Reactivos GPL

Barcelona, España

CE

-IRON FZ-

IRON
Ferrozine Colorimetric

Store at: +2+8ºC.

Presentation:
Cod. SU022 CONT: *R1 4 x 40 mL.+ R2 4 x 10 mL. +CAL 1 x 5 mL.*
Cod. SU022SP CONT: *R1 1 x 40 mL.+ R2 1 x 10 mL. + CAL 1 x 5 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:

$$\text{Transferrin } (Fe^{3+})_2 + e^- \xrightarrow{\text{Ascorbic acid}} 2\ Fe^{2+} + \text{Transferrin}$$

$$Fe^{2+} \xrightarrow{\text{FerroZine}} \text{Coloured complex}$$

The intensity of the colour 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 (Colour)	FERROZINE, ASCORBIC ACID	40 mmol/L.
IRON CAL	IRON AQUEOUS PRIMARY STANDARD	100 µg/dL

REAGENT PREPARATION AND STABILITY

All the reagents are ready to use.

Iron Cal: Proceed carefully with this product because due its nature it can get contaminated easily.

Signs of reagent deterioration:

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

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-25ºC, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.

SPECIMEN

Serum or heparinized plasma.
Fee of haemolysis and separated from cells as rapidly as possible.
Stability of the sample: 2-8ºC for 7 days[1].

MATERIAL REQUIRED BUT NOT PROVIDED

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

General laboratory equipment.

TEST PROCEDURE

1. **Assay Conditions**
   - Wavelength: ..... 562 nm. (530-590).
   - Cuvette: ..... 1 cm light path.
   - Temperature .....37ºC / 15-25ºC.
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette: (Note 3)

	Reagent Blank	Calibrator	Sample Blank	Sample
R1 (µL)	800	800	800	800
R2 (µL)	200	200	--	200
Distilled water (µL)	120	--	--	--
Calibrator (µL) (Note 2)	--	120	--	--
Sample (µL)	--	--	120	120

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

CALCULATIONS

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

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

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, Control H Normal Ref. QC003 and Control H Pathological Ref. QC004. 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 VALUES

Male:	65 - 175 µg/dL	≅	11,6 - 31,3 µmol/L (Note 4)
Female:	40 - 150 µg/dL	≅	7,6 – 26,85 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 haemoglobin production, molecule that transports oxygen inside red globules. Their deficit in the last causes the ferropenic anaemia. 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 7,23 µg/dL. to linearity limit of 3000 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*:

	Intra-assay n= 20		Inter-assay n= 20	
Mean (µg/dL)	113	250	111	249
SD	0.89	0.72	3.51	6.29
CV (%)	0.79	0.29	3.17	2.52

- *Sensitivity:*
1 µg/dL. = 0.00104
- *Accuracy:*
Results obtained using GPL reagents did not show systematic differences when compared with other commercial reagents.
The results obtained using 50 samples were the following:
Correlation coefficient $(r)^2$: 0.9934
Regression equation : y= 1.0243x – 3.877
The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCES

Haemolyzed 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

1. 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.
2. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.
4. **The reference values are strongly method dependent**.

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