COD 11509 4 x 50 mL
STORE AT 2-8ºC
Reagents for measurement of iron concentration Only for *in vitro* use in the clinical laboratory

IRON - FERROZINE

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PRINCIPLE OF THE METHOD

Transferrin-bound ferric ions in the sample are released by guanidinium and reduced to ferrous by means of ascorbic acid. Ferrous ions react with ferrozine forming a coloured complex that can be measured by spectrophotometry[1,2,3].

CONTENTS AND COMPOSITION

A. Reagent. 4 x 40 mL. Guanidinium chloride 1.0 molL, acetate buffer 0.4 mol/L, pH 4.0.

B. Reagent. 4 x 10 mL. Ferrozine 8 mmol/L, ascorbic acid 200 mmol/L.

S. Iron Standard. 1 x 5 mL. Iron 200 µg/dL (35.8 µmol/L). Aqueous primary standard.

STORAGE

Reagents: Store at 2-8ºC.

Standard (S): Store at 2-30ºC.

Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.080 at 560 nm.
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Standard (S) is provided ready to use.

Working Reagent: Transfer the contents of one Reagent B vial into a Reagent A bottle. Mix thoroughly. Other volumes can be prepared in the proportion: 1 mL Reagent B + 4 mL Reagent A. Stable for 6 months at 2-8ºC.

ADDITIONAL EQUIPMENT

- Analyzer, spectrophotometer or photometer able to read at 560 ± 20 nm.

SAMPLES

Serum or heparinized plasma collected by standard procedures.

Iron in serum or heparinized plasma is stable for 3 weeks at 4-8ºC[4].

PROCEDURE

1. Bring the Reagent to room temperature.
2. Pipette into labelled test tubes: (Notes 1, 2)

	Reag.Blank	Sample Blank	Sample	Standard
Distilled Water	200 µL	—	—	—
Sample	—	200 µL	200 µL	—
Iron Standard (S)	—	—	—	200 µL
Reagent (A)	—	1.0 mL	—	—
Working Reagent	1.0 mL	—	1.0 mL	1.0 mL

3. Mix thoroughly and let stand the tubes for 5 minutes at room temperature.
4. Read the absorbance (A) of the Sample Blanks at 560 nm against distilled water.
5. Read the absorbance (A) of the Samples and of the Standard at 560 nm against the Reagent Blank.

CALCULATIONS

The iron concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample} - A_{Sample\ Blank}}{A_{Standard}} \times C_{Standard} = C_{Sample}$$

REFERENCE VALUES

Serum and plasma[5]

Men:	70 – 180 µg/dL	=	12.5 – 32.2 µmol/L
Women:	60 – 180 µg/dL	=	10.7 – 32.2 µmol/L

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 6.17 µg/dL = 1.10 µmol/L. Quantification limit: 33.2 µg/dL = 5.94 µmol/L.
- Linearity limit: 1000 µg/dL iron = 179 µmol/L. Measuring range: 33.2 - 1000 µg/dL. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Precision:

Mean concentration	Repeatability (CV)	Within-laboratory (CV)
61.9 µg/dL = 11.1 µmol/L	7.5 %	7.9 %
98.8 µg/dL = 17.7 µmol/L	4.1 %	5.8 %
315 µg/dL = 56.4 µmol/L	1.6 %	2.1 %

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 3). Details of the comparison experiments are available on request.
- Interferences: Bilirubin (< 20 mg/dL) and lipemia (triglycerides < 15 g/L) do not interfere. Hemolysis interferes. Other drugs and substances may cause interference[7].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Iron is distributed in the body in a number of different compartments: hemoglobin, myoglobin, tissues (mainly in liver, spleen, bone marrow). Only 0.1% of total body iron is present in plasma.

Serum iron concentration is affected by many physiological or pathological conditions. Day-to-day variation is quite marked in healthy people.

Iron deficiency and iron overload are the major disorders of iron metabolism. However, altered iron metabolism is also related to a number of other diseases.

Serum iron is increased in hemochromatosis, in acute iron poisoning, in active cirrhosis or acute hepatitis and as a result of increased transferrin levels[6,8].

Serum iron concentration is decreased in many but not all patients with iron deficiency anemia and in chronic inflammatory disorders. Measurement of serum iron should not be used as a test for identification of iron deficiency[6,8].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
2. Contamination of glassware with iron will affect the test. Use acid-washed glassware or plastic tubes.
3. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

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