Fer-color

For serum iron determination

SUMMARY

Iron is distributed in the body in different ways, including hemoglobin, tissue iron and myoglobin. Iron transport from one organ to another is performed by a carrier protein called apotransferrin. This complex is known as transferrin.
Ferritin, present in most cells, constitutes an iron reservoir available for hemoglobin formation and further proteins containing the hemo group. Iron absorption is mainly produced in the duodenum. Both ferritin and transferrin are present in the intestinal mucous membrane cells and together they regulate iron absorption.
The main metabolism disorders are related to their deficiency or excess; however, alterations have been observed in many other diseases, including anemia, cardiovascular diseases, chronic hepatitis, renal diseases and infections.
One of the most frequently organic disorders found in clinical practice is the anemia caused by iron loss. It is usually observed in children, young and pregnant women, and the elderly. Gastric or duodenal ulcers and stomach carcinoma may also lead to ferropenic anemia.
On the contrary, the iron excess is associated to other disorders such as hemosiderosis, hemochromatosis and sideroblastic anemia.

PRINCIPLE

Serum iron is released from its specific carrier protein, transferrin, in pH 3.7 succinate buffer in the presence of a reducing agent, mercaptoacetic acid. Then it reacts with the color reagent, pyridyl bis-phenyl-triazine sulphonate (PBTS) producing a colored complex, which is measured at 560 nm.

PROVIDED REAGENTS

A. Reagent A: 0.25 mmol/l succinate buffer for pH 3.7.
B. Reagent B: self break ampoule containing 70% mercaptoacetic acid (reducing agent).
C. Reagent C: 50 mmol/l pyridyl bis-phenyl triazine sulphonate (PBTS) stabilized solution.
S. Standard: ferric ions (III) solution equivalent to 100 ug/dl.

NON-PROVIDED REAGENTS

Distilled water.

INSTRUCTIONS FOR USE

Reagent and **Standard:** ready to use.
Reagent A+B: transfer the contents of the Reagent B ampoule to the Reagent A bottle. Mix by inversion. Write down the date of preparation on the label.

WARNINGS

Reagents are for “in vitro” diagnostic use.
Reagent B is corrosive. H315+H320: Causes skin and eye irritation. P262: Do not get in eyes, on skin, or on clothing. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P302 + P352: IF ON SKIN: Wash with plenty of soap and water. P280: Wear protective gloves/protective clothing/eye protection/face protection.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: are stable at room temperature until the expiration date shown on the box.
Reagent A+B: is stable in refrigerator (2-10ºC) one year since preparation date. Bring to 18-30ºC before use.

INSTABILITY OR DETERIORATION OF REAGENTS

Variations in Blank or Standard readings show occasional contamination (water, glassware, etc.). An increase in Blank values indicates a contamination with iron.

SAMPLE

Serum
a) Collection: only serum must be used, since anticoagulants interfere with color reaction. The patient must be fasting and extractions should preferably be performed in the morning, always at the same time since physiological fluctuations are significant during the day.
b) Additives: not required.
c) Known interfering substances: hemoglobin interference is not observed up to 70 mg/dl, while hyperlipemia, Cu ions up to 200 ug/dl and bilirubin up to 400 mg/dl do not interfere. Although slight hemolysis does not interfere with this method, the International Committee for Standardization in Hematology (ICSH) recommends the use of serum free from hemolysis. See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: serum can be kept in refrigerator (2-10ºC) up to one week.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer and photocolorimeter.
- Micropipettes and pipettes for measuring the stated volumes
- Tubes or spectrophotometric cuvettes.

ASSAY CONDITIONS

- Wavelength: 560 nm in spectrophotometer or 540-560 nm

in photocolorimeter with green filter.
- Reaction temperature: room temperature.
- Reaction time: 10 minutes
- Sample volume: 500 ul
- Total reaction volume: 2.5 ml

PROCEDURE

In three tubes or cuvettes labeled B (Reagents Blank), S (Standard) and U (Unknown) add:

	B	S	U
Bidistilled water	500 ul	-	-
Standard	-	500 ul	-
Serum	-	-	500 ul
Reagent A+B	2 ml	2 ml	2 ml

Mix. Read U tube absorbance (Serum Blank, SB) in spectrophotometer at 560 nm or in photocolorimeter with green filter (540-560 nm) setting the instrument to zero with distilled water.

