Estrone RIA (CT)

Radio immunoassay for the quantitative determination of Estrone in human serum or plasma.

REF MG13001

Σ 96

2-8°C

EU: IVD CE

U.S.: For research use only. Not for use in diagnostic procedures.

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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 secreted and recorded 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 maintain the bone mineral density. In premenopausal women, excessive estrone 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 diagnostic test for 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:

\[
\text{E1} \quad \text{Ab} \quad \Rightarrow \quad \text{Bound} \quad \text{Ab} \quad \text{E1}
\]

Since the concentrations of \( ^{125}\text{I} \) - E1 and coated antibodies are constant, the advancing state of the equation depends on the concentration of E1. The amount of \( ^{125}\text{I} \) - 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 \( ^{125}\text{I} \) - 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. 1 bottle concentrated, 10 ml bottle concentrated buffered solution containing sodium azide (NaNO\(_3\) < 0.1 %). Poor the solution in 700 ml of distilled water.

3.3. 70 x concentrated, 10 ml bottle concentrated buffer solution containing sodium azide (NaNO\(_3\) < 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 clotting.

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.

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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:

Pipe 100 µl of each calibrator into the corresponding tubes.

2. Unknowns and control sera:

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

3. Add 400 µl of \( ^{125}\text{I} \) - 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 decant (before to decant, 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 decant carefully.

7. Repeat step 6.

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

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5.3. Data processing:

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

Calculate the ratio B/B0 as follows:

\[ B/B0 = \left[ \frac{\text{Calibrator or Smp cpm}}{\text{B0 (Calibrator 0) cpm}} \right] \times 100 \]

Draw the calibration 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. Example of a typical assay

<table>
<thead>
<tr>
<th>Contents (pg/ml)</th>
<th>cpm 1st duplicate</th>
<th>cpm 2nd duplicate</th>
<th>Mean count rate</th>
<th>B/Bo (%)</th>
<th>Estriol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total counts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal 0</td>
<td>0</td>
<td>20372</td>
<td>19950</td>
<td>20161</td>
<td>100</td>
</tr>
<tr>
<td>Cal 1</td>
<td>12.5</td>
<td>18690</td>
<td>18710</td>
<td>18850</td>
<td>92.5</td>
</tr>
<tr>
<td>Cal 2</td>
<td>25</td>
<td>36678</td>
<td>16386</td>
<td>36500</td>
<td>81.9</td>
</tr>
<tr>
<td>Cal 3</td>
<td>5</td>
<td>14221</td>
<td>14121</td>
<td>14171</td>
<td>70.3</td>
</tr>
<tr>
<td>Cal 4</td>
<td>125</td>
<td>10453</td>
<td>10753</td>
<td>10603</td>
<td>52.6</td>
</tr>
<tr>
<td>Cal 5</td>
<td>250</td>
<td>7715</td>
<td>7520</td>
<td>7618</td>
<td>37.8</td>
</tr>
<tr>
<td>Cal 6</td>
<td>750</td>
<td>3864</td>
<td>3569</td>
<td>3612</td>
<td>17.9</td>
</tr>
<tr>
<td>C 1 low</td>
<td>34.48</td>
<td>14994</td>
<td>14179</td>
<td>14656</td>
<td>43.6</td>
</tr>
<tr>
<td>C 2 high</td>
<td>170 - 220</td>
<td>8729</td>
<td>9301</td>
<td>9015</td>
<td>44.7</td>
</tr>
<tr>
<td>Sample 1</td>
<td></td>
<td>14025</td>
<td>14061</td>
<td>69.7</td>
<td>51.2</td>
</tr>
<tr>
<td>Sample 2</td>
<td></td>
<td>19155</td>
<td>19705</td>
<td>19430</td>
<td>96.4</td>
</tr>
</tbody>
</table>

