



1. NAME AND INTENDED USE

ESTR-US-CT is a highly sensitive radioimmunoassay coated tube kit for the direct quantitative determination of total Estradiol-17 β in human serum or plasma.

2. INTRODUCTION

2.1 Physiological background

A steroid hormone, estradiol-17B is the most active oestrogen in the peripheral circulation. It is mainly produced in the ovary by the Graafian follicle.

A small amount is also produced by the adrenal cortex and by different tissues via the peripheral transformation of estrone and testosterone. In non-pregnant women, the estradiol-17B concentration varies with the menstrual cycle, the highest values generally occurring one day before ovulation.

Its retro-control influence is vital in triggering the LH peak mid-cycle and subsequently ovulation. In the peripheral circulation, estradiol-17B is mainly present bound to proteins, the two most important being SHBG and albumin. The free form is physiologically active.

2.2 Clinical indications

The use of a highly sensitive estradiol-17B assay is necessary for young girls, peri- and post-menopausal women, and men. It is useful for testing young girls when precocious puberty is suspected. In post-menopausal women, concentrations of estradiol-17B are very low, similar to those found in men.

This low post-menopausal concentration increases the risk of osteoporosis and atherosclerosis, indicating the possible need for a hormone replacement therapy (HRT). In men, assays are useful in the investigation of feminizing syndromes (gynecomastia, oestrogen-secreting tumors). Serum assays are a helpful tool for establishing the etiology of an amenorrhoea and/or sterility, and in investigating oestrogen-secreting tumors in women.

Finally, it is useful in monitoring ovulation induction.

3. PRINCIPLE

The principle of the assay is based on a two step technique, using a solid phase.

In the first step, standards, controls and samples are incubated in tubes coated with a high affinity anti-Estradiol polyclonal antibody. After incubation, an amount of hormone proportional to the concentration of the Estradiol fraction present in the sample is bound to the bottom of the tubes.

In the second step, a ¹²⁵I-Estradiol solution is added in the tube to saturate the remaining free antibody sites. Any unbound tracer is then removed.

The quantity of labelled Estradiol bound to the antibody is inversely related to the amount of unlabelled Estradiol present in the sample.

4. REAGENTS

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

REAGENTS	QUANTITY	STORAGE
COATED TUBES: ready to use. Rabbit anti-Estradiol antibodies coated on the bottom of the tube.	100 tubes	2-8°C until the expiry date. Unused coated tubes removed from their bags should be stored in the original bag.
¹²⁵I-ESTRADIOL: concentrated solution. 125I labelled ESTRADIOL, buffer, dye, preservative and sodium azide. ≤ 85 kBq (≤ 2.3 μ Ci).	1 6 ml vial	2-8°C until the expiry date. 2-8°C up to 8 weeks after dilution. Return the tracer to 2-8°C immediately after use.
STANDARDS 0 to 5: ready to use. Estradiol, human serum, preservative and sodium azide. 0 - 10 - 25 - 100 - 500 - 2000 pmol/l (*)	6 2 ml vials	2-8°C until the expiry date.
DILUENT for tracer and incubation buffer: ready to use. Buffer and preservative.	1 45 ml vial	2-8°C until the expiry date.
WASHING SOLUTION: concentrated solution Distilled water, detergent and preservative.	1 20 ml vial	2-8°C until the expiry date 2-8°C stability up to 8 weeks after dilution.

(*) Standards are prepared gravimetrically.

5. PRECAUTIONS FOR USE

5.1. Safety measures

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However, as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

Do not pipette by mouth.

Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.

Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.

Avoid splashing.

Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.

The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.

The enforcement of the basic rules for handling radioactive products ensures adequate security.

A summary of these is given below :

Radioactive products must be stored in their original containers in a suitable area.

A record of the reception and storage of radioactive products must be kept up to date.

Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).

Do not eat, drink, smoke or apply cosmetics in a controlled zone.

Do not mouth-pipette radioactive solutions.

Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.

Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.

Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.

All radioactive waste disposal must be carried out according to the regulations in force.

5.3. Handling precautions

Do not use kit components beyond their expiry date.

Do not mix reagents from different batches.

Avoid any microbial contamination of the reagents or of the water used for washing.

Fully respect the incubation times and the washing instructions.

