ACCUCARE

IRON / UIBC REAGENT

Modified Ferrozine Method

Quantitative determination of iron and total iron-binding capacity in serum/plasma
Only for *In Vitro* Diagnostic use

ORDER INFORMATION

REF	Cont.
FE 100	2 X 50 ml
FE 200	2 X 100 ml

CLINICAL SIGNIFICANCE

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 intake, 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 loss of apotransferrin.

Method

Iron exists in serum complexed with transferrin, a transport protein. Most early procedures for iron determination involved dissociation of the iron from the iron-protein complex, precipitation of the proteins, and then 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, Persijn et al.1 presented a method using the chromagen ferrozine, described by Stookey.2 This method did not require protein precipitation and was more sensitive than previous methods. The present procedure is a modification of the Persijn method.

PRINCIPLE

Serum Iron: Transferrin-bound iron is released at an acid pH and reduced from ferric to ferrous ions. These ions react with ferrozine to form a violet colored complex which is measured spectrophotometrically at 560nm. The absorbance measured at this wavelength is proportional to serum iron concentration. 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 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

1. IRON BUFFER REAGENT — Hydroxylamine hydrochloride 220mM in acetate buffer, pH 4.5 with surfactant.
2. UIBC BUFFER REAGENT — Tris 500mM, pH 8.1 with surfactant, Sodium Azide 0.05% (w/v) as preservative.
3. IRON COLOR REAGENT — Ferrozine 16.7mM in hydroxylamine hydrochloride.
4. IRON STANDARD (500 µg/dL) — Ferrous chloride in hydroxylamine hydrochloride.

REAGENT PREPARATION

All reagents should be clear. Turbidity may indicate contamination and the reagent should not be used.

REAGENT STORAGE AND STABILITY

When stored at 2-8°C reagent is stable until the expiration date stated on the bottle and kit box label.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- NaCl solution 9 g/L
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

Serum or heparin plasma
It is very important to store the sample protected from light!
Stability: 1 Month at 4 – 8°C
2 months at –20°C in case of immediate freezing.
Freeze only once! Discard contaminated specimens!

ASSAY PROCEDURE

SERUM IRON

1. Label test tubes/cuvettes, “Blank”, “Standard”, “Control”, “Sample”, etc.
2. Add 1.0 ml Iron Buffer reagent to all tubes.
3. Add 0.25ml (250ul) sample to respective tubes. Mix. Note: Add 0.25ml (250ul) iron-free water to blank.
4. Zero spectrophotometer at 560nm with the reagent blank.
5. Read and record absorbances of all tubes. (A1 reading).
6. Add 0.02ml (20ul) Iron color reagent to all tubes. Mix.
7. Place all tubes in heating bath at 37°C for 10 minutes.
8. Zero instrument at 560nm with reagent blank.
9. Read and record absorbances of all tubes. (A2 reading).

SERUM IRON

	Blank	Standard	Sample Blank (A1)	Sample (A2)
Iron Buffer Reagent	1 ml	1 ml	1 ml	1 ml
Sample	-	-	250 µl	250 µl
Standard	-	250 µl	-	-
Iron free water	250 µl	-	-	
Color Reagent	20 µl	20 µl	-	20 µl

Mix & incubate for 10 min at 37°C. Zero instrument at 560 nm with reagent blank. Record the absorbance of all the tubes (A1 & A2 Reading)

CALCULATION

A = Absorbance
Std = Standard

$$\frac{A2\ Test - A1\ Test}{A2\ Std - A1\ Std} \times Conc.\ of\ Std = Total\ Iron\ (\mu g/dL)$$

Example: A1 Test = 0.08 A2 Test = 0.15
A1 Std = 0.00 A2 Std = 0.40

Then: $\frac{0.15 - 0.08}{0.40 - 0.00} \times 500 = 0.07 \times 500 = \frac{0.175}{0.40} \times 500 = 87.5\ \mu g/dL$

UIBC (Unsaturated Iron-Binding Capacity)

1. Label test tubes/cuvettes, “Blank”, “Standard”, “Control”, “Test”, etc.
2. Add 1.0ml UIBC buffer reagent to all tubes.
3. To “Blank” add 0.5 ml iron-free water. Mix.
4. To “Standard” add 0.25ml (250ul) iron-free water plus 0.25ml (250ul) standard. Mix.
5. To “Test” add 0.25ml (250ul) respective sample plus 0.25ml (250ul) Iron Standard. Mix.

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6. Zero spectrophotometer at 560nm with reagent blank.
7. Read and record the absorbance of all tubes. (A1 reading).
8. Add 0.025 (25ul) of Iron Color Reagent to all tubes. Mix.
9. Place all tubes in a heating bath at 37°C for ten minutes.
10. Zero spectrophotometer at 560nm with reagent blank.
11. Read and record the absorbance of all tubes. (A2 reading).

