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PROG-RIA-CT

KIP1458

LOT : 110218/1

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Read entire protocol before use.

PROG-RIA-CT

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of human Progesterone (PROG) in serum.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource PROG-RIA-CT Kit

B. Catalog number : KIP1458 : 96 tests

III. CLINICAL BACKGROUND

A. Biological Activity

Progesterone is a C-21 steroid hormone (molecular weight: 314.5) which is synthesized from cholesterol via pregnenolone in the granulosa and theca cells of the corpus luteum under the influence of LH. The major production sites are ovary and placenta and somewhat the adrenal cortex in both men and women. Progesterone is rapidly metabolized in the liver. Blood levels are very low during the follicular phase whereas one does observe a sharply increase during the luteal phase of menstrual cycles reaching a maximum some 5 to 10 days after the midcycle LH peak.

B. Clinical applications

Serum progesterone levels, which are low during the follicular phase, increase during the luteal phase of menstrual cycle. Unless pregnancy occurs, the progesterone level declines 4 days before the next menstrual period. Thus, the measurement of progesterone levels constitutes a well-established method for detection of ovulation. But there are many cases where the progesterone measurements are also of interest:

- To check the effectiveness of ovulation induction;
- To monitor the embryo transfer and progesterone replacement therapy;
- To detect the patients at risk for abortion during the beginning of pregnancy;
- To aid in the diagnostic of ectopic pregnancy;
- To detect all ovarian tumor (benign and malign) in postmenopausal women;
- To diagnose luteinized unruptured follicle by the dosage of 17 beta-estradiol and progesterone levels in peritoneal fluid;
- The steroid profiles of follicular fluids and the ratio of E2/PROG allow detecting a normal or a dysfunctional ovulation induction. (The empty follicular syndrome may reflect a dysfunctional ovulation induction).

IV. PRINCIPLES OF THE METHOD

A fixed amount of ^{125}I labelled steroid competes with the steroid to be measured present in the sample or in the calibrator for a fixed amount of antibody sites being immobilized to the wall of a polystyrene tube. Neither extraction nor chromatography is required. After 2 hours incubation at 37°C in a water bath, an aspiration step terminates the competition reaction. The tubes are then washed with 3 ml of wash solution and aspirated again. A calibration curve is plotted and the Progesterone concentrations of the samples are determined by dose interpolation from the calibration curve.

V. REAGENTS PROVIDED

Reagents	96 Test Kit	Colour Code	Reconstitution
 Tubes coated with anti PROG	2 x 48	silver	**Ready** for use
Ag | **^{125}I** TRACER: ^{125}Iodine labelled PROG (HPLC grade) in acetate buffer with bovine casein and azide (<0.1%)	1 vial 55 ml 220 kBq	red	**Ready** for use
CAL | **0** Zero Calibrator in human serum and azide (0.5%)	1 vial 1 ml	yellow	**Ready** for use
CAL | **N** Calibrators - N = 1 to 6 (see exact values on vial labels) in bovine serum and azide (0.5%)	6 vials 0.5 ml	yellow	**Ready** for use
WASH | **SOLN** | **CONC** Wash solution (TRIS-HCl)	1 vial 10 ml	brown	**Dilute** 70 x with distilled water (use a magnetic stirrer).
CONTROL | **N** Controls - N = 1 or 2 in human serum with thymol	2 vials lyophilised	silver	**Add** 0.5 ml distilled water

Note : Use the zero calibrator for sera dilutions.

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. Distilled water
2. Pipettes for delivery of: 50 µl and 500 µl (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Water bath at 37° ± 2°C
6. 5 ml automatic syringe (Cornwall type) for washing
7. Aspiration system (optional)
8. Any gamma counter capable of measuring ^{125}I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

A. **Controls**: Reconstitute the controls with 0.5 ml distilled water.

B. **Working Wash solution**: Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash Solution (70x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, controls are stable for 7 days at 2-8°C. For longer storage periods, aliquots should be made and kept at –20°C for maximum 3 months. Avoid subsequent freeze-thaw cycles.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, tracer is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

IX. SPECIMEN COLLECTION AND PREPARATION

- Serum samples must be kept at 2-8°C.
- If the test is not run within 48 hrs, storage at -20°C is recommended.
- Avoid subsequent freeze-thaw cycles.

X. PROCEDURE

A. Handling notes

Do not use the kit or components beyond expiry date.
Do not mix materials from different kit lots.
Bring all the reagents to room temperature prior to use. **Special attention should be taken to ensure that the Tracer is at room temperature.**
Thoroughly mix all reagents and samples by gentle agitation or swirling.
Use a clean disposable pipette tip for addition of each different reagent and sample in order to avoid cross-contamination. High precision pipettes or automated pipetting equipment will improve the precision.
Respect the incubation times.
Prepare a calibration curve for each run, do not use data from previous runs.

B. Procedure

1. Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes
2. Briefly vortex calibrators, controls and samples and dispense 50µl of each into the respective tubes.
3. Dispense 500 µl of ^{125}Iodine labelled PROG into each tube, including the uncoated tubes for total counts.
4. Shake the tube rack gently by hand to liberate any trapped air bubbles.
5. Incubate for 2 hours at 37°C in a water bath.
6. Aspirate the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
7. Wash tubes with 3 ml Working Wash solution (except total counts) and aspirate. Avoid foaming during the addition of the Working Wash solution.
8. Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
9. Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. Calculate the bound radioactivity as a percentage of the binding determined at the zero calibrator point (0) according to the following formula :

$$B/B0\,(\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

3. Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the (B/B0(%)) values for each calibrator point as a function of the PROG concentration of each calibrator point. Reject obvious outliers.
4. Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
5. By interpolation of the sample (B/B0 (%)) values, determine the PROG concentrations of the samples from the calibration curve.
6. For each assay, the percentage of total tracer bound in the absence of unlabelled PROG (B0/T) must be checked.

