SERUM IRON

Ref . 38

Intended use. End point reaction system for measurement of Serum Iron in blood samples.

Test principle. Ferric ions are dissociated of transferrin by the action of an acid pH buffer and reduced to ferrous ions by the action of hydroxylamine.

After the addition of Ferrozine®, a brilliant magenta complex is formed and its absorbance measured at 540 and 580 nm is proportional to the iron concentration in the sample.

Summary . Serum Iron Labtest systems associates operational simplicity and a great sensibility, allowing an increase of 20% on the final volume of the reaction, with a significant reduction of the sample volume.

The method has three main characteristics for its accuracy and precision: an excellent reaction of the chromogenic reagent for an appropriated colorimetric measurement, elimination of the deproteinization of the sample and an excellent comparison with an established method, indicating accuracy and specificity.

The data of recovery and dilution of matrix indicate that the system presents a right methodological accuracy and the repetitive studies show how robust the performance is day by day.

The proposed method uses a manual technique and is easily applied to most automated and semi-automated systems which are able to measure an end point reaction in a range of 540 and 580 nm.

Methodology. Goodwin modified.

Reagents

1. R 1 - Buffer - Store at 15 - 25 °C.

Reagent label bears expiration date. Buffer pH 4.0 (250 mmol/L), Hydroxylamine (144 mmol/L).

2. CAL - Standard 500 μg/dL - Store at 15 - 25 °C.

Reagent label bears expiration date. After handling, keep it tightly closed at 2-8°C

3. R3 - Ferrozine - Store at 15 - 25 °C.

Reagent label bears expiration date. Ferrozine (28 mmol/L).

Precautions and warnings

For in vitro diagnostic use.

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

The usual security cares should be applied on the reagent handling.

Stored glasses may accumulate residues which lead to false increased

Do not use the reagents if they are turbid or with contamination signs.

Storage and stability. Unopened reagents, when stored at indicated temperature, are stable up to expiration date shown on the label. In order to avoid evaporation of the Standard, keep the bottle tightly closed.

Deterioration. Microbial or chemical contamination may decrease reagents stability.

Specimen collection and preparation

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

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 preanalytical 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.

lonic detergents for cleaning materials are source of iron contamination.

Bilirubin up to 19 mg/dL and triglycerides up to 900 mg/dL do not interfere significantly.

Bilirubin over than 19 mg/dL and triglycerides over than 900 mg/dL provide false increased results.

Materials required not provided

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



Manual procedure

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	Standard
Buffer (nº 1)	1.0 mL	1.0 mL	1.0 mL
Serum		0.25 mL	
Standard (nº 2)			0.25 mL
Deionized water	0.25 mL		

Mix and measure the absorbance of the Unknown against Blank at 560 nm or green filter (540 - 580), obtaining the absorbance A_1 .

Blank		Unknown	Standard
Ferrozine® (nº 3)	0.025 mL	0.025 mL	0.025 mL

Mix, incubate at 37 $^{\circ}$ C for 10 minutes and measure the absorbance of the Unknown and Standard against Blank at 560 nm or green filter (540 - 580), obtaining the absorbance A_2 of the Unknown.

Quality control. For quality control use Qualitrol H Level 1 and Qualitrol H Level 2 or other suitable control material. 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

Iron (
$$\mu$$
g/dL) =
$$\frac{A_2 - A_1}{A_{standard}} \times 500$$

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

$$\text{Calibration factor} = \frac{500}{\text{A}_{\text{standard}}}$$

Iron ($\mu g/dL$) = (A₂ Unknown - A₁ Unknown) x Factor

Measurement/reportable range

Up to $1000 \mu g/dL$.

If iron concentration exceeds 1000 $\mu g/dL$, the sample must be diluted (1:5) with deionized water. Multiply the result by 5.

Expected range^{10,11} . 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)

Newborn		100 - 250
Infant		40 - 100
Child		50 - 120
Adults	Male	65 - 170
	Female	50 - 170

Conversion. Conventional Unit ($\mu g/dL$) x 0.179 = Unit IS ($\mu mol/L$).

Performance characteristics⁸

Recovery studies. In three samples with iron concentrations of 63, 216 and 389 μ g/dL were added different quantities of the analyte. Subsequent analyses provided recoveries ranging from 95 to 103%. The mean proportional systematic error at 50 μ g/dL decision level was 0.5 μ g/dL or 1.0%.

