FRT-CHECK-1

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

FOR EASY READER® AND EASY READER+® USE ONLY

Ref.: 25091

I- PRINCIPLE

Ferritin is the form in which higher animals store iron intracellularly. It is present in all cells, with the highest concentrations in the liver, spleen and bone marrow (1).

Ferritin is a protein consisting of 24 polypeptide subunits (Mr 450,000) assembled in the shape of a hollow sphere with an outer diameter of 12-14 nm. Stored iron is held within this sphere. Clinical trials have shown that serum Ferritin determination may be used for the diagnosis of iron deficiency anaemia (2, 3). As iron deficiency develops, iron stores are depleted before anaemia becomes apparent. Serum ferritin is useful in detecting iron store depletion as the concentration drops to very low values (4, 5, 6).

FRT-CHECK-1 test is a rapid quantitative assay for the detection of human FRT in serum, plasma or whole blood. The method employs a unique combination of monoclonal-dye conjugate and polyclonal solid phase antibodies to identify FRT 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 FRT forming an antibody-antigen complex. This complex binds to the FRT antibody in the positive reaction zone (T) and produces a pink-rose colour band.

In the absence of FRT, there is no line in the positive reaction zone (T). The reaction mixture continues flowing 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.

II- FRT-CHECK-1 KIT COMPONENTS

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

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

5- **Controls (Optional):**

Positive control (ref. V3300) and Negative control ref. (V3301): 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- FRT-CHECK-1 kit may be stored at any temperature between +4°C and +30°C.

2- Do not freeze the test kit.

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

IV- PRECAUTIONS

1- For *in vitro* diagnostic use and professional use only.

2- Read carefully instructions for use before using this test.

3- Do not use beyond the expiry date which appears on the package label.

4- Do not use a test from a damaged protective wrapper.

5- 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.

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

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

8- Avoid any contact between hands and eyes or nose during specimen collection and testing.

V- SPECIMEN COLLECTION AND PREPARATION

1. FRT-Check-1 is to be 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 hemolysis).

3- If anticoagulant is needed, only EDTA, citrate or heparin should be used.

4- Each specimen should be treated as if 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° 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.

VI- ASSAY PROCEDURE

a) Controls testing

- Wait for 15 minutes.
- 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.

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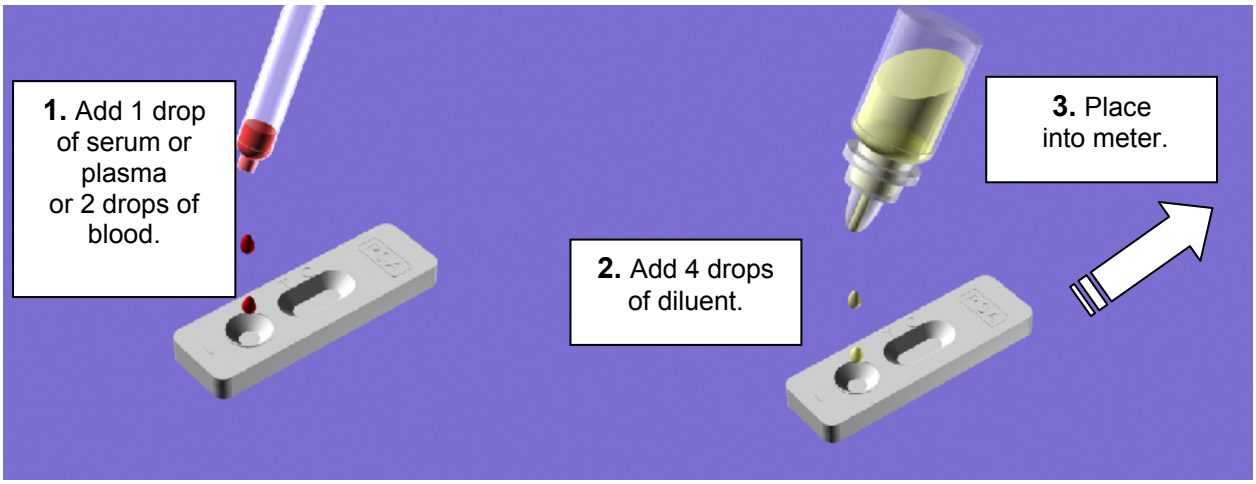
- The concentration range (**in ng/mL**) is indicated on the vial label and obtained result must be within the specified range. The confidence range can change slightly depending on lot number.
- The reconstituted vial should be kept between +2°C and +8°C and should be used within 1 week (7 days) after reconstitution.

b) Sample testing
Follow the below instructions or refer to the picture n°1.

1- Allow samples and FRT-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 specimens (serum, plasma or whole blood) and by holding it vertically, dispense one drop (25 µL) of serum or plasma 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 dropper bottle vertically and 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 15 minutes either using the immediate or countdown reading mode (see corresponding leaflet).

CAUTION
7- There is a matrix effect when assaying whole blood samples. Therefore, in order to get the right ferritin concentration level when using whole blood samples, the obtained value should be multiplied by 1.4 when using the Easy reader® instrument (ref. 36100). For example, if the obtained value for a whole blood sample is 145ng/mL, the real ferritin concentration is 203 ng/mL (145 x 1.4).
For serum/plasma samples, the result represents the real ferritin concentration and there is no need to modify the rendered value.
If the result is obtained with the Easy reader+® instrument (ref. 36200), the obtained value should not be modified whatever the sample type used as the Easy reader+ software is adapted for the sample selection (serum/plasma or whole blood sample).

