



# 17-OH-Progesterone Coated Tubes

Direct <sup>125</sup>I-Radioimmunoassay kit for the quantitative determination of serum 17-OH-Progesterone.

Cat. BL-01CT-100  
991221 - Rev. 03

## Summary and background of the test:

Five enzymes are involved in the conversion of cholesterol to testosterone (1). The initial reaction in this process involves side chain cleavage of cholesterol to form pregnenolone. The subsequent conversion of pregnenolone to testosterone involves an ordered series of enzymatic reactions, including side chain cleavage, reduction of 17-keto groups and A-ring oxidation. 17-OH-Progesterone is one of the steroids involved in the course of that process, and also a major step in the biosynthetic pathway leading to cortisol, by hydroxylation at both C<sub>11</sub> and C<sub>21</sub>.

C<sub>21</sub>-hydroxylase deficiency is the most common cause of ambiguous genitalia in infants as well as the most common form of congenital adrenal hyperplasia (CAH). Most CAH (2,3,4) result from an enzyme block producing very high plasma concentrations of 17-OH-Progesterone and androgen precursors, including androstenedione. Urinary excretion of pregnantriol, the metabolite of 17-OH-Progesterone, will also be increased. The diagnosis of C<sub>21</sub>-hydroxylase deficiency should always be considered in :

- patients with ambiguous genitalia.
- infants presenting severe dehydrated conditions.
- males and females with signs of virilization before puberty.

17-OH-Progesterone circulates bound to both transcortin and albumin. The elevation in concentration of plasma 17-hydroxyprogesterone is such a distinctive marker of 21-hydroxylase deficiency that prenatal diagnosis has been attempted by measuring its concentration in amniotic fluid (5).

Testes, ovaries, adrenals and placenta can produce 17-OH-Progesterone. Direct assays were described for the determination of 17-OH-Progesterone in small volumes of serum, saliva or blood spots (6-9). It should be kept in mind that some interference of steroids (17-OH-Pregnenolone,...) in some newborn sera might occur with radioimmunological determinations of 17-OH-Progesterone (10-11).

## Principle of the test :

Radioimmunoassay is based on the ability of a limited quantity of antibody to bind a fixed amount of radiolabelled antigen (<sup>125</sup>I-

Ag). The percentage of bound radiolabelled antigen is inversely related to the increasing concentration of unlabelled analyte in the sample. Separation of the bound and free radiolabelled antigen is necessary in order to determine the quantity of unlabelled antigen. The Bio-Line 17-OH-Progesterone kit utilizes the coated tubes methodology. The quantity of unlabelled antigen in an unknown sample is then determined by comparing the remaining radioactivity in the coated tubes with data established using known standards in the same assay system.

## Precautions:

1. Radioactive material: Radioactive material may be received, acquired, possessed and used only by physicians, clinical laboratories, or hospitals for "In-Vitro" clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals.

Compliance with these basic rules of radiation safety should provide adequate protection:

1. Do not eat, drink, smoke, or apply cosmetics in areas where radioactive material is used.
2. Do not pipet by mouth reagents containing radioactive materials.
3. Wear protective clothing; i.e., lab coats and disposable gloves, in order to avoid direct contact with radioactive reagents.
4. Work with radioactive materials should be performed in a designed area.
5. Radioactive materials should be stored in an acceptable location.
6. A log should be kept for receipt and disposal of radioactive materials.
7. Radioactive spills or accidents should be taken care of immediately according to established procedures.
8. Disposal of radioactive materials must comply with prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory.

2. Sodium azide: Sodium Azide, used as a bacteriostatic agent, is toxic in acid medium. In addition, it may form potentially explosive lead or copper azides. To avoid dangerous deposits, waste solutions should be flushed away with large volumes of water.

3. Hepatitis and Acquired Immune Deficiency Syndrome (HTLV-III): All Bio-Line reagents included in this kit have been

tested and found to be non reactive for hepatitis B surface antigen. They have also been screened and determined to be non-reactive for HTLV-III antibody. However, human serum products should be handled as if potentially capable of transmitting hepatitis, Acquired Immune Deficiency Syndrome, or other infectious agents.

### Materials provided:

Kit contains sufficient reagents for 100 determinations.

1. **17-OH-Progesterone human serum based standards & control:** 9 vials containing each 500 µl except Zero 1ml. Control:  $1.7 \pm 0.3$  ng/ml. Standards: 0-0.25-0.5-1-2-5-10-20 ng/ml.
2. **<sup>125</sup>I-17-OH-Progesterone tracer:** 1 vial (red solution) containing 31 ml. Activity < 4µCi or 148 kBq.
3. **Coated tubes:** 2 x 50 tubes, coated with Anti-17-OH-Progesterone antiserum (Rabbit).
4. **Wash solution concentrate:** 1 vial of 2 ml of concentrate, to be diluted into 250 ml NaCl 9‰ and stored at 4°C.

Reagents provided should be stored at 2° - 8° C.

Refer to the expiration date on the kit label for stability.

