CYSTATIN C-CHECK-1

Quantitative determination of Cystatin C in whole blood, plasma or serum samples

FOR EASY READER® AND EASY READER+® USE ONLY

Ref. 85091 (20 tests) / Ref. 85091-10T (10 tests)

I- INTENDED PURPOSE

The CYSTATIN C-CHECK-1 is a rapid screening test for the detection of cystatin C protein in whole blood, plasma or serum samples to be used as an aid by medical healthcare professionals assessing the status of the renal function (GFR). The measurement of the concentration range or determination of sole cystatin C is not sufficient to diagnose renal function failure or chronic kidney disease as application of Cystatin C in cancerology or cardiology field is also reported. The CYSTATIN C-CHECK-1 allows only the determination of normal or abnormal level in Cystatin C. Additional examination such as the determination of total protein in urine and/or MRI (Magnetic Resonance Imagery) can be necessary.

II- PRINCIPLE

Glomerular filtration rate (GFR) is the best indicator of renal function. GFR is usually estimated by serum creatinine or the creatinine clearance calculated on urine collected over 24 hours (1). Serum creatinine has a very poor sensitivity and is dependent of muscular mass, sex and age (2).
Cystatin C is a low molecular weight protein, acting as a protease inhibitor, which is synthesized in all nucleated cells (3) and eliminated freely in urine by glomerular filtration in human kidney. This protein is quite completely catabolized in the proximal tubules and therefore its plasmatic concentration might be used to estimate GFR in the context of the limitation of creatinine clairance determination.
Cystatin C also increases in the case of malignant melanoma and in patients infected by HIV.
As cystatin C levels are independent of sex, age (from 1 to 50 years), diet or muscular mass (4), it should be considered the best alternative to serum creatinine for the evaluation of renal function.
In case of renal dysfunction, the concentration increase 8 to 12 hours after the beginning of kidney failure before creatinine level increase could be detected.
In healthy individuals, normal values of cystatin C vary from 0.5 to 1mg /L.

CYSTATIN C-CHECK-1 is a rapid quantitative test for the detection of cystatin C in whole blood, serum or plasma samples. The method employs a unique combination of monoclonal dye conjugate and polyclonal-solid phase antibodies to identify cystatin C in the test samples with a high degree of specificity.
Depending on the CYSTATIN C-CHECK-1 concentration, different lines will appear in the reading window, allowing the quantitative measurements of CYSTATIN C-CHECK-1, when used in combination with the EASY READER® or EASY READER+® rapid test readers.

III- CYSTATIN C-CHECK-1 KIT COMPONENTS

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

1- CYSTATIN C-CHECK-1 reaction devices	10	20
2- Tubes filled with 2mL of dilution buffer	10	20
3- Disposable plastic pipettes	10	20
4- Instruction leaflet	1	1

5- Positive and negative controls (ref. V8500 and ref. V8501) (optional) 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 0.5 mL). The concentration range is indicated on the vial label.

Material required but not provided
1- Automatic precision pipette for sampling (10 µL and 20 µL for samples).
2- Timer.

IV- STORAGE AND STABILITY

1-All CYSTATIN C-CHECK-1 kit components, including optional control before reconstitution with distilled water, should be stored at any temperature between +4°C and +30°C in the sealed pouch.
2-**Do not freeze the test kit**.
3-The CYSTATIN C-CHECK-1 kit is stable until the expiry date stated on the package label.

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.

CE

VI- SPECIMEN COLLECTION AND PREPARATION

1- CYSTATIN C-CHECK-1 rapid test is to be performed on human whole blood, plasma or serum samples.
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 lithium heparinate or EDTA should be used. Do not use citrated samples.
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- 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.

