IRON

FERROZINE

Intended use:
In vitro test for the quantitative determination of iron in human serum and plasma.

Summary:
Ingested iron is mainly absorbed in the form of Fe2+ in the duodenum and upper jejunum. The trivalent form and the heme-bound Fe2+-component of iron in food has to be reduced by vitamin C. About 1 mg of iron is assimilated daily. Upon reaching the mucosal cells, Fe2+ ions become bound to transport substances. Before passing into the plasma, these are oxidized by ceruloplasmin to Fe3+ and bound to transferrin in this form. The transport of Fe ions in blood plasma takes place via transferrin-iron complexes. A maximum of 2 Fe3+ ions per protein molecule can be transported.Serum iron is almost completely bound to transferrin. Iron (non-heme)measurements are used in the diagnosis and treatment of diseases such as iron deficiency anemia, hemochromatosis (a disease associated with widespread deposit in the tissue of the two iron-containing pigments, hemosiderin and hemofuscin, and characterized by pigmentation of the skin), and chronic renal disease. Iron determinations are performed for the diagnosis and monitoring of microcytic anemia (e.g. due to iron metabolism disorders and hemoglobinopathy),macrocytic anemia (e.g. due to vitamin B12 deficiency, folic acid deficiency and drug-induced metabolic disorders of unknown origin) as well as normocytic anemias such as renal anemia (erythropoetin deficiency), hemolytic anemia, hemoglobinopathy, bone marrow disease and toxic bone marrow damage.
Numerous photometric methods have been described for the determination of iron.All have the following in common:
- Liberation of Fe3+ ions from the transferrin complex using acids or detergents.
- Reduction of Fe3+ ions to Fe2+ ions.
- Reaction of the Fe2+ ions to give a colored complex.
The method described here is based on the FerroZine® method without deproteinization.

Test principle:
Colorimetric assay

$$\text{Transferrin-Fe-complex} \xrightarrow{\text{pH < 2,0}} \text{apotransferrin} + Fe3+$$

$$Fe3+ \xrightarrow{\text{ascorbate}} Fe2+$$

$$\text{FerroZine®} + Fe2+ \longrightarrow \text{colored complex}$$

Under acidic conditions, iron is liberated from transferrin. Lipemic samples are clarified by the detergent. Ascorbate reduces the released Fe3+ ions to Fe'2+ ions which react with FerroZine to form a colored complex. The color intensity is directly proportional to the iron concentration and can be measured photometrically.

Reagent Concentration:

R1:	
Citric acid pH 1.8	200 mmol/l
Thiourea	115 mmol/l
Na-ascorbate	150 mmol/l
detergent	< 1 %
R2:	
Citric acid pH 6.4	200 mmol/l
FerroZine®	6 mmol/l
Stabilizers/ detergents	< 1 %

Preparation and stability:

R1: Ready for use
R2: Ready for use
Unopened kit components: Up to the expiration date at +2°C to +8°C.
Onboard stability:
R1: 28 days at +2°C to +8°C.
R2: 28 days at +2°C to +8°C.
Store protected from light.

Specimen:
Collect serum using standard sampling tubes. Heparinized plasma.
Stability: 7 days at +20°C to +25°C
3 weeks at +4°C to +8°C
several years at -20°C
Separate serum or plasma from the clot or cells within 1 hour. EDTA and oxalate plasma cause decreased values.

Limitation interference:
Criterion: Recovery within ± 10% of initial values.
Icterus: No significant interference up to an index I of 90 (approximate conjugated and unconjugated bilirubin concentration: 90 mg/dl). Hemolysis: No significant interference up to an index H of 70 (approximate hemoglobin concentration: 70 mg/dl). Higher hemoglobin concentrations lead to false-positive values due to contamination of the sample with hemoglobin-bound iron.Lipemia (Intralipid): No significant interference up to an index L of 500 (approximate triglycerides concentration: 1000 mg/dl). There is poor correlation between turbidity and triglycerides concentration. In patients treated with Deferoxamine, the drugbound serum iron does not react in the test, resulting in falsely lowered values.

