CEA-CHECK-1

Quantitative determination of CarcinoEmbryonic Antigen in whole blood, serum or plasma samples FOR EASY READER+® USE ONLY Ref.: 45091 (20 tests) / Ref.: 45091-10T (10 tests)

I-INTENDED PURPOSE

The CEA-CHECK-1 is a manual quantitative immunochromatographic screening test used in combination with VEDALAB's EASYREADER+® instrument for the determination of carcinoembryonic antigen (CEA) concentration in serum, plasma (citrate, EDTA or heparin) or whole blood samples. It can be used by medical healthcare professionals (not for self-testing nor near-patient testing) as an aid to assess the patient CEA level. The CEA-CHECK-1 only allows the determination of normal or abnormal CEA level. Additional investigations are necessary for the final diagnosis.

II- PRINCIPLE

Carcinoembryonic antigen (CEA) as well as alpha-fetoprotein (AFP) is produced during fetal development. After birth, the production of CEA stops and remains undetectable in normal healthy adults. However, onco-fetal antigens may appear due to the de-repression of genes that were normally expressed only during early life. As secreted antigens (CEA and AFP) do not contribute significantly in immunity against tumours, the role of these antigens, called neo-antigens, in immuno-surveillance is questionable. CEA levels in normal non smoker people range from 2.5 ng/mL up to 10 ng/mL for smokers group or women but become elevated (> 20 ng/mL) in a variety of cancers: uterine (1), lung (2), breast (3), colorectal (4) and typically among tobacco smokers (5). Patient management is the main use of these tumour neo-antigens. The presence of CEA in the circulation has been exploited for both diagnostic and therapeutic purposes. The measurement of CEA does not indicate necessarily cancer development as levels of this antigen may also rise in some non-malignant conditions, such as chronic cirrhosis, pulmonary emphysema or heavy smoking. Where curative surgery is contemplated, pre-operative CEA levels may have prognosis significance. High levels of CEA in gallbladder can indicate the presence of hepatic metastases in patient undergoing curative operation for colorectal carcinoma (6). Furthermore, serum CEA level was found to be a valuable prognostic indicator for advanced breast cancer (7). CEA is classically measured using colorimetric ELISA sandwich methods and its determination requires specific equipment, trained operators and is time consuming.

CEA-CHECK-1 is a rapid quantitative test for the measurement of CEA in serum, plasma or whole blood samples. The method relies on binding between anti-CEA monoclonal antibodies fixed on the dye conjugate and CEA in the sample. When CEA is present in the sample, the conjugate-antigen complex binds to the monoclonal antibodies coated on the test membrane. A sample that contains sufficient level of CEA will induce the formation of a pink-rose coloured band in the test region (T) while a negative sample will only allow the formation of the pink-rose control band (C) indicating that the test is functioning correctly.

Depending on the CEA concentration level, different lines of different intensities will appear on the reading window allowing the quantitative measurement of CEA when used in combination with the VEDALAB's reader.

III- CEA-CHECK-1 TEST KIT COMPONENTS

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

1- CEA-CHECK-1 reaction devices:	10	20
2- Disposable plastic pipettes:	10	20
3- Diluent in a dropper bottle containing saline buffer, detergent and sodium azide (NaN_3 < 0.1%):	2.5mL	5mL
4- Instruction leaflet:	1	1

5- **Controls (Optional):**

Positive control ref. V10000 and Negative control ref. V10001: 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.

Material required but not provided:

- Timer

IV- STORAGE AND STABILITY

1- All CEA-CHECK-1 test components, including optional control before reconstitution, should be stored at any temperature between +4°C and +30°C in their original package.

2- Do not freeze the test kit.

3- CEA-CHECK-1 test is stable until the expiry date stated on the package label.

4- The positive and negative control should be stored at +2°C to +8°C and should be used within 7 days after reconstitution.

V- 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 or if one of the component is damaged.

VI- SPECIMEN COLLECTION AND PREPARATION

1- CEA-CHECK-1 is to be 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- Samples showing cloudiness, high viscosity or particulate matter, should not be tested.

