EN

REF 17639

UIBC Liquid

Determination of UIBC (unsaturated iron-binding capacity) in serum

REAGENT 1a: 2 x 40 mL - REAGENT 1b: 1 x 10 tablets
REAGENT 2: 1 x 11 mL STANDARD: 1 x 10 mL

IVD

CE

STANDARD/CALIBRATOR: the term refers to the standard / the calibrator
REAGENT: the term refers to the single reagent **CONTROL**: the term refers to the control

SUMMARY
Transferrin is the transport protein in blood, normally 20% to 50% saturated in its two iron-binding sites. The additional amount of iron that can be bound is the unsaturated iron-binding capacity (UIBC). The sum of serum iron and UIBC represents the total iron-binding capacity (TIBC). UIBC is usually determined directly by saturating the transferrin at an alkaline pH with a known but excess amount of iron. UIBC measurements are used in the diagnosis and treatment of anemia.

PRINCIPLE
Serum is added to an alkaline buffer/reductant solution containing a known concentration of iron to saturate the available binding sites on the transferrin. The iron that remains free after transferrin saturation is reduced to ferrous state and then complexed by ferene-S to form a stable complex which colour intensity is measured at 580-600nm. UIBC is therefore determined by subtracting the quantity of unbound iron from the total added quantity.

REAGENTS
Reagents, stored at 2-8 °C in unopened vials, are stable up to expiry date indicated on the package.
Reagents must be limpid; do not use if turbid.
Components of the kit and initial concentration of reactive components:
- **REAGENT 1a**
  tris buffer 0.25 mol/L pH 8.6
- **REAGENT 1b**
  reducing agent: ascorbic acid in tablets
- **Plastic Forceps**
- **REAGENT 2**
  ferene-S* ≥ 22.0 mmol/L

***** = *3-(2-pyridil)-5,6-bis-[2-(5-furylsulfonic acid)]-1,2,4-triazine*
- **STANDARD**
  iron solution 500 µg/dL (90 µmol/L)

Barcode and bottle code number, if printed on reagent labels, are referred to the use of the product on Hitachi 911/912 analyzers. Please refer to the application and detailed information available upon request.

NOTES AND LIMITATIONS
1) Use disposable test tubes and glassware washed with hydrocloric acid 1N solution and distilled water.
2) REAGENTS PECULIAR INFORMATION:
   - the STANDARD value is verified using a NIST (National Institute of Standards and Technology) traceable reference standard.
   - working solutions must be limpid; do not use if turbid.

Preparation of reagent solutions
R1 Solution: pick up 4 tablets of REAGENT 1b with plastic forceps and put them into one vial of REAGENT 1a. Mix until complete dissolution. Stability: 60 days at 2-8 °C if contamination avoided and vial recapped immediately after use.

REAGENT 2: ready to use. Reagent in unopened vial is stable up to expiry date indicated on the package. Stability: 60 days at 2-8 °C after opening, if contamination avoided and vial recapped immediately after use.
STANDARD: ready to use. Reagent in unopened vial is stable up to expiry date indicated on the package. Stability: 120 days at 2-8 °C after opening, if contamination avoided and vial recapped immediately after use.

QUALITY CONTROL
The use of following control materials at different levels of analyte is recommended to verify test accuracy:
Clin Chem Control 1 REF **16150** **6x5mL**
Lyophilized control serum. For use, follow the instructions contained in the kit.
Clin Chem Control 2 REF **16250** **6x5mL**
Lyophilized control serum. For use, follow the instructions contained in the kit.

SAMPLE
Serum not haemolyzed. Separate serum from clot within 1 hour after collection. Collect samples in accordance with the NCCLS procedure reported in bibliography (1). Stability of the sample: 7 days at 2-8 °C.

WARNING AND PRECAUTIONS
- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Safety Data Sheets are available at www.sentineldiagnostics.com or contact your local representative.
- ⚠ **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens[2], Biosafety Level 2[3] or other appropriate biosafety practices[4,5] should be used for materials that contain or are suspected of containing infectious agents.

Instrumentation and materials required but not provided
- Usual laboratory equipment
- Filters photometer or spectrophotometer

ANALYTICAL PROCEDURE

Wavelength:	593 (580-600) nm
Pathlength:	1 cm
Temperature:	37 °C
Sample/R1 Solution/REAGENT 2:	1/10/1
Reaction:	end-point (increase)

Allow reagents to reach working temperature before using.
A proportional variation of the reaction volumes indicated in the analytical procedure does not change the result.

