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User's Manual

17-OH-Progesterone ELISA

Enzyme immunoassay for the in-vitro diagnostic quantitative determination of 17-OH-Progesterone in human serum and plasma

IVD

CE

REF **DEM-DEH3322**

Σ **96 Wells**

CONTENTS

1 INTRODUCTION

1.1 Intended Use

Enzyme immunoassay for the in-vitro diagnostic quantitative determination of 17-OH-progesterone in human serum and plasma.

1.2 Summary and explanation

The steroid hormone 17-OH-progesterone (17-OHP) is produced in the adrenal cortex and in the gonads. Gestagenic effects exerted by 17-OHP are only small. Nevertheless, this hormone is of clinical significance because it represents the ultimate precursor of 11β-desoxycortisol (compounds, CpS). CpS is formed by hydroxylation of the carbon atom C 21. Enzyme activity of 21-hydroxylase in the adrenal cortex may thus be monitored by analyzing the level of 17-OHP in the blood.
Deficiencies in 21-hydroxylase, most commonly found in congenital adrenal hyperplasia, result in excessive secretion of 17-OHP and consequently in enhanced blood levels. Deficiencies in 11-hydroxlase, however, merely lead to moderately increased values of 17-OHP. The analysis of this steroid hormone, therefore, plays a significant role in the differential diagnosis of congenital adrenal hyperplasia.
In adult non-pregnant women, 17-OHP levels in the blood depend on the phase of the menstrual cycle. Like progesterone, 17-OHP is secreted by the mature follicle and the corpus luteum. Concentrations are generally higher after ovulation.
In addition, levels of 17-OHP are influenced by daytime rhythms which correlate with the adrenal secretion of cortisol. Maximal levels are found in samples collected between midnight and 8.00 a.m..
In adult men, there are few indications of similar fluctuations of 17-OHP levels.
During pregnancy, large amounts of 17-OHP are produced by the fetus, the placenta and the adrenal cortex. The hormone is secreted into the fetal and the maternal blood circulation. Maternal values of 17-OHP strongly increase after the 32. week of pregnancy reaching 4-fold higher levels than during the luteal phase of the menstrual cycle. 17-OHP may also be found in the umbilical cord of newborns.

2 PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labeled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. After the substrate reaction the intensity of the developed colour is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

3 WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. 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.
3. 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.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. 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.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. 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.
9. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
10. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
11. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
15. 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.
16. Avoid contact with *Stop Solution*. It may cause skin irritation and burns.
17. 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.
18. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
19. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
20. Safety Data Sheets for this product are available upon request directly from Demeditec Diagnostics GmbH. The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

4 REAGENTS

4.1 Reagents provided

1. **SORB MT Microtiterplate**, 12x8 (break apart) strips, 96 wells;
   Wells coated with an anti-17-OH-progesterone antibody (polyclonal).
2. **CAL 0 Calibrator 0,** 1 vial, 1 ml, ready to use;
3. **CAL 1-5 Calibrator (Calibrator 1-5),** 5 vials, 0.5 ml each, ready to use;
   Concentrations: 0.1 - 0.4 - 1.6 - 6.5 - 25 ng/ml,
4. **CONTROL 1-2 Control low** / **Control high**, 2 vials, 0.5 ml each, ready to use;
   containing 17-OH-progesterone in serum.
   For control values and ranges please refer to QC-Datasheet.
5. **ENZ CONJ Enzyme Conjugate**, 1 vial, 11 ml, ready to use;
   17-OH-progesterone conjugated to horseradish peroxidase;
6. **SUB TMB Substrate Solution**, 1 vial, 22 ml, ready to use;
   Tetramethylbenzidine (TMB).
7. **STOP SOLN Stop Solution**, 1 vial, 7 ml, ready to use;
   contains 2 N acidic solution.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
8. **WASH SOLN 10x Wash Solution**, 1 vial, 50 ml (**10X** concentrated);
   see „Preparation of Reagents“.

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

4.2 Material required but not provided

- Microcentrifuge
- A microtiter plate calibrated reader (450±10 nm)
- Microplate mixer operating at about 600 rpm, optionally
- Vortex mixer
- Calibrated variable precision micropipettes (25 µl, 50 µl 100 µl, 200 µl).
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 Storage conditions

When stored at 2 °C to 8 °C unopened reagents will be stable until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2°C to 8°C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

4.4 Reagents preparations

Wash Solution
Add deionized water to the 10X concentrated *Wash Solution*.
Dilute 50 ml of concentrated *Wash Solution* with 450 ml deionized water to a final volume of 500 ml.
The diluted Wash Solution is stable for 3 months at room temperature.