Example of a typical assay, do not use for calculations

6. PERFORMANCE CHARACTERISTICS:

6.1. Specificity

<table>
<thead>
<tr>
<th>Steroid</th>
<th>% Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone</td>
<td>100.00</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.03</td>
</tr>
<tr>
<td>Estradiol sulphate</td>
<td>0.005</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>0.0003</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>N.D</td>
</tr>
<tr>
<td>Progesterone</td>
<td>N.D</td>
</tr>
<tr>
<td>Testosterone</td>
<td>N.D</td>
</tr>
<tr>
<td>Estrone Sulfate</td>
<td>N.D</td>
</tr>
<tr>
<td>17 O H Progesterone</td>
<td>N.D</td>
</tr>
</tbody>
</table>

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 addition of ESTRONE, a satisfactory correlation between added and assayed ESTRONE is obtained.

<table>
<thead>
<tr>
<th>Added E1 (pg/ml)</th>
<th>0</th>
<th>25</th>
<th>125</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assayed E1 (pg/ml)</td>
<td>92.8</td>
<td>57.3</td>
<td>115.3</td>
</tr>
<tr>
<td>% recovery</td>
<td>-</td>
<td>97.3</td>
<td>106</td>
</tr>
</tbody>
</table>

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
  - 89.6
- **Expected E1 (pg/ml)**
  - 89.6
  - 44.8
- **% recovery**
  - 97.3
  - 99 104.5

6.5. Reproducibility:

<table>
<thead>
<tr>
<th>Mean value (pg/ml)</th>
<th>Within assay variation (% CV) 10 replicates</th>
<th>Between assay variation (% CV) 5 Separate assays in duplicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool 1</td>
<td>27.03</td>
<td>5.0</td>
</tr>
<tr>
<td>Pool 2</td>
<td>114.02</td>
<td>3.0</td>
</tr>
<tr>
<td>Pool 3</td>
<td>227.2</td>
<td>10.9</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Estrogen (pg/ml)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>10 - 60</td>
</tr>
<tr>
<td>Females</td>
<td></td>
</tr>
<tr>
<td>Follicular phase</td>
<td>50 - 100</td>
</tr>
<tr>
<td>Luteal phase</td>
<td>100 - 300</td>
</tr>
<tr>
<td>Menopausal</td>
<td>10 - 60</td>
</tr>
</tbody>
</table>

9. WARNING AND PRECAUTION

For IN VITRO DIAGNOSTIC use only

**CAUTION - Radioactive material**

This kit contains 125I (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 radionuclides.

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 (NaN₃ < 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-immunocassay 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

Symbols / Symbole / Symbôles / Símbolos / Σύµβολα

<table>
<thead>
<tr>
<th>REF</th>
<th>Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθµός-Κατ.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot-№:</td>
<td>Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθµός-Παραγωγή:</td>
</tr>
<tr>
<td>Use by:</td>
<td>Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιµοποιείται από:</td>
</tr>
<tr>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συµπύκνωµα</td>
<td></td>
</tr>
<tr>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Λυοφιλιασµένο</td>
<td></td>
</tr>
<tr>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για Ιν-βίτρο Διάγνωση.</td>
<td></td>
</tr>
<tr>
<td>Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluación. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.</td>
<td></td>
</tr>
<tr>
<td>Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell’uso. / Διαβάστε τις οδηγίες πριν την χρήση.</td>
<td></td>
</tr>
<tr>
<td>Keep away from heat or direct sunlight. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l’abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερµότητα και άµεση επαφή µε το φως του ηλίου.</td>
<td></td>
</tr>
<tr>
<td>Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:</td>
<td></td>
</tr>
<tr>
<td>Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:</td>
<td></td>
</tr>
<tr>
<td>Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!</td>
<td></td>
</tr>
</tbody>
</table>

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύµβολα των συστατικών του κιτ συµβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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LIABILITY: Complaints will be accepted in each mode –written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

Symbols Version 3.5 / 2012-01-20