VII- ASSAY PROCEDURE

IMPORTANT: Switch the reader on and allow it to warm up for at least 30 minutes before performing any measurements.

a) Controls testing

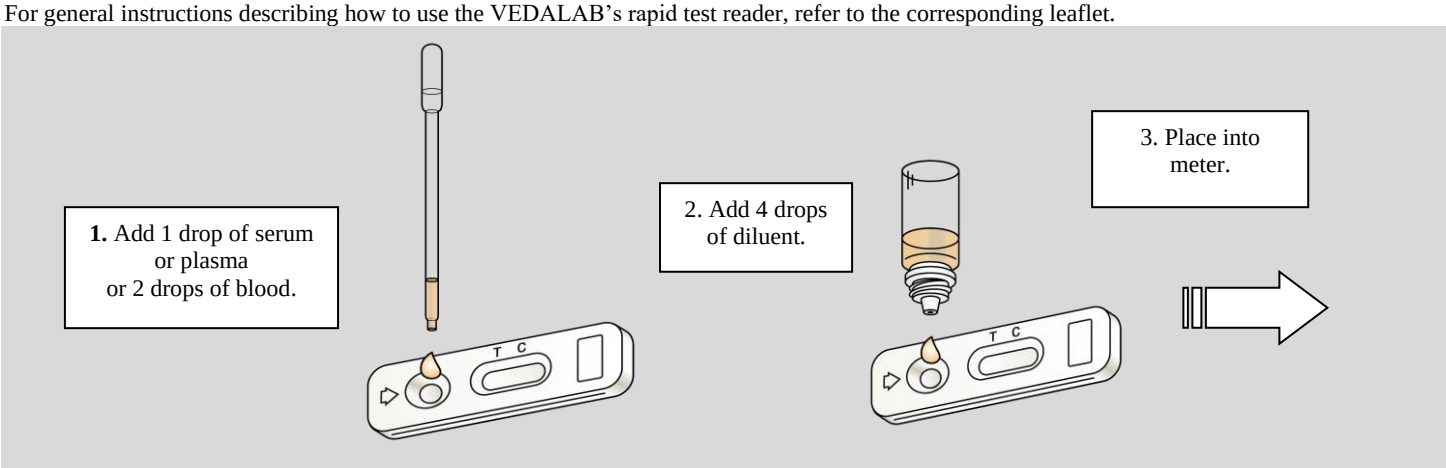
- Wait for 15 minutes after the freeze-dried control has dissolved.
- Add the requested volume (25µL) with **lab pipette (disposable tips)** into the sample well of the cassette and proceed in the same way as for a patient's sample.
- The expected concentration level (**in ng/mL**) is indicated on the vial label and obtained result must match the indicated value. The concentration level can change slightly depending on lot number.
- **The reconstituted vial should be kept at +2°C to +8°C and should be used within 7 days after reconstitution**.

b) Samples testing

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

1- Allow samples and CEA-CHECK-1 components 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 with specimen (serum or plasma) and by holding it vertically, dispense one drop (25 µL) into sample well (▷). If the whole blood is used as a sample, dispense two drops (50 µL) into the sample well (▷) **and wait for the blood sample to be completely absorbed before adding diluent**.
5– Hold the diluent vial vertically and slowly add exactly 4 drops of diluent (150 µL) in the sample well (▷) **with an interval of 2-3 seconds between each drop.**
6- Read the result **(in ng/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 reader, refer to the corresponding leaflet.



Picture n°1

VIII- PERFORMANCES CHARACTERISTICS

a) Linearity

A study has been performed using serum samples obtained from dilutions of CEA W.H.O reference material (international standard n° 73/601). The dose response obtained with the CEA-CHECK-1 quantitative test fits a linear regression in the range of 5 to 250 ng/mL:

$$Y = 1.008x - 0.3078$$

Linear regression coefficient (R^2) =0.9994

The measuring range is 5 - 250 ng/mL.
For CEA concentration below 5 ng/mL, the result will be given as " < 5 ng/mL".
For CEA concentration over 250 ng/mL, the result will be given as " > 250 ng/mL"

b) Accuracy

Serial dilution of CEA W.H.O reference material (International standard n°73/601) in CEA negative serum pool have been tested using CEA-CHECK-1 quantitative test. The obtained results are summarized in Table 1.

