detection buffer binds to Progesterone in the blood sample and unbound antibody binds to Progesterone covalently coupled to BSA that has been immobilized on test strip as sample mixture migrates through the nitrocellulose matrix. Thus the more progesterone in blood, the less unbound fluorescence labeled antibodies accumulated on the test strip. The fluorescence intensity of the anti-progesterone antibody reflects the amount of antigen captured and is processed in ichroma™ Reader to determine the progesterone concentration in the specimen. Progesterone is a C-21 steroid hormone secreted by granulosa cells of the ovary.

COMPONENTS AND REAGENTS
ichroma™ Progesterone consists of a ‘Test cartridge’, an ‘ID chip’, and a ‘Detection buffer’.
- The test cartridge consists of a test strip; on the membrane of which, Progesterone-BSA conjugate and chicken IgY have been immobilized at the test line and the control line respectively.
- Each test cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed test cartridges are packed in a box which also contains an ID chip.
- The detection buffer pre-dispensed in tube contains fluorochrome-labeled anti-progesterone antibodies, fluorescent-labeled anti-chicken IgY, bovine serum albumin (BSA) as a stabilizer and less than 0.1% sodium azide in phosphate buffered saline (PBS) as a preservative.

Storage
The detection buffer is packed in a separate box which is further packed in a styrofoam box provided with ice packs for the purpose of shipment.

STORAGE AND STABILITY
- The test cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4 - 30°C.
- The detection buffer dispensed in a tube is stable for 20 months if stored at 2 - 8°C.
- After the test cartridge pouch is opened, the test should be performed immediately.

LIMITATIONS OF THE TEST SYSTEM
ichroma™ Progesterone provides accurate and reliable results subject to the following constraints:
- ichroma™ Progesterone should be used only in conjunction with ichroma™ Reader.
- The test should always be performed on freshly collected sample(s).
- Any anticoagulants other than heparin should be avoided.
- The test sample must be at room temperature prior to testing. If the test samples are shipped for the purpose of this test, appropriate precautions must be exercised.
- The test should not be performed on hemolysed samples. If a test sample appears to be hemolysed, fresh blood sample should be obtained, processed and tested.

- Effectiveness of the test is highly dependent on storage of test components and test samples at prescribed optimal conditions.
- The test may yield false positive result(s) due to cross-reactions of some components of serum with the capture/detector antibodies.
and/or non-specific adhesion of certain components having similar epitopes to bind with these antibodies.

- The test may also yield false negative results; the most common factor being non-responsiveness of the antigen to the antibodies due to its epitopes being masked by some unknown components such that the antigen cannot be detected or captured by the antibodies. False negative results may also be obtained due to instability or degradation of the progesterone antigen with time and/or temperature making it unrecognizable by the antibodies.
- Other factors interfering with the test and causing erroneous results include technical/procedural errors, degradation of the test components/reagents as well as presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.

SAMPLE COLLECTION AND PROCESSING

- Serum (including serum collected in serum separator tubes) or plasma collected in heparin may be used in the ichroma™ Progesterone assay.
- Ensure that complete clot formation has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- If testing will be delayed more than 24 hours, serum or plasma should be separated from the clot or red blood cells. If using serum separator tubes, remove serum from the separator within 48 hours. Samples may be stored for up to a week at 2-8°C prior to being tested. If testing will be delayed more than a week, samples should be frozen at -20°C or below. Samples stored frozen at -20°C or below for 3 months showed no performance difference.
- Patient samples should be mixed and centrifuged after any freeze-thaw cycle or to remove red blood cells or platelet matter.
- Multiple freeze-thaw cycles should be avoided. Samples must be mixed thoroughly after thawing by low speed vortex or by gently inverting, and centrifuged prior to use to remove particulate matter and to ensure consistency in the results. Inspect all samples for bubbles. Remove bubbles prior to analysis.
- When shipped, samples must be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical samples and etiologic agents.
- It is recommended to avoid using severely hemolyzed specimens whenever possible. If a specimen appears to be severely hemolyzed, another specimen should be obtained and tested.

MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>REF</th>
<th>CFPO-21</th>
</tr>
</thead>
</table>

Components of ichroma™ Progesterone

- Test Cartridge Box: 25
  - Sealed Test Cartridges 25
  - ID Chip 1
  - Package Insert 1
- Box containing Detection Buffer Tube 25
  - Detection Buffer Tubes

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from ichroma™ Progesterone. Please contact our sales division for more information.

