IRON & TIBC KIT

(Ferrozine method)

For the determination of Iron and Total Iron Binding Capacity in serum.

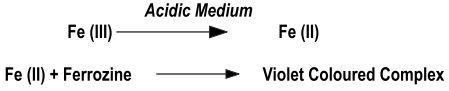
(For Invitro Diagnostic Use Only)

Summary

Iron found in blood is mainly present in the hemoglobin of the RBC's. It's role in the body is mainly in the transportation of oxygen and cellular oxidation. Iron is absorbed in the small intestine, and bound to a globulin in the plasma, called transferrin,and transported to the bone marrow for the formation of hemoglobin. Increased serum levels are found in hemolytic anemias, hepatitis, lead and iron poisoning. Decreased serum levels are found in anemias caused by iron deficency due to insufficient intake or absorption of iron, chronic blood loss, late pregnancy and cancer. Increase in TIBC is found in Iron defecient anemias and pregnancy. Decrease in TIBC is found in hypoproteinemia, hemolytic /pernicious / sicklecell anemias, inflammatory diseases and cirrhosis.

Principle

Iron, bound to Transferrin, is released in an acidic medium and the Ferric ions are reduced to Ferrous ions. The Fe (II) ions react with Ferrozine to form a violet coloured complex. Intensity of the complex formed is directly proportional to the amount of Iron present in the sample. For TIBC, the serum is treated with excess of Fe (II) to saturate the iron binding sites on transferrin. The excess Fe (II) is adsorbed and precipitated and the Iron content in the supernatant is measured to give the TIBC.



Normal reference values

Serum Iron (Males)	:	60 - 160 µg/dl
(Females)	:	35 - 145 µg/dl
(Neonates)	:	150 - 220 µg/dl
TIBC	:	250 - 400 µg/dl
UBIC	:	160 - 360 µg/dl

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents	**35 ml**	**75 ml**	**150 ml**
Iron Reagents			
L1 : Iron Buffer Reagent	35 ml	75 ml	150 ml
L2 : Iron Colour Reagent	2 ml	4 ml	8 ml
S : Iron Standard (100 µg/dl)	2 ml	2 ml	4 ml
TIBC Reagents			
L1 : TIBC Saturating Reagent	10 ml	20 ml	40 ml
L2 : TIBC Precipitating Reagent	1 gm	2 gm	4 gm

Storage / stability

Contents are stable at 2-8^0 C till the expiry mentioned on the labels.

Reagent Preparation

Reagents are ready to use.

Sample material

Serum, free from hemolysis. Iron is reported to be stable in serum for 7 days at 2-8^{0}C.

Procedure

Wavelength / filter	:	570 nm (Hg 578 nm)/ Yellow
Temperature	:	R.T.
Light path	:	1 cm

Iron Assay :

Pipette into clean dry test tubes labelled as Blank (B), Standard (S), Sample Blank (SB) and Test (T) :

Addition Sequence	**B** (ml)	**S** (ml)	**SB** (ml)	**T** (ml)
Iron Buffer Reagent (L1)	1.0	1.0	1.05	1.0
Distilled water	0.2	-	-	-
Iron Standard (S)	-	0.2	-	-
Sample	-	-	0.2	0.2
Iron Colour Reagent (L2)	0.05	0.05	-	0.05

Mix well and incubate at R.T. for 5 min. Measure the absorbances of the Blank (Abs.B), Standard (Abs.S), Sample Blank (Abs.SB) and Test Sample (Abs.T) against D.W.

TIBC Assay :

Pipette into a clean dry test tube

Serum	0.5 ml
TIBC Saturating Reagent (L1)	1.0 ml
Mix well and allow to stand at R.T. for 10 min. and add	
TIBC Precipitating Reagent (L2)	Approx. 50 mg

Mix well and allow to stand at R.T. for 10 min. Centrifuge at 2500 - 3000 rpm for 10 min. to obtain a clear supernatant. Determine the Iron content in the supernatant as above mentioned in the Iron Assay.

Calculations

$$\text{Iron in } \mu g/dl = \frac{\text{Abs.T - (Abs. SB + Abs.B)}}{\text{Abs. S - Abs. B}} \times 100$$

$$\text{TIBC in } \mu g/dl = \frac{\text{Abs.T - (Abs. SB + Abs.B)}}{\text{Abs. S - Abs. B}} \times 300$$

$$\text{UBIC in } \mu g/dl = \text{TIBC in } \mu g/dl - \text{Iron in } \mu g/dl$$

Linearity

This procedure is linear upto 1000 µg/dl. If values exceed this limit, dilute the sample with distilled water and repeat the assay. Calculate the value using the proper dilution factor.

Notes

Hemolysis interferes with the test as the hemoglobin present in the RBC's have a very high iron content. All glassware being used for the test should first be rinsed with 1% or 0.1 N HCl and then with good quality deionised water before use.

References

Siedel, J., et. al. (1984) Clin Chem. 30 : 975

System Parameters

Reaction	:	End Point + S.B.
Wavelength	:	578 nm
Zero Setting	:	Deionised water
Incub. Temp.	:	R.T.
Incub. Time	:	5 min.
Delay Time	:	---
Read Time	:	---
No. of read.	:	---
Interval	:	---
Sample Vol.	:	0.2 ml
Reagent Vol.	:	1.05 ml
Standard	:	100 µg/dl
Factor	:	---
React. Slope	:	Increasing
Linearity	:	1000 µg/dl
Units	:	µg/dl

IRT(Ferr).02(P)

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