MEDTEST DX
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Total Iron Reagent Set

Intended Use
For the quantitative determination of iron in serum using the Mindray BS-480 analyzer. 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 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:

Signal Word: Danger

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.


Signal Word: Danger

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.

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.
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]

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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. Mindray BS-480 Analyzer
2. BS-480 Operation manual
3. Chemistry Calibrator, catalog number CHEC480
4. Chemistry control, catalog number CHEQ480

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
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 Mindray BS-480 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 Mindray BS-480 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[7]

Within Day

Sample	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%

Total

Sample	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, 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).

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CHEMISTRY PARAMETERS

Chem:	FE	No.:	221	Sample Type:	Serum
Chemistry:	Iron (Ferrozine)			Print Name:	FE
Reaction Type:	End Point			Reaction Direction:	Positive
Pri Wave:	546			Sec Wave:	660
Unit:	µg/dL			Decimal	0
Blank Time:	47 49			Reaction Time:	80 82

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	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
	☐ Sample Blank	☑ Auto Rerun		R4: --- ul	--- ul

Slope/Offset Adjustment

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:			☐ Enzyme Linear Extension	

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

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CALIBRATION PARAMETERS

Calibrator Definition

Calibrator: * Lot No.: *

Exp Date: *

Carousel	**Pos**
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	FE	0	µg/dL
Chemistry Calibrator	*	*	*	FE	*	µg/dL

Calibration Setup

Chem: FE

Calibration Settings

Math Model: Two-Point Linear

Factor: Replicates: 2

Acceptance Limits

Cal Time: * Hour

Slope Diff: --- SD: ---

Sensitivity : --- Repeatability: ---

Deter Coeff: ---

Auto Calib.

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

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

REF IRO480

Manufactured for MedTest DX
5449 Research Drive Canton, MI 48188

2°C 8°C

IVD

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code	REF Catalog number	Manufacturer
Temperature limitation	Consult instructions for use	IVD *In vitro* diagnostic medical device	