Alinity i Estradiol-08

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BIENNIAL REVIEW:

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity i Estradiol assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of estradiol in serum and plasma on the Alinity i analyzer.

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, 3, 4, 5]

If conception occurs, estradiol levels continue to rise, reaching levels of 1000-5000 pg/mL during the first trimester, 5000-15 000 pg/mL during second trimester, and 10 000-40 000 pg/mL during third trimester.[6, 7, 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

This assay is a delayed one-step immunoassay for the quantitative determination of estradiol in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Sample, anti-estradiol (rabbit, monoclonal) coated paramagnetic microparticles, specimen diluent, and assay diluent are combined and incubated. The estradiol present in the sample binds to the anti-estradiol coated microparticles. Estradiol acridinium-labeled conjugate is added to create a reaction mixture. The reaction mixture is incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

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 system optics.

For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3.

REAGENTS

Kit Contents

Alinity i Estradiol Reagent Kit 07P50

NOTE: This product is composed of 4 components, which are packaged as a 2 cartridge reagent set. Both cartridges are required to perform the assay.

Volumes (mL) listed in the table below indicate the volume per cartridge set.

REF	07P5020	07P5030
Tests per cartridge set	100	500
Number of cartridge sets per kit	2	2
Tests per kit	200	1000
MICROPARTICLES	8.3 mL	33.8 mL
CONJUGATE	6.1 mL	26.5 mL
ASSAY DILUENT	6.1 mL	26.5 mL
SPECIMEN DILUENT	10.4 mL	47.1 mL

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

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

ASSAY DILUENT Surfactant in citrate buffer. Preservative: ProClin.

SPECIMEN DILUENT TRIS buffer with protein (bovine) stabilizer. Preservative: sodium azide.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY

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], [11], [12], [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 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/and SDS folder.

For a detailed discussion of safety precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 8.**

Reagent Handling

NOTE: Alinity i Estradiol Conjugate and Assay Diluent bottles do not contain a septum by design.

Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.

–Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.

- After mixing, place reagent cartridges in an upright position for 8 hours before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 7.

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright,

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
			gently invert the cartridge 10 times and place in an upright position for 8 hours before use.
On board	System Temperature	30 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, **refer to the Alinity ci-series Operations Manual, Section 5.**

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10**.

INSTRUMENT PROCEDURE

The Alinity i Estradiol assay requires that the Alinity Trigger Solution be stored on-board for no longer than 10 days after the day the reagent is installed. The system automatically tracks the on-board stability of the Trigger solution in the replacement bottle and the reservoir.

NOTE: If the on-board stability for the Trigger solution expires perform the Empty the bulk solution reservoirs (i-series) procedure found in Section 10 of the Operations Manual.

The Alinity i Estradiol assay file must be installed on the Alinity i analyzer prior to

performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 2.**

For information on printing assay parameters**, refer to the Alinity ci-series Operations Manual, Section 5.**

For a detailed description of system procedures, **refer to the Alinity ci-series Operations Manual.**

Alternate Result Units

Edit assay parameter "Result 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

The specimen types listed below were verified for use with this assay.

Other specimen types and collection tube types have not been verified with this assay.

Specimen Types	Collection Tubes
Serum	Serum
	Serum separator
Plasma	Lithium heparin
	Plasma separator
	Potassium EDTA

Specimen Conditions

Do not use:

–heat-inactivated specimens

–specimens with obvious microbial contamination

- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by gently inverting prior to recentrifugation.

Prepare frozen specimens as follows:

- Frozen specimens must be completely thawed before mixing.
- Mix thawed specimens thoroughly by low speed vortex or by gently inverting.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Recentrifuge specimens.

Recentrifugation of Specimens

- Transfer specimens to a centrifuge tube and centrifuge.
- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

Specimen Storage

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/Plasma	2 to 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.[14]

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.

PROCEDURE

Materials Provided

07P50Alinity i Estradiol Reagent Kit

Materials Required but not Provided

- Alinity i Estradiol assay file
- 07P5001 Alinity i Estradiol Calibrators
- 07P5010 Alinity i Estradiol Controls or other commercially available controls
- 07P5040 Alinity i Estradiol Manual Diluent Kit
- Alinity Trigger Solution
- Alinity Pre-Trigger Solution
- Alinity i-series Concentrated Wash Buffer

For information on materials required for operation of the instrument, **refer to the Alinity ci-series Operations Manual, Section 1.**

For information on materials required for maintenance procedures, **refer to the Alinity ci-series Operations Manual, Section 9.**

Assay Procedure

The Alinity i Estradiol assay requires that the Alinity Trigger Solution be stored on-board for no longer than 10 days after the day the reagent is installed. The system automatically tracks the on-board stability of the Trigger solution in the replacement bottle and the reservoir.