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 10 – 630 ng/mL.
For ferritin concentration below 10 ng/mL, the result will be given as "< 10 ng/mL".
For ferritin concentration over 630 ng/mL, the result will be given as "> 630 ng/mL".
For samples whose concentration is higher than 630 ng/mL, dilute with saline and repeat the assay as per instructions of Part. VI.

b) Accuracy
A study has been performed using serum samples obtained from dilutions of ferritin W.H.O. reference material n° 94/572. Covering a range of 0 to 630 ng/mL. Optical densities expressed as a function of ferritin concentrations are described by following linear curve:

$$Y = 43.15 + 0.9691 \times (r = 0.9742)$$

The results show a good correlation ($r > 0.95$) of the values obtained with FRT-CHECK-1 on VEDALAB's readers.

c) Sensitivity
Concentrations close to 5ng/mL are detected by FRT-CHECK-1 test.
In these cases, results will be rendered as "< 10 ng/mL".

Levels found outside of the concentrations range reported below are generally considered as abnormal:

- Men : 12-300 ng/mL
- Women : 10-150 ng/mL
- Children (6 months-15 years) : 7-142 ng/mL

d) Precision
A panel of 30 human sera samples pre-assayed on the BAYER CENTAUR analyser have been tested with FRT-CHECK-1 rapid test. Results were read using the VEDALAB's readers. Results are shown in table I.

Table I

Panel samples ID	[FERR] in ng/mL BAYER CENTAUR		[FERR] in ng/mL FRT-CHECK-1	Panel samples ID	[FERR] in ng/mL BAYER CENTAUR		[FERR] in ng/mL FRT-CHECK-1
	Target values	Range			Target values	Range	
V1091477	143	95.91 – 190.19	117.02	V1091492	377	252.59 – 501.41	418.1
V1091478	27	18.09 – 35.91	35.16	V1091493	244	163.48 – 324.52	219.94
V1091479	247	165.49 – 328.51	182.66	V1091494	66	44.22 – 87.78	55.03
V1091480	248	166.16 – 329.84	313.67	V1091495	122	81.74 – 162.26	98.44
V1091481	160	107.20 – 212.80	144.57	V1091497	144	96.48 – 191.52	141.33
V1091482	98	65.66 – 130.34	84.94	V1091498	243	162.81 – 323.19	200.49
V1091483	305	204.35 – 405.65	304.18	V1091499	3	2.01 – 3.99	< 10
V1091484	106	71.02 – 140.98	79.74	V1091500	309	207.03 – 410.97	304.18
V1091485	431	288.77 – 573.23	325.08	V1091501	158	105.86 – 210.14	166.45
V1091486	4	2.68 – 5.32	< 10	V1091502	246	164.82 – 327.18	239.38
V1091487	248	166.16 – 329.84	243.44	V1091503	251	168.17 – 333.83	211.02
V1091488	13	8.71 – 17.29	13.37	V1091504	25	16.75 – 33.25	35.78
V1091489	242	162.14 – 321.86	211.02	V1091505	143	95.81 – 190.19	132.41
V1091490	9	6.03 – 11.97	< 10	V1091506	350	234.5 – 465.5	315,07
V1091491	183	122.61 - 243.39	180.23	**V1091496**	**98**	**65.66 – 130.34**	**249.28**

Discrepancy was obtained only with one sample (identified in bold font). Further investigation has showed that a high level of CRP was found in this sample indicating an acute infectious status and the probable presence of poly-specific reactive antibodies that could interfere in the FRT-CHECK-1 test in the same way as rheumatoid factor as specified in the technical leaflet. In most of cases, negative, borderline and pathological samples were clearly detected. Considering values within 10 to 291 ng/mL for women and 22 to 322 ng/mL for men as reference intervals (BAYER CENTAUR analyser), data from the above table show that 100 % (30/30) of the results obtained with the VEDALAB rapid test correlate with the results obtained on the BAYER CENTAUR analyser.

e) Intra-assay reproducibility
Within run precision was evaluated by using 26 replicates of three commercially available references containing 17.56, 66.32 and 184.83 ng/mL of ferritin as determined with quantitative FRT-CHECK-1 for VEDALAB's readers.
The obtained CVs (coefficient of variation) were respectively equal to 9.58%, 8.86% and 8.92%.

f) Hook effect
There was no observed hook effect up to a ferritin concentration of 10,000 ng/mL.

VIII- LIMITATIONS
1- As for any diagnostic procedure, the physician should evaluate data obtained by the use of this test in the light of other clinical information available.
2- Some serum specimens with a high rheumatoid factor concentration may yield a no specific positive results during testing. Such cases should be identified before testing.
3- The test is designed to eliminate the potential interference of human antibodies to murine IgG (HAMA). However, high level of HAMA could give falsely positive results.
4- <u>Use only fresh whole blood samples (< 4 hours) when test is performed with blood samples. Finger prick samples should be assayed just after collection.</u>
5- This format of test is to be only used with VEDALAB's readers.
6- If the reading time (15 minutes) is not strictly respected, wrong results will be obtained.

7- It is very important, when assaying whole blood samples, to multiply the obtained result by 1.4 in order to get the right ferritin concentration in the sample.
8- This format of test should not be used for visual reading.
9- As it is true 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

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of iron in bone marrow in anemia of chronic disease". Clin. Chem. 37 : 560-563.
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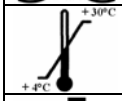
	Read the instructions before use		For *in vitro* diagnostic use
	Temperature limitations		Do not reuse
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