FOB-CHECK-2

Quantitative determination of fecal occult blood in faeces samples
FOR EASY READER® AND EASY READER+® USE ONLY
PATENTED TEST
Ref. 4091-3L (20 tests) & Ref. 4091-3L-10T (10 tests)

I- INTENDED PURPOSE

The FOB-CHECK-2 is a rapid screening test for the detection of blood in faeces as an aid to assess colorectal abnormal bleeding disorders by medical healthcare professionals. The presence of blood in faeces only is not sufficient to diagnose cancerous lesions. Additional examination such colonoscopy is necessary for the final diagnosis.

II- PRINCIPLE

Colorectal cancer has an annual incidence worldwide of more than 600,000 cases and ranks as the third most common cancer (1).
As with all cancers, early detection of lesions increases considerably the survival rate of the patient (2). Among people over 45 years old, 10% have colorectal polyps of which 1% will become malignant (3). Relying on the fact that many polyps larger than 0.5 cm can bleed, fecal occult blood testing appears to be a simple and cheap screening method for colorectal cancer when compared to colonoscopy. Therefore, haemoglobin was selected as the marker of choice for faecal occult blood detection.

For many years, chemical techniques based on the pseudoperoxidase activity of haemoglobin were used with the drawback of low sensitivity and poor specificity (4).
Immunological methods with improved sensitivity and specificity for human blood are starting to be used in spite of their greater technical complexity when compared to guaiac tests (5).

A recent publication suggests that there is a direct correlation between the haemoglobin concentration in faeces and colorectal cancer (6).
The FOB-CHECK-2 test is a quantitative immunochromatographic assay for the detection of fecal occult blood in feces.

The method employs a unique combination of gold conjugate and solid phase monoclonal antibodies to selectively identify human haemoglobin with a high degree of sensitivity and specificity.
After faeces sample collection in a specific collection device containing extraction solution, a few drops of the mixture are added to the sample well of the reaction device. As the liquid flows through the absorbent device, the labelled antibody-dye conjugate binds to the haemoglobin antigen (when present in the faeces sample) forming an antibody antigen complex.

Depending on the blood concentration level, different lines of different intensities will appear on the reading window allowing the quantitative measurement of haemoglobin when used in combination with the VEDALAB's readers EASY READER® or EASY READER+®.

III- FOB-CHECK-2 KIT COMPONENTS

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

1- FOB-CHECK-2 test devices	10	20
2- Sample collection devices (syringe) containing 2 mL of solution	10	20
3- Instructions leaflet	1	1

4- Controls (Optional):

Positive control (ref. V080, freeze-dried) and Negative control (ref. V081, liquid): the controls are prepared from non-infectious human blood, tested and found negative for anti-HIV, anti-HCV and HBs antigen, containing 0.05 % sodium azide and optionally available as a positive and negative control (1x 0.5 mL). The concentration range is indicated on the vial label.

IV- STORAGE AND STABILITY

1- All FOB-CHECK-2 kit components should be stored between +4°C and +30°C.
2- <u>Do not freeze the test kit</u>.
3- FOB-CHECK-2 is stable until the expiry date stated on the package label.

V- PRECAUTIONS

1- FOB-CHECK-2 test is designed for *in vitro* diagnostic use and for professional use only.
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) FOB-CHECK-2 rapid test is performed on human faeces.
Feces can be stored for 72 hours between +2°c and +8°C if testing is delayed.

2) Write the patient's name and date on the label of the sample collection device.

3) Unscrew the top of the sample collection device containing the sample collection probe.

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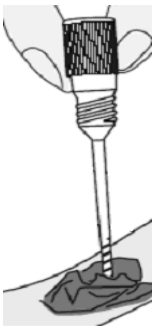
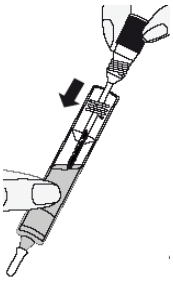
4) Collect the stool sample with the top of the collection device by deeping it into 3 different places of the same stool sample and put it in the collection device.

