UIBC

cobas®

Unsaturated Iron-Binding Capacity

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04536355 190	Unsaturated Iron-Binding Capacity 100 tests	System-ID 07 3763 1	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
12146401 216	Fe Standard (1 x 75 mL)	Code 566	
10171743 122	Precinorm U (20 x 5 mL)	Code 300	
10171735 122	Precinorm U (4 x 5 mL)	Code 300	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	

English

System information

For **cobas c** 311/501 analyzers:

UIBCI: ACN 779

For **cobas c** 502 analyzer:

UIBCI: ACN 8779

Intended use

In vitro test for the quantitative determination of the unsaturated iron-binding capacity in human serum and plasma on Roche/Hitachi **cobas c** 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]

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

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

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 photometrically.

Reagents - working solutions

R1 Ferrous chloride: 62 µmol/L; sodium hydrogen carbonate: 75 mmol/L; TRIS buffer: 375 mmol/L, pH 8.4; preservative

R3 FerroZine: 20 mmol/L; hydroxylamine: 160 mmol/L; pH 2.5

R1 is in position A and R3 is in position C. Position B contains H_2O for technical reasons.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
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 dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.

Response:

P362 + P364	Take off contaminated clothing and wash it before reuse.

Product safety labeling primarily follows EU GHS guidance.

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

Reagent handling

Ready for use

Note: Put a new cassette direct from the refrigerator on the analyzer, do not allow cassette to come to room temperature.

Storage and stability

UIBC

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

Li-heparin plasma values are approximately 6 % lower than serum values.

UIBC

Unsaturated Iron-Binding Capacity

The binding of iron to transferrin is strongly influenced by anions, specifically bicarbonate.[6] In order to avoid drift of UIBC recovery in samples over time the environmental concentration of CO_2 in the laboratory should be kept as constant as possible.

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.

Centrifuge samples containing precipitates before performing the assay.

Stability:[7,8] 4 days at 15-25 °C
7 days at 2-8 °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

cobas c 311 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 23-57	
Wavelength (sub/main)	700 / 546 nm	
Reaction direction	Increase	
Units	µmol/L (µg/dL, mg/L)	
Reagent pipetting		Diluent (H_2O)
R1	55 µL	70 µL
R3	25 µL	20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	20 µL	–	–
Decreased	20 µL	–	–
Increased	10 µL	–	–

cobas c 501/502 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 34-70	
Wavelength (sub/main)	700 / 546 nm	
Reaction direction	Increase	
Units	µmol/L (µg/dL, mg/L)	
Reagent pipetting		Diluent (H_2O)
R1	55 µL	70 µL
R3	25 µL	20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	20 µL	–	–
Decreased	20 µL	–	–
Increased	10 µL	–	–

Calibration

Calibrators	S1: H_2O
	S2: Iron Standard
Calibration mode	Linear
Calibration frequency	2-point calibration after reagent lot change and as required following quality control procedures

Enter the correction value for the calibration with Iron Standard as instrument factor y = ax + b, where a = -1.0 and b = 0.

Traceability: This method has been standardized against a primary reference material (weighed in purified material) through iron.

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. 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

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	µmol/L x 5.59 = µg/dL
	µmol/L x 0.0559 = mg/L
	µg/dL x 0.179 = µmol/L
	µg/dL x 0.010 = mg/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at an iron concentration of 60 µmol/L (335 µg/dL).

Icterus:[9] 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:[9] No significant interference up to an H index of 40 (approximate hemoglobin concentration: 24.8 µmol/L or 40 mg/dL).

Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid):[9] No significant interference up to an L index of 300. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

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.[10, 11]

Exception: Oxytetracycline causes artificially high UIBC values at the tested drug level.

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

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

UIBC

Unsaturated Iron-Binding Capacity

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 values.

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.

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).[12]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[13]

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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

3-125 µmol/L (16.8-700 µg/dL, 0.17-7 mg/L)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Lower detection limit of the test

3 µmol/L (16.8 µg/dL, 0.17 mg/L)

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 the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Note: The technical limits for this assay are defined as -125 µmol/L (-700 µg/dL, -7 mg/L) for the lower limit and -3 µmol/L (-16.8 µg/dL, -0.17 mg/L) for the upper limit. This is due to the instrument factor for UIBC (a = -1; see also above chapter "Calibration"). Results under the lower limit of the measuring range will be flagged with ">TEST". Results above the upper limit of the measuring range will be flagged with "<TEST".

Expected values[14]

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

Serum/plasma iron levels are dependent on diet and subject to circadian variation.

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 (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	µmol/L (µg/dL)	*µmol/L (µg/dL)*	*%*
PCCC1	39.9 (223)	0.5 (3)	1.2
PCCC2	56.0 (313)	0.6 (3)	1.1
Human serum A	46.1 (258)	0.6 (3)	1.3
Human serum B	102 (570)	0.5 (3)	0.4
Human serum C	15.5 (86.6)	0.7 (3.9)	4.3

Intermediate precision	*Mean*	*SD*	*CV*
	µmol/L (µg/dL)	*µmol/L (µg/dL)*	*%*
Precinorm U	23.7 (132)	0.8 (5)	3.5
Precipath U	41.7 (233)	0.7 (4)	1.7
Human serum 3	16.5 (92.2)	0.8 (4.5)	4.7
Human serum 4	24.3 (136)	0.8 (5)	3.1

Method comparison

Serum

UIBC values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi MODULAR P analyzer (x).

Sample size (n) = 58

Passing/Bablok[15]	Linear regression
y = 1.01x + 1.86 µmol/L	y = 1.03x + 1.23 µmol/L
τ = 0.956	r = 0.997

The sample concentrations were between 7.50 and 80.1 µmol/L (41.9 and 448 µg/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
GTIN	Global Trade Item Number

CE

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