Ferritin Quantitative Test

A Rapid "Sandwich" Immunochromatographic Test for Quantitative Detection of total Ferritin in human finger-prick Blood or Serum

CE REF **1179Q-25**

For in vitro Diagnostic use only ***Read Instructions before use***

INTENDED USE

Affimedix's **TestNOW® - Ferritin Quantitative Test** is an immunochromatography-based one step *in vitro* test. It is designed for the quantitative determination of total Ferritin in human finger-prick blood or serum for iron deficiency anemia. This assay provides a preliminary diagnostic test result and can be used for screening of Ferritin level. The liquid chromatography with tandem mass spectrometry (LC-MS/MS) assays or other quantitative immunoassays are recommended to further confirm the diagnostic test results.

SUMMARY AND EXPLANATION

Iron depletion is a progressive process with anemia as the final phase. Thus, screening for iron deficiency using hemoglobin will only identify the most severe cases. Moreover, hemoglobin is not specific for iron deficiency or iron deficiency anemia. Diagnosing patients for iron deficiency with Ferritin will identify early-stage of iron deficiency and will potentially result in iron therapy, preventing iron deficiency anemia. Ferritin is a universal intracellular protein that stores iron and releases it in a controlled fashion. Ferritin is an indirect marker of the total amount of iron stored in the body; hence serum ferritin is used as a diagnostic test for iron deficiency anemia.

Anemia due to iron depletion is widely held in children and women of all ages but mainly in women who still have their periods (at least 20% suffer from iron deficiency). Main signs are paleness, feeling tired, headaches, faster heartbeat, or shortness of breath during exercise. They may appear gradually and could go unnoticed. Iron deficiency occurs when blood does not contain enough red blood cells and thus low levels of hemoglobin, which is the major protein involved in oxygen transport in whole body. An important component of hemoglobin is iron. Depletion of iron, which can happen during pregnancy, growth, in case of insufficient iron intake, inadequate absorption or blood loss (period, abnormal bleedings, ulcers, etc.) has tremendous effects on health. Low ferritin may also indicate hypothyroidism, vitamin C deficiency or celiac disease. Low ferritin levels are seen in some patients with restless legs syndrome, not necessarily related to anemia, but perhaps due to low iron stores short of anemia.

The purpose of a Ferritin screening is to determine whether your body is storing a normal amount of iron before symptoms develop. A ferritin test can help to diagnose or rule out Iron deficiency anemia, Iron overload, Liver disease, Restless legs syndrome (RLS). Monitoring ferritin levels is important for people receiving iron supplementation therapy for iron deficiency anemia.

Therefore, now detecting Ferritin level is considered as a very important test to maintain and improve overall health and well-being. Multiple guidelines for Ferritin level have been published by various health organizations; but a common recommendation remained to be established. Recent literature has suggested the following ranges for the classification of Ferritin status:

Ferritin Level	Reference Range (ng/ml) - Men	Reference Range (ng/ml) - Women
Low	< 24	< 11
Normal	24 - 336	11 - 307
High	> 336	> 307

TEST PRINCIPLE

TestNOW® - Ferritin Quantitative Test utilizes the principle of Immunochromatography, a unique two-site "Sandwich" immunoassay on a membrane. The test employs an "Exclusive" pair of anti-Ferritin Monoclonal Antibodies; one conjugated with colloidal gold and another one immobilized on the solid phase. This will selectively detect Ferritin with a high degree of sensitivity and specificity. As the test sample flows through the membrane assembly within the test device, the colored anti-Ferritin-colloidal gold conjugate complexes with Ferritin from the sample. This complex moves further on the membrane by the capillary action to the test region (T) where it is immobilized by another anti-Ferritin coated on the membrane, leading to formation of a pink / purple colored band, which confirms a positive test result. The intensity of colored band in the test line region is Ferritin concentration-dependent, higher the concentration of Ferritin in the tested sample, the stronger the colored band is. A control line is present in the test window to work as procedural control. This colored band should always appear on the control line region (C) if the test device is stored in good condition and the test is performed appropriately.

MATERIALS PROVIDED

1. **TestNOW® - Ferritin Quantitative Test** (Kit Size: 25 Tests/Box)
2. 20µl MICROSAFE® Capillary Tube (inside each test pouch)
3. Sample Buffer bottle – 1
4. RFID Card (provides result in ng/ml) - 1
5. Instructions for use – 1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Timer or clock
2. Lancet
3. Alcohol Swab
4. ***RapiRead***™ CUBE Reader (CE Marked) – To be purchased separately

STORAGE AND STABILITY

The test device should be stored at 4ºC to 30ºC and will be effective until the expiration date stated on the package. The product is humidity-sensitive and should be used immediately after being open. Any improperly sealed product should be discarded.

PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Do not use the product beyond the expiration date.
3. Handle all specimens as potentially infectious.
4. Humidity sensitive product, do not open foil pouch until it is ready to be tested.
5. **TestNOW® - Ferritin Quantitative Test** device must be quantified with ***RapiRead™*** CUBE Reader only.
6. RFID Card is Lot Specific and cannot be interchanged with another Lot.

QUALITY CONTROL

Good Laboratory Practice recommends the frequent use of control materials to validate the reliability of test device. If control values do not fall within established range, assay results are invalid.

The **TestNOW® - Ferritin Quantitative Test** device provides a built-in process control with a different antigen/antibody reaction at the control region (C). This control line should always appear regardless the presence of Ferritin. If the control line does not appear, the test device should be discarded, and the obtained result is invalid. The presence of this control band in the control region serves as 1) verification that sufficient sample volume is added, 2) that proper sample flow is obtained.

CAUTION!

TestNOW® - Ferritin Quantitative Test device has been designed for "Decision-Point" Finger-prick Blood (or Serum) samples ONLY. NO Anticoagulated Blood or Plasma samples should be used for testing **TestNOW® - Ferritin Quantitative Test** device as Anticoagulants may impact the test results.

FINGER-PRICK BLOOD SPECIMEN COLLECTION AND PREPARATION

1. Wash the patient's hand with soap and warm water or clean with an alcohol pad. Allow to dry.
2. Message the non-dominant hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
3. Using sterile safety lancet puncture the side of your finger.
4. Collect 20 µl blood using MICROSAFE® Capillary Tube (See instructions below) and perform testing immediately.

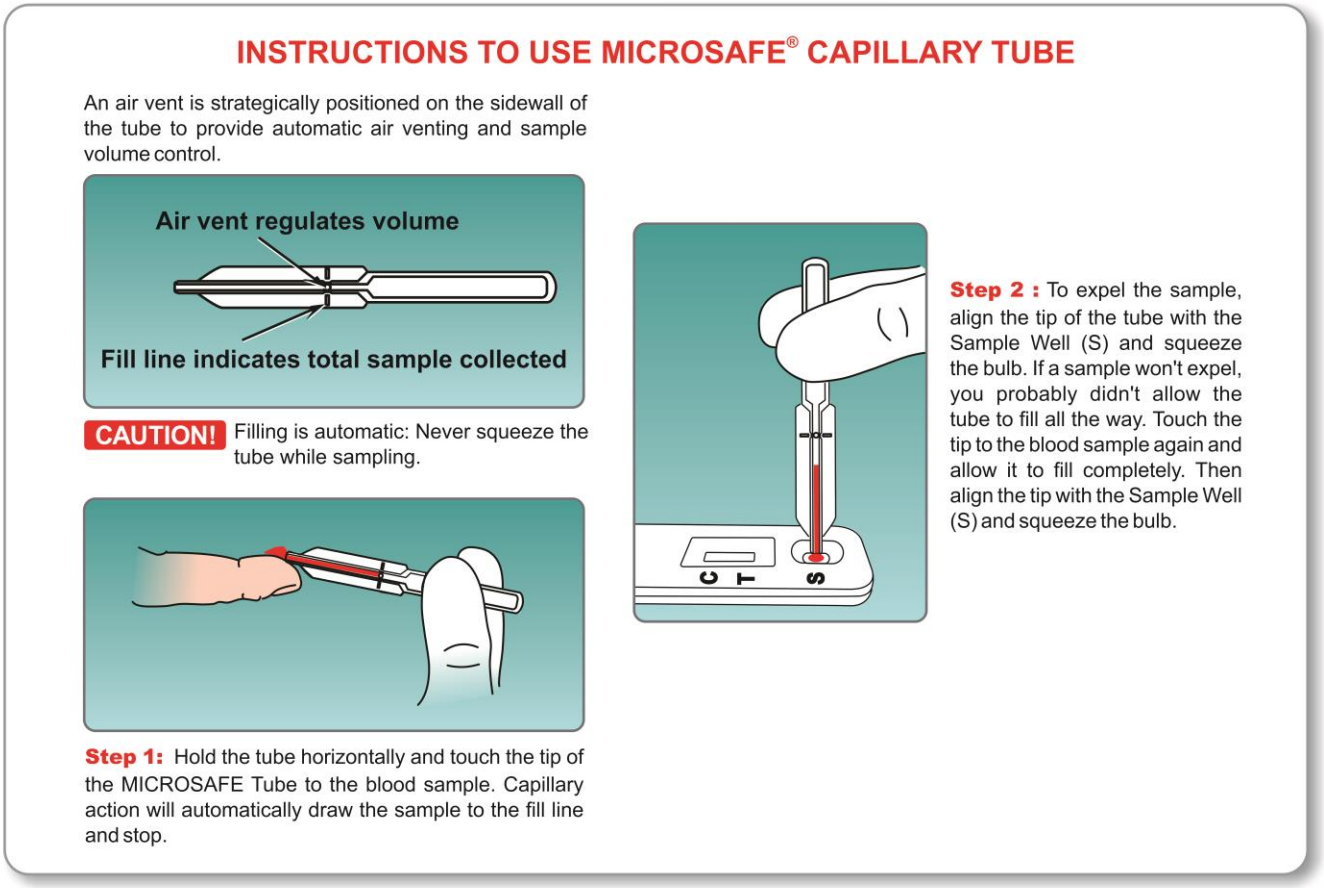
PROCEDURE:

