

# IRON FZ

FE F245 CH	12 x 20 ml
FE F400 CH	8 x 50 ml

## SUMMARY OF TEST

Serum iron concentration denotes the Fe(III) bound to serum transferrin and does not include the iron contained in serum as free hemoglobin. Serum iron concentration is decreased in many but not all patients with iron deficiency anemia and in chronic inflammatory disorders such as acute infection, immunization, and myocardial infarction. Serum iron concentration diminishes markedly in patients who are beginning to respond to specific hematinic therapy for anemias of other causes, for example, treatment of pernicious anemia with vitamin B12. Acute or recent hemorrhage, including that due to blood donation, results in low serum iron concentration. Serum iron concentration decreases during menstruation. Use of hormonal contraceptives raises serum iron concentration, but on cessation of contraceptive hormone intake, serum iron concentration decreases as much as 30% concurrent with uterine bleeding. Greater than normal concentrations of serum iron occur in iron loading disorders such as hemochromatosis, in acute iron poisoning in children, and after oral ingestion of iron medication or parenteral iron administration or acute hepatitis. For example, one 0.3 g tablet of ferrous sulfate ingested by an adult may raise the serum iron concentration by 50 to 90 µmol/l (300-500 µg/dl). Results exceeding 35 µmol/l (200 µg/dl) may also be observed in women who are taking progesterone-related contraceptive medication or when iron has contaminated the syringe or specimen container. Because normally only about one third of the iron binding sites of transferrin are occupied by Fe(III), serum transferrin has considerable reserve iron binding capacity. This is called the serum unsaturated iron binding capacity (UIBC). TIBC is a measurement of the maximum concentration of iron that serum proteins, principally, transferrin, can bind. The serum TIBC varies in disorders of iron metabolism. It is often increased in iron deficiency and decreased in chronic inflammatory disorders or malignancies, and it is often decreased also in hemochromatosis. In the common methods of assay, iron is released from transferrin by decreasing the pH of the serum; it is reduced from Fe(III) to Fe(II) and it then complexes with a chromogen that contains the reactive group -N=C-C=N- (this is the same structure as in the commonly used anticoagulant EDTA.) Metal cations are chelated between the two nitrogens. The iron-chromogen complex has extremely high absorptivity. Absorbance is proportional to iron concentration. Two chromogens that have been widely used for this purpose are bathophenanthroline and ferrozine, but the Fe(II) complex with the second one has a higher molar absorptivity.

## PRINCIPLE OF THE METHOD

Serum iron bound to transferrin is released in acidic environment. Fe(III) ions are then reduced to Fe(II), which reacts with ferrozine to give a violet colored complex. The absorbance measured at 560 nm is directly proportional to the amount of iron in the sample.

## KIT COMPONENTS

### For in vitro diagnostic use only.

The components of the kit are stable until expiration date on the label.

Keep away from direct light sources.

**Reagent A** F245: 12 x 16 ml (liquid) blue cap  
F400: 8 x 40 ml (liquid) blue cap

Composition: acetate buffer 500 mM pH 4.50, thiourea ≥ 50 mM, guanidine hydrochloride ≥ 100 mM, surfactant.

**Reagent B** F245: 2 x 24 ml (liquid) red cap  
F400: 2 x 40 ml (liquid) red cap

Composition: ferrozine 6 mM.

**Reagent B1** F245: 2 vials powder for 24 ml  
F400: 2 vials powder for 40 ml

Composition: sodium ascorbate ≥ 50 mM.

**Standard:** iron(III) solution 200 µg/dl - 5 ml

Store all components at 2-8°C.

## MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution. Calibrator.

## REAGENT PREPARATION

Reagent A: ready to use.

Reagent B: add all the content of reagent B1 and let to stay 20 minutes, mixing occasionally by inversion. Do not shake. Stable 90 days at 2-8°C.

Caution: keep well closed and refrigerated.

Stability of unmixed reagents:

up to expiration date on labels at 2-8°C.

Stability of unmixed reagents since first opening of vials: ≥ 60 days at 2-8°C.

## PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.

Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

## SPECIMEN

Serum, plasma heparinate.

Samples are stable 7 days at 15-25°C, 3 weeks at 2-8°C and several months at -20°C.

Separate serum/plasma from clot within 1 hour.

Anticoagulants as EDTA or oxalate could yield too low recovery values.

## TEST PROCEDURE

Wavelength:	560 nm (allowed 540 ÷ 580 nm)
Lightpath:	1 cm
Temperature:	25, 30 or 37°C

dispense:	blank	calibrator	sample
reagent A	2 ml	2 ml	2 ml
water	500 µl	-	-
standard	-	500 µl	-
sample	-	-	500 µl

Mix, incubate at 25, 30 or 37°C for 5 minutes.

Read absorbances of standard (Ac<sub>1</sub>) and samples (Ax<sub>1</sub>) against reagent blank.

dispense:	blank	calibrator	sample
reagent B	500 µl	500 µl	500 µl

Mix, incubate at 25, 30 or 37°C for 5 minutes.

Read absorbances of calibrator (Ac<sub>2</sub>) and samples (Ax<sub>2</sub>) against reagent blank.

## RESULTS CALCULATION

serum/plasma sample:

$$\text{iron } \mu\text{g/dl} = \frac{Ax_2 - Ax_1}{Ac_2 - Ac_1} \times 200 \text{ (standard value)}$$

## EXPECTED VALUES

men	59 - 158 µg/dl	(10.6 - 28.3 µmol/l)
women	37 - 145 µg/dl	(6.60 - 26.0 µmol/l)

Each laboratory should establish appropriate reference intervals related to its population.

## QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:

**QN 0050 CH** QUANTINORM CHEMA 10 x 5 ml  
with normal or close to normal control values

**QP 0050 CH** QUANTIPATH CHEMA 10 x 5 ml  
with pathological control values.

If required, a multiparametric, human based calibrator is available:

**AT 0030 CH** AUTOCAL H 10 x 3 ml

Please contact Customer Care for further information.

## TEST PERFORMANCE

### Linearity

the method is linear up to 1000 µg/dl.

If the limit value is exceeded, it is suggested to dilute sample 1+9 with distilled water and to repeat the test, multiplying the result by 10.

### Sensitivity/limit of detection (LOD)

the limit of detection is 5 µg/dl.

### Interferences

no interference was observed by the presence of:

hemoglobin	≤ 100 mg/dl
bilirubin	≤ 19 mg/dl
lipids	≤ 1700 mg/dl

### Precision

intra-assay (n=10)	mean (µg/dl)	SD (µg/dl)	CV%
sample 1	106.41	2.12	1.99
sample 2	178.48	1.54	0.86

inter-assay (n=14)	mean (µg/dl)	SD (µg/dl)	CV%
sample 1	107.69	6.65	6.20
sample 2	179.15	4.65	2.60

### Methods comparison

a comparison between Chema and a commercially available product gave the following results:

Iron FZ Chema = x  
Iron competitor = y  
n = 100

$$y = 0.947x + 0.387 \mu\text{g/dl} \quad r = 0.973$$

## WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

## REFERENCES








Paul Carter - Anal. Biochem. 40, 450-458 (1971).

Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).

## MANUFACTURER

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## SYMBOLS

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