Method comparison . A group of 80 sera were assayed by the proposed method and a similar technique. Serum iron values ranged from 4 - $727~\mu g/dL$. The comparisons yielded a correlation coefficient of 0.999 and regression equation was y=1.039x-2.247. The mean total systematic error (proportional and constant) at 50 $\mu g/dL$ decision level was $0.3~\mu g/dL$ or 0.6~%.

Imprecision - Within Run

	N	Mean (µg/dL)	SD (µg/dL)	(%) CV
Sample 1	20	53	2.41	4.5
Sample 2	20	219	5.75	2.6

Imprecision - Run-to-Run

	N	Mean (µg/dL)	SD (µg/dL)	(%) CV
Sample 1	20	54	2.43	4.5
Sample 2	20	226	6.50	2.9

Analytical sensitivity. Detection limit: 1.02 μg/dL corresponding to an absorbance of 0.001

The detection limit represents the lowest measurable iron concentration that can be distinguished from zero.

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 "www.fxol.org" in order to review physiopathological source and drugs interference in results and methodology.

References

- 1. Goodwin J. Murphy B. Guillemette M. Clin Chem 1966: 12:47.
- Henry RJ, Cannon DC, Winkelman JW. Clinical Chemistry, Principles and Technics, 2nd ed. New York, Harper & Row, 1974.
- 3. Ooi DS. Perkins SL. Tokessy NE. Clin Chem 1992:38/4:541-544.
- 4. Stookev L. Anal Chem 1970:42:779.
- Tonks DB. Quality Control in Clinical Laboratories, Warner-Chilcott Laboratories, Diagnostic Reagents Division, Scarborough, Canada, 1972.
- 6. Westgard J O, Barry PL, Hunt MR. Clin Chem 1981;27:493-501.
- 7. Williams HL. Johnson DJ. Haut MJ. Clin. Chem 1977:23:237-240.
- Sociedad Española de Bioquímica Clínica y Patología Molecular, Base de Datos de Variación Biológica. Avaiable in: http://www.seqc.es/article/articleview/330/1/170 (accessed on 2006/04).
- Basques JC. Especificações da Qualidade Analítica. Labtest Diagnóstica 2005.
- Ferraz MHC, Delgado RB. Valores de Referência para Exames Laboratoriais. In: Leão E, Corrêa EJ, Viana MB, Mota JAC (Ed). Pediatria Ambulatorial. 3.ed. Belo Horizonte: Coopmed, 1988. P.837-848.

- Burtis CA, Ashwood ER. Textbook of Clinical Chemistry, 2^a edição, Philadelphia: W.B. Saunders, 1986:2175-2211.
- 12. Labtest: Data on file.

Presentation

Product	Reference	Contents	
Serum Iron	38-80	R 1 1 X 80 mL	
		CAL 1 X 5 mL	
		R 2 1 X 2.5 mL	

Application procedures using Serum iron are available for various automated systems.

Consumer 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.

Labtest Diagnóstica S.A.

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Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro Symbols used with ivd devices

\sum	Conteúdo suficiente para < n > testes Contenido suficiente para < n > tests Contains sufficient for < n > tests	曼	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)	CE	Marca CE Marcado CE CE Mark
CAL	Material Calibrador Material Calibrador Calibrator Material		Tóxico Tóxico Poison
CAL	Material Calibrador Material Calibrador Calibrator Material	R	Reagente Reactivo Reagent
-	Limite de temperatura (conservar a) Temperatura limite (conservar a) Temperature limitation (store at)	•••	Fabricado por Elaborado por Manufactured by
EC REP	Representante Autorizado na Comunidade Europeia Representante autorizado en la Comunidad Europea Authorized Representative in the European Community	LOT	Número do lote Denominación de lote Batch code
Ţį	Consultar instruções de uso Consultar instrucciones de uso Consult instructions for use	CONTROL	Controle Control Control
REF	Número do catálogo Número de catálogo Catalog Number	CONTROL -	Controle negativo Control negativo Negative control
	Adições ou alterações significativas Cambios o suplementos significativos Significant additions or changes	CONTROL +	Controle positivo Control positivo Positive control
IVD	Produto diagnóstico in vitro Dispositivo de diagnóstico in vitro In vitro diagnostic device	CONTROL	Controle Control Control
LYOPH	Liofilizado Liofilizado Lyophilized		Corrosivo Corrosivo Corrosive
	Período após abertura Período post-abertura Period after-opening	(V)	Uso veterinário Uso veterinario Veterinary use
ĪN	Instalar até Instalar hasta Install before		Ref.: 140214

