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| --- | --- | --- | --- |
|  | **Sensitive Estradiol****CL-CH187** | **Dept:** | Clinical Core Lab-Chemistry Section |
| **Effective Date:** | 12/11/2019 |
| **Revised Date:** |  |
| **Contact:** | Clinical ChemistryManagement |
| **Name & Title:** Gregory J. Pomper, MD  Medical Director of Pathology Laboratories | **Date:** |  |
| **Signature:** |

1. **General Procedure Statement:**
	1. **Scope:** To provide laboratory testing personnel with instructions for performing laboratory procedures as deemed appropriate by industry practices and regulatory agencies to assist in quality patient care.
	2. **Responsible Department/Party/Parties:**
		1. Procedure owner: Clinical Chemistry Laboratory Management
		2. Procedure: Clinical Chemistry Laboratory Personnel
		3. Procedure prepared by: Johanna Waldron
		4. Supervision: Clinical Chemistry Management

Clinical Core Laboratory Specialist and Designees

Medical Director Clinical Chemistry

1. Implementation: Clinical Chemistry Laboratory Management

Clinical Core Laboratory Specialist and Designees

Medical Director Clinical Chemistry

1. **Definitions:**
2. **Procedure:**

This procedure is valid for the following Chemistry analyzers:

* Beckman Coulter Unicel DxI 800

Principle

Principles of the Procedure

The Access Sensitive Estradiol assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of estradiol levels in human serum and plasma using the Access Immunoassay Systems.

**Summary and Explanation**

Estradiol (17β-estradiol) is a natural estrogen with a molecular mass of 272.4 daltons.[1](#bibB844258_3054) Most circulating estradiol is strongly bound to sex hormone binding protein and loosely bound to albumin. It is estimated that only 1-5% of estradiol is free (unbound).[2](#bibB844257996) 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.[3](#bibB844257997)

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.[4](#bibB844257998),[5](#bibB844257999)

Levels of estradiol are used to monitor ovulatory status.[2](#bibB844257996) Because estradiol levels reflect follicular maturation, the measurement of estradiol as cited in the scientific literature has been used as a valuable tool in the assessment of sexual development in children, anovulation and/or amenorrhea, polycystic ovary syndrome and causes of infertility and menopause.[3](#bibB844257997),[4](#bibB844257998),[6](#bibB844258_30_30_30)

During in vitro fertilization, estradiol levels are routinely measured after gonadotropin stimulation to determine follicular status.[7](#bibB844258_30_301) Estradiol also affects areas other than reproductive tissues such as cardiovascular, immune and central nervous systems.[8](#bibB844258_30_302) For this reason estrogen has been investigated in the pathogenesis of cardiovascular disease, hormone-dependent cancers and osteoporotic fracture.[9](#bibB844258_30_303) Abnormally high levels in males are indicative of feminizing syndromes such as gynecomastia.[10](#bibB844258_30_304)

**Methodology**

The Access Sensitive Estradiol assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel with displacer and an anti-estradiol conjugate (anti-estradiol sheep monoclonal antibody conjugated to alkaline phosphatase in a MES-buffered protein solution). After incubation paramagnetic particles coated with estradiol analog are added. Estradiol in the sample competes with the estradiol analog on the paramagnetic particles for binding sites on a limited amount of specific anti-estradiol conjugate. The resulting estradiol analog-antibody complexes are bound 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 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.

**Specimen**

**Specimen Storage and Stability**

| Specimen | Type | Room Temperature(hours) | 2°C to 8°C(days) |
| --- | --- | --- | --- |
| Serum | Gel,No gel | 8 | 7 |
| Plasma | Lithium heparin, Sodium heparin, EDTA | 8 | 7 |

**Sample Collection and Preparation**

1. The role of preanalytical factors in laboratory testing has been described in a variety of published literature.[11](#bibB844252_3081),[12](#bibB844255859) To minimize the effect of preanalytical factors observe the following recommendations for handling and processing blood samples:[11](#bibB844252_3081)

a. Collect all blood samples observing routine precautions for venipuncture.

i. Follow blood collection tube manufacturer’s recommendations for centrifugation.

ii. Ensure residual fibrin and cellular matter has been removed prior to analysis.

b. Allow serum samples to clot completely before centrifugation in a vertical, closure-up position.

i. Nonanticoagulated tubes containing gel separator should be stored in an upright position as soon as the mixing is complete.

ii. Precentrifugation serum/cells contact time is according to tube manufacturer’s recommendations. Clotting may be slowed at cooler temperatures or if patient is on anticoagulant therapy.

