Fer-color

AA

Direct colorimetric method for the determination of iron in serum or plasma

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 4.5 acetate buffer and in the presence of a reducing agent (ascorbic acid). Then it reacts with the color reagent, pyridyl bis-phenyl triazine sulfonate (ferrozine) producing a magenta color complex measured at 560 nm.

PROVIDED REAGENTS

A. Reagent A: 150 mmol/l acetate solution for pH 4.5 containing guanidine.
B. Reagent B: ascorbic acid.
C. Reagent C: ferrozine stabilized solution.
S. Standard: ferric ions solution (III) equivalent to 100 ug/dl.

Final concentrations

Acetate	150 mmol/l, pH 4.5
Ferrozine	0.2 mmol/l
Ascorbic acid	0.03 mol/l
Guanidine	4.0 mol/l

NON-PROVIDED REAGENTS

- Wiener lab.'s **Calibrador A plus.**
- Distilled water.

INSTRUCTIONS FOR USE

Reagent C: ready to use.
Standard: ready to use
Working Reagent: transfer to the Reagent B vial the amount of Reagent A stated on the label. Date.

WARNINGS

Reagents are for "in vitro" diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
All reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: are stable at room temperature (< 25ºC) until the expiration date shown on the box.
Working Reagent: stable for 3 months from preparation date stored in refrigerator (2-10ºC) or for 10 days at room temperature (18-25ºC).

INSTABILITY OR DETERIORATION OF REAGENTS

Variations in Blank and/or Standard readings show occasional contamination (water, glassware, etc.). An increase in Blank values will indicate contamination with iron.

SAMPLE

Serum or plasma
a) Collection: the patient must be fasting and extractions should be performed always at the same time (preferably in the morning) since physiological fluctuations are significant during the day.
b) Additives: use heparin as anticoagulant whenever plasma is used as sample.
c) Known interfering substances: hemoglobin interference is not observed up to 90 mg/dl, bilirubin up to 15 mg/dl and heparin up to 50 IU/ml. Interference is observed with 3,72 g/l triglycerides.
Lipemic samples may yield erroneous results.
Although light 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 or heparinized plasma may be stored up to one week in refrigerator (2-10ºC).

REQUIRED MATERIAL (non-provided)

- Spectrophotometer or photocolorimeter.
- Micropipettes and pipettes for measuring the stated volumes
- Spectrophotometric tubes or cuvettes.

ASSAY CONDITIONS

- Wavelength: 560 nm in spectrophotometer or 540-560 nm in photocolorimeter with green filter.
- Reaction temperature: room temperature
- Reaction time: 5 minutes
- Sample volume: 200 ul
- Final reaction volume: 1.4 ml

PROCEDURE

In three photocolorimeter tubes labeled B (Reagent Blank), S (Standard) and U (Unknown) place:

	B	S	U
Bidistilled water	200 ul	-	-
Standard	-	200 ul	-
Serum	-	-	200 ul
Working Reagent	1 ml	1 ml	1 ml

Mix. Read absorbance of U Tube (Serum Blank: SB) in spectrophotometer at 560/580 nm or in photocolorimeter with green filter (540/560 nm) setting the instrument to zero with water. Then add:

Reagent C	200 ul	200 ul	200 ul

Mix at once. Reread each tube after 5 minutes, setting the instrument to zero with water.

STABILITY OF FINAL REACTION

Tubes must be read within 5 and 60 minutes after completing the procedure steps.

CALCULATIONS

Correct S and U readings, subtracting the corresponding Blanks:

S - B = corrected S
U - (B + SB) = corrected U
Fe (ug/dl) = 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: oligo-elements determination requires prevention from possible water and reagent contamination. The Reagent Blank, tested according to the Instruction Insert, should not be higher than 0.150 O.D. Also the water contribution to the blank should be insignificant. Hence, the use of proven quality water is recommended (conductivity below 0.02 uOhms).
- Cleaning of the material: 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 numerous washes steps using iron-free water. Dry the material at a temperature not above 80°C in stainless steel or vinyl coated baskets. This material should be exclusively used for iron determination.

PERFORMANCE

a) Reproducibility: when replicates of the same samples were assayed on the same day, the following results were obtained:

Level	S.D.	C.V.
146.5 ug/dl	± 1.93 ug/dl	1.32 %
324.6 ug/dl	± 1.76 ug/dl	0.54 %

Performing the same assay on different days, the following results were obtained:

Level	S.D.	C.V.
142.8 ug/dl	± 2.50 ug/dl	1.75 %
314.0 ug/dl	± 3.93 ug/dl	1.25 %

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 1000 ug/dl.
d) Detection limit: 6.05 ug/dl.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user manual of the autoanalyzer in use. For calibration, Wiener lab. **Calibrador A plus** should be used, according to the autoanalyzer requirements.

WIENER LAB. PROVIDES

- 5 x 20 ml (100 ml Reagent A) (Cat. Nº 1492003).

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

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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