

Summary and background of the test:

Estrone, estradiol and estriol, which are produced in ovaries, placenta, testes, adrenals, liver and adipose tissue, are the main estrogenic steroids of clinical interest.

During the menstrual cycle, estrone fluctuations are similar to those of estradiol. About 60% of daily estrogen production consists of estradiol, which arises from ovarian secretion, while 40%, in the form of estrone, results mainly from the conversion of androstenedione secreted by both adrenals and ovaries. Factors influencing the conversion of androstenedione to estrone are weight, age, liver function, heart failure and thyroid dysfunction.

After menopause, as a result of the cessation of cyclic ovarian function, estradiol only originates from the adrenals and the peripheral conversion of estrone, and is therefore present in the plasma at very low concentrations. The major estrogen in the blood circulation is estrone, at levels that are, however, insufficient to prevent estrogen deprivation from target organs such as hypothalamus, pituitary, uterus, vagina and breasts.

Disorders of the ovary and female reproductive tract may result in hyperestronemia in women with polycystic ovary syndrome, or with ovarian tumors.

Principle of the test :

Radioimmunoassay is based on the ability of a limited quantity of antibody to bind a fixed amount of radiolabelled antigen (¹²⁵I-Ag). The percentage of bound radiolabelled antigen is inversely related to the concentration of unlabelled analyte in the sample. Separation of the bound and free radiolabelled antigen is necessary in order to determine the quantity of unlabelled antigen. The Bio-Line Estrone kit utilizes the coated tubes methodology. The quantity of unlabelled antigen in an unknown sample is then determined by comparing the remaining radioactivity in the coated tubes with data established using known standards in the same assay system.

Precautions:

1. Radioactive material: Radioactive material may be received, acquired, possessed and used only by physicians, clinical laboratories, or hospitals for "In-Vitro" clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals.

Compliance with these basic rules of radiation safety should provide adequate protection:

1. Do not eat, drink, smoke, or apply cosmetics in areas where radioactive material is used.
2. Do not pipet by mouth reagents containing radioactive materials.
3. Wear protective clothing; i.e., lab coats and disposable gloves, in order to avoid direct contact with radioactive reagents.
4. Work with radioactive materials should be performed in a designed area.
5. Radioactive materials should be stored in an acceptable location.
6. A log should be kept for receipt and disposal of radioactive materials.
7. Radioactive spills or accidents should be taken care of immediately according to established procedures.
8. Disposal of radioactive materials must comply with prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory.

2. Sodium azide: Sodium Azide, used as a bacteriostatic agent, is toxic in acid medium. In addition, it may form potentially explosive lead or copper azides. To avoid dangerous deposits, waste solutions should be flushed away with large volumes of water.

3. Hepatitis and Acquired Immune Deficiency Syndrome (HTLV-III): All Bio-Line reagents included in this kit have been tested and found to be non reactive for hepatitis B surface antigen. They have also been screened and determined to be non-reactive for HTLV-III antibody. However, human serum products should be handled as if potentially capable of transmitting hepatitis, Acquired Immune Deficiency Syndrome, or other infectious agents.

Materials provided for 100 tests:

1. **Estrone standards & control:** 7 vials containing each 1.0 ml.
Standards range: 0-1500 pg/ml. Refer to vial labels for accurate standards & control concentrations.
2. **¹²⁵I-Estrone tracer:** 1 vial (violet solution) containing 51 mL. Activity < 4μCi or 148 kBq.
3. **Wash solution concentrate:** 1 vial of 2 ml of concentrate, to be diluted into 250 ml NaCl 9 % and stored at 4°C.
4. **Coated tubes:** 2 x 50 tubes, coated with anti-Anti-Estrone antiserum (Rabbit).

Reagents provided should be stored at 2° - 8° C.
Refer to the expiration date on the kit label for stability.

Materials required but not provided:

1. Pipets, micropipets, repeating syringes and repeating pipettors.
2. Gamma counter.
3. Logit log graph paper.
4. Horizontal shaker recommended (type IKA-VIBRAX-VXR), but a rotator could be used.
5. Test tube racks.
6. Vortex mixer.
7. 9 % NaCl saline solution.

