SPECTRUM

The Creative Approach to Bioscience

Total Iron and Total Iron Binding Capacity Reagent Set

REF: 270 001 (50 test)

Reagent 1 1 x 45 ml
Reagent 1B 1 x 50 ml
Reagent 2 1 x 6 ml
Reagent 3 1 x 50 ml
Reagent 4 1 x 2.5 g

REF: 270 002 (100 test)

Reagent 1 1 x 90 ml
Reagent 1 1 x100 ml
Reagent 2 1 x 11 ml
Reagent 3 1 x 50 ml
Reagent 4 1 x 2.5 g

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 and total iron binding capacity (TIBC) reagent set is intended for the in-vitro quantitative, diagnostic determination of total iron and total iron binding capacity 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.

Total Iron Binding Capacity

The transferrin in the specimen is saturated with iron by exposure to excess ferric ions; then unbound iron is removed by addition of light magnesium carbonate and centrifugation. The iron bound to protein in the supernatent is measured by the Principle applied to total iron described above.

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 2: Ferrozine 60 mmol/L

Reagent 3: Ferric chloride 120 µmol/L

Reagent 4: Magnesium carbonate powder.

Reagent Preparation

REF: 270 001 Prepare the working solution by adding 5 ml of chromogen (R2) to one bottle of buffer (R1).

REF: 270 002 Prepare the working solution by adding 10 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 and1 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 1	------	------	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		TIBC	
	Level 1	Level 2	Level 1	Level 2
n	20	20	20	20
Mean (µg/dL)	159	344	200	299
SD	2.1	1.9	2.12	1.36
CV%	2.3	0.57	1.06	0.45

Run to run (Reproducibility)

	Total Iron		TIBC	
	Level 1	Level 2	Level 1	Level 2
n	20	20	20	20
Mean (µg/dL)	162	351	203	303
SD	2.9	2.6	2.19	1.42
CV%	2.9	0.68	1.12	0.51

Methods Comparison

A comparison between Spectrum Diagnostics Iron and TIBC reagents 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 and 10 µg/dL for TIBC.

Linearity

The reaction is linear up to iron concentration of 500 µg/dL, and TIBC up to concentration of 1000 µg/dl .Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result × 2).

Procedure B- (TIBC)

1.Pipette into a labeled tube 1 ml Reagent 3 and 0.5 ml test specimen.
2.Mix thoroughly and let stand for 5–30 minutes at 15 – 25 ^{o}C.
3.Add to each tube one spoonful of reagent 4, mix thoroughly and leave for 30 minutes at 15 – 25 ^{o}C and mix several times during the incubation time.
4.Centrifuge at 3000 r.p.m for 10 minutes.
5.Carefully remove the supernatent into a clean tube and determine the serum iron as described above in procedure A within one hour.

Calculation

Total iron binding capacity = Iron in supernatent x 3 (dilution).

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 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.

Others
Pathological albumin levels more than 7 g/dL decrease the TIBC levels.

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)

TIBC

1 day	:	134 – 318 µg/dL	(24 - 57 µmol/L)
1 week	:	190 – 324 µg/dL	(34 - 58 µmol/L)
Infants	:	151 – 340 µg/dL	(27 - 61 µmol/L)
3 – 12 months	:	290 – 436 µg/dL	(52 - 78 µmol/L)
1 – 10 years	:	262 – 497 µg/dL	(47 - 89 µmol/L)
11 – 16 years	:	290 – 441 µg/dL	(49 - 89 µmol/L)
Adults Women	:	274 – 497 µg/dL	(49 - 89 µmol/L)
Men	:	291 – 430 µg/dL	(52 - 77 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

Iron : 5 – 500 µg/dl (0.9 – 89.5 µmol/ L).
TIBC : 10 – 1000 µg/dl (1.8 – 179.1 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
270 001	50 Test
270 002	100 Test

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