UIBC (UNSATURATED IRON BINDING CAPACITY)

Diagnostic reagent for determination of UIBC (Unsaturated Iron Binding Capacity) concentration.
Liquid. Dual reagents *(Ratio: R1/R2: 4/1)*. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Package	Ref No	Package	Ref No	Package	Ref No	Package
At305	248 mL	KUIB11	300 mL	MDIB10	250 mL	PL2316	130 mL
A300N	500 mL	KUIB10	600 mL	MUIB10	512 mL	RCU300	54,5 mL
A301N	250 mL	LM182	266 mL	MUIB11	255 mL	RDU300	300 mL
BYU300	675 mL	LM183	200 mL	M3IB10	250 mL	RDU301	150 mL
BYU301	450 mL	LM184	200 mL	M3IB11	200 mL	S2411	250 mL
DMU10	342 mL	LUIB10	675 mL	M3IB12	37,5 mL	S2410	250 mL
D2320	375 mL	LUIB11	300 mL	M4IB10	512 mL	TBUIB10	300 mL
D2321	250 mL	LUIB12	200 mL	M4IB11	255 mL	TBUIB11	150 mL
HN370	300 mL	LUIB13	250 mL	MS-100	100 mL	8AU300	675 mL
HN371	225 mL					8AU301	450 mL
						MH-282	75 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for quantitative determination of unsaturated iron-binding capacity (UIBC) in human serum and plasma.

GENERAL INFORMATION

Iron (Fe) is an essential element for almost all organisms, including humans, and its level is tightly controlled in nearly all biological processes.[1] Most of the iron (Fe) in the body (3-5 g) including also hemoglobin (2.5 g) and myoglobin (130 mg) is found in oxygen transport and storage proteins containing a heme molecule, Only small amounts of Fe, as little as 150 mg, are found in enzymes such as peroxidase, catalase, ribonucleotide reductase, crebs cycle and electron transport chain enzymes that contain heme or Fe-sulfur clusters in the active site. Most of the Fe (1 g in adult males) not included in the heme molecule is stored in macrophages and hepatocytes as ferritin or hemosiderin. Only a very small amount (3 mg) of Fe binds to the circulating serum protein transferrin (TF).[2]

Circulating Fe binds to free sites on TF, an iron transport protein in plasma. TF keeps Fe unreactive in the circulation and extravascular fluid, and transmits it to the cells via TF receptors. Iron binding capacity means TF's binding capacity to Fe. Iron binding capacity is of two types: total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC).[2]

Iron status can be assessed by several natural serum-based indicators, including serum ferritin, TF and TF receptor.[3] Abnormal serum ferritin level indicates iron storage disorder, while both TF and soluble Transferrin Receptor (sTfR) tests are used to diagnose iron deficiency anemia or iron overload.[4,5]

TF can be measured directly using immunologic techniques,[6] but some laboratory tests are also used to determine serum TF status, including serum iron, TIBC, transferrin saturation (TS) and UIBC.[7] While TIBC measures the iron binding capacity of TF in blood, TS measures the calculated iron-filled TF percentage using "serum iron / TIBC x 100" ratio. UIBC is a rapid, reliable multistep alternative to TS that measures the empty iron binding capacity of TF.[8] Ferrozine used in UIBC measurements is a compound that can form a red colored complex by forming chelates with $Fe2^{+}$,[9] and therefore can be measured colorimetrically.[10]

When Fe stores are depleted, TF levels in the blood rise. Since only one third of TF is saturated with Fe, TF in serum has an extra binding capacity (67%). This refers to UIBC.[11] TIBC is the sum of serum Fe and UIBC.[11,12]

A practical and chemical method for the determination of TIBC from serum or plasma samples was first developed in 1957.[13] In 1978, this method of measurement was further developed by the International Committee for Standardization in Haematology[14] and subsequently revised and defined as a recommended procedure in 1990.[15] Instead of this three-step colorimetric method, more direct measurement methods were developed in the 1990s.[16,17]

Most biochemistry analyzers nowadays detect TIBC computationally via UIBC measurement, which is more easily adapted to the automated systems.[12,18] TIBC value can then be obtained from the sum of the UIBC and Fe measured values.[19] In one study, it was reported that the UIBC test generally produced more accurate results than TF, TS and Fe measurements in the diagnosis of empty iron stores.[20]

However, conditions such as hemolysis, which alters serum content, and liver diseases such as cirrhosis and hepatitis, in which TF production is altered, can have adverse effects on UIBC results.[21,22]

Iron binding studies are important in the diagnosis of iron deficiency and iron overload. In iron-deficient states, the TS value declines below 15% and the UIBC and TIBC values are high due to the relative increase in transferrin content compared to iron content.[23] When Fe overload occurs in the body, as in hemochromatosis, the amount of free transferrin in the blood decreases and UIBC and TIBC values decline accordingly.[23] Low serum iron and TIBC values (ferrozine measurement method) occur in anemia due to chronic diseases, malignant tumors and infections. In liver diseases such as cirrhosis, iron binding capacity is reduced as transferrin is synthesized by the liver. TIBC levels may be low in multifactorial anemias or anemias of chronic inflammation. In iron overload states, including conditions associated with transfusion dependence seen in myeloid disorders or thalassemias (which can occur in later years with increased ability to absorb and store iron), TIBC levels are low, with proportional increases in iron saturation levels (UIBC).[11] It has been reported that UIBC and TIBC levels decreased significantly in patients with preeclampsia compared to the control group.[24]