### Materials required but not provided:

1. Pipets, micropipets, repeating syringes and repeating pipettors.
2. Gamma counter.
3. Logit log graph paper.
4. Horizontal shaker recommended (type IKA-VIBRAX-VXR), but a rotator could be used.
5. Test tube racks.
6. Vortex mixer.
7. 9‰ NaCl saline solution.

### Specimen collection and preparation:

Sera should be separated from blood cells immediately after collection. Sera are stable for at least 7 days at 4° C and for longer periods of time when stored frozen.

### Assay procedure:

Bring reagents to room temperature and mix before use. Label tubes for total counts (Tc), standards, control sera and unknowns.

1. Pipet 25 µl of standards, samples and controls into the corresponding tubes.
2. Add 300 µl of tracer solution (red) to each tube. Mix well (sideways shaking of whole rack).
3. Mix well, cover and incubate 3 hours at 37°C.
4. Aspirate (or decant). Wash twice, adding 1 ml of wash solution to each tube, except Tc. Aspirate or decant.
5. Record the counts per minute (cpm) for each tube. Count all tubes for one minute.

### 17-OH Progesterone Coated Tubes Flow chart

Tubes Reagents	Tc	B0	Stds., Control	samples
Standards or samples (µl)	-	25	25	25
Tracer (µl)	300	300	300	300
Mix well (sideways shaking of the whole rack) and incubate 3h at 37°C				
Wash solution	-	2 x 1 ml		
Aspirate or decant. Count 1 min.				

## Data table (example)

Tube	Duplicate cpm		Mean cpm	%B/B <sub>0</sub>	Conc. ng/ml
Tc	53 087	54 339	53 713	-	
Zero	33 837	34 043	33 940	100 %	
Std 0.25	27 063	27 014	27 038	79.7 %	
Std 0.50	20 320	21 200	20 760	61.2 %	
Std 1.0	16 289	16 417	16 353	48.2 %	
Std 2.0	11 347	11 194	11 270	33.2 %	
Std 5.0	4 908	5 086	4 997	14.7 %	
Std 10.0	2 426	2 509	2 467	7.3 %	
Std 20.0	1 344	1 404	1 374	4.1 %	
Control	12 520	12 782	12 651	37.3 %	1.61
Sample 1	8 511	8 548	8 529	25.1 %	2.62

### Calculation of results:

Determine the average counts for each set of duplicate tubes. Divide this value by the average net counts of the B<sub>0</sub>, and multiply by 100 to yield the % B/B<sub>0</sub>

$$\% \text{ B/B}_0 = \frac{\text{cpm (Stds, Controls or unknowns)}}{\text{cpm (B}_0\text{)}} \times 100$$

Plot % B/B<sub>0</sub> for each standard vs its concentration in ng/ml on semi-log graph paper. The concentration of 17-OH-Progesterone in the unknown samples may be read directly from the standard curve.

### Expected Values:

	Range (ng/ml)
cord blood	7 - 20
newborn	1 - 7
adult male	0.3 - 2
adult female Foll. phase	0.1 - 1.2
adult female LH peak	> 1
luteal phase	0.3 - 3
postmenopausal	0.1 - 0.8
children with CAH	> 50

Each laboratory should analyze normal samples to establish its own normal ranges.  
Conversion factor: 1 ng/ml = 3 nmol/l

### Specific performance characteristics:

#### 1. Specificity:

The relative percent of cross-reactivity by weight of 17-OH-Progesterone and various related compounds was evaluated for the antibody used in this assay. Cross-reactivities are expressed as the amount of 17-OH-Progesterone required to reduce the binding of <sup>125</sup>I-17-OH-Progesterone by 50%, relative to the amount of a related compound required to do the same.

$$\text{Cross-reactivity of } x = 100 \times \frac{\text{conc. 17-OH-P at 50\% B/B}_0}{\text{conc. compound } x}$$

Compound x	Cross-reactivity (%)
17-OH-Progesterone	100 %
17-OH-Pregnenolone	< 2 %
Progesterone	< 1 %
11-Deoxycortisol	< 1 %
Pregnenolone	< 0.50 %
Cortisone	< 0.05 %
Corticosterone	< 0.05 %

Deoxycorticosterone	< 0.05 %
DHEA	< 0.05 %
DHEA-S	< 0.05 %
Estriol	< 0.05 %
Estrone	< 0.05 %
Hydroxycortisone	< 0.05 %
Testosterone	< 0.05 %

#### 2. Sensitivity:

The lowest detectable concentration of 17-OH-Progesterone that can be reliably distinguished from zero with this kit has been evaluated to be  $\leq 0.09$  ng/ml.

#### 3. Precision and reproducibility:

Assays variations of two serum samples are mentioned in the following table.

	Sample 1	Sample 2
Mean	2.9 ng/ml	10.2 ng/ml
Within assay variation	3.8 %	4.3 %
Between assay variation	4.2 %	7.9 %

#### 4. Linearity:

The results obtained when diluting a serum with elevated 17-OH-Progesterone concentration with a 17-OH-Progesterone-free serum are summarized in the following table

Dilution factor	Expected values	Experimental values
1:1	19.92 ng/ml	-
1:2	9.96 ng/ml	9.82 ng/ml
1:4	4.98 ng/ml	5.07 ng/ml
1:8	2.49 ng/ml	2.84 ng/ml

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