ARCHITECT Estradiol

REF 7K72-25
REF 7K72-20
REF 7K72-35

en
Estradiol
7K72
G67636R10
B7K720

Read Highlighted Changes: Revised May 2019.

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

NAME

ARCHITECT Estradiol

INTENDED USE

The ARCHITECT Estradiol assay is a Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of estradiol in human serum and plasma.

SUMMARY AND EXPLANATION OF THE TEST

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 men and 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, 6.8×10^8 and 6×10^4.[1] 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.[2-5] 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.[6-8] At menopause, estradiol levels remain low.[2]

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.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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.

1. Sample, specimen diluent, assay diluent, and anti-estradiol (rabbit, monoclonal) coated paramagnetic microparticles are combined. Estradiol present in the sample binds to the anti-estradiol coated microparticles.
2. After incubation, estradiol acridinium-labeled conjugate is added to the reaction mixture.
3. After further incubation and washing, Pre-Trigger and Trigger Solutions are added to the reaction mixture.
4. 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.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Kit Contents

ARCHITECT Estradiol 7K72

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT iSystems. Please contact your local distributor.

REF	7K72-25	7K72-20	7K72-35
Σ	100	400	500
MICROPARTICLES	1 x 8.3 mL	4 x 8.3 mL	1 x 30.88 mL
CONJUGATE	1 x 5.9 mL	4 x 5.9 mL	1 x 26.27 mL
ASSAY DILUENT	1 x 5.9 mL	4 x 5.9 mL	1 x 26.27 mL
SPECIMEN DILUENT	1 x 10.0 mL	4 x 10.0 mL	1 x 50.90 mL

MICROPARTICLES Anti-Estradiol (rabbit, monoclonal) coated Microparticles in TRIS/BIS-TRIS buffer with protein (rabbit) stabilizers. Minimum Concentration: 0.0657% solids. Preservative: ProClin.

CONJUGATE Estradiol acridinium-labeled Conjugate in citrate buffer with surfactant stabilizers. Minimum concentration: 63.36 ng/mL. Preservative: ProClin.

ASSAY DILUENT Estradiol Assay Diluent containing surfactant in citrate buffer. Preservative: ProClin.

SPECIMEN DILUENT Estradiol Specimen Diluent containing TRIS buffer with protein (bovine) stabilizers. Preservative: Sodium Azide.

Other Reagents

PRE-TRIGGER SOLUTION ARCHITECT Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

TRIGGER SOLUTION ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide.

WASH BUFFER ARCHITECT Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

NOTE: Bottle and volume varies based on order.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[10-13]

The following warnings and precautions apply to: MICROPARTICLES / CONJUGATE / ASSAY DILUENT

WARNING:	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: SPECIMEN DILUENT

WARNING:	Contains diethylenetriamine pentaacetic acid (DTPA) and sodium azide.
H361	Suspected of damaging fertility or the unborn child.
H316*	Causes mild skin irritation.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P201	Obtain special instructions before use.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P308+P313	IF exposed or concerned: Get medical advice / attention.
P332+P313*	If skin irritation occurs: Get medical advice / attention.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

* Not applicable where regulation EC 1272/2008 (CLP) or OSHA Hazard Communication 29 CFR 1910.1200 (HCS) 2012 have been implemented.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Reagent Handling

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between kits.**
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **Mixing Instructions** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
- **ARCHITECT Estradiol requires the use of List Number 4D18-02 or higher septums.**
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
- Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.

For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Mixing Instructions

- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, discard the cap and place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Reagent Handling** section of this package insert.
- ARCHITECT Estradiol Calibrators and Controls should be mixed by gentle inversion prior to use.

