

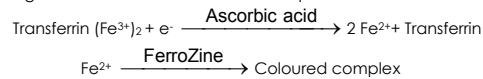
IRON



Iron. FerroZine. Colorimetric Ref.:IRO-023
Quantitative Determination of iron
 Only for *in vitro* use in clinical laboratory
 Store at 2-8°C 4 x 50 mL

PRINCIPLE OF THE METHOD

The iron is dissociated from transferring-iron complex in weakly acid medium. Liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions give with FerroZine a coloured complex:



The intensity of the color formed is proportional to the iron concentration in the sample^{1,2}.

CLINICAL SIGNIFICANCE

The iron is the component of a great number of enzymes. The myoglobin, muscular protein, contains iron, as well as the liver. Iron is necessary for the hemoglobin production, molecule that transports oxygen inside red globules. Their deficit in the last causes the ferropenic anemia. High levels of iron are found in hemochromatosis, cirrhosis, hepatitis and in increased transferrin levels. The variation day to day is quite marked in healthy people^{1,5,6}. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1	Buffer	Acetate pH 4.9	100 mmol/L
R 2	Reductant	Ascorbic acid	99.7%
R 3	Color	FerroZine	40 mmol/L
IRON CAL	Iron aqueous primary standard 100 µg/dL		

PREPARATION

Working reagent (WR):
 - Ref: 1001247. Dissolve (→) the contents of one tube R 2 Reductant in one bottle of R 1 Buffer.
 - Ref:1001248. Dissolve (→) one dose (dispense using the enclosed spoon) of R 2 Reductant in one bottle of R 1 Buffer.
 Cap and mix gently to dissolve contents.
 Stability: 3 months at 2-8°C or 1 month at 15-25°C.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.

IRON CAL

Once open is stable up to 1 month when stored tightly closed at 2-8°C, protected from light and contaminations are prevented during their use.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 562 nm ≥ 0.020.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 562 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment (Note 1).

SAMPLES

Serum or heparinized plasma.
 Fee of hemolysis and separated from cells as rapidly as possible.
 Stability of the sample: 2-8°C for 7 days¹.

PROCEDURE

- Assay conditions:
 Wavelength: 562 nm (530-590)
 Cuvette: 1 cm light path
 Temperature 37°C / 15- 25°C
- Adjust the instrument to zero with distilled water.
- Pipette into a cuvette:

	WR Blank	Standard	Sample Blank	Sample
WR (mL)	1.0	1.0	1.0	1.0
R 3 (drops)	1	1	--	1
Distilled water (µL)	200	--	--	--
Standard ^(Note 2,3) (µL)	--	200	--	--
Sample (µL)	--	--	200	200

- Mix and incubate 5 min at 37°C or 10 min at room temperature.
- Measure the absorbance (A) of Standard and sample against WR Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

$$\frac{(A)\text{Sample} - (A)\text{Sample Blank}}{(A)\text{Standard}} \times 100 (\text{Standard conc.}) = \mu\text{g/dL iron}$$

Conversion factor: µg/dL x 0.179 = µmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES⁵

Male 65 - 175 µg/dL ≅ 11.6 - 31.3 µmol/L^(Note 4)
 Female 40 - 150 µg/dL ≅ 7.16 - 26.85 µmol/L^(Note 4)

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 1.85 µg/dL to linearity limit of 1000 µg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
	Mean (µg/dL)	SD	CV (%)	
Mean (µg/dL)	102	190	107	193
SD	0.88	1.31	1.26	1.57
CV (%)	0.86	0.69	1.18	0.81

Sensitivity: 1 µg/dL = 0.0009 A.

Accuracy: Results obtained using BSM reagents did not show systematic differences when compared with other commercial reagents.

The results obtained using 50 samples were the following:

Correlation coefficient (r): 0.987.

Regression equation: y = 1,0052x - 2.3159.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemolyzed samples are rejected, since erythrocytes contain iron and therefore falsely elevate the serum results^{1,2}.

A list of drugs and other interfering substances with iron determination has been reported by Young et. al^{3,4}.

NOTES

- It is recommended to use disposable material. If glassware is used the material should be soaking for 6 h in diluted HCl (20% v/v) and then thoroughly rinsed with distilled water and dried before use.
- Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
- Use clean disposable pipette tips for its dispensation.
- Strongly method dependent.
- BSM has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

BIBLIOGRAPHY

- Perrotta G. Iron and iron-binding capacity. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1063-1065.
- Itano M M D. Cap Serum Iron Survey 1978 (70): 516-522.
- Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
- Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
- Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
- Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.