IRON

(Single Reagent – CAB Colorimetric Method)

Hemezyme

GENUINE QUALITY, RELIABLE RESULTS

INTENDED USE

Iron reagent is intended for the in-vitro quantitative, diagnostic determination of total iron in human serum or heparinized plasma on manual and automated systems.

BACK GROUND

The majority of iron in the body (~3 – 3.5 g) is found in the haemoglobin of the red blood cells or their precursors in the bone marrow. Plasma contains very small fraction of iron (~ 2.5 mg). Iron is transported from one organ to another as a complex formed of ferric ions and a protein called apotransferrin, This iron-protein complex is called transferrin. The major iron-storage compound in the body is ferritin; it occurs in almost all body cells but particulary in hepatocytes. Serum iron is measured by the quantity of iron bound to transferrin, while TIBC is a direct measurement to transferrin. Elevated serum iron levels have been found in cases of hemochromatosis, hepatitis, hepatic necrosis and hemolytic anemia. Decreased levels have been associated with iron deficiency anemia, chronic blood loss, chronic disorders and insufficient dietary iron. The TIBC varies in disorders of iron metabolism , so it is elevated in iron deficiency anemia.The measurements of both serum iron and TIBC is fundamental in evaluation and differential diagnosis of various types of anemia, liver disease and chronic illness.

METHOD

CAB Colorimetric Method.

ASSAY PRINCIPLE

Iron reacts with chromazurol B and cetyltrimethyl-ammonium bromide (CTMA) to form a coloured ternary complex with an absorbance measured at 630 nm. The intensity of the colour produced, is directly proportional to the concentration of iron in the sample.

REAGENTS

Standard Iron (ST)	200 µg/dL 17.9 µmol/L
Acetate buffer PH 4.7	50 mM
CAB	0.13 mM
CTMA	0.82 mM
Preservatives and Stabilizers	

PRECAUTIONS AND WARNINGS

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
Iron test is very sensitive against contamination: Use only distilled water.
Contaminated glass wares are a source of error.
Disposable plastic ware is recommended for the test.

REAGENT PREPARATION, STORAGE AND STABILITY

All reagents are supplied ready to use and stable until expiration date stated on label when stored at 2-8 °C.Once opened, the reagent and the standard vials are stable for 3 months at specified temperature.

DETERIORATION

Failure to recover control values within assigned range may indicate reagent deteriortion

SPECIMEN COLLECTION AND PRESERVATION

The recommended specimen is serum or heparinized plasma. Plasma specimens collected with EDTA, oxalate, or citrate as anticoagulants are unsatisfactory since they bind iron, preventing its reaction with the chromogen. Morning specimen is preferrable to avoid low result due to diurnal variation. The biological half life of iron in blood is few hours.

SYSTEM PARAMETERS

REACTION TYPE	End Point
WAVE LENGTH	630 nm
LIGHT PATH	1 cm
REACTION TEMPERATURE	37°c
BLANK / ZERO SETTING	Reagent
REAGENT VOLUME	1 ml
SAMPLE VOLUME	50 µl
INCUBATION TIME	5 Minutes
STANDARD CONCENTRATION	200 µg/dL
LOW NORMAL	37 µg/dL
HIGH NORMAL	158 µg/dL
LINEARITY	500 µg/dL

ASSAY PROCEDURE

	BLANK	STANDARD	SAMPLE
REAGENT	1ml	1ml	1ml
STANDARD		50 µl	
SAMPLE			50 µl

Mix, and incubate for 5 minutes at 37°C. Read the absorbance of the standard and sample against reagent blank.

CALCULATION

$$\text{Iron conc. (µg/dL)} = \frac{A_{sample}}{A_{standard}} \times 200$$

SI UNITS

(µg/dL) x 0.1791 = µmol/L

IMPORTANT NOTES

1. Make sure that the distilled (double distilled) water is absolutely iron free.
2. Do not use turbid or hemolytic sera or plasma.

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3. This iron test is very sensitive.
   To avoid contamination the glassware used must be iron free.
   We strongly recommend to use disposable laboratory materials when performing this test.
4. Bilirubin up to 15 mg/dL and copper up to 500 µg/dL do not interfere.

Methods Comparison
A comparison between Spectrum Iron reagent and a commercial reagent of the same methodology was performed on 200 human sera. A correlation of 0.983 was obtained.

Sensitivity

When run as recommended, the sensitivity of this assay is 12 µg/dL for serum iron.

Linearity

The reaction is linear up to iron concentration of 500 µg/dL, Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result × 2).

Interfering Substances

Haemolysis
No interference up to haemoglobin level of 5 g/L (0.3 mmol/L) in determining serum iron and up to 1 g/L for TIBC.

Icterus
No significant interference up to a bilirubin level of 30 mg/dL.

Lipemia
Lipemic specimens are not recommended since they may cause negative bias. Lipemic specimens can be diluted before assay and the dilution factor should be considered during calculation.

Anticoagulants
Citrate, EDTA, and oxalate should be avoided.

Expected values

1- Neonates	:	36 – 184 µg/dL	(6. 4 - 33 µmol/L)
2- < 7 months	:	37 – 145 µg/dL	(7.7 - 33 µmol/L)
3- Adults			
a) Women	:	37 – 145 µg/dL	(6.6 - 26 µmol/L)
b) Men	:	59 – 158 µg/dL	(10.6 - 28 µmol/L)

Waste Disposal
This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

References

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