ESTRADIOL (E2)

INTRODUCTION OF E2 IMMUNOASSAY

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.[1, 2, 3] 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 levelsincrease considerably, to well above the pre-ovulatory peak levels and high levels are sustained throughout pregnancy.[10]

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.[12] Estradiol levels have been reported to be increased in patients with feminizing syndromes,[14] gynaecomastia[15] and testicular tumors.[16] 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.[17,18]

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.[19].

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

PRINCIPLE OF THE TEST

The 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 25µL E2 standards, controls,patient samples, 100 µL Estradiol-HRP Conjugate Reagent and 50µL rabbit anti-Estradiol reagent at room temperature (18-25°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. Next, a solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled E2 in the sample. A standard curve is obtained by plotting the concentration of the standard versus the absorbance.

The E2 concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve

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
- Wash Buffer Concentrate (50X),15 mL
- TMB Substrate , 12 mL.
- Stop Solution , 12 mL.

Materials required but not provided

- Precision pipettes: 20~50 µL, 0.5~ 200 µL and 1.0 mL.
- Disposable pipette tips.
- Distilled and deionized water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Linear-linear graph paper.
- Microtiter plate reader.

SPECIME COLLECTION AND PREPARATION

1. Serum or EDTA plasma should be used. No special Pretreatment of sample is necessary.
2. Serum or plasma samples may be stored at 2-8°C for up to 24 hours,and should be frozen at −10°C or lower for longer periods. Do not use grossly hemolyzed or grossly lipemic specimens.
3. Please note: Samples containing sodium azide should not be used in the assay.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 O.D. at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

- All reagents should be brought to room temperature (18-22°C)before use.
- Samples with expected Estradiol concentrations over 1000 pg/mL may be quantitated by dilution with diluent available From the company.
- Dilute 1 volume of Wash buffer concentrate (50x) with 49 volumes of distilled water. For example, Dilute 15mL of Wash buffer concentrate(50x) into 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 25µL of standards, specimens and controls into appropriate wells.
3. Dispense 50 µL of rabbit anti-Estradiol(E2) reagent to each well.
4. Dispense 100µL of Estradiol-HRP Conjugate Reagent into each w
5. Thoroughly mix for 30 seconds. It is very important to mix them completely.
6. Incubate at room temperature (18-22°C) for 90 minutes.
7. Rinse and flick the microwells 5 times with washing buffer(1X).
8. Dispense 100 µL of TMB Substrate into each well. Gently mix for 10 seconds.
9. Incubate at room temperature (18-22°C) for 20 minutes.
10. Stop the reaction by adding 100 µL of Stop Solution to each Well.
11. Gently mix 30 seconds. It is important to make sure that all the blue Color changes to yellow color completely.
12. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

IMPORTANT NOTE

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

CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A450) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in pg/ml on a linear-linear graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of Estradiol in pg/ml from the Standard curve.
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against Estradiol concentrations shown in the X axis. Note: This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

E2 (pg/mL)	Absorbance (450 nm)
0	2.943
10	2.551
30	2.055
100	1.624
300	0.925
1000	0.571

PRESENTACIÓN:

CONT. 96 TEST CODIGO: RSET010

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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
Females: postmenopausal phase	< 18 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.
   - Hypoestrogenism in women: E2 measurements are frequently utilized in the assessment of hypoestrogenism in cases of delayed puberty, primary and secondary amenorrhea, and menopause. In hypoestrogenism women, E2 concentrations are usually <30 pg/mL.
2. Assessment of Excessive Estrogen Production in Women:In pregnant women, E2 concentrations will >1,000 pg/mL. In Non- pegnant 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.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
3. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
4. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot gurantee it will eliminate all the effects of that.

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