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ACCUCARE™
IRON / UIBC REAGENT
Modified Ferrozine Method

INTENDED USE

For the quantitative determination of iron and total iron-binding capacity in serum.

ORDER INFORMATION

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

METHOD HISTORY

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.

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.

REAGENTS

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 ug/dl): Ferrous chloride in hydroxylamine hydrochloride.

PRECAUTIONS

1. All reagents are toxic. Do not pipette by mouth. Avoid all contact.
2. UIBC buffer contains sodium azide and may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation.
3. This reagent is for *in vitro* diagnostic use only.

REAGENT STORAGE

Store all reagents refrigerated at 2-8℃.

REAGENT DETERIORATION

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

SPECIMEN COLLECTION AND STORAGE

1. Fresh, unhemolyzed serum is the specimen of choice.
2. Serum should be separated as soon as clot has formed.
3. Heparinized plasma may be used but other anticoagulants should not be used to avoid possible iron contamination.
4. Serum iron is reported to be stable for four days at room temperature (15-
5. 30℃) and seven days at 2-8℃.

INTERFERENCES

1. Certain drugs and other substances are known to influence circulating iron levels. See Young, et al.
2. Iron contained in hemoglobin does not react in this method, therefore, slight hemolysis will not interfere. However, gross hemolysis (pink or red specimens) will contribute to the absorbance measured at the wavelength used and should be avoided.3
3. To make tubes, pipettes, etc. iron free, they must be washed with hot, dilute (1:2) hydrochloric or nitric acid, followed by several rinsings with iron-free deionized or distilled water.

MATERIALS PROVIDED

1. Iron Buffer Reagent.
2. UIBC Buffer Reagent.
3. Iron Color Reagent.
4. Iron Standard (500ug/dl).

PROCEDURE (MANUAL)

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℃ 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℃. Zero instrument at 560 nm with reagent blank. Record the absorbance of all the tubes (A1 & A2 Reading)

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Calculation

A = Absorbance
Std = Standard

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

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

Then: $\frac{0.15 - 0.08}{0.40 - 0.00} \times 500 = 0.07 \times 500 = \frac{0.175}{0.40} \times 500 = 87.5\ ug/dl$

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.
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 Calculations

$$Conc.\ Of\ Std. \frac{-(A2\ Test - A1\ Test)}{(A2\ Std. - A1\ Std.)} \times Conc.\ of\ Std. = UIBC\ (ug/dl)$$

Example: Std. Conc. = 500ug/dl

A1 Test = 0.10 A2 Test = 0.20
A1 Std. = 0.00 A2 Std. = 0.40

Therefore: $500 \frac{-(0.20 - 0.10)}{(0.40 - 0.00)} \times 500 = UIBC\ (ug/dl)$

500 – (0.25 x 500) = 375 ug/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 (ug/dl)
SI Unit Conversion ug/dl x 0.179 = umo/L

Calibration

The procedure is calibrated with iron standard (500ug/dl) included in each kit.

Quality Control

Serum controls with known normal and abnormal values should be run routinely to monitor the validity of the reaction.

Expected Values

Iron, Total = 60 – 150 ug/dl
TIBC = 250-400 ug/dl
Iron Saturation = 20-55%
It is strongly recommended that each laboratory determine the normal range for its particular population.

Performance

Linearity: 500 ug/dl
Samples with values above 500 ug/dl must be diluted 1:1 with normal saline, re-assayed and result multiplied by two.

References

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