UIBC

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Unsaturated Iron-Binding Capacity - Application for Fe Standard

Order information

REF	CONTENT		Analyzers on which **cobas c** pack can be used
04536355190	Unsaturated Iron-Binding Capacity (100 tests)	System-ID 07 3763 1	COBAS INTEGRA 400 plus

Materials required (but not provided):

12146401216	Fe Standard (1 × 75 mL)	System-ID 07 7560 6	
12149435122	Precinorm U plus (10 × 3 mL)	System-ID 07 7999 7	
12149443122	Precipath U plus (10 x 3 mL)	System-ID 07 8000 6	
05117003190	PreciControl ClinChem Multi 1 (20 × 5 mL)	System-ID 07 7469 3	
05947626190	PreciControl ClinChem Multi 1 (4 × 5 mL)	System-ID 07 7469 3	
05117216190	PreciControl ClinChem Multi 2 (20 × 5 mL)	System-ID 07 7470 7	
05947774190	PreciControl ClinChem Multi 2 (4 × 5 mL)	System-ID 07 7470 7	

English

System information

Test UIBCI, test ID 0-564 on COBAS INTEGRA 400 plus systems

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]

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

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

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 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 C and SR is in position B.

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:

Warning

H317	May cause an allergic skin reaction.
H351	Suspected of causing cancer.

Prevention:

P201	Obtain special instructions before use.
P261	Avoid breathing mist or vapours.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.

Response:

P308 + P313	IF exposed or concerned: Get medical advice/attention.
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

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 at 10-15 °C	2 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
Plasma: 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]

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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 CO_2 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.

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

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

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_2O)
R1	80 µL	30 µL
Sample	30 µL	20 µL
SR	25 µL	15 µL
Total volume	200 µL	

Calibration

Calibrator	Standard 1 (high): deionized water (179 µmol/L or 1000 µg/dL)*
	Standard 2 (low): Fe Standard (89.5 µmol/L or 500 µg/dL)
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

*For technical reasons a virtual concentration of 179 µmol/L (1000 µg/dL) is assigned to the deionized water which is used as standard 1. This concentration is substracted from the results for serum and plasma using a Lab. Correlation Offset of -179 (-1000).

The calibrators must be placed on th CAL/QC rack with standard 1 (high, deionized water) first and standard 2 (low, FE Standard) last.

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 (weighed in purified material) through iron.

Quality control

Reference range	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

The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help.

Conversion factor: µmol/L x 5.59 = 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.

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 100 (approximate hemoglobin concentration: 62.1 µmol/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.

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

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10] *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 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 INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.
Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

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Limits and ranges

Measuring range

6.0-125 µmol/L (33.5-700 µg/dL)

Lower limits of measurement

Lower detection limit of the test:
6.0 µmol/L (33.5 µg/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 values[13]

Females:	24.2-70.1 µmol/L (135-392 µg/dL)
Males:	22.3-61.7 µmol/L (125-345 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

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 µmol/L	43.4 µmol/L
	(140 µg/dL)	(242 µg/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 400 analyzer with the COBAS INTEGRA UIBC reagent (y) were compared with those determined using the same reagents on a COBAS INTEGRA 800 analyzer (x) and those determined using the corresponding reagent on a Roche/Hitachi MODULAR P analyzer (x).

COBAS INTEGRA 800 analyzer	n = 73
Passing/Bablok[14]	Linear regression
y = 0.981x - 0.349 µmol/L	y = 0.988x - 0.278 µmol/L
τ = 0.940	r = 0.998

The sample concentrations were between 9.71 and 121 µmol/L (54.3 and 676 µg/dL).

Roche/Hitachi MODULAR P analyzer	n = 69
Passing/Bablok[14]	Linear regression
y = 1.04x + 3.73 µmol/L	y = 1.057x + 2.98 µmol/L
τ = 0.929	r = 0.994

The sample concentrations were between 6.00 and 87.6 µmol/L (33.5 and 490 µg/dL).

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, et al. 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 Harris WR. Thermodynamics of Anion Binding to Human Serum Transferrin. Biochemistry 1985;24:7412-7418.

7 Weissman N, Pileggi VJ. Inorganic ions. In: Henry RJ, Cannon DC, Winkelman JW, eds. Clinical Chemistry, Principles and Technics. 2nd ed. Hagerstown: Harper & Row 1974;639-754.

8 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

9 Breuer J. Report on the Symposium "Drug Effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

10 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

11 Tietz NW, Rinker AD, Morrison SR. When Is a Serum Iron Really a Serum Iron? A Follow-up Study on the Status of Iron Measurements in Serum. Clin Chem 1996;42(1):109-111.

12 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

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 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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 for reconstitution
GTIN	Global Trade Item Number

CE 0123

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