VIDAS® Estradiol II (E2II)

IVD

VIDAS® Estradiol II is an automated quantitative test for use on the VIDAS family of instruments for the quantitative measurement of total 17 β -estradiol in human serum or plasma (lithium heparin), using the ELFA technique (Enzyme Linked Fluorescent Assay). The VIDAS® E2 II assay is intended for use as an aid in the diagnosis and treatment of various hormonal sexual disorders and in assessing placental function in complicated pregnancy.

SUMMARY AND EXPLANATION

Estradiol is secreted primarily by the ovarian follicles during the female menstrual cycle, and by the placenta during pregnancy (1).
It is secreted to a lesser degree by the adrenal glands, testes and by peripheral conversion of androgens.
Estrogen receptors can be found in many tissues (bones, mammary glands, liver, endometrium, etc.). The synthesis and secretion of estradiol are regulated by the hypothalamic-pituitary axis, through LHRH, LH and FSH (2, 3, 4).
In cases of suspected hypofertility in either sex, evaluation of the estradiol concentration, in conjunction with LH, FSH, and progesterone assays, is useful for evaluating whether the hypothalamic-pituitary-gonadal axis functions correctly and is intact (5, 6).
Quantitative measurement of estradiol is useful for the evaluation and management of a number of sexual disorders: delayed or precocious puberty, menstrual cycle disorders, menopause, ovulation induction, and gynecomastia (7, 8, 9, 5).
During ovarian stimulation for the purpose of medically assisted procreation, the assay of estradiol enables follicular maturation to be monitored and assessed (8, 9, 10).
The estradiol assay, together with LH and FSH assays, is used to confirm a diagnosis of menopause (7).

PRINCIPLE

The assay principle combines a competition method with a final fluorescent detection (ELFA).
The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.
The sample is transferred into the well containing the conjugate, which is an alkaline phosphatase-labeled estradiol derivative. The estradiol present in the serum and the estradiol derivative in the conjugate compete for the anti-estradiol specific antibody sites coated to the inner surface of the SPR.
Unbound components are eliminated during the washing steps.
During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm.
The intensity of the fluorescence is inversely proportional to the concentration of antigen present in the sample.
At the end of the assay, the results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

CONTENT OF THE KIT (60 TESTS) - RECONSTITUTION OF REAGENTS

60 E2 II strips	STR	Ready-to-use.
60 E2 II SPRs 2 x 30	SPR	Ready-to-use. Interior of SPRs coated with polyclonal anti-estradiol immunoglobulins (rabbit).
E2 II control 1 x 3 mL (liquid)	C1	Ready to use. Human serum* + 17 β -estradiol + 1 g/L sodium azide. MLE data indicate the confidence interval in pg/mL (“Control C1 Dose Value Range”).
E2 II calibrator 2 x 4 mL (liquid)	S1	Ready to use. Human serum* + 17 β -estradiol + 1 g/L sodium azide. MLE data indicate the concentration in pg/mL (“Calibrator (S1) Dose Value”) and the confidence interval in "Relative Fluorescence Value” (“Calibrator (S1) RFV Range”).
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit, or • MLE bar code printed on the box label.		
1 Package insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

* This product has been tested and shown to be negative for HBs antigen and antibodies to HIV1, HIV2 and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

The SPR

The interior of the SPR® is coated during production with polyclonal anti-estradiol immunoglobulins (rabbit). Each SPR is identified by the E2II code. Only remove the required number of SPRs from the pouch and **carefully reseal the pouch after opening.**

The strip

The strip consists of 10 wells covered with a labeled foil seal. The label comprises a bar code which indicates the type of test performed, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorimetric reading is performed. The wells in the center section contain the various reagents required for the assay.

Description of the E2 II strip

Wells	Reagents
1	Sample well.
2 – 3 - 4	Empty wells.
5	Conjugate: alkaline phosphatase-labeled estradiol derivative + 0.9 g/L sodium azide (400 µl).
6	Empty well.
7 - 8	Wash buffer: Tris-NaCl (0.05 mol/L) pH 9 + 1 g/L sodium azide (600 µl).
9	Wash buffer: diethanolamine*(DEA) (1.1 mol/L, or 11.5%, pH 9.8) + 1 g/L sodium azide (600 µl).
10	Cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/L) + diethanolamine** (0.62 mol/L, or 6.6%, pH 9.2) + 1 g/L sodium azide (300 µl).

* Signal Word: **DANGER**

Hazard statement

H318: Causes serious eye damage.
H373: May cause damage to organs through prolonged or repeated exposure.
H315: Causes skin irritation.
H302: Harmful if swallowed.

Precautionary statement

P280: Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P309 + P311 : IF exposed or if you feel unwell: Call a POISON CENTER or doctor/physician.