TEST PRINCIPLE

Colorimetric Endpoint Measurement

To saturate existing binding sites on the transferrin molecule in the sample, an alkaline pH reagent containing a known concentration of ferrous (Fe^{+2}) iron is added to the sample. In an alkaline environment, ferrous (Fe^{+2}) iron binds to transferrin and converts to ferric (Fe^{+3}) form. After transferrin is completely saturated with iron, the unbound Fe^{2+} in the reagent reacts with ferrozine reagent to form a colored compound. The result is obtained by photometrically measuring the absorbance increase due to the color intensity formed as a result of the reaction. There is an inverse relationship between the color intensity and the corresponding absorbance increase and UIBC: More color intensity indicates more occupation of transferrin by iron (lower UIBC). Less color intensity indicates that transferrin has more binding capacity (high UIBC). As a result, the increase in absorbance due to color intensity is measured in two points, at wavelengths of 570 nm and 600 nm, and the amount of this increase is inversely proportional to UIBC.

$$Fe(II) + Transferrin \xrightarrow{Alkaline\ Buffer} Transferrin - Fe(III) + Fe(II)(excess)$$

$$Fe(II)(excess) + 3\ FerroZine\ Fe(II) \rightarrow Fe(II) - (FerroZine)_3\ (colored\ complex)$$

REAGENT COMPONENTS

Reagent 1

Buffer	≥ 0.2 mol/L pH : 8.45
Ferrous ammonium sulphate	≥ 8.4 µmol/L
Reducing chemical	≥ 0.1 mol/L
Nonionic surfactant	

Reagent 2

Ferrozine	≤ 24.3 mmol/L
Preservative	< 0.1%

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8˚C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[25]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. Multiple sample freezing and thawing should be avoided. The sample should be homogenized before testing.

UIBC Activity stability in serum and plasma:

7 days at +20/+25ºC ,
3 weeks at +2/+8ºC,
1 year at -20ºC.

Use serum or Li-heparin plasma. Do not use samples with EDTA, Oxalate or Citrate. Do not use hemolysed samples. Samples should be collected in the morning to avoid low results due to variations during the day.[35]

Unit Conversion

UIBC (µg/dL) = TIBC – Fe µg/dL
µmol/L x 5.59 = µg/dL
µmol/L x 0. 0559 = mg/L

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Unsaturated Iron Binding Capacity (UIBC) Calibrator or Arcal Auto Calibrator.

UIBC Calibrator
Ref.No: A302S
Ref.No: A301D
Ref.No: ZA107

Arcal Auto Calibrator
Ref.No: A39052

Ref.No: A39055 (For Olympus AU series.)

UIBC reagent must be calibrated with the calibrator included in the package. UIBC Calibrator values are lot specific.

For UIBC Calibrator, the calibrator value must be entered as a **negative** number. Arcal Auto Calibrator should be preferred for devices without negative number input.

Calibration *stability is 3 days*. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Arcon N Level 1 Control- Lyophilized
Ref.No: A3910
Ref.No: A3912 (For Olympus AU series.)
Ref.No: A3913 (For BS series.)
Ref.No: A3914 (For Erba.)

Arcon P Level 2 Control- Lyophilized
Ref.No: A3920
Ref.No: A3922 (For Olympus AU series.)
Ref.No: A3923 (For BS series.)
Ref.No: A3924 (For Erba.)

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

UIBC : 120 to 370 µg/dL
TIBC : 127 to 450 µg/dL

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.[26]

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, “Measuring Interval” refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte’s lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[27]

The determined analytic measuring interval for UIBC is 20-600 µg/dL.

Detection Capability

Limit of Detection (LoD): 10 µg/dL

Limit of Quantitation (LoQ): 20 µg/d

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[28]

Linearity

This method shows measurement linearity in the activities up to 600 µg/dL.

For the manual dilution procedure, dilute the sample 1:2 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[29]

Precision

Running system has been developed according to 20x2x2 “The Single Site” protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. [30]

Repeatability (Within Run) and Repeatability (Day to Day) SD and CV% values of UIBC have been given in the table 1 and 2 respectively.

Table 1. UIBC Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD*	CV%	n
127 µg/dL	4.31	3.40	80
243 µg/dL	4.89	2.01	80

***SD: Standard Deviation**

Note: This working system has been named “Within-Run Precision” in the previous CLSI - EP05-A2 manual.[31]

Table 2. UIBC Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
151 µg/dL	8.49	5.65	80
218 µg/dL	10.74	4.92	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[31]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablock equation:
y= 0.96x + 18 µg/dL
r=0.98

Interference

Endogenous interferant and analyte concentrations that have been used in the UIBC scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[32,33]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from UIBC interference scanning test is appropriate, is determined as ±10%.[34]

In UIBC test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	UIBC Target (µg/dL)	N*	Observed Recovery %
Hemoglobin 270 mg/dL	230	3	%108
Hemoglobin 990 mg/dL	421	3	%102
Bilirubin 30 mg/dL	117	3	%94
	275		%98
Lipemia 1000 mg/dL	117	3	%95
	275		%99

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[33]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[33]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

Danger

EUH032 :Releases a very toxic gas if contacts with acid.
H317 :May cause allergic skin reaction.

Precaution

P280 :Use protective gloves / clothes / glasses / mask.
P264 :Wash your hands properly after using.
P272 :Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352 :Wash with plenty of water and soap if it contacts with skin.
P333+P313 :Seek medical help if it irritates your skin or develops rash.
P362+P364 :Remove contaminated clothes and wash properly before using.

Disposal

P501 :Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests