



Conservar entre: +2+8°C.

Presentation:

Cod. SU022
Cal 1 x 5 mL

CONT: R1 4 x 50 mL. + R2 1 x 100 mg. + R3 1 x 10 mL. +

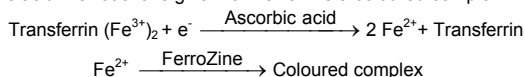
Procedure

Quantitative determination of iron.

Only for in vitro use in clinical laboratory (IVD)

TEST SUMMARY

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}.

REAGENTS COMPOSITION

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.

REAGENT PREPARATION AND STABILITY

- Working reagent (WR): Dissolve (→) the contents of one tube 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.

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 contamination prevented during their use.

Iron Cal:

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

Signs of reagent deterioration:

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

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8°C. Do not use reagents over the expiration date.

SPECIMEN

Serum or heparinized plasma.

Free of hemolysis and separated from cells as rapidly as possible.

Stability of the sample: 2-8°C for 7 days¹.

MATERIAL REQUIRED BUT NOT PROVIDED

- Spectrophotometer or colorimeter measuring at 562 nm.
- Matched cuvettes 1.0 cm. light path.

General laboratory equipment^(Note 1).

TEST PROCEDURE

- Assay Conditions
 - Wavelength : 562 nm. (530-590).
 - Cuvette: 1 cm light path.
 - Temperature37°C. 15-25°C.
- Adjust the instrument to zero with distilled water.
- Pipette into a cuvette:

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

- Mix and incubate 5 min at 37°C or 10 min at room temperature (15-25°C).
- Read the absorbance (A) of calibrator and sample against WR Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

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

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

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, Normal and Pathological. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES⁴

Male	65 - 175 µg/dL \equiv 11,6-31,3 µmol/L ^(Note 4)
Female	50 - 170 µg/dL \equiv 11,6-31,3 µmol/L ^(Note 4)

(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

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.

REAGENT PERFORMANCE

- Measuring Range:

From detection limit of 5.74 µg/dL. to linearity limit of 1000 µg/dL., under the described assay conditions.

If results obtained were greater than linearity limit, dilute the sample ½ with NaCl 9 g/L. and multiply result by 2.

- Precision:

Mean (µg/dL)	Intra-assay n= 20		Inter-assay n= 20	
	103	190	107	192
SD	3.02	1.31	1.38	1.64
CV (%)	2.91	0.69	1.29	0.85

- Sensitivity:

1 µg/dL. = 0.0002 A

- Accuracy:

Results obtained GPL reagents did not show systematic differences when compared with other commercial reagents.

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

INTERFERING SUBSTANCES

- Hemolyzed samples are rejected, since erythrocytes contain iron and therefore falsely elevate the serum results¹².
- 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.

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.

