ESTRONE-RIA-CT

KIPI9100

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ESTRONE-RIA-CT

Radioimmunoassay for the Quantitative Determination of Estrone in Human Serum or Plasma

KIPI9100

IN VITRO DIAGNOSTIC USE

en

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1. INTENDED USE:

For **IN VITRO** determination of serum or plasma ESTRONE levels.

The origin of plasma estrogens in women has been precisely studied by refined isotopic dilution techniques.

In normal women, most plasma estradiol is derived from the ovary, where theca cells secrete androstenedione, which is then converted to estrone and then to estradiol by the granulosa cells.

Little estrone is indeed formed and secreted by the ovary : most originates from a peripheral conversion of estradiol and from the aromatisation of androstenedione, a catalytic reaction essentially carried out in adipose tissue. In premenopausal women, androstenedione is secreted by the ovary and the adrenals. In pregnant women, the fetal adrenal gland provides a significant contribution to androstenedione production. In menopausal women, estrone is the essential estrogen found in the circulation, resulting from the conversion of adrenal androstenedione. An increase in estrogen formation occurs with aging and in correlation with the amount of adipose tissue. The estrogenic effects of estrone in menopausal women can produce endometrial hyperplasia and bleeding but also maintains the bone mineral content. In premenopausal women, excessive estrone blood levels can result from the conversion of large amounts of androstenedione produced in micropolycystic ovary syndrome and ovarian tumors. In such women, high estrone blood levels can participate in a disturbance of the menstrual cycle.

Estrone in the circulation is essentially bound to albumin. This is important in the interpretation of estrone-assay data. Indeed, and contrary to estradiol, total estrone levels are not significantly modified by SHBG concentration.

2. PRINCIPLE OF THE METHOD:

The ESTRONE (E1) CT RIA obeys the law of mass action according to the following equation :

$$\text{Free} \begin{cases} E1 \\ {}^{125}I\text{ - }E1 \end{cases} + Ab \rightleftharpoons \text{Bound} \begin{cases} Ab\text{ - }E1 \\ Ab\text{ - }{}^{125}I\text{ - }E1 \end{cases}$$

Since the concentrations of ^{125}I - E1 and coated antibodies are constant, the advancing state of the equation depends on the concentration of E1. The amount of ^{125}I - E1 bound to the coated tube is inversely proportional to the concentration of E1 in the sample.

Following the incubation, the tube is washed to remove excess of unbound ^{125}I - E1.

Patient samples concentration are read from a calibration curve.

3. MATERIAL PROVIDED AND STORAGE:

Stored at 2 - 8°C, the material can be used up to the expiration date printed on each label.

3.1. 2 x 48 Polystyrene tubes (12 x 75 mm) coated with anti-Estrone polyclonal antibodies.
Systematically allow the coated tubes to reach room temperature before use
Store the unused tubes at 2-8°C.

3.2. **Ag** | **125I** — yellow, 42 ml
1 bottle of ^{125}I-labelled ESTRONE in buffer with a stabilizer, a preservative (NaN_3 < 0.1 %) and a yellow dye.
Each bottle contains less than 185 kBq (5 µCi)

3.3. **CAL** | **N** — 1 ml in each vial except for Calibrator 0 : 2 ml
N = 0 to 5
6 vials of ESTRONE in serum containing preservative (NaN3< 0.1 %).
The concentrations are printed on the vial labels. Store at 2-8°C for up to 12 weeks. For longer periods, store at -20°C.

3.4. **CONTROL** | **N** — 2 vials, lyophilized - N=1 or 2
2 vials of human serum containing preservatives (NaN_3 < 0.1 %). The control sera are to be assayed along with the patient samples. The ranges for the control sera are printed on the vial labels.
Before use, reconstitute the content of the controls with 1 ml of distilled water.

3.5. **WASH** | **SOL** | **CONC** — 70 x concentrated, 10 ml
1 bottle concentrated buffered solution containing sodium azide (NaN_3 < 0.1 %). Poor the solution in 700 ml of distilled water.

4. MATERIAL REQUIRED BUT NOT PROVIDED:

- bench surfaces protected by absorbent paper to reduce the effects of radioactive spillage.
- waste disposal containers appropriately labelled and designed as suitable for solid or liquid radioactive materials.
- manual or automated precision micropipettes for dispensing samples or reagents without cross-contamination.
- absorbent paper.
- vacuum pump connected through a trap for aspiration
- horizontal shaker (max 300 rpm)
- a gamma scintillation counter.
- appropriate graph paper for plotting the results.

5. METHODOLOGY

5.1. Collection and handling of blood samples:

The blood sample may be collected into a dry tube or one containing an anticoagulant. If heparin is used, only the minimum required should be added to avoid cloting.

After separation from the red blood cells, plasma or serum samples may be assayed immediately, within 24 hours if stored at 2 - 8°C, or later, after period up to several months if stored at -20°C. Repeating freezing and thawing must be avoided.

5.2. Assay procedure :

Reagents stored at 2°- 8° C. must be brought at room temperature prior to use. Do not mix reagents of different lots. Label the tubes for T (« Total Counts » do not use coated tubes) calibrators, samples and control sera.

Perform the assay in duplicate. Calibrators, controls and samples must be assayed at the same time.

1. Calibrator curve:

   Pipette 100 µl of each calibrator into the corresponding tubes.

