DRG® 17-α-OH Progesterone ELISA (EIA-1292)

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Revised 25 June 2008 (Vers. 8.1) IVD

Introduction

Intended Use

The **DRG 17-α-OH Progesterone Enzyme Immunoassay Kit** provides materials for the quantitative determination of 17-α-OH Progesterone in serum.
This assay is intended for in vitro diagnostic use only.

Summary and Explanation

The steroid 17-α-Hydroxyprogesterone (17-α-OHP) is produced by both the adrenal cortex and gonads. Even though 17-α-OHP has relatively little progestational activity, it is of intense clinical interest because it is the immediate precursor to 11-desoxycortisol (Cpd-S). Because Cpd-S is produced by 21-hydroxylation of 17-α-OHP, measurement of 17-α-OHP is a useful indirect indicator of 21-hydroxylase activity. In congenital 21-hydroxylase deficiency, the most common variety of congenital adrenal hyperplasia (CAH), 17-α-OHP is secreted in abundant excess. It is moderately elevated in the 11-β-hydroxylase deficiency as well. Measurement of 17-α-OHP is therefore valuable in the initial diagnosis of CAH.

Clinical Physiology

Adult non-pregnant women:
In adult non-pregnant women in the childbearing age group, 17-α-OHP concentrations vary over the menstrual cycle with luteal phase concentrations being higher than follicular phase concentrations. This is because 17-α-OHP is secreted parallel with progesterone from maturing follicles or from the corpus luteum. There is also a diurnal variation of 17-α-OHP concentrations.
This rhythm is parallel with adrenal cortisol secretion such that maximum 17-α-OHP concentrations are measured in samples obtained between midnight and 8:00 am.

Adult males:
There is little information available on the systematic variability of 17-α-OHP concentration in adult males.

Pregnant women and newborn children:
The steroid 17-α-OHP is produced in large amounts by the fetus and the adrenals. It is secreted in abundance into both the fetal and maternal circulation. The maternal concentrations of 17-α-OHP increase very sharply after 32 weeks gestational age to about 4-fold above basal concentrations at term.

Clinical Applications

Congenital adrenal hyperplasia:
The principal application of the 17-α-OHP RIA is in the diagnosis of CAH in newborns with ambiguous genitalia and in virilized adolescent girls. Since 17-α-OHP is the immediate precursor to 11-desoxycortisol, basal 17-α-OHP concentrations are sharply elevated in patients with 21-hydroxylase deficiency and to a lesser degree in patients with 11-hydroxylase deficiency.
Because 17-α-OHP concentrations are so markedly elevated in newborns and adolescent girls afflicted with CAH, a single basal measurement is all that is normally required to make the diagnosis.

Late onset adrenal hyperplasia:
More recently, 17-α-OHP concentrations have been utilized in the evaluation of androgenized women where late onset of 21-hydroxylase deficiency is suspected. This condition is clinically very subtile and since the presentation is the same as classical polycystic ovarian disease, basal plasma 17-α-OHP concentrations, unlike classical congenital adrenal hyperplasia, are normal. The diagnosis is made by administration of an ACTH stimulation test.

Other applications:
Measurement of 17-α-OHP concentrations is also utilized in evaluation of both men and women with acne vulgaris, male pattern baldness and in some subtile forms of infertility. Experiences with these applications are very limited.

PRINCIPLE of the test
The DRG 17-α-OH Progesterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.
The microtiter wells are coated with a polyclonal antibody directed towards an antigenic site on the 17-α-OHP molecule. Endogenous 17-α-OHP of a patient sample competes with a 17-α-OHP-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.
The amount of bound peroxidase conjugate is inversely proportional to the concentration of 17-α-OHP in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of 17-α-OHP in the patient sample.

Warnings AND Precautions
1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.

13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin, BND and MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from DRG.

Reagents

Reagents provided

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with a anti-17-α-OHP antibody (polyclonal).
2. ***Standard (Standard 0-6)*****,** 7 vials, 1 mL, ready to use;
   Concentrations: 0; 0.15; 0.5; 1.5; 3; 7.5; 20 ng/mL
   0; 0.45; 1.5; 4.5; 9.1; 22.7; 60.6 nmol/L
   contain 0.03% Proclin 300 and 0.005% gentamycin sulfate as a preservatives.
3. ***Enzyme Conjugate***, 1 vial, 25 mL, ready to use;
   17-α-OHP conjugated to horseradish Peroxidase;
   * contain 0.03% Proclin 300, 0.015%BND and 0.010% MIT as a preservatives.
4. ***Substrate Solution***, 1 vial, 25 mL, ready to use;
   Tetramethylbenzidine (TMB).
5. ***Stop Solution***, 1 vial, 14 mL, ready to use;
   contains 0.5M H_2SO_4.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
6. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
   see „Preparation of Reagents“.

* BND = 5-bromo-5-nitro-1,3-dioxane
MIT = 2-methyl-2H-isothiazol-3-one

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Note: Additional *Standard 0* for sample dilution is available upon request.

Material required but not provided

- A microtiter plate calibrated reader (450±10 nm), (e.g. the DRG Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- distilled water
- Semi-logarithmic graph paper

Storage Conditions

When stored at 2 °C – 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C – 8 °C. Microtiter wells must be stored at 2 °C – 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for six weeks if stored as described above.

Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

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SPECIMEN Collection and Preparation
Serum can be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 24 hours at 2 °C – 8 °C prior to assaying.
Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) Dilution 1:10: 10 µL Serum + 90 µL *Standard 0* (mix thoroughly)
b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

Assay procedure

General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **25 µL** of each *Standard, Control* and samples with new disposable tips into appropriate wells.
3. Incubate for **5 minutes** at room temperature
4. Dispense **200 µL** *Enzyme Conjugate* into each well.
5. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
6. Incubate for **60 minutes** at room temperature.
7. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
8. Add **200 µL** of *Substrate Solution* to each well.
9. Incubate for **30 minutes** at room temperature.
10. Stop the enzymatic reaction by adding **100 µL** of *Stop Solution* to each well.
11. Read the OD at **450±10 nm** with a microtiter plate reader **within 10 minutes** after adding the *Stop Solution*.

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 ng/mL)	1.89
Standard 1 (0.15 ng/mL)	1.51
Standard 2 (0.5 ng/mL)	1.10
Standard 3 (1.5 ng/mL)	0.69
Standard 4 (3.0 ng/mL)	0.46
Standard 5 (7.5 ng/mL)	0.28
Standard 6 (20 ng/mL)	0.18

Expected values

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study using the DRG 17-α-OH Progesterone ELISA the following values are observed:

Newborns	girls	boys	boys and girls
1. month after birth	2.4 - 16.8 ng/mL	0.0 - 8.0 ng/mL	0 - 16.8 ng/mL
2. month after birth	1.6 - 9.7 ng/mL	3.6 - 13.7 ng/mL	1.9 - 9.8 ng/mL
3. month after birth	0.1 - 3.1 ng/mL	1.7 - 4.0 ng/mL	0.1 - 4.0 ng/mL

Children	3 - 14 years	0.07 - 1.7 ng/mL
Reproductive aged women	Follicular phase:	0.1 - 0.8 ng/mL
	Luteal phase:	0.6 - 2.3 ng/mL
	Ovulation:	0.3 - 1.4 ng/mL
	Post ACTH:	< 3.2 ng/mL
	Third trimester:	2.0 - 12 ng/mL
	Postmenopausal women	0.13 - 0.51 ng/mL
Normal men		0.5 - 2.1 ng/mL

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

Quality Control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or DRG directly.

Performance Characteristics

Assay Dynamic Range

The range of the assay is between 0,034 – 20 ng/mL.

Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Steroid	% Cross Reaction
17-α-OH Progesterone	100.0
Estriol	< 0.01
Estradiol 17β	< 0.01
Testosterone	< 0.01
Dihydrotestosterone	< 0.01
DOC	0.05
11-Desoxicortisol	1.4
Progesterone	1.2
DHEA	< 0.01
DHEAS	< 0.001
Cortisol	< 0.01

Corticosterone	< 0.05
Aldosterone	< 0.01
Androstendione	< 0.01
Dehydroepiandrosten sulfate	< 0.01
Prednison	< 0.01

Sensitivity

The analytical sensitivity was calculated from the mean minus two standard deviations of twenty (20) replicate analyses of *Standard 0* and was found to be 0.034 ng/mL.

Precision

Intra Assay

The within assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	20	0.53	5.40
2	20	2.79	6.42
3	20	7.23	5.54

Inter Assay

The between assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	12	0.53	7.21
2	12	2.95	6.17
3	12	7.80	6.87

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Recovery

Recovery of the DRG ELISA was determined by adding increasing amounts of the analyte to three different patient sera containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples.

Sample	Endogenous 17α OH-P ng/mL	Added 17α OH-P ng/mL	Measured Conc. 17α OH-P ng/mL	Expected Conc 17α OH-P ng/mL	Recovery (%)
1	11.18	0.00	11.18		
		10.00	13.48	15.59	86.5
		3.75	9.84	9.34	105.3
		1.50	6.09	7.09	85.9
		0.75	5.39	6.34	85.0
2	1.43	0.00	1.43		
		10.00	9.67	10.71	90.2
		3.75	4.66	4.46	104.3
		1.50	2.48	2.21	111.9
		0.75	1.66	1.46	113.1
3	4.68	0.00	4.68		
		10.00	11.18	12.34	90.6
		3.75	6.03	6.09	99.1
		1.50	3.90	3.84	101.5
		0.75	3.49	3.09	112.8

Linearity

Sample	Dilution	Measured Conc. 17α OH-P (ng/mL)	Expected Conc. 17α OH-P (ng/mL)	Recovery (%)
1	None	11.18	11.18	
	1:2	5.44	5.59	97.2
	1:4	2.47	2.80	88.4
	1:8	1.25	1.40	89.6
	1:16	0.72	0.70	102.4
2	None	1.43	1.43	
	1:2	0.68	0.71	94.7
	1:4	0.33	0.36	91.4
	1:8	0.16	0.18	86.9
	1:16	0.10	0.09	113.2
3	None	4.68	4.68	
	1:2	2.16	2.34	92.1
	1:4	1.00	1.17	85.2
	1:8	0.56	0.59	95.6
	1:16	0.29	0.29	98.8

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Limitations of Use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 7.5 mg/mL) have no influence on the assay results.

Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of 17-α-OH Progesterone in a sample.

High-Dose-Hook Effect

No hook effect was observed in this test.

Legal Aspects

Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid.
Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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