4.5 Disposal of the kits

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 Sheet.

4.6 Damaged test kits

In case of any severe damage of the test kit or components, DEMEDITEC have to be informed written, 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.

5 SPECIMEN COLLECTION AND PREPARATION

Serum, Plasma

<table>
<tr><td colspan="4">The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly haemolytic, icteric or grossly lipaemic specimens. Samples which appear turbid should be centrifuged before testing to remove any particulate material.</td></tr>
<tr><td rowspan="2">Storage:</td><td rowspan="2">2-8°C</td><td>≤ -20°C
(Aliquots)</td><td rowspan="2">Keep away from heat or direct sun light
Avoid repeated freeze-thaw cycles</td></tr>
<tr><td>Stability:</td><td>7 d</td><td>3 m</td></tr>
</table>

6 ASSAY PROCEDURE

6.1 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.
- Respect the incubation times as stated in this instructions for use.

6.2 Assay procedure

Each run must include a standard curve.

1. Secure the desired number of coated strips in the frame holder.
2. Dispense **25 µl** of each **Standard, Control and samples** with new disposable tips into appropriate wells.
3. Dispense **100 µl Enzyme Conjugate** into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at room temperature. Shaking on a horizontal shaker during incubation is not necessary, but it improves the sensitivity of the test slightly.
5. Briskly empty the contents of the wells by aspiration or by decanting.
   Rinse the wells 4 times with diluted Wash Solution (300 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!
6. Add **200 µl** of **Substrate Solution** to each well.
7. Incubate for **30 minutes** in the dark at room temperature.
8. Stop the enzymatic reaction by adding **50 µl** of **Stop Solution** to each well.
9. Determine the absorbance of each well at **450±10 nm**.
   It is recommended that the wells be read within 15 minutes.

6.3 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 calculation 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.

6.3.1 Example of typical calibrator curve

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

Calibrator		**Optical Density (450 nm)**
Calibrator 0	0.0 ng/ml	3.561
Calibrator 1	0.1 ng/ml	2.764
Calibrator 2	0.4 ng/ml	1.925
Calibrator 3	1.6 ng/ml	1.136
Calibrator 4	6.5 ng/ml	0.526
Calibrator 5	25 ng/ml	0.226

7 EXPECTED NORMAL VALUES

Apparently healthy subjects show the following values of 17-OH-Progesterone:

Newborn	5. – 30. day	< 0.7 - 2.5 ng/mL
	31. – 60. day (male)	0.8 - 5.0 ng/mL
	31. – 60. day (female)	0.5 - 2.5 ng/mL
Children	3 -14 years	0.05 - 2.0 ng/mL
Reproductive aged women	Follicular phase:	0.1 - 1.0 ng/mL
	Luteal phase:	0.6 - 2.5 ng/mL
	Ovulation:	0.3 - 1.5 ng/mL
	Post ACTH:	< 3.2 ng/mL
	Third trimester:	2.0 - 12 ng/mL
	Postmenopausal women:	0.13 - 0.6 ng/mL
Normal men		0.5 - 3 ng/mL

The results itself should not be the only reason for any therapeutically consequences. They have to be correlated to other clinical observations and diagnostic tests.
It is strongly recommended that each laboratory establishes its own range of normal values.

8 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 DEMEDITEC directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Analytical Sensitivity

The analytical sensitivity of the DEMEDITEC ELISA was calculated by subtracting 2 standard deviations from the mean of twenty (20) replicate analyses of *Calibrator 0*. The analytical sensitivity of the assay is 0.03 ng/ml.

9.2 Specificity (Cross Reactivity)

The following materials have been evaluated for cross reactivity.

Substance	**% Cross-reactivity**
Androsterone	< 0,1%
5α-Dihydrotestosterone	< 0,1%
Androstendione	< 0,1%
Testosterone	< 0,1%
Cortisol	< 0,1%
11-Desoxycortisol	1,9%
Progesterone	2,4%
Estradiol	< 0,1%
Estriol	< 0,1%
Pregnenolone	0,4%
Prednisolone	< 0,1%
Prednisone	< 0,1%

9.3 Assay Dynamic Range

The range of the assay is between 0 – 25 ng/ml.