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

3. Alternate collection types may be appropriate if the laboratory has established its own performance characteristics as defined by applicable law.

4. Avoid assaying lipemic or hemolyzed samples.

5. Thaw samples no more than three times.

6. If the assay will not be completed within 7 days, freeze at -20°C or colder.

**NOTE:** Specimens received in a standard 13x75 vacutainer tube are processed by the Beckman Power Processor and delivered to attached instruments robotically. Those samples which are of insufficient volume or are of any tube type other than a 13x75 vacutainer are centrifuged and processed manually.

DxI 800 Sample Programming and Processing

*Processing Bar Coded Samples*

1. Refer to Beckman Coulter's Primary Tube Sample Template to identify which rack(s) to load samples on and to determine if nesting cups are necessary.

Short samples and pediatric samples are spun by Central Processing and brought directly over to Chemistry. Central Processing places the bar code label for pediatric samples on a transport tube and bring both pediatric sample (microtainer) and bar coded transport tube directly over to Chemistry. Short samples and pediatric samples can be poured into a nesting cup that fits on top of a designated DxI 800 rack. These nesting cups are then loaded directly on the instrument. These samples will be manually programmed on the DxI since only a nesting cup and not the bar coded transport tube is loaded on the machine. Either write the sample ID or place a taglet on the nesting cup so that the sample will have an ID at all times. After testing, the nesting cup is placed on top of the pediatric transport tube or poured back into the tube in case of a short sample.

2. Load tube(s) into appropriate rack(s) with the bar code labels visible through the slot on the same side of the rack as the rack bar code label.

3. Remove caps from sample(s).

4. Place rack(s) in the onload area of the instrument. Close the SPU cover.

*Processing Samples Manually*

1. Refer to Beckman Coulter's Primary Tube Sample Template to identify which rack(s) to load samples on and to determine if nesting cups are necessary.

2. From the Sample Manager screen, select **New Request F3**.

3. Select **Patient/QC Requests F1** to display the Test Requests Screen.

4. Enter the rack ID in the **Enter ID** field and press **[Enter]**.

5. Enter the sample ID in the **Sample ID** field and press **[Enter]**.

6. Select the tests to be run on that sample by selecting their corresponding Test buttons.

7. To order additional samples, repeat steps 5-6 for remaining positions in the selected rack.

8. Select the **Back** tab to exit the Test Requests screen.

9. Load tube(s) into appropriate rack(s) as entered in step 4.

10. Remove caps from sample(s).

11. Place rack(s) in the onload area of the instrument. Close the SPU cover.

**Reagents**

Contents

Access Sensitive Estradiol Reagent Pack

Cat. No. B84493: 100 determinations, 2 packs, 50 tests/pack

**Access Estradiol Reagent Packs are located in Walk In #1.**

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 28 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.

| Well | Ingredients |
| --- | --- |
| **R1a:** | Dynabeads\* paramagnetic particles coated with streptavidin, biotin and estradiol analog coupled to biotin in a Tris buffer with proteins (fish), surfactant and 0.0125% Cosmocil CQ\*\*. |
| **R1b:** | Tris buffer with proteins (goat, bovine), surfactant, < 0.1% azide and 0.1% Proclin 300\*\*\*. |
| **R1c:** | Sheep monoclonal anti estradiol alkaline phosphatase conjugate in a MES buffer with proteins (goat, avian), surfactant, < 0.1% azide and 0.1% Proclin 300. |

\*Dynabeads is a registered trademark of Dynal A.S., Oslo, Norway.

\*\*Cosmocil is a trademark of Arch Chemicals, Inc.

