Iron and Total Iron Binding Capacity (TIBC), Procedure No. FT715

Quantitative Colorimetric Determination of Iron and Unstaurated Iron-Binding Capacity in Serum

Summary and Principle

Most procedures for serum iron are based upon its release from combination, in the ferric form, with transferrin, the transport protein which binds iron at body pH. The released iron, after reducion to the ferrous state, is combined with one of several reagents to form a colored complex, which is quantitated colormetrically. Since only about one-third of the serum iron is normally bound to the gobulin, transferrin, the "unsaturated iron-binding capacity" (UIBC), or the additional quantity of iron that can be bound by the serum, is determined by saturating the transferrin with a known excess of iron. Unused iron is estimated by the same technique and UIBC calculated by the difference.

The method presented is a modification of that reported by Persijn et al[1], using the chromogenic compound, Ferrozine, described by Stookey[2]. In addition to iron, copper is the only other trace metal found in serum reported to form a colored complex with Ferrozine.[3] Neocuproine is therefore used in the color reagent to prevent copper interference.

Iron is released from its combination with transferrin in acid medium, reduced to the ferrous form by hydroxylamine and reacted with Ferrozine to form a violet colored complex which is measured at 560 nm. A separate technique for serum unsaturated iron-binding capacity (UIBC) involves addition of a known excess of ferrous ions, which saturate available transferrin iron-binding sites. Excess (unbound) iron is then quantitated as described above, with UIBC being the difference in iron concentration between that added and that determined in the remaining excess. It follows that serum total iron-binding capacity (TIBC) is the sum of iron and UIBC.

Reagents

Iron Color Reagent, Cat. No. FT715a 0371

A solution containing:

Ferrozine……7.8 mmol/L
Neocuproine……14.4 mmol/L
Hydroxylamine Hydochloride ……220 mmol/L

Iron HA Buffer, Cat. No. FT715b 0372

A solution containing:
Hydroxylamine Hydochloride……220 mmol/L in an acetate buffer. Also contains surfactants.

Iron TRIS Buffer, Cat. No. FT715c 0373

A solution containing:
TRIS ..... ……500 mmol/L
in a buffer solution. Also contains surfactants.

Iron Standard (500 µg/dL), Cat. No. FT715d

Aqueous solution containing 500 µg/dL of ferric iron.

Precautions: *For In Vitro Diagnostic Use.*
Reagent Preparation: The reagents and standard are supplied ready-to-use.

Reagent Storage and Stability: Store HA buffer, TRIS buffer and standard at room temperature. Iron Ferrozine Color reagent should be stored at 2-8°C and protected from light. All reagents are stable until the expiration date on their respective labels.

Materials Required But Not Provided

Spectrophotometer, capable of absorbance readings at 550-570nm. Deionized or distilled water (Iron-free), Accurate pipetting devices, Cuvets, Interval Timer, Test tubes, Incubator block or Water bath (37°C)

Specimen Collection and Preparation

Serum is the preferred specimen since it avoids possible iron contamination from inorganic anticoagulants. Collection tubes should be iron free and serum should be separated as soon as the clot has formed.

Sample Stability: Iron in serum is reported to be stable 4 days at room temperature and approximately 7 days at 2-8°C. [4,5]

Interfering Substances: Hemolytic sera should be avoided. Young, et al[6] list interfering substances which can affect the accuracy of iron values obtained. Bilirubin concentrations up to 15 mg/dL and copper concentrations up to 500 µg/dL will not interfere. This iron test is very sensitive to contamination, therefore, any glassware used must be iron free. We recommend using disposable laboratory materials when performing this test.

