en

Immunoassay

REF **CMF0601/CMF0602/CMF0603/CMF0604/CMF0605**

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

PRG CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of PRG (Progesterone) concentration in human serum.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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CE IVD

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Introduction

PRG (progesterone) is a steroid hormone whose main role is to help prepare a female's body for pregnancy; it works in conjunction with several other female hormones.

In mammals, progesterone, like all other steroid hormones, is synthesized from pregnenolone, which in turn is derived from cholesterol.[1] Progesterone exerts its primary action through the intracellular progesterone receptor although a distinct, membrane bound progesterone receptor has also been postulated.[2,3] In addition, progesterone is a highly potent antagonist of the mineralocorticoid receptor.

Abnormal progesterone secretion has been implicated in premenstrual tension, irregular shedding of endometrium, dysmenorrhoea, and luteal insufficiency.[4]

In females, progesterone levels are relatively low during the preovulatory phase of the menstrual cycle, rise after ovulation, and are elevated during the luteal phase. Progesterone levels are relatively low in children and postmenopausal women. Progesterone levels may be ordered, along with other tests such as an FSH, LH, HCG, thyroid tests and clotting tests, to help determine the cause of abnormal uterine bleeding in non-pregnant women. And this test measures the level of progesterone in the blood.

Measurement Principle

This assay is based upon the one-step competitive method. The sample, mouse anti-sheep antibodies coated microparticles, Antibody Solution and enzyme labeled PRG are combined. During the incubation, enzyme labeled PRG and PRG present in the sample compete for binding to the antibodies in Antibody Solution, then the reaction mixture bind to the mouse anti-sheep antibodies coated on microparticles. After washing, a complex is generated between the solid phase, antibodies in Antibody Solution, PRG in the sample and enzyme-linked PRG by immunological reactions. The complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is inversely proportional to the amount of PRG in the sample.

Materials Provided

1. Calibrators

7 vials each containing 1.0 mL of calibrator A through G. The matrix is PBS buffer containing hormone-free human serum. Contains a selection of preservatives.

Calibrators provided ready to use.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3 mL*2	2.3 mL*5	1.2 mL*2
Enzyme Conjugate	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2
Antibody Solution	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2

- Microparticles Solution

Mouse anti-sheep antibodies coated microparticles in PBS buffer containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

Horseradish-peroxidase labeled PRG in MES buffer containing BSA. Contains a selection of preservatives.

- Antibody Solution

Goat monoclonal antibodies in Tris-NaCl buffer containing BSA. Contains ProClin 300® and Bronidox preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample cup(s) or tube(s) for sample containing
4. Chemiluminescent Substrate
5. System Wash for washing the pipetting needle
6. Wash Buffer used in washing procedure
7. Distilled or deionized water

Metrological Traceability of Calibrators

The measurand (analyte) in the Assay Analyzer PRG Calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511.

The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: the calibrators contain material of human origin, which has been tested and found non-reactive for HBsAg, HIV-1 and HIV-2, HCV and syphilis. It is recommended that all materials of human origin be considered potentially infectious. This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. Do not smoke, drink, eat or use cosmetics in the working area.
7. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
8. Conduct the assay away from bad ambient conditions. e. g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
9. Do not use reagents beyond the labeled expiry date.
10. Do not mix or use components from kits with different batch codes.
11. When storing the calibrators, be certain the vials are securely sealed.
12. Ensure the microparticles are resuspended before loading it on the analyzer.
13. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
14. Do not substitute any reagent in this kit from other manufacturers or

other lots.

15. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the remaining calibrators to 2-8 ℃, under which conditions the stability will be retained for 2 months, for longer use, store opened calibrators in aliquots and freeze at -20 ℃. Avoid multiple freeze-thaw cycles.

Sample

1. Collect serum samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples 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. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of the sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25 °C for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample cups or tubes on the sample rack, 50 μL of samples for each test. But considering the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution, Antibody Solution and Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of PRG in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve

- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample cups or tubes and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements.
  - A reagent kit and Chemiluminescent Substrate with new batch code is used.
  - Beyond the expiration date of a calibration curve.
  - Important parts of the analyzer are replaced or repaired.
- Refer to the Analyzer's system operations manual.

5. Dilute the sample

Samples with a PRG value exceeding 120 ng/mL may be diluted manually. Hormone-free serum or low-value sample can be used to dilute the samples. After dilution, multiply the result by the dilution factor.

- The concentration of the sample after dilution should not be less than 2 ng/mL.

