Hormone

ichroma™ Progesterone

INTENDED USE

ichroma™ Progesterone is a fluorescence Immunoassay (FIA) for the quantitative determination of progesterone in human serum/plasma. It is useful as an aid in management and monitoring 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 (supports gestation) and embryogenesis of humans and other species.[2] 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[3,4]; c) in the brain: neurobehavioral expression associated with sexual responsiveness[5] and d) in the bone: prevention of bone loss[6].

During the follicular phase of the cycle, progesterone levels remain low[7-9]. 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.[8] If pregnancy does not occur, progesterone levels decrease during the last four days of the cycle due to the regression of the corpus luteum.[7,8-13] 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.[7,14,15]

PRINCIPLE

The test uses a competitive immunodetection method.

In this method, the target material in the sample binds to the fluorescence (FL)-labeled detection antibody in detection buffer, to form the complex as sample mixture. This complex is loaded to migrate onto the nitrocellulose matrix, where the covalent couple of progesterone and bovine serum albumin (BSA) is immobilized on a test strip, and interferes with the binding of target material and FL-labeled antibody. If the more target material exists in blood, the less detection antibody is accumulated, resulting in the less fluorescence signal.

COMPONENTS

ichroma™ Progesterone consists of 'Cartridges', 'Detection Buffer Tubes' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has BSA-Progesterone conjugate at the test line, while streptavidin at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The detection buffer contains anti human progesterone-fluorescence conjugate, biotin-BSA-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is dispensed in a tube. 25 detection buffer tubes are packaged in a box and further packed in a Styrofoam box with ice-pack for the shipment.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, ID chip and detection buffer) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A detection buffer tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with the regulations. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, detection buffer and sample to be at room temperature for approximately 30 minutes.
- **ichroma™ Progesterone** as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used detection buffer tubes, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ Progesterone** will provide accurate and reliable results subject to the following conditions.

- Use **ichroma™ Progesterone** should be used only in conjunction with instrument for ichroma™ tests.
- Any anticoagulants other than **Sodium heparin, EDTA** should be avoided.

STORAGE AND STABILITY

- The 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 cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous

results, such as technical/procedural errors, degradation of the test components/reagents or 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.

MATERIALS SUPPLIED

REF CFPC-21

Components of **ichroma™ Progesterone**
- Cartridge Box:
  - Cartridges 25
  - ID Chip 1
  - Instruction For Use 1
- Box containing Detection Buffer tubes
  - Detection Buffer tubes 25

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ Progesterone**. Please contact our sales division for more information.
- Instrument for ichroma™ tests
  - **ichroma™ Reader** REF FR203
  - **ichroma™ II** REF FPRR021
  - **ichroma™ D** REF 13303
- **ichroma™ Printer** REF FPRR007
- **Boditech Hormone Control** REF CFPO-95

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Progesterone** is human serum/plasma.
- To avoid time related absorption, serum samples should not be stored in collection tube with gel separators.
- It is recommended to test the sample within 24 hours after collection.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- 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.
- Samples stored frozen at -20 °C for 2 months showed no performance difference.
- Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change of test values.

TEST SETUP

- Check the contents of **ichroma™ Progesterone**: Sealed Cartridge, Detection Buffer Tubes and ID Chip.
- Ensure that the lot number of the cartridge matches that of the ID chip as well as the detection buffer.
- Keep the sealed 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 cartridge on a clean, dust-free and flat surface.
- Turn on the instrument for ichroma™ tests.
- Insert the ID Chip into the ID chip port of the instrument for ichroma™ tests**.**
- Press the 'Select' button on the instrument for ichroma™ tests. (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

CAUTION

- To minimize erroneous test results, we suggest that the ambient temperature of the test cartridge should be 25 ℃ during the reaction time after loading sample mixture to the test cartridge.
- To maintain the ambient temperature to 25 ℃, you can use various devices such as an i-Chamber or an incubator and so on.

TEST PROCEDURE

1) Transfer 30 µL (Human serum/plasma/control) of sample 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. (The sample mixture must be used immediately.)
3) Pipette out 75 µL of a sample mixture and load it into the sample well on the cartridge.
4) Insert the sample-loaded cartridge into the slot of the i-Chamber or an incubator (25 °C).
5) Leave the sample-loaded cartridge in the i-Chamber or an incubator for 15 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.*
6) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
7) Press 'Select' button on the instrument for ichroma™ tests to start the scanning process.
8) Instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
9) Read the test result on the display screen of the instrument for ichroma™ tests.