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For further information, refer to the Material Safety Data Sheet.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Pipette with disposable tip to dispense 200 µL.
- Powderless, disposable gloves.
- For other specific materials and disposables, refer to the Instrument User Manual
- Instrument of the VIDAS® family.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- **This kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (see Laboratory Biosafety Manual - WHO - Geneva - latest Edition).**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use SPR®s if the pouch is pierced.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Do not use reagents after the expiration date indicated on the label.
- Do not mix reagents (or disposables) from different lots.
- Use powderless gloves as powder has been reported to cause false results for certain enzyme immunoassay tests.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- The wash buffer (well 9) contains a harmful agent (11.5% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- The optical cuvette with substrate (well 10) contains an irritant agent (6.6% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- Spills should be wiped up thoroughly after treatment with liquid detergent or a solution of household bleach containing at least 0.5% sodium hypochlorite. See the User Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (see the User Manual).

STORAGE CONDITIONS

- Store the VIDAS® E2II kit at 2-8°C.
- **Do not freeze reagents.**
- **Store all unused reagents at 2-8°C.**
- After opening the kit, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPRs.
- **Carefully reseal the pouch with the desiccant inside after use to maintain the stability of the SPRs and return the complete kit to 2-8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label.

SPECIMENS

Specimen type and collection

Use serum or plasma (lithium heparin). **Do not use tubes with EDTA.**

Clarify samples by centrifugation if necessary.

Before definitively using new sample collection tubes, it is recommended that the quality of the results is checked. In effect, in some cases it has been found that certain coagulation activators interfere with test results.
The use of heat-inactivated sera has not been established for this test - do not heat sera.
None of the following factors have been found to significantly influence this assay.
- hemolysis (after spiking samples with hemoglobin: 0 to 322.5 µmol/L (monomer)),
- lipemia (after spiking samples with lipids: 0 to 2 g/L or 2 mmol/L equivalent in triglycerides),
- bilirubinemia (after spiking samples with bilirubin: 0 to 361 µmol/L),
- heparin (after spiking samples with heparin: 0 to 50 IU/mL).

However, it is recommended not to use samples that are clearly hemolyzed, lipemic or icteric and, if possible, to collect a new sample.

Specimen stability

Samples can be stored at 2-8°C in stoppered tubes for up to 3 days. If longer storage is required, freeze the sera or plasma at -25 ± 6°C for up to 4 months. Avoid successive freezing and thawing.

INSTRUCTIONS FOR USE

For complete instructions, see the User Manual.

Reading Master lot data

Before each new lot of reagents is used, enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.

If this operation is not performed **before initiating the tests**, the instrument will not be able to print results.

Note: the master lot data need only be entered once for each lot.
It is possible to enter MLE data **manually or automatically** depending on the instrument (refer to the User Manual).

Calibration

Calibration, using the calibrator provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data have been entered. Calibration should then be performed every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.
The calibrator, identified by "S1", must be tested **in triplicate** (see User Manual). The calibrator value must be within the set RFV (Relative Fluorescence Value). If this is not the case, recalibrate as the mean will not be stored in memory.

Procedure

1. **Only remove the required reagents from the refrigerator and allow them to come to room temperature for 30 minutes before use.**
2. Use one "E2II" strip and one "E2II" SPR for each sample, control or calibrator to be tested. **Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.**
3. The test is identified by the "E2II" code on the instrument. The calibrator must be identified by "S1" and tested **in triplicate**. If the control needs to be tested, it should be identified by C1.
4. Mix the calibrator, control and samples with a vortex-type mixer (for serum or plasma separated from the pellet) in order to improve result reproducibility.
5. **For this test, the calibrator, control, and sample test portion is 200 µl.**
6. Insert the "E2II" SPR®s and "E2II" Reagent strips into the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent strips match.
7. Initiate the assay as directed in the User Manual. All the assay steps are performed automatically by the instrument.
8. Reclose the vials and return them to 2–8°C after pipetting.
9. The assay will be completed within approximately 60 minutes. After the assay is completed, remove the SPRs and the strips from the instrument.
10. Dispose of the used SPRs and strips into an appropriate recipient.

RESULTS AND INTERPRETATION

Once the assay is completed, results are analyzed automatically by the computer. Fluorescence is measured twice in the Reagent Strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the substrate is placed in contact with the SPR. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluorescence Value) is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.

The results are automatically calculated by the instrument using the calibration curves stored in memory (4-parameter logistic model) and then printed. The estradiol concentrations are expressed in pg/mL.
The VIDAS® Estradiol II assay is calibrated against the ID-GCMS (Isotope Dilution - Gas Chromatography Mass Spectrometry) technique.

