KAISER PERMANENTE®
COLORADO LABORATORY

ESTRADIOL TEST FOR BECKMAN / COULTER ACCESS II

PRINCIPLE

The Access Estradiol assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel with paramagnetic particles coated with goat anti-rabbit: rabbit anti-estradiol and a TRIS-buffered protein solution. After 20 minutes, estradiol alkaline phosphatase conjugate is added. Estradiol in the sample competes with the estradiol-alkaline phosphatase conjugate for binding sites on a limited amount of specific anti-estradiol antibody. Resulting antigen: antibody complexes are bound to the capture antibody on the solid-phase. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of estradiol in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Estradiol (17β - estradiol, 1,3,5 (10) - Estratrien - 3,17β - diol) is a natural estrogen with a molecular mass of 272.3 daltons. Most circulating estradiol is bound to protein. It is estimated that only 1–3% of estradiol is free (unbound). In non-pregnant women, estradiol is secreted by the ovary and the corpus luteum. The adrenals and testes (in men) are also believed to secrete minute amounts of estradiol.(1) Estradiol levels are lowest at menses and into the early follicular phase and rise in the late follicular phase to a peak just prior to the hLH (human Luteinizing Hormone) surge, initiating ovulation. As the hLH peaks, the levels of estradiol decrease before rising again in the luteal phase. Endometrial growth is stimulated by estradiol and progesterone (secreted by the corpus luteum) in preparation for implantation of a fertilized egg. If conception does not occur, the secretion of estradiol and progesterone by the corpus luteum decreases, initiating menses.(2)

Levels of estradiol are used to monitor ovulatory status. Because estradiol levels reflect follicular maturation, the measurement of estradiol as cited in the scientific literature has been used as a tool in the assessment of sexual development, etiology of amenorrhea, causes of infertility and menopause.(3,4) Abnormally high levels in males are indicative of feminizing syndromes such as gynecomastia.(5)

SCOPE

All Medical Technologists and Medical Laboratory Technicians working at the Franklin Laboratory.

SPECIMEN REQUIREMENTS

1. Serum and plasma (heparin) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
3. If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
4. If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.

5. Thaw samples only once.
6. Use the following guidelines when preparing specimens, unless instructed otherwise in the product insert:
   • Ensure residual fibrin and cellular matter has been removed prior to analysis.
   • Follow blood collection tube manufacturer's recommendations for centrifugation.

Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.

7. Frozen specimens can be stored up to six months before testing

- **ORDER CODES**

Cerner Code	Cerner Description	Health Connect Code	Health Connect Description
EST	ESTRADIOL	82670B	Estradiol

EQUIPMENT AND MATERIALS

1. Beckman Coulter Access II Analyzer(Franklin Facility ID# 982972/SN #504760)
2. Beckman Coulter Access reaction Vessels Ref#81901
3. Beckman Coulter Access Waste Bags Ref#81904
4. Transfer Pipettes Item # 9990 5580
5. Fiber-free polyester swabs
6. 2.0 mL sample cups(3 for maintenance)

REAGENTS

A. R1: Access Estradiol Reagent Pack
Cat. No. 33540: 100 determinations, 2 packs, 50 tests/pack.

Provided ready to use. Store upright and refrigerate at 2 to 10°C. Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument. Stable until the expiration date stated on the label when stored at 2 to 10°C. Stable at 2 to 10°C for 14 days after initial use. Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range. If the reagent pack is damaged (i.e., broken elastomer), discard the pack. All antisera are polyclonal unless otherwise indicated.

1. R1a:	Paramagnetic particles coated with goat anti-rabbit IgG: rabbit anti-estradiol in TRIS buffered saline, with bovine serum albumin (BSA), < 0.1% sodium azide.
2. R1b	TRIS, sodium chloride, protein (bovine, goat) and < 0.1% sodium azide.
3. R1c	-alkaline phosphatase conjugate (bovine), protein (BSA, rabbit) < 0.1% sodium azide.

B. Access Estradiol Calibrators
Cat. No. 33545: S0, 4.0 mL/vial; S1–S5, 2.5 mL/vial

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e. assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

Provided ready to use. Store upright and refrigerate at 2 to 10°C. Mix contents by gently inverting before use. Avoid bubble formation. Stable until the expiration date stated on the label when stored at 2 to 10°C. Signs of possible deterioration are control values out of range. Refer to calibration card and or vial labels for exact concentrations.

Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

1. S0: Human serum, < 0.1% sodium azide, and 0.025% Cosmocil** CQ. Contains 0 pg/mL (pmol/L) estradiol.

2. S1–S5: Estradiol (purified chemical compound) in human serum at levels of approximately 106, 570, 1800, 3100, and 4800 pg/mL (389, 2092, 6608, 11,380 and 17,621 pmol/L), respectively, with < 0.1% sodium azide, and 0.025% Cosmocil CQ.

3. Calibration Card: 1

C. Access Substrate
Cat. No. 81906: 4 x 130 mL

Provided ready to use. Refer to the following chart for storage conditions and stability. An increase in substrate background measurements may indicate instability.

Condition	Storage	Stability
Unopened	2 to 8°C	Until expiration date stated on the label
Equilibration prior to use (unopened)	15 to 30°C (room temperature)	Minimum 18 hours Maximum 14 days
In use (opened)	External fluids tray substrate position	Maximum 14 days

Refer to the appropriate system manuals and/or Help system for detailed instructions.

R2 Access Substrate: Lumi-Phos* 530 (buffered solution containing dioxetane Lumigen* PPD, fluorescer, and surfactant).

D. **Access, Access 2, SYNCHRON LXi:**
Access Wash Buffer II, Cat. No. A16792, 4 x 1950 mL
UniCel DxI:
Unicel DxI: Wash Buffer II, Cat. No. A16793, 1 x 10L

Provided ready to use. Stable until the expiration date stated on the label when stored at room temperature (15 to 30°C). An increase in substrate background measurements or increased relative light units for the zero calibrators in “sandwich”-type assays may indicate instability.

Refer to the appropriate system manuals and/or Help system for detailed instructions.

R3 Wash Buffer II: TRIS buffered saline, surfactant, < 0.1 sodium azide, and 0.1% ProClin*** 300.

E. Quality Control (QC) materials: commercial control material.

F. Access Estradiol Calibrator S0
Cat. No. 33546, 4 mL/vial

The analyte level in patient samples may exceed the level of the specific S5 calibrator. If a quantitative value is required, it will be necessary to dilute the samples in order to determine the analyte concentration.

Provided ready to use. Store upright and refrigerate at 2 to 10°C. Mix contents by gently inverting before use. Avoid bubble formation. Stable until the expiration date stated on the label when stored at 2 to 10°C.

Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value of the specific assay. If a sample contains more analyte than the stated value of the S5 calibrator, dilute the sample following dilution instructions in the labeling under "Limitations of the Procedure" in the reagent pack section. Refer to the appropriate system manuals and/or Help system for instructions on how to enter a sample dilution in a test request.

S0: Human serum, < 0.1% sodium azide, and 0.025% Cosmocil**CQ. Contains 0 pg/mL (pmol/L) Estradiol.

CALIBRATION- See Additional Access Calibration Procedure

QUALITY CONTROL- See Additional Access QC procedure.

PROCEDURE

- **Loading patient samples – Step by step instructions**

1. Place small LIS label on the 0.5 ml cup and place aliquot of plasma in cup (fill it to the top)
2. Use rack series 2500 for the 0.5 ml cups
3. Go to "sample manager" and barcode the rack.
4. Select "test request"
5. Use the barcode scanner and read the barcode on the plasma cup.
6. Select the test "Estradiol" and press ENTER.
7. Turn on batch mode to program the rest of the samples on the same rack for the same test.
8. Use the barcode scanner and read the barcode of all the samples you have on the rack.
9. Load the rack on the instrument – remember to tell, wait, do, done – then press RUN.
10. Check QC on log to make sure it is OK before reporting patient results.
11. The completed report will automatically print
12. Enter results in CERNER – see below
13. Cap and save samples in the back refrigerator and discard after 24 hours.

- **<u>Procedural Comments</u>**

A. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.

B. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.

C. Use thirty-five (35) µL of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.

D. The system default unit of measure for sample results is pg/mL. To change sample reporting units to the International System of Units (SI units), pmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply pg/mL by multiplication factor 3.671.

REPORTING RESULTS

1. Patient results are entered in the "ARE" application of CERNER – accession or instrument queue mode.
2. Enter the accession # (by typing or scanning the barcode) in the accession # field.
3. PERFORM then VERIFY.
4. Patient test results are determined automatically by the system software using a smoothing spline math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

5. Patient test results are determined automatically by the system software. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

REFERENCE RANGES

A. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.