Specimen collection and preparation:

Sera should be separated from blood cells immediately after collection. Sera are stable for at least 7 days at 4° C and for longer periods of time when stored frozen.

Assay procedure:

Bring reagents to room temperature and mix before use. Label tubes for total counts (Tc), standards, control sera and unknowns.

1. Pipet 100 µl of standards, samples and controls into the corresponding tubes.
2. Add 500 µl of Estrone Tracer solution (violet) to each tube.
3. **Mix well**, cover and incubate 120 minutes at room temperature, with horizontal shaking at 250 rpm.
4. Aspirate (or decant). Add 1 ml of wash solution to each tube, except Tc. Aspirate or decant.
5. Record the counts per minute (cpm) for each tube. Count all tubes for one minute.

Direct Estrone Coated Tubes Flow chart:

Tubes Reagents	Tc	B0	Stds. & Control	samples
Standards or samples (µl)	-	100	100	100
Tracer (µl)	500	500	500	500
Mix well and incubate 120 min. at RT on a horizontal shaker (250 rpm). Aspirate or decant.				
Wash solution	-	1 ml.		
Aspirate or decant. Count 1 min.				

Data table (example):

Tube	Duplicate cpm		Mean cpm	%B/B ₀
Tc	38 906	38 481	38 694	-
Zero	25 284	26 654	25 969	100 %
Std 25	23 178	23 778	23 478	90.4 %
Std 80	19 365	19 522	19 444	74.9 %
Std 340	11 560	11 483	11 522	44.4 %
Std 700	7 544	7 648	7 596	29.3 %
Std 1000	5 594	5 828	5 711	22.0 %
control	19 262	19 188	19 225	82.1 pg/mL

Calculation of results:

Determine the average counts for each set of duplicate tubes. Divide this value by the average net counts of the B₀, and multiply by 100 to yield the % B/B₀

$$\% B/B_0 = \frac{\text{cpm (Stds, Controls or unknowns)}}{\text{cpm (B}_0\text{)}} \times 100$$

Plot % B/B₀ for each standard vs its concentration in pg/ml on semi-log graph paper. The concentration of Estrone in the unknown samples may be read directly from the standard curve.

Expected Values:

	Range (pg/ml)
Male	20 - 80
Female	
-Foll. phase	10 - 75
-Mid cycle	70 - 185
-Lut. phase	40 - 120
-Postmenop.	< 80

Each laboratory should analyze normal samples to establish its own normal ranges.

Specific performance characteristics:

1. Specificity: The relative percent of cross-reactivity by weight of Estrone and various related compounds was evaluated for the antibody used in this assay. Cross-reactivities are expressed as the amount of Estrone required to reduce the binding of ¹²⁵I-Estrone by 50%, relative to the amount of a related compound required to do the same.

$$\text{Cross-reactivity of } x = 100 \times \frac{\text{conc. Estrone at 50\% B/B}_0}{\text{conc. compound } x}$$

Compound x	Cross-reactivity (%)
Estrone	100 %
17- α -Estradiol	0.2 %
17- β -Estradiol	< 0.08 %
Estriol	< 0.08 %
Equiline	5 %

2. Sensitivity: The lowest detectable concentration of Estrone that can be reliably distinguished from zero with this kit has been evaluated to be less than 12.5 pg/ml.

3. Precision and reproducibility: Within and between assay variations of two serum samples are mentioned in the following table:

	Sample 1	Sample 2
Mean	76 pg/ml	267 pg/ml
Within assay variation	6.8 %	7.2 %
Between assay variation	8.9 %	10.9 %

4. Linearity: The results obtained when diluting a serum with elevated Estrone concentration with an Estrone-free serum are summarized in the following table:

Dilution factor	Expected values	Experimental values
1:1	130 pg/ml	-
1:2	65 pg/ml	64 pg/ml
1:4	32 pg/ml	30 pg/ml
1:8	16 pg/ml	15 pg/ml

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