| **Estradiol** |
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| **Purpose** | This procedure provides instructions for performing ESTRADIOL on the Abbott Architect i1000SR. |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing on the Abbott Architect i1000SR. |
| **Principle** | The ARCHITECT Estradiol assay is a delayed one step immunoassay to determine the presence of estradiol in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex. Sample, specimen diluent, assay diluent, and anti-estradiol (rabbit, monoclonal) coated paramagnetic microparticles are combined. Estradiol present in the sample binds to the antiestradiol coated microparticles. After incubation, estradiol acridinium-labeled conjugate is added to the reaction mixture. After further incubation and washing, Pre-Trigger and Trigger Solutions are added to the reaction mixture. The resulting Chemiluminescent reaction is measured as relative light units (RLUs). There is an inverse relationship between the amount of estradiol in the sample and the RLUs detected by the ARCHITECT iSystem optics. |
| **Clinical Significance** | Estradiol is the most potent natural estrogen in humans. It regulates reproductive function in females, and, with progesterone, maintains pregnancy. Most estradiol is secreted by the ovaries (non-pregnant women), although the testes (in men) and adrenal cortex (in menand women) secrete small amounts. During pregnancy, the placenta produces most of the circulating estradiol. Estradiol and estrone interconvert *in vivo*. In normal non-pregnant women, estradiol synthesized by the ovary is the predominant source of both estrone and estriol. Virtually all circulating estradiol is protein-bound. Reported association constants for estradiol with sex hormone binding globulin and serum albumin are, respectively, 6.8 x **10**8 and 6 x **104** . One consequence of this binding is that the conditions of any assay for serum estradiol must release this steroid quantitatively from its binding partners. The amount and proportion of protein-bound and free estradiol vary by gender, and with pregnancy and menstrual phase in women.1 Normal estradiol levels are lowest at menses and into the early follicular phase (25-75 pg/mL) and then rise in the late follicular phase to a peak of 200-600 pg/mL just before the LH surge, which is normally followed immediately by ovulation. As LH peaks, estradiol begins to decrease before rising again during the luteal phase (100-300 pg/mL). If conception does not take place, estradiol falls further to its lowest levels, and menses begins shortly thereafter. If conception occurs, estradiol levels continue to rise, reaching levels of 1,000-5,000 pg/mL during the first trimester, 5,000-15,000 pg/mL during second trimester, and 10,000-40,000 pg/mL during third trimester. At menopause, estradiol levels remain low. Because the ovaries produce most estradiol in normal women, estimation of this hormone is sometimes a gauge of ovarian function.9 In addition, monitoring estradiol levels is important in evaluating amenorrhea, precocious puberty, the onset of menopause, and infertility in men and women. Monitoring estradiol levels is essential during *in vitro* fertilization, because the timing of recovery of oocytes depends on follicular development, which in turn depends on the estradiol level. |
| **Instrument** | **PRIMARY METHOD:** Abbott Architect i1000SR**Backup Method:** Mayo Medical Laboratories (DIOL) |
| **Sunquest Test Code** | ETDI |
| **Specimen** | **Preferred**: Serum Separator Tube (SST)Alternate Tube Types: Serum without gel, Lithium Heparin (gel or no gel), and K2 EDTA plasma are also acceptable**Recommended Draw Volume**: 1.2 mL (minimum 0.6 mL)**Minimum Processed volume:** 200 µL (0.2 mL) of serum/plasma**Stability:** Room Temperature 24 hours, 2 – 8 °C 7 Days, ≤-20°C for up to two years.**Rejection criteria:** Unlabeled tube, Unacceptable sample type**Preparation:** 1. Serum samples should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis.
2. Remove serum or plasma from the clot, red blood cells, or separator gel if stored longer than the maximum room temperature storage time.
3. Lipemic samples should be ultrafuged.
4. Specimens should be free of particulate matter.
5. Transfer serum or plasma to a properly labeled sendout tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
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| **Reagents** |

