| COD 11835 50 mL |
|---|
| STORE AT 2-8ºC |
| Reagents for measurement of UIBC concentration<br>Only for *in vitro* use in the clinical laboratory |

# UNSATURATED IRON BINDING CAPACITY (UIBC)

BioSystems
REAGENTS & INSTRUMENTS

UNSATURATED IRON BINDING CAPACITY (UIBC)

FERROZINE

## PRINCIPLE OF THE METHOD

A known concentration of $Fe^{2+}$ ions are added to the sample to saturate transferrin. Uncomplexed $Fe^{2+}$ ions react with ferrozine forming a coloured complex that can be measured by spectrophotometry[1,2,3]. The coloured intensity is inversely proportional to the unsaturated iron binding capacity (UIBC) in the sample. The sum of serum iron and UIBC represents total iron binding capacity (TIBC).

## CONTENTS AND COMPOSITION

A. Reagent. 40 mL. TRIS 215 mmolL, sodium hydrogen carbonate 84 mmol/L, iron (II) sulfate 36 µmol/L, pH 8.4.

*WARNING: H351: Suspected of causing cancer. P281: Use personal protective equipment as required. P309+P311: IF exposed or if you feel unwell: Call a POISON CENTER or doctor/physician.*

B. Reagent. 10 mL. Ferrozine 8 mmol/L, ascorbic acid 200 mmol/L.

## STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity.

## AUXILIARY REAGENTS

Biochemistry Calibrator (BioSystems cod. 18011) or Biochemistry Calibrator Human (BioSystems cod. 18044).

## REAGENT PREPARATION

Reagents are provided ready to use.

## ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer able to read at 560 ± 20 nm.

## SAMPLES

Serum or heparinized plasma collected by standard procedures. Anticoagulants other than heparin should not be used.

Unsaturated iron binding capacity in serum or heparinized plasma is stable for 7 days at 2-8ºC[4].

## PROCEDURE

1. Bring the Reagent and the photometer to 37ºC.
2. Pipette into a cuvette: (Notes 1, 2)

| | Reagent Blank | Sample/Calibrator |
|---|---|---|
| Destilled Water | 175 µL | — |
| Reagent A | 800 µL | 800 µL |
| Sample/Calibrator | — | 175 µL |

3. Mix and insert the cuvette into the photometer. Start the stopwatch. After 2.5 minutes, read the absorbance (A1) at 560 nm against distilled water.
4. Pipette into a cuvette:

| | | |
|---|---|---|
| Reagent B | 200 µL | 200 µL |

5. Mix. After 5 minutes, read the absorbance ($A_2$) at 560 nm.

## CALCULATIONS

The UIBC concentration in the sample is calculated using the following general formula: (Note 3)

$$\frac{(A_2 - 0.83 \times A_1)_{Blank} - (A_2 - 0.83 \times A_1)_{Sample}}{(A_2 - 0.83 \times A_1)_{Blank} - (A_2 - 0.83 \times A_1)_{Calibrator}} \times C_{Calibrator} = C_{Sample}$$

The total iron binding capacity (TIBC) concentration in the sample could be calculated using the following formula: (Note 4)

TIBC = UIBC + Iron

## REFERENCE VALUES

Serum and plasma[5]: 150 - 336 µg/dL = 26 - 60 µmol/L.

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

## QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

## METROLOGICAL CHARACTERISTICS

– Detection limit 19.0 µg/dL UIBC = 3.4 µmol/L UIBC.

– Linearity limit: 700 µg/dL UIBC = 125 µmol/L UIBC. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

| Mean UIBC concentration | CV | n |
|---|---|---|
| 174 µg/dL = 31.2 µmol/L | 2.1 % | 20 |
| 280 µg/dL = 50.1 µmol/L | 1.5 % | 20 |

– Reproducibility (run to run):

| Mean UIBC concentration | CV | n |
|---|---|---|
| 174 µg/dL = 31.2 µmol/L | 2.8 % | 25 |
| 280 µg/dL = 50.1 µmol/L | 2.4 % | 25 |

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Interferences: Bilirubin (< 30 mg/dL) does not interfere. Lipemia (triglycerides > 325 mgd/L) and hemolysis (> 400 mgd/L) may affect the results. Other drugs and substances may cause interference[6].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

## DIAGNOSTIC CHARACTERISTICS

Iron is distributed in the body in a number of different compartments: hemoglobin, myoglobin, tissues (mainly in liver, spleen, bone marrow). Only 0.1% of total body iron is present in plasma. Serum iron is transported from one organ to another as $Fe^{3+}$ by a plasma iron transport protein, apotransferrina. The apotransferrin-$Fe^{3+}$ complex is called transferrin. Normally, only about one third of the iron binding sites of transferrin are occupied by iron. The additional amount of iron that can be bound is the unsaturated iron binding capacity (UIBC).

A decrease in iron binding capacity may be due to an hemochromatosis, acute iron poisoning, active cirrhosis or acute hepatitis[7,8].

Iron binding capacity is usually increased in iron deficiency anemia, however, measurement of iron binding capacity or iron saturation should not be used as a test for iron deficiency[5,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

## NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
2. Contamination of glassware with iron will affect the test. Use acid-washed glassware or plastic tubes.
3. The factor 0.83 compensates the decrease of the absorbance in the reaction mix after addition of the RB. The compensation is necessary if a high sample volume is used.
4. The total iron binding capacity concentration also can be measured using the TIBC reagents cod 11554.

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BioSystems S.A. Costa Brava, 30. 08030 Barcelona (Spain)
www.biosystems.es

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