BECKMAN COULTER CE

AU

Instructions For Use

Iron
IRON

REF OSR6186 4 x 15 mL R1, 4 x 15 mL R2
OSR6286 4 x 30 mL R1, 4 x 30 mL R2

For *in vitro* diagnostic use only.

PRINCIPLE

INTENDED USE

Photometric colour test for the quantitative determination of iron in human serum and plasma on AU Beckman Coulter analysers.

SUMMARY AND EXPLANATION

Reference[1,2,3]

Iron participates in a variety of vital processes in the body varying from cellular oxidative mechanisms to the transport and delivery of oxygen to body cells. It is a constituent of the oxygen-carrying chromoproteins, haemoglobin and myoglobin, as well as various enzymes, such as cytochrome oxidase and peroxidases. The remaining body iron is present in the flavoproteins, the iron-sulphur proteins, as well as storage iron-ferritin and transport iron-transferrin. Measured serum iron concentration is principally the Fe (III) bound to serum transferrin and does not include the iron contained in serum as free haemoglobin.

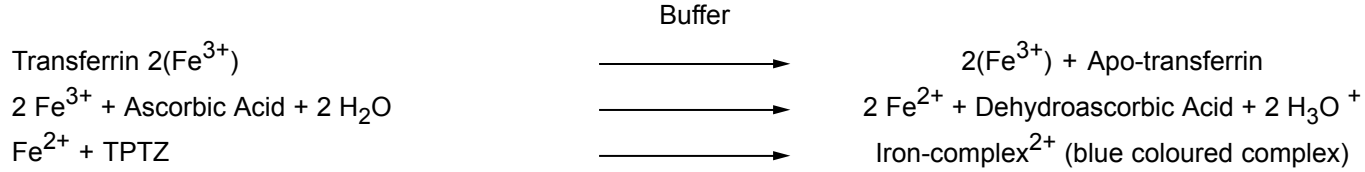
Serum iron concentration is decreased in many but not all patients with iron deficiency anemia; in acute or chronic inflammatory disorders such as acute infection, immunisation, and myocardial infarction; acute or recent haemorrhage; malignancy; kwashiorkor; late pregnancy; menstruation and nephrosis. Serum iron concentration diminishes markedly in patients who are beginning to respond to specific therapy for anemias of other causes e.g. treatment of pernicious anemia with Vit B_{12}. Greater than normal concentrations of serum iron occur in iron-overload disorders such as haemochromatosis and in acute iron poisoning following oral or parenteral iron administration. Iron levels may also be increased in acute hepatitis, lead poisoning, acute leukemia, thalassemia or oral contraception.

METHODOLOGY

Reference[4,5,6]

The method utilises TPTZ [2,4,6-Tri-(2-pyridyl)-5-triazine] as the chromogen. 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 coloured complex which can be measured bichromatically at 600/800 nm. The increase in absorbance is directly proportional to the amount of iron present.

CHEMICAL REACTION SCHEME



SPECIMEN

TYPE OF SPECIMEN

Serum and heparinised plasma. Do not use EDTA, oxalate or citrate plasma.

Stable in serum and plasma for 3 weeks when stored at 2…8°C and 7 days when stored at 15…25°C.[7]

Lipemic samples should be avoided. Remove serum from the red cells to minimize hemolysis as hemolyzed samples may produce erroneous results.

Samples should be taken in the morning from patients in a fasting state, since iron values can decrease by 30% during the course of the day.[8]

REAGENTS

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.

Dispose of all waste material in accordance with local guidelines.

REACTIVE INGREDIENTS

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
Preservative	

The concentrations of the reactive components of the reagents shown on the kit label are the actual concentrations in the individual R1/R2 vials. The reagent composition which is shown in the Instructions For Use is the final concentration of these components in the reaction cuvette after addition of R1, Sample, and R2.

GHS HAZARD CLASSIFICATION

Iron R1	DANGER	
		
	H314	Causes severe skin burns and eye damage.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
	P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
		Hydrochloric Acid < 0.1%
		Poly(oxy-1,2-ethanediyl), .alpha.-[3,5-dimethyl-1-(2-methylpropyl)hexyl]-.omega.-hydroxy- 1 - 2%
Iron R2	DANGER	
		
	H314	Causes severe skin burns and eye damage.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
	P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
		Hydrochloric Acid < 0.1%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

REAGENT PREPARATION

The reagents are ready for use and can be placed directly on board the instrument.

