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Immunoassay

REF **CMF0901/CMF0902/CMF0903/CMF0904/CMF0905**

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

17α-OHP CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of 17α-OHP (17α-Hydroxyprogesterone) 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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Contact your local dealer for all product-related questions in your local language.

Introduction

17α-Hydroxyprogesterone (17α-OHP), or hydroxyprogesterone (OHP) (INN, BAN), also known as 17α-hydroxypregn-4-ene-3,20-dione, is an endogenous progestogen steroid hormone related to progesterone.[1,2,3] It is also a chemical intermediate in the biosynthesis of many other endogenous steroids, including androgens, estrogens, glucocorticoids, and mineralocorticoids, as well as neurosteroids.

Measurements of levels of 17α-OHP are useful in the evaluation of patients with suspected congenital adrenal hyperplasia as the typical enzymes that are defective, namely 21-hydroxylase and 11β-hydroxylase, lead to a build-up of 17α-OHP. In contrast, the rare patient with 17α-hydroxylase deficiency will have very low or undetectable levels of 17α-OHP. 17α-OHP levels can also be used to measure contribution of progestational activity of the corpus luteum during pregnancy as progesterone but not 17α-OHP is also contributed by the placenta.

Measurement Principle

This assay is based upon the one-step competitive method. The secondary antibody coated microparticles and rabbit polyclonal antibody-linked antibody solution are added, antibodies are generated after they bind together, then 17α-OHP present in the sample and 17α-OHP antigen in the Enzyme Conjugate are added and complete to bind to the antibodies. After the washing, a complex is generated among the antibodies, the 17α-OHP within the sample and enzyme-linked antigens by immunological reactions. Chemiluminescent Substrate are then added and catalyzed by this complex, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is reversely proportional to the concentration of 17α-OHP in the patient sample.

Materials provided

1. Calibrators

6 vials each containing 1.0 mL of calibrator A through F. The matrix is Tris-NaCl buffer containing BSA (bovine serum albumin). Contains ProClin 300® preservative.

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	2.3mL*2	2.3mL*5	1.2mL*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

Goat anti-rabbit antibody coated microparticles in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains ProClin 300® and sodium azide preservatives.

- Enzyme Conjugate

HRP (horseradish peroxidase) labeled 17α-OHP antigen in MES buffer containing bovine serum. Contains ProClin 300® preservative.

- Antibody Solution

Rabbit polyclonal antibody in PBS buffer containing BSA (bovine serum albumin). Contains ProClin 300® preservative.

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 tube(s) or cup(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needle
7. Wash Buffer used in the washing procedure
8. Distilled or deionized water

Metrological Traceability of Calibrators

The analyte in the 17α-OHP 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 waste safely according to local requirement.
5. CAUTION: This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. Some reagents contain ProClin 300® may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately and show this container or label.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
10. 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.
11. Do not use reagents beyond the labeled expiry date.
12. Do not mix or use components from kits with different batch codes.
13. When storing the calibrators, be certain the vials are securely sealed.
14. Ensure the microparticles are resuspended before loading it on the analyzer.
15. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
16. Do not substitute any reagent in this kit from other manufacturers or other lots.
17. 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 at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 60 days.

Sample

1. Collect 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 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 or red blood cells.
6. Insufficient processing of 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 ℃ 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 vortexing 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 tubes or cups on the sample rack, 25 μL of samples and calibrators for each test. But consider 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, Enzyme Conjugate and Antibody Solution to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of 17α-OHP 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 tubes or cups 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 Assay Analyzer's operation manual.

5. Dilute the sample

Samples with the 17α-OHP value exceeding 30 ng/mL may be diluted via the program of the analyzer. Diluent Universal is used to dilute the samples. The software takes the dilution into account when reporting the result.

- The concentration of the sample after dilution should not be less than 2.0 ng/mL.

Measurement Results

The sample test results are determined automatically by the system software. The amount of 17α-OHP 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 data.

Control Procedure

The recommended control requirement for this assay is to purchase control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels. When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. It is recommended that each laboratory establish its accepted range to ensure proper test performance.

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. Performance of this test has not been established with neonatal samples.
4. This assay was designed and validated for use with human serum or plasma from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
5. This test measures concentrations within the range of 0.05-30 ng/mL. If 17α-OHP concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the maximum dilution is 1:9 of this test, allowing samples to be quantitated up approximately to 300 ng/mL.

Biological Reference Interval

A study of 843 normal adult population individuals was undertaken to determine reference intervals for this assay, with 5% and 95% as limit using percentile method. 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.

Population		Sample no.	Reference Interval (ng/mL)
Male		105	0.31-2.01
Females	Follicular Phase	110	0.05-1.02
	Luteal Phase	103	0.3-2.34
	Ovulatory Phase	92	0.1-1.4
	Postmenopausal Phase	90	<0.93
	Postconceptual Phase	112	2.28-9.24
Children (1 to 13-year-old)		108	<2.32
Infant (1 month to 1-year-old)		123	0.82-16.63

Performance Characteristics

1. Measurement Precision

This assay is designed to have a within-run precision of <10%. 2 human serum based panel members (1 and 2) were assayed, using 1 batch of reagents, in replicates of 10. Data from this study are summarized in the following table.

Panel Member	Batch	n	Mean	Within-run Precision SD	%CV
1	1	10	1.12	0.04	3.45
2	1	10	10.54	0.18	7.38

This assay is designed to have a between-run precision of <15%. 2 human serum based panel members (1 and 2) were assayed, using 1 batch of reagents, in replicates of 10, once per day across 3 testing days. Data from this study are summarized in the following table.

Panel Member	Batch	n	Mean	Between-run Precision SD	%CV
1	1	30	1.13	0.05	4.43
2	1	30	10.58	0.65	6.11

2. Analytical Sensitivity

Analytical sensitivity, defined as the concentration corresponding to the mean ALUs of 20 replicates of calibrator A plus 2 standard deviations, is ≤ 0.05 ng/mL.

3. Analytical Specificity

Cross reaction: This assay is designed to have an analytical specificity of less than 1% cross reactivity with the substances listed below, at the concentration levels listed, in calibrator diluent.

Substance	Concentration (ng/mL)	Measured Value (nmol/L)
E2	5000	<1%
Estriol	5000	<1%
Testosterone	2000	<1%
Cortisol	1000	<1%
Aldosterone	5000	<1%
DHEA	4000	<1%
17α-hydroxypregnenolone	100	<1%
Progesterone	500	<1%

Interference: No interference with 100 mg/dL of hemoglobin, 25 mg/dL of Bilirubin, 6000 mg/dL of Triglyceride.

4. Measurement Accuracy by Correlation

A study was performed where samples were tested using this assay and a 17α-OHP Test which was already available on the market. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	200	0.1137	0.5697	0.9864

Literature References

1. J. Elks (14 November 2014). The Dictionary of Drugs: Chemical Data: Chemical Data, Structures and Bibliographies. Springer. pp. 664–665.
2. I.K. Morton; Judith M. Hall (6 December 2012). Concise Dictionary of Pharmacological Agents: Properties and Synonyms. Springer Science & Business Media. pp. 146.
3. Index Nominum 2000: International Drug Directory. Taylor & Francis. January 2000. pp. 532.