LIQUID LINE

Fer-color

AA

Direct colorimetric method for iron determination 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.
Photometric techniques for iron determination in serum are based on a chromogen complex formation, among which ferrozine and bathophenanthroline have been widely used. The present method uses ferene, and additional chelating agent, to increase the colorimetric assay sensitivity. This compound presents a high molar absorptivity, is more sensitive than ferrozine and facilitates iron detection.

PRINCIPLE

Serum iron is released from the transferrin complex in acid medium and is reduced to Fe (II) with ascorbic acid. Then it reacts with the color reagent, ferene, yielding a blue color complex measured at 600 nm. The obtained absorbance is directly proportional to the iron concentration.

PROVIDED REAGENTS

A. Reagent A: 200 mM citric acid solution, 34 mM ascorbic acid, 100 mM thiourea and surfactant.
B. Reagent B: ferene stabilized solution > 3 mM.
S. Standard*: ferric ions solution (III) equivalent to 100 ug/dl.

NON-PROVIDED REAGENTS

- Wiener lab. **Calibrador A plus**.
- Deionized water.

INSTRUCTION FOR USE

Provided Reagents: ready to use.

WARNINGS

Reagents are for "in vitro" diagnostic use. Avoid ingestion and direct contact with eyes.
Reagent A contains thiourea. Research studies performed in animals using this drug have shown a possible carcinogenic effect.
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

Reagents A and B: stable at 2-10ºC until the expiration date stated on the box.
Standard: stable at room temperature (< 25ºC) until the expiration date stated on the box.

INSTABILITY OR DETERIORATION OF REAGENTS

Variations in Reagent Blank and/or Standard readings indicate occasional contamination (water, glassware, etc.). Any increase in Blank values exhibits iron contamination.

SAMPLE

Serum or heparinized plasma
a) Collection: the patient must be fasting and withdrawals 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: no interference has been observed with hemoglobin up to 300 mg/dl, conjugated bilirubin up to 12 g/dl and heparin up to 50 IU/ml. Triglycerides do not interfere until a concentration of 1000 mg/dl using autoanalyzer and 250 mg/dl by manual procedure.
Although light hemolysis does not interfere with this method, the International Committee for Standardization in Hematology (ICSH) recommends the use of hemolysis free serum.
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 at 2-10ºC or up to one year at -20ºC.

** Non-provided with all kit sizes*

REQUIRED MATERIAL (non-provided)
- Spectrophotometer or autoanalyzer.
- Micropipettes and pipettes for measuring the stated volumes.
- Spectrophotometric tubes or cuvettes.
- Stopwatch.

ASSAY CONDITIONS
- Wavelength: 600 nm
- Reaction temperature: room temperature (< 25ºC)
- Reaction time: 5 minutes
- Total reaction volume: 1.4 ml

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

	B	S	U
Bidistilled water	200 ul	-	-
Standard	-	200 ul	-
Sample	-	-	200 ul
Reagent A	1 ml	1 ml	1 ml
Mix. Measure absorbance of U Tube (Serum Blank: BB) in spectrophotometer at 600 nm setting the instrument to zero with water. Then add:			
Reagent B	200 ul	200 ul	200 ul
Mix at once. Remeasure each tube after 5 minutes, setting the instrument to zero with water.			

STABILITY OF FINAL REACTION
Tubes must be read within 5 and 30 minutes after completing the procedure steps.

CALCULATIONS
Correct S and U readings, substracting the corresponding Blanks:

S - B = corrected S
U - (B + SB) = corrected U
Fe (ug/dl) = corrected U x f

$$\text{where: } f = \frac{\text{100 ug/dl (Standard)*}}{\text{corrected S}}$$

* If **Calibrador A plus** is used, refer to the designed values since they are lot-specific. In this case, the calibrator reading should be corrected subtracting the corresponding blank.

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 require prevention from possible water and reagent contamination with iron. The Reagent Blank, tested according to the packaged insert, should not be higher than 0.05 O.D. The water contribution to the blank should also be insignificant. Hence, the use of proven quality water is recommended.
- Reagent A may developed a slight yellowish color in time, which does not affect its reactivity.
- Material cleaning process: the labware used should be iron-free, submerge it for 6 hours into 10% HCl, eliminating the acidity with numerous washing steps using iron-free water. This material should be exclusively used for iron determination.

PERFORMANCE
a) Reproducibility: using CLSI (former NCCLS) EP15-A document as guideline, the following results were obtained:

Intra-assay precision (n = 20)

Level	S.D.	C.V.
61.84 ug/dl	± 0.87 ug/dl	1.40 %
116.89 ug/dl	± 0.46 ug/dl	0.39 %
236.31 ug/dl	± 0.72 ug/dl	0.31%

Total precision (n = 20)

Level	S.D.	C.V.
61.82 ug/dl	± 0.88 ug/dl	1.42 %
116.89 ug/dl	± 1.30 ug/dl	1.11 %
236.31 ug/dl	± 2.83 ug/dl	1.20 %

b) Linearity: reaction is linear up to 1500 ug/dl in auto-analyzers and up to 1000 ug/dl using the manual technique.
c) Detection limit: the minimum detectable iron concentration using **Fer-color AA líquida** method is 4 ug/dl.
d) Quantification limit: the minimum iron concentration that may be quantitatively determined with acceptable precision and accuracy using **Fer-color AA líquida** method is 4 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

120 ml (Cat. Nº: 1492360): - 1 x 100 ml Reagent A
- 1 x 20 ml Reagent B
- 1 x 20 ml Standard

120 ml (Cat. Nº: 1009285): - 2 x 50 ml Reagent A
- 2 x 10 ml Reagent B

120 ml (Cat. Nº: 1009336): - 2 x 50 ml Reagent A
- 2 x 10 ml Reagent B

120 ml (Cat. Nº: 1009613): - 2 x 50 ml Reagent A
- 2 x 10 ml Reagent B

REFERENCES

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SYMBOLS

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