Unsaturated Iron-Binding Capacity

Order information

COBAS INTEGRA®	100 Tests	Cat. No. **20737631** 322
Unsaturated Iron-Binding Capacity		System-ID 07 3763 1
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
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 Unsaturated Iron-Binding Capacity cassette (UIBC) contains an in vitro diagnostic reagent system intended for use on COBAS INTEGRA systems for the quantitative determination of the unsaturated iron-binding capacity in serum and plasma (test UIBC, 0-063 on COBAS INTEGRA 700/800 systems, 0-463 on COBAS INTEGRA 400/400 plus 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]

$$\text{Fe(II)} + \text{transferrin} \xrightarrow{\textit{Alkaline Buffer}} \text{transferrin-Fe(III)} + \text{Fe(II) (excess)}$$

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

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 Saturating reagent in vial B (liquid).

R2 = SR FerroZine in vial C (liquid).

Active ingredients

Components	Concentrations			
	R1	SR	Test	
TRIS	375		150	mmol/L
Ferrous chloride	62		25	µmol/L
Sodium hydrogen carbonate	75		30	mmol/L
FerroZine		20	2.5	mmol/L
Hydroxylamine		160	20	mmol/L
Sodium azide	0.02		0.01	%
pH	8.4	2.5	8.4	

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

Precautions and warnings

Pay attention to all precautions and warnings listed in Chapter 1, Introduction.
This kit contains components classified as follows according to the European Directive 99/44/EC:

Xi	Irritant
R 43	May cause sensitization by skin contact.
S 24	Avoid contact with the skin.
S 37	Wear suitable gloves.

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 cassette
COBAS INTEGRA 400/400 plus analyzers	
On-board in use at 10 to 15°C	2 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 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]
When processing samples in primary tubes, follow the instructions of the tube manufacturer.

Stability:[6] 4 days at 15-25°C
7 days at 4°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-S-SR
Reaction direction	Increase
Wavelength A/B	552/629 nm
Calc. first/last	33/64
Test range	0-125 µmol/L (0-700 µg/dL)
Postdilution factor	No
Unit	µmol/L

Pipetting parameters

		Diluent (H_2O)
R1	80 µL	30 µL
Sample	30 µL	20 µL
SR	25 µL	15 µL
Total volume	200 µL	

COBAS INTEGRA 700/800 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
Test range	0-125 µmol/L (0-700 µg/dL)
Postdilution factor	No
Unit	µmol/L

Pipetting parameters

		Diluent (H_2O)
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.[7]

Quality control

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

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 × 5.587 = µg/dL

Limitations - interference

If the patient's serum iron exceeds the binding capacity of the transferrin, a negative UIBC value results.
Criterion: Recovery within ±10% of initial value.
Serum, plasma

Hemolysis	Avoid hemolyzed specimens. Hemoglobin levels higher than 0.06 mmol/L (1.0 g/L) increase the apparent UIBC value significantly.
Icterus	No significant interference.
Lipemia	No significant interference up to an Intralipid level of 200 mg/dL. 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.
Drugs	Of the drugs tested in vitro, methyldopa and oxytetracycline cause artificially high UIBC values at the tested drug level. Refer to Chapter 1, Introduction, for a list of tested drugs and their concentration.
Other	Pathologically high levels of albumin (7 g/dL) decrease the apparent UIBC value significantly. 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

Adults: 20-62 µmol/L (112-346 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[7]

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	25.0 µmol/L (140 µg/dL)	43.4 µmol/L (242 µg/dL)
CV within run	1.6%	0.8%
CV between run	1.7%	1.2%

Analytical sensitivity (lower detection limit)

4.2 µmol/L (23.5 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

UIBC values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Unsaturated Iron-Binding Capacity cassette were compared to those determined with reagents for UIBC on a COBAS MIRA system and a commercially available alternative clinical chemistry system. Samples were measured in duplicate. Sample size (n) represents all replicates.
Values ranged from 0.2 to 81.5 µmol/L (1.1 to 455.2 µg/dL).

		COBAS MIRA system	Alternative system
Sample size	(n)	238	230
Corr. coefficient	(r)	0.988	0.996
	(r_s)	0.979	0.994
Lin. regression		$y = 1.06x - 1.1$ µmol/L	$y = 0.93x + 1.7$ µmol/L
Passing/Bablok		$y = 1.08x + 1.0$ µmol/L	$y = 0.93x + 1.6$ 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. Lauber K, Peheim E, Perritaz R, Urbinelli R, Rietz P. Latente Eisenbindungskapazität und andere Eisenparameter im Plasma. GIT Labor Medizin 1991;14:95-96.
4. Stookey LL. FerroZine - a new spectrophotometric reagent for iron. Anal Chem 1970;42:779-781.
5. Persijn JP, Van der Slik W, Riethorst A. Determination of serum iron and latent iron-binding capacity (LIBC). Clin Chim Acta 1971;35:91-98.
6. Weissman N, Pileggi VJ. Inorganic ions. In: Henry RJ, Cannon DC, Winkleman W, eds. Clinical Chemistry, Principles and Techniques. 2nd ed. Hagerstown: Harper & Row 1974:639-754.
7. Data on file at Roche Diagnostics.

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