CRP-CHECK-1

Quantitative determination of C-Reactive Protein in whole blood, plasma or serum samples

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

(Laboratory version) Ref. 34091-3L (20 tests) & Ref. 34091-3L-10T (10 tests)

- PATENTED TEST -

I- PRINCIPLE

C-Reactive Protein (CRP) is a non specific, acute-phase reactant used to diagnose bacterial infectious disease and inflammatory disorders, such as acute rheumatic fever and rheumatoid arthritis (1, 2). CRP levels do not consistently rise with viral infections. CRP is an abnormal protein produced primarily by the liver during an acute inflammatory process (3). A positive test result indicates the presence, but not the cause, of an acute inflammatory reaction (4). The synthesis of CRP is initiated by antigen-immune complexes, bacteria, fungi, and trauma.

The CRP test is a more sensitive and rapidly responding indicator than the erythrocyte sedimentation rate (5, 6).

This test is also useful in evaluating patients with an acute myocardial infarction. The level of CRP correlates with peak levels of the MB isoenzyme of creatine kinase, but CRP peaks occur 1 to 3 days later. Failure of CRP to normalise may indicate ongoing damage to the heart tissue. Levels are not elevated in patients with angina.

CRP is classically measured using latex agglutination and nephelometric or turbidimetric methods. CRP-CHECK-1 is a rapid quantitative screening test for the detection of CRP in serum, plasma or whole blood samples.

Depending on the CRP concentration, different lines will appear in the reading window, allowing the quantitative measurements of CRP in serum, plasma or whole blood samples, when used in combination with the Easy Reader® or Easy Reader+® rapid test readers.

II- CRP-CHECK-1 KIT COMPONENTS

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

1- CRP-CHECK-1 test devices:	10	20
2- Disposable plastic pipettes:	10	20
3- Diluent bottle:	7.5mL	15mL
4- Instruction leaflet:	1	1

5- Controls (Optional):

Positive control (ref. V340) and Negative control (ref. V341): 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- MATERIAL REQUIRED BUT NOT PROVIDED

1- Automatic precision pipette for sampling (5 µL for serum/plasma samples or 10 µL for whole blood samples).
2- Plastic tubes.
3- Timer.

IV- STORAGE AND STABILITY

1- All CRP-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 CRP-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.

VI- SPECIMEN COLLECTION AND PREPARATION

1- CRP-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).
2- 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.

VII. ASSAY PROCEDURE

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

a) Control preparation (no dilution required)

1- Add 0.25 mL of distilled or tap water to the vial using a lab pipette and wait for 15 minutes after freeze-dried dissolving.
2- The expected concentration level (**in µg/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.
3- **The reconstituted vial should be kept between +2°C and +8°C and should be used within 7 days after reconstitution.**

b) Samples preparation (<u>dilution required</u>)

1) Standard dilution:

1.1- Using the diluent dropper bottle, dispense **<u>precisely</u>** 10 drops (0.3 mL) of diluent into a clean plastic tube previously labelled with the patient's name.

1.2- Using a precision pipette, add 5 µL of serum/plasma sample into the tube containing the diluent. If whole blood is to be used, add 10 µL of sample into the tube.

1.3- Mix well for a few seconds.

<u>Please do not discard the diluted sample, as it may be needed for further dilution.</u> (Cf. Part VIII- a) linearity range)

2) Additional dilution (To be performed only in case the CRP concentration is over 100 µg/mL) :

2.1- Label a new plastic tube with patient's name and dilution "1/19".

2.2- Add 3 drops (90 µL) of diluent, using the diluent dropper bottle.

2.3- Add 5 µL of **<u>diluted sample</u>** previously prepared for the first CRP testing.
(Cf. Part VII- b) Samples preparation – 1) *Standard dilution* (above).

2.4- Mix well for a few seconds.

Further dilution could be necessary for samples containing very high levels of CRP. In this case repeat the steps described in 2).

c) Controls and samples testing

<u>Follow the instructions below or refer to the picture n°1.</u>

1– Allow samples and CRP-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.