Reagent Storage

When stored and handled as directed, reagents are stable until the expiration date.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened/ Opened*	2-8˚C	Until expiration date	May be used immediately after removal from 2-8˚C storage. Store in upright position.
On board	System temperature	30 days	Discard after 30 days. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

* Reagents may be stored on or off the ARCHITECT iSystem. If reagents are removed from the system, store them at 2-8˚C (with septums and replacement caps) in an upright position. For reagents stored off the system, place them in their original trays and boxes to ensure they remain upright and protect from long-term exposure to light. If any reagent bottle does not remain upright (with a septum installed) while in 2-8˚C storage off the system, the reagent kit must be discarded. For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- **The ARCHITECT Estradiol assay requires that ARCHITECT Trigger Solution be stored on-board for no longer than 10 days after the day it is installed. Write the on-board expiration date on the trigger bottle (install date plus 10 days is the on-board expiration date).**

NOTE: Trigger can be used through the on-board expiration date. This date must not exceed the printed expiration date of the ARCHITECT Trigger Solution.

- Install the **_Estradiol** assay file from the ARCHITECT iSystem Assay CD-ROM on the ARCHITECT iSystem before performing this Estradiol assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.

For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.

For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

Alternate Result Units

Edit assay parameter "Result concentration units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default result unit	Conversion factor	Alternate result unit
pg/mL	3.67	pmol/L
	0.00367	nmol/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Validated specimen types to be used with this assay:

	Collection Tubes	
Specimen Types	**Glass**	**Plastic**
Human serum	Serum	Serum
	Serum separator tubes	Serum separator tubes
Human plasma	Lithium heparin	Lithium heparin
	Plasma separator tubes	Plasma separator tubes
	Potassium EDTA	Potassium EDTA

- Other anticoagulants have not been validated for use with the ARCHITECT Estradiol assay.
- Performance has not been established for the use of cadaveric specimens or the use of body fluids other than human serum or plasma.
- The instrument does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - obvious microbial contamination
- For optimal results, serum and plasma specimens should be free of fibrin, red blood cells or other particulate matter.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

Preparation for Analysis

- Refer to the specimen collection tube manufacturer's instructions as well as these package insert instructions for specimen collection and preparation for analysis. Further specimen handling information can be found in Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Guideline H18-A2.[14]
- Specimens must be mixed THOROUGHLY after thawing, by LOW speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Sample from the middle of the tube to avoid any particulates on the top or bottom of the specimen.
- Inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Specimen Storage

Specimen Type	Storage Temperature	Maximum Storage Time
Serum/Plasma	2-8°C	≤ 7 days

If testing will be delayed more than 24 hours, remove serum or plasma from the clot, separator, or red blood cells and store at 2-8°C.

If testing will be delayed more than 7 days, specimens should be frozen at -20°C or colder.[15]

Avoid multiple freeze/thaw cycles.

Specimen Shipping

- Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

Sample Volume

- Minimum sample cup volume is calculated by the system and printed on the Orderlist report. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

  Maximum number of replicates sampled from the same sample cup: 9
  - Priority:

    Sample volume for first test: 200 µL

    Sample volume for each additional test from same sample cup: 150 µL
  - ≤ 3 hours on board:

    Sample volume for first test: 200 µL

    Sample volume for each additional test from same sample cup: 150 µL
  - > 3 hours on board: Additional sample volume is required. Refer to the ARCHITECT System Operations Manual, Section 5 for information on sample evaporation and volumes.
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

PROCEDURE

Materials Provided

7K72 ARCHITECT Estradiol Reagent Kit

Materials Required but not Provided

- ARCHITECT Estradiol Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 7K72-01 ARCHITECT Estradiol Calibrators
- 7K72-10 ARCHITECT Estradiol Controls
- 7K72-50 ARCHITECT Estradiol Manual Diluent
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Reaction Vessels
- ARCHITECT Sample Cups
- ARCHITECT Septum
  List Number 4D18-02 or higher
- ARCHITECT Replacement Caps
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

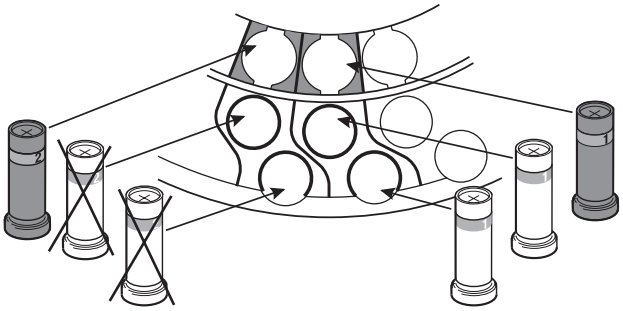
For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- **The ARCHITECT Estradiol assay requires that ARCHITECT Trigger Solution be stored on-board for no longer than 10 days after the day it is installed. Write the on-board expiration date on the trigger bottle (install date plus 10 days is the on-board expiration date).**

NOTE: Trigger can be used through the on-board expiration date. This date must not exceed the printed expiration date of the ARCHITECT Trigger Solution.

