



IRON/TIBC REAGENT SET

INTENDED USE

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

INTRODUCTION

The iron content of the human body may be divided into three classes: iron in storage, iron in use, and iron in transport. Iron in storage is reserved iron contained within the cells. Iron in use contained in hemoglobin, various enzymes, and several other types of proteins. Iron in transport is being moved to storage or is being removed from storage to be utilized in the formation of hemoglobin, etc. Iron in a free state is not only relatively insoluble, but it is toxic. Therefore, nearly all iron in the body is attached to some type of protein. It is of fundamental importance to note that a specimen should be analyzed for both iron and iron binding capacity because of the need for both values in the differential diagnosis of various types of anemia and liver diseases. For this reason, the current procedure is designed for the simultaneous determination of iron and iron binding capacity.

Serum iron assays measure transport iron bound to the protein transferrin. Increase in serum iron levels may indicate increased erythrocyte destruction, decreased erythrocyte formation, increased absorption, or defects in storage capabilities. Decrease in serum iron levels may indicate iron deficiency or inability to retrieve storage iron. Iron binding capacity is usually increased in iron deficient anemia and decreased in hemochromatosis, malignancies, rheumatic fever, Hodgkin's diseases, and collagen vascular disease.

Most successful iron methodologies remove iron from transferrin, reduce it to the ferrous state, bind it to a chromophore, and quantitate it by measuring the amount of color developed. The determination of Iron binding capacity involves adding sufficient iron to saturate transferrin and then determining either the total amount of bound iron or the excess unbound iron. The later procedure is applied to determine Iron Binding Capacity.

PRINCIPLE

The iron in serum is dissociated from its Fe (III) - transferrin complex by the addition of an acidic buffer containing hydroxylamine. This addition reduces the Fe (III) to Fe (II). The chromogenic agent, Ferrozine, forms a highly colored Fe (II) - complex that is measured photometrically at 560 nm.

The unsaturated iron binding capacity (UIBC) is determined by adding Fe (II) ion to serum so that they bind to the unsaturated iron binding sites on transferrin. The excess Fe (II) ions are reacted with Ferrozine to form the color complex, which measured photometrically. The difference between the amount of Fe (II) added and the amount of Fe (II) measured represents the unsaturated iron binding. The total iron binding capacity (TIBC) is determined by adding the serum iron value to the UIBC value.

REAGENTS

1. Iron buffer reagent: Acetate buffer containing 220 mM Hydroxylamine hydrochloride, pH 4.5 with surfactant.
2. UIBC buffer reagent: Tris buffer 0.5 M, pH 8.0, with surfactant, and sodium azide as a preservative.
3. Iron color reagent: Ferrozine (16.6 mM) in Hydroxylamine hydrochloride.
4. Iron standard (500 µg/dl): 500 µg Ferrous chloride in Hydroxylamine hydrochloride.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
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. Avoid ingestion of reagent, as toxicity has not yet been determined.
4. Specimens should be considered infectious and handled appropriately.

STORAGE AND STABILITY

All the reagents and standard should be stored at room temperature (18 – 30°C).

REAGENT DETERIORATION

1. Appearances of turbidity, possible mold growth, or crystal formation that will not readily dissolve are signs of reagent deterioration.
2. Failure to obtain accurate results in the assay of control materials may indicate reagent deterioration.

SPECIMEN COLLECTION AND STORAGE

1. Fresh, un-Hemolyzed 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 (18 - 30°C) and seven days at 2 - 8°C.

INTERFERING SUBSTANCES

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.¹
3. To make tubes, pipettes, etc. iron free, they must be washed with hot dilute 1:3 hydrochloric or nitric acid, followed by several rinsing with iron-free de-ionized or distilled water.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Spectrophotometer capable of reading at 560 nm
2. Iron-free de-ionized water
3. Pipetting devices
4. Test tubes /rack A2 Std - A1 Std
5. Timer
6. Heating bath/block *Example:*

MANUAL PROCEDURE

Serum Iron:

1. Label test tubes/cuvettes: "Blank", "Standard", "Control", "Sample", etc.
2. Add 2.5 ml Iron Buffer reagent to all tubes.
3. Add 0.5 ml (500µl) sample to respective tubes and mix. *NOTE: Add 500 µl iron-free water to blank.*
4. Zero spectrophotometer at 560 nm with the reagent blank.
5. Read and record the absorbencies of all tubes (A1 reading).
6. Add 0.05 ml (50 µl) Iron color reagent to all the tubes. Mix.
7. Place all the tubes in the heating bath at 37°C for 10 minutes.
8. Zero the instrument at 560 nm with the reagent blank. (Wavelength range: 520-560 nm).
9. Read and record absorbencies of all the tubes (A2 reading).

CALCULATIONS

A = Absorbance

Std = Standard

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

UIBC (Unsaturated Iron-Binding Capacity)

1. Label test tubes/cuvettes, "Blank", "Standard", "Control", "Test", etc.
2. Add 2.0 ml UIBC buffer reagent to all tubes.
3. To "Blank" add 1.0 ml iron-free water. Mix.
4. To "Standard" add 0.5 ml (500 µl) iron-free water plus 0.5 ml (500 µl) Iron standard. Mix.
5. To "Test" add 0.5 ml (500 µl) respective sample plus 0.5 ml (500 µl) Iron Standard. Mix.
6. Zero spectrophotometer at 560 nm with reagent blank.
7. Read and record the absorbance of all tubes (A₁ reading).
8. Add 0.05 ml (50 µl) of Iron Color Reagent to all tubes. Mix.
9. Place all tubes in a heating bath at 37°C for ten (10) minutes.
10. Zero spectrophotometer at 560 nm with reagent blank.
11. Read and record the absorbance of all tubes. (A₂ reading).

UIBC CALCULATIONS

$$\text{Conc. Of Std.} - \left\{ \frac{A2 \text{ Test} - A1 \text{ Test}}{A2 \text{ Std} - A1 \text{ Std}} \right\} \times \text{Conc. Of Std.} = \text{UIBC } (\mu\text{g/dl})$$

NOTE: The difference between A1 Test and A2 Test may sometimes be very small due to a high degree of unsaturation of transferrin with iron. The sample should be diluted with iron-free water and re-assayed. Multiply the result by the dilution factor.

CALCULATIONS

TIBC (Total Iron-Binding Capacity):

$$\text{Iron Level} + \text{UIBC} = \text{TIBC } (\mu\text{g/dl})$$

SI Unit Conversion: $\mu\text{g/dl} \times 0.179 = \text{mmol/L}$

CALIBRATION

The procedure is calibrated with iron standard (500 $\mu\text{g/dl}$) 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 $\mu\text{g/dl}$

TIBC = 250 - 400 $\mu\text{g/dl}$ Iron Saturation = 0 - 55%

It is strongly recommended that each laboratory determine the normal range for its particular population.

PERFORMANCE CHARACTERISTICS

1. Linearity: 500 $\mu\text{g/dl}$ Samples with values above 500 $\mu\text{g/dl}$ must be diluted 1:1 with normal saline, re-assayed and result multiplied by two.
2. Sensitivity: Based on an instrument of $A = 0.001$, this procedure has a sensitivity of 2 $\mu\text{g/dl}$.
3. Comparison study: A study performed between this procedure and a similar Serum Iron procedure resulted in a coefficient of correlation of 0.98 with a regression equation of $y = 1.0x - 4.86$. A study performed between this procedure and a similar UIBC procedure resulted in a coefficient of correlation of 0.97 with a regression equation of $y = 0.86x + 56$.

REFERENCES

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