NOTE: If the on-board stability for the Trigger solution expires perform the Empty the bulk solution reservoirs (i-series) procedure found in Section 10 of the Operations Manual.

For a detailed description of how to run an assay, **refer to the Alinity ci-series Operations Manual, Section 5.**

- If using primary or aliquot tubes, **refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present**.
- 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 the reagent and sample manager:

◦Sample volume for first test: 200 µL

◦Sample volume for each additional test from same sample cup: 150 µL

> 3 hours on the reagent and sample manager:

◦Replace with a fresh aliquot of sample.

- Refer to the Alinity i Estradiol calibrator package insert and Alinity i Estradiol control package insert for preparation and usage.
- For general operating procedures, **refer to the Alinity ci-series Operations Manual, Section 5.**
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, **Section 9. Perform maintenance more frequently when required by laboratory procedures.**

Sample Dilution Procedures

Samples with an estradiol value exceeding 1000 pg/mL (3670 pmol/L) are flagged with the code "> 1000 pg/mL" (> 3670 pmol/L) and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a **1:5** dilution of the sample and automatically calculates the

concentration by multiplying the result by the dilution factor.

After the automatic dilution is performed, if the sample concentration is > 5000 pg/mL (> 18 350 pmol/L), dilute the sample 1:10 and run using the Manual Dilution Procedure.

Manual Dilution Procedure

Suggested dilution**: 1:10**

Add 20 µL of the sample to 180 µL of Alinity i Estradiol Manual Diluent.

The operator must enter the dilution factor in the Specimen or Control tab of the Create Order screen. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result. The result should be > 100 pg/mL (367 pmol/L) before the dilution factor is applied.

If the operator does not enter the dilution factor, the result must be manually multiplied by the appropriate dilution factor before reporting the result. If a diluted sample result is less than 100 pg/mL (367 pmol/L), do not report the result. Rerun using an appropriate dilution.

For detailed information on ordering dilutions, refer to the Alinity ci-series Operations Manual, Section 5.

Calibration

For instructions on performing a calibration, **refer to the Alinity ci-series Operations Manual, Section 5.**

Each assay control must be tested to evaluate the assay calibration.

Once a 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.
- Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert.

–If **statistically-based quality control limits are not available**, then the calibration should not exceed **a 30-day limit for recalibration** frequency.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

The recommended control requirement for the Alinity i Estradiol assay is that a single sample of each control level be tested once every day testing performed.

To establish statistically-based control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level.

This can be accomplished by assaying **a minimum of 20 replicates over several (3-5) days** and using the reported results to establish the expected average (target) and variability about this average (range) for the laboratory. Sources of variation that should be included in this study in order to be representative of future system performance include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules (if applicable)
- Data points collected at different times of the day

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) Document C24-A3 or other published guidelines, for general quality control recommendations.*15*

- If quality control results do not meet the acceptance criteria defined by laboratory QC procedure, sample results may be suspect. Follow the established quality control procedures to troubleshoot. Recalibration may be necessary. For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10.**
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Commercial controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance.

For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

Quality Control Guidance

Refer to “Basic QC Practices” by James O Westgard, Ph.D. for guidance on laboratory quality control practices.*16*

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

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

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 Alinity ci-series Operations Manual, Section 5.

Measuring Interval

Measuring interval is defined as the range of values in pg/mL (pmol/L) which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity i Estradiol assay is **24 to 1000 pg/mL** (88 to 3670 pmol/L).

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.[17]

EXPECTED VALUES

This study was performed on the ARCHITECT i System.

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The manufacturers provided reference ranges adopted and difficult to verify

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.[18]

All cycles included in establishing reference ranges were ovulatory.

