PROCAL-CHECK-1

Quantitative determination of Procalcitonin in whole blood, plasma or serum samples

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

Ref. 33091 (20 tests) & Ref. 33091-10T (10 tests)

I- PRINCIPLE

Procalcitonin (PCT) is a small protein of 116 amino acid residues with a molecular weight of approximately 13kDa which was first described by Moullec et al. in 1984 (1) and which is the calcitonin precursor originating in the thyroid gland.

However, PCT is produced and secreted in a completely different manner from calcitonin. In response to mediators of an infectious stimulus, PCT is produced and constitutively secreted from nearly every cell type in the body (2) PCT has been a valuable marker for sepsis for nearly two decades, ever since it was initially discovered that PCT levels increased exponentially during bacterial infections and that there was a correlation between the amount of PCT and severity of the infection (3).

As such, Sepsis being a condition with high morbidity and mortality (4) it is of up most importance to detect it as early as possible to initiate an efficient therapy.

In healthy individuals or for local infections, the PCT concentration in blood is lower than 0.5ng/mL.

The PROCAL-CHECK-1 test is a quantitative rapid screening test for the detection of PCT in serum, plasma and whole blood samples.

The method employs a unique combination of monoclonal-dye conjugate and monoclonal solid phase antibodies to identify PCT in the test samples with a high degree of specificity.

As the test sample flows through the absorbent device, the labelled antibody-dye conjugate binds to the PCT forming an antibody-antigen complex. This complex binds to the anti-PCT antibody in the positive reaction zone (T) and produces a pink-rose colour band.

In the absence of PCT, there is no line in the positive reaction zone (T). The reaction mixture continues to flow through the absorbent device past the reaction zone (T) and control zone (C). Unbound conjugate binds to the reagents in the control zone (C) producing a pink-rose colour band, demonstrating that the reagents are functioning correctly.

Depending on the PCT concentration, lines of different colour density will appear in the reading window allowing the quantitative measurement of PCT when used in combination with the Easy Reader® rapid test reader.

II- PROCAL-CHECK-1 KIT COMPONENTS

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

1- PROCAL- CHECK-1 reaction devices:	10	20
2- Disposable plastic pipettes:	10	20
3- Diluent in a dropper bottle:	2.5 mL	5 mL
4- Instruction leaflet:	1	1

5- **Controls** (**Optional):**

Positive control ref. V0330 and Negative control ref. V0331: 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 PROCAL-CHECK-1 kit components should be stored at room temperature (+4°C to +30°C) in the sealed pouch.

2- ***Do not freeze the kit***.

3- The PROCAL-CHECK-1 kit is stable until the expiry date stated on the package label.

4- The control vials are stable 7 days when stored at +2°C to +8°C after reconstitution.

IV- PRECAUTIONS

1- This test is designed for *in vitro* diagnostic use and professional use only.

2- Read the instruction carefully before using this test.

3- Handle all specimens as if they contain 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 hands contact with 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- PROCAL-check-1 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 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.

CE

VI- ASSAY PROCEDURE

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

a) Controls testing

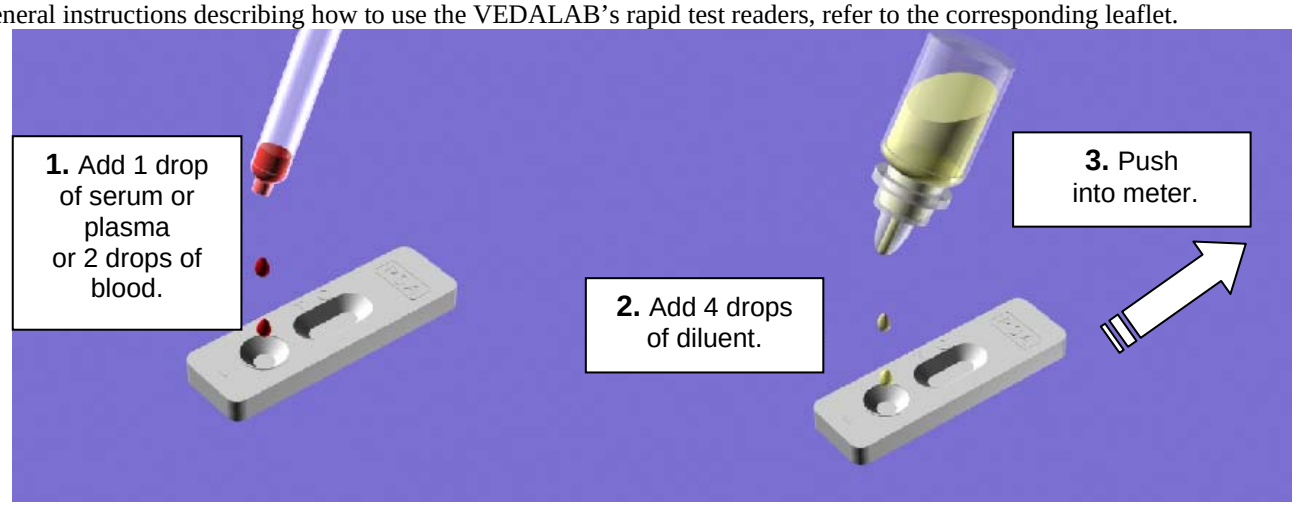
- After reconstitution, wait for 15 minutes for a complete dissolution of the lyophilized control. Mix gently.
- Add the requested volume (25µL) with **<u>lab pipette (disposable tips)</u>** into the sample well of the cassette and proceed in the same way as for a patient's sample.
- The expected concentration 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