\*\*\*ProClin™ is a trademark of The Dow Chemical Company (“Dow”) or an affiliated company of Dow.

WARNING 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.

•  For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

|  |
| --- |
|  CAUTIONSodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations. |
|

GHS HAZARD CLASSIFICATION

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| --- | --- | --- |
| Estradiol Dissociation buffer pH=7.6 (Compartment R1b) | WARNING |  |
|  |  |  |
|  | H316 | Causes mild skin irritation. |
|  | H317 | May cause an allergic skin reaction. |
|  | P280 | Wear protective gloves, protective clothing and eye/face protection. |
|  | P332+P313 | If skin irritation occurs: Get medical advice/attention. |
|  | P333+P313 | If skin irritation or rash occurs: Get medical advice/attention. |
|  | P362+P364 | Take off contaminated clothing and wash it before use. |
|  |  | Tris(hydroxymethyl)– aminomethane 1 - 5% |
|  |  | reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05% |
| W.S. Anti-Estradiol Conjugate coupled to rec-ALP (Compartment R1c) | WARNING |  |
|  |  |  |
|  | H317 | May cause an allergic skin reaction. |
|  | P280 | Wear protective gloves, protective clothing and eye/face protection. |
|  | P333+P313 | If skin irritation or rash occurs: Get medical advice/attention. |
|  | P362+P364 | Take off contaminated clothing and wash it before use. |
|  |  | reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05% |

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| --- | --- |
|  | Safety Data Sheet is available at techdocs.beckmancoulter.com |

Materials Needed But Not Supplied With Reagent Kit

1. Access Sensitive Estradiol Calibrators
 Provided at zero and approximately 11.0, 32.0, 292, 885 and 5,200 pg/mL
(0, 40.4, 117, 1,072, 3,249 and 19,089 pmol/L).
 Cat. No. B84494

 **Access Estradiol Calibrators are located in Walk In # 1.**

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.

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

3. Access Sensitive Estradiol Calibrator S0
 Cat. No. B97145

 **Access Estradiol Calibrator S0 is located in Walk In #1.**

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.

4. Access Substrate
Cat. No. 81906

**Access Substrate is located in Walk In #1.**

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 hoursMaximum 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).

5. UniCel DxI Wash Buffer II, Cat. No. A16793

 **Access Wash Buffer II is located in the Specials Area of the Chemistry Lab.**

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.

**Calibration**

**Calibration Information**

An active calibration curve is required for all tests. For the Access Sensitive Estradiol assay, calibration is required every 28 days. See calibrator Instructions For Use (IFU) for additional calibration information. Refer to the appropriate system manuals and/or Help system for information on calibration method, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Recalibration of this test is required when any of these conditions exist:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

Refer to DxI 800's Operator's Guide and/or Help system (question mark on DxI 800's instrument computer) for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

DxI 800 Calibration Procedure

1. From the Sample Manager screen, select **New Request F3** then **Calibration F2** to display the Request Calibration window.

2. Select the calibrator lot for this calibration request, and then select **OK F1**.

3. Pick 2 racks that are used for 2 mL nesting cups. (Racks 1-10).

4. On the test requests screen, enter the rack ID in the **Enter ID** field and press **[Enter]**. To enter a second rack ID, select **Rack ID F1**, then **Second F2**. enter the rack ID in the **Enter ID** field and press **[Enter]**.

5. (Optional) To change the reagent lot for this calibration request, select **Change Reag.** **Lot**. Select the desired reagent lot number from the list and then select **OK F1**.

6. Place the calibrators (10 drops per level) into the nesting cups in the racks and in the order indicated on the Rack button of the test requests screen.

7. Place racks in the SPU onload area of the instrument. Close the SPU cover.

Calibration reports will print out automatically upon completion. File all printouts in the provided calibration notebooks for each DxI.

Follow all passed calibrations with the appropriate quality control material for each immunoassay that was calibrated.

In the event of a calibration failure - troubleshoot according to calibration failure codes and assay calibration troubleshooting tables. For more information see Chapter 5.5 Troubleshooting Failed Calibrations in the Operator's Guide for the DxI 800.

