



## 1. NAME AND INTENDED USE

OHP-CT is a radioimmunoassay kit for the direct determination of 17  $\alpha$ -hydroxyprogesterone in human serum or plasma.

## 2. INTRODUCTION

17  $\alpha$ -hydroxyprogesterone is a steroid hormone with a molecular weight of 330.4.

Its assay enables the establishment of an early diagnosis and the adaptation of the treatment of congenital hyperplasia of the adrenal glands. This recessive autosomal hereditary condition is characterized by an enzymatic deficiency in the biosynthesis of cortisol. The compounds formed upstream of the block are over-synthesized and an increase in the 17-cetosteroids is observed. Inversely, the compounds below the block are usually decreased in the blood and in the urine.

The most frequent deficiency is that of 21-hydroxylase, which causes excessive production of suprarenal androgens with virilization and rapid bone maturation. Clinically, in addition to the hyperplasia of the adrenal glands, androgeny with feminine pseudo-hermaphroditism is observed in girls, and early pseudo-puberty is observed with boys. Biologically, there is a serum increase of 17  $\alpha$ -hydroxyprogesterone combined with an increase in the urinary levels of pregnanetriol and the 17 cetosteroids. In untreated subjects, the levels of 17  $\alpha$ -hydroxyprogesterone can reach 100 to 500 times the normal levels, whereas the metabolic clearance is practically normal.

## 3. PRINCIPLE

The principle of the assay is based on the competition between the 125 iodine labeled 17  $\alpha$ -hydroxyprogesterone and 17  $\alpha$ -hydroxyprogesterone contained in standards or specimens to be assayed for a fixed and limited number of antibody binding sites. After the incubation, the amount of labelled 17  $\alpha$ -hydroxyprogesterone bound to the antibody is inversely related to the amount of unlabelled 17  $\alpha$ -hydroxyprogesterone originally present in the sample. The method adopted for bound / free fraction separation is based on the use of coated tubes.

## 4. REAGENTS

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

REAGENTS	QUANTITY	STORAGE
<b>COATED TUBES:</b> ready to use. Rabbit anti-serum 17 $\alpha$ -hydroxyprogesterone antibodies coated on to the bottom of the tube.	100 (4 x 25) tubes	2-8°C until the expiry date. Tubes removed from their packs must be stored in the bag supplied at 2-8°C with a dessicant.
<b><sup>125</sup>I 17 <math>\alpha</math>-hydroxyprogesterone:</b> ready to use. 17 $\alpha$ -hydroxyprogesterone labelled with iodine, buffer and sodium azide. $\leq 97$ kBq ( $\leq 2.6$ $\mu$ Ci)	2 vials of 52 ml	2-8°C until the expiry date.
<b>O STANDARD:</b> ready to use. Human serum, preservative and sodium azide.	1 vial of 1 ml	After the first opening, freeze at - 15 C° until the expiry date. Repeated freezing will not harm the standards.
<b>STANDARDS 1 TO 6:</b> ready to use. Human serum, preservative, sodium azide and 17 $\alpha$ -hydroxyprogesterone at the following concentrations: 0.30 - 0.76 - 3 - 7.6 - 30.3 - 75.7 nmol/l* 0.10 - 0.25 - 1 - 2.5 - 10 - 25 ng/ml.	6 vials of 0.5 ml	After the first opening, freeze at - 15 C° until the expiry date. Repeated freezing will not harm the standards.

\* Conversion to nmol/l may be made using the following calculation: nmol 17  $\alpha$ -hydroxyprogesterone/l = ng/ml x 3.03

## 5. PRECAUTIONS FOR USE

### 5.1. Safety measures

Human blood derivatives contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all human blood derived products, including the samples to be assayed, must be treated as potentially infectious.

Do not pipette by mouth.

Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.

Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.

Avoid splashing.

Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

### 5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.

The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.

The enforcement of the basic rules for handling radioactive products ensures adequate security.

A summary of these is given below :

Radioactive products must be stored in their original containers in a suitable area.

A record of the reception and storage of radioactive products must be kept up to date.

Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).

Do not eat, drink, smoke or apply cosmetics in a controlled zone. Do not mouth-pipette radioactive solutions.

Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves. Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes. Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures. All radioactive waste disposals must be carried out according to the regulations in force.

**5.3. Handling precautions**

Do not use kit components beyond their expiry date.  
Do not mix reagents from different batches.  
Avoid any microbic contamination of the reagents or of the water used for washing.  
Fully respect the incubation times and the washing instructions indicated.

**6. SPECIMEN COLLECTION AND PREPARATION**

The assay is performed directly on serum or plasma (EDTA or Heparin). If the test is to be carried out within 24 hours, the samples should be refrigerated at 2-8°C. Otherwise, they should be divided into aliquots and deep frozen (-20°C) until needed. Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin have to be discarded.

**Dilutions**

Should elevated 17 α-hydroxyprogesterone levels be suspected, the 0 Standard found in the kit is used for dilution. It is recommended that disposable plastic tubes be used when carrying out the dilutions.

**Important remark:** for samples from pregnant women at the 3<sup>rd</sup> trimester and newborn children, 17 α-hydroxyprogesterone must be assayed immediately after an extraction step.

**7. ASSAY PROCEDURE**

**7.1. Material required**

Precision micropipettes or similar, with disposable tips, capable of dispensing 25 µl and 1 ml. Their calibrations should be checked regularly.  
Distilled water. Test tube rack. Vortex-type mixer.  
Water bath (37°C). Aspirating device.  
Gamma scintillation counter for 125 iodine.