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

1– Allow samples and PROCAL-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 plastic pipette with sample or control and, by holding it vertically, dispense one drop (25 µL) of serum or plasma or control into sample well (▷). If the whole blood is used, dispense two drops (50 µL) into the sample well (▷) **<u>and wait for the blood sample to be completely absorbed before adding diluent</u>**.
5– Hold the diluent vial vertically and slowly add exactly 4 drops of diluent (150 µL) in the sample well (▷) **<u>with an interval of 2-3 seconds between each drop.</u>**
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 readers, refer to the corresponding leaflet.



Picture n°1

VII- PERFORMANCES CHARACTERISTICS

a) Linearity

The measuring range is 0.3-100ng/mL.
For PCT concentration below 0.3ng/mL, the result will be shown as "< 0.3ng/mL".
For PCT concentration over 100ng/mL, the result will be shown as ">100ng/mL".
For samples whose concentration is higher than 100ng/mL, dilute with saline and repeat the assay as per instructions of Part. VI.

b) Accuracy

A study has been performed using serum samples pre-assayed on the BRAHMS KRIPTOR® analyser covering a range of 0.02 to 166.60ng/mL. Optical densities expressed as a function of PCT concentrations are described by following polynomial formulation:

$$Y = 0.685x^2 + 12.20x + 47.66.$$

The results show a good correlation ($r > 0.96$) of VEDALAB's reader and PROCAL-CHECK-1 rapid test versus BRAHMS KRIPTOR® analyser.

c) Clinical study

A study was performed within a population of 177 patients admitted in hospital (Walk in and emergency patients) who had body temperatures above 37°C. Serum samples were collected and pre-assayed using BIOMERIEUX VIDAS analyzer before being tested using PROCAL-CHECK-1 test and Easyreader+® instrument. The results showed a good coefficient correlation of 0.85 (95% CI [0.80 – 0.89]) in between the two methods (figure 1).

95% CI: 95% Confidence interval.

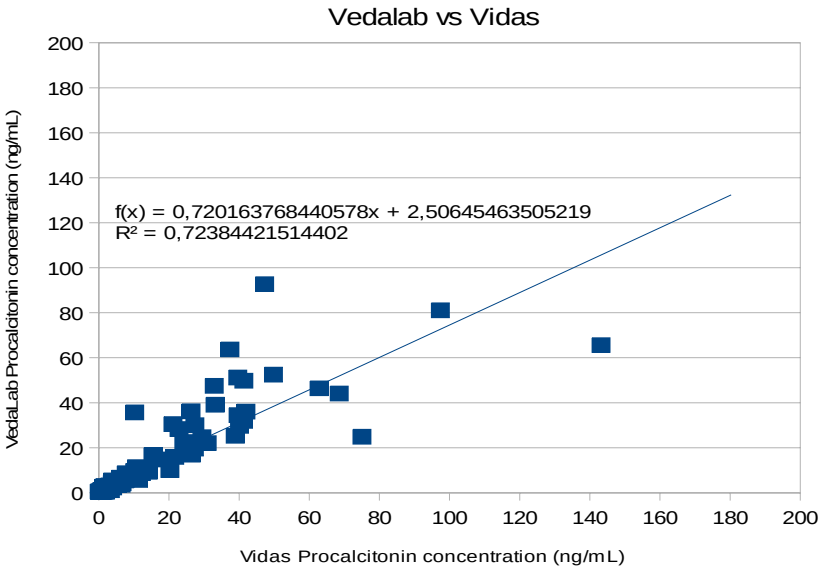


Figure 1: Results comparison

The diagnostic sensitivity, specificity, negative and positive predictive values were calculated considering the diagnostic decision values of 0.5ng/mL and 2.0ng/mL to respectively discriminate local infections and likely sepsis from real potential sepsis cases (tables 1 and 2).

