Lab sheet #3

Quantitative determination of serum iron, unsaturated iron binding capacity (UIBC), and total iron binding capacity (TIBC)

Method:

-Prepare 6 test tubes, the divide them in to two group one for each test (3 test tube for each test)

	Serum Iron test		
	Blank	Standard	Test
Iron buffer (pH 4.5)	2.5 ml	2.5 ml	2.5 ml
Iron Standard	-------	0.2 ml	-------
Sample	--------	--------	0.2 ml
Water	0.2 ml	--------	------

	UIBC test		
	Blank	Standard	Test
UIBC buffer	2 ml	2 ml	2 ml
Iron Standard	-------	0.2 ml	0.2 ml
Sample	--------	--------	0.2 ml
Water	0.4 ml	0.2 ml	------

Mix. Read the Abs. of Std. and test (IRON) at 565 nm against their blank, this is (A^0)
Also read the Abs. of Std. and test (UIBC) at 565 nm against their blank, this is (A')
Then add:

Iron color reagent	0.05 ml	0.05 ml	0.05 ml	0.05 ml	0.05 ml	0.05 ml

Mix and incubate at 37 ^{0}C for 10 min. Read the Abs. of Std. and test (IRON) at 565 nm against their blank, this is (A^1). Also read the Abs. of Std. and test (UIBC) at 565 nm against their blank, this is (A'').

Results:

	Before adding the iron color reagent	Abs. at 565nm
(IRON) (A^o)	Absorbance of (Standard)	
	Absorbance of (Test)	
(UIBC) (A')	Absorbance of (Standard)	
	Absorbance of (Test)	

	After adding the iron color reagent	Abs. at 565nm
(IRON) (A^1)	Absorbance of (Standard)	
	Absorbance of (Test)	
(UIBC) (A'')	Absorbance of (Standard)	
	Absorbance of (Test)	

Calculations:

Serum iron conc. in test (μ g/dl) =

$[(A^1 - A^0)$ test$/(A^1 - A^0)$ std] x Std. iron conc.

Serum UIBC in test (μ g/dl) =

Std. iron conc. – { [(A" – A') test/(A" – A') std] x Std. iron conc.}

Serum TIBC in test (μ g/dl) = Serum iron + Serum UIBC

Transferrin saturation (%) = [Serum iron concentration/ TIBC] x 100

*** The std iron conc. = 500 μ g/dl*

Normal range:

- **Serum iron (50 -160 µg/dl)**
- **TIBC (250 - 450 µg/dl)**
- **Transferrin saturation (20 – 55 %)**