- Order tests.
- Load the reagent kit on the ARCHITECT iSystem.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
  - The reagent carousel has color coded rings which match the color bands on the reagent bottle labels.
  - The color bands on the reagent bottle labels have a #1 or #2 designation.
  - The diagram on the inside of the reagent carousel cover and the following diagram can be used to facilitate reagent loading.



  - Place the bottle with the yellow color band #1 (Assay Diluent) in the yellow ring of the carousel.
  - Place the bottle with the pink color band #1 (Microparticles) in the pink ring of the carousel.
  - Place the bottle with the green color band #1 (Specimen Diluent) in the green ring of the carousel.
  - Place the bottle with the yellow color band #2 (Conjugate) in the yellow ring of the carousel to the **left** of the bottle with the #1 designation.

    NOTE: Estradiol Reagent Kits do not contain bottles with a green and pink #2 designation.
  - Do not place Estradiol reagent bottles with a #1 designation in reagent carousel position 25 and reagent bottles with a #2 designation in reagent carousel position 1.
  - Prepare ARCHITECT Estradiol Calibrators and Controls.
    - Mix calibrator(s) and controls by gentle inversion before use.
    - Hold bottles **vertically** and dispense recommended volumes into each respective sample cup.
    - Recommended volumes:
      for each calibrator: 15 drops (400 µL)
      for each control: 10 drops (250 µL)
  - After each use tightly close the caps, place bottles in carton to protect from light and return the calibrators and controls to 2-8°C storage.
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For information on ordering patient specimens, calibrators and controls, and general operating procedures refer to the ARCHITECT System Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Specimen Dilution Procedures

Specimens with an estradiol value exceeding 1,000 pg/mL are flagged with the code ">1000" and may be diluted using the Automated Dilution Protocol.

Automated Dilution Protocol

The system performs a 1:5 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result.

Specimens with an estradiol value exceeding 5,000 pg/mL are flagged with the code ">5000" when run using the Automated Dilution Protocol. These specimens may be diluted with the Manual Dilution Procedure.

Manual Dilution Procedure

Suggested dilution: 1:10

1. For example, add 20 µL of the patient specimen to 180 µL of ARCHITECT Estradiol Manual Diluent.
2. The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result. The result should be > 100 pg/mL before the dilution factor is applied.

For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- Test Calibrators A through F in duplicate. The calibrators should be priority loaded.

  A single sample of each control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the respective control package insert.
- Calibration Range: 0 - 1000 pg/mL.
- Once an ARCHITECT Estradiol calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used or
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

Quality Control Procedures

The recommended control requirement for the ARCHITECT Estradiol assay is that a single sample of each control level be tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures.

