TIBC / UIBC

METHOD – MODIFIED FERROZINE
PRODUCT CODE – LI01

ANAMOL
THE ORIGINAL MAKERS

INSTRUCTIONS FOR USE

INTENDED USE: Test for estimation of Iron and Total Iron Binding Capacity in human serum.

SUMMARY

Iron exists in serum complexed with transferrin, a transport protein. Most early procedures for iron determination involved dissociation of the iron from the iron-protein complex, precipitation of the proteins, and then measurement of the iron content of the protein free filtrate. Many chromogens have been used in the determination including thiocyanate o-phenanthroline, bathophenanthroline and TPTZ. In 1971, Persijn et al. presented a method using the chromogen ferrozine, described by Stookey. This method did not require protein precipitation and was more sensitive than previous methods. The present procedure is a modification of the Persijn method. In most cases, both serum iron and UIBC values are necessary for greatest diagnostic significance. Low serum iron values are seen in chronic blood loss, insufficient intake or absorption of iron, and increased demand on the body stores (e.g., pregnancy). Elevated serum iron values are seen in increased red cell destruction, decreased red cell synthesis, increased iron intake, or increased iron stores release. Increase in the UIBC may be due to increased production of apotransferrin (e.g., chronic iron deficiency) or an increased release of ferritin, as in hepatocellular necrosis. Decreases in the UIBC can occur with cirrhosis and hemochromatosis due to a deficiency in ferritin, or in nephrosis due to loss of apotransferrin.

PRINCIPLE

Serum Iron: Transferrin-bound iron is released at an acid pH and reduced from ferric to ferrous ions. These ions react with ferrozine to form a violet-coloured complex which is measured spectrophotometrically at 560nm. The absorbance measured at this wavelength is proportional to serum iron concentration. Total Iron-Binding Capacity (TIBC): A known number of ferrous ions are added to serum at an alkaline pH. The ferrous ions bind with transferrin at unsaturated iron-binding sites. The additional unbound ferrous ions are measured using the ferrozine reaction. The difference between the number of ferrous ions added and the unbound ions measured is the unsaturated iron-binding capacity (UIBC). The TIBC is equal to the serum iron concentration plus the UIBC.

KIT COMPONENTS

Reagent 1 : Iron Buffer Reagent
Reagent 2 : UIBC Buffer Reagent
Reagent 3 : Iron Colour Reagent
Reagent 4 : Iron Standard (500 µg/dL)

REAGENT STORAGE & STABILITY

Store all reagents stored at 2-8 ^{0}C. All reagents are stable till the expiry date indicated on the label. All reagents should be clear. Turbidity may indicate contamination and the reagent should not be used.

PRECAUTIONS & HANDLING

The reagents/samples should be handled by qualified personnel only. Discard reagent/sample as per good laboratory practices and local regulatory requirements. Read the instructions given on the labels and instructions for use carefully before using the kit. The kit is intended for in-vitro diagnostic use only. Don't freeze the reagent. Do not shake the reagent vigorously. Discard the reagent if the absorbance of the reagent exceeds 0.200 O.D. against D/W at 560 nm. Contamination of the reagent should be avoided.

MATERIALS REQUIRED BUT NOT PROVIDED

Test tubes, Micropipette with tips, Analyzer, Controls, Incubation chamber.

SPECIMEN COLLECTION & PRESERVATION

Fresh, unhaemolysed serum is the specimen of choice. Serum should be separated as soon as clot has formed. Heparinized plasma may be used but other anticoagulants should not be used to avoid possible iron contamination. Serum iron is reported to be stable for four days at room temperature (15-30 ^{0}C) and seven days at 2-8 ^{0}C.

COMPONENTS OF REAGENT

Component	Concentration
Hydroxylamine Hydrochloride	220 mmol/L
Tris	500 mmol/L
Ferrozine	16.7 mmol/L
Stabilizers and inactive ingredients.	-

TEST PARAMETERS

Name	Iron
Reaction Type	End Point
Wavelength Primary	560 nm
Flow Cell Temp.	37 °C
Blank setting	Reagent
Standard Conc.	500 µg/dL

Reagent 1 Vol	1000 µl
Sample Volume	250 µl
Incubation Temp.	37 ^{0}C
Incubation Time	10 mins
Linearity	500 µg/dL

ASSAY PROCEDURE

SERUM IRON

	Blank	Standard	Sample Blank (A1)	Sample Blank (A2)
Iron Buffer Reagent	1 ml	1 ml	1 ml	1 ml
Sample	-	-	250 µl	250 µl
Standard	-	250 µl	-	-
Iron free water	250 µl	-	-	
Color Reagent	20 µl	20 µl	-	20 µl

