

Iron (Total) Assay

CATALOGUE NUMBER: 102-25
102-15

SIZE: 2 x 500 mL + 1 x 250 mL
2 x 250 mL + 1 x 125 mL

INTENDED USE

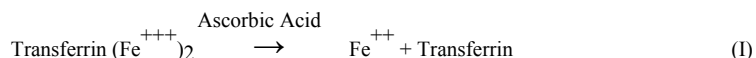
For the IN VITRO quantitative measurement of iron in serum.

TEST SUMMARY

In 1970, Stookey ⁽¹⁾ 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. The major advantages of ferrozine are the high molar absorptivity of the ferrous ferrozine complex (28,000), its water solubility, and stability over the pH range of 4-9. It also can be manufactured economically and isolated in a state of high purity. Gibbs ⁽²⁾ has further characterized ferrozine with respect to its use as a ferrous iron indicator. Carter ⁽³⁾ reported the use of ferrozine for the determination of serum iron. This procedure for iron is a modification of the method of Carter.

TEST PRINCIPLE

In an acidic medium transferrin bound iron dissociates into free ferrous and ferric ions. Ascorbic acid is then used to reduce the ferric iron to the ferrous state. Ferrozine reacts with ferrous iron to form a magenta complex which absorbs at 560 nm. The absorbance is directly proportional to the amount of iron in the serum.



REAGENTS

Acid Dissociating Reagent (R1): a solution of HCl containing at least 26.2 mmol/L thiourea.

Acid Dissociating Diluent (1a): a solution of HCl and a surfactant.

Iron Color Reagent (R2): a solution of HCl containing 26.2 mmol/L thiourea, 10 mmol/L Ferrozine[®], and a surfactant.

Ascorbic Acid (1b): Cat. No. 102-25: 1 vial containing 1.5 g ascorbic acid
Cat. No. 102-15: 1 vial containing 0.75 g ascorbic acid

WARNINGS & PRECAUTIONS FOR USE

S24/25: Avoid contact with skin and eyes.

The reagent provided is for in vitro diagnostic use. Do not pipette the reagent by mouth and avoid contact with eyes. Thiourea is considered to be an animal carcinogen by NTP and IARC.

See Material Safety Data Sheet for additional information.

REAGENT PREPARATION, STORAGE & STABILITY

R1 Working Reagent

Combine the Acid Dissociating Reagent (R1) with the Acid Dissociating Diluent (1a). Dissolve the Ascorbic Acid (1b) in this solution. Allow sufficient time for the Ascorbic Acid to completely dissolve before beginning the serum iron assay. The R1 working reagent is stable for one week at 2-8°C.

The R2 Color Reagent is ready for use. The reagents included are stable until the expiry date stated on the labels at 18-26°C. The Color Reagent (R2) should be protected from light.

Stability claims are based on real time studies.

REAGENT DETERIORATION

The reagent solutions should be clear. Turbidity would indicate deterioration.

DISPOSAL

Reagents must be disposed of in accordance with all Federal, Provincial, State, and local regulations.

SPECIMEN

Fresh, clear, unhemolysed serum. No preservatives are necessary. The tube for iron analysis should be collected before tubes containing anticoagulants to avoid contamination.

If the sample is to be stored for longer than 8 hours, storage at 2-8°C is recommended. The samples should be drawn in the morning following a 12 hour fast. Blood collecting glassware should be free of iron contamination.

GLASSWARE PREPARATION

All glassware and equipment used in an iron or unsaturated iron binding 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.

SAMPLE STORAGE

If the sample is to be stored for longer than 8 hours, storage at 2-8°C is recommended.

ANALYTICAL SPECIFICITY

Cross Contamination studies have not been performed on automated instruments. Certain reagent / instrument combinations used in sequence with this assay may interfere with reagent performance and test results. The existence of, or effects of, any potential cross contamination issues are unknown.

Copper is the only cation of the trace metals normally present in serum and capable of forming a colored complex with ferrozine. Copper interference with ferrozine has been studied by Duffy and Gaudin.⁽⁴⁾ Ninety-five percent of copper interference is eliminated by using thiourea which forms a Cu⁽¹⁾ thiourea complex.

Interferences from icterus, lipemia and hemolysis were evaluated for this iron method on a Roche/Hitachi® analyzer using a significance criterion of >10% variance from control. Interference data was collected in serum.

Concentration of Analyte		Substance Tested	Concentration of Interferent Where Interference is Insignificant	
Conventional Units	SI Units			
98 µg/dL	18 µmol/L	Hemoglobin	500 mg/dL	77.5 µmol/L
93 µg/dL	17 µmol/L	Bilirubin	60 mg/dL	1026 µmol/L
97 µg/dL	17 µmol/L	Intralipid	500 mg/dL	1500 mg/dL (17.0 mmol/L) Simulated Triglycerides

The information presented above is based on results from Genzyme Diagnostics' studies and is current at the date of publication.