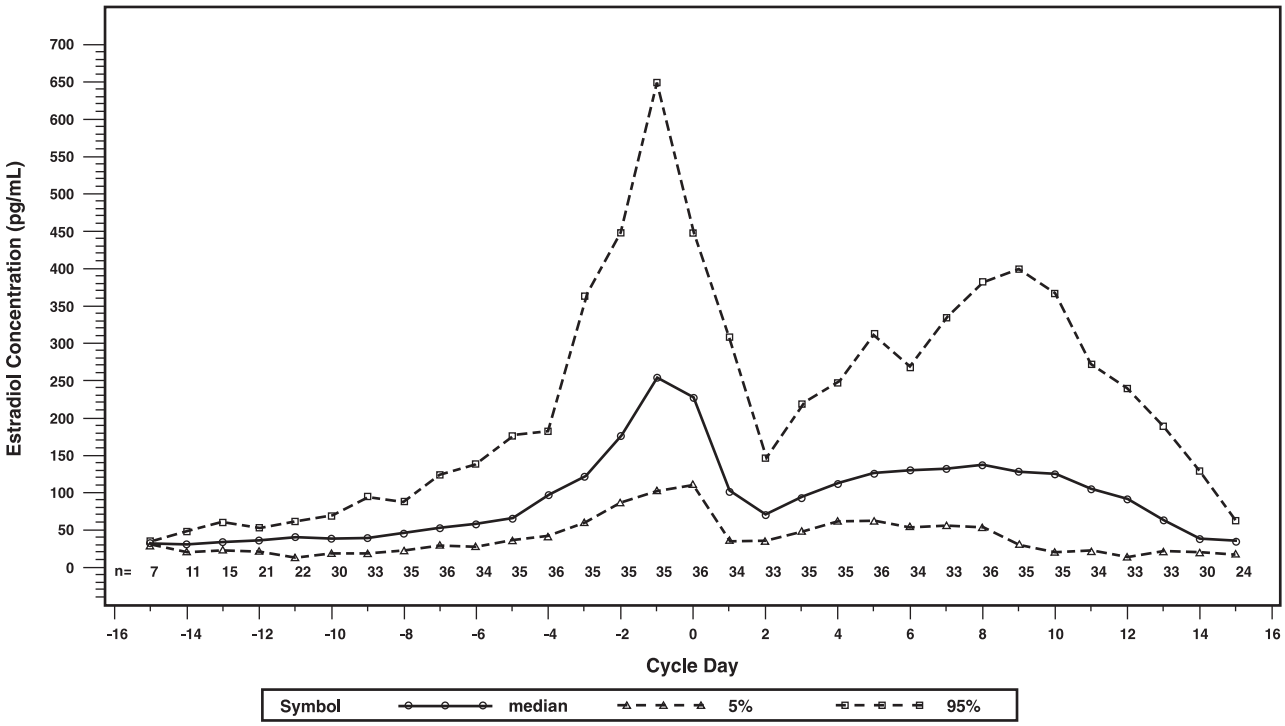
Ensure that assay control values are within the concentration ranges specified in the control package insert.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B.

The ARCHITECT Estradiol assay belongs to method group 1.

ARCHITECT Estradiol Profile During the Normal Menstrual Cycle



RESULTS

Calculation

The ARCHITECT Estradiol assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration curve.

For information on alternate result units, refer to the **INSTRUMENT PROCEDURE, Alternate Result Units** section of this package insert.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- 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.[16]

EXPECTED VALUES

The expected ranges for the ARCHITECT Estradiol assay were obtained by testing specimens drawn from 101 males, 72 postmenopausal females and normal menstruating females. For the normal menstruating female ranges, specimens were obtained from 36 women drawn throughout their cycle, resulting in a total of 956 specimens. Variations in cycle length were normalized by aligning the cycles based on Day 0 as the day of the LH peak (same day as the FSH peak and same day or one day after the estradiol peak). To establish cycle-specific reference ranges, the specimens were categorized as follicular phase, mid-cycle phase and luteal phase. Follicular phase was defined as the period of time from 15 days to 2 days prior (-15 to -2) to the period of the mid-cycle gonadotropin surge (Days -1 to +1). The luteal phase was defined as +2 days to +15 days.[17] All cycles included in establishing reference ranges were ovulatory.

The results are presented below.*

		Estradiol Concentration Value	
Population	n	Median (pg/mL)	Central 95% Range (pg/mL)
Normal Menstruating Females			
Follicular Phase	385	54	21 - 251
Mid-Cycle Phase	105	196	38 - 649
Luteal Phase	466	99	21 - 312
Postmenopausal Females not on HRT	50	<10	<10 - 28
Postmenopausal Females on HRT**	22	28	<10 - 144
Males	101	23	11 - 44

* Representative data; results in individual laboratories may vary from these data.

