

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 increasing 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 second antibody methodology.

The quantity of unlabelled antigen in an unknown sample is then determined by comparing the radioactivity of the isolated precipitate with data established using known standards in the same assay system.

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 each containing 0.5 ml, except Zero 1 ml.
Standards range: 0-1500 pg/ml. Refer to vial labels for accurate standards & control concentrations.
2. **¹²⁵I-Estrone tracer** : 1 vial (red solution) containing 21.0 ml. Activity < 4 μ Ci or 148 kBq.
3. **Anti-Estrone antiserum (Rabbit)** : 1 vial (blue solution) containing 21.0 ml.
4. **Second antibody (Sheep a-Rabbit)** : 1 vial (yellow suspension) containing 51 ml.
5. **NSB reagent** : 1 vial containing 2.0 ml.

Materials required but not provided:

1. Pipets, micropipets, repeating syringes and repeating pipettors.
2. Gamma counter.
3. Logit log graph paper.
4. 37°C water bath incubator.
5. Test tube racks and Glass tubes.
6. Vortex mixer.
7. 9‰ NaCl saline solution.

Assay procedure:

Bring reagents to room temperature and mix before use. Label Glass disposable tubes for total counts (Tc), non specific binding (NSB), standards, control sera and unknowns.

1. Pipet 50 µl of standards, samples and controls into the corresponding tubes.
2. Add 200 µl of tracer solution (red) to each tube. Add 200 µl of NSB reagent to NSB tubes.
3. Add 200 µl of anti-Estrone antiserum solution (blue) to each tube, except Tc and NSB.
4. Mix well and incubate 60 minutes at 37°C.
5. Add 500 µl of second antibody (yellow suspension) to each tube, except Tc. Maintain moderate magnetic stirring of the suspension during the transfer.
6. Mix and incubate for 15 minutes at room temperature.
7. Centrifuge all tubes , except Tc, for 15 minutes at 1300-1500g at 4°C.
8. Decant all tubes once, discarding the supernatant into a radioactive waste container. While tubes are inverted, gently blot the final drop onto absorbent paper. Do not aspirate. Do not let stand inverted.
9. Record the counts per minute (cpm) for each tube. Count all tubes for one minute.

Reagents provided should be stored at 2° - 8° C.

Refer to the expiration date on the kit label for stability.

Specimen collection and preparation:

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

Direct Estrone Liquid Phase Flow chart:

Reagents	Tubes	Tc	NSB	B0	Stds. & Control	samples
Standards or samples (µl)		-	50	50	50	50
NSB buffer		-	200	-	-	-
Tracer (µl)		200	200	200	200	200
1st Ab (µl)		-	-	200	200	200
Mix well and incubate 60 min. at 37°C						
2 nd Ab		-		500		500
Incubate 15 minutes at RT. Centrifuge. Aspirate or decant. Count 1 min.						

Data table (example):

Tube	Duplicate cpm		Mean cpm	%B/B ₀
Tc	40 839	40 042	40 441	-
NSB	1 562	1 651	1 607	4.0 %
Zero	17 978	17 422	17 700	100 %
Std 25	15 118	14 878	14 998	83.2 %
Std 80	12 674	12 919	12 797	69.5 %
Std 340	8 433	8 401	8 417	42.3 %
Std 700	6 516	6 557	6 537	30.6 %
Std 1000	5 219	5 330	5 275	22.8 %
control	12 852	13 019	12 935	75.2 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

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

Plot % B/B₀ for each standard vs its concentration in ng/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	0 - 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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