POINTE SCIENTIFIC, INC.
5449 Research Drive • Canton MI 48188 • USA

Total Iron Reagent Set

Intended Use
For the quantitative determination of iron and total iron-binding capacity in serum. For *in vitro* diagnostic use only.

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 (R1) Reagent: Hydroxylamine hydrochloride 220mM in acetate buffer, pH 4.5 with surfactant.
2. Iron Color (R2) Reagent: Ferrozine 3.6mM 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 R1 Reagent
2. Iron Color R2 Reagent

Materials Required but not Provided
1. Controls
2. Calibrator
3. Beckman Coulter AU™ analyzer
4. Application and instrument manuals

Procedure (Beckman Coulter AU™400 application)

SPECIFIC TEST PARAMETERS

TEST NUMBER: **#** TEST NAME: **Iron** ∇ TYPE: **Serum** ∇ OPERATIONAL: **Yes** ∇
SAMPLE VOL.: **9** DIL. VOL.: **0** PRE-DILUTION RATE: **1**
REAGENTS: R1 VOLUME: **150** DIL. VOL.: **0** MIN. OD MAX. OD
R2 VOLUME: **30** DIL. VOL.: **0** L H
REAGENT OD LIMIT:
WAVELENGTH: PRI. **570** ∇ SEC. **800** ∇ FIRST L: **-0.100** FIRST H: **1.500**
METHOD: **END** ∇ LAST L: **-0.100** LAST H: **1.500**
REACTION SLOPE: **+** ∇ DYNAMIC RANGE:
MEASURING POINT 1: FIRST: **0** LAST: **12** L: **#** H: **#**
MEASURING POINT 2: FIRST: **0** LAST: **10** CORRELATION FACTOR:
LINEARITY: % A: **1.000** B: **0.000**
NO LAG TIME: ∇ ON BOARD STABILITY PERIOD: **#**

SPECIFIC TEST PARAMETERS

VALUE FLAG: **#** ∇ LEVEL L: **#** LEVEL H: **#**

NORMAL RANGES:	SEX	AGE L YEAR	AGE L MONTH	AGE H YEAR	AGE H MONTH	L	H
○ 1.	# ∇	#	#	#	#	#	#
○ 2.	# ∇	#	#	#	#	#	#
○ 3.	# ∇	#	#	#	#	#	#
○ 4.	# ∇	#	#	#	#	#	#
○ 5.	# ∇	#	#	#	#	#	#
○ 6.	# ∇	#	#	#	#	#	#
7. NONE SELECTED						#	#
8. OUT OF RANGE		L	H			#	#

PANIC VALUE: **#** **#** UNIT: **ug/dl** DECIMAL PLACES: **0**

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CALIBRATION SPECIFIC PARAMETERS

CAL TYPE: **AB** ∇ FORMULA: **Y=AX+B** ∇ COUNTS: **2** PROCESS: **CONC.** ∇

	CAL. NO.	OD	CONC.	FAC/OD-L	FAC/OD-H
POINT 1.	#		#	**-9999999**	**9999999**
POINT 2.					
POINT 3.					
POINT 4.					
POINT 5.					
POINT 6.					
POINT 7.					

1-POINT CAL. POINT: ○ WITH CONC-0

MB TYPE FACTOR: CALIBRATION STABILITY PERIOD: **#**

#: User-Defined

The above reagent parameters are intended to serve as a guide for use with Pointe Scientific, Inc. reagent. The parameters are based on data generated by Pointe Scientific, Inc. Please note: These parameters should be used in conjunction with your laboratory Quality Control Program for validation.

NOTE: For other instrument specific applications please contact Pointe Scientific, Inc. Technical Service Department at 1-800-445-9853

Calculations

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

Aqueous standards can be used to calibrate the procedure or an appropriate serum calibrator. The procedure should be calibrated according to the instrument manufacturer's instructions. If control results are found to be out of range, the procedure should be re-calibrated.

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 comparison study performed between the Beckman Coulter AU™400 and Roche Hitachi 717 using this method resulted in a correlation coefficient of r = 0.996 with a regression equation of y= 0.984x – 8.3. (n =40 , range 11-513)
3. Precision:
   Within - day precision study was performed using three levels of material.
   Between - day precision study was performed using two levels of control material assayed over a 20 day period with 2 runs per day and 2 replicates per run.

Within Day (N=20)			Day to Day		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
66	3.3	5.0	51	2.9	5.7
129	2.1	1.6	211	2.1	1.0
197	2.8	1.4			

Precision and Linearity studies were performed following modifications of CLSI Protocols EP5 and EP6[7] using a Beckman AU™400 analyzer

References

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).
7. CLSI protocols EP5-A2, Vol. 24 No. 25, 2nd Ed, (2004) and CLSI EP6-A, Vol. 23 No. 16 (2003)

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