Estradiol (E2) is secreted into the bloodstream where 98% of it circulates bound to sex hormone binding globulin (SHBG). To a lesser extent it is secreted by the adrenal cortex, and the male testes.

Introduction of E2 Immun assay

Immunospec Estradiol (E2) is a C18 steroid hormone with a phenolic A ring. This steroid hormone has a molecular weight of 272.4. It is the most potent natural Estrogen, produced mainly by the ovary, placenta, and in smaller amounts by the adrenal cortex, and the male testes. Estradiol (E2) is secreted into the blood stream where 98% of it circulates bound to sex hormone binding globulin (SHBG). To a lesser extent it is bound to other serum proteins such as albumin. Only a tiny fraction circulates as free hormone or in the conjugated form. Estrogenic activity is effected via estradiol-receptor complexes which trigger the appropriate response at the nuclear level in the target sites. These sites include the follicles, uterus, breast, vagina, urethra, hypothalamus, pituitary and to a lesser extent the liver and skin.

In non-pregnant women with normal menstrual cycles, estradiol secretion follows a cyclic, biphasic pattern with the highest concentration found immediately prior to ovulation. The rising estradiol concentration is understood to exert a positive feedback influence at the level of the pituitary where it influences the secretion of the gonadotropins, follicle stimulating hormone (FSH), and luteinizing hormone (LH), which are essential for follicular maturation and ovulation, respectively (8,9). Following ovulation, estradiol levels fall rapidly until the luteal cells become active resulting in a secondary gentle rise and plateau of estradiol in the luteal phase. During pregnancy, maternal serum Estradiol levels increase considerably, to well above the pre-ovulatory peak levels and high levels are sustained throughout pregnancy.

Serum Estradiol measurements are a valuable index in evaluating a variety of menstrual dysfunctions such as precocious or delayed puberty in girls(11) and primary and secondary amenorrhea and menopause. Estradiol levels have been reported to be increased in patients with feminizing syndromes,(10,11) gynae comastia (12) and testicular tumors. (13) In cases of infertility, serum Estradiol measurements are useful for monitoring induction of ovulation following treatment with, for example, clomiphene citrate, LH-releasing hormone (LH-RH), or exogenous gonadotropins. (14,15) During ovarian hyperstimulation for in vitro fertilization (IVF), serum estradiol concentrations are usually monitored daily for optimal timing of human chorionic gonadotropin (hCG) administration and oocyte collection. (16)

Immunospec Estradiol (E2) EIA kits are designed for the measurement of total Estradiol in human serum or plasma.

Principle of the test

Immunospec E2 EIA is based on the principle of competitive binding between E2 in the test specimen and E2-HRP conjugate for a constant amount of rabbit anti-Estradiol. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 25 µl E2 standards, controls, patient samples, 100 µl Estradiol-HRP Conjugate Reagent and 50 µl rabbit anti-Estradiol reagent at room temperature (10-25°C) for 90 minutes. During the incubation, a fixed amount of HRP-labeled E2 competes with the endogenous E2 in the standard, sample, or quality control serum for a fixed number of binding sites of the specific E2 antibody. Thus, the amount of E2 peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of E2 in the specimen increases. Unbound E2 peroxidase conjugate is then removed and the wells washed. A solution of chemiluminescent substrate is then added and read relative light units (RLU) with a Luminometer. The intensity of the emitting light is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled E2 in the sample. By reference to a series of E2 standards assayed in the same way, the concentration of E2 in the unknown sample is quantified.

Materials and components

Materials Provided with Test Kit
- Goat Anti-Rabbit IgG-coated microtiter wells, 96 wells
- Estradiol Reference Standards: 0, 10, 30, 100, 300, and 1000 pg/ml
- Liquid, 0.50 ml each, ready to use.
- Rabbit Anti-Estradiol Reagent, 7.0 ml
- Estradiol-HRP Conjugate Reagent, 12 ml
- 50x Wash Buffer Concentrate, 15 ml
- Chemiluminescence Reagent A, 6.0 ml
- Chemiluminescence Reagent B, 6.0 ml
- Controls, Level 1 & 2 (Optional)

Materials Required but not Provided
- Distilled water.
- Precision pipettes: 0.05ml, 0.1ml, 0.2ml
- Disposable pipette tips.
- Glass tube or flasks to mix Chemiluminescence Reagent A and B.
- Microtiter well luminometer.
- Vortex mixer or equivalent.
- Absorbent paper.
- Graph paper.

