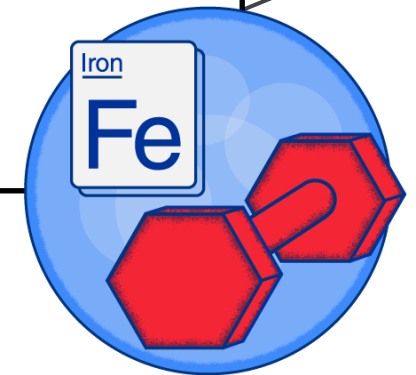


Blood Biochemistry BCH 471[Practical]

**Lab (7) Quantitative Determination of Serum Iron, (UIBC),  
and (TIBC)**



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- **Objectives**

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- - To determine the normal level of serum iron.

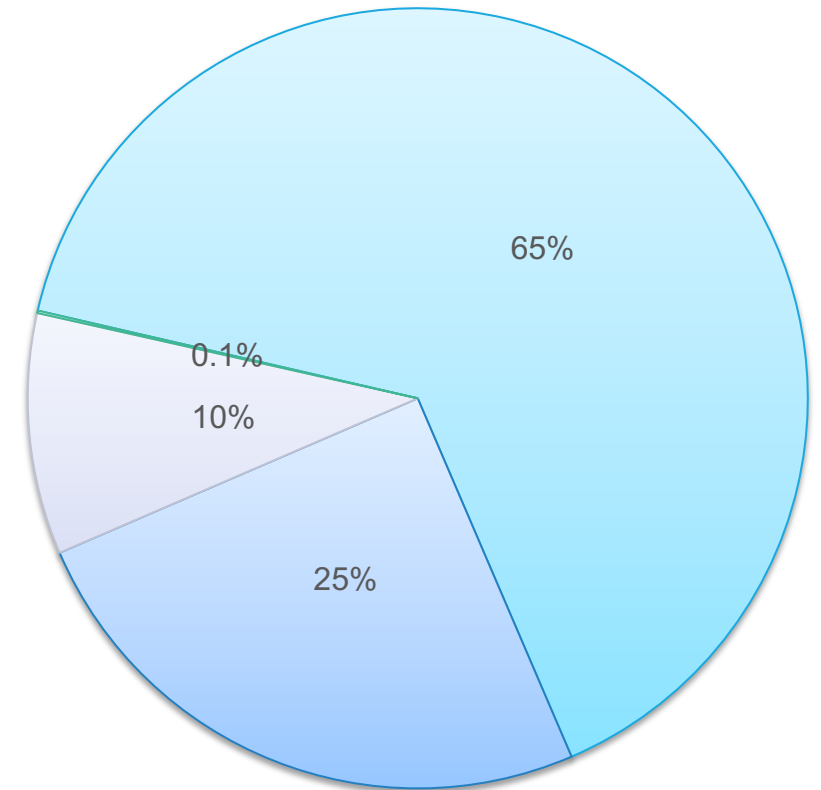
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- - To determine the use of this test in diagnosis of anemia (iron deficiency).

# Iron in The Body

- **Iron** is the **metal** component of **hemoglobin, myoglobin, cytochromes** and some proteins of the electron transport chain.
- The total iron of an adult **male is 4-5g** and of a **female is 3-4g**.
- **Serum iron** is defined as Fe (III) bound to transferrin and to a much lesser extent to some other serum proteins.

Iron distribution in the body

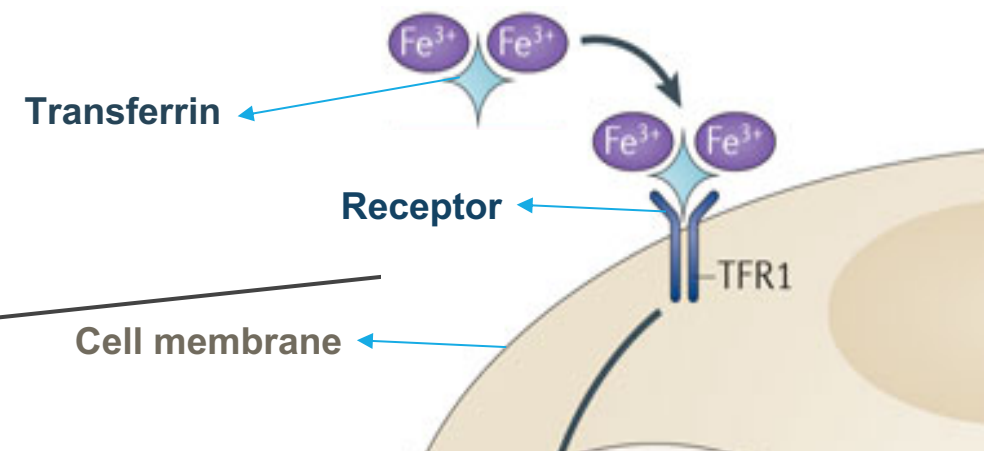


- hemoglobin
- stored iron ( ferritin and hemosiderin )
- other forms ( myoglobin, cytochromes etc. )
- serum iron