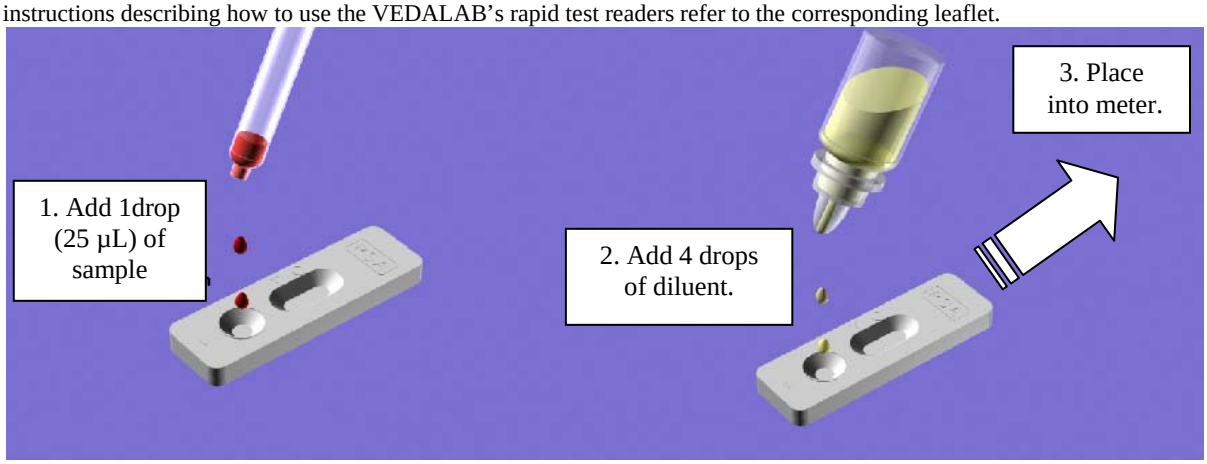
4.1- **<u>Controls:</u>** Dispense 25µL of **<u>undiluted control</u>** (**preparation described in VII. a**) with **<u>lab pipette (disposable tips)</u>** into the sample well of the cassette (▷).

4.2- **<u>Samples:</u>** Fill the supplied plastic pipette with **<u>diluted sample</u>** **(preparation described in VII. b**) and, by holding it vertically, dispense one drop (25 µL) into the sample well (▷).

5– Hold the diluent vial vertically and slowly add exactly 4 drops of diluent (120 µL) in the sample well (▷) **<u>with an interval of 2-3 seconds between each drop.</u>**

6– Read the result **(in µg/mL**) after 10 minutes, either using the immediate or countdown reading mode (See MD-361018 Part V. Assay procedure).

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 2.5 to 400 µg/mL and results will be given as per the table hereunder.

CRP concentration (µg/mL)	Reader results (µg/mL)
0 - 2.5	"< 2.5 µg/mL"
2.5 - 100	Quantitative results
100 - 200	"100 – 200 µg/mL"
200 - 400	"200 – 400 µg/mL"
400 and over	"> 400 µg/mL"

The linear measuring range being 2.5 – 100 µg/mL, a second measurement with an additional 1/19 dilution of the diluted sample (Cf. Part VII- b) *2) Additional dilution*) will be necessary, in case an exact CRP value is needed for samples over 100 µg/mL or even over 400 µg/mL.

b) Accuracy

A study has been performed using a range of standards prepared by dilution of international W.H.O. standard
Nr 85-506 in a serum depleted in CRP and covering a range of 0 to 400 µg/mL. Optical densities expressed as a function of CRP concentrations are described by following curve:

$$Y = \frac{570\ x}{(37.8+x)} \quad (r = 0.96).$$

c) Sensitivity

The CRP-CHECK-1 is allowing to detect CRP concentration of 2.5µg/mL, according to WHO 1st CRP International Standard Nr 85-506.
Levels higher than 8µg/mL are generally considered as abnormal values.

d) Precision

A panel of 33 human sera pre-assayed on BECKMAN analyser has been evaluated using the CRP-CHECK-1 quantitative rapid device. Results are read with the Easy Reader photometer and reported in table I.

