IRON2

cobas®

Iron Gen.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03183696 122	Iron Gen.2, 200 tests	System-ID 07 6596 1	**cobas c** 311, **cobas c** 501/502
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
10171743 122	Precinorm U (20 x 5 mL)	Code 300	
10171778 122	Precipath U (20 x 5 mL)	Code 301	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	

English

System information

For **cobas c** 311/501 analyzers:

IRON2: ACN 661

For **cobas c** 502 analyzer:

IRON2: ACN 8661

Intended use

In vitro test for the quantitative determination of iron in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5]

Ingested iron is mainly absorbed in the form of Fe^{2+} in the duodenum and upper jejunum. The trivalent form and the heme-bound Fe^{3+} 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, Fe^{2+} ions become bound to transport substances. Before passing into the plasma, these are oxidized by ceruloplasmin to Fe^{3+} 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 Fe^{3+} 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 B_{12} deficiency, folic acid deficiency and drug-induced metabolic disorders of unknown origin) as well as normocytic anemias such as renal anemia (erythropoetin 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 Fe^{3+} ions from the transferrin complex using acids or detergents.
- Reduction of Fe^{3+} ions to Fe^{2+} ions.
- Reaction of the Fe^{2+} ions to give a colored complex.

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

Test principle

Colorimetric assay.

$$\text{Transferrin-Fe-complex} \xrightarrow{\text{pH} < 2.0} \text{apotransferrin} + Fe^{3+}$$

$$Fe^{3+} \xrightarrow{\text{ascorbate}} Fe^{2+}$$

$$Fe^{2+} + \text{FerroZine} \longrightarrow \text{colored complex}$$

Under acidic conditions, iron is liberated from transferrin. Lipemic samples are clarified by the detergent. Ascorbate reduces the released Fe^{3+} ions to Fe^{2+} ions which then react with FerroZine to form a colored complex. The color intensity is directly proportional to the iron concentration and can be measured photometrically.

Reagents - working solutions

R1	Citric acid: 200 mmol/L; thiourea: 115 mmol/L; detergent
R3	Sodium ascorbate: 150 mmol/L; FerroZine: 6 mmol/L; preservative

R1 is in position A and R3 is in position B.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Hazardous components: Isotridecyl(PEG-Ether)n,n=8
Citric acid monohydrate
Thiourea

Danger

H314	Causes severe skin burns and eye damage.

Prevention:

P264	Wash skin thoroughly after handling.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.

Response:

P301 + P330 + P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.

P303 + P361 + P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P304 + P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
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
P363	Wash contaminated clothing before reuse.

Storage:

P405	Store locked up.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Contact phone: all countries: +49-621-7590

Reagent handling
Ready for use

Storage and stability

IRON2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	6 weeks

When removing the **cobas c** pack from the instrument during use, please immediately store at 2-8 °C.
Do not shake the **cobas c** pack to avoid foaming.
Store protected from light.

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.
Centrifuge samples containing precipitates before performing the assay.

Stability:[6,7]	7 days at 15-25 °C
	3 weeks at 2-8 °C
	several years at (-15)-(-25) °C

Materials provided
See "Reagents – working solutions" section for reagents.

Materials required (but not provided)
See "Order information" section
General laboratory equipment

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.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 311 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 23-27	
Wavelength (sub/main)	700/570 nm	
Reaction direction	Increase	
Units	µmol/L (µg/dL, mg/L)	
Reagent pipetting		Diluent (H_2O)
R1	100 µL	–
R3	20 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	8.5 µL	–	–
Decreased	4.0 µL	–	–
Increased	8.5 µL	–	–

cobas c 501 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 34-42	
Wavelength (sub/main)	700/570 nm	
Reaction direction	Increase	
Units	µmol/L (µg/dL, mg/L)	
Reagent pipetting		Diluent (H_2O)
R1	100 µL	–
R3	20 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	8.5 µL	–	–
Decreased	4.0 µL	–	–
Increased	8.5 µL	–	–

cobas c 502 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 34-42	
Wavelength (sub/main)	700/570 nm	
Reaction direction	Increase	
Units	µmol/L (µg/dL, mg/L)	
Reagent pipetting		Diluent (H_2O)
R1	100 µL	–
R3	20 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)

Normal	8.5 µL	–	–
Decreased	4.0 µL	–	–
Increased	17.0 µL	–	–

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after **cobas c** pack change • after 7 days on board • as required following quality control procedures

Traceability: This method has been standardized against a primary reference material (SRM 937).

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

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 defined limits.

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

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	µmol/L x 5.59 = µg/dL µmol/L x 0.0559 = mg/L µg/dL x 0.179 = µmol/L µg/dL x 0.010 = mg/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at an iron concentration of 26.9 µmol/L (150 µg/dL).

Icterus:[8] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[8] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 125 µmol/L or 200 mg/dL). Higher hemoglobin concentrations lead to artificially increased values due to contamination of the sample with hemoglobin-bound iron.

Lipemia (Intralipid):[8] No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

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 artificially low values.

In the presence of high ferritin concentrations > 1200 µg/L the assumption that serum iron is almost completely bound to transferrin is not valid anymore. Therefore, such iron results should not be used to calculate Total Iron Binding Capacity (TIBC) or percent transferrin saturation (% SAT).[11]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[12]

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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1+2/SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

0.90-179 µmol/L (5.00-1000 µg/dL, 0.05-10.0 mg/L)

Determine samples having higher concentrations via the rerun function. For samples with higher concentrations, the rerun function decreases the sample volume by a factor of 2.1. The results are automatically multiplied by this factor.

Lower limits of measurement

Lower detection limit of the test

0.90 µmol/L (5.00 µg/dL, 0.05 mg/L)

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

Expected values[13]

Adults:	5.83-34.5 µmol/L (33-193 µg/dL)

The concentration of iron in serum/plasma is dependent on ingestion of iron and is subject to circadian variations.[14]

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 the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	µmol/L (µg/dL)	*µmol/L (µg/dL)*	*%*
Precinorm U	19.8 (111)	0.1 (0.6)	0.6
Precipath U	32.8 (183)	0.2 (1.1)	0.6
Human serum 1	11.3 (63.2)	0.2 (0.6)	1.3
Human serum 2	54.5 (305)	0.5 (3)	0.8

Intermediate precision	*Mean*	*SD*	*CV*
	µmol/L (µg/dL)	*µmol/L (µg/dL)*	*%*
Precinorm U	20.1 (112)	0.3 (2)	1.5
Precipath U	33.5 (187)	0.5 (3)	1.5
Human serum 1	11.8 (66.0)	0.2 (1.1)	1.8
Human serum 2	55.1 (308)	0.7 (4)	1.3

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Method comparison

Iron values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).
Sample size (n) = 85

Passing/Bablok[15]	Linear regression
y = 1.002x + 0.290 µmol/L	y = 1.000x + 0.367 µmol/L
τ = 0.986	r = 1.000

The sample concentrations were between 3.50 and 162 µmol/L (19.6 and 906 µg/dL).

References

1 Wick M, Pinggera W, Lehmann P, ed. Eisenstoffwechsel, Diagnostik und Therapie der Anämien. 3rd ed. Wien/New York: Springer Verlag 1996.

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT → Contents of kit

Volume after reconstitution or mixing

CE

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Roche