INTERPRETATION OF TEST RESULT

- Instrument for ichroma™ tests calculates the test result automatically and displays progesterone concentration of the test sample in terms of nmol/mL and ng/mL.
- Reference range

Type		Mean (nmol/L)	Range (nmol/L)
Males		2.67	0.46-6.55.
Non-pregnant Females	Mid-follicular phase	2.19	0.99-4.83
	Mid-luteal phase	36.32	16.4-59.02
	Post menopausal	0.80	<0.25-2.48
Pregnancy	First trimester	70.50	15.04-161.35
	Second trimester	94.54	61.72-144.05

*SI : nmol/L = 3.18 X ng/mL
- Working range : 4.45-127.2 nmol/L and 1.4-40 ng/mL

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.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided with **ichroma™ Progesterone**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance. (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**

Limit of Blank	(LoB)	1.2 nmol/L
Limit of Detection	(LoD)	1.7 nmol/L
Limit of Quantification	(LoQ)	4.45 nmol/L

- **Analytical specificity**

- Cross-reactivity

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

Cross reactivity materials	Concentration of cross reactivity materials	Cross reactivity (%)
17-α-OH-progesterone	2 μg/ml	1.2
17β-estradiol(oestradiol)	2 μg/mL	0.1
5 α-prognane-3, 20-dione	0.2 μg/mL	5.5
Hydrocortisone	2 μg/mL	0.1
Danasol	20 μg/mL	*N/D
Estriol	2 μg/mL	0.2
Testosterone	2 μg/mL	0.1
Dexamethasone	2 μg/mL	*N/D
Estrone	2 μg/mL	0.2
Transferrin	2 μg/mL	*N/D

*N/D: Not Detection

- Interference

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

Interference materials	Concentration of interference materials	Interference (%)
D-glucose	600 mM/L	1.4
L-Ascorbic acid	2 mM/L	1.6
Bilirubin [unconjugated]	4 mM/L	0.6
Hemoglobin[human]	20 g/L	1.1
Cholesterol	130 mM/L	2.8
Triglyceride	100 mg/mL	1.6

- **Precision**

- Intra-assay

For testing intra-assay precision, one person tested three different lots of **ichroma™ Progesterone**, ten times at each concentration of the control standard.

Progesterone [nmol/L]	Lot 1		Lot 2		Lot 3	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
5.39	5.3	12.6	5.4	13.6	5.1	8.6
34.7	35.4	4.7	34.2	8.3	32.8	6.7
108.0	101.4	6.3	102.8	4.2	99.2	5.1

- Inter-assay

For testing inter-assay precision under the same conditions, three persons tested three different lots of **ichroma™ Progesterone** during five days; six times at each concentration of the control standard.

Progesterone [nmol/L]	Mean	Within run	Between run	Between day	Total CV(%)
		CV(%)	CV(%)	CV(%)	
5.39	5.6	11.8	11.6	9.2	9.8
34.7	34.0	7.3	6.6	7.4	7.3
108.0	103.4	5.3	5.2	6.9	6.5

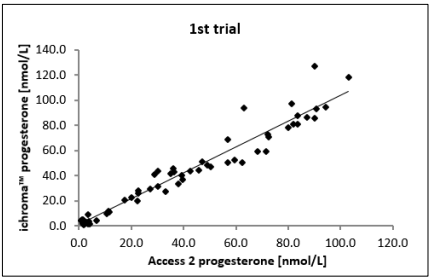
- **Accuracy**

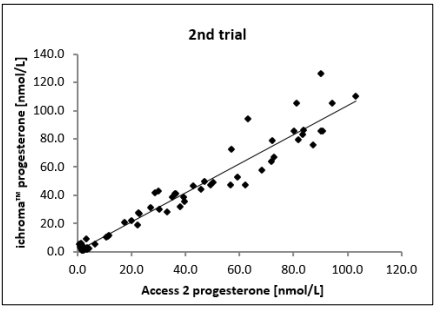
The accuracy was confirmed by 3 different lots testing six times each different concentrations.

Progesterone [nmol/L]	Lot 1	Lot 2	Lot 3	AVG	SD	CV(%)	Bias(%)
5.4	5.3	5.7	5.5	5.5	0.65	11.8	101.9
34.7	35.5	32.7	33.5	33.9	3.02	8.9	97.7
108.0	103.1	107.0	111.1	107.1	5.53	5.2	99.2

- **Comparability:** Progesterone concentrations of 95 clinical samples were quantified independently with **ichroma™ Progesterone** and Beckman Coulter Access2 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.

	Regression	Correlation coefficient(R)
1st trial	y = 1.0347x – 0.6367	0.9847
2nd trial	y = 1.046x – 0.5723	0.9841





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

Σ	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

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