**7.2. Protocol**

All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use. Dispensing of the reagent into the tubes is also to be carried out at room temperature. The assay requires the following groups of tubes:  
T group, for the total activity determination.  
Standard groups, to establish the standard curve.  
Sx groups, for the test samples.  
It is recommended that the samples be assayed in duplicate.  
Strictly respect the order in which reagents are to be added:  
**Dispense** 25 µl of standards and samples to be assayed into the correspondingly-labeled coated tubes. Refreeze the standards at below – 15°C between usage.  
**Add** 1000 µl of 17 α-hydroxyprogesterone <sup>125</sup>I to each tube and to the T tubes.  
**Mix** each tube gently with a Vortex-type mixer.  
**Incubate** for 2 h 30 minutes at 37°C.  
**Aspirate** the contents of the coated tubes as completely as possible or discard the solution by overturning the tubes and tap the top of each tube firmly onto absorbent paper (except T tubes).  
**Measure** the remaining radioactivity bound to the tubes with a gamma scintillation counter.

**8. QUALITY CONTROL**

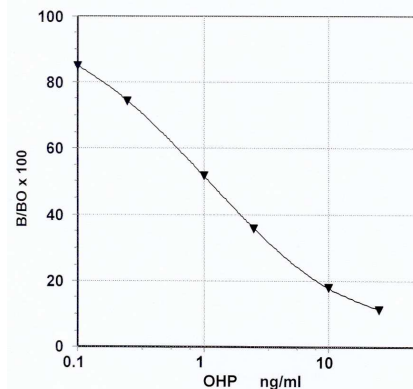
Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

**9. RESULTS**

Draw up the standard curve by plotting the B/Bo % versus concentrations (ng/ml or nmol/l). Determine the B/Bo % value for each patient sample. Using the standard curve, determine the 17 α-hydroxyprogesterone concentrations for each patient sample. Read the sample values directly from the curve, correcting the read value by the dilution factor, if necessary.

**Typical standard curve** (example only): this data must not be substituted for results obtained in the laboratory.

Group of tubes	Mean cpm	B/Bo x 100	Concentration	
			ng/ml	nmol/l
T	37587			
Standard 0	18228	100	0	0
Standard 1	15493	85.0	0.1	0.30
Standard 2	13562	74.4	0.25	0.76
Standard 3	9406	51.6	1	3
Standard 4	6526	35.8	2.5	7.6
Standard 5	3226	17.7	10	30.3
Standard 6	2060	11.3	25	75.7



## 10. PROCEDURAL LIMITATIONS

Do not extrapolate sample values beyond the last standard. Dilute the samples concerned and re-assay.

## 11. EXPECTED VALUES

It is recommended that each laboratory establish its own range of normal values. For example, the following results have been obtained:

### Reproductive aged women

<b>Follicular phase</b>	0.10 - 0.80 ng/ml	or	0.303 - 2.42 nmol/l
<b>Luteal phase</b>	0.27 - 2.90 ng/ml	or	0.82 - 8.78 nmol/l
<b>Post ACTH</b>	< 3.2 ng/ml	or	< 9.69 nmol/l

### Normal men

0.31 - 2.17 ng/ml	or	0.94 - 6.57 nmol/l
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### Third trimester pregnant women\*

2 - 12 ng/ml	or	6.06 - 36.36 nmol/l
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### Newborn\*

< 0.7 - 2.5 ng/ml	or	< 2.12 - 7.5 nmol/l
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\* Note : These samples require an extraction step prior to assaying.

## 12. SPECIFIC CHARACTERISTICS OF THE ASSAY

### 12.1. Imprecision

This has been assessed using pools of assay samples with different concentrations. They were tested 24 times in the same series of assay and in 20 independent assays.

Samples	Within-run	
	Mean nmol/l	CV %
1	0.345	12.3
2	2.74	7.8
3	13.21	8.3

Samples	Between-run	
	Mean nmol/l	CV %
4	2.94	12
5	12.30	9.8
6	22.81	12.8

### 12.2. Dilution

Samples were diluted up to 1:16 using standard 0. Recovery percentages were found from 83% to 109%.

### 12.3. Spiking test

Recovery percentages are from 88% to 112%.

### 12.4. Specificity

The antibodies used in the test present the cross-reactions below:

Compound	Cross-reaction (%)	Compound	Cross-reaction (%)
17 $\alpha$ -Hydroxyprogesterone	100.00 %	Androsterone	< 0.01
11-Desoxycortisol	2.1 %	DHEA	< 0.01
17 $\alpha$ -Hydroxypregnenolone	2.2	Androstenedione	< 0.01
Progesterone	0.49	Etiocholanolone	< 0.01
Desoxycorticosterone	0.02	Dihydroxytestosterone	< 0.01
Pregnenolone	0.01	Estriol	< 0.01
Pregnenolone-SO 4	0.01	Estradiol-17 $\beta$	< 0.01
Corticosterone	< 0.01	Estrone	< 0.01
Aldosterone	< 0.01	Testosterone	< 0.01

### 12.5. Detection limit

The detection limit is defined being the smallest concentration different from zero with a probability of 95%. It has been assessed as being 0.09 nmol/l.

### ASSAY FLOW-CHART

Tubes	Standards $\mu$ l	Samples $\mu$ l	<sup>125</sup> I 17 $\alpha$ -hydroxyprogesterone $\mu$ l	Mix Incubate for 2 hours 30 minutes at 37°C	Aspirate or decant	C O U N T
T	-	-	1000			
Standards	25	-	1000			
Samples	-	25	1000			