VIDAS® CA 125 II™ (125)

IVD

VIDAS CA 125 II is an automated quantitative test for use on the VIDAS family instruments, for the measurement of OC 125 antigenic determinants in human serum or plasma (lithium heparin or EDTA) using the ELFA technique (Enzyme Linked Fluorescent Assay).

SUMMARY AND EXPLANATION

Tumor-associated OC 125 reactive antigenic determinants are carried by high molecular weight glycoproteins. The serum tumor marker assay, VIDAS CA 125 II, is a second generation test that uses monoclonal OC 125 antibodies and a second monoclonal antibody, M11, which can recognize another OC 125 antigenic determinant epitope [7, 8, 5, 2].

An increase in the assay value of CA 125 II is frequently found in certain types of cancer (ovary, breast…), but also in certain non-cancerous pathologies [3, 10, 11, 6, 4]. Certain publications also describe the OC 125 antigenic determinant as a marker for monitoring endometriosis [1, 9].

The CA 125 II assay level can decrease after therapy and increase in cases of relapse, residual disease and metastasis. The VIDAS CA 125 II assay is used as an additional test for the prognosis and therapeutic monitoring of patients with diagnosed malignant tumors. A decrease in the CA 125 II assay level can indicate a positive response to therapy and therefore good prognosis. A constant increase in the CA 125 II assay value often reflects evolution of the tumor and a poor response to therapy.

PRINCIPLE

The assay principle combines a 2-step enzyme immunoassay sandwich 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 cycled in and out of the SPR several times. This operation enables the M11 antibody fixed onto the interior wall of the SPR to capture the reactive antigenic determinants present in the sample. Unbound components are eliminated during the washing steps. Alkaline phosphatase-labeled OC 125 antibody (conjugate) is then incubated in the SPR where it binds with the OC 125 reactive antigenic determinants. Unbound conjugate is then 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 proportional to the concentration of OC 125 reactive antigenic determinants present in the sample.

At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

CONTENT OF THE KIT (30 TESTS) – RECONSTITUTION OF REAGENTS:

30 CA 125 II strips	STR	Ready-to-use.
30 CA 125 II SPRs	SPR	Ready-to-use. Interior of SPRs coated with a monoclonal M 11 antibody (mouse).
CA 125 II control 1 x 2 ml (lyophilized)	C1	Reconstitute using 2 ml of distilled water. Wait for 5 to 10 minutes before mixing. **After reconstitution, stable for 48 hours at 2-8°C or 7 months at - 25 ± 6°C**. 5 freeze/thaw cycles are possible. Human serum* + OC 125 antigenic determinants (human origin) + chemical stabilizers. MLE data indicate the confidence interval in U/mL ("Control C1 Dose Value Range").
CA 125 II calibrator 1 x 3 ml (lyophilized)	S1	Reconstitute using 3 ml of distilled water. Wait for 5 to 10 minutes before mixing. **After reconstitution, stable for 48 hours at 2-8°C or 7 months at - 25 ± 6°C**. 5 freeze/thaw cycles are possible. Human serum* + OC 125 antigenic determinants (human origin) + chemical stabilizers. MLE data indicate the concentration in U/mL ("Calibrator (S1) Dose Value") and the confidence interval in "Relative Fluorescence Value" ("Calibrator (S1) RFV Range").
CA 125 II diluent 1 x 5 ml (liquid)	R1	Ready-to-use. Bovine albumin + 0.9 g/l sodium azide.
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 mouse monoclonal M11 antibody. Each SPR is identified by the "125" code. Only remove the required number of SPRs from the pouch and **carefully reseal the pouch after opening**.

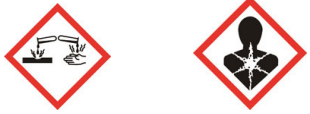
The strip

The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, 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 fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the CA 125 II strip:

Wells	Reagents
1	Sample well.
2 - 3 - 4	Empty wells.
5	Conjugate: Alkaline phosphatase-labeled OC 125 antibody + 0.9 g/l sodium azide (400 µl).
6 - 7	Wash buffer: Tris (0.01 mol/l, pH 7.4) + Tween (0.05%) + NaCl (0.4 mol/l) + 0.9 g/l sodium azide (600 µl).
8	Diluent: Potassium phosphate (0.045 mol/l) + calf serum (10%) + 0.9 g/l sodium azide (400 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.

