



Serum Iron-SL Assay

CATALOGUE NUMBER: 157-01
157-02
157-10
157-30

SIZE: R1: 1 x 1000 mL
R2: 1 x 250 mL
R1: 1 x 100 mL + R2: 1 x 25 mL
R1: 3 x 100 mL + R2: 1 x 75 mL

INTENDED USE

For the IN VITRO quantitative determination of iron in serum.

WARNINGS AND PRECAUTIONS

S24/25: Avoid contact with skin and eyes.

REAGENTS

Acid Dissociating Reagent (R1): a buffer solution (pH 4.5 at 25°C) containing a surfactant, preservatives, and stabilizers.

Iron Color Reagent (R2): a solution containing 6 mmol/L ferene and stabilizers.

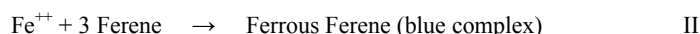
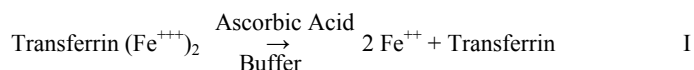
Iron Standard: 1 x 10 mL of a solution containing 35 µmol/L (195 µg/dL) iron (included with 157-10 and 157-30 only).

SUMMARY OF THE TEST

Iron measurements are used in the diagnosis and treatment of diseases and conditions such as iron deficiency anemia, hemochromatosis (characterized by a progressive increase in iron stores leading to organ impairment), chronic inflammatory disorders, hepatitis, and lead poisoning (1).

Various photometric methods have been used to measure serum iron. In 1970 Stookey (2) reported the synthesis of 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine, monosodium salt (Ferrozine®) which complexed with ferrous iron to form a tris ferrozine/iron, Fe(Fz)₃ complex. An additional feroin type compound called 5,5'-(3-(2-pyridyl)-1,2,4-triazine-5,6 diyl)-bis-2-furansulfonic acid, disodium salt (Ferene®) has become available (3,4,5) and is used in this reagent. Ferene® is a superior iron chelating agent forming a tris complex with ferrous iron with a maximum absorption at 593 nm and a molar absorptivity of 35,500. The compound has a 27% higher molar absorption than ferrozine, absorbs at a longer wavelength and has the other advantages of ferrozine; namely, its solubility and stability over the pH range 4-9.

PRINCIPLE



In an acidic medium transferrin bound iron dissociates into ferric ions which are reduced to ferrous ions in the presence of ascorbic acid. The ferrous iron reacts with the chromogen Ferene® to form a blue chromophore which absorbs at 595 nm. The absorbance is directly proportional to the serum iron concentration.

REAGENT PREPARATION, STORAGE AND STABILITY

The reagents are provided in a ready to use format.

The reagents and standard included are stable until the expiry date stated on the labels at 2-8°C. The R2 Color Reagent should be protected from light.

Stability claims are based on real time studies.

REAGENT DETERIORATION

The reagents should be clear. Turbidity would indicate deterioration.

EQUIPMENT AND MATERIALS

Materials provided

The reagents necessary for the determination of iron are provided.

Materials Required

1. Spectrophotometer or analyzer capable of accurately measuring absorbance at 595 nm.
2. 1 cm cuvettes or a flow cell capable of transmitting light at 595 nm.
3. Test tubes of the appropriate size.
4. Pipettes of the appropriate size.
5. Deionized water.
6. An appropriate timer.
7. An appropriate water bath.

SPECIMEN

Freshly drawn, clear, unhemolysed serum from fasting patients is the specimen of choice. Avoid anticoagulants. Serum should be separated from the cells promptly to minimize hemolysis. It is recommended that specimen collection be carried out in accordance with NCCLS document H4-A3 (6). Serum iron is reported to be stable for 4 days at 18-26°C or 7 days at 2-8°C (7).

GLASSWARE PREPARATION

All glassware and equipment used in an iron assay must be free of contaminating iron. Glassware may be prepared by soaking overnight in 1 N HCl or sulfuric acid-dichromate cleaning solution. If stronger concentrations of HCl are used, the time necessary for decontamination may be decreased. The glassware should be rinsed with deionized water before it is used.

LIMITATIONS AND INTERFERENCE

Copper is the only cation of the trace metals normally present in serum capable of forming a colored complex with ferene. Copper interference with ferene is similar to that encountered with ferrozine and studied by Duffy and Gaudin (8). Ninety-five percent of the copper interference is eliminated by chelation of free copper.

Interferences from icterus, lipemia, and hemolysis were evaluated for this iron method on a Hitachi analyzer using a significance criterion of > 10% variance from control.

No significant icteric or lipemic interference was found for this method. Bilirubin levels of 0-684 µmol/L (0-40 mg/dL) were studied using a 13.4 µmol/L (75 µg/dL) iron sample. Lipemic interference was studied using Intralipid samples of 0-1000 mg/dL (0-3000 mg/dL triglyceride) using a 12.4 µmol/L (69 µg/dL) iron sample.

Hemoglobin levels of 0-155 µmol/L (0-1000 mg/dL) were studied with acceptable results to a level of 15.5 µmol/L (100 mg/dL). At a hemoglobin level of 15.5 µmol/L (100 mg/dL), a 6.9% positive interference was displayed in a 13.1 µmol/L (73 µg/dL) iron sample. Unhemolysed samples are recommended.