Note: If liquid feces, please add 20µL of feces into the collection device using a micropipette.



5) Put the sample collection probe loaded with sample back in place on collection device and screw it down firmly.



6) Store the sample collection device at +2°C to +8°C.

VII- 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

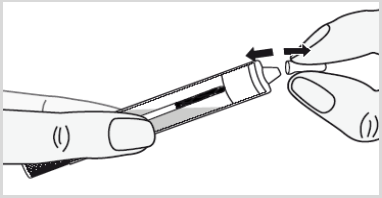
- <u>Positive control:</u> Wait for 15 minutes after freeze-dried dissolving.
- <u>Negative control:</u> Ready to use.
- Add the requested volume (150µL) with **<u>lab pipette (disposable tips)</u>** into the sample well of the cassette and proceed in the same way as for a patient's sample.
- The concentration range (**in ng/mL**) 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.

- <u>Positive control:</u> The reconstituted vial should be kept between +2°C and +8°C and should be used within 7 days after reconstitution.
- <u>Negative control:</u> After each use, promptly replace the stopper and keep the vial between +4°C to +30°C until the expiry date indicated on the label.

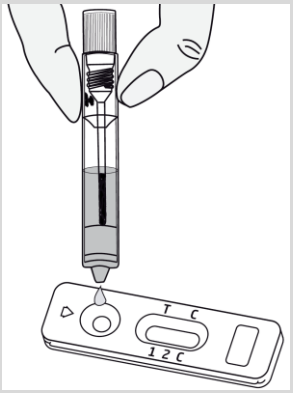
b) Samples testing

1) Allow the samples and reagents to return to room temperature prior to testing
2) Remove the test device from the pouch.
3) Break the tip of the collection device and while holding it vertically, add 6 drops (150 µL) of extracted sample into the sample well (▷) of the reaction device with **an interval of 1-2 sec in between each drop**.

1. Break tip of the collection device.



2. Add 6 drops of the extracted sample.



3. Place into meter.

4) Read the results after 10 minutes using either the immediate or countdown reading mode (see corresponding leaflet). Results will be express in ng/mL and in µg/g of feces.

VIII- PERFORMANCES

a) Linearity
Results are expressed in ng of haemoglobin per mL of extraction solution and in µg/g of feces. The measuring range is 10 to 500 ng/mL (1.43 – 71.5 µg/g) and results will be given as the chart hereunder.

Haemoglobin concentration		Reader result	
ng/mL	**µg/g**	**ng/mL**	**µg/g**
0 – 9	0 – 1.29	"Hb < 10 ng/mL"	"Hb <1.43 µg/g
10 – 499	1.43 – 71.3	Quantitative result	Quantitative result
500 – 5,000	71.5 - 715	"Hb = 500 – 5,000 ng/mL"	"Hb = 71.5 – 715 µg/g"
> 5,000	>715	"Hb > 5,000 ng/mL"	"Hb > 715 µg/g"

b) Accuracy
A study was performed on a panel of 24 faeces samples supplied by the Bradford Hospital for the Yorkshire External Quality Assurance Scheme (YEQAS) from United Kingdom.
These samples, which are containing haemoglobin at known concentrations (in µg/g of faeces), have been assayed both on HEM-CHECK-2 visual rapid test and on FOB-CHECK-2 quantitative test.
The results show a perfect correlation of results between HEM-CHECK-2 visual test and FOB-CHECK-2 quantitative test. In addition, the FOB-CHECK-2 quantitative results correlate well with the quantity of haemoglobin (data supplied by YEQAS and expressed in µg of Hb per gram of faeces), as samples have been correctly assayed in all cases.

c) Sensitivity
Concentrations close to 5 ng/mL (0.71 µg/g) are detected using FOB-CHECK-2.
In these cases, results will be rendered as "< 10 ng/mL" (<1.43 µg/g).
Levels higher than 100 ng/mL (14.3 µg/g) are generally considered as abnormal values.

