POINTE SCIENTIFIC, INC.
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Total Iron Reagent Set

Intended Use
For the quantitative determination of iron in serum.

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.

Clinical Significance[3]
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 hemochromatosis 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. IRON COLOR REAGENT: Ferrozine 16.7mM in hydroxylamine hydrochloride.

Precautions
1. All reagents are toxic. Do not pipette by mouth. Avoid all contact.
2. This reagent is for *in vitro* diagnostic use only.

Reagent Storage
Store all reagents refrigerated at 2-8°C.

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]
4. Serum iron is reported to be stable for four days at room temperature (15-30°C) and seven days at 2-8°C.[4]

Interferences
1. Certain drugs and other substances are known to influence circulating iron levels. See Young, et al.[5]
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. Iron Color Reagent.

Materials Required but not Provided
1. Accurate pipetting devices
2. Test tubes/rack
3. Timer
4. Spectrophotometer able to read at 560 nm.
5. Iron-free deionized water.
6. Heating bath/block (37°C).

Procedure (Automated)
Refer to specific instrument application instructions.

Procedure (Manual)

Serum Iron
1. Label test tubes/cuvettes, “Blank”, “Standard”, “Control”, “Sample”, etc.
2. Add 2.5ml Iron Buffer reagent to all tubes.
3. Add 0.5ml (500ul) sample to respective tubes. Mix. Note: Add 500ul iron-free water to blank.
4. Zero spectrophotometer at 560nm with the reagent blank.
5. Read and record absorbances of all tubes. (A_1 reading).
6. Add 0.05ml (50ul) 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. (A_2 reading).

Calculation
A = Absorbance STD = Standard

$$\frac{A_2 \text{ Test} - A_1 \text{ Test}}{A_2 \text{ STD} - A_1 \text{ STD}} \times \text{Conc. of STD} = \text{Total Iron (ug/dl)}$$

Example: A_1 Test = 0.08, A_2 Test = 0.15
A_1 STD = 0.00, A_2 STD = 0.40

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

Calibration
The procedure is calibrated with an aqueous iron standard (500ug/dl) or an appropriate serum calibrator.

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

Performance

1. 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.
2. Comparison: A study performed between this procedure and a similar Serum Iron procedure resulted in a coefficient of correlation of .993 with a regression equation of y= 1.02x + 7.0
3. Precision: Total Iron

Within Run			Run to Run		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
110	3.0	2.7	109	5.1	4.7
184	3.1	1.7	177	3.8	2.1
251	3.3	1.3	249	4.5	1.8

References

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