

# CALPROTECTIN-CHECK-1

## Quantitative determination of calprotectin in faeces samples FOR EASY READER® AND EASY READER+® USE ONLY

Ref. 63091 (20 tests) / Ref. 63091-10T (10 tests)

### I- INTENDED PURPOSE

The CALPROTECTIN-CHECK-1 is a quantitative rapid screening test for the detection of calprotectin protein in human faeces to be used as an aid by medical healthcare professionals in assessing chronic inflammatory diseases of the intestine. The sole measurement of calprotectin concentration is not sufficient to diagnose haemorrhagic recto colitis, Crohn's disease or severe enterocolitis. Further examination as endoscopy or biopsies can be necessary.

### II- PRINCIPLE

Calprotectin is a 36.5 kDa protein belonging to the S100 group of proteins (1). It represents up to 60% of the soluble protein content in the neutrophile granulocytes cytosol (2). Due to its ability to sequester manganese and zinc, calprotectin has recognized bacteriostatic and mycostatic activity which is comparable to antibiotics. Elevated levels of faecal calprotectin indicate the migration of neutrophils to the intestinal mucosa which occurs during intestinal inflammation.

The main diseases that cause or increased excretion of faecal calprotectin are inflammatory bowel diseases, coeliac diseases, infectious colitis, intestinal cystic fibrosis and colorectal cancer (3,4).

As the protein is resistant to enzymatic degradation, it has become a marker of choice which could be easily measured in faeces (5).

CALPROTECTIN-CHECK-1 is a quantitative immunochromatographic assay for the detection of calprotectin antigen in the faeces.

After faeces collection in a tube containing extraction solution, a few drops of this extract are added to the sample well (▷) of the reaction device.

As the test sample flows through the absorbent device, the anti calprotectin monoclonal antibody-gold conjugate binds to the calprotectin antigen (when present in the sample), forming an antibody antigen complex.

This complex binds to another anti calprotectin monoclonal antibody in the positive reaction window, producing a rose-pink coloured band.

In the absence of calprotectin, there is no line in the positive reaction zone (T). The reaction mixture continues flowing through the absorbent device, past the positive reaction zone (T) and control zone (C).

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

### III- CALPROTECTIN-CHECK-1 KIT COMPONENTS

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

1- CALPROTECTIN-CHECK-1 test devices	10	20
2- Faeces sample collection devices containing 3.5 mL of solution	10	20
3- Disposable plastic pipettes	10	20
4- Diluent in dropper bottle vial	2.5 mL	5 mL
5- Instructions leaflet	1	1

### 6- Controls (Optional):

**Positive control (ref. V6300) and Negative control (ref. V6301):** 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

- Automatic precision pipette.
- Plastic tubes
- Timer.

### IV- STORAGE AND STABILITY

1- All CALPROTECTIN-CHECK-1 kit components should be stored between +4°C and +30°C.

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

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

### V- PRECAUTIONS

1- CALPROTECTIN-CHECK-1 test is designed for *in vitro* diagnostic use and for professional use only and not for self-testing use.

2- Read the instruction notice carefully 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- The extraction reagents may cause irritation to skin, eyes and mucus membranes. Avoid any contact between hands and eyes or nose during specimen collection and testing.

**Rinse immediately with water if extraction reagents come into contact with skin.**

5- Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.

6- Do not eat, drink or smoke in the area where specimens and kit reagents are handled.

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) CALPROTECTIN-CHECK-1 rapid test is performed on human faeces. **The faeces must be collected in a clean, dry container without any detergent.**

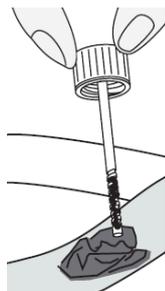
**Faeces can be stored for 72 hours undiluted between +2°C and +8°C if testing is delayed.**

2) Indicate the patient's name and date on the sample collection device.

3) Unscrew the **white cap** of the sample collection device containing the sample collection probe.

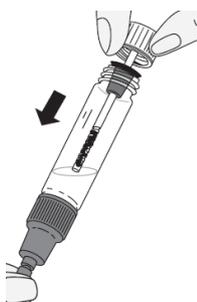


4) Collect the stool sample using the tip of the collection device by dipping it into 3 different places of the same stool sample.  
For solid faeces, it represents a sample weight of 20 µg of faeces.



For liquid faeces, add approximately 20 µL of faeces into the collection device using a micropipette.

5) Put the white cap with the collection tip containing the faeces sample back in place on collection device and screw it down firmly. Shake thoroughly for 10 seconds to resuspend the faeces sample into the diluent and assay the dilution sample.



**Important note:**

Please assay the diluted faeces sample **immediately** after dilution into faeces collection device. Delaying the assay will lead to inaccurate results.

**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 freeze-dried dissolving. Add the requested volume (150 µL) with **lab pipette (disposable tips)** into the sample well of the cassette and proceed in the same way as for a patient's sample.

