Diagnostics Biochem Canada

Total Estrogens ELISA

REF CAN-E-630

ASSAY PROCEDURE

Bring kit components to room temperature. Mix gently by inversion. Prepare working wash buffer.

Pipette 50 µL of each calibrator, control and specimen sample.

Incubate on a microplate shaker for 30 minutes at room temperature.

Pipette 150 µL of Estrogen-HRP conjugate.

Incubate on a microplate shaker for 120 minutes at room temperature.

Wash 3 times.

Pipette 150 µL of TMB substrate.

Incubate on a microplate shaker for 30 minutes at room temperature.

Pipette 50 µL of stopping solution. Tap gently on microplate frame to mix.

Read the plate on a microplate reader at 450 nm.

Total estrogens comprise the total quantity of estrone, estradiol, and estriol. The estrogens are involved in the development of female sex organs and secondary sex characteristics. Before the ovum is fertilized, the main action of the estrogens is on the growth and function of the reproductive tract to prepare it for the fertilized ovum.

During the follicular phase of the menstrual cycle, the total estrogens level shows a slight increase. The production of total estrogens then increases markedly to peak at around day 13. The peak is of short duration and by day 16 of the cycle levels will be low. A second peak occurs at around day 21 of the cycle. If fertilization does not occur, the production of total estrogens decreases.

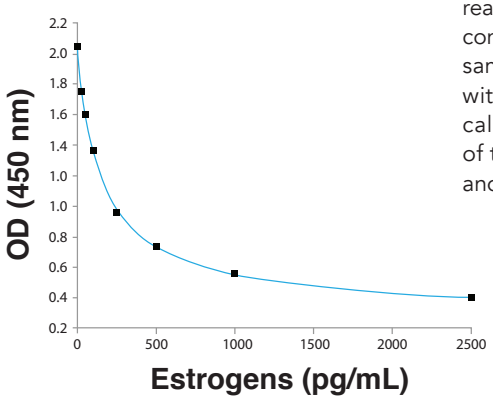
In post-menopausal women, the concentration of all estrogens decreases substantially and estrone becomes the predominant estrogen. In pregnant women, the concentration of all estrogens escalates and estriol becomes the predominant estrogen.

A total estrogens test is commonly indicated to:

- *Aid in diagnosis of sex steroid metabolism related conditions, for example, **premature or delayed puberty**, and **aromatase** and **17 alpha-hydroxylase deficiencies**.*
- *Follow-up female hormone replacement therapy in post-menopausal women.*
- *Prognose antiestrogen therapy, for example, aromatase inhibitor therapy.*

PRINCIPLE OF THE TEST

The total estrogens ELISA is a competitive immunoassay. Competition occurs between total estrogens (estrone, estradiol, and estriol) present in calibrators, controls and patient samples and an enzyme-labelled antigen (conjugate) for a limited number of anti-estrogen antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the enzyme substrate is added, and approximately 30 minutes later the enzymatic reaction is terminated by addition of stopping solution. The resulting optical density (OD), measured with a microplate reader, is inversely proportional to the concentration of total estrogens in the sample. A calibrator curve is plotted with a provided set of calibrators to calculate directly the concentration of total estrogens in patient samples and controls.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit was calculated following EP17-A2. Sixty replicates of the matrix and a low concentration sample were run in independent tests with two lots of the kit. The Limit of Background was determined to be 5.4 pg/mL and the Limit of Detection was determined to be **12.4 pg/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The cross-reactivity was evaluated in relation to estrogens reacting at 100%.

Compound	% Cross-Reactivity	Compound	% Cross-Reactivity
Estrone	100	DHEA	0.3
17β-Estradiol	100	DHEAS	0.004
Estriol	100	DHT	0.5
11-Deoxycorticosterone	0.4	Equilin	6.3
17-Hydroxyprogesterone	0.3	Estradiol sulfate	0.1
17α-Estradiol	5.3	Estrone sulfate	0.07
Aldosterone	0.2	Prednisone	0
Androstenedione	0.2	Pregnenolone	< 0.1
Androsterone	0.2	Pregnenolone sulfate	< 0.1
Cholesterol	0	Progesterone	< 0.1
Corticosterone	< 0.01	Testosterone	0.3
Cortisol	< 0.1		

INTERFERENCES

Hemoglobin up to 2 g/L, Bilirubin conjugated and unconjugated up to 10 mg/dL, Triglycerides up to 5 mg/mL, Biotin up to 2.4 µg/mL, HAMAS up to 1.2 µg/mL, and Rheumatoid Factor up to 1500 IU/mL did not interfere with the assay.