Put into cuvette	Reagent Blank	Sample	Standard
Distilled water	0.2 mL	-	0.1 mL
Sample	-	0.1 mL	-
STANDARD	-	0.1 mL	0.1 mL
R1 Solution	1.0 mL	1.0 mL	1.0 mL

Mix carefully and incubate for 5 minutes at working temperature. Read the absorbance of Sample (ABS) against Reagent Blank. Add:

REAGENT 2	0.1 mL	0.1 mL	0.1 mL

Mix carefully and incubate for 5 minutes at working temperature. Read the absorbance of Sample (AS) and Standard (AST) against Reagent Blank. Final colour is stable for at least 1 hour.

CALCULATION

UIBC (µg/dL) = 500 - { 500 x [(AS - ABS)/AST] }

To obtain the TIBC (Total Iron-Binding Capacity) value, add the serum iron concentration to the UIBC value (expressed in the same measure unit):
TIBC = UIBC + Serum iron concentration.

Conversion Factor:
UIBC: [µg/dL] x 0.179 = UIBC [µmol/L]

REFERENCE VALUES

UIBC: 110-370 µg/dL (19.7-66.2 µmol/L)

The serum iron level can show a 30% diurnal variation, with a peak early in the morning.
It is recommended that each laboratory establish its own expected range.

PERFORMANCES

Interferences: the test is not affected by the presence of conjugated and non-conjugated bilirubin up to 50 mg/dL and total lipids up to 1000 mg/dL. Ascorbic acid > 20 mg/dL and hemoglobin >0.2 g/dL interfere in the test.
Measuring range: 9 - 500 µg/dL. Samples with UIBC concentration higher than 500 µg/dL (90 µmol/L) must be diluted 1:10 with normal saline and result multiplied by 10.
Intra-Assay Precision: it was determined on Hitachi 704 Instrument on 20 replicates of three different samples (3 levels of UIBC tested - L1/L2/L3). Results were as follows:
L1: average 141 µg/dL, SD 2.64, CV% 1.87 / L2: average 114 µg/dL, SD 2.21, CV% 1.94 / L3: average 145 µg/dL, SD 4.01, CV% 2.80.
Inter-Assay Precision: it was determined on Hitachi 704 Instrument for 10 days on 2 replicates of each control - 3 different levels (L1/L2/L3). Results were as follows:

	Mean µg/dL	Within Run SD	Within Run CV%	Run to Run SD	Run to Run CV%	Total SD	Total CV%
L1	135	2.61	1.93	6.63	4.91	7.12	5.28
L2	102	4.35	4.25	6.63	6.48	7.92	7.75
L3	134	4.42	3.29	7.25	5.40	8.49	6.33

Sensitivity: 9 µg/dL. Sensitivity was calculated on 10 replicates of normal saline and reported as the "mean zero value + 3 SD".
Accuracy: this UIBC test (y) was compared with a commercially available method (x). Results were as follows:
N = 40, r = 0.99658, y = 1.0991 x -6.4903

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

1) NCCLS Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard - Fifth Edition (H3-A5). Wayne, PA: The National Committee for Clinical Laboratory Standards, 2003.
2) US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. Bloodborne Pathogens.
3) US Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories, 5th Ed. Washington,DC: US Government Printing Office, January 2007.
4) World Health Organization. Laboratory Biosafety Manual, 3rd ed.Geneva: World Health Organization, 2004.
5) Sewell DL, Bove KE, Callihan DR, et al. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline — Third Edition (M29-A3). Wayne, PA: Clinical and Laboratory Standards Institute, 2005.
6) Kaplan, L.A., Pesce, A.J.: “Clinical Chemistry”, Mosby Ed. (1996).
7) EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of Good Laboratory Practice as specified in Council Directive 87/18/EEC.
8) Jakobs, D.S., Kasten, Jr., B.L., Demmott, W.R., Wolfson, W.L.: “Laboratory Test Handbook”, Lexi-Comp and Williams & Wilkins Ed. (2nd Edition - 1990).
9) Levy A., Vitacce P.: "Direct determination and binding capacity of serum iron", Clin.Chem. 7:241-248 (1961).
10) Schade et Al.: " Bound iron and unsaturated iron binding capacity of serum, rapid and reliable quantitative determination", Proc.Soc.Exp.Med. 67:442 (1954).

Explanation of symbols

REAGENT	The term refers to the single reagent
IVD	*In vitro* Diagnostic Medical Device
REF	Catalogue number
LOT	Batch code
Cont.	Contents of kit
	Caution, consult accompanying documents
	Consult instructions for use
	Use by (last day of the month)
xxx	Contains sufficient for <n> tests
	Temperature limitation
	Manufacturer

Note: changes in comparison to the previous version are indicated by a vertical bar in the text margin.