2. Unknowns and control sera:

   Pipette 100 µl of each sample or control sera into the corresponding tubes.

3. Add 400 µl of ^{125}I - ESTRONE tracer to each tube.

4. Vortex, cover and incubate 2 hours at room temperature on a horizontal shaker (max. 300 rpm).

5. Carefully aspirate or decante (before to decante, add 2 ml of washing solution to each tube.) the solution of all tubes. (Except total counts tubes).

6. Add 2 ml of washing solution to each tube. Aspirate or decante carefully.

7. Repeat step 6.

8. Count the radioactivity fixed in each tube for at least 60 seconds.

5.3. Data processing:

Determine the mean count rate for each set of duplicate tubes.

Calculate the ratio B/B0 as follows :

$$B/B0\ \% = [\text{ Calibrator or Smp cpm / B0 (Calibrator 0) cpm}] \times 100$$

Draw the calibrator curve on semilogarithmic paper by plotting the ratio B/B0 % (linear scale) obtained for each calibrator versus its respective concentration expressed in pg/ml (logarithmic scale). ESTRONE concentrations in samples may be read directly from the calibrator curve.

If a computer is used to calculate the results, the data can be fitted to the appropriate equation : weighed 4 PL.

5.4. Example of a typical assay:

	Contents (pg/ml)	cpm 1st duplicate	cpm 2nd duplicate	Mean count rate	B/Bo (%)	Estrone (pg/ml)
Total counts	-	50158	50055	50107	-	-
Cal 0	0	15459	14618	15039	100.0	-
Cal 1	15	13641	13125	13383	89.0	-
Cal 2	55	11572	10720	11146	74.1	-
Cal 3	115	9103	9337	9220	61.3	-
Cal 4	260	6787	7207	6997	46.5	-
Cal 5	815	3705	3578	3642	24.2	-
C 1 low	34 - 48	11973	11582	11777	78.3	42.3
C 2 high	170 - 220	7450	8064	7757	51.6	198.5
Sample 1	-	12485	12612	12549	83.4	28.1
Sample 2	-	8490	8512	8502	56.5	149,1

Example of a typical assay, do not use for calculations

6. PERFORMANCE CHARACTERISTICS:

6.1. Specificity

Steroid	% Cross-reactivity
Estrone	100.00
Estradiol	0.03
Estriol	0.005
DHEA-S	0.0003
Androstenedione	N.D
Progesterone	N.D
Testosterone	N.D
Estrone - Sulfate	N.D
17 OH Progesterone	N.D

6.2. Minimum detectable concentration of ESTRONE:

The minimum detectable concentration has been assayed at 3.2 pg/ml and corresponds to the concentration given by two standards deviations below the mean cpm of 20 replicate determinations of the zero calibrators.

6.3. Recovery test:

When sera of known ESTRONE contents have their ESTRONE supplemented by addtion of ESTRONE, a satisfactory correlation between added and assayed ESTRONE is obtained.

Added E1 (pg/ml)	0	25	125
Assayed E1 (pg/ml)	92.8	57.3	115.3
% recovery	-	97.3	106

6.4. Dilution test :

The dilution test indicates that there is immunological identity between the ESTRONE present in the sample and the ESTRONE used to calibrate the calibrator curve.

Dilution Factor	1	1/2	1/4	1/8
Assayed E1 (pg/ml)	179.1	87.2	44.4	23.4
Expected E1 (pg/ml)	-	89.6	44.8	22.4
% recovery	-	97.3	99	104.5

6.5. Reproducibility:

	Mean value (pg/ml)	Within assay variation (% CV) 10 replicates	Between assay variation (% CV) 5 Separate assays in duplicate
Pool 1	27.03	5.0	10.55
Pool 2	114.02	3.0	6.29
Pool 3	227.2	10.9	8.89

7. LIMITATION OF THE PROCEDURE

7.1. The results obtained from this or any other diagnostic kit should be used and interpreted only in the context of an overall clinical picture.

7.2. Do not use lipemic, haemolyzed, icteric or turbid specimens.

8. EXPECTED VALUES

It is recommended that each laboratory establishes its own reference values.

	Estrone (pg/ml)
Males	10 - 60
Females	
Follicular phase	50 - 100
Luteal phase	100 - 300
Menopausal	ND - 60

9. WARNING AND PRECAUTION

For IN VITRO DIAGNOSTIC use only

CAUTION : Radioactive material

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.

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

WARNING : Sodium azide

Some components contain sodium azide as preservative agent (NaN_3 < 0.1%). Dispose of the reagents by flushing with large amount of water through the plumbing system.

WARNING : Potentially infectious material

Handle all components (and all patient samples) as if capable of transmitting viral diseases such as hepatitis B and C and the acquired immunodeficiency syndrome (AIDS).

Source material derived from human body fluids or organs and used in the preparation of this kit were tested and found negative for HBsAg and anti-HCV by immunoassay. However, no known test can guarantee that such material does not contain the causative agent of viral hepatitis.

Likewise, all human materials used in the preparation of this kit were screened for the presence of antibodies against HIV-1 and -2 by enzyme-immunoassay and were found negative. However, absence of this antibody cannot guarantee the absence of the viral agent responsible for the acquired immunodeficiency syndrome.

10. BIBLIOGRAPHY

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