Testing procedure:
Applications for automated systems are available on request.
Materials provided
• Working solutions as described above
Additional materials required
• Calibrators and controls as indicated below
• 0.9% NaCl

Manual Testing Procedure:	
Wavelength:	Hg 578 nm (560nm)
Reaction temperature:	+37°C
Cuvette:	1 cm light path
Zero adjustment	One reagent blank for each series
R1	800 µl
Sample/ Calibrator	160 µl
Mix and incubate 5 minutes. Read the absorbance A1. Than add:	
R2	200 µl
Mix and incubate 5 minutes. Read the absorbance A2	
Calculation: A2 Sample – A1 Sample → x Calibrator conc.= Iron in µg/dl A2 Calibrator – A1 Calibrator SI Units: (µg/dl) x 0.1791 = µmol/l	

Measuring range:
10 -500 µg/dl (0.895-179 µmol/l)
Determine samples with higher concentrations µg/dl via the rerun function. On instruments without rerun function, manually dilute these samples with 0.9% NaCl (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).

Reference value:

Serum/plasma
Men: 70 – 180 g/dL = 12.5 – 32.2 mol/L

Women: 55 – 180 g/dL = 10.7 – 32.2 mol/L

The concentration of iron in serum/plasma is dependent on the diet and is subject to circadian variations.
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes the iron results should always be assayed in conjunctionwith the patient's medical history, clinical examinations and other findings.

Analytical sensitivity (lower detection limit):
Detection limit: 10 µg/dl
The lower detection limit represents the lowest measurable iron concentration that can be distinguished from zero.

Imprecision:
Reproducibility was determined using controls within run (n = 20).
The following results were obtained

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Within run			
Sample	Mean (µmol/l)	SD (µmol/l)	CV (%)
Sample 1	120.97	2,36	1,95
Sample 2	183.32	1,75	0,95
Sample 3	191.58	3,02	1,58

Reproducibility was determined using controls in an internal protocol between day (n = 10). The following results were obtained:

Between day			
Sample	Mean (µmol/l)	SD (µmol/l)	CV (%)
Sample 1	112.82	1.72	1,52
Sample 2	178.01	4.63	2.60
Sample 3	190.22	3.50	1,84

Method comparison:

A comparison of the BIOANALYTIC Fe-FZ (y) with a commercial obtainable assay (x) gave with 78 samples the following result (µmol/l):
y = 1.001x – 0.493; r =0.997

Quality Control:

Control Serum:

BIOCON N	5 x 5 ml	#B10814
BIOCON P	5 x 5 ml	#B10817

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calibration:

S1: 0.9% NaCl

S2: BIOANALYTIC UIBC+IRON STD 5 x 2 ml #B11944

Calibration frequency:

A two-point-calibration is recommended in case of:
1-change of lot
2- quality control requirements

Literature:

1. Bablok W et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.
2. Bernat I. Eisenresorption. In: Bernat I (Hrsg.). Eisenstoffwechsel. Stuttgart/New York: Gustav Fischer,l981:36-37.
3. de Jong G, von Dijk IP van Eijk HG. The biology of transferrin. Clin Chim Acta 1990;190:1-46.
4. Einer G, Zawta B. Präanalytikfibel, 2nd. Leipzig/Heidelberg: Verlag AW Barth, 1991 .
5. Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-474.
6. Guder WG, Narayanan S, Wisser H, Zawta B. List of Analytes Preanalytical Variables. Broschüre in: Samples: From the Patient to the Laboratory. Darmstadt: GIT Verlag,l996.

Order information (Cat No.) :

CC440	BIRO250	B25211	B28212	B33210	B37210
OL440	B21210	B25212	B30210	B33211	B37211
AB440	B21211	B27210	B30211	B33212	B42210
KL440	B21212	B27211	B30212	B34210	B80210
SH440	B22210	B27212	B31210	B35210	B80211
CR440	B24210	B28210	B32210	B36210	B80212
BIRO500	B25210	B28211	B32211	B36211	B80213

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SYMBOLS

IVD	for in vitro diagnostic use only
LOT	lot of manufacturing
REF	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	Read the directions