Fe Liquiform
Insert

Ref.: **91**

Serum Iron

Intended use . Bi-reagent system for measurement of iron in blood samples by end point reaction.

Professional use.

[For in vitro diagnostic use].

Test principle . Iron is dissociated of transferrin by the action of an acid pH buffer. Ascorbic acid presented in Reagent 2 reduces ferric ions to ferrous ions that afterwards form a brilliant magenta complex with Ferrozine®. Its absorbance is measured at 540 and 580 nm and is proportional to the iron concentration in the sample.

Summary . Fe Liquiform Labtest System allows a simple and fast procedure for the serum iron determination in serum sample. Reagent 1 has acid pH and chaotropic agent that causes the iron dissociation of the transferrin. Reagent 2 contains ascorbic acid that causes the iron reduction, and Ferrozine® that forms a stable complex with the reduced iron allowing an appropriated colorimetric measurement at 540 and 580 nm. Fe Liquiform system characteristics confer high accuracy on serum iron determinations.

The method is not interfered by heparin, fibrinogen, and copper conferring excellent accuracy to the results.

Fe Liquiform System guarantees traceability to the reference method proposed by CLSI[7] and allows the measurement of samples with iron concentration up to 1000 µg/dL minimizing the necessity of dilution in samples with high concentration.

The proposed method was developed for automated equipments that are able to measure an end point reaction in a range of 540 and 580 nm. However, Fe Liquiform System may also be applied to most semi-automated equipments or manually, in photometer.

Methodology . Labtest Ferrozine®

Reagents

1. R 1 - Reagent 1 - Store at 2-8ºC.
Reagent label bears expiration date. Buffer pH 4.5 (400 mmol/L); thiourea (30 mmol/L) and surfactants.

2. R 2 - Reagent 2 - Store at 2-8ºC.
Reagent label bears expiration date. Buffer pH 4.0 (50 mmol/L); Ferrozine® 10 mmol/L; ascorbic acid (32.6 mmol/L).

3. CAL - Calibrator - Store at 2-8ºC.
Reagent label bears expiration date and concentration. Bovine serum lyophilized with iron concentration traceable to the reference method proposed by CLSI[7].

Precautions and warnings

Disposal of all waste material should be in accordance with local guidelines.

The usual security cares should be applied on the reagent handling.
In case of eyes contact, immediately flush eyes with plenty of water and get medical assistance.

Storage and stability . Unopened reagents, when stored at indicated temperature, are stable up to expiration date shown on the label.

Deterioration . Microbial or chemical contamination may decrease reagents stability.
Do not use the reagents if they are turbid or with contamination signs.

Materials required not provided

1. A constant temperature water bath (37ºC).
2. Photometer capable of measuring absorbance at 540 - 580 nm.
3. Pipettes to measure reagents and samples.
4. Chronometer.

Sample

Use serum (non-hemolytic) collected in fasting period. Iron is reportedly stable for about 4 days at 15-25ºC and 6 days at 2-8ºC.

No known test method can offer complete assurance that human blood samples will not transmit infectious diseases. Therefore, all blood derivatives should be considered potentially infectious.

Interference

Pre-analytical factors are the most important causes of wrong determination of serum iron. The contamination may occur at the moment of the collection, the transport and during the sample process. Studies indicate that 60% of the errors occurred in the assay are due to pre-analytical errors.

The sample must be collected during the morning in order to get the optimum conditions of the results because the day-time variation may result up to 30% reduction of the values.

Age, sex, pregnancy period, use of oral contraceptive, and estrogen, alter iron concentrations. Biologic variation is an independent event of analytical error and indicates that serum iron concentration may suffer variation up to 26.5% around the homeostatic point in each individual.

Conjugated and non-conjugated bilirubin up to 20 mg/dL and triglycerides up to 1000 mg/dL do not interfere significantly.

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Hemoglobin presence yields significantly increased results. In case it is impossible to collect a non-hemolytic sample, this interference may be minimized according to the following procedure:

1. Measure the iron concentration (Fe) in the hemolytic sample;

2. Evaluate the approximated hemoglobin (Hb) concentration in the hemolytic sample;

3. Multiply the obtained value for the hemoglobin concentration by 0.26 and subtract the obtained value for serum ion (Fe) concentration. The obtained result corresponds to the approximated concentration of iron in the sample.

Serum iron corrected (mg/dL) = Fe - (0.26 x Hb)

Preparing the reagents

Reagent 1 and Reagent 2 are ready to use.

Calibrator . Using volumetric pipette, add 3.0 mL of deionized water to the calibrator bottle contents. Wait it resting for 30 minutes. Mix by inversion avoiding foam.
Stable 5 days at 2-8ºC and 30 days if stored at temperature ≤-8ºC in a hermetically closed bottle. In order to avoid repetitive freezing and melting, it is suggested to aliquot the calibrator in 0.5 mL to 1.0 mL before freezing. In order to avoid evaporation of the material, it is suggested to use appropriated tubes for freezing (cryotubes).

Procedure

This procedure does not apply for semi-automated equipments that use only flow cuvette.
See notes 1, 2 and 3.

The material used in the procedure must be iron contamination free in order to avoid getting incorrect results.

Set up three tubes and proceed as follows:

	Blank	Unknown	Calibrator
Reagent 1	0.8 mL	0.8 mL	0.8 mL
Serum	----	0.1 mL	----
Calibrator	----	----	0.1 mL
Deionized water	0.1 mL	----	----

Mix and measure the absorbance of the Unknown and Calibrator against water at 560 nm (540 - 580), obtaining the absorbance A_1.

	Blank	Unknown	Calibrator
Reagent 2	0.2 mL	0.2 mL	0.2 mL

Mix, incubate at 37ºC for 5 minutes and measure the absorbance of the Unknown and Calibrator against Blank at 560 nm or green filter (540 - 580), obtaining the absorbance A_2.

