Manual Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12480 R 1 x 50 ml
For 50 tests

Cat. No. 12481 R 2 x 50 ml
For 100 tests

Iron (Nitro - PAPS)

Colorimetric Method

Liquid Reagent

Test principle

Transfenirn-Fe-complex → Apotransferrin + Fe^{3+}

Fe^{3+} —(Reducing agent)→ Fe^{2+}

Nitro PAPS + Fe^{2+} → Colored Complex

Iron ions are dissociated from its carrier protein (transferring) in an acid medium and are reduced to the ferrous form by a reducing factor. The ferrous ions react with the chromogen Nitro-PAPS to form a colored complex highly specific.The absorbance of the color is directly proportional to the iron concentration.

Concentrations in the test

Reagent R		
Guanidine hydrochloride pH = 4.8	2.2	mol/L
Nitro PAPS	26	µmol/L
Thio urea	60.0	mmol/L
Reducing agent		
Detergent		

Standard : The concentration as indicated on vial.

Stability of reagent

Reagent R: *liquid, ready to use.*
The reagent is stable up to expiry date given on the label when stored at + 2 ➔ + 8 °C.

Note: Don't use if the reagent is turbid.

Specimen collection and handling

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

Calibrator / Standard

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
Spectrophotometer	582 nm
Cuvette	1 cm light path
Temperature	37 °C / 20 - 25°C
Measurment	against reagent blank
Reaction	end point

Assay

	Blank	Calibrator / Standard	Sample
Double dist. water	100 µl	--	--
Calibrator / Standard	--	100 µl	--
Sample	--	--	100 µl
Reagent	1000 µl	1000 µl	1000 µl
Mix, incubate for 5 min. at 37°C or 10 min. at 20 - 25°C. Read the absorbance (A) .The final color is stable for 1 hour.			

Calculation

$$\text{Conc.}_{\text{Iron}}\ (\mu g/dl) = \frac{A_{\text{Sample}}}{A_{\text{Cal./STD}}} \times \text{Conc.}_{\text{Cal./STD}}\ (\mu g/dl)$$

µmol/L ⇄ µg/dl (X 5.58 / 0.179 X)

Linearity

Up to 400 µg/dl
If the result exceeds 400 µg/dl, repeat the test using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Interferences

1. Contaminated glassware is the greatest source of error. Disposable material is recommended to avoid iron contamination.
2. Triglycerides up to 400 mg/dl have been found to significantly influence this assay.
3. To make glass material iron-free rinse with diluted (1+2) hydrochloric or nitric acid followed by several rinsings with iron free deionized or distilled water.
4. Certain drugs and other substances are known to influence circulating iron levels.

Precautions

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

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. Makino et al :Clinica Chemica Acta, 171 (1988), 19 – 28.
2. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.