Samples with estradiol concentrations greater than 3,000 pg/mL must be retested after manual dilution 1/2 in a specimen collected from a male patient.

Any concentration values of estradiol obtained should be used for diagnosis in association with additional information gathered by the physician (patient questioning, current drug therapy, ultrasound scan, clinical observations, other examinations, etc.).

In cases of estrogen therapy, and particularly that of hormone replacement therapy (menopause), overestimated results may be obtained.

Interpretation of test results should be made taking into consideration the patient's history, and the results of any other tests performed.

QUALITY CONTROL

A control is included in each VIDAS® Estradiol II kit. This control must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using this control. The instrument will only be able to check the control value if it is identified by C1.
Results cannot be validated if the control value deviates from the expected values.

Note

It is the responsibility of the user to perform Quality Control in accordance with any applicable local regulations.

LIMITATIONS OF THE METHOD

- **Do not use the VIDAS® Estradiol II assay to measure estradiol levels in patients undergoing Fulvestrant therapy.**
- Interference may be encountered with certain sera containing antibodies directed against reagent components. For this reason, assay results should be interpreted taking into consideration the patient's history, and the results of any other tests performed.

RANGE OF EXPECTED VALUES

In a clinically healthy population, the following values were found:

Men (n = 173)	< 62 pg/mL
Women:	
- Follicular phase: (n = 129):	18 - 147 pg/mL
- Pre-ovulatory peak (n = 33):	93 - 575 pg/mL
- Luteal phase(n = 131):	43 - 214 pg/mL
- Menopause (n = 26):	< 58 pg/mL

These figures are given as a guide. It is recommended that each laboratory establishes its own reference values from a rigorously selected population.

Conversion factors:
From pmol/L to pg/mL (ng/L), multiply by 0.272
From pg/mL (ng/L) to pmol/L, multiply by 3.67

PERFORMANCE

Studies performed using VIDAS® Estradiol II gave the following results:

Measurement range

The measurement range of the VIDAS® E2II reagent is 9-3000 pg/mL.

Detection limits

The analytical detection limit, defined as the smallest concentration of estradiol which is significantly different from the zero concentration with a probability of 95% is **9 pg/mL.**

The functional detection limit, defined as the smallest measurable concentration of estradiol with a coefficient of variation equal to 20%, is 25 pg/mL.

Precision

Within-run reproducibility

Five samples were tested 30 times in the same run.

Number	Mean (pg/mL)	CV (%)
30	38.1	7.5
30	174.1	3.9
30	594.3	2.4
30	1290.0	2.2
30	2863.0	4.6

Between-run reproducibility

Five samples were tested singly in 29 different runs, on the same VIDAS instrument, over a period of 8 weeks.

Number	Mean (pg/mL)	CV (%)
29	39.0	9.5
29	173.0	5.1
29	586.0	6.4
29	1258.0	7.4
29	2800.0	3.2

Specificity

Tested compounds	% cross-reactivity
17 β - Estradiol	100
Estrone	23.5
Estriol	1.15
Ethynyl estradiol	0.33
Progesterone	< 0.003
Testosterone	< 0.003
Norethindrone	< 0.003
Corticosterone	< 0.003
Danazol	< 0.003
Diethylstilbestrol	< 0.003
17 ß - Estradiol glucuronide	0.18

Accuracy: Dilution test: 3 samples were diluted in human serum and tested singly in 3 runs. The ratio of the mean concentration measured over the expected concentration is expressed as a mean recovery percentage.

Serum samples	Dilution factor	Expected mean concentration (pg/mL)	Measured mean concentration (pg/mL)	Mean recovery percentage
1	1/1	2324	2324	100.0
	1/2	1176	1219	103.6
	1/4	603	623	103.3
	1/8	316	318	100.8
2	1/1	2014	2014	100.0
	1/2	1022	1015	99.4
	1/4	525	542	103.2
	1/8	277	276	99.6
3	1/1	2788	2788	100.0
	1/2	1409	1408	100.0
	1/4	719	710	98.8
	1/8	374	391	104.6

Comparison with another test method

Correlation was established between the VIDAS® E2II kit (Y) and a commercially available enzyme immunoassay kit (X) using 166 samples:

Y = 1.145 X + 14.28 r =0.996 (n = 166)

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

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INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalog number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

WARRANTY

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REVISION HISTORY

Change type categories:

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release date	Part Number	Change Type	Change Summary
2015/01	08098M	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT (60 TESTS) – RECONSTITUTION OF REAGENTS WARNINGS AND PRECAUTIONS INSTRUCTIONS FOR USE
2016/08	08098N	Technical	LIMITATIONS OF THE METHOD

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