Please refer to the DxI 800's Operator's Guide Chapter 5 for additional information on:

* Assay Calibration Overview
* Setting Up Calibrators
* Running a Calibration
* Reviewing Calibration Data
* Troubleshooting Failed Calibrations

Corrective action to resolve calibration failures includes recalibration, new reagent and/or control/calibration material, lot change discrepancies and if necessary field service.

Quality Control

Quality control materials simulate the characteristics of samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a “random access” format rather than a “batch” format, quality control materials should be included in each 24-hour time period.[13](#bibB8442538_308) Include commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

**NCBH's Quality Control Material for Sensitive Estradiol (SNSE2):**

Bio-Rad Liquichek Immunoassay Plus Control, Levels 1, 2 and 3, Item Nos. 361, 362 and 363. Unopened controls are stable until expiration date on package when stored unopened at -70˚ C. Once thawed and stored unopened at 2 to 8˚ C, this liquid product is stable for 30 days. Once thawed and open, all analytes will be stable for 14 days at 2 to 8˚ C. Exceptions are PSA which is stable for 3 days and Folate which is stable for 14 days. Once thawed, do not refreeze control material. Frozen controls are located in Freezer #17. Thawed controls are located in Refrigerator #4. Quality Control is verified using Westgard Rules programmed into the LIS system, Beaker. Corrective action to resolve control failures includes repeat analysis, recalibration, new reagent and/or control material, lot change discrepancy investigation, and if necessary, field service. Once Quality Control is deemed acceptable, specimen processing may proceed.

Beaker Quality Control Codes: **C-IA1I1, C-IA2I1, C-IA3I1, C-IA1I2, C-IA2I2** and **C-IA3I2**

DxI 800 Quality Control Procedure

1. From the Sample Manager screen, select **New Request F3**, then select **Patient/QC Requests F1**.

2. Determine which rack is to be used depending on what size tubes the controls are in.

3. Enter the rack ID in the **Enter ID** field and press **[Enter]**.

4. Select **QC F5**.

5. The request QC window is displayed. Select up to four quality controls, then select **OK F1**. Each quality control selected is displayed in one available sample position on the Test Request screen.

6. Select the first quality control that was requested in the previous step.

7. Select the appropriate Test buttons.

* If using a multi-analyte quality control, multiple tests can be selected to run for each sample. A pre-determined QC panel can also be selected.
* To change a reagent lot for a certain test - select **Change Reag.** **Lot**. Select the desired reagent lot number from the list and then select **OK F1**.

8. Repeat steps 6 and 7 for each quality control test requested in step 5.

9. Complete the test request by exiting the Test Request Screen.

10. Place the QC samples (found in refrigerator # 4) in the rack entered in step 3. Place the rack in the onload area of the instrument. Close the SPU cover.

Testing Procedure

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.

a. 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.

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 (30) µL of sample for each determination in addition to the sample container and system dead volumes when requesting the Access Sensitive Estradiol assay. Use one hundred and fifty-five (155) μL of sample in addition to the sample container and system dead volumes for each determination run with the DxI system onboard dilution feature (test name: dSNE2). Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.

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

a. Select SNSE2 as the test name for assaying samples containing estradiol concentrations up to the concentration of the Access Sensitive Estradiol S5 calibrator.

b. UniCel DxI users may use the UniCel DxI onboard dilution feature (test name: dSNE2) for assaying samples containing estradiol concentrations greater than the Access Sensitive Estradiol S5 calibrator.

Limitations

1. 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 human anti-animal antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other antibodies such as human anti-goat antibodies may be present in patient samples.[14](#bibB844252_3082),[15](#bibB844252_3083) Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

2. Other potential interferences in the patient sample could be present and may cause erroneous results in immunoassays. Some examples that have been documented in literature include rheumatoid factor, endogenous alkaline phosphatase, fibrin, and proteins capable of binding to alkaline phosphatase.[16](#bibB844252694) Carefully evaluate results if the sample is suspected of having these types of interferences.

3. The Sensitive 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.

Results Interpretation

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. 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.