** For n=22, the central 95% range is the same as the range from minimum to maximum.

It is recommended that each laboratory establish its own expected ranges which may be unique to the population it serves depending on the geographical, patient, dietary or environmental factors.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT Estradiol assay precision is ≤ 5 pg/mL (total SD) for concentrations in the range of the low control (target 45 pg/mL), and ≤ 7% (total CV) for concentrations in the range of the medium control (target 190 pg/mL) and the high control (target 600 pg/mL).

A study was performed for the ARCHITECT Estradiol assay based on guidance from CLSI protocol EP5-A.[18] Three control levels (low, medium, and high) were assayed, using three lots of reagents, in replicates of two at two separate times per day for 20 days on two instruments. Data from this study are summarized below.*

	Reagent			Mean Conc. Value	Within Run		Total	
Control	Lot	Instrument	n	(pg/mL)	SD	%CV	SD	%CV
Low	1	1	80	48	2.6	5.5	3.3	6.7
Low	1	2	80	47	2.4	5.0	2.8	6.0
Low	2	1	80	45	2.9	6.4	3.3	7.4
Low	2	2	80	46	2.1	4.6	2.6	5.6
Low	3	1	80	45	2.7	6.0	3.0	6.6
Low	3	2	80	47	2.3	4.8	3.3	7.1
Medium	1	1	80	188	3.5	1.9	4.0	2.1
Medium	1	2	80	188	3.6	1.9	4.3	2.3
Medium	2	1	80	188	4.2	2.3	4.6	2.4
Medium	2	2	80	190	3.3	1.7	4.3	2.3
Medium	3	1	80	183	3.7	2.0	4.5	2.5
Medium	3	2	80	192	3.0	1.5	4.3	2.3
High	1	1	80	596	10.5	1.8	10.5	1.8
High	1	2	80	593	8.5	1.4	10.6	1.8
High	2	1	80	606	9.8	1.6	11.5	1.9
High	2	2	80	604	10.3	1.7	10.9	1.8
High	3	1	80	590	10.9	1.8	13.7	2.3
High	3	2	80	591	10.7	1.8	15.2	2.6

* Representative data; results in individual laboratories may vary from these data.

Analytical Sensitivity

The analytical sensitivity of the ARCHITECT Estradiol assay is ≤ 10 pg/mL. Analytical sensitivity is defined as the concentration at two standard deviations from the mean RLU value of the ARCHITECT Estradiol Calibrator A (0 pg/mL) and represents the lowest measurable concentration of estradiol that can be distinguished from zero.

Functional Sensitivity

The functional sensitivity of the ARCHITECT Estradiol assay is ≤ 25 pg/mL.

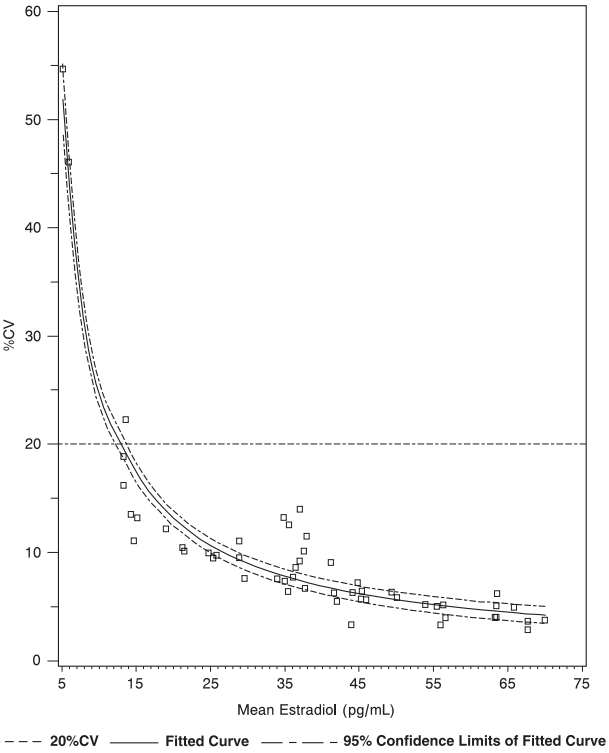
Functional sensitivity is defined as the lowest concentration that can be measured with a coefficient of variation (CV) less than or equal to 20%.

