CA-125-CHECK-1

Quantitative determination of ovarian cancer marker CA-125 in whole blood, plasma or serum samples

Ref. 74091

- FOR EASY READER® AND EASY READER+®USE ONLY -

I. PRINCIPLE

Cancer Antigen (CA 125) is a surface antigen associated with epithelial ovarian cancer. In serum, CA-125 is associated with a high molecular weight glycoprotein. Published studies have indicated that elevated serum CA 125 levels can be found in individuals with serious endometroid, clear-cell and undifferenciated ovarian carcinoma (1, 2, 3). The serum CA 125 concentration is greater than 35 U/mL in 60 % of women with ovarian cancer. Most of the studies recommend this level of 35 U/mL as the decision level (4). The serum CA 125 is elevated in 1 % of normal healthy women, 3 % of normal healthy women with benign ovarian diseases, 6 % of patients with non-neoplastic conditions (5) (including but not limited to first trimester pregnancy, menstruation, endometriosis, uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum, pericardium or pleura). Serial determinations of serum CA 125 as well as pelvic examination increase the test specificity. Serum CA 125 concentration may be useful in monitoring treatment and distinguishing between good response to treatment and progressive malignant disease with poor therapeutic response. CA 125 is a serum tumour marker for monitoring response to chemotherapy, detecting disease recurrence and distinguishing malignant from benign pelvic masses. A rapid fall in CA 125 during the chemotherapy process predicts a favourable prognosis.

To date, CA 125 is the most sensitive marker for residual epithelial ovarian cancer. CA 125 may also be elevated in patients with lung, cervical, fallopian tube, and uterine cancer and endometriosis (6, 7).

The CA-125-CHECK-1 is a rapid quantitative assay for the detection of ovarian cancer antigen in serum, plasma or whole blood to be used as a screening test. The method employs a unique combination of monoclonal dye conjugate and polyclonal-solid phase antibodies to identify CA 125 in the test samples with a high degree of specificity.

As the sample flows through the absorbent device, the labelled antibody-dye conjugate binds to the CA 125 contained in sample. This complex migrates on the membrane and is bound by the polyclonal anti-CA-125 antibodies coated on the solid phase in the reaction zone (T). The colour intensity of the test band is proportional to the CA 125 concentration. The mixture continues flowing through the absorbent device past the reactive zone (T) and control zone (C). Unbound conjugate binds to the reagents in the control zone (C), producing a pink colour band and demonstrating that the reagents are functioning correctly.

II- CA-125-CHECK-1 KIT COMPONENTS

Each kit contains everything needed to perform 10 or 20 tests.

1- CA-125-CHECK-1 reaction devices:	10	20
2- Disposable plastic pipettes:	10	20
3- Diluent dropper bottle:	2.5mL	5 mL
4- Instructions leaflet:	1	1

5- Controls (Optional):

Positive control (ref. V7400) and Negative control (ref. V7401): a freeze-dried preparation of a non-infectious compound in diluted human serum, tested and found negative for anti-HIV, anti-HCV and HBs antigen, containing 0.05 % sodium azide is optionally available as a positive and negative control (1x 0.25 mL). The concentration range is indicated on the vial label.

III- STORAGE AND STABILITY

1- All CA-125-CHECK-1 kit components should be stored at room temperature (+4°C to +30°C) in the sealed pouch.
2- ***Do not freeze the test kit.***
3- The CA-125-CHECK-1 kit is stable until the expiry date stated on the package label.

IV- PRECAUTIONS

1- This test is designed for *in vitro* diagnostic use and professional use only.
2- Read carefully the instructions before using this test.
3- Handle all specimens as if they contained infectious agents. When the assay procedure is completed, dispose of specimens carefully after autoclaving them for at least one hour. Alternatively, they can be treated with 0.5% to 1% solution of sodium hypochlorite for one hour before disposal.
4- Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.
5- Do not eat, drink or smoke in the area where specimens and kit reagents are handled.
6- Avoid any contact between hands and eyes or nose during specimen collection and testing.
7- Do not use beyond the expiry date which appears on the package label.
8- Do not use a test from a damaged protective wrapper.

V- SPECIMEN COLLECTION AND PREPARATION

1- CA-125-CHECK-1 rapid test is performed on human serum, plasma or whole blood.
2- The specimen should be collected under the standard laboratory conditions (aseptically in such a way as to avoid haemolysis).
3- If anticoagulant is needed, only citrate, EDTA or heparin should be used.
4- Each specimen should be treated as if potentially infectious.
5- Whole blood samples should be tested immediately (< 4 hours). Finger prick samples should be assayed just after collection.
6- If the test is to be run within 48 hours after collection the specimen should be stored in the refrigerator (+2°C to +8°C). If testing is delayed more than 48 hours, the specimen should be frozen. The frozen specimen must be completely thawed, thoroughly mixed and brought to room temperature prior to testing. Avoid repeated freezing and thawing.
7- In case of cloudiness, high viscosity or presence of particulate matter into the serum specimen, it should be diluted with equal volume (V/V) of diluting buffer (not provided but available upon request) before testing.