Reporting Results

Samples can be accurately measured within the analytical range of the lower limit of detection and the highest calibrator value (approximately 15.0 - 5,200 pg/mL [55.1 - 19,089 pmol/L]).

•  If a sample contains less than the lower limit of detection (LoD) for the assay, report the result as less than that value (i.e. < 15.0 pg/mL [55.1 pmol/L]). When the DxI system onboard dilution feature is used, the system will report results as less than 3,400 pg/mL (12,481 pmol/L).

•  If a sample contains more than the stated value of the highest Access Sensitive Estradiol Calibrator (S5), report the result as greater than that value (i.e., > 5,200 pg/mL [> 19,089 pmol/L]). Alternatively, dilute one volume of sample with one volume of Access Sensitive Estradiol Calibrator S0.

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.

Manual Dilutions on the DxI 800

Whether or not a test needs to be diluted will be determined by the technologist manning the Remisol software. There are several tests that we do not dilute. They are: B12, BNP, FOL, FT4, IPTH, PTHIO, RFOL, TT4, and TU. These tests are sent out as greater than their linear range (see individual test procedures for ranges).

To make a dilution: Refer to the DxI Test Information chart located in the NCBH IFU for the DxI 800 to determine what diluent is needed for the test to be diluted. Decide what size of dilution to make, i.e. x5. Into a 0.5mL nesting cup, using an MLA pipettor, pipet the appropriate amount of diluent. Then add the appropriate amount of patient sample. Mix well. For example, to make a x5 TSH dilution, use 200 µL Sample Diluent A and 50 µL patient sample. Make sure that the nesting cup has the patient ID and dilution factor written on it. Load nesting cup onto a designated 0.5 mL rack and then order the manual dilution on the DxI using the following instructions:

1. From the Sample Manager screen, select **New Request F3**.

2. Select **Patient/QC Requests F1** to display the Test Requests Screen.

3. Enter the rack ID in the **Enter ID** field and press **[Enter]**.

4. Enter the sample ID in the **Sample ID** field and press **[Enter]**.

5. Select the tests to be run on that sample by selecting their corresponding Test buttons.

6. Enter the dilution factor in the **Dilution** field and press **[Enter]**.

7. Place the diluted sample in the rack selected in step 3.

8. Place rack in the onload area of the instrument. Close the SPU cover.

After testing is completed, the system reports the results adjusted for the dilution factor. Discard dilution cup when testing is complete.

Expected Results

**NCBH Reference Ranges:**

|  |  |
| --- | --- |
| **Sex / Age** | **Reference Range** |
| **Female 0 up to 1 year** | **15.0-38.2 pg/mL** |
| **Female 1 up to 12 years** | **15.0-16.0 pg/mL** |
| **Female 12 up to 19 years** | **36.5-196.0 pg/mL** |
| **Female 19 up to unspecified** | **15.0-517.0 pg/mL** |
| **Male 0 up to 1 year** | **15.0-38.2 pg/mL** |
| **Male 1 up to 12 years** | **<15.0 pg/mL** |
| **Male 12 up to 19 years** | **19.5-34.8 pg/mL** |
| **Male 19 up to unspecified** | **15.0-31.5 pg/mL** |

**Additional comments to be added to results for female patients:**

**Early follicular Phase: 22.4-115 pg/mL**

**Mid-Follicular Phase: 25.0-115 pg/mL**

**Mid-Luteal Phase: 36.5-246 pg/mL**

**Postmenopause: <15.0-25.1 pg/mL**

**Ovulatory Peak: 32.1-517 pg/mL**

1. Each laboratory should determine its own reference intervals appropriate to the laboratory’s patient population; including age of the patient.