A study was conducted on two instruments and three reagent lots using human panels which were prepared at target concentrations ranging from 0 to 70 pg/mL. The panels were assayed in replicates of three over five days for a total of 15 replicates per panel, instrument and lot combination. Mean and %CV were calculated for each of the 15 replicate combinations. For each instrument, the calculated %CVs were plotted against the corresponding means for all panels and all three reagent lots. A reciprocal curve (Y=a+b/X) was fitted through the data and the functional sensitivity was estimated as the concentration corresponding to the 20% CV level of the fitted curve.

The following functional sensitivities were determined:*

Instrument	Functional Sensitivity
A	13 pg/mL
B	14 pg/mL

The precision profile corresponding to the functional sensitivity determination for instrument A is shown below.



* Representative data; results in individual laboratories may vary from these data.

Specificity

The specificity of the ARCHITECT Estradiol assay was determined by studying the compounds listed below in either the absence or presence of estradiol using guidance from CLSI protocol EP7-A.[19]

Table A*

A study was performed in which synthetic specimens containing essentially no residual estradiol were supplemented with potential cross reactants at the concentrations listed and tested for estradiol. The percent cross reactivity is shown below:

Cross Reactant	Concentration Cross Reactant	% Cross Reactivity
17β-Estradiol 3-sulfate	50 ng/mL	0.1%
Estrone	1500 pg/mL	0.7%

Cross Reactivity of the following compounds was undetectable at the concentrations listed below:

Cross Reactant	Concentration Cross Reactant
Aldosterone	10 µg/mL
5α-Androstan-3β,17β-diol	10 ng/mL
5α-Androstandione	10 ng/mL
Androstenedione	100 ng/mL
Clomiphene citrate	60 ng/mL
Corticosterone	570 ng/mL
Cortisone	500 ng/mL
Deoxycorticosterone acetate	500 ng/mL
11-Deoxycortisol	500 ng/mL
Dexamethasone	12,770 ng/mL
DHEA	120 ng/mL
DHEAS	8 µg/mL
5β-Dihydrocorticosterone	500 ng/mL
DHT (Dihydrotestosterone)	2 ng/mL
Equilin	0.6 ng/mL
Equilin Sulfate	5 ng/mL
Estetrol	2.4 ng/mL
17α Estradiol	0.3 ng/mL
17β-Estradiol-3-glucuronide	4.8 ng/mL
17β-Estradiol 17-valerate	1 ng/mL
17β-Estradiol 17-propionate	1 ng/mL
17β-Estradiol 3-sulfate17-glucuronide	50 ng/mL
Estriol	2500 pg/mL
Estriol 16α-(β-D-glucuronide)	106 ng/mL
Estriol 3-sulfate	300 ng/mL
Estriol 3-(β-D-glucuronide)	106 ng/mL
Estrone 3-sulfate	0.4 ng/mL
Ethynodiol diacetate	1 µg/mL
Ethynylestradiol	0.4 ng/mL
Hydrocortisone	500 ng/mL
16α-Hydroxyestrone	1 ng/mL
17α-Hydroxypregnanolone	480 ng/mL
17α-Hydroxyprogesterone	1200 ng/mL
Medroxyprogesterone	12.3 ng/mL
Mestranol	0.4 ng/mL
Norethindrone	16 ng/mL
Norethindrone acetate (Norethisterone acetate)	14 ng/mL
Pregnanolone	59 ng/mL
Progesterone	500 ng/mL
Tamoxifen	183 ng/mL
Testosterone	20 ng/mL

* Representative data; results in individual laboratories may vary from these data.

This assay should NOT be used to assess estradiol levels for patients undergoing Fulvestrant or Mifepristone treatment. Structural and functional analogues of steroid hormones, including the estradiol molecule, have the potential to cause interference/cross reactivity with the ARCHITECT Estradiol assay. Samples from patients administered medications which inhibit tumour cell proliferation (e.g. CDK 4/6 inhibitors) may be subject to interference/cross reactivity with the ARCHITECT Estradiol assay. In addition, drugs which interfere with or activate production of steroid hormones (e.g. Aromatase inhibitors) may also interfere or cross react with the ARCHITECT Estradiol assay. In such cases, an alternate method such as chromatography should be used.