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

PROG		cpm	B/Bo (%)
Total count		86718	
Calibrator	0.00 ng/ml	33822	100.0
	0.12 ng/ml	30680	90.7
	0.90 ng/ml	21353	63.1
	3.00 ng/ml	13831	40.9
	7.90 ng/ml	7899	23.4
	15.00 ng/ml	4849	14.3
	36.00 ng/ml	2180	6.5

XIII. PERFORMANCE AND LIMITATIONS

A. Detection limit

Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations below the average counts at zero binding, was 0.05 ng/ml.

B. Specificity

The percentage of cross-reaction estimated by comparison of the concentration yielding a 50% inhibition are respectively:

Compound	Cross-Reactivity (%)
20-α-Dihydroprogesterone	0.03
20-β-Dihydroprogesterone	3.27
5-α-Pregnan-3,20 dione	3.51
17-α-Hydroxyprogesterone	1.50
Pregnonelone	0.38
Cortisol	0.003
21-Deoxycortisol	0.01
11-Deoxycortisol	0.05
Corticosterone	0.30
11-Deoxycorticosterone	0.83
Androstenedione	0.12
Testosterone	0.03
Estradiol	< 0.0012
Danazol	< 0.0012
DHEA	0.02
DHEA-SO4	0.004

Note: this table shows the cross-reactivity for the anti PROG

C. Precision

INTRA-ASSAY PRECISION

Serum	N	<X> ± SD (ng/ml)	CV (%)
A	20	1.27 ± 0.05	4.1
B	20	4.08 ± 0.13	3.3

INTER-ASSAY PRECISION

Serum	N	<X> ± SD (ng/ml)	CV (%)
A	11	1.17 ± 0.10	8.6
B	11	3.93 ± 0.26	6.5

SD: Standard Deviation; CV: Coefficient of variation

D. Accuracy

DILUTION TEST

Sample	Dilution	Theoretical Concent. (ng/ml)	Measured Concent. (ng/ml)
A	1/1	-	33.86
	1/2	16.93	17.16
	1/4	8.47	8.20
	1/8	4.23	3.88
	1/16	2.12	1.96
	1/32	1.06	1.11
	1/64	0.53	0.57

Sample was diluted with zero calibrator.

RECOVERY TEST

Sample	added PROG (ng/ml)	Recovered PROG (ng/ml)	Recovered (%)
1	22.23	21.95	99
	8.14	8.59	106
	2.68	2.76	103
	0.86	1.04	121
	0.31	0.29	94

Conversion factor :

From ng/ml to nmol/L : x 3.18
From nmol/L to ng/ml : x 0.314

The concentrations of the calibrator are traceable to an internal reference preparation.

E. Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 40 minutes after the calibrator has been added to coated tubes.

TIME DELAY

Serum ng/ml	0'	15'	20'	30'	40'
C 1	1.28	1.18	1.24	1.15	1.24
C 2	3.40	3.03	2.89	3.45	3.37

XIV. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises.

XV. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

	Concentration range (ng/ml)	Number of subjects
Males	0.60 – 2.11	50
Females		
. Follicular phase	0.70 – 1.78	34
. Ovulatory phase	0.79 – 3.95	29
. Luteal phase	4.57 – 17.56	39
. Menopause	0.43 – 2.13	50

(*) The range is based on 2.5 % and 97.5 % percentiles

XVI. PRECAUTIONS AND WARNINGS

Safety

For *in vitro* diagnostic use only.

This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations.

This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area. away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HbsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

XVII. BIBLIOGRAPHY

1. ALPER M. et al (1987)
Comparison of follicular fluid hormones in patients with one or two ovaries participating in a program of in vitro fertilization.
Fertil. and Steril. 48, 1, 94-97

2. GIDLEY-BAIRD A. et al. (1986)
Failure of implantation in human in vitro fertilization and embryo transfer patients : the effects of altered progesterone/estrogen ratios in humans and mice.
Fertil. Steril. 45, 1, 69-74.

3. HEINONEN P. et al (1985)
Elevated progesterone levels in serum and ovarian venous blood in patients with ovarian tumors.
Acta Obstet.Gynecol. Scand. 64, 649-652.

4. JANSSEN-CASPERS H. et al (1986)
Diagnosis of luteinized unruptured follicle by ultrasound and steroid hormone assays in peritoneal fluid : a comparative study.
Fertil. Steril.,46, 5, 823-827.

5. KO-ENHUANG et al. (1986)
Serum progesterone levels in women treated with human menopausal gonadotropin and human chorionic gonadotropin for in vitro fertilization.
Fertility and Sterility, 46, 5, 903-906.

6. KRUITWAGEN R. et al (1987)
Oestradiol-17b and progesterone level changes in peritoneal fluid around the time of ovulation.
British Journal of Obstetrics and Gynecology, 94, 548-553.

7. MATTHEWS C. (1986)
Serum progesterone levels as an aid in the diagnosis of ectopic pregnancy.
Obstetrics and Gynecology, 68, 390-394.

8. OSKOWITZ S. et al (1986)
Luteal phase serum progesterone levels after follicle aspiration with and without clomiphene citrate treatment.
Fertil. Steril., 46, 3, 461-465.

XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS µl	CALIBRATORS µl	SAMPLE (S) CONTROLS µl
Calibrators (0 to 6) Samples, Controls Tracer	- - 500	50 - 500	- 50 500
Incubation	2 hours at 37°C in a water bath		
Separation Working Wash solution Separation	-	Aspirate 3.0 ml Aspirate	
Counting	Count tubes for 60 seconds		

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