IRON

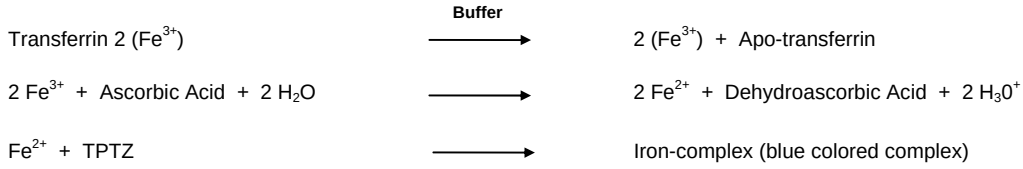
OSR6186	**4 x 15 mL**	**R1**
	4 x 15 mL	**R2**
OSR6286	**4 x 30 mL**	**R1**
	4 x 30 mL	**R2**

Intended Use
System reagent for the quantitative determination of Iron in human serum on Beckman Coulter AU analyzers.

Summary
Iron (non-heme) measurements are used in the diagnosis and treatment of diseases such as iron deficiency anemia, hemochromatosis (a disease associated with widespread deposit in the tissues of two iron-containing pigments, hemosiderin and hemofuscin, and characterized by pigmentation of the skin), and chronic renal disease. Transferrin is the major iron carrying protein in the serum.

Methodology
In 1954, Schade et al.[1] introduced a method for the direct determination of serum iron. The iron level was determined by incubating the serum in a phosphate buffer with ascorbic acid and terpyridine. Goodwin,[2] in 1966, proposed a direct method for serum iron using an acetate buffer and bathophenanthroline. These modifications eliminated random iron contamination from phosphate buffers and enhanced color development by using a more sensitive iron chromogen.

This Beckman Coulter method utilizes a variation of these methods using TPTZ [2,4,6-Tri-(2-pyridyl)-5-triazine] as the chromogen.[3] In an acidic medium, transferrin-bound iron dissociates into free ferric ions and apo-transferrin. Hydrochloric acid and sodium ascorbate reduce the ferric ions to the ferrous state. The ferrous ions then react with TPTZ to form a blue colored complex which can be measured bichromatically at 600/800 nm. The increase in absorbance is directly proportional to the amount of transferrin bound iron present.



System Information
For AU400/400^e/480, AU600/640/640^e/680 and AU2700/5400 Beckman Coulter Analyzers.

Reagents
Final concentration of reactive ingredients:

Glycine buffer (pH 1.7)	215 mmol/L
L-Ascorbic Acid	4.7 mmol/L
2,4,6-Tri-(2-pyridyl)-5-triazine	0.5 mmol/L

Also contains preservatives

Precautions
1. For in vitro diagnostic use.
2. WARNING! CORROSIVE! Do not pipet by mouth. Avoid contact with eyes, skin or clothing. In case of contact, immediately flush affected area with plenty of water for 15 minutes. Obtain medical attention immediately for eye contact or ingestion.

Preparation of Reagents
The Iron Reagents are ready for use. No preparation is required.

Storage and Stability
The reagents are stable, if unopened, up to the stated expiration date when stored at 2 - 8°C. Opened reagents are stable for 60 days when stored in the refrigerated compartment of the analyzer.
The color of R1 turns to brown during the course of the shelf life. This does not restrict any function of this reagent as long as reagent OD results on the analyzer are within specified limits.

Indications of Deterioration
Visible signs of microbial growth, turbidity or precipitation or any change in the color of the reagent may indicate degradation and warrant discontinuance of use.

Specimen Collection and Preparation
Serum or heparinized plasma samples, free from hemolysis, are the recommended specimens. Remove serum from the red cells to minimize hemolysis as hemolyzed samples may produce erroneous results. Plasma specimens collected with EDTA, oxalate, or citrate are unsatisfactory, since they bind iron, preventing its reaction with the chromogen. Samples should be taken in the morning from patients in a fasting state, since iron values decrease by 30% during the course of the day[4] and there can be significant interference from lipemia.