STORAGE AND STABILITY

The reagents are stable, unopened, up to the stated expiry date when stored at 2…8°C. Once open, reagents stored on board the instrument are stable for 60 days.

Some discoloration may be observed in R1 as the reagent ages. This does not affect the performance of the reagent.

CALIBRATION

CALIBRATOR REQUIRED

System Calibrator Cat. No. 66300.

The calibrator iron value provided in the calibrator package insert is traceable to a Beckman Coulter Master Calibrator.

Recalibrate the assay when the following occur:

Change in reagent lot or significant shift in control values;

Major preventative maintenance was performed on the analyser or a critical part was replaced.

QUALITY CONTROL

Control Cat. No. ODC0003 and ODC0004 or other control materials with values determined by this Beckman Coulter system may be used.

Each laboratory should establish its own control frequency however good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration is performed.

The results obtained by any individual laboratory may vary from the given mean value. It is therefore recommended that each laboratory generates analyte specific control target values and intervals based on multiple runs according to their requirements. These target values should fall within the corresponding acceptable ranges given in the relevant product literature.

If any trends or sudden shifts in values are detected, review all operating parameters.

Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

TESTING PROCEDURE(S)

Refer to the appropriate Beckman Coulter AU analyser User Guide/Instructions For Use (IFU) for analyser-specific assay instructions for the sample type as listed in the Intended Use statement. The paediatric application is suitable for use with small volume serum/plasma samples.

CALCULATIONS

The Beckman Coulter analysers automatically compute the iron concentration of each sample.

REPORTING RESULTS

REFERENCE INTERVALS

Reference[9,10]		
Serum (Adults)	Male	12.5 – 32.2 µmol/L (70 – 180 µg/dL)
	Female	10.7 – 32.2 µmol/L (60 – 180 µg/dL)
Serum (Children)	Newborn	17.90 – 44.8 µmol/L (100 – 250 µg/dL)
	Infant	7.2 – 17.9 µmol/L (40 – 100 µg/dL)
	Child	9.0 – 21.5 µmol/L (50 – 120 µg/dL)

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval

according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

PROCEDURAL NOTES

LIMITATIONS

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.[11]

INTERFERENCES

Results of studies conducted to evaluate the susceptibility of the method to interference were as follows:

Icterus:	Interference less than 3% up to 40 mg/dL or 684 µmol/L bilirubin
Haemolysis:	Interference less than 10% up to 1 g/L haemoglobin
Lipemia:	Interference less than 10% up to 100 mg/dL Intralipid
Copper:	Interference less than 10% up to 1 mg/dL or 0.157 mmol/L copper
Globulin:	Interference less than 10% up to 5 g/dL or 50 g/L
Triglyceride:	Interference less than 10% up to 300 mg/dL or 3.4 mmol/L triglyceride

In very rare cases gammopathy, especially monoclonal IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Refer to Young[12] for further information on interfering substances.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

LINEARITY

The test is linear within a concentration range of 2 – 179 µmol/L (10 – 1,000 µg/dL).

SENSITIVITY

The lowest detectable level in serum on an AU5800 analyser was estimated at 0.4 µmol/L.

The lowest detectable level represents the lowest measurable level of iron that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

METHODS COMPARISON

Patient serum samples were used to compare this Iron OSR6186 assay on the AU600 against another commercially available iron assay. Results of linear regression analysis were as follows:

y = 1.002x – 0.304	r = 0.999	n = 96	Sample range = 1.9 – 41.6 µmol/L

PRECISION

The following data was obtained on an AU5800 using 3 serum pools analysed over 20 days.

n = 80	Within-run		Total	
Mean µmol/L	**SD**	**CV%**	**SD**	**CV%**
10.76	0.12	1.1	0.22	2.0
27.44	0.21	0.8	0.30	1.1
89.59	0.44	0.5	0.62	0.7

ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	Description
FE-1N	Iron (Serum)
FE-1NP	Iron (Serum Paediatric)

Setting Sheet Footnotes
User defined
† System Calibrator Cat. No.: 66300
* Values set for working in SI units (µmol/L). To work in µg/dL multiply by 5.585.

REVISION HISTORY

Added new languages

Preceding version revision history

IFU updated to add Vietnamese language.

Updated Additional Information section

REFERENCES

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11. Bakker AJ. Influence of monoclonal immunoglobulins in direct determinations of iron in serum. Clin Chem 1991;37:690-4.
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