6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed on human sera or plasma (heparin or EDTA). Do not use citrate plasma samples. Serum bilirubin concentrations $\leq 170 \mu\text{mol/l}$ or haemoglobin concentrations $\leq 10000 \text{ mg/l}$ have no effect on the estradiol concentrations measured. Hyperlipemic samples should not be used. If the test is to be carried out within two days, the samples must be refrigerated at 2-8°C. Otherwise, they should be divided into aliquots, and stored deep frozen (-20°C) until needed (maximum 1 month). They must be thawed only just before using. Do not refreeze samples for later use.

Dilution

If elevated Estradiol levels are suspected, the standard "0" should be used for dilution. It is recommended that disposable plastic tubes be used when carrying out the dilutions.

7. ASSAY PROCEDURE

7.1. Material required

Precision micropipettes or similar, with disposable tips, capable of dispensing 100 μl , 200 μl , 250 μl and 2 ml. Their calibrations should be checked regularly.

Reagent dispenser 1 ml (for washing)

Distilled water.

Standard 0 for dilution (ESTR-US-STD0)

Vortex type mixer.

Absorbent paper.

Water bath (37°C).

Parafilm (optional).

Disposable plastic test-tubes.

Gamma scintillation counter calibrated for 125 Iodine.

7.2. Tracer dilution

Dilute the tracer 2.5 fold with the diluent (e.g. 1 ml tracer + 1.5 ml diluent). Recap the vial. Mix gently to ensure complete dilution.

It is recommended that only the required volume of tracer be diluted at any time.

It must be refrigerated immediately after use.

7.3. Dilution of the washing solution

Dilute the washing solution 1:6 with distilled water (e.g. 20 ml of concentrated solution with 100 ml of distilled water, to make a total volume of 120 ml). Recap the vial. Mix gently to ensure complete dilution.

7.4. Protocol

All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use. Dispensing of reagents is also carried out at room temperature.

The assay requires the following groups of tubes:

T group, for the total activity determination.

Standard groups, to establish the standard curve.

Reference group for the external controls.

Sx groups, for the test samples.



It is recommended to perform the assay in duplicate for the standard groups, controls and samples. Strictly respect the order in which reagents should be added.

Dispense 200 µl of standards, controls and samples to be assayed into the correspondingly-labeled coated tubes.

Add 250 µl of diluent to each tube (except T group).

Mix each tube gently with a Vortex-type mixer.

Incubate 1 hour at 37°C after covering the tubes with plastic film.

Add 100 µl of ¹²⁵I-Estradiol to each tube (and T group). Return the remaining tracer to the refrigerator.

Mix each tube gently with a Vortex-type mixer.

Cover the tubes with plastic film and **Incubate** 1 hour at 37°C.

Decant liquid from each assay tube and **tap** the head of each tube firmly onto absorbent paper (except T tubes).

Wash once with 1 ml of washing solution, shaking the rack by hand.

Empty the tubes and tap firmly onto absorbent paper. Let the tubes stand upside down at least 5 min. (except T tubes).

Measure (for at least 2 minutes) the remaining radioactivity bound to the tubes with a gamma scintillation counter calibrated for 125 Iodine.

8. QUALITY CONTROL

Good laboratory practices require the use of quality control samples in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. RESULTS

For each group of tubes, compute the mean counts. Calculate B/Bo values. Draw up the standard curve by plotting the B/Bo of the standards against their concentrations. Read sample values directly from the standard curve, and correct the read value for the dilution factor, if necessary.

This data must not be substituted for results obtained in the laboratory.

Conversion to pg/ml may be accomplished by using the following equation: Estradiol (pg/ml) = Estradiol (pmol/l) x 0.2724

Typical standard curve (example of results): this data must not be substituted for results obtained in the laboratory.

GROUPS OF TUBES		Mean CPM	B/Bo x 100	Concentration pmol/l
T		1337	-	
Standard 0	0 pmol/l	4730	100	
Standard 1	10 pmol/l	4230	89,4	
Standard 2	25 pmol/l	3741	79,1	
Standard 3	100 pmol/l	2624	55,5	
Standard 4	500 pmol/l	875	18,5	
Standard 5	2000 pmol/l	349	7,6	
Sample A		2548	53,9	107
Sample B		575	12,2	844

10. PROCEDURAL LIMITATIONS

Strict following of the procedures described in this package insert and careful handling of the reagents will enable reliable results to be obtained with the ESTR-US-CT kit.

