

REF	CONTENT	System-ID	Analyzers on which cobas c pack can be used
04536355 190	Unsaturated Iron-Binding Capacity (100 tests)	System-ID 07 3763 1	COBAS INTEGRA 400 plus COBAS INTEGRA 800
10759350 190	Calibrator f.a.s. (12 × 3 mL)	System-ID 07 3718 6	
12149435 122	Precinorm U plus (10 × 3 mL)	System-ID 07 7999 7	
12149443 122	Precipath U plus (10 × 3 mL)	System-ID 07 8000 6	
10171743 122	Precinorm U (20 × 5 mL)	System-ID 07 7997 0	
10171735 122	Precinorm U (4 × 5 mL)	System-ID 07 7997 0	
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	System-ID 07 7469 3	
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	System-ID 07 7469 3	
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	System-ID 07 7470 7	
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	System-ID 07 7470 7	

English

System information

Test UIBC, test ID 0-463 on COBAS INTEGRA 400 plus analyzer; test ID 0-063 on COBAS INTEGRA 800 analyzer

Intended use

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

Summary^{1,2,3}

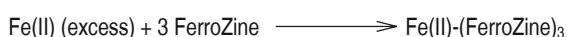
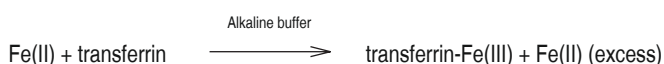
The prosthetic group of hemoglobin is the iron complex of protoporphyrin IX (heme) in which the centrally located iron atom acts as a stabilizer of oxyhemoglobin. Numerous enzymes and coenzymes require iron, e.g. peroxidases, catalases, cytochromes (which are also heme proteins), many of the enzymes 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 in 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. Normally only about one third of the iron-binding sites of transferrin are occupied by Fe(III). The additional amount of iron that can be bound is the unsaturated (or latent) iron-binding capacity (UIBC). The sum of the serum iron and UIBC represents total iron-binding capacity (TIBC). TIBC is a measurement for the maximum iron concentration that transferrin can bind.

The serum TIBC varies in disorders of iron metabolism. In iron-deficiency anemia the TIBC is elevated and the transferrin saturation is lowered to 15 % or less. Low serum iron associated with low TIBC is characteristic of the anemia of chronic disorders, malignant tumors, and infections.

Test principle

Direct determination with FerroZine^{4,5}



The color intensity is directly proportional to the unbound excess iron concentration and indirectly proportional to the unsaturated iron-binding capacity. It is determined by measuring the increase in absorbance at 552 nm.

Reagents - working solutions

R1	Ferrous chloride: 62 µmol/L; sodium hydrogen carbonate: 75 mmol/L; TRIS buffer: 375 mmol/L, pH 8.4; preservative
SR	FerroZine: 20 mmol/L; hydroxylamine: 160 mmol/L, pH 2.5

R1 is in position A and SR is in position C. Position B contains H₂O for technical reasons.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

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

Hazardous component: Hydroxylammonium chloride



Warning

H317 May cause an allergic skin reaction.

H351 Suspected of causing cancer.

Prevention:

P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.

Response:

P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P308 + P313	IF exposed or concerned: Get medical advice/attention.
P321	Specific treatment (see supplemental first aid instructions on this label).
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P363	Wash contaminated clothing before reuse.

Storage:

P405	Store locked up.
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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

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

COBAS INTEGRA 400 plus systems

On-board in use at 10-15 °C 2 weeks

COBAS INTEGRA 800 systems

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 plasma, 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 binding of iron to transferrin is strongly influenced by anions, specifically bicarbonate.⁶ In order to avoid drift in UIBC recovery in samples over time the environmental concentration of CO₂ in the laboratory should be kept as constant as possible.

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:⁷ 4 days at 15-25 °C
7 days at 2-8 °C

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 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	552/629 nm
Calc. first/last	33/64
Unit	µmol/L

Pipetting parameters

		Diluent (H ₂ O)
R1	80 µL	30 µL
Sample	30 µL	20 µL
SR	25 µL	15 µL
Total volume	200 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	552/629 nm
Calc. first/last	43/97
Unit	µmol/L

Pipetting parameters

		Diluent (H ₂ O)
R1	80 µL	30 µL
Sample	30 µL	20 µL
SR	25 µL	15 µL
Total volume	200 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) through iron.

