Iron

Order information

COBAS INTEGRA Iron	150 Tests	Cat. No. **20737585** 322 System-ID 07 3758 5
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 System-ID 07 7999 7
Precipath U plus	10 × 3 mL	Cat. No. **12149443** 122 System-ID 07 8000 6

● Indicates analyzer(s) on which cassette can be used

COBAS INTEGRA 400/400 plus	COBAS INTEGRA 700	COBAS INTEGRA 800
●	●	●

Intended use

The COBAS INTEGRA Iron cassette (IRON) contains an in vitro diagnostic reagent system intended for use on COBAS INTEGRA systems for the quantitative determination of the iron concentration in serum and plasma (test IRON, 0-058).

Summary[1,2]

The prosthetic group of hemoglobin is the iron complex of protoporphyrin IX (heme) in which the centrally located iron atom acts as a stabilizer of oxyhemoglobin. Numerous enzymes and coenzymes require iron, e.g. peroxidases, catalases, and cytochromes which are also heme proteins, many of the enzymes of the Krebs cycle, and monoamine oxidase, which is involved in neurotransmission.

The total iron content of the body is about 3 to 3.5 g. Of this amount about 2.5 g is contained within erythrocytes or their precursors in the bone marrow. Plasma contains only about 2.5 mg of iron. Iron is transported as Fe(III) bound to the plasma protein apotransferrin. The apotransferrin-Fe(III) complex is called transferrin. Iron is stored mainly in hepatocytes bound to ferritin and hemosiderin. The total body requirements vary from 1 to 2 mg per day depending on age and sex.

Serum iron concentration may be decreased in patients with iron deficiency anemia and in acute or chronic inflammatory disorders, such as acute infection, immunization, and myocardial infarction. Acute or recent hemorrhage, including that due to blood donation, results in low serum iron concentration. Serum iron concentration also drops at the time of menstruation.

Increased concentrations of serum iron occur in iron-overload disorders such as hemochromatosis, in acute hepatitis, in acute iron poisoning in children, and following oral ingestion of iron medication or parenteral iron administration.

Test principle

Guanidine/FerroZine method.[3,4,5,6]
Fe(III) is released from transferrin by guanidine hydrochloride and reduced to Fe(II) by ascorbate and hydroxylamine. Bivalent iron ions form a red-colored chelate complex with FerroZine. To prevent copper interference, cupric ions are bound to thiourea.

$$\text{Transferrin-Fe(III)} \xrightarrow{\textit{Guanidine-HCl}} \text{apotransferrin + Fe(III)}$$

$$\text{Fe(III)} \xrightarrow{\textit{Reducing agents}} \text{Fe(II)}$$

$$\text{Fe(II) + 3 FerroZine} \longrightarrow \text{Fe(II)-(FerroZine)}_3$$

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

Reagents - working solutions

R1 Guanidine hydrochloride in vial A (liquid).
R2 Ascorbate in vial B (granulate).
R3 = SR FerroZine in vial C (liquid).

Active ingredients

Components	Concentrations (reconstituted)				
	R1	R2	SR	Test	
Guanidine-HCl	4.5			1.2	mol/L
Hydroxylamine	300			78	mmol/L
Thiourea	120			31	mmol/L
Ascorbate		225		13	mmol/L
FerroZine			40	1.5	mmol/L
Acetate			200	7.4	mmol/L
pH	4.5		5.0	4.5	

Reagent R1 contains a nonreactive surfactant.
Please see cassette label for reagent filling volumes.

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.
This kit contains components classified as follows according to the European directive 88/379/EEC:

Xn	R1 contains guanidine hydrochloride 38% w/w. Harmful
R 22	Harmful if swallowed.
R 36/38	Irritating to eyes and skin.
S 22	Do not breathe dust.

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

Reagent handling

COBAS INTEGRA 400/400 plus systems

Before insertion of the cassette pierce the aluminium foil of the reagent bottles using the tip of the unlock rack tool. After insertion of the cassette, granulate R2 is automatically reconstituted with the appropriate volume of water. Place the reconstituted cassette on the Cassette Mixer and mix for 10 minutes.

COBAS INTEGRA 700/800 systems

Granulate R2 is automatically reconstituted and mixed within approximately 5 minutes with the appropriate volume of water.

Storage and stability

Shelf life at 2 to 8°C	See expiration date on cassette
COBAS INTEGRA 400/400 plus analyzers	
On-board in use at 10 to 15°C	4 weeks
COBAS INTEGRA 700/800 analyzers	
On-board in use at 8°C	8 weeks

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 and lipemia).
Plasma (free from hemolysis and lipemia): Li-heparin plasma.
Do not use EDTA, oxalate, or citrate as anticoagulants, since they bind iron ions, preventing their reaction with the chromogen.
Specimens should be collected in the morning to avoid low results due to diurnal variation.[1]

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:[7]	7 days at 20-25°C
	3 weeks at 4-8°C
	Several years at -20°C

Centrifuge samples containing precipitates before performing the assay.

