IRON

CHEMPAK

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(Ferrozine Method)

CLINICAL SIGNIFICANCE :

Iron is usually bound to protein. Approximately 73% of the total iron is circulating in the erythrocyte bound to haemoglobin. The normal body contains approximately 51 to 73 mmol (3.2 to 4.3 gms) of iron and as free iron is toxic for body, approximately 27% is stored in the liver, spleen or bone marrow associated with the iron storage compound ferritin. Only 51-73 µmol (3.2 to 4.3 mg) of the total body iron is circulating in the serum bound to the transport protein transferrin. The remaining iron is incorporated into myoglobin, iron containing enzymes and cytochromes.

Increased iron concentrations occur in iron loading disorders such as haemochromatosis, acute iron poisoning in children and acute hepatitis among others. Decreased iron concentrations are seen in many but all patients with iron deficiency, anaemia and chronic inflammatory disorders.

PRINCIPLE :

Transferrin bound iron breaks into free ferric ions in an acidic medium. These ferric ions react with Hydroxylamine Hydrochloride reduces into ferrous ions which react with Ferrozine to form a purple coloured complex measured at 560 nm. The difference before and after the addition of ferrozine is proportional to iron concentration reaction in the specimen.

REAGENT COMPOSITION:

Active Ingredients	Concentration
Reagent-1	
* Sodium Acetate	50 mmol/L
* Hydroxylamine Hydrochloride	150 mmol/L
* Detergent	5 mmol/L
pH 4.5 ± 0.1 at 25^0 C	
Reagent-2	
* Ferrozine	4 mmol/L
* Hydroxylamine Hydrochloride	150 mmol/L
Reagent-3	
* Enhancer	10 mmol/L
Iron Standard (80 µmol/L)	

Also contains non-reactive fillers and Stabilizers.

PRESENTATION :

	No. of Bottles
Store all reagents at 2-8^{0}C	20 tests
1 Iron (Buffer Reagent)	2
2 Iron (Color Reagent)	1
3 Iron (Enhancer)	2
Iron Standard (80 µmol/L)	1

PREPARATION OF WORKING REAGENT-1 :

Carefully transfer the content of one vial of 3 Iron to one bottle of 1 Iron. Mix to dissolve. Wait for 15 minutes before use.

SPECIMEN COLLECTION AND HANDLING :

Fresh clear serum with no hemolysis should be used. Plasma should not be used. Specimens are stable for one day at room temperature or one week at 2-8^{0}C.

STABILITY AND STORAGE OF REAGENT :

When stored, refrigerated at 2-8^{0}C and protected from light, the reagents are stable until the expiry date stated on the bottle and kit box labels.

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PRECAUTIONS :

It is essential that all the glasswares used for assay should be Iron-free. Glasswares should be soaked in 0.1N HNO_3 or HCl & rinsed thoroughly with iron-free deionized water.

REACTION PARAMETERS :

Monochromatic

Temperature	: 37^0 C
Wavelength	: 560 nm
Type of reaction	: End point (Increase)
Incubation	: 10 min at 37^{0}C.
Std. Concentration	: 80 µmol/L (446 µg/dl)
Std./Sample volume	: 200 µl (0.200 ml)
Reagent 1 Volume	: 1.0 ml
Reagent 2 Volume	: 0.050 ml (50 µl)
Light path	: 1.0 cm
Zero setting with	: Reagent blank

Bichromatic

Other parameters as above	
Wavelength	: 560 nm and 630 nm
Sample Blank	: No
Zero setting with	: Distilled water

Set the instrument using above system parameters

PROCEDURE :

A) Monochromatic Method:

PIPETTE INTO TEST TUBE		BLANK	STD.	TEST	
				Sample Blank(A1)	Sample Blank(A2)
Rgt.-1	(ml)	1.0	1.0	1.0	1.0
Sample	(ml)	-	-	0.2	0.2
Standard	(ml)	-	0.2	-	-
Dis. Water	(ml)	0.2	-	-	-
Rgt.-2	(ml)	0.05	0.05	-	0.05

Mix and allow to stand for 10 minutes at 37^{0}C. Read absorbance of test (A1& A2) and standard against reagent blank at 560 nm.

B) Bichromatic Method :

PIPETTE INTO TEST TUBE		STANDARD	TEST
Reagent-1	(ml)	1.0	1.0
Sample	(ml)	-	0.2
Standard	(ml)	0.2	-
Reagent-2	(ml)	0.05	0.05

Mix and allow to stand for 10 minutes at 37^{0}C. Read absorbance of test and standard against distilled water at 560 nm & 630 nm.

TEST RESULTS :

$$\text{Serum Iron (µmol/L)} = \frac{\text{Abs or } \Delta \text{ Absorbance of test x 80 (µmol/L)}}{\text{Absorbance of standard}}$$

Where

Δ Abs. = (A2-A1)

80 µmol/L = Concentration of Standard

To convert (µg/dl) = µmol/L x 5.585

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FORMULA :

% Saturation of Transferrin = $\frac{\text{Serum Iron}}{\text{TIBC}}$ X 100

EXPECTED VALUES :

Serum Iron :

Male : 12.5 - 32.2 µmol/L (70-180 µg/dl)
Female : 10.7 - 32.2 µmol/L (60-180 µg/dl)

% Saturation of Transferrin :

Male : 20 - 50 %
Female : 15 – 50 %

LIMITATIONS :

a) Haemolysis causes falsely elevated results.
b) Iron medications (oral, intravenous or intravascular) affect serum levels.

LINEARITY :

This procedure is linear upto 89 µmol/L (500 µg/dl). For sample values higher than 89 µmol/L (500 µg/dl), dilute the sample suitably with 0.9% saline and repeat the assay. Apply dilution factor to obtain test results.

REFERENCES :

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3/7,B.I.D.C., Gorwa, Vadodara 390 016 (INDIA)
Web: www.reckondiagnostics.com
Phone: +91·265·2281631
Email: mail@reckondiagnostics.com

RECKON DIAGNOSTICS P. LTD.