Do not attempt to extrapolate sample values beyond the last standard. Dilute the samples and re-assay.

11. EXPECTED VALUES

Each laboratory must establish its own range of normal values.

Estradiol values were followed during one menstrual cycle in 25 healthy, normally menstruating women. The ovulation days were established by measuring the LH value of each sample. Day 0 is the day of the LH peak. Results are shown in table below:

Phases	Days from the LH peak	n	Mean (pmol/l)	Range (pmol/l)
Follicular phase	-8	10	150	105-217
	-4	15	343	207-1000
Mid-cycle	-1	24	709	416-1399
Luteal phase	9	17	399	165-788

Serum Estradiol values were also measured in samples from 42 post-menopausal women without hormone replacement therapy, 42 pre-pubertal girls and 117 men. Results are shown in the tables below :

Group	Age	n	Mean (pmol/l)	Range (pmol/l)
Women				
Postmenopausal women	47-66 years	42	26	11-50
Pre-pubertal girls	3-5 years	42	17	ND-36

Men	
n =	117
Mean =	117 pmol/l
Reference interval*	34-226 pmol/l
Confidence intervals	0.90
Lower reference limit	23 – 51 pmol/l
Upper reference limit	191 – 266 pmol/l

ND = non detectable

* Reference interval = 0.025 and 0.975 fractiles.

12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Precision

This was evaluated using 8 samples with different concentrations assayed 10 times in the same series or in duplicate in 10 different series.

Samples	Within-run		Samples	Between-run	
	Mean value (pmol/l)	CV (%)		Mean value (pmol/l)	CV (%)
1	17	18.1	5	12	17.6
2	87	2.8	6	94	5.8
3	311	3.5	7	260	8.1
4	1021	5	8	1799	9.7

12.2. Recovery test

Known quantities of Estradiol were added to different serum pools. The recovery percentages of Estradiol obtained were in the range from 88% to 117%, with a mean value of 102%.

12.3. Specificity

This was determined from equivalent displacement measurements at 50% binding. The antiserum used in the test showed the following cross-reactions:

Compound	Cross reactivity (%)	Compound	Cross reactivity (%)
Estradiol	100	Estrone-glucuronide, sodium salt	0.004
Equilenin	8.9	Norethisterone acetate	0.002
Ethinylestradiol	1.4	Mesterolone	0.002
Equilin	1.1	Norgestrel	0.001
Estrone	0.97	Estradiol-3, 17-disulphate, disodium salt	<0.001
16-Oxoestradiol	0.86	Estradiol-17-sulphate, sodium salt	<0.001
Estradiol-3-glucuronide, sodium salt	0.61	Testosterone	< 0.001
Estriol	0.44	Cortisol	< 0.001
16-hydroxyestrone	0.26	Cortisone	< 0.001
Estradiol-17-glucuronide, sodium salt	0.25	Ethisterone	< 0.001
Estradiol-17-valerate	0.16	Danazol	< 0.001
Progesterone	< 0.05	3,17-Beta-D-glucuroconjugate	0.007
Corticosterone	0.02	Androstenediol	< 0.0003
Estradiol-3-sulfate	0.02	Oestrone-3-Beta-D-glucuronide	0.0003
2-hydroxyestradiol	0.01	Oestrone-3-sulfate	<0.0003
Norethisterone	0.01	17 α -Estradiol	0.005

12.4. Detection limit

The detection limit is defined as being the smallest concentration different from the zero with a probability of 95%. It has been assessed as being 5 pmol/l.

ASSAY FLOW-CHART

Groups of tubes	Standards, Controls Samples μ l	Diluent μ l		125I-Estradiol μ l		Washing solution μ l	
T	---			100		---	
Standards	200	250	Mix ---	100	Mix ---	1000	Empty the tubes ---
Controls and samples	200	250	Incubate 1h at 37°C	100	Incubate 1h at 37°C ---	1000	Measure for at least 2 minutes

12.5 Measurement range

10-2000 pmol/l.