VII- ASSAY PROCEDURE

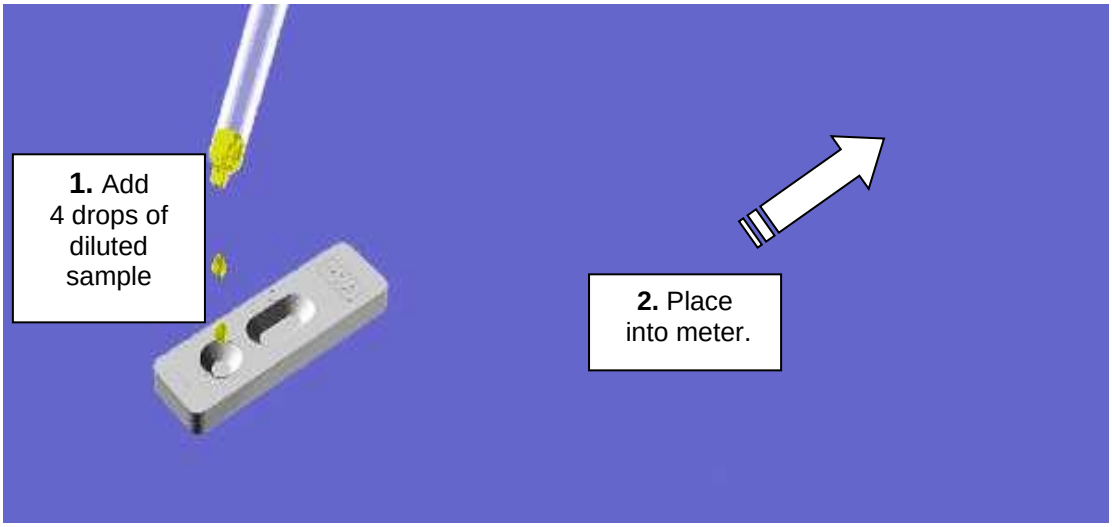
IMPORTANT: Switch the reader on and allow it to warm up for at least 30 minutes before performing any measurements.

a) Samples dilution
1/ Label one tube containing the dilution buffer with patient's name.
2/ Using a precision pipette, add 10 µL of serum/plasma into the tube. (If whole blood, add 20 µL into the dilution buffer).
3/ Mix well for a few seconds.

b) Control preparation (no dilution required)
Wait for 15 minutes after freeze dried dissolving and proceed as indicated in "c) samples and controls testing".
The expected concentration level in mg/L is indicated on the vial label and obtained result must match the indicated value.
The concentration level can change slightly depending on the lot number.
The reconstituted vial should be kept between +2°C and +8°C and should be use within 1 week after reconstitution.

c) Samples and control testing
Please follow the below instructions or refer to the picture n°1.
1– Allow samples and CYSTATIN C-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.
4.1 **Controls**: Dispense 150µL of **undiluted control** (preparation described in VII b) with **lab pipette (disposable tips)** into the sample well (▷).
4.2 **Sample:** Fill in, using the supplied plastic pipette, with diluted sample and, by holding it vertically, dispense 4 drops (150 µL) into the cassette sample well (▷) **with an interval of 2-3 seconds between each drop.**
5– Read the result **(in mg/L)** after 10 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

VIII- PERFORMANCES CHARACTERISTICS

a) Linearity
The measuring range is 0.1 – 8mg/L.
For Cystatin C concentration below 0.1mg/L, the result will be shown as "<0.1mg/L".
For Cystatin C concentration over 8mg/L the result will be shown as ">8mg/L".

b) Accuracy
A study has been performed using the European Cystatin C reference material n° ERM -DA471/IFCC, covering a range of 0 to 8mg/L. Optical densities expressed as a function of Cystatin C concentrations are described by following polynomial curve:

$$Y= 20.2788 + 175.3314x -30.5443x^2 + 1.8857x^3$$

The results show a good correlation (r>96) of the values obtained with CYSTATIN C-CHECK-1 on VEDALAB's readers.

c) Analytical sensitivity and abnormal values
The analytical sensitivity of CYSTATIN C-CHECK-1 is 0.1mg/L when using the European Cystatin C reference material.
Levels over 1 mg/L are considered as abnormal values.

d) Precision
A panel of 51 human serum samples pre-assayed on BNII SIEMENS nephelometer has been tested using the CYSTATIN C-CHECK-1 rapid test. Results were read with the VEDALAB's reader and showed a coefficient of correlation of 98.7% between both methods (CI 95% [97.7-99.3]*).

e) Hook effect
No hook effect was observed up to a Cystatin C concentration of 130 mg/L. The reader result was shown as : ">8mg/L".