Table B*

The ARCHITECT Estradiol assay recovery in the presence of the following compounds is 100 ± 40% at the concentrations listed below:

A study was performed in which synthetic specimens containing estradiol (600 pg/mL) were supplemented with potential interferents at the concentrations listed and tested for estradiol. The percent recovery is shown below:

Interferent	Concentration Interferent	% Recovery
Equilin	1.2 ng/mL	98.4
Equilin Sulfate	10 ng/mL	92.6
Ethynylestradiol	0.8 ng/mL	88.6
Mestranol	0.8 ng/mL	100.5
Norethindrone	32 ng/mL	76.9
Norethindrone acetate (Norethisterone acetate)	28 ng/mL	99.5

The ARCHITECT Estradiol assay recovery in the presence of the following compounds is 100 ± 10% at the concentrations listed below:

A study was performed in which synthetic specimens containing estradiol (600 pg/mL) were supplemented with potential interferents at the concentrations listed and tested for estradiol. The percent recovery is shown below:

Interferent	Concentration Interferent	% Recovery
Aldosterone	10 µg/mL	100.1
5α-Androstan-3β,17β-diol	10 ng/mL	98.6
5α-Androstandione	10 ng/mL	99.6
Androstenedione	100 ng/mL	100.1
Clomiphene citrate	60 ng/mL	98.8
Corticosterone	570 ng/mL	99.1
Cortisone	500 ng/mL	98.4
Deoxycorticosterone acetate	500 ng/mL	98.9
11-Deoxycortisol	500 ng/mL	100.4
Dexamethasone	12,770 ng/mL	100.5
DHEA	120 ng/mL	99.8
DHEAS	8 µg/mL	100.2
5β-Dihydrocorticosterone	500 ng/mL	100.9
DHT (Dihydrotestosterone)	2 ng/mL	100.9
Estetrol	2.0 ng/mL	93.0
17α Estradiol	0.3 ng/mL	100.7
17β-Estradiol-3-glucuronide	4.8 ng/mL	98.8
17β-Estradiol 17-valerate	1 ng/mL	100.4
17β-Estradiol 17-propionate	1 ng/mL	100.1
17β-Estradiol 3-sulfate	50 ng/mL	105.1
17β-Estradiol 3-sulfate17-glucuronide	50 ng/mL	99.6
Estriol 16α-(β-D-glucuronide)	106 ng/mL	101.3
Estriol 3-sulfate	300 ng/mL	97.9
Estriol 3-(β-D-glucuronide)	106 ng/mL	100.1
Estrone 3-sulfate	0.4 ng/mL	100.1
Ethynodiol diacetate	1 µg/mL	97.7
Hydrocortisone	500 ng/mL	99.4
16α-Hydroxyestrone	1 ng/mL	100.2
17α-Hydroxypregnanolone	480 ng/mL	100.0
17α-Hydroxyprogesterone	1200 ng/mL	98.9
Medroxyprogesterone	12.3 ng/mL	99.1
Pregnanolone	59 ng/mL	100.2
Progesterone	500 ng/mL	100.5
Tamoxifen	183 ng/mL	100.9
Testosterone	20 ng/mL	98.1

* Representative data; results in individual laboratories may vary from these data.

This assay should NOT be used to assess estradiol levels for patients undergoing Fulvestrant or Mifepristone treatment. Structural and functional analogues of steroid hormones, including the estradiol molecule, have the potential to cause interference/cross reactivity with the ARCHITECT Estradiol assay. Samples from patients administered medications which inhibit tumour cell proliferation (e.g. CDK 4/6 inhibitors) may be subject to interference/cross reactivity with the ARCHITECT Estradiol assay. In addition, drugs which interfere with or activate production of steroid hormones (e.g. Aromatase inhibitors) may also interfere or cross react with the ARCHITECT Estradiol assay. In such cases, an alternate method such as chromatography should be used.

