TRANSFERRIN-CHECK-1

Quantitative determination of transferrin in faeces samples
FOR EASY READER® AND EASY READER+® USE ONLY
Ref. 67091

I- PRINCIPE

Transferrin is a glycoprotein of around 80kDa which consists of a polypeptide strand with 2 N-glycosidically linked oligosaccharide chains (1).
The main role of transferrin is iron transport from the absorption centers in the duodenum and white blood cell macrophages to all tissues (2). Therefore it is considered as an extremely sensitive indicator of iron deficiency (3).
Further more, transferrin is present in the blood in relatively high concentration and is much more stable than haemoglobin especially in faeces samples. Therefore, transferrin has also been used recently to detect gastrointestinal bleedings occurring in the upper digestive tract cancers such as stomach and colorectal cancers (4).
The TRANSFERRIN-CHECK-1 test is a quantitative immunochromatographic assay for the detection of transferrin in faeces.
The method employs a unique combination of gold conjugate and solid phase monoclonal antibodies to selectively identify human transferrin 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 transferrin antigen (when present in the faeces sample) forming an antibody antigen complex.

In the absence of transferrin, 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 transferrin concentration level, different lines of different intensities will appear on the reading window allowing the quantitative measurement of transferrin when used in combination with the VEDALAB's readers EASYREADER® or EASYREADER+®.

II- TRANSFERRIN-CHECK-1 KIT COMPONENTS

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

1- TRANSFERRIN-CHECK-1 test devices	10	20
2- Faeces sample collection devices containing 2 mL of solution	10	20
3- Instructions leaflet	1	1

III- STORAGE AND STABILITY

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

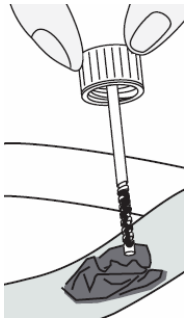
IV- PRECAUTIONS

1- TRANSFERRIN-CHECK-1 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.

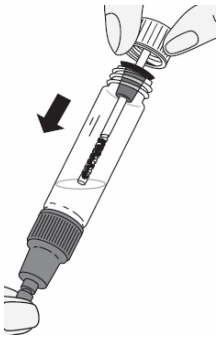
CE

V- SPECIMEN COLLECTION AND PREPARATION

1) TRANSFERRIN-CHECK-1 rapid test is performed on human faeces.
Faeces can be stored for 72 hours 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 tip.



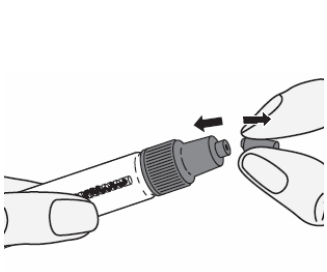
4) Collect the stool sample using the tip of the collection device by dipping it into 3 different places of the same stool sample.
For liquid faeces, add approximately 20µL of faeces into the collection device using a micropipette.

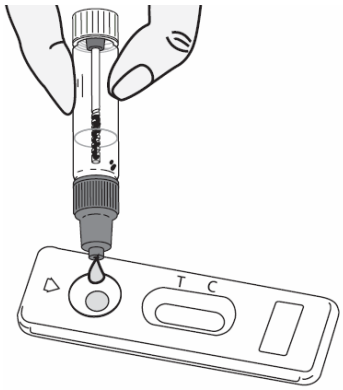


5) Put the white cap with the collection tip containing the faeces back in place on collection device and screw it down firmly.

VI- ASSAY PROCEDURE

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 purple tip of the collection device, and dispense 5 drops (150 µL) of the mixture into the sample well (▷) of the reaction device.





4) Enter the cassette into the reader and measure the result (in ng/mL) 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).

VII- PERFORMANCES

a) Linearity

The results are expressed in ng of transferrin per mL of extraction solution. The measuring range is 4 ng/mL – 300 ng/mL. It corresponds to a real transferrin concentration of 0.4 µg/gram to 30 µg/gram in faeces.
For transferrin concentration below 4 ng/mL, the result will be shown as "<4 ng/mL".
For transferrin concentration exceeding 300 ng/mL the result will be shown as ">300 ng/mL".

b) Accuracy

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

$$Y= 7E\text{-}06x^3\text{-}0.0057x^2+2.273x + 6.4127$$

The result show an excellent correlation ($r>0.99$) of the transferrin obtained values using VEDALAB's instrument.

c) Analytical sensitivity

The test analytical sensitivity has been adjusted to 4 ng/mL using a purified transferrin antigen. This is the transferrin concentration which is considered as being the cut-off for gastrointestinal bleeding requiring further clinical investigations.

d) Precision

A comparative study was performed using serial dilutions of 3 human sera pre-assayed on SEBIA Capillarys analyser. Results showed an overall coefficient of correlation of 98.5% between TRANSFERRIN-CHECK-1 quantitative rapid tests and reference methods.

e) Hook effect

No hook effect was observed up to a transferrin concentration of 13 000 ng/mL.

f) Intra-assay reproducibility

Within run precision was evaluated by using 25 replicates of 3 samples containing 200, 50 and 10 ng/mL of transferrin prepared by serial dilution of commercially available transferrin antigen.
The obtained CVs (coefficient of variation) were respectively equal to 9.89%, 17.48% and 19.55%. For low transferrin concentration (10 ng/mL and 50 ng/mL, the CVs are higher as expectable.

g) Diagnostic significance (interpretation)

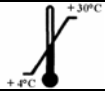
As stressed in the part VIII. Limitations, there are many causes for the presence of blood in faeces and the physician should confirm the results obtained with TRANSFERRIN-CHECK-1 with other clinical methods like colonoscopy.
But a transferrin concentration of 4 ng/mL or over is to be considered as gastrointestinal bleeding requiring further clinical investigations.

VIII- LIMITATIONS

1- TRANSFERRIN-CHECK-1 is specifically designed for the quantitative determination of transferrin in faeces for the detection of gastrointestinal bleeding.
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. Bleeding from the upper part of the digestive tract (for example in the case of stomach or duodenal ulcers) may be better detected using TRANSFERRIN-CHECK-1 than classical FOB tests due to the higher stability of transferrin antigen when compared to haemoglobin.
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.

IX- BIBLIOGRAPHY

1. "Transferrin Structure". St. Edward's University. 2005-07-18. Retrieved 2009-04-24.
2. **Macedo MF, de Sousa M** (Mar 2008). "Transferrin and the transferrin receptor: of magic bullets and other concerns". Inflammation & Allergy Drug Targets 7 (1): 41-52. doi:10.2174/187152808784165162. PMID 18473900.
3. **Miller JL.** Iron Deficiency Anemia: A Common and Curable Disease. Cold Spring Harbor perspectives in medicine. 2013;3(7):10.1101/cshperspect.a011866 a011866.doi:10.1101/cshperspect.a011866.
4. **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

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

 Manufactured by VEDALAB - France