Quality control

Reference range	Precinorm U, Precinorm U plus or PreciControl ClinChem Multi 1
Pathological range	Precipath U plus or PreciControl ClinChem Multi 2
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. 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

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 plus/800 analyzers).

Conversion factor: $\mu\text{mol/L} \times 5.59 = \mu\text{g/dL}$

Limitations - interference

If the patient's serum iron exceeds the binding capacity of the transferrin, a negative UIBC value results.

Criterion: Recovery within $\pm 10\%$ of initial value.

Icterus:⁸ No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 $\mu\text{mol/L}$ or 60 mg/dL).

Hemolysis:⁸ No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62.1 $\mu\text{mol/L}$ or 100 mg/dL).

Avoid hemolyzed specimens. Hemoglobin levels higher than 0.06 mmol/L (1.0 g/L) increase the apparent UIBC value significantly.

Lipemia (Intralipid):⁸ No significant interference up to an L index of 200. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration. Lipemic specimens may cause negative values and/or high absorbance flagging. Dilute the sample with NaCl 0.9 % and rerun the assay. Calculate the result with the appropriate dilution factor.

Anticoagulants: Complexing anticoagulants such as EDTA, oxalate, and citrate must not be used.

Therapeutic drug interference was tested according to the recommendations of the VDGH^{a)}. No interferences were found.

Exceptions: Methyldopa and oxytetracycline cause artificially high UIBC values at the tested drug level.

The physiological function of deferoxamine containing drugs is to bind iron to facilitate its elimination from the body. Therefore, any deferoxamine concentration interferes with the UIBC assay.

Pathologically high levels of albumin (7 g/dL) decrease the apparent UIBC value significantly.

In the presence of high ferritin concentrations > 1200 $\mu\text{g/L}$ 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).⁹

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.¹⁰

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

a) Verband der Diagnostica und Diagnostica Geräte Hersteller. Refer to section 1 / Introduction of this Method Manual, for a list of drugs tested and their concentrations.

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

6.0-125 $\mu\text{mol/L}$ (33.5-700 $\mu\text{g/dL}$)

Lower limits of measurement

Lower detection limit of the test:

6.0 $\mu\text{mol/L}$ (33.5 $\mu\text{g/dL}$)

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 a zero sample (zero sample + 3 SD, repeatability, n = 30).

Expected values¹¹

Females: 24.2-70.1 $\mu\text{mol/L}$ (135-392 $\mu\text{g/dL}$)

Males: 22.3-61.7 $\mu\text{mol/L}$ (125-345 $\mu\text{g/dL}$)

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

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 and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained:

	Level 1	Level 2
Mean	25.0 $\mu\text{mol/L}$ (140 $\mu\text{g/dL}$)	43.4 $\mu\text{mol/L}$ (242 $\mu\text{g/dL}$)
CV repeatability	1.6 %	0.8 %
CV intermediate precision	1.7 %	1.2 %

Method comparison

UIBC values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA UIBC reagent (y) were compared with those determined using reagents for UIBC on a COBAS MIRA system (x) and a commercially available alternative clinical chemistry system (x). Samples were measured in duplicate. Sample size (n) represents all replicates.

		COBAS MIRA system
Sample size	(n)	238
Corr. coefficient	(r)	0.988
	(r _s)	0.979
Lin. regression		$y = 1.06x - 1.1 \mu\text{mol/L}$
Passing/Bablok ¹²		$y = 1.08x + 1.0 \mu\text{mol/L}$
		Alternative system
Sample size	(n)	230
Corr. coefficient	(r)	0.996
	(r _s)	0.994
Lin. regression		$y = 0.93x + 1.7 \mu\text{mol/L}$
Passing/Bablok ¹²		$y = 0.93x + 1.6 \mu\text{mol/L}$

The sample concentrations were between 0.2 and 81.5 $\mu\text{mol/L}$ (1.1 and 455.2 $\mu\text{g/dL}$).

References


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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

COBAS, COBAS C, COBAS INTEGRA, COBAS MIRA, PRECINORM, PRECIPATH and PRECICONTROL are trademarks of Roche.

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Significant additions or changes are indicated by a change bar in the margin.

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