

## Intended Use

For the quantitative determination of iron in serum using the Yumizen C560 analyzer. **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.<sup>1</sup> presented a method using the chromagen ferrozine, described by Stookey.<sup>2</sup> 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<sup>3</sup>

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 and Hazards

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

### Hazards:

**Buffer:** Hazard Classifications: Skin Corrosion/Irritation (Category 2), Eye Damage/Irritation (Category 2), Specific Target Organ Toxicity, Repeat Exposure; Blood and Central Nervous System (Category 1), Skin Sensitizer (Category 1), Carcinogen (Category 2)

Hazard Statements: H315: Causes skin irritation, H317: May cause an allergic skin reaction, H319: Causes serious eye irritation, H351: Suspected of causing cancer, H372: Causes damage to organs through prolonged or repeated exposure

Precautionary Statements: **Prevention:** P202: Do not handle until all safety precautions have been read and understood. P260: Do not breathe dust/fume/gas/mist/vapors/spray. P264: Wash skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection. **Response:** P314: Get medical advice/attention if you feel unwell. P362: Take off contaminated clothing and wash before use P302 + P352: IF ON SKIN: wash with plenty of soap and water. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P308 + P313: IF exposed or concerned: Get medical advice/attention. P332 + P313: IF SKIN irritation occurs: Get medical advice/attention. P337 + P313: IF eye irritation persists: Get medical advice/attention. **Storage:** P404: Store in a closed container. **Disposal:** P501: Dispose of contents to an approved waste disposal plant.

**Color:** Hazard Classifications: Skin Sensitizer (Category 1), Specific Target Organ Toxicity, Repeat Exposure; Blood and Central Nervous System (Category 1), Carcinogen (Category 2)

Hazard Statements: H317: May cause an allergic skin reaction, H351 Suspected of causing cancer, H372: Causes damage to organs through prolonged or repeated exposure.

Precautionary Statements: **Prevention:** P202: Do not handle until all safety precautions have been read and understood. P260: Do not breathe dust/fume/gas/mist/vapors/spray. P264: Wash skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection. **Response:** P314: Get medical advice/attention if you feel unwell. P363: Wash contaminated clothing before reuse. P302 + P352: IF ON SKIN: wash with plenty of soap and water. P308 + P313: IF exposed or concerned: Get medical advice/attention. P333 + 313: IF SKIN irritation or rash occurs: Get medical advice/attention. **Storage:** P404: Store in a closed container. **Disposal:** P501: Dispose of contents to an approved waste disposal plant. **Refer to the Safety Data Sheet for this product (SDS-HI904) available by calling 1-734-487-8300.**



Signal Word: Danger



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## Reagent Storage and Stability

Store all reagents refrigerated at 2-8°C. The reagents are stable until the expiration date appearing on the label when stored as directed. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

## 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.

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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.<sup>4</sup>

## Interferences

1. Certain drugs and other substances are known to influence circulating iron levels. See Young, et al.<sup>5</sup>
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.<sup>3</sup>
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 C560 Analyzer
2. Yumizen C560 Operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

## 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 test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

## 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<sup>6</sup>

Iron, Total = 60 – 150 ug/dl

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

## Performance

1. Assay Range: 2 - 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 C560 and a similar analyzer using this method, resulting in a the following:

Method	Iron
N	93
Mean Iron (µg/dL)	90.2
Range (µg/dL)	0-333
Standard Deviation	71.0
Regression Analysis	$y = 0.967x + 9.9$
Correlation Coefficient	0.9885

3. Precision: Precision studies were performed using the Yumizen C560 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.<sup>7</sup>

Sample	Within Day		
	LOW	MID	HIGH
N	20	20	20
Mean	80.4	222.2	492.1
Standard Deviation	1.0	1.7	4.0
Coefficient of Variation (%)	1.3%	0.8%	0.8%

Sample	Total		
	LOW	MID	HIGH
N	40	40	40
Mean	75.4	211.4	482.0
Standard Deviation	2.4	8.9	16.9
Coefficient of Variation (%)	3.2%	4.2%	3.5%

4. Sensitivity: 2 SD Limit of Detection (95% Con Int): 2 µg/dL

## 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, 2<sup>nd</sup> 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", 2<sup>nd</sup> Ed. (1992).

### CHEMISTRY PARAMETERS

Chem:	IRON	No.:	221	Sample Type:	Serum
Chemistry:	Iron (Ferrozine)	Print Name:	IRON	Reaction Direction:	Positive
Reaction Type:	End Point	Sec Wave:	660	Decimal	0
Pri Wave:	546	Reaction Time:	80	82	
Unit:	µg/dL	Reagent Vol.	Diluent		
Blank Time:	47	49			
	Sample Vol.	Aspirated	Diluent		
Standard:	7.2 ul	-- ul	-- ul	R1:	120 ul -- ul
Decreased:	-- ul	-- ul	-- ul	R2:	24 ul -- ul
Increased:	-- ul	-- ul	-- ul	R3:	-- ul -- ul
	<input type="checkbox"/> Sample Blank	<input checked="" type="checkbox"/> Auto Rerun		R4:	-- ul -- ul
<b><u>Slope/Offset Adjustment</u></b>					
Slope: 1		Offset: 0			

Linearity Range (Standard)	2	500	Linearity Limit:
Linearity Range (Decreased)	---	---	Substrate Depletion:
Linearity Range (Increased)	---	---	Mixed Blank Abs:
R1 Blank Abs:	---	---	Uncapping Time
Blank Response:	---	---	Reagent Alarm Limit:
Twin Chemistry:			<input type="checkbox"/> Enzyme Linear Extension
<input type="checkbox"/> Prozone Check		<input type="checkbox"/> Rate Check	<input type="checkbox"/> Antigen Addition
Q1:	Q2:	Q3:	Q4:
PC:	ABS:		

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## CALIBRATION PARAMETERS

<b>Calibrator Definition</b>						
Calibrator:	*		Lot No.:	*		
Exp Date:	*					
<b>Carousel</b>		<b>Pos</b>				
Sample Carousel 1	*					
Sample Carousel 2						
Sample Carousel 3						
<b>Reagent/Calibration</b>						
<u>Calibrator</u>	<u>Pos</u>	<u>Lot No</u>	<u>Exp Date</u>	<u>Chem</u>	<u>Conc</u>	<u>Unit</u>
Water	W	*	*	IRON	0	µg/dL
Chemistry Calibrator	*	*	*	IRON	*	µg/dL
<b>Calibration Setup</b>						
Chem:	IRON					
<u>Calibration Settings</u>						
Math Model:	Two-Point Linear					
Factor:		Replicates:	2			
<u>Acceptance Limits</u>						
Cal Time:	334	Hour				
Slope Diff:	---	SD:	---			
Sensitivity :	---	Repeatability:	---			
Deter Coeff:	---					
<u>Auto Calib.</u>						
<input type="checkbox"/> Bottle Changed	<input type="checkbox"/> Lot Changed	<input type="checkbox"/> Cal Time				

It is recommended that two levels of control material be assayed daily.  
\* Indicates user defined parameter.

**REF** 14-HI904-350



Manufactured by  
HORIBA Instruments Incorporated-Pointe Brand  
5449 Research Drive Canton, MI 48188



### 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.

Manufactured by HORIBA Instruments Incorporated – Pointe Brand  
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### Symbol Key



Use by (YYYY-MM-DD)



Lot and batch code



Catalog number



Manufacturer



Temperature limitation



Consult instructions for use



*In vitro* diagnostic medical device **Rx Only:** Prescription Use Only