PROGESTERONE

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 EIA kits are designed for the measurement of total progesterone in human serum or plasma.

PRINCIPLE OF THE TEST

The progesterone EIA is based on the principle of competitive binding between progesterone in the test specimen and progesterone-HRP conjugate for a constant amount of rabbit antiprogesterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 25 µl progesterone standards, controls, patient samples, 100 µl progesterone-HRP Conjugate Reagent and 50 µl rabbit anti-progesterone reagent at room temperature (18-25°C) for 90 minutes. During the incubation, a fixed amount of HRP-labeled progesterone competes with the endogenous progesterone in the standard, sample, or quality control serum 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. Next, a solution of TMB Reagent is then added and incubated at room temperature for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of Stop Solution, and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled progesterone in the sample. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The progesterone concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve.

MATERIALS AND COMPONENTS

Materials Provided with Test Kit

1. Goat Anti-Rabbit IgG-coated microtiter wells, 96 wells.
2. Progesterone Reference Standards: 0, 0.5, 3.0, 10, 25 and 50 ng/ml. Liquids, 0.5 ml each, ready to use.
3. Rabbit Anti-Progesterone Reagent, 7 ml.
4. Vortex mixer or equivalent.
5. Absorbent paper or paper towel.
6. Linear-linear graph paper.
7. Microtiter plate reader.

SPECIMEN COLLECTION AND PREPARATION

1. Serum or EDTA plasma should be used. No special pre-treatment of sample is necessary.
2. Serum or plasma samples may be stored at 2-8°C for up to 24 hours, and should be frozen at −10°C or lower for longer periods. Do not use grossly hemolyzed or grossly lipemic specimens.
3. Please note: Samples containing sodium azide should not be used in the assay.

STORAGE OF TEST KIT AND INSTRUMENTATION

- Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2.5 O.D. or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

- All reagents should be brought to room temperature (18-22°C) before use.
- To prepare Working Progesterone-HRP Conjugate Reagent, add 0.1 ml of Progesterone-HRP Conjugate Concentrate (11x) to 1.0 ml of Progesterone-HRP Conjugate Diluent (1:10 dilution) and mix well. The amount of conjugate diluted depends on your assay size. Discard the excess after use.
- Dilute 1 volume of Wash Buffer Concentrate (50x) with 49 volumes of distilled water. For example, dilute 15 ml of Wash Buffer Concentrate (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 25 µl of standards, specimens and controls into appropriate wells.
3. Dispense 50 µl of rabbit anti-progesterone reagent to each well.
4. Dispense 100 µl of **Working Progesterone-HRP Conjugate Reagent** into each well.
5. Thoroughly mix for 30 seconds. ***It is very important to mix them completely.***
6. Incubate at room temperature (18-22°C) for 90 minutes.
7. Rinse and flick the microwells 5 times with washing buffer (1X).
8. Dispense 100 µl of TMB Substrate into each well. Gently mix for 10 seconds.
9. Incubate at room temperature (18-22°C) for 20 minutes.
10. Stop the reaction by adding 100 µl of Stop Solution to each well.
11. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
12. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

Important Note:

- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
- If there are bubbles existing in the wells, false readings will be created. Please use distilled water to remove the bubbles before adding the substrate.

CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A450) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on a linear-linear graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of Progesterone in ng/ml from the standard curve.
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against Progesterone concentrations shown in the X axis. Note: This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is runing at the same time.

Progesterone (ng/ml)	Absorbance (450 nm)
0	2.719
0.5	1.937
3	1.391
10	0.828
25	0.528
50	0.291

EXPECTED VALUES AND SENSITIVITY

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. The following information is cited from reference #9.

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 menopausa	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 the Progesterone ELISA assay as measured by 2 SD from the mean of a zero standard is estimated to be 0.2 ng/ml.

CLINICAL APPLICATION

1. Documentation of Ovulation:

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Monitoring the progesterone concentration during the menstrual cycle is useful in the documentation of ovulation. Progesterone concentration > 3.0 ng/ml will be a strong presumptive evidence of ovulation.

2. Normal vs. Abnormal Progesterone Levels:

Greater-than-normal levels may indicate pregnancy. High level can also indicate adrenal cancer or ovarian cancer, a molar pregnancy, or overproduction of hormones by the adrenal glands. However, levels of progesterone are higher during a multiple pregnancy than during a single pregnancy. Lower-than-normal levels may indicate amenorrhea. Abnormally low levels of progesterone can also indicate problems with ovulation. In a pregnant woman, progesterone levels fall to <5 ng/mL may indicate a threatened miscarriage.

3. Ectopic Pregnancy:

Progesterone can also be useful in ectopic pregnancy diagnosis. For values <25 ng/ml during pregnancy, fetus viability need to be established by ultrasound. However, progesterone <5 ng/ml in the first trimester indicates a nonviable pregnancy regardless of location of the fetus.

LIMITATIONS OF THE PROCEDURE

There are some 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. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects of that.
3. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
4. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
5. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

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PRESENTACIÓN:

CONT. 96 TEST CODIGO: RSET016