Calibration

The Calibrator iron concentration is traceable to the reference method proposed by CLSI[7].

Manual calibrations
Perform a new calibration after reagent lot change or when the internal quality control indicates.

Automatic systems
Blank of reagents: water or 0.85% NaCl;
Standards: use calibrator (Ref.: 91.3).

Calibration frequency
Blank calibration when using a new bottle of reagent;
Two point calibration (blank and calibrator) when the internal quality control indicates or when using a new lot of reagent.

Quality control . The limits and control interval must be adapted to the laboratory requirements. Each laboratory should establish corrective actions to be taken if values fall outside the control limits.

Calculations . A_1 of Unknown and Calibrator must be corrected for the final volume of the reaction obtaining A_{1cor}.

A_{1cor} Unknown = A_1 Unknown x 0.82

A_{1cor} Calibrator = A_1 Calibrator x 0.82

$$\text{Iron } (\mu g/dL) = \frac{\text{Unknown } (A_2 - A_{1cor})}{\text{Calibrator } (A_2 - A_{1cor})} \times \text{Ccal}$$

Ccal: Calibrator concentration

Due the great reproductive results of the assay system, it is possible to use the factor method:

$$\text{Calibration factor} = \frac{\text{Ccal}}{\text{Calibrator } (A_2 - A_{1cor})}$$

Iron (μg/dL) = Unknown $(A_2 - A_{1cor})$ x Factor

Measurement/reportable range

Up to 1000 μg/dL.

If iron concentration exceeds 1000 μg/dL, the sample must be diluted with 0.85% NaCl. Multiply the result by the dilution factor.

Expected values[11,12] . Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

Serum iron (μg/dL)

Newborns		100 - 250
Infant		40 - 100
Pre-school and school		50 - 120
Adults	Men	65 - 170
	Women	50 - 170

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Conversion . Conventional Unit (μg/dL) x 0.179 = Unit IS (μmol/L)

Performance characteristics[8]

Recovery studies . In samples with iron concentrations of 50, 213 and 409 μg/dL were added different quantities of the analyte. Subsequent analyses provided recoveries ranging from 99 to 103%. The mean proportional systematic error at 50, 220 and 400 μg/dL concentration were 1.29, 2.71, and 1.38 % respectively.

Method comparison . A group of 40 sera were assayed by the proposed method and a similar technique. Serum iron values ranged from 26 - 330 μg/dL. The comparisons yielded a correlation coefficient of 0.994 and regression equation was y = 1.041x - 1.079. The mean total systematic error (proportional and constant) at 50, 220 and 400 μg/dL decision level were 1.91, 3.58 and 3.80 %, respectively.

Imprecision - Within Run

	N	Mean	SD	(%) CV
Sample 1	20	49	1.71	3.25
Sample 2	20	213	1.28	0.80
Sample 3	20	387	1.63	0.47

Imprecision - Run-to-Run

	N	Mean	SD	(%) CV
Sample 1	20	49	1.10	4.05
Sample 2	20	213	3.38	1.74
Sample 3	20	387	4.36	1.21

Analytical sensitivity . Detection limit: 1.25 μg/dL. The detection limit represents the lowest measurable serum iron concentration that can be distinguished from zero. It is calculated as two standard deviations of 20 replicates of one sample without iron.

Effects of matrix dilution . Two samples with values equal to 1214 and 1058 μg/dL were used to evaluate the system response in the matrix dilutions with 150 mmol/L NaCl (0.85%). Using dilution factors ranging from 2 to 16, the mean recovery was 99.5%.

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The deionized or distilled water in the laboratory to prepare reagents, use in the measurements and for final glass washing must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsiems/cm and silicates concentration must be <0.1mg/L.

3. It is suggested to consult http://www.fxol.org in order to review physiopathological source and drugs interference in results and methodology.

References

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Presentation

Product	Reference	Contents	
Fe Liquiform	91-2/50c	R 1	2 X 40 mL
		R 2	2 X 10 mL
		CAL	1 X 3 mL
Fe Liquiform Labmax 560/400	91-4/35	R 1	4 X 26 mL
		R 2	4 X 9 mL
		CAL	1 X 3 mL

Application procedures using Fe Liquiform are available for various automated systems.

The number of tests in automated systems **depends of the programmed parameters.**

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Customer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label since the application procedures and storage conditions, indicated on the label and in this insert, have been followed correctly.

CE

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Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro

Symbols used with ivd devices

	**Conteúdo suficiente para < n > testes** Contenido suficiente para < n > tests Contains sufficient for < n > tests		**Risco biológico** Riesgo biológico Biological risk
	**Data limite de utilização (aaaa-mm-dd ou mm/aaaa)** Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)		**Marca CE** Marcado CE CE Mark
	**Material Calibrador** Material Calibrador Calibrator Material		**Tóxico** Tóxico Poison
	**Material Calibrador** Material Calibrador Calibrator Material		**Reagente** Reactivo Reagent
	**Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limitation (store at)		**Fabricado por** Elaborado por Manufactured by
	**Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community		**Número do lote** Denominación de lote Batch code
	**Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use		**Controle** Control Control
	**Número do catálogo** Número de catálogo Catalog Number		**Controle negativo** Control negativo Negative control
	**Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes		**Controle positivo** Control positivo Positive control
	**Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device		**Controle** Control Control
	**Liofilizado** Liofilizado Lyophilized		**Corrosivo** Corrosivo Corrosive
	**Período após abertura** Período post-abertura Period after-opening		**Uso veterinário** Uso veterinario Veterinary use
	**Instalar até** Instalar hasta Install before		Ref.: 140214

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