Reagent preparation
1. All reagents should be allowed to reach room temperature (18-25°C) before use, and mixed by gently inverting or swirling prior to use. Do NOT induce foaming.
2. To prepare substrate solution, make a 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

Assay procedure
1. Secure the desired number of coated wells in the holder.
2. Dispense 25 µl of standards, specimens and controls into appropriate wells.
3. Dispense 50 µl of rabbit anti-Estradiol (E2) reagent to each well.
4. Dispense 100 µl of Estradiol-HRP Conjugate Reagent into each well.
5. **Thoroughly mix for 30 seconds. It is very important to mix them completely.**
6. Incubate at room temperature (18-25°C) for 90 minutes.
7. Rinse and flick the microwells 5 times with washing buffer(1X).
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.
9. Dispense 100 µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
10. Read wells with a chemiluminescence microwell reader 5 minutes later. (between 5 and 20 min. after dispensing the substrates).

**Important Note:**
1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. If there are bubbles existing in the wells, the false readings will be created. Please use distilled water to remove the bubbles before adding the substrate.

**CALCULATION OF RESULTS**
Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.

We recommend using proper software to calculate the results. The best reference standards, control, and samples.

**EXAMPLE OF STANDARD CURVE**
Results of a typical standard run are shown below. This standard curve is for the purposes of illustration only, and should not be used to analyze unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is running at the same time.

<table>
<thead>
<tr>
<th>E2 (pg/ml)</th>
<th>Relative Light Units (RLU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15.2</td>
</tr>
<tr>
<td>10</td>
<td>12.5</td>
</tr>
<tr>
<td>30</td>
<td>9.6</td>
</tr>
<tr>
<td>100</td>
<td>6.7</td>
</tr>
<tr>
<td>300</td>
<td>3.6</td>
</tr>
<tr>
<td>1000</td>
<td>1.8</td>
</tr>
</tbody>
</table>

**EXPECTED VALUES AND SENSITIVITY**
Each laboratory should establish its own normal range based on the patient population. The Estradiol EIA was performed on randomly selected outpatient clinical laboratory samples. The results of these determinations are as follows:

**Males:**
- < 60 pg/ml
- Ovulating, early follicular: 30-100 pg/ml
- Late follicular: 100-400 pg/ml
- Luteal phase: 60-150 pg/ml
- Pregnant, normal up to: 35,000 pg/ml
- Prepubertal children, normal: < 10 pg/ml

The minimum detectable concentration of the Estradiol ELISA assay as measured by 2 SD from the mean of a zero standard is estimated to be 5 pg/ml.

**CLINICAL APPLICATION**
1. **Assessment of Female Menstrual Dysfunctions:**
   - Hyperestrogenism in girls:
     - Elevated E2 can be used in the evaluation of precocious puberty in girls. However, extensive ancillary aids are required for specific diagnoses.
   - Hyperestrogenism in women:
     - E2 measurements are frequently utilized in the assessment of hyperestrogenism in cases of delayed puberty, primary and secondary amenorrhea, and menopause. In hyperestrogenism women, E2 concentrations are usually <30 pg/ml.
2. **Assessment of Excessive Estrogen Production in Women:**
   - Elevated E2 can be used in the evaluation of precocious puberty in girls.
   - Elevated E2 can be used in the evaluation of precocious puberty in girls.

In pregnant women, E2 concentrations will >1,000 pg/ml. In non-pregnant women, excessive estrogen may indicate ovarian neoplasms.

3. **Monitoring Ovulation:**
E2 is often measured to monitor ovulation induction and for patient follow-up during infertility therapy, e.g. in vitro fertilization (IVF).

4. **Estradiol Measurement in Male:**
E2 measurement is used in the differential diagnosis gynecomastia, feminizing syndromes, hypogonadism and testicular tumors.

**REFERENCES**
19.

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