9.4 Reproducibility

9.4.1 Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of 3 serum samples within one run using the DEMEDITEC ELISA. The within-assay variability is shown below:

	Sample 1	Sample 2	Sample 3
Mean (ng/ml)	0.72	5.77	16.58
SD (ng/ml)	0.05	0.19	0.60
CV (%)	7.0	3.3	3.6
n =	20	20	20

9.4.2 Inter-Assay

The inter-assay variation was determined by duplicate measurements of 3 serum samples in 10 different runs using the DEMEDITEC ELISA. The inter-assay variability is shown below:

	Sample 1	Sample 2	Sample 3
Mean (ng/ml)	0.91	4.98	16.34
SD (ng/ml)	0.06	0.38	0.69
CV (%)	6.6	7.5	4.2
n =	10	10	10

9.5 Recovery

Using the Calibrator matrix a spiking solution was prepared (1000 ng/ml). Aliquots of 1, 2 and 3 µl, respectively, were spiked into 500 µl of three different sera, leaving the serum matrix of the spiked samples relatively intact. All samples were then measured by the 17-OH-Progesterone assay procedure. The percentage recoveries were determined by comparing expected and measured values of the samples.

Serum	Spiking	Measured concentration	Expected concentration	Recovery %
1	-	3,60	-	-
	2 ng/ml	5,99	5,60	107%
	4 ng/ml	7,54	7,60	99%
	6 ng/ml	10,43	9,60	109%
2	-	7,60	-	-
	2 ng/ml	9,95	9,60	104%
	4 ng/ml	12,82	11,60	110%
	6 ng/ml	15,68	13,60	115%
3	-	3,62	-	-
	2 ng/ml	6,01	5,62	107%
	4 ng/ml	7,76	7,62	102%
	6 ng/ml	9,71	9,62	101%

9.6 Linearity

Three serum samples containing different amounts of analyte were assayed undiluted and diluted with the calibrator matrix. The percentage recovery was calculated by comparing the expected and measured values for 17-OH-progesterone.

Serum	Dilution	Measured concentration	Expected concentration	Recovery %
1	-	19,86	-	-
	1 in 2	10,62	9,93	107%
	1 in 4	5,56	4,97	112%
	1 in 8	2,85	2,48	115%
2	-	26,28	-	-
	1 in 2	13,38	13,14	102%
	1 in 4	6,46	6,57	98%
	1 in 8	3,36	3,28	102%
3	native	3,97	-	-
	1 in 2	1,91	1,99	96%
	1 in 4	1,01	0,99	102%
	1 in 8	0,56	0,50	112%

10 LIMITATIONS OF PROCEDURE

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.

10.1 High-Dose-Hook Effect

No hook effect was observed in this test

10.2 Drug Interferences

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

11 LEGAL ASPECTS

11.1 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 DEMEDITEC.

11.2 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.

11.3 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.

12 REFERENCES

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SYMBOLS USED WITH DEMEDITEC ELISA´S

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Contenu	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Numéro	Volumen/Número	Volume/Quantità
Microtiterwells	Microtiterwells	Mikrotiterwells	Plaques de micro-titration	Placas multipocillo	Micropozzetti
Antiserum	Antiserum	Antiserum	Antisérum	Antisuero	Antisiero
Enzyme Conjugate	Enzyme Conjugate	Enzymkonjugat	Conjugué enzymatique	Conjugado enzimático	Tracciante enzimatico
Enzyme Complex	Enzyme Complex	Enzymkomplex	Complexe enzymatique	Complex enzimático	Complesso enzimatico
Substrate Solution	Substrate Solution	Substratlösung	Solution substrat	Solución de sustrato	Soluzione di substrato
Stop Solution	Stop Solution	Stopplösung	Solution d'arrêt	Solución de parada	Soluzione d´ arresto
Zero Standard	Zero Standard	Nullstandard	Standard 0	Estándar 0	Standard zero
Standard	Standard	Standard	Standard	Estándar	Standard
Control	Control	Kontrolle	Contrôle	Control	Controllo
Assay Buffer	Assay Buffer	Assaypuffer	Tampon d'essai	Tampón de ensayo	Tampone del test
Wash Solution	Wash Solution	Waschlösung	Solution de lavage	Solución de lavado	Soluzione di lavaggio
1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH (idrossido di sodio 1N)
1 N HCl	1 N HCl	1 N HCl	1N HCl	1 N HCl	
Sample Diluent	Sample Diluent	Probenverdünnungs-medium	Solution pour dilution de l'échantillon	Solución para dilución de la muestra	Diluente dei campioni
Conjugate Diluent	Conjugate Diluent	Konjugatverdünnungs-medium	Solution pour dilution du conjugué	Solución para dilución del conjugado	Diluente del tracciante