Materials provided

See "Reagents - working solutions" section for reagents.

Assay

For optimal performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator 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-R2-S-SR
Reaction direction	Increase
Wavelength A/B	552/652 nm
Calc. first/last	49/69
Test range	0-150 µmol/L (0-839 µg/dL)
with postdilution	0-1500 µmol/L (0-8385 µg/dL)
Postdilution factor	10 recommended
Unit	µmol/L

Pipetting parameters

		Diluent (H_2O)
R1	35 µL	10 µL
R2	8 µL	10 µL
Sample	25 µL	35 µL
SR	4 µL	8 µL
Total volume	135 µL	

COBAS INTEGRA 700/800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-R2-S-SR
Reaction direction	Increase
Wavelength A/B	552/652 nm
Calc. first/last	43/97
Test range	0-150 µmol/L (0-839 µg/dL)
with postdilution	0-1500 µmol/L (0-8385 µg/dL)
Postdilution factor	10 recommended
Unit	µmol/L

Pipetting parameters

		Diluent (H_2O)
R1	35 µL	10 µL
R2	8 µL	10 µL
Sample	25 µL	35 µL
SR	5 µL	7 µL
Total volume	135 µL	

Calibration

Calibrator	Calibrator f.a.s. Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

Traceability: This method has been standardized against a primary reference material (weighed in purified material).[8]

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 the 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.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Chapter 7, Data Analysis, User Manual (COBAS INTEGRA 700 analyzer), or to Data Analysis in the Online Help (COBAS INTEGRA 400/400 plus/800 analyzers).

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

Limitations - interference

Criterion: Recovery within ±10% of initial value.
Serum, plasma

Hemolysis	No significant interference up to an H index of 500 (approximate hemoglobin concentration: 500 mg/dL or 311 µmol/L).
Icterus	No significant interference up to an I index of 40 (approximate conjugated and unconjugated bilirubin concentration: 40 mg/dL or 684 µmol/L).
Lipemia	Do not use lipemic specimens.
Other	No significant interference up to an albumin level of 7 g/dL and a γ-globulin level of 4 g/dL. 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. 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.

Expected values[9]

Females	6.6-26.0 µmol/L	(37-145 µg/dL)
Males	11-28 µmol/L	(61-157 µg/dL)

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[8]

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

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within run n = 20, between run n = 20). The following results were obtained:

	Level 1	Level 2
Mean	7.7 µmol/L (43 µg/dL)	42.4 µmol/L (237 µg/dL)
CV within run	2.4%	0.97%
CV between run	2.9%	1.8%

Analytical sensitivity (lower detection limit)

0.24 µmol/L (1.34 µg/dL)
The 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 a zero sample (zero sample + 3 SD, within run precision, n = 30).

Method comparison

Iron values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Iron cassette were compared to those determined with commercially available reagents for iron on a COBAS MIRA instrument and an alternative manufacturer's clinical chemistry system. Samples were measured in duplicate. Sample size (n) represents all replicates. Values ranged from 1.3 to 63.4 µmol/L (7 to 354 µg/dL).

		COBAS MIRA analyzer	Alternative system
Sample size	(n)	236	226
Corr. coefficient	(r)	0.998	0.998
	(r_s)	0.997	0.996
Lin. regression		$y = 1.02x + 0.1$ µmol/L	$y = 0.95x + 0.5$ µmol/L
Passing/Bablok		$y = 1.03x + 0.1$ µmol/L	$y = 0.95x + 0.4$ µmol/L

References

1. Fairbanks VF, Klee GG. Biochemical aspects of hematology. In: Tietz NW, ed. Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders 1987:789-824.
2. Bauer JD. Hemoglobin, porphyrin, and iron metabolism. In: Kaplan LA, Pesce AJ, eds. Clinical Chemistry, theory, analysis, and correlation. St. Louis: Mosby Company 1984:611-655.
3. Stookey LL. FerroZine - a new spectrophotometric reagent for iron. Anal Chem 1970;42:779-781.
4. Williams HL, Johnson DJ, Haut MJ. Simultaneous spectrophotometry of Fe^{2+} and Cu^{2+} in serum denatured with guanidine hydrochloride. Clin Chem 1977;23:237-240.
5. Eisenwiener HG, Rietz P, Schläpfer PJ. Die Bestimmung des Eisens mit der Guanidiniumchloride/Ferrozin-Methode. J Clin Chem Clin Biochem 1979;17:149.
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7. Guder WG, Narayanan S, Wisser H, Zawta B. List of Analytes; Pre-Analytical Variables. Brochure in: Samples: From the Patient to the Laboratory. Darmstadt: GIT Verlag 1996.
8. Data on file at Roche Diagnostics.
9. Heil W, Koberstein R, Zawta B. Reference Ranges for Adults and Children, Pre-Analytical Considerations. 6th ed. 1999 (Published by Roche Diagnostics).

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