(Ferrozine Method)

CLINICAL SIGNIFICANCE:

Measurement of Unsaturated Iron-binding Capacity (UIBC) are used to assist in the diagnosis and treatment of **anaemia.**

PRINCIPLE:

Excess ferrous ions bind specifically with available iron-binding sites of transferrin and saturating the molecules with iron in alkaline medium.

Ferrozine reacts with the remaining unbound iron to form a strongly purple coloured complex which is measured at 560 nm. The difference between the known excess amount of iron added and the remaining unbound iron is equivalent to unsaturated iron-binding capacity (UIBC). Total iron binding capacity (TIBC) is calculated as serum iron plus UIBC.

REAGENT COMPOSITION:

Active Ingredients	Concentration
Reagent-1	
* Buffer	100 mmol/L
* Detergent	5 mmol/L
pH 4.5 ± 0.1 at 25^0 C	
Reagent-2	
* Ferrozine	4 mmol/L
* Hydroxylamine Hydrochloride	150 mmol/L
Reagent-3	
* Enhancer	10 mmol/L
UIBC Standard (80 µmol/L)	

Also contains non-reactive fillers and Stabilizers.

PRESENTATION:

	No. of Bottles
Store all reagents at 2-8^{0}C	20 Test
1 UIBC (Buffer Reagent)	2
2 UIBC (Colour Reagent)	1
3 UIBC (Enhancer)	2
UIBC standard (80 µmol/L)	1

PREPARATION OF WORKING REAGENT -1:

Carefully transfer the content of one vial of 3 UIBC to one bottle of 1 UIBC. Mix to dissolve. Wait for 15 minutes before use.

REAGENT STORAGE AND STABILITY:

When stored, refrigerated at 2-8^{0}C and protected from light, the reagents are stable until the expiry date stated on the bottle and kit box labels. The working reagent is stable for 2 months at 2-8^{0}C.

SPECIMEN COLLECTION AND HANDLING:

Fresh clear serum or plasma with no hemolysis should be used. Specimens are stable for one day at room temperature or one week at 2-8^{0}C.

PRECAUTION:

It is essential that all the glasswares used for assay should be Iron-free. Glasswares should be soaked in 0.IN HNO_3 or HCl & rinsed thoroughly with iron-free deionized water.

REACTION PARAMETERS :

Monochromatic

Type of reaction	: End point (Increase)
Wavelength	: 560 nm
Temperature	: 37^{0}C
Incubation	: 10 min at 37^{0}C
Std. Concentration	: 80 µmol/L (446 µg/dL)
Std./Sample Volume	: 200 µl (0.200 ml)
Reagent 1	: 1.0 ml
Reagent 2	: 0.050 ml (50 µl)
Light path	: 1.0 cm
Zero setting with	: Reagent blank

Bichromatic

Other parameters as above	
Wavelength	: 560 nm and 630 nm
Sample Blank	: No
Zero setting with	: Distilled water

Set the instrument using above system parameters

PROCEDURE:

A) Monochromatic Method :

Pipette into test tube	Blank	Std.	Test	
			Sample Blank (A1)	Sample Test (A2)
Rgt.-1 (ml)	1.0	1.0	1.0	1.0
Sample (ml)	-	-	0.2	0.2
Standard (ml)	-	0.2	0.2	0.2
Dist. Water (ml)	0.4	0.2	-	-
Rgt.-2 (ml)	0.05	0.05	-	0.05

Mix and allow to stand for 10 minutes .at 37^{0}C. Read absorbance of test (A_1& A_2) and standard against reagent blank at 560 nm.

B) Bichromatic Method:

Pipette into Test tube	Std.	Test
Reagent -1 (ml)	1.0	1.0
Sample (ml)	-	0.2
Standard (ml)	0.2	0.2
Dist. Water (ml)	0.2	-
Reagent-2 (ml)	0.05	0.05

Mix and allow to stand for 10 minutes .at 37^{0}C. Read absorbance of test and standard against distilled water at 560 nm & 630 nm.

TEST RESULTS:

$$\text{Excess Iron (µmol/L)} = \frac{\text{Abs or } \Delta \text{ Absorbance of test x 80 (µmol/L)}}{\text{Absorbance of Std.}}$$

Where

Δ Absorbance = (A2-A1)

80 µmol/L = Concentration of Standard

UIBC (µmol/L) = 80 - excess Iron (µmol/L).

TIBC (µmol/L) = Serum Iron (µmol/L) + UIBC (µmol/L).

To convert (µg/dl) = µmol/L x 5.585

EXPECTED VALUES:

UIBC: 28.6 -64.5 µmol/L (160-360 µg/dl)

TIBC: 44.7 -71.6 µmol/L (250-400 µg/dl)

LIMITATIONS:

1) Hemolysis causes falsely elevated results.
2) Iron medications (oral, intravenous or intravascular) affect serum levels.

LINEARITY:

This procedure is linear upto 89 µmol/L (500 µg/dl). For sample values higher than 89 µmol/L (500 µg/dl), dilute the sample suitably with 0.9% saline and repeat the assay. Apply dilution factor to obtain test results.

REFERENCES:

1. Tietz NW "Text book of clinical chemistry 2nd Edition" Tetz NW (Ed) WB Saunders company Philadelphia 1994; 2059.
2. CaO G.and Prior R.L. Chemistry Anthocyanins and iron metabolism in human serum 1999b; 574-76.
3. National committee for Clinical Laboratory Standards. User evaluation of precision performance of clinical chemistry Devices. NCCLS, 1984 NCCLS Publication EP5-T.

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