** Signal Word: **DANGER**

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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 2 ml, 3 ml and 200 µl.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User's 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 Bosafety 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 or inhale).
- Do not use the SPRs 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 in well 9 contains a harmful agent (11.5% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- The substrate in 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's 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's Manual).

STORAGE CONDITIONS

- Store the VIDAS CA 125 II kit at 2-8°C.
- **Do not freeze the strips, SPRs or diluent.**
- **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 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. Refer to the kit composition table for special storage conditions.

SPECIMENS

Specimen type and collection:

Human serum or plasma (lithium heparin or EDTA).
It is recommended that each laboratory checks the compatibility of collection tubes used.
Samples containing impurities must be centrifuged before analysis.
None of the following factors have been found to significantly influence this assay:
- hemolysis (after spiking samples with hemoglobin, 0 to 300 µmol/l (monomer)),
- lipemia (after spiking samples with lipids, 0 to 10 mg/ml equivalent in triglycerides),
- bilirubinemia (after spiking samples with bilirubin, 0 to 364 µmol/l).

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 48 hours, or freeze the sera or plasma for 12 months at - 25 ± 6°C. Avoid successive freezing and thawing.

INSTRUCTIONS FOR USE

For complete instructions, see the User's 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's 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 **duplicate** (see User's Manual). The calibrator value must be within the set RFV "Relative Fluorescence Value" range. If this is not the case, recalibrate.

Procedure

1. **Only remove the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes.**
2. Use one "125" strip and one "125" 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 "125" code on the instrument. The calibrator must be identified by "S1", and tested in **duplicate**. If the control is to be tested, it should be identified by "C1".
4. Mix the calibrator and/or the control and samples using a vortex-type mixer (for serum or plasma separated from the pellet).
5. **For this test, the calibrator, control, and sample test portion is 200 µl.**
6. Insert the "125" SPRs and "125" 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's Manual. All the assay steps are performed automatically by the instrument.
8. Reclose the vials and return them to the required temperature after pipetting.
9. The assay will be completed within approximately **60 minutes.** After the assay is completed, remove the SPRs and 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 SPR is introduced into the substrate. 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 calibration curves which are stored by the instrument (4-parameter logistic model) and the concentrations are expressed in U/ml.

Samples with a CA 125 II test value concentration greater than 600 U/ml should be reassayed after being diluted as weakly as possible with the CA 125 II diluent (R1). If the dilution factor has not been entered when the Work List was created (see User's Manual), multiply the result by the dilution factor to obtain the sample concentration.

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 CA 125 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 these controls. 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 local applicable regulations.

LIMITATIONS OF THE METHOD

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.
The serum tumor marker assay, VIDAS CA 125 II, is based on the use of the monoclonal antibodies OC 125 and M11 supplied exclusively by Fujirebio Diagnostics, Inc., their distributors and licensed organizations. Methods using antibodies other than OC 125 and M11 may give different clinical results.
The VIDAS CA 125 II assay cannot be used as a screening test for cancer.
It is advised not to perform CA 125 II assays in patients who have received a contrast agent in the previous 24 hours [12].

RANGE OF EXPECTED VALUES

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

The expected values were determined using 1181 samples collected from 451 healthy subjects, 520 subjects with a diagnosed malignant pathology, and 211 subjects with a diagnosed non-malignant pathology.