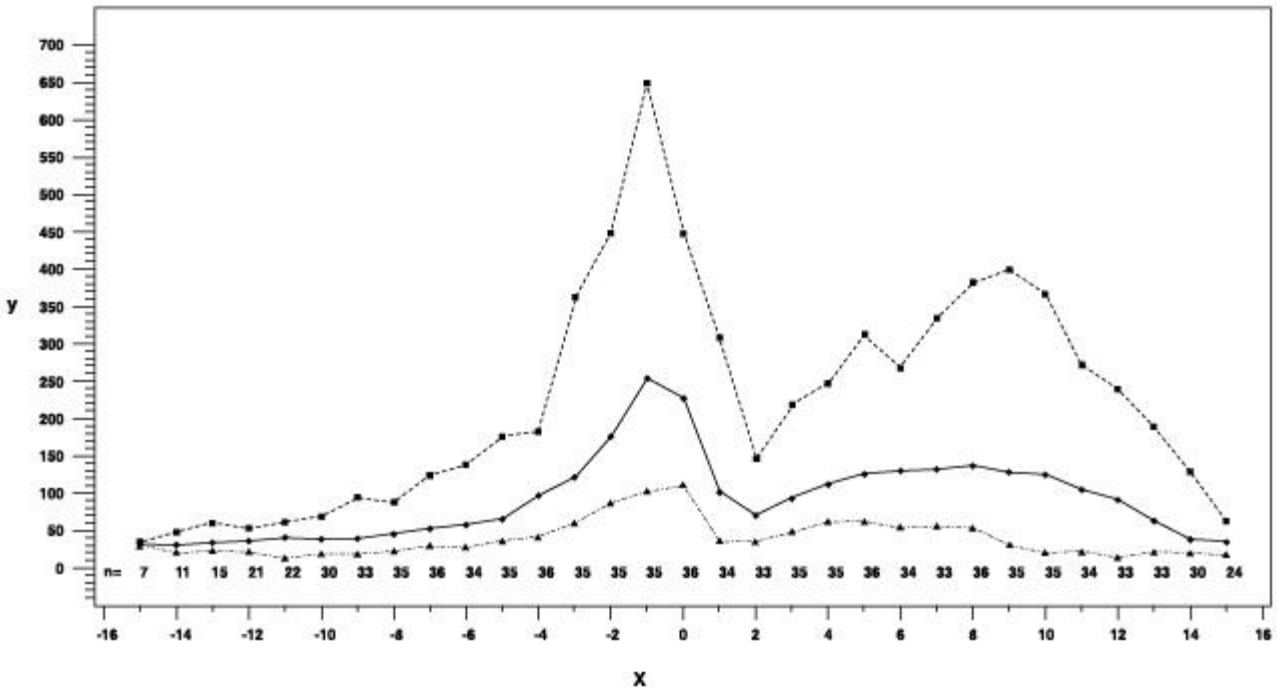
The results are presented below.

Population	n	Estradiol Concentration Value	
		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

HRT = Hormone Replacement Therapy

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

ARCHITECT Estradiol Profile During the Normal Menstrual Cycle



x	Cycle Day		Median
y	Estradiol Concentration (pg/mL)		5%
			95%

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity i analyzer and the ARCHITECT i System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity i analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2.[19] Testing was conducted using 1 lot of the Alinity i Estradiol Reagent Kit, 1 lot of the Alinity i Estradiol Calibrators, and 1 lot of the Alinity i Estradiol Controls and 1 instrument. Three controls were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

Sample	n	Mean (pg/mL)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Low Control	120	49	3.5	7.1	3.8	7.7
Medium Control	120	188	4.5	2.4	4.9	2.6
High Control	120	587	12.8	2.2	15.2	2.6

[a]Includes within-run, between-run, and between-day variability.

Sample	n	Mean (pmol/L)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Low Control	120	180	13.0	7.2	13.9	7.7
Medium Control	120	688	16.4	2.4	17.9	2.6
High Control	120	2156	47.1	2.2	55.9	2.6

[a]Includes within-run, between-run, and between-day variability.

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2.[20] Testing was conducted using 3 lots of the Alinity i Estradiol Reagent Kit on each of 2 instruments over a minimum of 3 days. The maximum observed Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below.

	pg/mL	pmol/L
LoB[a]	13	48
LoD[b]	20	73
LoQ[c]	**24**	88

[a]The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b]The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ was determined from n ≥ 60 replicates of low-analyte level samples and is defined as the lowest concentration at which a maximum allowable precision of 20 %CV was met.

Linearity

A study was performed based on guidance from CLSI EP06-A.[21]

This assay is linear across the measuring interval of **24 to 1000 pg/mL** (88 to 3670 pmol/L).

Analytical Specificity

This study was performed on the ARCHITECT i System.

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.[22]

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

Cross Reactant	Concentration Cross Reactant
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

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 Alinity i 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 Alinity i 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 Alinity i 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

Interferent	Concentration Interferent	% Recovery
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

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 Alinity i 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 Alinity i 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 Alinity i 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

Interference

This study was performed on the ARCHITECT i System.

Potentially Interfering Endogenous Substances

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.[22]

Potentially Interfering Substance	Interferent Level Conventional Units
Hemoglobin	500 mg/dL
Bilirubin	20 mg/dL
Triglycerides	1000 mg/dL
Protein	4 and 12 g/dL
Cholesterol	240 mg/dL

Method Comparison

A study was performed based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method.[23]

		Units	n	Correlation Coefficient	Intercept	Slope	Concentration Range
Alinity i Estradiol vs ARCHITECT Estradiol	Serum	pg/mL (pmol/L)	120	0.99	2.97 (10.69)	1.07	12-873 (44-3202)

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