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Estradiol Reagent | 7K72 | **Store at:** 2 – 8 °C**Unopened/Opened:** Manufacturer expiration date.**On-board:** 30 Days |
| Estradiol Calibrator | 7K72-01 | **Store at:**  2 – 8 °C**To Use**: Gently mix after removal from fridge**Unopened**: Manufacturer expiration date.**Opened**: Store at 2 – 8 °C, use manufacturer expiration date |
| Multiassay Diluent | 7D82-50 | Refer to Supply Status on Analyzer |
| Pre-Trigger Solution | 06E23-65 | Refer to Supply Status on Analyzer |
| Trigger Solution | 06C55-60 | Refer to Supply Status on Analyzer |
| Wash Buffer | 06C54-58 | Refer to Supply Status on Analyzer |
| Reaction Vessels  | 07C15 (-02 or -03) | N/A |
| Lyphochek Immunoassay Plus (LYIP) | Level 1 - 371Level 2 - 372Level 3 - 373 | **Unopened storage:** 2-8°C**To Use:** Reconstitute with exactly 5.0 mL of DI water. Let vials sit for 15 minutes, swirling occasionally to ensure homogeneity. Do not allow to sit at room temperature longer than 20 minutes.**Once Opened, Store:** 2-8°C**Stability:** 3 Days |
| Estradiol Manual Diluent | 7K72-50 | **Unopened storage:** 2-8°C**Once Opened, Store:** 2-8°C**Stability:** Manufacturer expiration date |

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| **Risk and Safety:** | **Reagent:** Contains methylisothiazolones. May cause an allergic skin reaction. Avoid breathing mist / vapors / spray. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves / protective clothing / eye protection.**Estradiol Manual Diluent:** Contains diethylenetriamine pentaacetic acid (DTPA) and sodium azide. Contact with acids liberates very toxic gas. Wear protective gloves / protective clothing / eye protection. Recap vial with a used (black or white) reagent cap and dispose of in correct hazardous waste bin. |
| Calibration/ Verification/AMR |

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| Analytical Measuring Range: | 10 - 1000 pg/mL |
| Reference Material: | Estradiol Calibrator |
| Suggested Calibration Levels | A – 0.0 pg/mLB – 50.0 pg/mLC – 100.0 pg/mLD – 250.0 pg/mLE – 500.0 pg/mLF – 1000.0 pg/mL |
| Verification Scheme: | n=6 |
| Verification Frequency: | * For each new lot of reagent
* After major maintenance or service, if indicated by quality control results
* As indicated in laboratory quality control procedures
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| AMR | Verification of AMR is accomplished with each calibration.* Cal Verification and AMR verification are performed at least once every six (6) months by way of reagent calibration.
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| **Quality Control** | Bio-Rad Lyphochek Immunoassay Plus Levels 1,2 and 3**Frequency:** Three levels each day of use.**Stability:** 3 Days at 2-8°C.**Preparation**: Reconstitute with exactly 5.0 mL of DI water. Let vials sit for 15 minutes, swirling occasionally, to ensure homogeneity. Do not allow to sit at room temperature longer than 20 minutes.**Sunquest Control names:** Level 1 = C-LYIP1, Level 2 = C-LYIP2, Level 3= C-LYIP3**Acceptable ranges:** * Ranges are current in Sunquest and the instrument. Refer to the Quality Control in Chemistry procedure for QC exception codes.
* If a control value is outside the confidence interval, the determination must be repeated. If the repeat determination confirms the deviation, a new reference curve should be established.
* Do not release patient results until the cause of deviation has been identified and corrected
* When a new lot of assayed control is received, validate the manufacturer’s insert range by running the new lot in parallel with the current lot, and confirming that the results obtained are within the stated range
* When a new lot of unassayed control is received, verify new ranges by running the new lot in parallel with the current lot 30 times, and calculate a new range using the method mean ± 3 SD. Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes.
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| **Interferences** | Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions. If the estradiol results are inconsistent with clinical evidence, additional testing is suggested to confirm the result. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis. |
| **Reference Range** | **Male** Tanner Stage Reference Values:Stage I: (>14 days and prepubertal) Undetectable to 13 pg/mLStage II: (mean age 12 years) Undetectable to 16 pg/mLStage III: (mean age 13.6 years) Undetectable to 26 pg/mLStage IV: (mean age 15 years) Undetectable to 38 pg/mLStage V: (18 years) 10-40 pg/mL