EXPECTED VALUES	**No. of subjects**	**Zone of values in U/ml**									
		0-35		**35-50**		**50-250**		**250-600**		**> 600**	
		%	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**
HEALTHY SUBJECTS	**451**	**99.1%**	**447**	**0.2%**	**1**	**0.7%**	**3**	**0.0%**	**0**	**0.0%**	**0**
Malignant pathologies											
Ovarian	252	36.9	93	4.8	12	19.8	50	9.5	24	29.0	73
Ovarian at initial stage	25	12.0	3	0.0	0	16.0	4	20.0	5	52.0	13
Breast	75	88.0	66	4.0	3	6.7	5	1.3	1	0.0	0
Colorectal	41	70.7	29	2.4	1	17.1	7	2.4	1	7.3	3
Pulmonary	26	38.5	10	11.5	3	38.5	10	3.8	1	7.7	2
Other gynecological disorders	45	91.1	41	2.2	1	0.0	0	2.2	1	4.4	2
Others	56	51.8	29	7.1	4	23.2	13	8.9	5	8.9	5
TOTAL	**520**	**52.1%**	**271**	**4.6%**	**24**	**17.1%**	**89**	**7.3%**	**38**	**18.8%**	**98**
Non malignant pathologies											
Gastric	26	100.0	26	0.0	0	0.0	0	0.0	0	0.0	0
Pulmonary	16	93.8	15	6.3	1	0.0	0	0.0	0	0.0	0
Dialysis	30	86.7	26	13.3	4	0.0	0	0.0	0	0.0	0
Diabetes	34	85.3	29	0.0	0	14.7	5	0.0	0	0.0	0
Asthma	23	100.0	23	0.0	0	0.0	0	0.0	0	0.0	0
Cirrhosis	10	10.0	1	10.0	1	10.0	1	30.0	3	40.0	4
Hepatitis	41	78.0	32	0.0	0	17.1	7	2.4	1	2.4	1
Gynecological	31	93.5	29	3.2	1	0.0	0	0.0	0	3.2	1
TOTAL	**211**	**85.8%**	**181**	**3.3%**	**7**	**6.2%**	**13**	**1.9%**	**4**	**2.8%**	**6**

PERFORMANCE

Studies performed using VIDAS CA 125 II gave the following results:

Measurement range

The measurement range of the VIDAS CA 125 II kit is: 4-600 U/ml.

Analytical detection limit

Defined as the smallest concentration of OC 125 reactive antigenic determinants which is significantly different from the zero concentration with a probability of 95%: < **4 U/ml.**

Hook effect

No hook effect was found up to OC 125 reactive antigenic determinant concentrations of 200,000 U/ml.

Precision

Within-run reproducibility

Four samples and the control were tested n times in the same run.

Sample	1	2	3	4	5
n	30	30	29	29	28
Mean (U/ml)	33.5	29.4	94.3	231.7	566.1
CV %	3.4	3.6	3.1	3.7	3.9

Between-run reproducibility

Four samples and the control were tested singly in n different runs on the same VIDAS instrument.

Sample	1	2	3	4	5
n	30	30	29	30	29
Mean (U/ml)	33.8	28.6	93.1	225	561.3
CV %	4.7	4.6	3.8	4.7	4.1

Comparison with other test methods

The concentration of OC 125 reactive antigenic determinants in a sample, determined using the kits from different manufacturers, may vary depending on the test methods used. If the test method is changed and in the case of patient monitoring, laboratories should confirm the concentrations previously found.

Correlation was established between VIDAS CA 125 II and a radioimmunoassay (X).
VIDAS CA 125 II = 1.13 X – 19.30 r = 0.97
(n = 137)

WASTE DISPOSAL

Dispose of used or unused reagents and 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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9. NILOFF J.M., BAST R.C., SCHAETZL E.M. et al. Predictive value of CA 125 antigen levels in second-look procedures for ovarian cancer. Am J Obstet Gynecol 1985 ; 151: 981-986.
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12. WATANABE N. and al. In vitro effect of contrast agents during immunoradiometric assay for tumor-associated antigens. Nuclear Medicine Communications, 1998, 19, 63-70.

INDEX OF SYMBOLS

Symbol	Meaning
	Catalog number
	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
	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	08587H	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT (30 TESTS) – RECONSTITUTION OF REAGENTS WARNINGS AND PRECAUTIONS INSTRUCTIONS FOR USE

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