2. Estradiol concentrations were measured in human serum samples from apparently healthy adult male, female and pediatric subjects. Adult female subjects were not currently pregnant, not using hormonally based birth control in the three months prior to study, not using hormonal therapy in the six months prior to the study, had regular menstrual cycles (cycled females) and no previous history of ovarian surgery. Post-menopausal women were not on hormone replacement therapy. Pediatric age ranges were partitioned based on a reference by Karbasy, et. al.[17](#bibB844258_30_307)Serum samples were analyzed using multiple Access Immunoassay Systems with the Access Sensitive Estradiol assay following the CLSI EP28-A3c guideline.[18](#bibB844256_3012) The observed ranges of estradiol concentrations are shown below for each population represented:

| Non-pregnant females | Range of days from hLH peak (day 0) | N | Median(pg/mL) | Lower 2.5th Percentile Limit: (95% CI) pg/mL | Upper 97.5th Percentile Limit:(95% CI)pg/mL |
| --- | --- | --- | --- | --- | --- |
| Early Follicular | -14 to -10 | 49 | 36.9 | 22.4(20.0-25.1) | 115(83.9-157) |
| Mid Follicular | -9 to -4 | 49 | 53.0 | 25.0(20.7-30.1) | 115(95.5-139) |
| Ovulatory Peak | 0 | 50 | 202 | 32.1(19.3-53.5) | 517(429-622) |
| Mid Luteal | +4 to +11 | 50 | 127 | 36.5(25.8-51.8) | 246(217-279) |

| Post-Menopausal Females | N | Median(pg/mL) | Upper 95th Percentile Limit: (95% CI) pg/mL |
| --- | --- | --- | --- |
| Not on hormone therapy | 160 | < 15.0 | 25.1(19.9-30.0) |

One-sided test is used due to skewed data distribution. Normal reference interval is defined as the values ≤ upper 95th percentile limit.

| Males | N | Median(pg/mL) | Lower 2.5th Percentile Limit pg/mL | Upper 97.5th Percentile Limit: (95% CI)pg/mL |
| --- | --- | --- | --- | --- |
| ≥ 19 years old | 129 | 22.2 | < 15.0 | 31.5(29.8-33.1) |

| Pediatric | Age Range (years) | N | Median (pg/mL) | Upper 95th Percentile Limit: (95% CI) pg/mL |
| --- | --- | --- | --- | --- |
| Pediatric (male and female) | 0 to < 1 | 114 | < 15.0 | 38.2(32.6-43.7) |
| Pre-puberty female | 1 to < 12 | 117 | < 15.0 | 16.0(< 15.0-20.3) |
| Puberty female | 12 to < 19 | 150 | 36.5 | 196(162-230) |
| Pre-puberty male | 1 to < 12 | 99 | < 15.0 | < 15.0 |
| Puberty male | 12 to < 19 | 58 | 19.5 | 34.8(30.8-38.7) |

One-sided test is used due to skewed data distribution. Normal reference interval is defined as the values ≤ upper 95th percentile limit.

Performance Characteristics

Performance Characteristics

Representative data is provided for illustration only. Performance obtained in individual laboratories may vary.

Methods Comparison

A comparison of 135 values using the Access Sensitive Estradiol assay on an Access Immunoassay System and mass spectrometry including comparison to the ID/GC/MS reference measurement procedure (RMP) developed at Ghent University and an LC-MS tandem method gave the following statistical data using Passing-Bablok regression and Pearson's correlation based on the CLSI EP09-A3 guideline:[19](#bibB84425798_30)

| N | Range of Observations (pg/mL) | Intercept (pg/mL) [95% CI]  | Slope  [95% CI]  | Correlation Coefficient (r) |
| --- | --- | --- | --- | --- |
| 135 (combined GC-MS and LC-MS) | 15.7 to 4,838 | -2.957 [-8.46 to 0.63] | 0.98 [0.95 to 1.00] | 0.99 |
| 48 (GC-MS only) | 15.7 to 4,838 | 3.224 [-0.44 to 8.10] | 0.99 [0.97 to 1.03] | 0.99 |
| 87 (LC-MS only) | 17.3 to 2,119 | -5.121 [-14.35 to 2.95] | 0.91 [0.83 to 1.04] | 0.97 |

Linearity

NCBH’s REPORTABLE RANGE: 15.0 – 5200.0 pg/mL. Results reading greater than 5200 pg/mL are to be diluted.