- ichroma™ Reader REF FR203
- ichroma™ Universal Control REF CFPO-25
- ichroma™ Printer REF PPR007

TEST SETUP

1. Check the contents of ichroma™ Progesterone: Sealed Test Cartridge, ID Chip, and Detection Buffer Tube.
2. Ensure that the lot number of the test cartridge matches that of the ID chip as well as the detection buffer tube.
3. Keep the sealed test cartridge (if stored in refrigerator) and the detection buffer tube at room temperature for at least 30 minutes just prior to the test. Place the test cartridge on a clean, dust-free and flat surface.
4. Turn on the ichroma™ Reader.
5. Insert the ID Chip into the ID chip port of the ichroma™ Reader.
6. Press the ‘Select’ button on the ichroma™ Reader. (Please refer to the ‘ichroma™ Reader Operation Manual’ for complete information and operating instructions.)

* Recommended operation condition for ichroma™ Progesterone

Temperature: 20 – 30 °C
Humidity: < 70%

CAUTION!

Operation temperature for ichroma™ Progesterone.
Acceptable Temperature: 20-32 °C

1. Description
- The ambient temperature is displayed in upper right corner of the screen of the ichroma™ Reader.
- If the ambient temperature is not within the acceptable range, 20-32 °C, “Caution” message will be displayed.
- “Low Temperature, Continue: Press S, Back: Press R” - If the ambient temperature is below 20 °C.
- “High Temperature, Continue: Press S, Back: Press R” – If the ambient temperature is above 32 °C.
- “S” is “Select” button and “R” is “Reset” button.

2. Corrective Action
- Please wait until the ambient temperature reaches to the operation temperature, 20-32 °C.
- Do not arbitrarily increase or lower the temperature displayed on display.

3. If the operation temperature is not within the range, the test results may not be accurate.

4. If the temperature displayed in the screen is abnormal or there are some problems during the testing, please contact our technical support team.

TEST PROCEDURE

1. Transfer 30µl of samples (serum or plasma or control) using a transfer pipette to a tube containing the detection buffer.
2. Close the lid of the detection buffer tube and mix the sample thoroughly by shaking it about 10 times.
3. Pipette out 75ul of a sample mixture and dispense it into the sample well on the test cartridge.
4. Leave the sample-loaded test cartridge at room temperature for 15 minutes.
5. For scanning the sample-loaded test cartridge, insert it into the test cartridge holder of the ichroma™ Reader. Ensure proper orientation of the test cartridge before pushing it all the way inside the test cartridge holder. An arrow has been marked on the test cartridge especially for this purpose.
6. Press ‘Select’ button on the ichroma™ Reader to start the scanning process.
7. ichroma™ Reader will start scanning the sample-loaded test cartridge immediately.
8. Read the test result on the display screen of the ichroma™ Reader.

INTERPRETATION OF TEST RESULT

- ichroma™ Reader calculates the test result automatically and displays progesterone concentration of the test sample in terms of ng/ml and nmol/L.
- Working range of ichroma™ Progesterone is 1.4 - 40 ng/mL (4.45-127.2 nmol/L).
- ichroma™ Progesterone test should be considered as a screening tool only. Please consult a physician to discuss the test result. The physician may decide further course of action.

* Conversion factor as unit of nmol/L
  - 1 ng/ml = 3.18 nmol/L
**QUALITY CONTROL**

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- Before testing a clinical sample using a new test lot, control reagents should be tested to confirm the test procedure, and to verify whether the test produces the expected results.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control reagents are not provided with ichroma™ Progesterone. For more information regarding obtaining the control reagents, contact Boditech Med Inc.’s Sales Division for assistance.
- ichroma™ Progesterone test has a built-in internal control that satisfies the routine quality control requirements. This internal control test is performed automatically each time a clinical sample is tested. An invalid result from the internal control leads to display an error message on the ichroma™ Reader indicating that the test should be repeated.

**PERFORMANCE CHARACTERISTICS**

1. **Specificity:** Study of interference and cross reactivity showed the following results.

<table>
<thead>
<tr>
<th>Cross reactivity materials</th>
<th>Concentration of cross reactivity materials</th>
<th>Cross reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17α-OH-progesterone</td>
<td>2 μg/ml</td>
<td>3.5</td>
</tr>
<tr>
<td>17β-estradiol(oestradiol)</td>
<td>2 μg/mL</td>
<td>0.1</td>
</tr>
<tr>
<td>5 α-progesterone-3, 20-dione</td>
<td>0.2 μg/L</td>
<td>8.0</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>2 μg/ml</td>
<td>0.3</td>
</tr>
<tr>
<td>Danasol</td>
<td>20 μg/mL</td>
<td>ND</td>
</tr>
<tr>
<td>Estriol</td>
<td>2 μg/ml</td>
<td>ND</td>
</tr>
<tr>
<td>Testosterone</td>
<td>2 μg/ml</td>
<td>ND</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>2 μg/mL</td>
<td>ND</td>
</tr>
<tr>
<td>Estrone</td>
<td>2 μg/mL</td>
<td>ND</td>
</tr>
<tr>
<td>Transferrin</td>
<td>2 μg/mL</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND : Not Detected

