CORTISOL-CHECK-1

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

Ref.:110091 (20 Tests)/Ref.: 110091-10T (10Tests)

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

I. INTENDED PURPOSE

The CORTISOL-CHECK-1 test is a rapid screening test for the detection of cortisol in human whole blood, serum or plasma to be used as an aid for medical healthcare professionals assessing disorders of corticosteroids metabolism. The sole measuring of cortisol level is not sufficient to diagnose dysfunction corticosteroids secretion causing potentially hyper hypercortisolism or pathological deficiency of adrenal glands.
Additional medical examination is necessary to get a definitive diagnosis.

II. PRINCIPLE

Cortisol is a steroid hormone produced in humans by the adrenal cortex (1). This hormone has a molecular weight of 363.5 Da.
The release of cortisol is controled by the hypothalamus through the feedback control system of ACTH (and CRH) hormones (2; 3).
The secretion of cortisol is the body response to stress and low blood glucose concentration (4).
It fonctions to increase blood sugar through glucogenesis, to suppress the immune system and to aid in the metabolism of fat, protein and carbohydrates (5).
Diurnal cycles of cortisol levels are found in humans, the highest level being achieved in the morning (approximately 8 a.m) and the lowest around midnight (6).
Cortisol measurement is a powerful tool for the evaluation of suspected abnormalities in glucocorticoid production: Cushing's Syndrome (hypercortisolism), Addison's disease or secondary adrenal insufficiency (hypocortisolism).
The CORTISOL-CHECK-1 is a rapid quantitative assay for the detection of cortisol in serum, plasma or whole blood. The method employs a unique combination of monoclonal dye conjugate and cortisol antigen coated on solid phase to identify cortisol in the test samples with a high degree of specificity.
As the sample flows through the absorbent device, the labelled antibody-dye conjugate binds competitively to the cortisol contained in sample and to the cortisol coated in the reaction zone (T). The colour intensity of the band appearing in the test zone (T) is inversely proportional to the concentration of cortisol in the sample. The mixture continues flowing through the absorbent device past the reactive zone (T) and control zone (C).
Depending on the cortisol concentration level, different lines of different intensities will appear on the reading window allowing the quantitative measurement of cortisol when used in combination with the VEDALAB's readers Easy Reader® or Easy Reader+®.

III- CORTISOL-CHECK-1 KIT COMPONENTS

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

1- CORTISOL-CHECK-1 test devices :	10	20
2- Disposable plastic pipettes :	10	20
3- Diluent in a dropper bottle :	2.5mL	5 mL
4- Instructions leaflet :	1	1

5- Controls (Optional):
Positive control (ref. V11000) and Negative control (ref. V11001): Positive and negative control (1x0.25 mL) 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.

IV- STORAGE AND STABILITY

1- All CORTISOL-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 CORTISOL-CHECK-1 kit is stable until the expiry date stated on the package label.
4- After reconstitution, the control is stable for 7 days when stored at +2°C and +8°C.

V- 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.

CE

VI- SPECIMEN COLLECTION AND PREPARATION

1- CORTISOL-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.

VII- ASSAY PROCEDURE

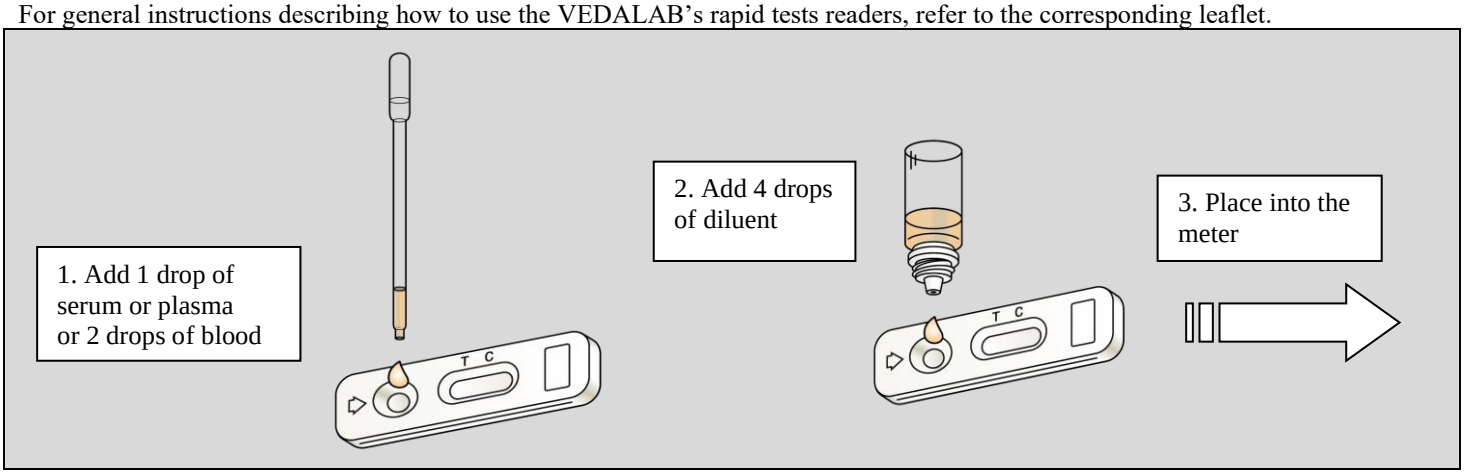
IMPORTANT: Switch the reader on and allow it to warm up for at least 30 minutes before performing any measurements.

a) Controls testing
- Reconstitute each the positive and negative control vial with 0.25 mL of distilled water.
- After reconstitution, wait for 15 minutes after freeze-dried dissolving. Slowly shake the vials.
- 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 levels (**in ng/mL**) are 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 preparation

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

1- Allow the sample and CORTISOL-CHECK-1 test device to return to room temperature prior to testing.
2- Remove the reaction device from its protective wrapper by tearing along the line.
3- Label the device with the patient's name or control number.
4- Fill the serum dropper with specimens (serum or plasma) and by holding it vertically, dispense one drop (25 µL) into sample well. If the whole blood is used, 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 10 minutes either using the immediate or countdown reading mode (see corresponding leaflet).
For general instructions describing how to use the VEDALAB's rapid tests readers, refer to the corresponding leaflet.