**Important:** the reconstituted positive control vial should be kept between +2°C and +8°C and should be used **within 30 minutes after reconstitution.**

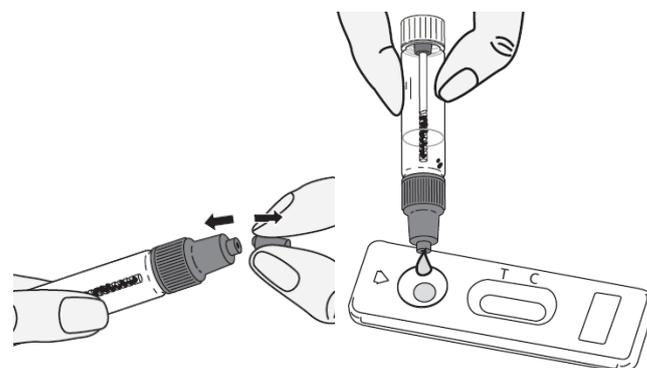
The reconstituted negative control vial should be kept between +2°C and +8°C and should be used within 7 days after reconstitution.

The concentration range (in µg/g) is indicated on the vial label and obtained result must be within the specified range. The confidence range can change slightly depending on lot number.

**b) Samples testing**

**A) Adults (measuring range: 25 µg/gr to 350 µg/gr)**

- 1) Allow the samples and reagents to return to room temperature prior to testing.
- 2) Remove the test device from the pouch and place one plastic tube (not supplied) on a rack.
- 3) Break the purple tip of the collection device, and dispense 5 drops (150 µL) of mixture into the sample well (▷) of the reaction device.



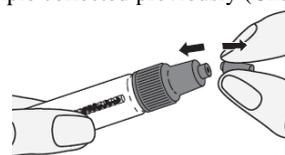
- 4) Enter the cassette into the reader and measure the result (in µg/g) after 10 minutes using either immediate or count down mode (refer to the corresponding leaflet for the general instructions on how to use VEDALAB's rapid tests readers).

**Note:** Using the procedure means that quantitative results will be given within the measuring range of 25 µg/g to 350 µg/g. However, in case quantitative results are requested up to 700 µg/g or even 1,050 µg/g (For neonates as an example), there is a need to further dilute the collected sample (see Chapter VII. B).

**B) Procedure for extending the measuring range**

In some cases (for example neonates), there is a need of an additional dilution of the collected sample to extend the measuring range up to 700 µg/g or 1,050 µg/g. Proceed as follows:

- 1) Allow the samples and reagents to return to room temperature prior to testing
- 2) Remove the test device from the pouch and place one plastic tube (not supplied) on a rack.
- 3) Break the purple tip of the faeces collection device containing the faeces sample collected previously (Chapter VII. A).



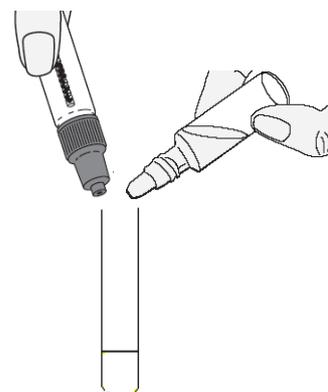
- 4) a/ **For an extended measuring range up to 700 µg/g, proceed as follows:**

Dispense 4\* drops (150 µL) of mixture and 5\* drops (150 µL) of supplied diluent using the dropper bottle into the plastic tube.

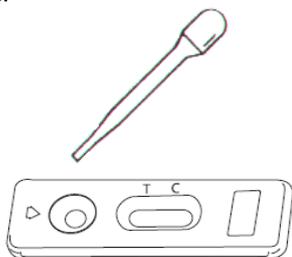
- b/ **For an extended measuring range up to 1,050 µg/g, proceed as follows:**

Dispense 4\* drops (150 µL) of mixture and 10\* drops (300 µL) of supplied diluent using the dropper bottle into the plastic tube.

(\* ) **The number of drops is different due to the different drop size delivered through both dropper systems.**



- 5) Mix well and using the supplied disposable plastic pipette, add 4 drops (150 µL) of mixture into the sample well (▷) of the reaction device.



- 6) Enter the cassette into the reader and measure the result (in µg/g) after 10 minutes using either immediate or count down mode (refer to the corresponding leaflet for the instructions for use of VEDALAB's rapid tests readers).

**Important:** The obtained Calprotectin value, as seen on the display or on the printed paper, should be modified as follows (due to the additional faeces dilution):

- either doubled (x2) in the case the 700 µg/g extended range procedure is applied
- or tripled (x3) in the case 1,050 µg/g extended range procedure is applied

## VIII- PERFORMANCES

### a) Linearity

The results are expressed in µg of calprotectin per gram of faeces. The measuring range is 25 µg/g to 350 µg/g.

For calprotectin concentration below 25 µg/g, the result will be shown as "<25 µg/g".

For calprotectin concentration exceeding 350 µg/g the result will be shown as ">350 µg/g".

### b) Accuracy

A study has been performed using a diluted calprotectin antigen. Optical densities expressed as a function of calprotectin concentration are described by following polynomial curve:

$$Y = 2.246E - 5x^3 - 0.0167x^2 + 4.438x - 33.19$$

The results show an excellent correlation ( $r > 0.99$ ) of the obtained calprotectin values using VEDALAB's reader.