The Access Sensitive Estradiol assay demonstrated acceptable linearity throughout the analytical measuring range of 15.0 pg/mL (55.1 pmol/L) to approximately 5,200 pg/mL (19,089 pmol/L). Based on CLSI EP06-A[20](#bibB84425222_30), one high sample [approximately 5,200 pg/mL and one low sample [approximately 8.8 pg/mL] were mixed to make 9 sample concentrations evenly distributed across the analytical measuring range. Four replicates of the 7 mixed samples, 8 replicates of the low sample and 4 replicates of the high sample were tested on multiple Access Immunoassay Systems.

Imprecision

The Access Sensitive Estradiol assay exhibits within laboratory (total) imprecision ≤ 10% at concentrations greater than 30.0 pg/mL (110.1 pmol/L), and within laboratory (total) standard deviation (SD) ≤ 5.00 pg/mL (18.36 pmol/L) at ≤ 30.0 pg/mL (110.1 pmol/L).

One study, using six serum-based samples on one Access Immunoassay System generating a total of 40 assays, two replicates per assay, over 10 days with 4 runs per day provided the following data, calculated based on CLSI EP05-A3[21](#bibB844257981) guidelines.

|   | Within-Run | Between-Run | Between-Day | Within Lab (Total Imprecision) |
| --- | --- | --- | --- | --- |
| Sample | N | Grand Mean (n=40)(pg/mL) | SD (pg/mL) | CV (%) | SD (pg/mL) | CV (%) | SD (pg/mL) | CV (%) | SD (pg/mL) | CV (%) |
| Sample 1 | 82 | 23.3 | 2.50 | NA | 2.16 | NA | 1.13 | NA | 3.49 | NA |
| Sample 2 | 84 | 41.7 | 2.00 | 5% | 1.75 | 4% | 1.16 | 3% | 2.90 | 7% |
| Sample 3 | 84 | 173 | 3.23 | 2% | 2.95 | 2% | 3.11 | 2% | 5.36 | 3% |
| Sample 4 | 84 | 799 | 13.62 | 2% | 18.96 | 2% | 6.05 | 1% | 24.11 | 3% |
| Sample 5 | 84 | 1,608 | 35.26 | 2% | 35.89 | 2% | 25.31 | 2% | 56.32 | 4% |
| Sample 6 | 84 | 3,477 | 168.34 | 5% | 225.36 | 6% | 99.55 | 3% | 298.39 | 9% |

Interfering Substances

Serum samples containing estradiol concentrations of 150 and 500 pg/mL were spiked with multiple concentrations of the substances below and run on multiple Access Immunoassay Systems. Values were calculated as described in CLSI EP07-A2.[22](#bibB844257982)Interference was determined by testing controls (no interfering substance added) and matched test samples (with interfering substance added). Of the compounds tested, none were found to cause significant interference (as defined by a shift in dose greater than 10%) using the highest test concentrations indicated in the table below.

| Substance | High Concentration |
| --- | --- |
| Acetaminophen | 20 mg/dL |
| Acetylsalicylic acid | 65 mg/dL |
| Ascorbic acid | 342 µmol/L |
| Bilirubin (conjugated) | 43 mg/dL |
| Bilirubin (unconjugated) | 40 mg/dL |
| Biotin | 200 ng/mL |
| Fulvestrant | 25,000 pg/mL |
| Hemoglobin | 300 mg/dL |
| Heparin | 3,000 U/L |
| 2-hydroxy-Ibuprofen | 50 mg/dL |
| Multi Vitamin (Centrum liquid) | 0.38% (v/v) |
| Tamoxifen | 4 µmol/L |
| Triglyceride (Triolein) | 37 mmol/L |
| Total Protein | 12 g/dL |
| Uric Acid | 1.4 mmol/L |

Cross Reactivity

A study was performed to evaluate the potential cross-reactivity of the assay with other substances that are similar in structure to estradiol. Serum samples containing estradiol concentrations of approximately 150 and 500 pg/mL were spiked with multiple concentrations of the substances below and run on multiple Access Immunoassay Systems. Values were calculated as described in CLSI EP07-A2. The percent cross-reactivity observed for the following substances is indicated below.