Three samples identified in bold typo are showing discrepant results when compared to the reference method.

But in the three cases, both methods lead to the same clinical diagnosis profile (positive).

Therefore negative, borderline and positive samples are all correctly identified (a correlation of 98.2% (CI 95% [96.3-99.1]*) has been established between VEDALAB rapid test and BECKMAN) using CRP-CHECK-1.

**CI : 95% confidence interval.*

Table I

Human sera identification	[CRP] in µg/mL Expected values BECKMAN	[CRP] in µg/mL Obtained values CRP-CHECK-1
1	<1	<2.5
2	4.2	5.02
3	10.7	9.14
4	58	57.58
5	132	100-200
6	1.6	<2.5
7	2	<2.5
8	7.3	8.7
9	17.9	18.69
10	34.1	38.12
11	74.3	64.25
12	91	100-200
13	113	93.85
14	227	200-400
15	397	200-400
16	3.7	3.36
17	**9.9**	**7.4**
18	13.9	12.7
19	**29.4**	**22.27**
20	74	75
21	80	89.21
22	81	76.4
23	82	79.1
24	88	89.9
25	90	90.5
26	91	90.9
27	93	97.1
28	**93**	**72**
29	130	100-200
30	134	100-200
31	163	100-200
32	166	100-200
33	193	200-400

e) Hook effect

A sample containing 3,010 µg/mL gave a result of ">400 µg/mL" on the VEDALAB meter indicating that no hook effect has been observed up to rather 500 times the normal values.

f) Intra-assay reproducibility

Within run precision was evaluated by using 35 replicates of two commercially available sera containing 10.97 and 50.65 µg/mL of CRP as determined with quantitative CRP-CHECK-1 for VEDALAB reader. The obtained CVs (coefficient of variation) were respectively equal to 12.55% and 11.20%.

g) Interferences and matrix effect

The quantitative CRP-CHECK-1 test is an assay requiring high dilution (1/61 for serum/plasma sample and 1/31 for whole blood sample) of the sample into a specific diluent. Considering this high dilution step, the probability of non specific reactions or matrix effect in CRP-CHECK-1 test is not significant.

IX- LIMITATIONS

1- An equivocal result could indicate the beginning of an immune response.

2- A questionable result can also be observed after therapy and an overcome infection.

3- As for any diagnostic procedure, the physician should evaluate the data obtained using this kit in the light of the other clinical available information.

4- Use only fresh whole blood samples (< 4 hours) when test is performed with blood samples. Finger prick samples should be assayed just after collection.

5- This format of test is to be used only with VEDALAB's rapid test readers (Easy Reader® or Easy Reader+®).

6- If the reading time (10 minutes) is not strictly respected, wrong results will be obtained.

7- This format of test should not be used for visual reading.

8- 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.

9-Do not use the reader for measurements before at least 30 minutes warm-up after having switched it on.

X- BIBLIOGRAPHY

1- **Van Lente F**, "The Diagnostic Utility of C-Reactive Protein", Hum Pathol, 1982 13(12) : 1061-3.

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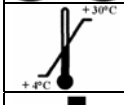
3- **Dowton SR and Colten HR**, "Acute Phase Reactants in Inflammation and Infection", Semin Hematol, 1988, 25(2):84-90.

4- **Shaw AC**, "Serum C-Reactive Protein and Neopterin Concentrations in Patients with Viral or Bacterial Infection", J Clin Pathol, 1991, 44(7): 596-9.

5- **Wu TT, Lee YH, Tzeng WS, et al** "The Role of C-Reactive Protein and Erythrocyte Sedimentation Rate in the Diagnosis of Infected Hydronephrosis and Pyonephrosis", J Urol, 1994, 152(1): 26-8.

6- **Gambino R.** "C-Reactive Protein (CRP) - How much Proof do we need?" Lab Rep, 1994, 16(11) : 83-5.

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

Manufactured by VEDALAB - France