Serum UIBC

	Blank	Standard	Sample Blank (A1)	Sample (A2)
UIBC Buffer Reagent	1 ml	1 ml	1 ml	1 ml
Sample	-	-	250 µl	250 µl
Standard	-	250 µl	250 µl	250 µl
Iron free water	500 µl	250 µl	-	
Color Reagent	25 µl	25 µl	-	25 µl

Mix & incubate for 10 min at 37°C. Zero instrument at 560 nm with reagent blank. Record the absorbance of all the tubes (A1 & A2 Reading)

UIBC CALCULATION

Conc. Of Std. $\frac{\text{– (A2 Test - A1 Test)}}{\text{(A2 Std. – A1 Std.)}}$ X Conc. of std = UIBC (µg/dL)

Example: Std. Conc. = 500µg/dL

A1 Test = 0.10 A2 Test = 0.20

A1 Std. = 0.00 A2 Std. = 0.40

Therefore: 500 $\frac{\text{- (0.20 – 0.10)}}{\text{(0.40 – 0.00)}}$ x 500 = UIBC (µg/dL)

500 – (0.25 x 500) = 375 µg/dL (UIBC)

NOTE: The difference between A1Test and A2 Test may sometimes be very small due to a high degree of unsaturation of transferrin with iron. The sample should be diluted 1:1 with iron-free water and re-assayed. The result is then multiplied by two.

CALCULATION

TIBC (Total Iron-Binding Capacity)

Iron Level + UIBC = TIBC (µg/dL)

SI Unit Conversion µg/dL x 0.179 = umo/L

SAMPLE DILUTIONS

- This method is linear upto a concentration of 500 µg/dL.
- Dilute samples above this concentration 1:1 with 0.9% saline
- Repeat assay. Multiply the result by 2.

CLIBRATORS AND CONTROLS

For the calibration of automated photometric systems the commercially available suitable multi-calibrator is recommended.
The assigned values of the calibrator have been made traceable to the NIST-SRM®937reference material.

It is recommended to run a normal and a pathological control serum which is commercially available to verify the performance of the measured procedure. The value of controls should fall within the established limit.

Each laboratory should establish corrective action in case of deviations in control recovery.

PERFORMANCE CHARACTERISTICS

WITHIN RUN

Sample	Mean Concentration	SD	CV %
Iron			
Level 1	80.93	2.65	3.27%
Level 2	220.63	2.49	1.13%
UIBC			
Level 1	149.89	2.09	1.39%
Level 2	250.85	2.49	0.99%

RUN TO RUN

Sample	Mean Concentration	SD	CV %
Iron			
Level 1	82.29	2.78	3.38%
Level 2	220.57	4.49	2.15%
UIBC			
Level 1	151.86	2.17	1.43%
Level 2	250.33	5.32	2.32%

LINEARITY

The method is linear upto a concentration of IRON is 500µg/dL. Dilute samples above this concentration 1:1 with 0.9% saline solution and repeat assay. Multiply the result by 2.

Limit of detection: The limit of detection for Iron is 6 µg/dL.

METHOD COMPARISON

A comparison of Accucare Iron/UBIC with a commercially available assay (x) using 20 samples gave following results: $R^2 = 0.9900$

REFERENCE VALUES

Iron, Total = 60 – 150 ug/dl
TIBC = 250-400 ug/dl
Iron Saturation = 20-55%
The reference values are to be considered as indicative only. Every laboratory should establish its own normal range.

LIMITATION OF THE PROCEDURE

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

INTERFERENCE

- Hemoglobin: No interference found upto 100 mg/dL.
- Bilirubin: No interference found upto 50 mg/dL.
- Lipemia: No interference found upto 2000 mg/dL.
- These characteristics have been obtained using an automatic analyzer. Results may vary if a different instrument or a manual procedure is used.

BIBLIOGRAPHY

1. Persijn, J.P., et al, Clin. Acta 35:91, (1971).
2. Stookey, L.L., Anal. Chem. 42:779, (1970).
3. Tietz, N.W., Fundamentals of Clinical Chemistry Philadelphia, W.B. Saunders, pp. 923-929, (1976).
4. Weissman, N., Pileggi, V.J., in Clinical Chemistry: Principles and Technics, 2nd Ed., R.J. Henry et al, editors, Hagerstown (MD), Harper & Row, pp. 692- 693, (1974).
5. Young, D.S. et al, Clin. Chem. 21:1D, (1975).
6. Henry, J.B., Clinical Diagnosis and Management by Laboratory Methods, Philadelphia, W.B. Saunders, p. 1434, (1984).

GLOSSARY OF SYMBOL

	Consult Instruction for Use	LOT	Lot Number
REF	Catalog Number		Date of Manufacturing
	Store between		Use By or Expiration Date
	Manufacturer	IVD	For *in vitro* Diagnostic use only
	Keep away from sunlight	CONT	Content of the kit

LAB-CARE DIAGNOSTICS (INDIA) PVT. LTD.
C1 Type, Shed No.: 3225, Chemical Zone,
GIDC Sarigam – 396155, Dist. Valsad, Gujarat, India.
Tel.: +91 22 2554 2109 /1558
Email: accucare@labcarediagnostics.com;
Website: www.labcarediagnostics.com

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