BECKMAN COULTER

AU US

Instructions For Use

UIBC
UNSATURATED IRON BINDING CAPACITY

REF OSR61205 4 x 27 mL R1
4 x 3 mL R1a
4 x 6 mL R2
4 x 2 mL R2a

For *in vitro* diagnostic use only.

For Rx use only

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

System reagent for the quantitative determination of Unsaturated Iron Binding Capacity (UIBC) in human serum and plasma on Beckman Coulter AU analyzers.

SUMMARY AND EXPLANATION

Iron (non-heme) measurements are used in the diagnosis and treatment of diseases such as iron deficiency anemia, hemochromatosis (a disease associated with widespread deposit in the tissues of two iron-containing pigments, hemosiderin and hemofuscin, and characterized by pigmentation of the skin), and chronic renal disease. Transferrin is the major iron carrying protein in the serum.

Serum iron concentration connotes the Fe (III) bound to serum transferrin and does not include the iron contained in serum as free hemoglobin. Because normally only about one third of the iron binding sites of transferrin are occupied by Fe (III), serum transferrin has considerable reserve Iron Binding Capacity. This is called the serum Unsaturated Iron Binding Capacity (UIBC).[1]

METHODOLOGY

In this UIBC procedure intended for use on the Beckman Coulter automated AU analyzers, ferrous-Iron (Fe^{2+}) at alkaline pH, added to serum, binds specifically with transferrin at unsaturated iron binding sites. Remaining unbound ferrous iron reacts with the Nitroso-PSAP [2-Nitroso-5-(N-propyl-N-sulfopropylamino)phenol] to form an intense green complex. The difference between the resulting change in the measured absorbance and the absorbance from the total amount added to serum is equivalent to the quantity bound to transferrin. This is the Unsaturated Iron Binding Capacity (UIBC).

SPECIMEN

SPECIMEN STORAGE AND STABILITY

Serum iron is stable for 7 days at 2 – 8°C or 4 days at room temperature (15 – 25°C) after the serum is separated from red cells.[2]

Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

Additional handling conditions as designated by this laboratory:

SPECIMEN COLLECTION AND PREPARATION

Serum or heparinized plasma samples, free from hemolysis, are the recommended specimens. Remove serum from the red cells to minimize hemolysis. Plasma specimens collected with EDTA, oxalate, or citrate are unsatisfactory, since they bind iron, preventing its reaction with the chromogen. Samples should be taken in the morning from patients in a fasting state, since iron values can decrease by 30% during the course of the day.[2]

Additional instructions for patient sample preparation as designated by this laboratory:

Additional type conditions as designated by this laboratory:

REAGENTS

CONTENTS

Unsaturated Iron Binding Capacity reagent

Reagent storage location in this laboratory:

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.

Dispose of all waste material in accordance with local guidelines.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

Tris buffer pH 8.1 (20°C)	180 mmol/L
Iron	6.9 µmol/L
Nitroso-PSAP	400 µmol/L
Also contains preservatives	

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

UIBC R1	WARNING	
	H316	Causes mild skin irritation.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
		Tris(hydroxymethyl)– aminomethane 1 - 5%
UIBC R1a	WARNING	
		
		
		
	H316	Causes mild skin irritation.
	H317	May cause an allergic skin reaction.
	H351	Suspected of causing cancer.
	H373	May cause damage to organs through prolonged or repeated exposure.
	H411	Toxic to aquatic life with long lasting effects.

	P201	Obtain special instructions before use.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
	P391	Collect spillage.
		Hydroxylamine Hydrochloride 1 - 5%
UIBC R2	WARNING	
		
		
	H316	Causes mild skin irritation.
	H317	May cause an allergic skin reaction.
	H351	Suspected of causing cancer.
	P201	Obtain special instructions before use.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
	P332+P313	If skin irritation occurs: 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 use.
		Tris(hydroxymethyl)– aminomethane 1 - 5%
		Hydroxylamine Hydrochloride 0.1 - 0.2%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

Chemistry Calibrator (Cat # DR0070)

Storage location of the Calibrator in this laboratory:

EQUIPMENT AND MATERIALS

For AU400/400^{e}/480, AU640/640^{e}/680, AU2700/5400/AU5800 and DxC 700 AU Beckman Coulter Analyzers.

Storage location of test tubes or sample cups in this laboratory:

REAGENT PREPARATION

1. UIBC R1: Pour an aliquot of R1 buffer into R1a bottle and mix gently by inversion. Pour entire contents of R1a into R1 bottle and mix gently by inversion.
2. UIBC R2: Pour an aliquot of R2 buffer into R2a bottle and mix gently by inversion. Pour entire contents of R2a into R2 bottle and mix gently by inversion.

Note: It is important that the entire contents of the R2a color reagent be transferred to R2 and thoroughly mixed. Failure to do so will result in high bottle to bottle imprecision of control and patient recovery.