| Substance | Highest Concentration Tested (μg/mL) | Highest Cross-Reactivity Observed (%) | Criteria (%)  ≤ |
| --- | --- | --- | --- |
| Aldosterone | 100 | 0.000030 | 0.90 |
| Androstenediol | 53.7 | 0.0030 | 1.40 |
| Androstenedione | 50 | 0.000020 | 0.50 |
| Estradiol valerate | 0.7 | 0.40 | 1.00 |
| 17α-Estradiol | 2 | 0.0040 | 1.00 |
| 17β-estradiol 3 glucuronide | 0.3 | 0.10 | 1.00 |
| 3.17β-Estradiol diglucuronide | 56.3 | 0.0030 | 1.00 |
| Estriol | 4.2 | 0.050 | 1.00 |
| Estriol-3-sulfate | 3.7 | 0.00030 | 1.00 |
| Estriol-17-sulfate | 0.3 | 0.00200 | 1.00 |
| Estrone | 0.4 | 0.40 | 2.0 |
| Estrone-3-glucuronide | 100 | 0.0020 | 1.00 |
| Estrone-3-Sulfate | 14.1 | 0.0010 | 1.70 |
| Ethinyl estradiol | 2.5 | 0.030 | 1.0 |
| Norgestrel | 100 | 0.000070 | 0.40 |
| Testosterone | 34.2 | 0.00060 | 0.21 |

Analytical Sensitivity

| Parameter | Total Samples | Total Replicates Tested Per Study | Total Runs Per Study | Range of Observed Results | Criteria |
| --- | --- | --- | --- | --- | --- |
| **Limit of Blank (LoB)** (highest measurement result that is likely to be observed in a blank sample) | 4 | 120 | 3 | 5.0-7.5 pg/mL (18.5-27.6 pmol/L) | ≤ 10.0 pg/mL (≤ 36.7 pmol/L) |
| **Limit of Detection (LoD)** (lowest concentration of analyte that can be consistently detected) | 5 | 225 | 5 | 9.4-12.4 pg/mL (34.5-45.4 pmol/L) | ≤ 15.0 pg/mL (≤ 55.1 pmol/L) |
| **Limit of Quantitation (LoQ) ≤ 20% within lab (total) CV** | 11 | 495 | 5 | 10.4-15.1 pg/mL (38.3-55.5 pmol/L) | ≤ 19.0 pg/mL (≤ 69.7 pmol/L) |

*Per the study design the results were calculated using a protocol based on CLSI EP17-A2*[23](#bibB844255852)with three reagent lots and one calibrator lot on multiple Access Immunoassay Systems for a total of six studies.

Additional Information

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Symbols Key

Glossary of Symbols is available at techdocs.beckmancoulter.com (document number C02724)

1. **Review/Revision/Implementation:**
	1. Review Cycle: 2 years
	2. Office of Record: Department of Clinical Core Laboratory-Chemistry
	3. All new procedures and procedures that have major revisions must be signed by the Laboratory Director.
	4. All reviewed procedures and procedures with minor revisions can be signed by the designated section medical director.
2. **Related Procedures:**
3. **References, National Professional Organizations, etc.:**

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21. Approved Guideline - Evaluation of Precision Performance of Quantitative Measurement Methods, EP05-A3. August 2014. Clinical and Laboratory Standards Institute.

22. Approved Guideline - Interference Testing in Clinical Chemistry, EP07-A2. November 2005. Clinical and Laboratory Standards Institute.

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Beckman Coulter, Inc. Access Estradiol product insert, Brea, CA 92821, P/N A56077.

Beckman Coulter, Inc. Access Substrate product insert, Brea, CA 92821, P/N 386966.

Beckman Coulter, Inc. Access Wash Buffer II product insert, Brea, CA 92821, P/N A16534.

Beckman Coulter, Inc. UniCel DxI Wash Buffer II product insert, Brea, CA 92821, P/N A16543.

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1. **Attachments:**
2. **Revision Dates:**

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| **Review Date** | **Revision Date** | **Signature** |
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