Mix & incubate for 10 min at 37 ^{0}C. Zero instrument at 560 nm with reagent blank. Record the absorbance of all the tubes (A1 & A2 Reading)

CALCULATION FOR SERUM IRON

$$\text{Total Iron (µg/dL)} = \frac{(\text{A2 of Test} - \text{A1 of Test}) \times \text{Conc. Of Standard}}{(\text{A2 of Std.} - \text{A1 of Std.})}$$

UIBC (UNSATURATED IRON BINDING CAPACITY)

	Blank	Standard	Sample Blank (A1)	Sample Blank (A2)
UIBC Buffer Reagent	1 ml	1 ml	1 ml	1 ml
Sample	-	-	250 µl	250 µl
Standard	-	250 µl	250 µl	250 µl
Iron free water	500 µl	250 µl	-	-
Color Reagent	25 µl	25 µl	-	25 µl

Mix & incubate for 10 min at 37 ^{0}C. Zero instrument at 560 nm with reagent blank. Record the absorbance of all the tubes (A1 & A2 Reading)

CALCULATION FOR UIBC

$$\text{UIBC (µg/dL)} = \frac{\text{(A2 of Test – A1 of Test) x Conc. Of Standard}}{\text{(A2 of Std. – A1 of Std.)}}$$

NOTE: The difference between A1 Test and A2 Test may sometimes be very small due to a high degree of unsaturation of transferrin with iron. The sample should be diluted 1:1 with iron-free water and re-assayed. The result is then multiplied by two.

TIBC CALCULATION

TIBC (Total Iron-Binding Capacity) **=** Iron Level + UIBC
SI Unit Conversion µg/dL x 0.179 = µmol/L

REFERENCE VALUES FOR NORMAL PEOPLE

Iron, Total = 60 - 150 µg/dL
TIBC = 250 - 400 µg/dL
Iron Saturation = 20 - 55%
It is strongly recommended that each laboratory determine the normal range for its particular population.

PERFORMANCE CHARACTERISTICS

Measuring Range: The assay is linear between 15-500 µg/dL. If the TIBC/UIBC value exceeds linearity limit (above 500 µg/dL), dilute the specimen suitably with normal saline and repeat the assay. In that case, assay value should be multiplied with the dilution factor to obtain correct glucose value of the specimen.

Interference: Iron contained in haemoglobin does not react in this method, therefore, slight haemolysis will not interfere. However, gross haemolysis (pink or red specimens) will contribute to the absorbance measured at the wavelength used and should be avoided. To make tubes, pipettes, etc. iron free, they must be washed with hot, dilute (1:2) hydrochloric or nitric acid, followed by several rinsing with iron-free deionized or distilled water.

Precision: Precision studies has been carried out using quality control sera as shown below:

For TIBC:

(n=10)	Within Run			Between Run		
Specimen Material	Mean (µg/dL)	SD (µg/dL)	CV %	Mean (µg/dL)	SD (µg/dL)	CV %
Low Value Serum	184	0.94	0.5	194	1.2	0.6
High Value Serum	270	1.4	0.5	301	1.0	0.3

For UIBC:

(n=10)	Within Run			Between Run		
Specimen Material	Mean (µg/dL)	SD (µg/dL)	CV %	Mean (µg/dL)	SD (µg/dL)	CV %
Low Value Serum	92	1.2	1.4	110	0.82	0.7
High Value Serum	143	0.94	0.7	152	0.88	0.6

Note: We recommend all the laboratories to establish its own accuracy and precision data.

QUALITY CONTROL

Inclusion of a normal value and abnormal value chemistry control serum in each test run ensures optimum quality control. Consistent use of same type and methodology of control serum provides between run precision and accuracy data for TIBC/UIBC. We recommend to produce such data on daily basis for greater accuracy in assay system which include reagents, instrument, apparatus and operator.

PRECAUTIONS

All reagents are toxic. Do not pipette by mouth. Avoid all contact. UIBC buffer contains sodium azide and may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation. This reagent is for *in vitro* diagnostic use only.

BIBLIOGRAPHY

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Symbol	Explanation	Symbol	Explanation
	Manufactured By		In Vitro Diagnostic Use
	Lot Number		Read Instructions Before Use
	Catalogue Number		Storage Temperature
	Manufacturing Date		Number of Tests / Volume
	Expiry Date		Do Not Reuse
	Protect from Sunlight		Keep Dry

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