f) Intra-assay repeatability
Within run precision was evaluated by using 20 replicates of three commercially available references containing 1, 2 and 5 mg/L of cystatin C as determined with quantitative CYSTATIN C-CHECK-1 for VEDALAB's readers.
The obtained CVs (coefficient of variation) were respectively equal to 12.7%; 11.4% and 14.0%.

g) Inter-assay reproducibility
Between run reproducibility was determined by performing three serum samples containing 0.46, 1.64 and 3.44 mg/L of cystatin C respectively measured using three different lots of CYSTATIN C-CHECK-1 (3 replicates/lot). The obtained coefficients of variation (CV) are 11.57, 9.18 and 14.95% respectively.

h) Matrix effect
30 whole blood samples and their corresponding plasma fractions were tested using the CYSTATIN C-Check-1 test. The correlation between the whole blood values and plasma levels is 98% (95% CI [97.6 – 99.5]*) showing no matrix effect between plasma or whole blood samples.

*CI 95% : 95% confidence interval.

i) Effect of anticoagulants
3 plasma samples of different cystatin C concentration levels were tested in presence of different anticoagulants (heparin, EDTA and sodium citrate) or in absence of any anticoagulant. Each sample was tested in triplicate using CYSTATIN C-CHECK-1 test. Table I reports the results and acceptance ranges obtained with the different assays after 10 minutes.

	Acceptance Range in mg/L (no anticoagulant)	Heparin	EDTA	Citrate
Sample 1	[0.5 – 0.8]	0.6	0.6	0.4
Sample 2	[1.2 – 2.0]	1.8	1.5	1.5
Sample 3	[3.7 – 6.1]	5.3	4.4	3.0

Table I: Effect of anticoagulants

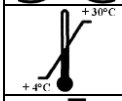
There is no effect of heparin or EDTA while citrate lowers the concentration values. **The sodium citrate must not be used to collect samples when anticoagulant is needed.**

IX- LIMITATIONS

1- The CYSTATIN C-CHECK-1 test is useful to evaluate the renal function in patients from both sexes.
2- A progressive increase of cystatin C concentration is seen in patients over the age of 60 years.
3- The cystatin C level does not change in dialyzed patients in case of the use of a low flux membrane but can be lowered by 30 to 50% in case the dialysis is performed using a high flux membrane (1).
4- As for any diagnostic procedure, the physician should evaluate the data obtained using this kit in the light of the other clinical information available.
5- 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.
6**- When the test is to be performed with whole blood, only fresh samples should be used (<4 hours). Finger prick samples should be assayed just after collection.**
7- High level of RF (Rheumatoid factor) or HAMA (Human anti-mouse antibodies) could give false positive result.
8- High level of CRP (C-reactive protein) indicates inflammatory process related to infection and thus increased concentration in poly-specific antibodies that could give false positive result in some cases.
9-Do not use the reader for measurements before at least 30 minutes warm-up after having switched on.
10- This format of test is to be only used with VEDALAB rapid test reader (EASY READER® or EASY READER+®).
11- If the reading time (10 minutes) is not strictly respected, wrong results will be obtained.
12- This format of test should not be used for visual reading.
13- Do not use citrated samples.

X- BIBLIOGRAPHY

1- **Delanaye, P., Chapelle, J.P., Gielen, J., Krzesinski, J.M. and Rorive, G.** L'intérêt de la cystatine C dans l'évaluation de la fonction rénale, Néphrologie, 2003, 24(8) : 457-468.
2- **Inker, L.A. et al.** Estimating glomerular flitartion rate from serum creatinine and cystatin C, N. Engl. J. Med., 2012, 367 : 20-29.
3- **Biomnis**. Cystatine C. Précis de biopathologie analyses médicales spécialisées, 2012 : 1-2.
4- **Séronie-Vivien et Coll.** Cystatine C : point d'étape et perspectives. Ann. Biol. Clin., 2008, 66(3) : 301 – 323.

	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	**Changes description**
Technical change	- Chap VI no citrate - Chap VIII d) and h) CI 95% g) Inter-assay reproducibility h) Matrix effect i) Effect of anticoagulants - Chap IX no citrate
Administrative change	- Intended purpose (Chap I) - Changes description

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