Add one drop of Reagent C to every tube holding the dropper bottle in vertical position. Mix immediately each tube and read all tubes at 560 nm between 6 and 20 minutes, setting the instrument to zero with water.

STABILITY OF FINAL REACTION

Tubes must be read between 6 and 20 minutes after having completed the Procedure.

CALCULATIONS

Correct readings of S and U, subtracting the corresponding Blanks:

S - B = corrected S

U - (B + BS) = corrected U x f

$$\text{Where: } f = \frac{100 \text{ ug/dl}}{\text{Corrected S}}$$

QUALITY CONTROL METHOD

Test two levels of a quality control material (**Standatrol S-E 2 niveles**) with known iron concentration for each determination.

THEORETICAL VALUES

Men: 65 to 175 ug/dl (11.6 - 31.3 umol/l)
Women: 50 to 170 ug/dl (9 - 30.4 umol/l)

REFERENCE VALUES

Among a group of 20 healthy women and 20 healthy men, between 18 and 51 years of age, a range of 55-175 ug/dl* was observed, obtaining the following mean values:

Men: 114.6 ug/dl (20.5 umol/l)
Women: 103.3 ug/dl (18.5 umol/l)

* Reference values obtained from Wiener lab. records.
Each laboratory should establish its own references values.

SI SYSTEM UNITS CONVERSION

Iron (ug/dl) x 0.179 = Iron (umol/l)

PROCEDURE LIMITATIONS

- Acceptable Blank values: oligos determination requires prevention from possible water and reagent contamination. The Reagent Blank, tested according to the Package Insert, should not be higher than 0.150 O.D. Also the water contribution to the Blank should be insignificant. To control this, the reading of a Reagent Blank is recommended (2 ml Reagent A+B + 0.5 ml water + 1 Reagent C drop) against a Reagent A Blank (2.5 ml Reagent A+B + 1 Reagent C drop): the Reagent Blank reading should be lower or equal to the Reagent A Blank. Hence, the use of proven quality water is recommended (conductivity below 0.02 uOhms) On the other hand, if the Reagent A Blank reading is higher than 0.150 O.D. it indicates reagent's contamination, which should be discarded. Perform such control periodically.
- Mixing: the tubes contents can be mixed with a rod or by gently shaking. Do not mix by inversion so as to avoid contamination.
- Labware washing instructions: the labware used should be iron-free, so it should be submerged for 6 hours into 10-15% analytical grade hydrochloric acid, eliminating the acidity with several rising with iron-free water. Let the material dry at a temperature not above 80ºC in stainless steel or vinyl coated racks. This material should be exclusively used for iron determination.

PERFORMANCE

a) Reproducibility: by processing the same sample in 10 different days, a 4.2% coefficient variation was obtained for a 129 ug/dl serum iron level.

b) Recovery: a recovery between 90 and 103% was obtained by adding known amounts of Fe (II) to aliquots of the same serum.

c) Linearity: reaction is linear up to 500 ug/dl.

WIENER LAB. PROVIDES

Kit for 100 tests (Cat. Nº 1492001).

REFERENCES

- Dixon, K. - Ann. Clin. Biochem. 10/5:127 (1973).
- I.C.S.H. - Am. J. Clin. Path. 56/4:543 (1971).
- Zak, B.; Baginski, E.S.; Epstein, E. y Wiener, L.M.- Clin. Toxicol. 4/4:621 (1971).
- Rojkín, M. L.; Olguín de Mariani, M. C.; Drappo, G. A. y Albarracín, A. - III Congreso Argentino de Bioquímica - Buenos Aires (1975).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

Symbols

The following symbols are used in the packaging for Wiener lab. diagnostic reagent kits.

CE — This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Σ — Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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