1. Bring all materials and specimens to room temperature (between 21°C – 24°C).
2. Remove the test card from the sealed foil pouch and place it on a hard flat surface.
3. Follow Instructions to use MICROSAFE® Capillary Tube.
4. After applying finger-prick blood, add 3 drops of Sample Buffer into the sample well (S).
5. Read and record the results at 15 Minutes by ***RapiRead™*** CUBE Reader.

SERUM PROTOCOL:

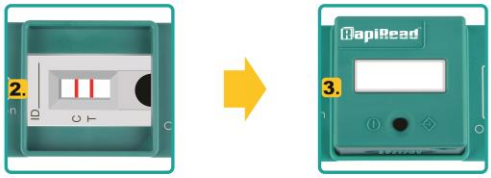
TestNOW® - Ferritin Quantitative Test has been designed for human finger-prick blood. However, Ferritin Serum sample can be used for testing. After applying 10 µl Serum sample using Micropipette (not provided with the Kit) to sample well (S), add 3 drops of Sample Buffer into the sample well (S). Read and record the results at 15 Minutes by ***RapiRead™*** CUBE Reader.

Important Note: ***Result after 15 minutes may not be accurate.***



QUANTITATIVE DETECTION USING *RapiRead*™ CUBE READER

1. Check the "Correct Orientation" shown on the Adaptor for the Test Device and *RapiRead*™ CUBE Reader.



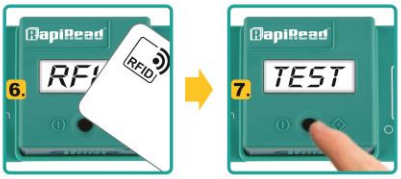
2. Place the Adapter on top of the Test Device correctly.

3. Place the *RapiRead*™ CUBE Reader on top of the Adaptor correctly
- For Non-Timer Protocol: After 15 Minutes of testing.
- For Timer Protocol: After adding 3 drops of pre-mix blood into the Sample Well (S) of the Test Cassette.

NON-TIMER PROTOCOL

4. Turn-on the *RapiRead*™ by pressing the black button. Reader runs a self-test, during the self-test "WAIT" is displayed. After an audible beep signal, "ON" is displayed. To perform a reading, press the black button again once for 1 second.

5. The display will show "RFID".



6. Place the Lot specific RFID Card provided with the Kit onto the top side of the *RapiRead*™. This will upload Ferritin test specific Calibration data from RFID Card to *RapiRead*™.

7. Following an audible beep signal, "TEST' is displayed. Press the black button, the Reader displays "RUN".

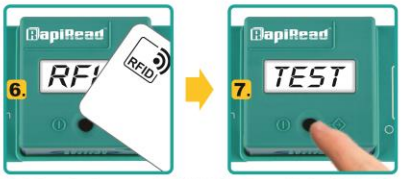
8. After successful data transmission the measurement will start.

9. Ferritin concentration is displayed as ng/ml with an audible beep signal.

TIMER PROTOCOL

4. Turn-on the *RapiRead*™ by pressing the black button. Reader runs a self-test, during the self-test "WAIT" is displayed. After an audible beep signal, "ON" is displayed. Keep pressing black button till display shows RFID.

5. The display will show "RFID".



6. Place the Lot specific RFID Card provided with the Kit onto the top side of the *RapiRead*™. This will upload Ferritin test specific Calibration data from RFID Card to *RapiRead*™.