1) Diagnostic sensitivity and specificity

	Value [95 % Confidence interval]	
	0.5ng/mL	2ng/mL
Diagnostic specificity	94.9 [85.9 – 98.9]%	98 [93.1 – 99.8]%
Diagnostic sensitivity	82.2 [74.1 – 88.6]%	89.3 [80.1 – 95.3]%
Overall agreement	86.4 [80.5 – 91.1]%	94.4 [89.9 – 97.3]%

Table 1: 95% confidence interval for diagnostic sensitivity, specificity, and correlation for both cut-offs

2) Negative and positive predictive values

	Value [95 % Confidence interval]	
	0.5ng/mL	2ng/mL
Negative Predictive Value (NPV)	72.7 [61.4 – 82.3]%	92.6 [93.1 – 99.8]%
Positive Predictive Value (PPV)	97 [91.5 – 99.4]%	97.1 [89.9 – 99.6]%

Table 2: 95% confidence interval for diagnostic predictive values and for both cut-offs

Although absolute concentration values can be somewhat different when comparing to the reference method (MiniVIDAS), the final diagnosis is identical when considering the reference values and in clinical case where sepsis must be suspected for level higher than 0.5ng/mL and in cases where sepsis is likely for level greater than 2ng/mL. For these decision values the PROCAL-CHECK-1 shows a respective positive predictive value of 97% and 97.1%. In addition, the quantitative rapid test is useful in screening patients not developing sepsis. The difference between healthy patients and patient with local infections could then be done when concentration level is lower than 0.5ng/mL.

d) Analytical sensitivity

Concentrations close to 0.2ng/mL could be detected by PROCAL-CHECK-1 test. However, results will be shown as "<0.3ng/mL" in such cases. Levels over 0.5ng/mL and particularly over 2.0ng/mL are generally considered as abnormal values and could indicate a possible systemic infection (sepsis).

e) Precision

A panel of 69 serum samples pre-assayed using the BRAHMS KRIPTOR® analyser has been measured using the PROCAL-CHECK-1 quantitative rapid test and VEDALAB's readers.
The result shows an excellent coefficient of correlation of 98.6%.

f) Hook effect
Spiked serum samples having a PCT concentration up to 5000 ng/mL were tested and showed consistently positive results.

g) Intra-assay reproducibility
The intra-assay reproducibility was evaluated by running 25 replicates of three serum samples having a PCT concentration of 1.0, 7.2 and 42.7 ng/mL. The obtained CVs (coefficient of variation) were 15%, 12.8% and 12.5% respectively.

h) Inter-assay reproducibility
Between lots reproducibility was determined by using two specimens containing 1.0 and 15.9ng/mL tested in 3 independent assays with three different lots of devices. The obtained coefficients of variation (CV) were respectively 10.7% and 9.1%.

i) Interferences
1- Rheumatoid factor (RF):
Negative serum samples spiked either with 1,633 IU/mL and 1,682 IU/mL in RF did not show any interfering effect.

2- Human anti-mouse antibodies (HAMA):
As well positive samples in HAMA type 1 and type 2 positive and procalcitonin negative samples have been tested with the PROCAL-CHECK-1 test
Negative serum samples in presence of HAMA type 2 did not show any interference while in presence of HAMA type 1, interference was observed. This may lead to false positive results in exceptional cases.

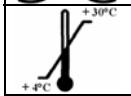
3- Anticoagulants
Three negative whole blood samples collected in presence of EDTA, heparin and citrate and containing respectively 0.5, 5 and 10 ng/mL of procalcitonin were tested as well as the corresponding plasma fractions. There is no interference detected using these anticoagulants both in whole blood or plasma samples.

j) Reference values

PCT Concentration	Patients status
<0.05 ng/mL	Healthy
0.05 – 0.49 ng/mL	Local infections
0.50 – 1.99 ng/mL	Systemic infection (sepsis) possible
2.00 – 9.99 ng/mL	Systemic infection (sepsis) likely
>10.0 ng/mL	Severe sepsis or Septic shock

VIII- LIMITATIONS
1- As for any diagnostic procedure, the physician should evaluate the data obtained using this kit in the light of the other clinical information available.
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- This format of test is to be only used with VEDALAB's rapid test readers.
4- If the reading time (15 minutes) is not strictly respected, wrong results will be obtained.
5- This format of test should not be used for visual reading.
6- 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.
7- It is recommended that each laboratory establish its own references ranges based on representative patient population in order to test the validity of the supplied data. Therefore, the data given should only be intended as orientational guidelines.
8- In case of a positive result, it is recommended to confirm the exact PCT concentration through another method should the precise PCT concentration be required for clinical reasons.
9- Elevated PCT levels could also be seen in some infections cases which should be identified before initiating any therapy.
10- Do not use the reader for measurements before at least 30 minutes warm-up after having switched it on.

IX- BIBLIOGRAPHY
1- **Le Moullec JM,** and **al.** The complete sequence of human procalcitonin. *FEBS letters* (1984) 167(1), 93-97
2- **Müller B, White JC** and **al.** Ubiquitons Expression of the clocatinin –I gene in Multiple Tissue in Response to sepsis, of clinical endocrinology and metabolosim (2001) 86, 396-404.
3- **B Müller** and **KL Becker**, "Procalcitonin : How a Hormone Became a Marker and Mediator of Sepsis" *Swiss Medical Weekly* 131 (2001) : 595-602.
4- **KA Wood** and **DC Angus**, "Pharmacoeconomic Implications of New Therapies in Sepsis", *Pharmaco Economics* 22, no.14 (2004) : 898-906.

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