Test Procedure - Serum Iron

1. Pipet into cuvets the following volumes (mL) and mix well:

	Reagent Blank (RB)	Standard (S)	Unknown (U)
Iron HA Buffer	2.3	2.3	2.3
Distilled Water	0.30	—	—
Standard	—	0.30	—
Sample (or controls)	—	—	0.30

2. Incubate for 1 minute at room temperature.
3. Measure the absorbance (A^1) of the Standard, Controls, and Sample against the Reagent Blank at 560 nm.
4. Add 0.1 mL (100 µL) Iron Color Reagent to each cuvet, mix well and incubate for 10 minutes at 37°C.
5. Measure the absorbance (A^2) of the Standard, Controls, and Sample against the Reagent Blank at 560 nm. Read within 30 minutes.

Test Procedure - Iron Binding Capacity

1. Pipet into cuvets the following volumes (mL) and mix well:

	Reagent Blank (RB)	Standard (S)	Unknown (U)
TRIS Buffer	2.2	2.2	2.2
Distilled Water	0.60	0.30	—
Standard	—	0.30	0.30
Sample (or controls)	—	—	0.30

2. Incubate for 1 minute at room temperature.
3. Measure the absorbance (A^1) of the Standard, Controls, and Sample against the Reagent Blank at 560 nm.
4. Add 0.1 mL (100 µL) Iron Color Reagent to each cuvet, mix well and incubate for 10 minutes at 37°C.
5. Measure the absorbance (A^2) of the Standard, Controls, and Sample against the Reagent Blank at 560 nm. Read within 30 minutes.

Quality Control: Two levels of control material with known Iron content, determined by this method or an Iron Ferrozine procedure should be analyzed each day of testing.

Results

1) Values are derived by the following equation:

$$\text{Serum Iron (µg/dL)} = \frac{A^2\text{-}A^1 \text{ Unknown}}{A^2\text{-}A^1 \text{ Standard}} \times 500$$

Iron Binding Capacity

$$\text{a. Excess Iron (µg/dL)} = \frac{A^2\text{-}A^1 \text{ Unknown}}{A^2\text{-}A^1 \text{ Standard}} \times 500$$

b. UIBC (µg/dL) = 500 (the total iron added in µg/dL) - Excess Iron (µg/dL)

c. TIBC (µg/dL) = Serum Iron (µg/dL) + UIBC (µg/dL)

NOTE: Samples having Iron values greater than 1000 µg/dL are diluted 1:2 (1+1) with distilled water, the assay repeated and results multiplied by the dilution factor 2.

Expected Values[7]

	Serum Iron	Serum TIBC
Male:	65 - 170 µg/dL	250 - 450 µg/dL
Female:	50 - 170 µg/dL	250 - 450 µg/dL
Child:	50 - 120 µg/dL	250 - 400 µg/dL
Infant:	40 - 100 µg/dL	100 - 400 µg/dL
Newborn:	100 - 250 µg/dL	-

For conversion to SI Units: µg/dL x 0.179 = µmol/L.
It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories and local populations.

Performance Characteristics[8]

Correlation: Serum iron determinations were performed on 20 sera (range 16 - 221 µg/dL) by the method described (y) and by an automated procedure (x), utilizing the chromogen (TPTZ). Statistical analysis showed a correlation coefficient (r) of 0.991 and the regression equation to be y=0.963x - 1.875. Using the same specimens and techniques, serum UIBC values were derived (range 10 - 437 µg/dL), yielding an r value of 0.989 and a regression equation y = 0.937x + 0.311.

Precision: Each of two serum pools having mean iron levels of 64 and 272 µg/dL, were subjected to 20 replicate assays over a 5 day period. Standard deviations were calculated to be 7.2 and 12.1, respectively. CV's were 11.2 and 4.4%, respectively. Mean UIBC values for these samples were 181 and 150 µg/dL, with the same assay program yielding respective SD's of 8.8 and 9.8 µg/dL and CV values of 4.9 and 6.5% respectively.

Linearity: When performed as directed the serum iron method is linear to 1000 µg/dL and the UIBC method to 500 µg/dL.

References

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7. Burtis, CA and Edward Ashford, Editors, Tietz Textbook of Clinical Chemistry,2nd Ed., Saunders, 1994, pp 2195
8. Interchim Laboratory Data

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