Sample Storage and Stability
Serum iron is stable for 7 days when stored at 2 - 8°C or 4 days at room temperature (15 - 25°C) after the serum is separated from red cells.[5]

Iron

Interfering Substances
Results of studies[6] show that the following substances interfere with this iron procedure when tested at 150 µg/dL Iron.

The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin: No significant interference up to 40 mg/dL Bilirubin
Copper: No significant interference up to 1 mg/dL Copper
Globulin: No significant interference up to 5 g/dL Human Gamma Globulin
Lipemia: No significant interference up to 400 mg/dL Intralipid*
Hemolysis**

* Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.
** Hemolyzed samples should not be tested. Hemolyzed samples may react with the reagent producing results with a negative bias.

Note: In rare instances, extremely high concentrations of monoclonal immunoglobulins, due to monoclonal gammopathies, may cause turbidity in the reaction cuvette and elevate direct colorimetric iron assays.[10]
The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young for a compilation of reported interferences with this test.[7]

Procedure

A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided
Iron Reagent

Materials Required But Not Provided
Chemistry Calibrator (Cat # DR0070)

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration
The frequency of calibration is 30 days. Calibration of this iron procedure is accomplished by use of the Chemistry Calibrator (Cat # DR0070), which is traceable to the National Institutes of Standards and Technology (NIST) Standard Reference Material (SRM) 1598 and 937.

Recalibration of this test is required when any of these conditions exist:
1. A reagent lot number has changed or there is an observed shift in control values.
2. A fresh bottle of reagent is used for testing.
3. Major preventative maintenance was performed on the analyzer or a critical part was replaced.

Quality Control
During operation of the Beckman Coulter AU analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration, with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results
Automatically printed out for each sample in µg/dL at 37°C. For SI units (µmol/L) multiply result by 0.179.

Dynamic Range

The Iron procedure is linear from 10 to 1000 µg/dL. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically by utilizing the AUTO REPEAT RUN.

Expected Values

Adult:[8] 50 - 212 µg/dL

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the Iron Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision[11]
Estimates of precision, based on CLSI recommendations[9], are consistent with typical performance. The within run precision is less than 3% CV and total precision is less than 5% CV.
Assays of control sera were performed and the data reduced following CLSI guidelines above:

N = 60	Within run		Total	
Mean, µg/dL	SD	CV%	SD	CV%
53.6	0.54	1.02	1.12	2.09
158	1.05	0.66	2.8	1.77
589	3.81	0.65	7.23	1.23

Method Comparison[11]

Patient samples were used to compare this Iron Reagent. The table below demonstrates representative performance on AU analyzers.

Y Method	AU640
X Method	OSR6123/6223
Slope	1.040
Intercept	2.23
Correlation Coeff. (r)	0.995
No. of Samples (n)	96
Range (µg/dL)	11.60-253

Sensitivity

Typical change in absorbance for 1 µg/dL of Iron is 0.2 mAbsorbance.

References

1. Schade, A., Ogama, J., Reinhart, R., and Miller, J., Proc. Soc. Exp. Biol. Med., 87: 442, 1954.
2. Goodwin, J., Murphy, B., and Guillemette, M., Clin. Chem., 12: 47, 1966.
3. Diehl, H., and Smith, G.F., "The Iron Reagents, Bathophenanthroline, 2,4,6-Tripyridyl-s-triazine, Phenyl-2-pyridylocine", GF Smith Chem Co, 1960.
4. Tietz, N.W. Textbook of Clinical Chemistry, WB. Saunders, 1580, 1986.
5. Henry, R.J. et al. Clinical Chemistry: Principles and Technics, Harper and Row, 1974.
6. CLSI/NCCLS, Interference Testing in Clinical Chemistry EP7-P, 1986.
7. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 5th Edition, AACC Press, 2000.
8. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
9. CLSI/NCCLS Evaluation Protocol, EP5-T2, 1992.
10. Bakker, A.J., Clin. Chem. 37:690,1991.
11. Data is on file for specific AU analyzers.

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