d) Hook effect
No hook effect was observed up to a Haemoglobin concentration of 2 mg/mL.

e) Cross-reactions
FOB-CHECK-2 did not show any cross reactions with haemoglobin from bovine, pig, rabbit, horse and sheep sources.

f) Intra-assay reproducibility
Within run precision was evaluated by using 25 replicates of two commercially available samples containing 27.7 and 109 ng/mL of Haemoglobin as determined with quantitative FOB-CHECK-2 for VEDALAB's reader.
The obtained CVs (coefficient of variation) were respectively equal to 8.5% and 11.4%.

g) Diagnostic significance (interpretation)

As stressed in the part VIII. Limitations, there are many causes for the presence of blood in faeces and the physician should confirm the results obtained with FOB-CHECK-2 with other clinical methods like colonoscopy.
An evaluation study was performed on 54 real faeces samples tested both on FOB-CHECK-2 quantitative test and on HEM-CHECK-2 visual test. After comparison of the results obtained on both tests, it appears that any haemoglobin concentration lower than 100 ng/mL (14.3 µg/g) should be interpreted as negative, any concentration in between 100 and 200 ng/mL (14.3 and 28.6 µg/g) as borderline positive and any concentration over 200 ng/mL (28.6 µg/g) as positive. Nevertheless, and even if the haemoglobin concentration is lower than 100 ng/mL (14.3 µg/g), it is recommended to consult a physician in case the symptoms are persisting.

h) Conversion factor
The FOB concentration is expressed in ng/mL and in µg/g of feces. The conversion factor is:

µg/g = ng/mL x 0.143

IX- LIMITATIONS

1- FOB-CHECK-2 is specifically designed for the quantitative determination of human blood (haemoglobin) in faeces.
2- The presence of blood in stools may be due to several causes, besides colorectal bleeding such as haemorrhoids, blood in urine or stomach irritations. Blending from the upper part of the digestive tract (for example in the case of stomach or duodenal ulcers) may not be detected all of the time due to further protein digestion and the difficulty of antibodies to recognise the haemoglobin antigen after proteolysis.
3- All colorectal bleeding may not be due to pre-cancerous or cancerous polyps.
4- As with any diagnostic procedure, the physician should confirm the data obtained by the use of this test with other clinical methods, such as barium enema, sigmoïdoscopy or colonoscopy.
5- Negative results do not exclude bleeding since it can be intermittent.
6- Colorectal polyps at a very early stage may not bleed. This is the reason why it is safe to periodically control (once a year) people over the age of 45.
7- This format of test is to be only used with VEDALAB rapid test readers (EASYREADER® or EASYREADER+®).
8- If the reading time (10 minutes) is not strictly respected, wrong results will be obtained.
9- This format of test should not be used for visual reading.
10- 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.
11-Do not use the reader for measurements before at least 30 minutes warm-up after having switched on.

X- BIBLIOGRAPHY

1- D.M. Parkin, E. Laara and C.S. Muir. "Estimates of the worldwide frequency of sixteen major cancers in 1980." Int. J. Cancer, Volume 41 : 184-197. 1988
2- Sidney J. Winaver, Paul Sherlock, David Schottenfeld and Daniel G. Miller. "Clinical trends and topics : Screening for colon cancer." Gastroenterology, volume 70 : 783-789. 1976
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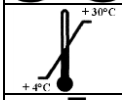
4- D.J. Frommer, A. Kapparis and M.K. Brown. "Improved screening for colorectal cancer by immunological detection of occult blood." British medical journal, Volume 296 : 1092-1094. 1988
5- Jerome B. Simon. "Occult blood screening for colorectal carcinoma: a critical review." Gastroenterology, Volume 88 : 820-837. 1985
6- Fraser, C.G., Mathew, C.M., McKay, K., Carey, F.A. and Steele, R.J.C. "Automated immnochemical quantitation of haemoglobin in faeces collected on cards for screening colorectal cancer. Gut, Volume 57 : 1256-1260. 2008

	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	- Chap I Intended purpose - Chap VII Procedure - Changes description

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