

Iron Gen.2

Order information

COBAS INTEGRA Iron Gen.2	200 Tests	Cat. No. 03183696 122 System-ID 07 6596 1	● Indicates analyzer(s) on which cobas c pack can be used
Calibrator f.a.s.	12 × 3 mL	Cat. No. 10759350 190	
Calibrator f.a.s. (for USA)	12 × 3 mL	Cat. No. 10759350 360 System-ID 07 3718 6	
Precinorm U	20 × 5 mL	Cat. No. 10171743 122 System-ID 07 7997 0	
Precipath U	20 × 5 mL	Cat. No. 10171778 122 System-ID 07 7998 9	
Precinorm U plus	10 × 3 mL	Cat. No. 12149435 122	
Precinorm U plus (for USA)	10 × 3 mL	Cat. No. 12149435 160 System-ID 07 7999 7	
Precipath U plus	10 × 3 mL	Cat. No. 12149443 122	
Precipath U plus (for USA)	10 × 3 mL	Cat. No. 12149443 160 System-ID 07 8000 6	

COBAS INTEGRA 400/400 plus	COBAS INTEGRA 800
●	●

System information

COBAS INTEGRA Iron Gen.2 (IRON2)
Test IRON2, test ID 0-596

Intended use

In vitro test for the quantitative determination of iron in human serum and plasma on COBAS INTEGRA systems.

Summary^{1,2,3,4,5}

Ingested iron is mainly absorbed in the form of Fe²⁺ in the duodenum and upper jejunum. The trivalent form and the heme-bound Fe³⁺-component of iron in food has to be reduced by vitamin C. About 1 mg of iron is assimilated daily. Upon reaching the mucosal cells, Fe²⁺ ions become bound to transport substances. Before passing into the plasma, these are oxidized by ceruloplasmin to Fe³⁺ and bound to transferrin in this form. The transport of Fe ions in blood plasma takes place via transferrin-iron complexes. A maximum of 2 Fe³⁺ ions per protein molecule can be transported. Serum iron is almost completely bound to transferrin.

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 tissue of the two iron-containing pigments, hemosiderin and hemofuscin, and characterized by pigmentation of the skin), and chronic renal disease.

Iron determinations are performed for the diagnosis and monitoring of microcytic anemia (e.g. due to iron metabolism disorders and hemoglobinopathy), macrocytic anemia (e.g. due to vitamin B12 deficiency, folic acid deficiency and drug-induced metabolic disorders of unknown origin) as well as normocytic anemias such as renal anemia (erythropoietin deficiency), hemolytic anemia, hemoglobinopathy, bone marrow disease and toxic bone marrow damage.

Numerous photometric methods have been described for the determination of iron. All have the following in common:

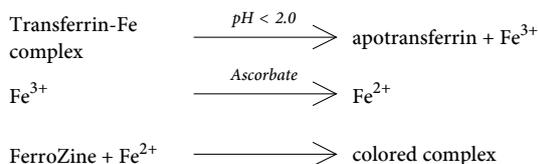
- Liberation of Fe³⁺ ions from the transferrin complex using acids or detergents.
- Reduction of Fe³⁺ ions to Fe²⁺ ions.
- Reaction of the Fe²⁺ ions to give a colored complex.

The method described here is based on the FerroZine method without deproteinization.

Test principle

FerroZine method.

Under acidic conditions, iron is liberated from transferrin. Lipemic samples are clarified by the detergent. Ascorbate reduces the released Fe³⁺ ions to Fe²⁺ ions which then react with FerroZine to form a colored complex.



The color intensity is directly proportional to the iron concentration. It is determined by monitoring the increase in absorbance at 552 nm.

Reagents - working solutions

Components	Concentrations			Test	
	R1	R2			
Citric acid	200		125		mmol/L
Thiourea	115		71.9		mmol/L
Sodium ascorbate		150	18.8		mmol/L
FerroZine		6	0.75		mmol/L

Reagent R1 contains a nonreactive surfactant, R2 contains preservative.

Precautions and warnings

Pay attention to all precautions and warnings listed in this Method Manual, Chapter 1, Introduction.

WARNING: This reagent contains thiourea, a substance known to the State of California to cause cancer or reproductive harm. It may also cause skin reactions. In the event of contact, flush affected areas with copious amounts of running water. Get immediate medical attention for contact with the eyes or if ingested.

INTEGRA 400/800

Contact phone: all countries: +49-621-7590,
USA: +1-800-428-2336

Reagent handling

Ready for use.

Storage and stability

Shelf life at 2 to 8 °C See expiration date on
cobas c pack label

COBAS INTEGRA 400/400 plus systems

On-board in use at 10 to 15 °C 6 weeks

COBAS INTEGRA 800 systems

On-board in use at 8 °C 6 weeks

When removing the cobas c pack during use from the instrument, please immediately store at 2 to 8 °C.