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| Age | Range |
| 15 days – 1 year | 0-25 pg/mL |
| 1 - 11 years | 0-13 pg/mL |
| 11 -13 years | 0-26 pg/mL |
| 13 – 15 years  | 0-28 pg/mL |
| 15 - 19 years  | 0-38 pg/mL |

**Female** Tanner Stage Reference ValuesStage I: (>14 days and prepubertal) Undetectable to 20 pg/mLStage II: (mean age 10.5 years) Undetectable to 24 pg/mLStage III: (mean age 11.6 years) Undetectable to 60 pg/mLStage IV: (mean age 12.3 years) 15-85 pg/mLStage V: (14.5 years) 15-350pg/mL (will vary widely throughout menstrual cycle)

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| Age | Range |
| 15 days – 1 year | 0-25 pg/mL |
| 1 - 9 years | 0-10 pg/mL |
| 9 -11 years | 0-48 pg/mL |
| 11 – 12 years  | 0-94 pg/mL |
| 12 – 14 years  | 11-172 pg/mL |
| 14-19 years | 10-255 pg/mL |

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| **Critical Values** | None specified |
| **Limitations** | The instrument reporting system contains error messages to warn the operator of specific malfunctions. Refer to Operator’s Manual for troubleshooting specific error messages. |
| **Dilutions** | Samples with an estradiol value exceeding 1000.0 pg/mL are diluted with the automation dilution protocol on the Abbott Architect. The system performs a 1:5 dilution of the specimen and automatically calculates the concentration.For specimens >5000 pg/mL, perform a manual dilutionMaximum Manual Dilution factor: 1:101. Add exactly 20.0 μL of patient sample to exactly 180.0 μL of Architect Estradiol Manual Diluent
2. The operator must manually program the sample with the manual dilution factor. (See Abbott Architect Operating Procedure for details about manually programming a patient sample) [Abbott Architect i1000SR Operating Procedure](https://starnet.childrenshc.org/References/labsop/chem/procedure/ch5.106-abbott-architect-operating-procedure.pdf)
3. The analyzer will use this dilution factor to calculate the concentration of the patient sample before dilution, and report the result.
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| **Result Reporting** | * Results between 10 – 300 pg/mL without an error messages are released
* Results less than 10 are reported as <10 rather than the numerical value
* Results greater than 1000 pg/mL are diluted on board the analyzer at a dilution factor of 1:5
* Results greater than 5000 pg/mL are manually diluted with Estradiol Manual Diluent at a dilution factor of 1:10
* Results greater than 10,000 pg/mL are reported as >10,000 pg/mL rather than the numerical value
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| **Specimen Storage** | Specimens are stored in Special Chemistry Freezer in Minneapolis. Specimens will be stored for 7 days before being discarded. |
| **References** | 1. Abbott Architect Estradiol reagent package insert Abbott Laboratories, Abbott Park, IL, 60064. Revised Date June 2016
2. Abbott Architect Estradiol calibrator package insert Abbott Laboratories, Abbott Park, IL 60064. Revised June 2015.
3. Abbott Architect Safety Data Sheet, Abbott Diagnostics, Abbott Park, IL 60064. Revised 2016-05-04.
4. Abbott Architect Estradiol manual diluent package insert, Abbott Diagnostics, Abbott Park, IL 60064. Revised June 2015.
5. Bio-Rad Lyphochek Immunoassay Plus Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618 May 2017
6. Mayo Medical Laboratories, Tanner Stages Reference Values, [Mayo Medical Laboratories](https://www.mayomedicallaboratories.com/test-catalog/Clinical%2Band%2BInterpretive/81816).
7. [CALIPER reference studies](http://www.sickkids.ca/Caliperproject/index.html), accessed 4/20/2018.
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| **Historical Record** |

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| **Version** | **Written/Revised By** | **Effective Date** | **Summary of Revisions** |
| 1 | Kelsi Brown/Erin Bartos | 4/24/2018 | New Procedure |
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