SPECTRUM

The Creative Approach to Bioscience

Total Iron - Ferrozine

REF: 272 001 (100 test)

Reagent 1 2 x 45 ml
Reagent 1B 2 x 50 ml
Reagent 2 1 x 11 ml

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Temperature Limitation
IVD	For in-vitro diagnostic use		Use by/Expiration Date
LOT	Batch Code/Lot number		CAUTION. Consult instructions for use
REF	Catalogue Number		
	Consult instructions for use		Manufactured by

Intended Use

Spectrum Diagnostics iron is intended for the in-vitro quantitative, diagnostic determination of total iron in human serum.

Background

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 defeciency anemia, chronic blood loss, chronic disorders and insufficient dietary iron. The TIBC varies in disorders of iron metabolism so, TIBC is elevated in iron deffeciency 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

Guanidine / Ferrozine method.

Assay Principle

Iron
Ferric ions are released from transferrin by guanidine hydrochloride and reduced to ferrous state by hydroxylamine. Ferrous ions react with ferrozine forming a coloured complex. To prevent copper interference, cupric ions are bound to thiourea.

Transferrin-Fe(III) —*Guanidine-HCL*→ Apotransferrin +Fe(III)

Fe(III) —*Hydroxylamine*→ Fe(II)

Fe(II) + Ferrozine ⟶ Colored complex

The color intensity is directly proportional to the iron concentration and is determined by monitoring the increase in absorbance at 546 nm.

Reagents

Standard Iron (ST)	200 µg/dL 35.8 µmol/L
Reagent 1 (buffer pH 4.5)	
Acetate buffer	0.4 mol / L
Guanidine hydrochloride	1.5 mol / L
Hydroxylamine hydrochloride	0.6 mol / L
Thiourea	100 mmol/L
Reagent 1B (buffer pH 4.5)	
Acetate buffer	0.4 mol / L
Guanidine hydrochloride	1.5 mol / L
Hydroxylamine hydrochloride	0.6 mol / L
Thiourea	100 mmol/L
Reagent 2: Ferrozine	60 mmol/L

Reagent Preparation

Prepare the working solution by adding 5 ml of chromogen (R2) to one bottle of buffer (R1). Or prepare the working solution according to the number of tests required by mixing 9 volumes of R1 and 1 volume of R2, e.g. 900 µl R1+100 µlR2.

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.

Reagent Storage and Stability

All reagents are stable until expiration date stated on label when stored refrigerated at 2 - 8 ^{o}C.
Working solution is stable for 6 months at 2 – 8 ^{o}C.

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 diurinal variation. The biological half life of iron in blood is few hours.

Stability: 7 days at 15 –25 ^{o}C ; 3 weeks at 2 – 8 ^{o}C;
1 year at -20 ^{o}C

Procedure A-(Iron)

	Reagent Blank	Standard	Sample Blank	Sample
Working Solution	1.0 ml	1.0 ml	------	1.0 ml
Dist.water	200µl	------	------	------
Standard	------	200µl	------	------
Sample	------	------	200µl	200µl
Reagent 1B	------	------	1.0 ml	------

Mix, and incubate for 5 to 10 minutes at 20 – 25 ^{o}C. Read the absorbance of the standard and sample against reagent blank, and the absorbance of sample blank against distilled water within 30 minutes at 546 nm.

Calculation

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

Quality Control

Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

Performance Characteristics

Precision

Within run (Repeatiblity)

	Total Iron	
	Level 1	Level 2
n	20	20
Mean (µg/dL)	159	344
SD	2.1	1.9
CV%	2.3	0.57

Run to run (Reproducibility)

	Total Iron	
	Level 1	Level 2
n	20	20
Mean (µg/dL)	162	351
SD	2.9	2.6
CV%	2.9	0.68

Methods Comparison

A comparison between Spectrum Diagnostics Iron reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.983 was obtained.

Sensitivity

When run as recommended, the sensitivity of this assay is 5 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

Serum, plasma

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 .

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.

Others
Pathological albumin levels more than 7 g/dL .

Expected values

Iron

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

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature reference.

Analytical Range

5 – 500 µg/dl (0.9 – 89.5 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

1. Bauer JD. Haemoglobin, porphyrin, and iron metabolism. In:Kaplan LA, Pesce AJ, ed. Clinical Chemistry, theory, analysis, and correlation. ST. Louis:Mosby Company:1984:611-655.
2. Fairbanks VF,Klee GG. Biochemical aspects of hematology. In : Tietz NW, ed. Fundamentals of clinical chemistry. 3rd ed. Philadelphia: WB saunders:1987:789-824.
3. Stookey LL. Ferrozine-a new spectrophotometric reagent for iron. Anal Chem. 1970;42:779-781.
4. Viollier MA, Gschwind H, Schläpfer P. Neue serumeisenbestimmung auf dem GSA II. Lab Med.1980;4:240-244.
5. Williams HL, Johnson DJ, Haut MJ. Simultaneous spectrophotometry of Fe2+ and Cu2+ in serum denatured with guanidine hydrochloride. Clin Chem.1977;23:237-240.

ORDERING INFORMATION

CATALOG NO.	QUANTITY
272 001	100 Test

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