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Pointe Total Iron Reagent Set

Intended Use
For the quantitative determination of iron in serum using the Yumizen C230 and Yumizen C240 analyzers. For *in vitro* diagnostic use only. **Rx 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. The reagents are stable until the expiration date appearing on the label when stored as directed.

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. 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. Yumizen C230 / Yumizen C240 Analyzer
2. Yumizen C230 / Yumizen C240 Operation manual
3. Pointe Chemistry Calibrator, catalog number C7506-50
4. Pointe Chemistry control, catalog number C7592-100

Test Parameters

Test:	Iron	Chemistry: Iron	
Chemistry No.:	221	Print Name: Iron	
Reaction Type:	Endpoint	Reaction Direction: Positive	
Pri. Wave:	546 nm	Sec. Wave 670 nm	
Decimal.:	0	Samp. Type: Serum	
Blank Time:	-2 -1	Reaction Time: 32	34
Unit:	ug/dL	Incubation Time: 3	

	Sample Vol.	Aspirated	Diluent		Reagent Vol.	Diluent	
Standard;	6	uL	uL	uL	120	uL	uL
Decreased;		uL	uL	uL	20	uL	uL
Increased;		uL	uL	uL			

Linearity Range (Standard);	0-500		Linearity Limit:		
Linearity Range (Decreased):			Substrate Depletion:		
Linearity Range (Increased):			Mixed Blank Abs.:	-40000	40000
R1 Blank Abs.:	-40000	40000	On-board Stability:	30	Day (s)
Blank Response	-40000	40000	Reagent Alarm Limit:	5	
Twin Chemistry:					

Prozone Check:		
Q1:	Q2:	Q3:
Q4:	PC:	ABS:

Use Qualitative Result:		
	Range:	Flag:

Slope Offset:			
	Slope	Offset	Unit
	1	0	ug/dL

Pretreatment:			
Pretreat Sample Vol.:	uL	Pretreat Reagent Vol.:	uL

Ref. Range:					
Sample Type:	Gender:	Age Range:	Ref. Range:	Critical Range:	Unit:

Pointe
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Calibration Setup Parameters

Chem: Iron
Calibration Setting
Math Model: Two-Point Linear
Factor: Replicates: 2
Acceptance Limits
Cal Time: 336 hr.
Slope Diff: SD:
Sensitivity: Repeatability: * User Defined
Deter Coeff:
Auto Calib.
☐ Cal Time

Calibrator	Conc.	Pos	Lot No.
Water	0.0	W	
Chem Cal	*	*	

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

Use an NIST-traceable 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. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values[6]

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 was performed between the Yumizen 200 series analyzers and a similar analyzer using this method, resulting in a correlation coefficient of.994 with a regression equation of y= 1.072x – 3.1.
3. Precision: Precision studies were performed using the Yumizen 200 series analyzers following a modification of the guidelines which are contained in NCCLS document EP5-T2.[7]

Within Run			Day to Day		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
81.5	3.6	4.4	78.6	2.1	2.7
289.4	6.2	2.1	280.7	5.6	2.0

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. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
IVD *In vitro* diagnostic medical device	Temperature limitation
Consult instructions for use	**Rx Only:** Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

REF 12-HI904-144

Manufactured by
HORIBA Instruments Incorporated - Pointe Brand
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IVD

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Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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