DXI ESTRADIOL

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PURPOSE

To provide instruction on how to perform Estradiol testing on the DXI instrument.

BACKGROUND

Principle

The Estradiol reagent, when used in conjunction with the Beckman Access or DXI Systems and Access Calibrators, is intended for quantitative determination of Estradiol concentration in human serum or plasma.

Clinical Significance

Estradiol 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. 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.

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. Abnormally high levels in males are indicative of feminizing syndromes such as gynecomastia.

Methodology

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

RELATED DOCUMENTS

R-PO-CH0810	Quality Control Program General Laboratory
R-PO-CH0809	Quality Control Westgard Rules Statistics

R-PR-AD0540	Specimen Rejection/Cancellation Protocol
J-F-CG0824	DXI & Access Controls
J-F-CH0825	DXI Calibrators
R-F-CH2000	Access 2 and DXI Analytical Measurement Range

SPECIMEN

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn serum or plasma (heparin) are the preferred specimens.

Specimen Storage and Stability

1. Tubes of blood are to be kept closed and in a vertical position at all times. It is recommended that the serum or plasma be physically separated from contact with cells within two hours of the time of collection.

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

Sample Type	Volume	Sample Stability
Serum	0.5mL	• Separate serum from cells within 2 hours. • Room Temp 8 hours • Refrigerated 48 hours • Frozen 3 months.

Criteria for Unacceptable Specimens

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens. See also Related Documents: Specimen Rejection/Cancellation Protocol

Sample Volume

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the Primary Tube Sample Template for your system.

REAGENTS

1. R1: Access Estradiol Reagent Pack
   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.

   - 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.

- R1b: TRIS, sodium chloride, protein (bovine, goat) and < 0.1% sodium azide.
- R1c: Estradiol-alkaline phosphatase conjugate (bovine), protein (BSA, rabbit) < 0.1% sodium azide

2. Access Estradiol Calibrator
   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.

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

3. Access Substrate
   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

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

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

4. Access, Access 2, SYNCHRON LXI
   Access Wash Buffer II
   UniCel DXI;

   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.

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

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

5. Access Estradiol Calibrator S0
   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.

6. Access Immunoassay System and supplies

7. Warnings and Precautions

   - For *in vitro* diagnostic use.
   - Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
   - Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.
   - Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.
   - Each serum/plasma pool used in the preparation of this product has been tested and found negative for the presence of fibrinogen.
   - Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.

CALIBRATION

An active calibration curve is required for all tests. For the Access Estradiol assay, calibration is required every 14 days. 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.

The Access Estradiol Calibrators are provided at six levels - zero and approximately 106, 570, 1800, 3100, and 4800 pg/mL – prepared from synthetic estradiol and human serum. Assay calibration data are valid up to 14 days.

Run the Access Estradiol S0 and S1 Calibrators in quadruplicate, and the S2–S5 Calibrators in duplicate.

QUALITY CONTROL

See Related Documents: DXI & Access Controls

PROCEDURE STEPS

1. Access Instrument
   Refer to the appropriate system manuals and/or Help system for preparation and operation.

2. Assay Procedure
   Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

RESULTS

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

PERFORMANCE CHARACTERISTICS

Reference Range

Male:	0 - 56 pg/mL
Female:	
Follicular	0 - 160 pg/mL
Mid-follicular	0 - 84
Late-follicular	34 - 400
Luteal	27 - 246
Post-menopausal	0 - 35
Post-menopausal, treated	0 - 93

Analytic Measurement Range (AMR)

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)(> 4800 pg/mL), 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.

LIMITATIONS

- 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.
- Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
- 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.
- 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. The lowest detectable level of estradiol distinguishable from zero (Access Estradiol Calibrator S0) with 95% confidence is 20 pg/mL (73 pmol/L).
- 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. Control materials or survey samples containing high levels of estriol may be similarly affected.
- The following potential cross-reactants of substances that are similar in structure to estradiol did not exceed 5% cross-reactivity.

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

PROCEDURAL NOTES

1. 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.

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

3. 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.

4. 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.

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