### c) Analytical sensitivity

The analytical sensitivity is 25 µg of calprotectin/g of faeces corresponding to about 150 ng of calprotectin/mL of diluent taking into account the dilution factor of the faeces sample. A calprotectin concentration of 50 µg of calprotectin/g of faeces is considered as being the cut off concentration requiring further clinical investigations in adults (10-59 years old). Calprotectin is significantly higher in children (less than 10 years old) and in elderly people (over 59 years old).

### d) Diagnostic sensitivity and specificity and overall correlation

A panel of 117 faeces samples pre-assayed using different CE certified reference methods, have been evaluated using the CALPROTECTIN-CHECK-1 rapid test. A summary of obtained results/performances is reported in the table 1 and 2.

CALPROTECTIN-CHECK-1	Reference methods			
		Positive	Negative	Total
	Positive	79	3	82
Negative	8	27	35	
Total	87	30	117	

Table 1: Summary of results

	CALPROTECTIN-CHECK-1
Diagnostic sensitivity	$(79/87) \times 100 = 90.80\%$ [91.91-96.13] IC95%
Diagnostic specificity	$(27/30) \times 100 = 90.00\%$ [72.15-98.75] IC95%
Global correlation	$((79+27)/117) \times 100 = 90.60\%$ [83.16-95.34] IC95%
Positive predictive value (PPV)	$(79/(79+3)) \times 100 = 96.34\%$ [79.17-99.52] IC95%
Negative predictive value (NPV)	$(27/(27+8)) \times 100 = 77.14\%$ [57.29-90.90] IC95%
Positive likelihood ratio (LR+)	$(79/(79+8))/(3/(27+3)) = 9.08$
Negative likelihood ratio (LR-)	$(8/(79+8))/(27/(27+3)) = 0.1$

Table 2: Summary of performances

\*CI 95%: 95% Confidence interval

### e) Hook effect

No hook effect was observed up to a calprotectin concentration of 8,000 µg/g.

### f) Intra-assay reproducibility

Within run precision was evaluated by using 25 replicates of 3 samples containing 35 µg/g, 100 µg/g and 250 µg/g of calprotectin prepared by serial dilution of commercially available calprotectin antigen.

The obtained CVs (coefficient of variation) were respectively equal to 14.5%, 14.7% and 14.3%.

### g) Inter-assay reproducibility

Between run reproducibility was determined by performing 2 samples containing 35 µg/g and 250 µg/g of calprotectin prepared by serial dilution of commercially available calprotectin antigen. These samples were measured using three different lots of CALPROTECTIN-CHECK-1. The obtained coefficients of variation (CV) are 12.37% and 14.68% respectively.

### h) Expected values

The reference calprotectin concentration in healthy patients varies depending on the age (3) and (6):

Patient age	Upper limit concentration (µg/g faeces)
< 1 year	350 µg/g
2-9 years	166 µg/g
10-59 years	51 µg/g
≥ 60 years	112 µg/g

## IX- LIMITATIONS

- 1- CALPROTECTIN-CHECK-1 is specifically designed for the quantitative determination of calprotectin in faeces.
- 2- Results will be accurate as long as the diluted faeces sample is assayed immediately after dilution into the faeces collection device.
- 3- As with any diagnostic procedure, the physician should confirm the data obtained by the use of this test with other clinical methods.
- 4- A negative result does not exclude the presence of calprotectin if the concentration is below the detection limit of the test.
- 5- This format of test is to be only used with VEDALAB 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 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 on.

## X- BIBLIOGRAPHY

- 1- **Brophy Megan Brunjes; Nolan Elizabeth M.** (16 January 2015). "Manganese and Microbial Pathogenesis: Sequestration by the Mammalian Immune System and Utilization by Microorganisms" ACS Chemical Biology:150116125412006. doi:10.1021/cb500792b.
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- 3- **Joshi S., Lewis S.J., Creanor S., Ayling R.M.** (2009). "Age-related faecal calprotectin, lactoferrin and tumour M2-PK concentrations in healthy volunteers". Annals of Clinical Biochemistry **47** (Pt3):259-263. doi:10.1258/acb.2009.009061. PMID 19740914.
- 4- **Vaos G., Kostakis I.D., Zavras N., Chatzemichael A.** (2013). "The role of calprotectin in pediatric disease". Biomed Res Int (Review) **2013**: 542363. doi:10.1155/2013/542363. PMC:3794633. PMID 24175291.
- 5- **Tibble J., Teahon K., Thjodleifsson B., Roseth A., Sigthorsson G., Bridger S., Foster R., Sherwood R., Fagerhol M., Bjarnason I.** (2000). "A simple method for assessing intestinal inflammation in Crohn's disease". Gut **47** (4):506-513. doi:10.1136/gut.47.4.506. PMC:1728060. PMID 10986210.
- 6- **Ezri J. and Nydegger A.** (2011). La calprotectine fécale en pédiatrie : utilisation et interprétation. Rev. Med. Suisse (7) : 69-70.

	Read the instructions before use		For <i>in vitro</i> 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 of required material but not provided
Technical change	Chap VIII: modification of d)

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