UIBC

FERROZINE

Intended use:
In vitro test for the quantitative determination of total iron binding capacity in human serum.

Summary:
Iron exists in serum complexed with transferring, a transport protein. Most early procedures for iron determination involved dissociation of the iron from the iron-protein complex, precipitation of the proteins, an the measurement of the iron content of the protein free filtrate.
Many chromagens have been used in the determination including thiocyanate o-phenanthroline , bathophenanthroline and TPTZ. In 1971 Presijn et al. 1 presented a method using the chromogen ferrozine, described by Stookey.2 This method did not require protein precipitation and was more sensitive then previous methods. The present procedure is a modification of the Presijn method.
In most cases, both serum iron and TIBC values are necessary for greatest diagnostic significance. Low serum iron values are seen in chronic blood loss, insufficient intake or absorption of iron and increased demand on the body stores (e.g. pregnancy). Elevated serum iron values are seen in increased red cell destruction, decreased red cell synthesis, increased iron take, or increased iron stores release.
Increase in the TIBC may be due to increased production of apotransferrin (e.g.chronic iron deficiency) or an increased release of ferritin, as in hepatocellular necrosis.
Decreases in the TIBC can occur with cirrhosis and hemachromatosis due to a deficiency in ferritin, or in nephrosis due to a loss of apotransferrin.

Test principle:
Photometric test using chromagen ferrozine.
Total Iron-Binding Capacity(TIBC): A known amount of ferrous ions are added to serum at an alkaline pH. The ferrous ions bind with transferrin at unsaturated iron-binding sites. The additional unbound ferrous ions are measured using the ferrozine reaction. The difference between the amount of ferrous ions ions added and the unbound ions measured is the unsaturated iron-binding capacity (UIBC). The TIBC is equal to the serum iron concentration plus the UIBC.

Reagent concentration:
R1 Tris buffer : ≥ 0.2 mol/l, pH 8.45; Ferrous ammonium sulfate: ≥ 8.4 µmol/ l; Hydroxylamine hydrochloride: ≥0.1 mol/l ; Nonionic surfactant ; thiourea; Dilute sulfuric acid

R2 FerroZine: 20.3 mmol/l ; Preservative

R3 Uibc Calibrator (550 ug/dl)

Preparation and stability:
All reagents ready to use.
On reagent stability: R1: 28 days
R2: 28 days

Specimen:
Serum free of hemolysis.

Stability:		
	8 hours	at +20°C to +25°C
	24 hours	at +2°C to +8°C
	1 month	at -20°C

Avoid plasma. If necessary, use heparin-plasma.
Specimen are not allowed to contain any iron chelating agent (EDTA).
The supernatant used in the determination of iron-binding capacity must be absolutely clear. Otherwise it must be decanted and centrifuged again, Use only swing-out centrifuges, not with a fixed angle rotor.

Notes:
For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.

Limitations - interference:
Iron chelating agents (e.g. EDTA) interfere with the MgCO3 - precipitation reaction. Iron bleeding or contaminated materials interfere.
Interferences with the iron concentration measurement:
See manual instruction of BIOANALYTIC UIBC-FZ

Testing procedure:
Materials provided
• Working materials as described above

Manual Testing	
Wavelength:	Hg 575 nm (side wavelength 700 nm)
Reaction temperature:	+37°C
Cuvette:	1 cm light path
Zero adjustment	Sample blank
	Sample/Calib./Stand.
Sample/ Calib./Stand.	40 µl
R1	400 µl
Mix well and incubate at: 37°C for 5 minutes. And read blank absorbance A1,	
R2	100 µl
Incubate at 37°C for 5 minutes. Read sample absorbance A2 Calculate = A2(sample)-A1(blank)	

Measuring /reportable range:
20 -700 µg/dl
Determine samples with concentrations > 500µg/dl via the rerun function. On instruments without rerun function, manually dilute these samples with 0.9% NaCl (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).

Expected Values:
Serum/Plasma :
UIBC : 150 to 336 µg/dl
TIBC=Iron+UIBC

Analytical sensitivity (lower detection limit)
Detection limit: 20 µg/dl
This limit results from the concentration determination with the Fe-FZ method.

Imprecision:
Reproducibility was determined using human samples (n=20). The following results were obtained:

	Within run			Between day		
Sample	Mean	SD	%CV	Mean	SD	%CV
	µg/dl	µg/dl		µg/dl	µg/dl	
Human serum 1	184.0	3.01	1.6	387.6	13.75	5.1
Human serum 2	361.0	2.65	0.7	119.0	5.70	4.8
Control	93.0	2.95	3.2	89.4	4.86	5.4

Quality Control:
Control Serum:
BIOCON N 5 x 5 ml #B10814

Calibration:
S1: 0.9% NaCl
S2: BIOANALYTIC UIBC STD 5 x 2 ml #B11944

Calibration stability

It is suggested to use Calibrator products produced by Bioanaliytic. It is suggested to use supplementary calibrator (pure water or 0.9% NaCl) to conduct 2-point calibration. The calibration curve is formed automatically. When lot number is changed or QC is invalid, calibration shall be conducted again. Recalibrate the assay every 30 days under ideal conditions, or when the following occur:
Change in reagent lot or significant shift in control values;
Major preventative maintenance was performed on the analyser or a critical part was replaced(Halogen Lamp)

Literature:

1. Kunesh JP and Small LL. Adaptation of the Zak-Epstein automated micromethod for serum iron to determine iron-binding capacity and urinary iron. Clin Chem 1970:16: 148-149.
2. Tietz N.W. Clinical Guide to Laboratory Tests, 3rd Philadelphia: W.B. Saunders Company, 1995:2059-2072.
3. Yamanishi H., Iyama S. et al. Total Iron-binding Capacity Calculated from Serum Transferrin Concentration or Serum Iron Concentration and Unsaturated Iron-binding Capacity Clin Chem 2003:49: 175-178.

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Order information (CatNo.) :

CC485	AB485	B21287	B27286	B30287	B33287	**B37285**
CC486	AB486	B22285	B27287	B31285	B34285	B37286
OL485	BTIB500	B24285	B28285	B31286	B34286	B80285
OL486	BTIB250	B25285	B28286	B32285	B35285	B80286
KL485	BTIB125	B25286	B28287	B32286	B35286	B80288
KL486	B21285	B25287	B30285	B33285	B36285	B80288
CR485-486	B21286	B27285	B30286	B33286	B36286	

SYMBOLS

IVD	for in vitro diagnostic use only
LOT	lot of manufacturing
REF	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	Read the directions

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