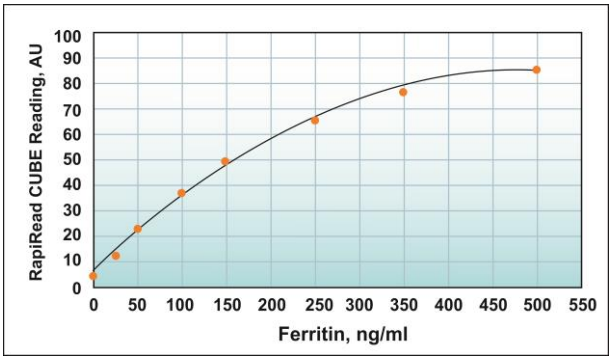
7. Following an audible beep signal, "TEST' is displayed. Press the black button, the countdown timer will start.

8. Countdown timer display will start.

9. After 15 minutes Ferritin concentration is automatically displayed as ng/ml with an audible beep signal.

STANDARD CURVE USING *RapiRead*™ CUBE READER

A typical standard curve is illustrated on right side. The reading AU is automatically converted into ng/ml in RapiRead™ Reader. The serum calibrators were prepared from WHO International Standard Ferritin / NIBSC code: 94/572.



INTERPRETATION OF RESULTS:

The ***RapiRead***™ CUBE analyzer automatically determines the final result by comparing the AU for each sample against a pre-established calibration curve. Ferritin levels are expressed in ng/ml. Please refer to Table on Page-1.

PERFORMANCE CHARACTERISTICS:

Sensitivity:

The sensitivity of **TestNOW® - Ferritin Quantitative Test** device is 3.0 ng/ml (LOD).

Detection Range:

The Detection Range of **TestNOW® - Ferritin Quantitative Test** with ***RapiRead***™ CUBE Reader is from 3.0 ng/ml to 500 ng/ml.

Accuracy:

The accuracy of **TestNOW® - Ferritin Quantitative Test** was evaluated using human finger-prick blood samples in comparison with a reference Ferritin ELISA assay using corresponding serum samples. The comparison result showed a linear regression with slope of 0.91 and Correlation Coefficient of 94%. In conclusion, **TestNOW® - Ferritin Quantitative Test** results of human blood samples showed good agreement with the ELISA results of corresponding serum samples.

The accuracy of **TestNOW® - Ferritin Quantitative Test** was also evaluated using 20 serum samples in comparison with LC-MS/MS Assay ("Gold Standard" for Ferritin measurement). The comparison result showed a linear regression with the slope of 0.93 and Correlation Coefficient of 97%. In conclusion, **TestNOW® - Ferritin Quantitative Test** results agree closely to the true values generated from LC-MS/MS assay.

Precision:

Intra Lot

Sample	No. of Lot	No. of Replicates	Mean ng/ml	Coefficient Variation (CV)
Serum -1	3	20	114	7.9%
Serum -2	3	20	391	13.5%
Blood - 1	3	10	330	11.2%

Inter Lot

Sample	No. of Lot	No. of Replicates	Mean ng/ml	Coefficient Variation (CV)
Serum -1	3	60	114	8.2%
Serum -2	3	60	391	14.5%
Blood - 1	3	15	330	9.3%

Specificity:

30 Ferritin free serum samples were tested, and all showed negative results: suggesting 100% Specificity.
No interference and cross reactivity was observed with Bilirubin, Triglycerides, Cholesterol, Vitamin B12 and Vitamin C.

EXPECTED RESULTS

TestNOW® - Ferritin Quantitative Test is a Rapid Quantitative assay. The test is intended to use for screening individuals to identify Ferritin level. This assay provides only a preliminary analytical test result. The liquid chromatography with tandem mass spectrometry (LC-MS/MS) assays or quantitative immunoassays are recommended to confirm the analytical result.

REFERENCES

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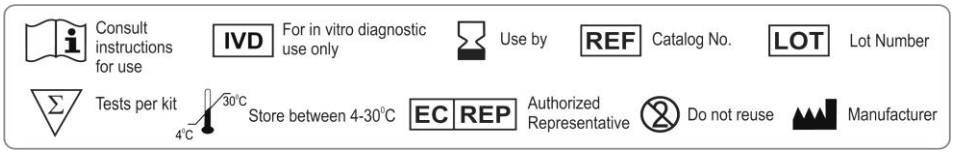
SHELF LIFE: 18 Months

INDEX OF CE SYMBOLS



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