Picture n° 1

VIII- PERFORMANCES CHARACTERISTICS

a) Linearity
The measuring range is 25-250ng/mL.
For cortisol concentration below 25 ng/mL, the result will be given as "< 25 ng/mL".
For cortisol concentration over 250 ng/mL, the result will be given as "> 250 ng/mL".
For samples whose concentration is higher than 250 ng/mL, dilute with saline and repeat the assay as per instructions of Part. VI.

b) Accuracy
A study has been performed using preassayed serum samples containing Cortisol concentration in a range of 0 to 250 ng/mL. Optical densities expressed as a function of cortisol concentrations are described by following polynomial curve:

$$Y = 1E\text{-}05x^3\text{-}0.0048x^2\text{-}0.9134x+667.99$$

The results show a good correlation ($r > 0.99$) of the values obtained with CORTISOL-CHECK-1 on VEDALAB's reader.

c) Sensitivity
Concentrations close to 15 ng/mL are detected by CORTISOL-CHECK-1 test. In these cases, results will be rendered as "< 25ng/mL".

d) Precision
A comparative study was performed using a panel of 14 human sera preassayed on the Roche Cobas ECLIA® analyser and another panel of 32 human sera preassayed on Biomerieux Mini-Vidas analyser. Results showed an overall coefficient of correlation of 96.7% (IC95% [94.1-98.2] *) between CORTISOL-CHECK-1 quantitative rapid tests and reference methods.

e) Hook effect
The CORTISOL-CHECK-1 quantitative test is a competitive assay showing an OD decrease for increasing concentration of cortisol.
Therefore, there is no possibility of a hook effect in this assay.

f) Intra assay reproducibility
Within run precision was evaluated using 25 replicates of 3 samples containing 50, 100 and 200 ng/mL of cortisol by serial dilutions of commercially available antigen. The obtained CVs (coefficient of variation) were respectively equal to 24.2%, 12.82% and 8.77%. For low cortisol concentration (50 ng/mL), the CV is higher as expected.

g) Inter-assay reproducibility
Within run precision was evaluated using 3 replicates of 3 samples containing 58, 129 and 187 ng/mL of cortisol by 3 serial dilutions of commercially available antigen. The obtained CVs (coefficient of variation) were respectively equal to 14.8%, 8.2% and 11.1%.

h) Expected values
It is recommended that each laboratory should determine its own reference ranges. The expected values (table 1) are representative of values as indicated in the literature.

Group	Time	Values	
		(ng/mL)	(nmol/L)
Male / Female	9.00 am	51-225	140-616
	Midnight	29-127	79-348

Table 1 : expected values

i) Reference values

The Cortisol concentration could be expressed either in ng/mL or in nmol/L.
The conversion factor is:

- nmol/L = concentration in ng/mL X 2.74.

j) Interferences

1- Bilirubin, triglycines and hemoglobin
6 samples containing respectively 0; 50; 100; 200; 500 and 10,000 ng/mL, of cortisol have been spiked with, bilirubin (0.2g/L), triglycerides(15g/L) or hemoglobin (1.5g/L). Each sample has been 3 times by using the quantitative rapid test CORTISOL-CHECK-1.
The obtained results shown that bilirubin, triglycerides and hemoglobin have no interferences at a respectively concentration of 0.2 g/L, 15 g/L and 1.5 g/L.

2- Anticoagulants
Three negative whole blood samples collected in presence of EDTA, heparin and citrate and containing respectively 50, 100, 200 and 500 ng/mL of cortisol were tested as well as the corresponding plasma fractions. There is no interference detected using these anticoagulants both in whole blood or plasma samples.

k) Cross reactions
The following substances (table 2 hereunder) were tested for cross reactivity (Data supplied by the anti-cortisol antibody manufacturer).

Substances	Cross reactivity
Cortisol	100%
11-deoxycortisol	0.9%
Prednisolone	5.6%
Corticosterone	0.6%
11-deoxycorticosterone	<0.1%
Progesterone	<0.1%
17-hydroxyprogesterone	<0.1%
Testosterone	<0.1%
Estradiol	<0.1%
Estriol	<0.1%
Danazol	<0.01%

Table 2: Cross reactivity

IX. 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 rapid test readers (EASY READER® or EASY READER+®).
4- If the reading time (10 minutes) is not strictly respected, wrong results will be obtained.
5- This format of test should not be used for visual reading.
6- For better results, it is recommended to strictly follow the proceeding temperature recommendations as well as to warm up the reader for 30 minutes before starting measurements.
7- 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.
8- 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.

X. BIBILIOGRAPHY

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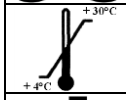
	Read the instructions before use		For *in vitro* diagnostic use
	Temperature limitations		Do not reuse
	Manufacturer		

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 : - Ref 10Tests -Chap I
Technical change	Addition : -a) in Chap VII - CI 95%, g), j) in Chap VIII -IX and Reader Warm up (30mins)

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