A summary of the influence of drugs on clinical laboratory tests may be found by consulting Young, D.S.⁽⁵⁾

ANALYTICAL PROCEDURE

MATERIALS PROVIDED

Genzyme Diagnostics' Iron reagents.

MATERIALS REQUIRED (BUT NOT PROVIDED)

1. Automated analyzer capable of accurately measuring absorbance at appropriate wavelength as per instrument application.
2. Calibration material. Calibrators used with this procedure must be protein based. Aqueous calibrators with no buffering agent should NOT be used.
3. Quality Control materials.

TEST CONDITION

For the data presented in this insert, studies using this reagent were performed on an automated analyzer using an endpoint test mode, with a sample to reagent ratio of 1:12.5:2.5 and a wavelength reading of (primary/secondary) 570/700 nm. For assistance with applications on automated analyzers within Canada and the U.S., please contact Genzyme Diagnostics Technical Services at (800)565-0265. Outside Canada and the U.S., please contact your local distributor.

CALIBRATION

A protein based calibrator should be used to calibrate the procedure. Aqueous calibrators with no buffering agent should not be used with this reagent. The frequency of calibration, if necessary, using an automated system is dependent on the system and the parameters used.

QUALITY CONTROL

A normal and abnormal concentration control should be analyzed as required. The results should fall within the acceptable range as established by the laboratory.

CALCULATIONS

The analyzer automatically calculates the iron concentration of each sample.

TEST LIMITATIONS

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

REFERENCE INTERVALS⁽⁶⁾

Male: 65-170 µg/dL (11.6-30.4 µmol/L)
Female: 50-170 µg/dL (8.9-30.4 µmol/L)

These values are suggested guidelines. It is recommended that each laboratory establish its own expected range.

There is a diurnal variation in iron concentrations. They are normal in the morning, low in mid-afternoon, and very low near midnight.

PERFORMANCE CHARACTERISTICS

Data presented was collected on an automated analyzer unless otherwise stated.

RESULTS

Iron concentration is reported as µg/dL (µmol/L).

REPORTABLE RANGE

The linearity of the procedure described is 1000 µg/dL (179 µmol/L).

PRECISION STUDIES

Total precision data was collected on two concentrations of control sera in 40 runs conducted over 20 days.

Concentration		Total SD		Total CV %	Within Run SD		Within Run CV %
µg/dL	µmol/L	µg/dL	µmol/L		µg/dL	µmol/L	
58.1	10.4	2.0	0.36	3.5	1.3	0.23	2.2
152.4	27.3	3.3	0.59	2.2	1.9	0.34	1.2

ACCURACY










The performance of this method (y) was compared with the performance of a similar method (x) on a Roche/Hitachi® 704. Forty-seven patient serum samples ranging from 12-704 µg/dL (2-126 µmol/L) gave a correlation coefficient was 0.999. Linear regression analysis gave the following equation:

$$\text{This method} = 1.07 (\text{reference method}) - 1.0 \mu\text{g/dL} (0.16 \mu\text{mol/L}).$$

The information presented was based on results from Genzyme Diagnostics' studies and is current at the date of publication.

REFERENCES

1. Stookey, L. L., Ferrozine - A New Spectrophotometric Reagent for Iron, Anal. Chem. 42,779 (1970).
2. Gibbs, C. R., Characterization and Application of Ferrozine Iron Reagent as a Ferrous Iron Indicator, Anal. Chem. 48, 1197-1200 (1976).
3. Carter, P., Spectrophotometric Determination of Serum Iron at the Sub-pMicrogram Level with a New Reagent (Ferrozine), Anal. Biochem. 40, 450 (1971).
4. Duffy, J. R., Gaudin, J., Copper Interference in the Determination of Iron in Serum Using Ferrozine, Clin. Biochem. 10, 122-123 (1977).
5. Young, D. S., Effects of Drugs on Clinical Laboratory Tests, AACCC Press, Washington, Third Edition, 1990.
6. Burtis, C.A., Ashwood, E.R. (Editors), Tietz Textbook of Clinical Chemistry, Second Edition, W.B. Saunders Company, p. 2195.

Definitions for Symbols	
	This product fulfills the requirements of the European Directive for In Vitro Diagnostic Medical Devices.
 Batch code	 Use by YYYY-MM-DD or YYYY-MM
 Manufacturer	 Catalog number
 Consult instructions for use	 Authorized representative in the European Community
 <i>In vitro</i> diagnostic medical device	 Temperature limitation

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