	CEA concentration (ng/mL)					
International standard theorical concentration	6.25	12.5	25	50	100	200
Mean of CEA-CHECK-1 quantitative test results	6.6	12.9	25.5	46.5	102.4	201.1
Bias	**+5.6 %**	**+3.2 %**	**+2.0 %**	**-7.0 %**	**+2.4 %**	**+0.6 %**

CV Coefficient of variation*

Table 1: Accuracy

The bias between nominal and measured values is statistically (95% t-test) non-significant and CEA concentrations determined using CEA-CHECK-1 test are accurately measured when compared to W.H.O reference material.

c) Analytical sensitivity
The analytical sensitivity of CEA-CHECK-1 is 5 ng/mL when using 73/601 WHO Standard.

d) Diagnostic sensitivity and specificity and overall correlation
A panel of 100 human pre-assayed serum samples (Beckman-Coulter Access or Cobas Roche) is assayed using the CEA-CHECK-1 quantitative test.
A summary of obtained results (using VEDALAB reader) is reported in the table 1 (negative samples correspond to samples for which the CEA concentration is < 5 ng/mL and positive samples correspond to samples for which the CEA concentration is ≥ 5 ng/mL).

		Reference methods		
		Positive	Negative	**Total**
CEA-CHECK-1	Positive	49	2	**51**
	Negative	1	48	**49**
	Total	**50**	**50**	**100**

Table 1: Summary of results

	CEA-CHECK-1
Diagnostic sensitivity	(49/(49+1)) x 100 = 98.00% (CI 95%* [88.78 – 100.00])
Diagnostic specificity	(48/(48+2)) x 100 = 96.00% (CI 95% [85.59 – 100.00])
Global correlation	((49+48)/100) x 100 = 97.00% (CI 95% [91.06 – 99.61])
Positive predictive value	(49/(49+2)) x 100 = 96.08% (CI 95% [85.86 – 100.00])
Negative predictive value	(48/(48+1)) x 100 = 97.96% (CI 95% [88.56 – 100.00])
Positive likelihood ratio (LR+)	(49/(49+1))/(2/(48+2)) = 24.50
Negative likelihood ratio (LR-)	(1/(49+1))/(48/(48+2)) = 0.02

Table 2: Summary of results

On the other hand, the coefficient of correlation between quantified results of CEA-CHECK-1 test and reference methods results is 98.8% (CI* 95% [98.1 – 99.2]).
**CI 95%: 95% Confidence interval*

e) Interferences
1- Rheumatoid factor (RF)
A serum sample having a RF concentration of 1,750 IU/mL has not shown any false positive result. Therefore, there is no interference of the CEA-CHECK-1 quantitative test up to a RF concentration of 1,750 IU/mL.

2-HAMA
A HAMA (anti-mouse human antibody) positive serum samples (type 1 or 2) have not shown any false positive results. Therefore, there is no interference of the CEA-CHECK-1 quantitative test on HAMA type 1 and type 2 positive samples.

3-Anticoagulants
Negative (0 ng/mL), weak positive (20 ng/mL) and strong positive (100 ng/mL) CEA samples, spiked with EDTA dipotassium (final concentration: 1.8 mg/mL), citrate trisodic (final concentration: 32 mg/mL) or heparin lithium (final concentration: 17 U/mL) did not show any effect on CEA-CHECK-1 quantitative test results (negative or positive).

4-Hemoglobin, bilirubin and triglycerides
Negative (0 ng/mL), weak positive (10 ng/mL) and strong positive (50 mIU/L) CEA samples, spiked with hemoglobin (final concentration: 5g/L), bilirubin (final concentration: 5 mg/L) or triglycerides (final concentration: 3 g/L) did not show any effect on CEA-CHECK-1 quantitative test results (negative or positive).

f) Matrix effect
Results between serum, plasma and whole blood samples show an excellent correlation. There is no matrix effect on CEA-CHECK-1 quantitative test when using plasma, serum or whole blood samples.

g) Hook effect
No hook effect has been observed up to 5,000 ng/mL CEA international standard concentration.

h) Intra-assay repeatability
Within run precision was evaluated by using 26 replicates of two commercially available references containing 25.69 and 48.43 ng/mL of CEA assayed using quantitative CEA-CHECK-1 for VEDALAB's reader.
The obtained CVs (coefficients of variation) were respectively equal to 8.49% and 8.55%.

i) Inter-assay reproducibility
Between lots reproducibility was determined by using two control serum samples containing 7.9 and 18.4 ng/mL of CEA respectively measured using three different lots of CEA-CHECK-1 (3 replicates /lot). The obtained CVs (coefficients of variation) are 11.3% and 11.9% respectively.

j) Expected values
The expected values are indicated in the table 3.