VI. ASSAY PROCEDURE

a) Control testing

- Wait for 15 minutes after freeze-dried dissolving.
- Add the requested volume (25µL) with **lab pipette (disposable tips)** into the sample well of the cassette and proceed in the same way as for a patient's sample.
- The concentration range (**in U/mL**) is indicated on the vial label and obtained result must be within the specified range. The confidence range can change slightly depending on lot number.

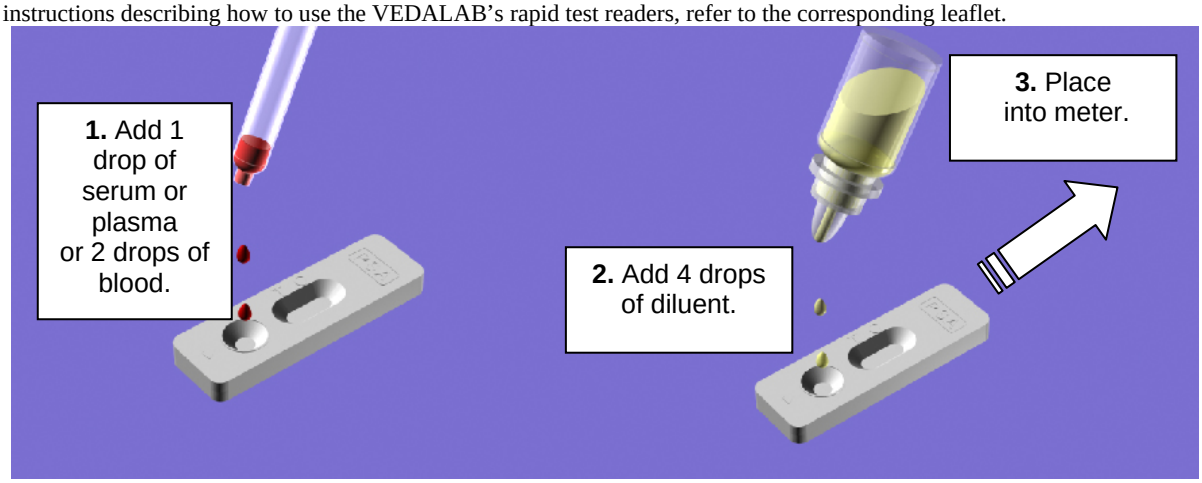
- The reconstituted vial should be kept between +2°C and +8°C and should be used within 7 days after reconstitution.

b) Samples testing

Follow the below instructions or refer to the picture n°1.

1- Allow the samples and CA-125-CHECK-1 test devices to come to room temperature prior to testing.
2- Remove the reaction device from its protective wrapper by tearing along the split.
3- Label device with the patient's name or control number.
4- Fill the dropper plastic pipette with specimens (serum, plasma or whole blood) and by holding it vertically, dispense one drop (25 µL) into the sample well. If whole blood is used, dispense 2 drops (50 µL) into the sample well **and wait for the blood sample to be completely absorbed before adding diluent**.
5- Add exactly 4 drops of diluent into the sample well.
6- Read the result **(in U/mL)** after 15 minutes either using the immediate or countdown reading mode (see corresponding leaflet).

For general instructions describing how to use the VEDALAB's rapid test readers, refer to the corresponding leaflet.



Picture n° 1

VII. PERFORMANCES CHARACTERISTICS

a) Linearity

The measuring range is 15 – 750 U/mL.
For CA 125 concentration below 15 U/mL, the result will be given as "< 15 U/mL".
For CA 125 concentration over 750 U/mL, the result will be given as "> 750 U/mL".

For samples whose concentration is higher than 750 U/mL, dilute with saline and repeat the assay as per instructions of Part. VI. In that case, **the obtained concentration must be multiplied by the dilution factor in order to get the right value: for example, the concentration value obtained from a 10 times (1 volume of sample + 9 volumes of saline) diluted sample must be multiplied by 10 to get the sample CA 125 level before dilution.**

b) Accuracy

A study has been performed using serum sample obtained from dilutions of CA 125 internal reference material covering the range of 15 to 750 U/mL. Optical densities expressed as a function of CA 125 concentrations are described by the following polynomial curve:

$Y = 9.553 + 0.5611X - 1.873.10^{-4} X^2$ (r = 0.997).

