Manual Procedure

Automated procedure on request

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12881	**B-R1**	**1**	**Buffer 50**	**ml**
For 50 tests	**P-R1**	**5 powder for**	**10**	**ml**
	R2	**1 x**	**10**	**ml**

Iron (Ferrozine)

Colorimetric test

Powder liquid reagents

Test Principle

Ferric ions (Fe^{+3}) are released from transferring under acidic conditions and reduced to ferrous state (Fe^{+2}) by a strong reducing agent. Ferrous ions then react with Ferrozine to form a colored complex which can be measured photometrically. The intensity of the color produced is proportional to the iron concentration in the sample. Lipemic samples are clarified by the detergent.

Transferrin-Fe-complex $\xrightarrow[PH=4.5]{Guanidinium}$ apotransferrin + Fe^{3+}

Fe^{3+} $\xrightarrow{Ascorbic\ acid}$ Fe^{2+}

Ferrozine + Fe^{2+} $\longrightarrow$ colored complex

Concentrations in the test

Buffer Reagent B-R1		
Good,s Buffer	800	mmol/L
Guanidinium hydrochloride	2.2	mol/L
Thiourea	0.60	mmol/L
Preservative		
Powder Reagent P-R1		
Ascorbic acid		
Reagent R2		
Ferrozine	2.0	mmol/L
Preservative		

Stability and preparation of working reagent

Buffer Reagent B-R1: *liquid*
Powder Reagent P-R1: *powder.*
Reagent R2: *liquid, ready to use*

Working Reagent

Add 10 ml reagent Buffer B-R1 to one vial powder reagent P-R1 and mix gently for 15 minutes before use.
Stability: 2 weeks at 20 – 25 °C
1 month at 2 – 8 °C
All reagents are stable up to expiry date given on label when stored at 2 – 8 °C

Note: All reagents should be clear solutions. Don't use if the reagent is turbid.

Specimen Collection and Handling

1. Non-hemolyzed serum is the specimen of choice.
2. Serum should be separated from blood clot as soon as possible.
3. Heparinized plasma could only be used, other anticoagulants should not be used.
4. Serum iron is reported to be stable for 4 days at 20 – 25°C and 7 days at 2 – 8 °C.

Calibrator

MediCal U Cat. No. 15011
Iron STD. Cat. No. 16131

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 578 nm (550–570nm)
Spectrophotometer	562nm
Cuvette	1 cm light path
Temperature	37°C / 20 – 25 °C
Measurment	against reagent blank
Reaction	end point

Assay

	Blank	Standard	Sample blank	Sample
dist. water	200 µl	--	200 µl	--
Standard	--	200 µl	--	--
Sample	--	--	200 µl	200 µl
W. R.	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Mix, incubate for 5 minutes at 20 - 25° C				
Reagent R2	200 µl	200 µl	--	200 µl
Mix, incubate for 10 minutes at 20 - 25°C, or 5 minutes at 37°C. Read the absorbance of sample blank against distilled water, and Read the absorbance of standard/sample against reagent blank.				

Calculation

$$Conc._{Iron} = \frac{(A_S - A_{SB})_{Sample}}{A_{standard}} \times Conc._{Standard}$$

$$\mu mol/L \xrightarrow{X\ 5.58} \mu g/dl$$

$$\mu g/dl \xrightarrow{0.179\ X} \mu mol/L$$

Linearity

Up to 1000 µg/dl (179 µmol/L);
If result exceeds 1000 µg/dl, repeat test using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply result by 2.

Interference

1. Certain drugs and other substances are known to influence circulating iron levels.
2. To make tubes, pipettes, etc. iron free, they must be washed with diluted (1+2) hydrochloric or nitric acid followed by several rinsings with iron free deionized or distilled water.

Precautions

1. The reagent is toxic, don't pipette by mouth, avoid all contacts.
2. Use only disposable plastic containers or iron free tubes and cuvettes. Avoid any contamination by the use of clean laboratory material.

Iron ferrozine method

Reference range

Serum

New born		36 - 184	µg/dl
≤ 6 mth.		36 - 156	µg/dl
≥ 7 mth.		43 - 184	µg/dl
Adults	women	37 - 145	µg/dl
	men	59 - 158	µg/dl

Urine

Urine /24 hr.	< 98	µg/ 24 hr.

References

1. Garoc A., Clin. Chem. Acta 94, 115 (1979).
2. Brivio et coll., La Ricerca Clin. Lab. 18, 523 (1986).
3. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.