Table C*

The ARCHITECT Estradiol assay recovery in the presence of the following compounds is 100 ± 10% at the concentrations listed below:

A study was performed in which synthetic specimens containing estradiol (concentrations listed below) were supplemented with potential interferents at the concentrations listed and tested for estradiol. The percent recovery is shown below:

Interferent	Concentration Estradiol	Concentration Interferent	% Recovery
Estrone	750 pg/mL	300 pg/mL	93.9
Estrone	4000 pg/mL	1500 pg/mL	92.8
Estriol	4000 pg/mL	1500 pg/mL	98.4
Estriol	150 pg/mL	2500 pg/mL	92.1

* Representative data; results in individual laboratories may vary from these data.

Interference

Potential interference in the ARCHITECT Estradiol assay from hemoglobin, bilirubin, triglycerides, protein, and cholesterol at the levels indicated below is ≤ 10%. Interference was evaluated in a study based on guidance from CLSI protocol EP7-A.[19]

- Hemoglobin at 500 mg/dL
- Bilirubin at 20 mg/dL
- Triglycerides at 1000 mg/dL
- Protein at 4 and 12 g/dL
- Cholesterol at 240 mg/dL

Method Comparison

The slope of the ARCHITECT Estradiol assay versus isotope dilution-gas chromatography/mass spectrometry (ID-GCMS)[20] is 1.00 ± 0.10 from 10 to 1000 pg/mL and 1.00 ± 0.15 from 10 to 5,000 pg/mL. The correlation coefficient is ≥ 0.95 for both ranges.

A study was performed based on guidance from CLSI EP9-A2.[21] The ARCHITECT Estradiol assay was compared to isotope dilution-gas chromatography/mass spectrometry (ID-GCMS). Specimens with known GCMS concentrations were assayed using the ARCHITECT Estradiol assay. Due to the limited number of GCMS specimens, each specimen was tested with three different reagent lots; one replicate per lot. The mean of the three replicates for each specimen was calculated. The calculated mean was used for the pooled regression analysis. In addition, the correlation coefficient from individual replicates vs. GCMS and the regression statistics from each reagent lot were also calculated. The regression analysis was performed by both Least Squares and Passing- Bablok[22]. Data from this study are summarized below.*

Abbott ARCHITECT Estradiol vs. ID-GCMS (Individual Specimens)

Range	n	Min GCMS Conc.	Max GCMS Conc.	Correlation Coefficient
10 to 1000 pg/mL	315	13.3	945.2	0.99
10 to 5000 pg/mL	393	13.3	4269.2	0.99

Regression Method	n	Pooled Intercept	Pooled Slope	Slope per Lot (Lot 1, Lot 2, Lot 3)
10 to 1000 pg/mL Least Squares	105	0	1.03	0.99, 1.03, 1.08
Passing-Bablok**	105	2	1.02	0.98, 1.02, 1.06
10 to 5000 pg/mL Least Squares	131	-31	1.09	1.08, 1.08, 1.12
Passing-Bablok**	131	0	1.04	1.00, 1.03, 1.07

* Representative data; results in individual laboratories may vary from these data.

** A linear regression method with no special assumptions regarding the distribution of the samples and the measurement errors.

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Key to Symbols

ISO 15223 Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
REF	List Number
SN	Serial number

Other Symbols

ASSAY DILUENT	Assay Diluent
CONJUGATE	Conjugate
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTROL NO.	Control Number
DISTRIBUTED IN THE USA BY	Distributed in the USA by
INFORMATION FOR USA ONLY	Information needed for United States of America only
MICROPARTICLES	Microparticles
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCT OF IRELAND	Product of Ireland
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SPECIMEN DILUENT	Specimen Diluent
TRIGGER SOLUTION	Trigger Solution
WARNING: REPRODUCTIVE HAZARD	Warning: Reproductive Hazard
WARNING: SENSITIZER	Warning: May cause an allergic reaction.
WASH BUFFER	Wash Buffer

Abbott Ireland
Diagnostics Division
Lisnamuck, Longford
Co. Longford
Ireland
+353-43-3331000

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Revised May 2019.

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