Measurement Results

The sample test results are determined automatically by the system software. The amount of PRG in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing the stored results.
The default unit for this assay is ng/ml.
Conversion formula: 1ng/mL × 3.18 = 1nmol/L

Control Procedure

Controls for the various concentration ranges should be run individually when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
4. Because of pulsatile secretion, samples obtained within the same day from the same patient may fluctuate widely within the reference interval, reflecting physiological variation rather than errors in technique or methodology.
5. This assay was designed and validated for use with human serum from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
6. This test measures concentrations within the range of 0.05 - 120 ng/mL. If PRG concentrations above the measuring range to be expected, it is recommended to dilute samples with hormone-free serum or low-value sample, the maximum dilution is 1:4 of this test, allowing samples to be quantitated up to approximately 600 ng/mL.

Biological Reference Interval

The suggested normal range (central 95% interval) was obtained by testing serum samples from 120 normal males, 45 normal cycling females and 52 postmenopausal females. For this study, the follicular phase was defined as the period of time from 10 to 4 days prior to the mid-cycle peak. The luteal phase was defined as the period of time from 4 to 10 days following the mid-cycle peak. Cycle days were synchronized to the mid-cycle peak, the day on which the LH concentration was most elevated. The results are presented in the following table. It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

		N	Mean Value (ng/mL)	Reference Interval (ng/mLl)
Males		120	0.90	0.1-2.0
Normally Menstruating Females	Follicular Phase	145	1.10	0.2-2.4
	Mid-cycle Peak	45	1.95	0.5-3.6
	Luteal Phase	140	12.52	6.0-20.5
Postmenopausal Females		52	0.80	0.1-1.8

Performance Characteristics

1. Measurement Precision

3 samples were assayed in replicate of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean	Within-run %CV	Total %CV
1	80	1.71	5.78	6.67
2	80	18.38	3.61	4.36
3	80	48.82	4.22	5.93

*Representative data; results in individual laboratories may vary from these data

2. Analytical Sensitivity

Limit of Blank=0.05ng/mL
Limit of Detection=0.15ng/mL
Limit of Quantitation= 0.45ng/mL with a coefficient of variation of ≤ 20%.

3. Analytical Specificity

Cross reaction: this assay is designed to have an analytical specificity of less than 0.15 ng/mL cross reactivity with the substances listed below, at the concentration levels listed, in hormone-free human serum and the following cross-reactivities (%) were found:

Substances	Concentration	Cross reactivity
Dehydroepiandrosterone	40 μg/mL	0.08%
Estradiol	50 μg/mL	0.00%
Testosterone	20 μg/mL	0.05%
Pregnenolone	10 μg/mL	0.01%
Prednisolone	10 μg/mL	0.00%
Cortisone	100 μg/mL	0.00%
Hexadecadrol	10 μg/mL	0.00%
21-hydroxyprogesterone	10 μg/mL	0.98%
Corticosterone	10 μg/mL	0.01%
17α -Hydroxyprogesterone	10 μg/mL	1.41%
Deoxycortisol	10 μg/mL	0.00%
Androsterone	50 μg/mLl	0.07%
Dehydroepiandrosterone-S	100 μg/mL	0.02%
Danazol	1 μg/mL	no cross reaction
Androstenedione	0.1 μg/mL	0.24%
Norgestrel	1 μg/mL	no cross reaction
Mesterolone	0.1 μg/mL	no cross reaction
11 - keto testosterone	0.05 μg/mL	no cross reaction
11 - hydroxy testosterone	6.25 ng/mL	no cross reaction
Dihydrotestosterone	0.1 μg/mL	no cross reaction

Interference: this assay is designed to have no interference with the substances listed below, at the concentration levels listed, in serum samples.

Interferent	Concentration
Bilirubin	20 mg/dL
Hemoglobin	500 mg/dL
Triglyceride	250 mg/dL

4. Measurement Accuracy by Correlation

A comparison study was performed where samples were tested using this assay and a PRG assay which was already CE marked. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	150	0.2022	0.9837	0.9951

Literature References

1. Allen WM. THE ISOLATION OF CRYSTALLINE PROGESTIN. *Science*. 1935;82(2118):89-93.

2. Luconi M, Bonaccorsi L, Maggi M, et al. Identification and characterization of functional nongenomic progesterone receptors on human sperm membrane. *J. Clin. Endocrinol. Metab.* 1998;83(3):877-885.

3. Jang S, Yi LSH. Identification of a 71 kDa protein as a putative non-genomic membrane progesterone receptor in boar spermatozoa. *J. Endocrinol.* 2005;184(2):417-425.

4. Stamatelou F, Deligeoroglou E, Farmakides G, Creatsas G. Abnormal progesterone and corticotropin releasing hormone levels are associated with preterm labour. Ann. Acad. Med. Singap. 2009;38 (11):1011-1016.