The results show a good correlation (r > 0.99) of the values obtained with the CA-125-CHECK-1 on VEDALAB's readers with the expected concentration values.

c) Sensitivity

Concentrations close to 10 U/mL are detected by CA-125-CHECK-1 test. In that case, result will be rendered as "< 15 U/mL".
Levels higher than 35 U/mL are generally considered as abnormal values.

d) Precision
A panel of 30 human sera pre-assayed on CENTAUR XP analyser has been assayed using the CA-125-CHECK-1 rapid test. Results are read with the VEDALAB's readers and reported in table 1.

Table 1

Human sera identification	**[CA125] in U/mL Expected values (CENTAUR XP)**	**Confidence range**		**[CA125] in U/mL Obtained values (CA125-CHECK-1)**
		Lower limit	**Upper limit**	
1	31	23.25	38.75	25
2	**31**	**23.25**	**38.75**	**18.5 (d)**
3	31	23.25	38.75	23.3
4	40	30	50	40.1
5	**38**	**28.5**	**47.5**	**26.6 (d)**
6	41	30.75	51.25	50.4
7	33	24.75	41.25	26.6
8	50	37.5	62.5	45.3
9	33	24.75	41.25	28.3
10	**32**	**24**	**40**	**106.2 (d)**
11	99	74.25	123.75	106.2
12	80	60	100	79.7
13	99	74.25	123.75	77.9
14	72	54	90	59.2
15	**99**	**74.25**	**123.75**	**72.8 (d)**
16	100	75	125	95.1
17	55	41.25	68.75	53.9
18	**51**	**38.25**	**63.75**	**137.3 (d)**
19	58	43.5	72.5	71.1
20	63	47.25	78.75	47.8
21	131	98.25	163.75	106.2
22	144	108	180	141.2
23	134	100.5	167.5	133.4
24	3317	2487.8	4146.3	>750
25	162	121.5	202.5	129.8
26	133	99.75	166.25	100.3
27	174	130.5	217.5	180.1
28	**132**	**99**	**165**	**343.6 (d)**
29	132	99	165	117.8
30	127	95.25	158.75	121.7

(d): discrepant.

Discrepancies are obtained with serum samples N°2, 5, 10, 15, 18 and 28 identified by **bold typo**.
Samples N°2 and 5 levels are close to the decision cut-off and therefore they can be considered as borderline samples.
Additional assays showed that serums n° 10 and n° 28 have a high level of rheumatoid factor (RF) which can interfere as indicated in part VIII-Limitations.
The results with samples n° 15 and n° 18 will not lead into any risk for the patient as both methods indicate the same clinical diagnosis profile (positive).
Assuming that serum samples n° 10 and n° 28 are not taken into account, negative, borderline and positive samples are correctly detected (a correlation of 88.5% has been established between VEDALAB rapid test and CENTAUR XP SIEMENS).

e) Hook Effect
No hook effect was observed up to a CA 125 concentration of 3317 U/mL. The reader result was: "> 750 U/mL".

f) Intra-assay reproducibility

Within run precision was evaluated by using 25 replicates of three commercially available sera containing 24.5, 85 and 432.8 U/mL of CA 125 as determined with quantitative CA-125-CHECK-1 for VEDALAB's readers.
The obtained CV (coefficient of variation) were respectively equal to 14%, 12.3% and 9.1%.

VIII. LIMITATIONS

1- As with any diagnostic procedure, the physician should confirm the data obtained using this test by other clinical methods.
2- **Use only fresh whole blood samples (< 4 hours) when test is performed with blood samples. Finger prick samples should be assayed just after collection.**
3- In case of high RF (rheumatoid factor) or CRP (C-reactive protein) concentrations (high levels indicate acute infections), the test could exceptionally show a positive result.
4- The test is designed to eliminate the potential interference of human antibodies to murine IgG (HAMA). However, high level of HAMA could give falsely positive results.
5- This format of test is to be only used with VEDALAB rapid test readers (EASY READER® or EASY READER+®).
6- If the reading time (15 minutes) is not strictly respected, wrong results will be obtained.
7- This format of test should not be used for visual reading.
8- As for any diagnostic method or for any measurements through analysers, there is a variability of the obtained result. Therefore, a confidence range of +/-25% should be considered for the final value and for the clinical significance of the result.

IX. BIBILIOGRAPHY

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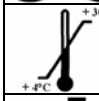
	Read the instructions before use		For *in vitro* diagnostic use
	Temperature limitations		Do not reuse
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