B. Estradiol concentrations were measured in human serum samples from apparently healthy adult male and female subjects using the Access Estradiol assay. The observed ranges of estradiol concentrations are shown below for each population represented:

Reference Group	n	Median (pg/mL)	Lower Limit (95% Confidence Interval) (pg/mL)	Upper Limit (95% Confidence Interval) (pg/mL)
Males	129	23	< 20	47 (43–53)
Post menopausal females†	122	< 20	< 20	40 (33–48)
Non-pregnant females				
- mid-follicular phase††	96	57	27 (23–31)	122 (107–139)
- mid-luteal phase†††	100	126	49 (42–57)	291 (250–338)
- Peri-ovulatory phase††††	92	198	95 (83–109)	433 (378–495)

† not on hormone therapy
†† range represents days -6, to -8 from the hLH peak (day 0)
††† range represents days +6, to +8 from the hLH peak (day 0)
†††† range represents day -1 from hLH peak (day 0)

Laboratory	Analytical Measurement Range	Reportable Range
Franklin (Estradiol)	20 – 4800 pg/mL	20 – 4800 pg/mL

LIMITATIONS OF THE PROCEDURE

A. Samples can be accurately measured within the analytical range of the lower limit of detection and the highest calibrator value (approximately 20–4800 pg/mL [73–17,621 pmol/L]).

- If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e. < 20 pg/mL [< 73 pmol/L]).

- If a sample contains more than the stated value of the highest Access Estradiol Calibrator (S5), report the result as greater than that value (i.e. > 4800 pg/mL [> 17,621 pmol/L]). Alternatively, dilute one volume of sample with one volume of Access Estradiol Calibrator S0 (zero) which is also available as Access Estradiol Calibrator S0 Cat. No. 33546. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.

B. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.(10,11)

   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

C. The Access Estradiol results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information.

D. Serum samples containing up to 10 mg/dL (171 μmol/L) Bilirubin, hemolyzed samples up to 1 g/dL (10 g/L) hemoglobin and lipemic samples containing the equivalent of 1800 mg/dL (20.32 mmol/L) triglycerides do not affect the concentration of estradiol assayed utilizing a serum sample containing approximately 1089 pg/mL estradiol.

E. The following potential cross-reactants of substances that are similar in structure to estradiol did not exceed 5% cross-reactivity:

- Estrone sulfate
- Estrone
- Estriol
- Estriol 3-sulfate
- Estriol 17-sulfate
- Ethinyl estradiol
- Estradiol valerate
- Aldosterone
- Testosterone
- Androstenediol
- 17-α-estradiol
- 17β-Estradiol 3 glucuronide
- 3,17β-Estradiol diglucuronide
- Norgestrel
- Estrone-3-glucuronide

Data observed for analytical specificity/interferences testing are provided below:

Substance	Amount Added (pg/mL)	Amount Observed (pg/mL)	Apparent Cross-Reactivity (%)
Estrone sulfate	1,000,000	63.35	0.01
Estrone	20,000	395.28	1.98
Estriol	10,000	49.69	0.50
Estriol 3-sulfate	2,500,000	0.00	ND†
Estriol 17-sulfate	2,500,000	59.61	0.002
Ethinyl estradiol	50,000	184.76	0.37
Estradiol valerate	1,000,000	2850.97	0.29
Aldosterone	100,000,000	0.00	ND†
Testosterone	10,000,000	446.89	0.004
Androstenediol	2,000,000	22.53	0.001
17-α-estradiol	100,000	353.81	0.35
17β-Estradiol 3 glucuronide	2,000,000	574.19	0.029
3,17β-Estradiol diglucuronide	2,000,000	9.97	ND†
Norgestrel	100,000,000	506.14	0.00051
Estrone-3-glucuronide	100,000,000	634.80	0.0006

† ND: no significant statistical difference was observed.

F. The lowest detectable level of estradiol distinguishable from zero (Access Estradiol Calibrator S0) with 95% confidence is 20 pg/mL (73 pmol/L).

G. Estradiol values from pregnant females may be affected by high levels of estriol such as are present in the second and third trimesters of pregnancy (12). Control materials or survey samples containing high levels of estriol may be similarly affected.

REFERENCES

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