Sensitive 17-OH-Progesterone

Direct ^{125}I-Radioimmunoassay kit for the quantitative determination of serum 17-OH-Progesterone.

Cat. BL-01-100
981209 - Rev. 05

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_{11} and C_{21}.
C_{21}-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_{21}-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(^{125}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 second antibody methodology. The quantity of unlabelled antigen in an unknown sample is then determined by comparing the radioactivity of the isolated precipitate 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 ± 0.3 ng/ml.
   Standards: 0-0.25-0.5-1-2-5-10-20 ng/ml.
2. **^{125}I-17-OH-Progesterone tracer**: 1 vial (red solution) containing 21 ml. Activity < 4µCi or 148 kBq.

3. **Anti-17-OH-Progesterone antiserum (Rabbit)**: 1 vial (yellow solution) containing 10.5 ml.
4. **Second antibody (Sheep a-Rabbit)**: 1 vial (white suspension) containing 10.5 ml.

All reagents are ready for use and 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. Test tubes.
3. Cooling centrifuge capable of developing 1300 to 1500g.
4. Gamma counter.
5. Logit log graph paper.
6. 37°C waterbath.
7. Test tube racks.
8. Vortex mixer.
9. Magnetic stirrer and stir bars.
10. 9 ‰ NaCl saline solution.

The use of some plastic tubes may result in higher NSB values, without affecting the results. However, glass tubes are recommended for the Bio-Line 17-OH-Progesterone assays.

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 **glass disposable tubes** for total counts (Tc), non specific binding (NSB), standards, control sera and unknowns.

1. Pipet 20 µl of standards, samples and control into the corresponding tubes.
2. Add 200 µl of tracer solution (red) to each tube.
3. Add 100 µl of anti-17-OH-Progesterone antiserum (yellow) to each tube, except Tc and NSB tubes. Add 100 µl of NaCl 9 ‰ to the NSB tubes.
4. Mix and incubate for 1 hour at 37° C.
5. Add 100 µl of second antibody (white suspension) to each tube, except Tc. Maintain moderate magnetic stirring of the suspension during the transfer.
6. Mix and incubate for 10-15 minutes at room temperature.
7. Add 2 ml of saline (NaCl 9 ‰) to each tube, except Tc. Centrifuge at 4°C all tubes, except Tc, for 15 minutes at 1300-1500g.
8. Decant all tubes as a whole, discarding the supernatant into a radioactive waste container. While tubes are inverted, gently blot the final drops onto absorbent paper. Do not aspirate, do not let stand inverted.
9. Record the counts per minute (cpm) for each tube.

17-OH Progesterone Flow chart

Tubes Reagents	Tc	NSB	B0	Stds., Control	samples
Standards or samples (µl)	-	20	20	20	20
Tracer (µl)	200	200	200	200	200
1^{st}Ab (µl)	-	-	100	100	100
NaCl 9 ‰(µl)	-	100	-	-	-
Mix and incubate 60 min. at 37°C					
2nd Ab (µl)	-	100			
Mix and incubate 10 min.RT					
NaCl 9‰	-	2 ml	2 ml	2 ml	2 ml
Centrifuge 15 min. at 4°C, decant, count 1 min.					

Data table (example)

Tube	Duplicate cpm		Mean cpm	%B/B0	Conc. ng/ml
Tc	35 406	33 861	34 634	-	
NSB	1 635	1 428	1 532	4.4 %	
Zero	27 644	26 771	27 208	100 %	
Std 0.25	22 308	22 208	22 258	81.8 %	
Std 0.50	18 284	17 777	18 031	66.3 %	
Std 1.0	13 918	13 990	13 954	51.3 %	
Std 2.0	10 541	10 394	10 468	38.5 %	
Std 5.0	5 627	5 417	5 522	20.3 %	
Std 10.0	3 278	3 215	3 247	11.9 %	
Std 20.0	1 802	1 718	1 760	6.5 %	
Control	11 427	11 432	11 430	42.0 %	1.70
Sample 1	23 345	24 167	23 756	87.3 %	0.28

Calculation of results:

Data need not be expressed as counts per minute (cpm) but the counting period must be the same for all tubes that are counted.
Determine the average counts for each set of duplicate tubes. Subtract the NSB average counts from the average counts of samples and standards. Divide this value by the average net counts of the Bo, and multiply by 100 to yield the % B/Bo

$$\% B/B_0 = \frac{\text{cpm (Stds, Controls or unknowns) - cpm (NSB)}}{\text{cpm } (B_0) \text{ - cpm (NSB)}} \times 100$$

Plot % B/Bo 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 ^{125}I-17-OH-Progesterone by 50%, relative to the amount of a related compound required to do the same.

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

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 less than 0.125 ng/ml.

3. Precision and reproducibility:
Assays variations of two serum samples are mentioned in the following table.

	Sample 1	Sample 2
Mean	1.8 ng/m	8.7 ng/ml
Within assay variation	4.6 %	5.9 %
Between assay variation	6.4 %	8.1 %

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	18.60 ng/ml	-
1:2	9.30 ng/ml	9.1 ng/ml
1:4	4.65 ng/ml	4.9 ng/ml
1:8	2.33 ng/ml	2.6 ng/ml

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