Do not shake the cobas c pack to avoid foaming.

Specimen collection and preparation

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable. Serum (free from hemolysis).

Plasma (free from hemolysis): Li-heparin plasma.

Do not use EDTA or oxalate plasma.

Separate serum or plasma from the clot or cells within 1 hour.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Stability:⁶ 7 days at 15-25 °C

3 weeks at 2-8 °C

several years at (-15)-(-25) °C

Centrifuge samples containing precipitates before performing the assay.

Materials provided

See "Reagents - working solutions" section for reagents.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Application for serum and plasma**COBAS INTEGRA 400/400 plus test definition**

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S- -SR
Reaction direction	Increase
Wavelength A/B	552/659 nm
Calc. first/last	49/55
Unit	µmol/L

Pipetting parameters

		Diluent (H ₂ O)
R1	100 µL	
Sample	8.5 µL	11.5 µL
SR	20 µL	20 µL
Total volume	160 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-R2 (late)
Reaction direction	Increase
Wavelength A/B	552/659 nm
Calc. first/last	103/113
Unit	µmol/L

Pipetting parameters

		Diluent (H ₂ O)
R1	100 µL	
Sample	8.5 µL	11.5 µL
SR	20 µL	20 µL
Total volume	160 µL	

Calibration

Calibrator	Calibrator f.a.s. Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each cobas c pack and every 7 days and as required following quality control procedures.

Traceability⁷: This method has been standardized against an internal method traceable to a primary reference material (SRM937).

Quality control

Reference range	Precinorm U or Precinorm U plus
Pathological range	Precipath U or Precipath U plus
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the "Order information" section. Other suitable control material can be used in addition.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits.

Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400/400 plus/800 analyzers).

Conversion factor: µmol/L x 5.59 = µg/dL

Limitations - interference⁸

Criterion: Recovery within ± 10 % of initial value	
Icterus	No significant interference.
Hemolysis	No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L or 200 mg/dL).
Lipemia (Intralipid)	No significant interference.
Albumin	No significant interference.
γ-Globulin	No significant interference.
Anticoagulants	Complexing anticoagulants such as EDTA, oxalate, and citrate must not be used.

Drugs No interference was found at therapeutic concentrations using common drug panels.^{9,10}

In patients treated with iron supplements or metal-binding drugs, the drug-bound iron may not properly react in the test, resulting in falsely low results.

Other In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special wash programming: The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the Method Manual, Introduction, Extra Wash Cycles for further instructions. Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

0.9-179 µmol/L (5-1000 µg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Lower detection limit of the test:

0.9 µmol/L (5.00 µg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values¹¹

Females 6.6-26.0 µmol/L (37-145 µg/dL)
Males 10.6-28.3 µmol/L (59-158 µg/dL)

Serum/plasma iron levels are dependent on diet and subject to circadian variation.¹²

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol. Repeatability^a (n = 21), intermediate precision^b (1 aliquot per run, 1 run per day, 21 days). The following results were obtained:

Sample	Mean µmol/L (µg/dL)	Repeatability ^a		CV %
		SD µmol/L (µg/dL)		
Precinorm U	19.6 (110)	0.17 (0.950)		0.9
Precipath U	30.4 (170)	0.16 (0.894)		0.5
Serum low	18.2 (102)	0.19 (1.06)		1.0

a) repeatability = within-run precision

Intermediate precision^b

Sample	Mean	SD	CV
	µmol/L (µg/dL)	µmol/L (µg/dL)	%
Precinorm U	19.9 (111)	0.25 (1.40)	1.3
Precipath U	30.7 (172)	0.40 (2.24)	1.3
Serum low	11.2 (62.6)	0.25 (1.40)	2.3

b) intermediate precision = total precision / between-run precision / between-day precision

Method comparison

Iron values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Iron Gen.2 reagent (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x) and with the previous IRON reagent on a COBAS INTEGRA 700 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 60
Passing/Bablok ¹³	Linear regression
$y = 1.019x - 0.09 \mu\text{mol/L}$	$y = 1.026x - 0.26 \mu\text{mol/L}$
$\tau = 0.988$	$r = 1.00$
SD (md 95) = 0.71	$Sy.x = 0.32$
The sample concentrations were between 2.0 and 173 µmol/L (11 and 967 µg/dL).	

COBAS INTEGRA 700 analyzer	Sample size (n) = 59
Passing/Bablok ¹³	Linear regression
$y = 0.997x + 0.13 \mu\text{mol/L}$	$y = 1.041x - 0.56 \mu\text{mol/L}$
$\tau = 0.980$	$r = 0.999$
SD (md 95) = 3.86	$Sy.x = 1.47$
The sample concentrations were between 1.8 and 144 µmol/L (10 and 805 µg/dL).	

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