IRON2

cobas®

Iron Gen.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057931190	Iron Gen.2 (700 tests)	System-ID 2077 001	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

IRON2: ACN 20770

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.

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

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

Fe^{2+} + FerroZine $\longrightarrow$ 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 B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

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:

EUH 208 Contains 1-[1,3-bis(hydroxymethyl)-2,5-dioxoimidazolidin-4-yl]-1,3-bis(hydroxymethyl)urea. May produce an allergic reaction.

Danger

H314 Causes severe skin burns and eye damage.

Prevention:

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.

Response:

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

P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.

P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.

P305 + P351 + P338 + P310 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.

Disposal:

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

Product safety labeling follows EU GHS guidance.

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

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 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.

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.

Plasma: 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.

See the limitations and interferences section for details about possible sample interferences.

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

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/570 nm		
Reagent pipetting		Diluent (H_2O)	
R1	75 µL	–	
R3	15 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (NaCl)
Normal	6.4 µL	–	–
Decreased	9.0 µL	25 µL	50 µL
Increased	6.4 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change
	1-point recalibration using S1 - after **cobas c** pack green change - after 7 days on-board

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

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. It is recommended to perform quality control always after lot calibration and subsequently at least every 12 weeks.

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

cobas c systems automatically calculate the analyte concentration of each sample in the unit µmol/L (µg/dL, mg/L).

Conversion factors:	µmol/L x 5.59 = µg/dL
	µmol/L x 0.0559 = 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 **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet for information. For further instructions refer to the operator's manual.

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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.9 µmol/L (5.03 µg/dL)
Limit of Detection	= 0.9 µmol/L (5.03 µg/dL)
Limit of Quantitation	= 0.9 µmol/L (5.03 µg/dL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 20 %. It has been determined using low concentration iron samples.

Expected values[13]

µmol/L

Adults:	5.83-34.5 µmol/L

µg/dL

Adults:	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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean µmol/L*	*SD µmol/L*	*CV %*
PCCC1[a)]	18.6	0.111	0.6
PCCC2[b)]	41.4	0.163	0.4
Human serum 1	2.37	0.0817	3.4
Human serum 2	6.01	0.0830	1.4
Human serum 3	35.1	0.135	0.4
Human serum 4	89.2	0.307	0.3
Human serum 5	158	0.655	0.4

Intermediate precision	*Mean µmol/L*	*SD µmol/L*	*CV %*
PCCC1[a)]	18.6	0.212	1.1
PCCC2[b)]	41.6	0.369	0.9
Human serum 1	2.32	0.120	5.2
Human serum 2	5.95	0.149	2.5
Human serum 3	35.1	0.187	0.5
Human serum 4	89.2	0.337	0.4
Human serum 5	158	0.673	0.4

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s).

Method comparison

Iron values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 74

Passing/Bablok[15]	Linear regression
y = 1.004x - 0.0354 µmol/L	y = 1.003x - 0.00110 µmol/L
τ = 0.985	r = 1.000

The sample concentrations were between 1.20 and 169 µmol/L.

Iron values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 98

Passing/Bablok[15]	Linear regression
y = 1.011x - 0.0750 µmol/L	y = 1.011x - 0.0772 µmol/L
τ = 0.993	r = 1.000

The sample concentrations were between 1.72 and 172 µmol/L.

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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13 Löhr B, El-Samalouti V, Junge W, et al. Reference Range Study for Various Parameters on Roche Clinical Chemistry Analyzers. Clin Lab 2009;55:465-471.

14 Burtis CA, Ashwood ER, Bruns DE (eds.). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th ed. St Louis, Missouri; Elsevier Saunders 2006;1190.

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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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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