PATHOZYME DIAGNOSTICS

LIQVIPATH IRON

(Ferrozine Method)

CAT NO.: IRN

Reagent for quantitative estimation of IRON in human Serum.

DIAGNOSTIC 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 ⌈mol (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.

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 578nm & 630nm (Bichromatic Mode).

SPECIMEN COLLECTION:

Fresh clear serum with no hemolysis should be used. **Plasma should not be used.**

KIT PRESENTATION:

Pack Size	25 Test	50 Test
R1 – IRON	1 X 12.5 ml	1 X 25 ml
R2 - IRON	1 X 0.70 ml	1 X 1.40 ml
IRON Std.(100 µg/dl)	1 X 1 ml	1 X 2 ml

REAGENT STORAGE & STABILITY:

IRON reagents and standard are stable at 2-8^{0}C until the expiry date indicated on the label.

PRECAUTION:

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

ASSAY PARAMETERS:

Reaction	: End point	Sample Vol.	: 100 µl
Wavelength 1	: 578 nm	R1 + R2 Vol.	: 500 µl + 25 µl
Wavelength 2	: 630 nm	Std Conc.	: 100 µg /dl
Zero Setting	: Distilled Water	Linearity	: 500 µg /dl
Incub. Time	: 5+10 mins at 37 ^{0}C	Unit	: µg /dl

PROCEDURE:

Pipette into TT	Standard	Test
R1 - IRON	500 µl	500 µl
IRON Std (**100 µg/dl**)	100 µl	--
Sample (Test)	--	100 µl
Mix & Incubate for 5 minutes at 37 ^{0}C		
R2 – IRON	25 µl	25 µl
Mix & Incubation for 10 minutes at 37 ^{0}C. Read absorbance of Standard (S) and Test (T) against Distilled Water at **578 nm & 630 nm.**		

CALCULATION:

IRON Conc. (**µg/dl**) = $\frac{\text{Absorbance of Test X 100}}{\text{Absorbance of Standard}}$

Unit Conversion

µg/dl ÷ 5.585 = µMol/L

NORMAL VALUES:

Male : 70 – 180 µg/dl (12.5 – 32.2 µMol/L)
Female : 60 – 180 µg/dl (10.7 – 32.2 µMol/L)
Each laboratory should establish its own reference range.

LINEARITY:

The method is linear up to 500 µg/dl (89 µMol/L). For sample values higher than 500 µg/dl (89 µMol/L), dilute the sample suitably with 0.9% saline and repeat the assay. Apply dilution factor to result.

REFERENCES:

1. CaO G.and Prior R.L. Clinical Chemistry Anthocyanins and iron metabolism in human serum 1999b; 574-76.
2. Tietz NW "Text book of clinical chemistry 2nd Edition" Tietz NW (Ed) WB Saunders company Philadelphia 1994; 2059.

IFU No.: 030/CB | Rev. No.: 00/120723

Expiry Date | IVD In-Vitro Diagnostics Use | Storage | Mfg. Date | LOT Batch Number | REF Catalogue Number | See Package Insert

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PATHOZYME DIAGNOSTICS

LIQVIPATH DIRECT TIBC

(DIRECT COLORIMETRIC METHOD)

CAT No.: TIB

Reagent kit for quantitative estimation of TIBC in Serum.

DIAGNOSTIC SIGNIFICANCE:

Total iron-binding capacity (TIBC) is the measure of the maximum concentration of iron that the serum proteins can bind. Together with the total serum iron concentration, the TIBC is used in the diagnosis and treatment of iron deficiency anemia, other disorders of iron metabolism, and chronic inflammatory disorders. As an index of nutritional status, TIBC reflects the degree of transferrin saturation by serum iron. Serum TIBC is increased in iron deficiency, and decreased in anemia that is due to chronic disease.

PRINCIPLE:

Reagent 1 (R1), an acidic buffer containing an iron binding dye and ferric chloride, is added to the serum sample, the low pH of R1 releases iron from transferrin. The iron then forms a colored complex with the dye present in Reagent 2 (R2). The serum transferrin rapidly binds to the iron by forming a dye-iron complex is directly proportional to the total iron binding capacity of serum sample.

SPECIMEN COLLECTION:

Serum without hemolysis.

KIT PRESENTATION:

Pack Size	25 Test	50 Test
R1-TIBC (Buffer Reagent)	1 X 12.5 ml	1 X 25 ml
R2-TIBC (Colour Reagent)	1 X 2.5 ml	1 X 5.0 ml
TIBC Calibrator	1 No	1 No

REAGENT & CALIBRATOR PREPARATION:

All reagents are ready to use.

To reconstitute the Calibrator, refer the Calibrator vial label. After reconstitution Calibrator stable for 14 days at 2-8^{0}C if store & handle properly.

REAGENT STORAGE & STABILITY:

All reagents included in the kit are stable at 2-8^{0}C until the expiry date stated on the label.

LIMITATION:

Greater than 460 µg/dL of iron (ferrous sulphate) causes significantly decreased TIBC results.

ASSAY PARAMETERS:

Reaction	**: End point**	**Sample Volume**	**: 10 µl**
Wavelength	**: 620 (600-630) nm**	**R1 + R2 Volume**	**: (500+100) µl**
Incub. Temp	**: 37 ^{0}C**	**Calibrator Conc.**	**: As Per Vial**
Zero Setting	**: Reagent Blank**	**Linearity**	**: 700 µg/dl**
Incubation	**: 5 mins + 7 mins.**	**Unit**	**: µg/dl**

PROCEDURE:

Pipette into TT	Blank	Calibrator	Test
R1-TIBC (Buffer Reagent)	500 µl	500 µl	500 µl
TIBC Calibrator	--	10 µl	--
Sample (Test)	--	--	10 µl
Mix & incubate for 5 minutes at 37 ^{0}C			
R2-TIBC (Colour Reagent)	100 µl	100 µl	100 µl
Mix & Incubation for 7 minutes at 37 ^{0}C. Read absorbance of Test (T) and Calibrator (C) against Reagent Blank at 620 (600-630) nm.			

CALCULATION:

$$\text{TIBC (µg/dl)} = \frac{\text{Abs. of Test X Cocn. of Calibrator}}{\text{Abs. of Calibrator}}$$

NORMAL VALUES:

250-450 µg/dl

LINEARITY:

The method is linear up to 700 µg/dl.

REFERENCE:

1.Tietz NW (ed). Textbook of Clinical Chemistry, ed. 3. Philadelphia, PA: WB Saunders; 1701-1703;1999.

2.NCCLS. Determination of Serum Iron and Total Iron Binding Capacity; Proposed Standard, NCCLS Document H17-P. Wayne, PA: NCCLS, Vol.10 No.4;1990.

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