Groups	**CEA concentration (ng/mL)**
Normal people	< 5 ng/mL
Smokers, obesity, alcohol drinkers, hypertension, age (>60)	< 10 ng/mL
Extensive cancers, cirrhosis, hepatitis	> 20 ng/mL

Table 3: Expected CEA values

IX- LIMITATIONS
1- Even if CEA level increase generally occurs in certain malignancies, it may also rise in some non-malignant conditions such as chronic cirrhosis, pulmonary emphysema and heavy smoking.
2- CEA results should not be interpreted as absolute evidence for the presence or absence of malignant disease but should be used in conjunction with information from other test procedures and from clinical evaluations of the tested patient. CEA is not a screening test for occult cancer but a monitoring test for patients with various types of malignancies or for evaluating response to therapy and a potential indicator of recurrence and prognosis.
3- CEA level lower than 5 ng/mL could be obtained in patients with early carcinoma.
4- Positive results can be obtained in patients who are on antineoplastic drugs medication (the hepatotoxicity of these drugs may permit release of CEA into the circulation). Radiation therapy may also induce a transient rise in CEA.
5- High levels of RF (Rheumatoid factor) or CRP (C-reactive protein) may create interferences and therefore lead to false positive results.
6- 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.
7- **Use only fresh whole blood samples (< 4 hours) when test is performed with blood samples. Finger prick samples should be assayed just after collection.**
8- This format of test is to be only used with VEDALAB's rapid test reader.

9- If the reading time (15 minutes) is not strictly respected, wrong results will be obtained.
10- This format of test should not be used for visual reading.
11- As it is true 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.
12- Do not use the reader for measurements before at least 30 minutes warm-up after having switched on.

X- BIBLIOGRAPHY

1- Juang, C.M., Wang, P.H., Yen, M.S., Lai, C.R. et al. Application of tumor markers CEA, TPA, and SCC-Ag in patients with low-risk FIGO stage IB and IIA squamous cell carcinoma of the uterine cervix. Gynecol. Oncol. 2000, 76 : 103-106.

2- Lee, Y.C., Yang, P.C., Kuo, S.H. and Luh, K.T. Tissue polypeptide antigen and carcinoembryonic antigen as tumor markers in lung cancer. J. Formos. Med. Assoc. 1991, 90 : 631-636.

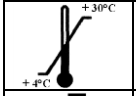
3- Molina, R., Jo, J., Filella, X., Zanon, G., Farrus, B. et al. C-erbB-2, CEA, and CA 15.3 serum levels in the early diagnosis of recurrence of breast cancer patients. Anticancer Res. 1999, 19 : 2551-2555 .

4- Holubec, L. Jr., **Topolcan, O., Pikner, R. et al.** The significance of CEA, CA19-9 and CA72-4 in the detection of colorectal carcinoma recurrence. Anticancer Res. 2000, 20 : 5737-5244.

5- Padros, M.C., Alvarez-Sala, R., Terreros Caro, F.J. et al. The concentrations of five tumor markers in both BAL fractions in lung cancer patients in relation to cigarette smoking. Tumori.1999, 85 : 454-457.

6- Waisberg, J., Palma, R.T., Neto, L.C. et al. Biliary carcinoembryonic antigen levels in diagnosis of occult hepatic metastases from colorectal carcinoma. World J. Gastroenterol. 2003, 9(7) : 1589-1593.

7- Pathak, K.A., Khanna, R., Khanna, S. et al. Carcinoembryonic antigen : an invaluable marker for advanced breast cancer. JPGM. 1996, 42(3) : 68-71.

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

 Manufactured by VEDALAB - France

CHANGES DESCRIPTION

Changes type:

- N/A	Not Applicable (creation)
- Technical change	Addition, revision and/or removal of information related to the product.
- Administrative	Implementation of non-technical changes noticeable to the end-user.

Changes type	**Change description**
Administrative	Addition: Chap I
Technical change	Modifications in Chap VIII a), b), c), d), e), f) and g) Addition of Changes description

Note: Minor typographical, grammar, spelling and formatting changes are not reported in the change details.