STORAGE AND STABILITY

The unopened reagents are stable until the expiration date printed on the label when stored at 2 – 8°C.

Working reagents are stable for 30 days when stored in the refrigerated compartment of the analyzers.

INDICATIONS OF DETERIORATION

Visible signs of microbial growth, turbidity or precipitate, or any change in reagent color may indicate degradation and warrant discontinuance of use.

Additional storage requirements as designated by this laboratory:

STABILITY OF FINAL REACTION MIXTURE

The Beckman Coulter AU analyzers automatically compute every determination at the same interval.

CALIBRATION

CALIBRATION INFORMATION

The frequency of calibration for the Unsaturated Iron Binding Capacity procedure is every 14 days. Calibration of this Unsaturated Iron Binding Capacity procedure is accomplished by use of Chemistry Calibrator (Cat # DR0070).

Recalibration of this test is required when any of these conditions exist:

1. A reagent lot number has changed or there is an observed shift in control values.
2. A fresh bottle of reagent is used for testing.

3. Major preventative maintenance was performed on the analyzer or a critical part was replaced.

QUALITY CONTROL

During operation of the Beckman Coulter AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter AU analyzer User Guide/Instructions For Use (IFU). Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Location of controls used at this laboratory.

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

A complete list of test parameters and operational procedure can be found in the User Guide/IFU appropriate to the Beckman Coulter AU analyzer.

RESULTS INTERPRETATION

Automatically printed out for each sample in µg/dL at 37°C.

REPORTING RESULTS

EXPECTED RESULTS

Adults:[3] 155 - 355 µg/dL

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Expected reference ranges in this laboratory:

INTERVALS	SAMPLE TYPE	UNITS

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

INTERFERENCES

Results of studies[4] show that the following substances interfere with this Unsaturated Iron Binding Capacity procedure. The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin:	No significant interference up to 40 mg/dL Bilirubin
Copper:	No significant interference up to 1 mg/dL Copper
Hemoglobin:	No significant interference up to 200 mg/dL Hemolysate
Lipemia:	No significant interference up to 1,000 mg/dL Intralipid*

*Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

Gross, abnormal high amounts of trace metals may interfere in this assay.

Eltrombopag and its metabolites may interfere with this assay causing erroneously high patient results.

The information presented is based on results from Beckman Coulter studies and is current at the time of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[5] for a compilation of reported interferences with this test.

Laboratory specific procedure notes:

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

The following data was obtained using the UIBC Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

DYNAMIC RANGE / ANALYTICAL MEASURING RANGE

The Unsaturated Iron Binding Capacity assay is linear from 55 – 450 µg/dL. Samples exceeding the upper limit of linearity should be diluted and repeated. On the analyzer the sample may be diluted, repeated and multiplied by the dilution factor automatically by utilizing the AUTO REPEAT RUN.

SENSITIVITY

The typical change in absorbance for 1 µg/dL of UIBC is 0.32 mAbsorbance.

METHODS COMPARISON

Reference [6]

Patient samples were used to compare this UIBC Reagent. The table below demonstrates representative performance on the AU analyzers.

Y Method	AU640
X Method	Method 2
Slope	0.975
Intercept	+6.920
Correlation Coeff. (r)	0.993
No. of Samples (n)	120
Range (µg/dL)	63 – 433

PRECISION

Reference[7]

Estimates of precision, based on CLSI recommendations, are consistent with typical performance. The within run precision is less than 8% CV and total precision is less than 10% CV. Assays of serum pools and control sera were performed and the data reduced following CLSI guidelines.

N = 80	**Within-run**		**Total**	
Mean, µg/dL	**SD**	**CV%**	**SD**	**CV%**
141	2.62	1.86	4.96	3.53
201	2.25	1.12	4.99	2.48
413	1.87	0.45	5.22	1.27

ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	**Description**
UBC1U	UIBC (Serum)

Setting Sheet Footnotes

User defined

Lot or Lot + Bottle

† Beckman Coulter System Calibrator Cat No.: DR0070

* Values set for working in µg/dL. To work in SI units (µmol/L) divide by 5.6

REVISION HISTORY

Revised GHS section

Revised Interferences section.

Preceding version revision history

Revised GHS section

REFERENCES

1. Burtis, C.A. and Ashwood, E.R., Tietz Textbook of Clinical Chemistry, 2nd Edition, W.B. Saunders Co., 1994.
2. Perrotta, G., Iron and Iron-Binding Capacity, In: Pesce, A.J., Kaplan, L.A. eds., Methods in Clinical Chemistry, C.V. Mosby, St. Louis, 1258 - 1261, 1987.
3. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
4. CLSI/NCCLS, Interference Testing in Clinical Chemistry, EP7-A2, 2005.
5. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 5th Edition, Washington, DC, AACC Press, 2000.
6. Data is on file for specific AU analyzers.
7. CLSI/NCCLS Evaluation Protocol EP5 - A2, 2004.

Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.
www.beckmancoulter.com