<table>
<thead>
<tr>
<th>Interference materials</th>
<th>Concentration of interference materials</th>
<th>Interference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-glucose</td>
<td>600 mM/L</td>
<td>1.4</td>
</tr>
<tr>
<td>L-Arscorbic acid</td>
<td>2 mM/L</td>
<td>1.6</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>4 mM/L</td>
<td>0.6</td>
</tr>
<tr>
<td>Hemoglobin[human]</td>
<td>20 g/L</td>
<td>1.1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>130 mM/L</td>
<td>2.8</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>100 mg/mL</td>
<td>1.6</td>
</tr>
</tbody>
</table>

There was no significant interference and cross reactivity from these materials with the ichroma™ Progesterone test measurements.

2. **Imprecision:** For studying intra-assay, 10 replicates of each of the three concentrations of control reagent were tested using different 3 lots of ichroma™ Progesterone. For studying inter-assay, 6 replicates of each of the three concentrations of control reagent were tested by different persons with 2 different ichroma™ Readers during 5 days using different 3 lots of ichroma™ Progesterone.

<table>
<thead>
<tr>
<th>Progesterone [nmol/L]</th>
<th>Intra-assay</th>
<th>Lot 1</th>
<th>Lot 2</th>
<th>Lot 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV (%)</td>
<td>Mean</td>
<td>CV (%)</td>
<td>Mean</td>
</tr>
<tr>
<td>5.39</td>
<td>5.3</td>
<td>12.6</td>
<td>5.4</td>
<td>13.6</td>
</tr>
<tr>
<td>34.7</td>
<td>35.4</td>
<td>437</td>
<td>34.2</td>
<td>8.3</td>
</tr>
<tr>
<td>108.0</td>
<td>101.4</td>
<td>6.3</td>
<td>102.8</td>
<td>4.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Progesterone [nmol/L]</th>
<th>Mean</th>
<th>Within run CV(%)</th>
<th>Between run CV(%)</th>
<th>Between day CV(%)</th>
<th>Total CV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.39</td>
<td>5.3</td>
<td>CV(%)</td>
<td>CV(%)</td>
<td>CV(%)</td>
<td>CV(%)</td>
</tr>
<tr>
<td>34.7</td>
<td>35.4</td>
<td>CV(%)</td>
<td>CV(%)</td>
<td>CV(%)</td>
<td>CV(%)</td>
</tr>
<tr>
<td>108.0</td>
<td>101.4</td>
<td>CV(%)</td>
<td>CV(%)</td>
<td>CV(%)</td>
<td>CV(%)</td>
</tr>
</tbody>
</table>

3. **Comparability:** Progesterone concentrations of 95 serum samples were quantified independently with ichroma™ Progesterone and Access 2 as per prescribed test procedures. Test results were compared and their comparability was investigated with linear regression and coefficient of correlation (R). This study was performed 2 times using same serum samples. Linear regression and coefficient of correlation between the two tests were as below.

<table>
<thead>
<tr>
<th>Regression</th>
<th>Correlation coefficient(R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st trial</td>
<td>y = 1.0347x – 0.6367</td>
</tr>
<tr>
<td>2nd trial</td>
<td>y = 1.046x – 0.5723</td>
</tr>
</tbody>
</table>

**REFERENCES**

1. Potential use of single measurement of serum progesterone in detecting early pregnancy failure Hanita O MD, MPATH, Hanisah AH MD, MPATH


Note: Please refer to the table below to identify various symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="READ" /></td>
<td>Read instructions for use</td>
</tr>
<tr>
<td><img src="image" alt="USE" /></td>
<td>Use by</td>
</tr>
<tr>
<td><img src="image" alt="LOT" /></td>
<td>Batch code</td>
</tr>
<tr>
<td><img src="image" alt="REF" /></td>
<td>Catalog number</td>
</tr>
<tr>
<td><img src="image" alt="CAUTION" /></td>
<td>Caution</td>
</tr>
<tr>
<td><img src="image" alt="MANUFACTURER" /></td>
<td>Manufacturer</td>
</tr>
<tr>
<td><img src="image" alt="AUTHORIZED" /></td>
<td>Authorized representative of the European Community</td>
</tr>
<tr>
<td><img src="image" alt="IVD" /></td>
<td>In vitro diagnostic medical device</td>
</tr>
<tr>
<td><img src="image" alt="TEMPERATURE" /></td>
<td>Temperature limit</td>
</tr>
<tr>
<td><img src="image" alt="DO NOT REUSE" /></td>
<td>Do not reuse</td>
</tr>
<tr>
<td><img src="image" alt="PRODUCT" /></td>
<td>This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices</td>
</tr>
</tbody>
</table>

For technical assistance, please contact:
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