Note on Fulvestran

Estradiol immunoassays have been reported to show interference from the drug Fulvestran (Faslodex®). This cross-reactivity can cause falsely elevated estrogen levels in patients under Fulvestrant treatment.

The following results were obtained with theTotal Estrogens ELISA kit after pooled serum samples from three cohorts were spiked to a concentration of 25 ng/mL of Fulvestran.

Sample	Unspiked Sample (pg/mL)	Sample Spiked to 25 ng/mL Fulvestran (pg/mL)
Pool 1	106.8	128.6
Pool 2	87.8	105.8
Pool 3	326.4	377.6

The Cmax has been reported as 11.4 ng/mL (Robertson and Harrison, 2004) and 25.1 ng/mL (AstraZeneca Canada, 2017).

References

1. Robertson JFR and Harrison M. Fulvestran Pharmacokinetics and pharmacology. *British Journal of Cancer*. 2004; 90:S7–S10.
2. Faslodex® Product Monograph. AstraZeneca Canada, 2017.

PRECISION

The experimental protocol used a nested components-of-variance design with 10 testing days, two runs per scientist per day, and two replicate measurements per run (a 10 x 2 x 2 x 2 design) for each sample. The results were analyzed with a two-way nested ANOVA and summarized in the table below:

Sample	Mean	Within Run SD	Within Run CV%	Between Run SD	Between Run CV%	Total SD	Total CV%
1	104.6	6.6	6.3	8.3	8.0	11.9	11.4
2	56.5	5.3	9.3	7.0	12.4	8.8	15.5
3	377.2	17.6	4.7	10.8	2.9	24.4	6.5
4	83.3	4.7	5.7	4.2	5.0	7.1	8.5
5	100.2	6.0	6.0	7.5	7.4	9.9	9.9
6	251.8	10.3	4.1	13.3	5.3	17.0	6.8
7	365.9	16.8	4.6	52.2	14.3	54.8	15.0
8	1276.7	78.9	6.2	46.8	3.7	98.0	7.7

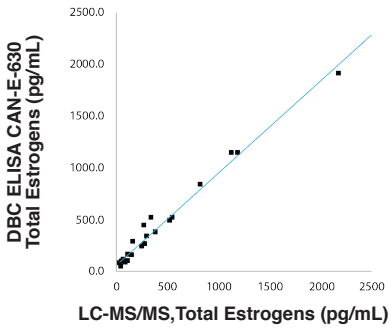
LINEARITY

The linearity study was performed with four human serum samples covering the range of the assay and following CLSI guideline EP06-A. The samples were diluted in calibrator A at several equidistant concentration levels and up to ten percent (1:10), tested in duplicate, and the results compared to the predicted concentration. The statistical analysis shows that the assay is sufficiently linear up to a 1:10 dilution when using calibrator A as the diluent.

COMPARATIVE STUDIES

The DBC Total Estrogens ELISA kit (y) was compared to Liquid Chromatography-Tandem Mass Spectrometry (x) Estrogens method. The comparison of 27 serum samples yielded the following linear regression results:

$y = 0.89x + 62$, $r = 0.99$



REFERENCE RANGES

Reference ranges (95%) were established using samples obtained from individuals of diverse races. Each laboratory shall establish their own range of reference values.

Group	N	Median (pg/mL)	95% Reference Range (pg/mL)
Pre-menopausal Females, cycle			
1–10 days	40	120	16–328
11–20 days	40	136	34–501
21–30 days	40	168	48–350
Post-menopausal Females	120	74	40–244
Adult Males	120	104	56–213

Ordering Information: REF CAN-E-630

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