A summary of the influence of drugs on clinical laboratory tests may be found by consulting Young, D.S. (9).

A sample with an iron level exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.

PROCEDURE

Conditions

Follow the guidelines provided for adaptation to specific automated analyzers or contact DCL Technical Services at (800)565-0265 for instrument specific parameters.

	Generic	Automated Analyzer
Wavelength.....	595 nm	600/700 nm
Temperature.....	37°C	37°C
Pathlength.....	1 cm	
Mode.....	Endpoint	Endpoint
Reaction Time.....	5 minutes	5 minutes
Sample Volume.....	60 µL	15 µL
Reagent Volume.....	R1: 1.0 mL; R2: 0.25 mL	R1: 225 µL; R2: 50 µL
Total Volume.....	1.31 mL	0.290 mL
Sample to Reagent Ratio.....	1:17:4	1:15:3

CALIBRATION

An iron standard is included with Cat. Nos. 157-10 and 157-30 and is used as directed to calibrate the procedure. An iron standard is not included with Cat. Nos. 157-01 or 157-02, however, one should be used as directed to calibrate the procedure.

QUALITY CONTROL

A normal and abnormal level control should be analyzed as required. The results should fall within plus or minus two standard deviations of the established value.

NOT INTENDED FOR USE WITH BOVINE BASED CONTROL SERUM.

CALCULATION

The analyzer calculates the iron concentration of each sample.

REFERENCE INTERVALS (10)

Male: 11.6-30.4 $\mu\text{mol/L}$ (65-170 $\mu\text{g/dL}$)

Female: 8.9-30.4 $\mu\text{mol/L}$ (50-170 $\mu\text{g/dL}$)

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located. Serum iron concentrations show a diurnal variation with peak values seen in the early morning.

PERFORMANCE CHARACTERISTICS

These performance characteristics were generated in DCL laboratories on a Hitachi analyzer.

Results

Iron concentration is reported as $\mu\text{mol/L}$ ($\mu\text{g/dL}$).

Recovery Study

Iron was added to pooled human sera to increase the iron concentration by 8 $\mu\text{mol/L}$ (46 $\mu\text{g/dL}$) and 30 $\mu\text{mol/L}$ (167 $\mu\text{g/dL}$). Recovery of the added iron was 103% and 99% respectively.

Reportable Range (NCCLS EP6-P)

The linearity of the procedure described is 179 $\mu\text{mol/L}$ (1000 $\mu\text{g/dL}$). The lower limit of detection of the procedure described is 2 $\mu\text{mol/L}$ (8 $\mu\text{g/dL}$). This data results in a reportable range of 2-179 $\mu\text{mol/L}$ (8-1000 $\mu\text{g/dL}$).

Precision Studies (NCCLS EP5-T2)

Data was collected on two levels of control sera using a single lot of reagent in 40 runs conducted over 20 days.

Level		Total SD		Total CV %	Within Run SD		Within Run CV %
$\mu\text{mol/L}$	$\mu\text{g/dL}$	$\mu\text{mol/L}$	$\mu\text{g/dL}$		$\mu\text{mol/L}$	$\mu\text{g/dL}$	
15	84	0.25	1.4	1.6	0.13	0.7	0.8
50	277	0.54	3.0	1.1	0.18	1.0	0.3

Accuracy (NCCLS EP9-P)

The performance of this method (y) was compared with the performance of a similar commercially available method (x) on a Hitachi analyzer. Forty-five patient serum samples ranging from 2-52 $\mu\text{mol/L}$ (9-288 $\mu\text{g/dL}$) gave a correlation coefficient of 0.9995. Linear regression analysis gave the following equation:

$$\text{This method} = 1.00 (\text{reference method}) - 1 \mu\text{mol/L} (5 \mu\text{g/dL}).$$

DISPOSAL

Dilute with large volumes of water; dispose into sewer system in accordance with all **Federal, Provincial, State, and local regulations**.

LITERATURE REFERENCES

1. Burtis, Carl A., Ashwood, Edward R. (Ed), Tietz Textbook of Clinical Chemistry, Second Edition, W.B. Saunders Company, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, p. 2062, 1994.
2. Stookey, L.L., Ferrozine - A New Spectrophotometric Reagent for Iron, Anal. Chem. 42, 779 (1970).
3. Artiss, J.D., Vinogradov, S., Zak, B., Spectrophotometric Study of Several Sensitive Reagents for Serum Iron, Clin. Biochem. 14, 311-315 (1981).
4. Higgins, T., Novel Chromogen for Serum Iron Determinations, Clin. Chem. 27, 1619 (1981).
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6. NCCLS Document H4-A3, "Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture", 3rd ed. (1991).
7. Weissman, N., Pileggi, VJ, In Clinical Chemistry Principles and Techniques, 2nd ed., R.J. Henry, D.C. Cannon, J.W. Winkelman, Editors, Harper & Row, Hagerstown (MD), 1974, pp 684, 685, 695.
8. Duffy, J.R., Gaudin, J., Copper Interference in the Determination of Iron in Serum Using Ferrozine, Clin. Biochem. 10, 122-123 (1977).
9. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, AACC Press, Third Edition, Washington, 1990.
10. Burtis, Carl A., Ashwood, Edward R. (Ed), Tietz Textbook of Clinical Chemistry, Second Edition, W.B. Saunders Company, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, p. 2195, 1994.

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