# Iron Transportation

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- **Iron** is carried in  $\text{Fe}^{3+}$  state bound to a specific iron transport protein known as **transferrin**.
- **Transferrin** are iron-binding blood plasma glycoproteins that control the level of free iron in biological fluids.
- It contains **two** specific high-affinity Fe(III) binding sites.
- Largely synthesized by the **liver**.
- Transferrin distributes iron to those tissues which have a demand for its utilization.
- The **transferrin–iron complex** enters the cell through specific receptors and the iron ions are released for metabolic functions.



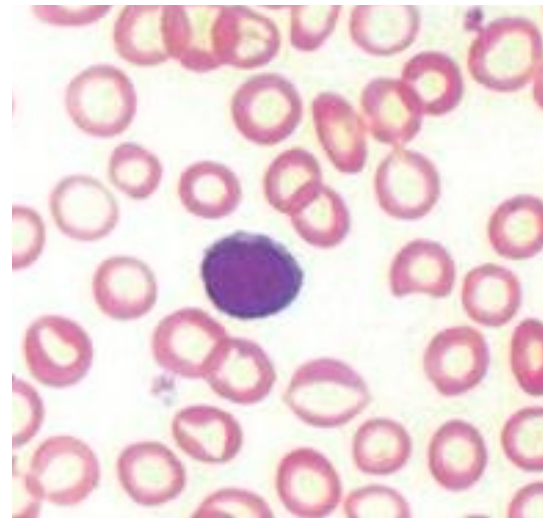
# Iron Transportation

- When iron stores become **low**, **transferrin** levels will **increase**.
- When there is **too much** iron, **transferrin** levels are **low**.
- Individuals who lack transferrin show severe **microcytic hypochromic anemia** and are also susceptible to bacterial and viral infections.
- **microcytic hypochromic anemia**, is a type of anemia in which the circulating RBCs are smaller than the usual size of RBCs (microcytic) and have decreased red color (hypochromic).

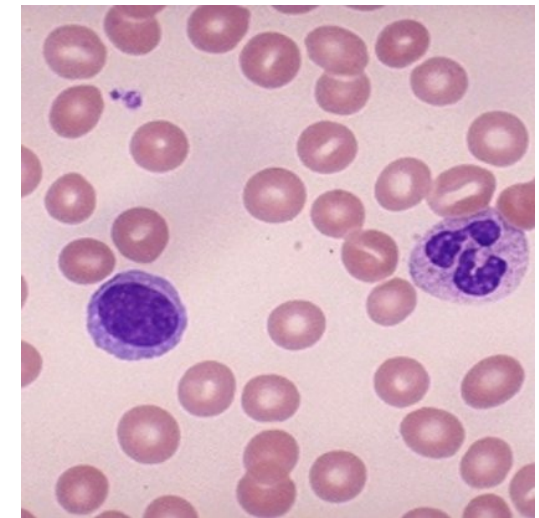
**Normally:**

Stored Iron ↑ **transferrin** ↓

Stored Iron ↓ **transferrin** ↑



**Fig. 2.** microcytic hypochromic anemia



**Fig. 3.** normal blood smear

# Iron Level in Blood

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- It is important to measure **iron** and **iron-binding capacity** to detect iron deficiency or overload.

## Tests used:

### 1. Serum Iron

### 2. Total iron-binding capacity (TIBC)

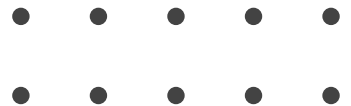
- TIBC or UIBC may be ordered along with serum iron when it appears that a person has **iron deficiency or overload**.
- These tests may be ordered when there are signs of **anemia**, especially when a CBC is performed and shows red blood cells that are **microcytic** and **hypochromic** and the **hemoglobin** and **hematocrit levels are low**.

**Note:** Serum iron on its own provides no complete information on iron level

# Total Iron-binding Capacity (TIBC)

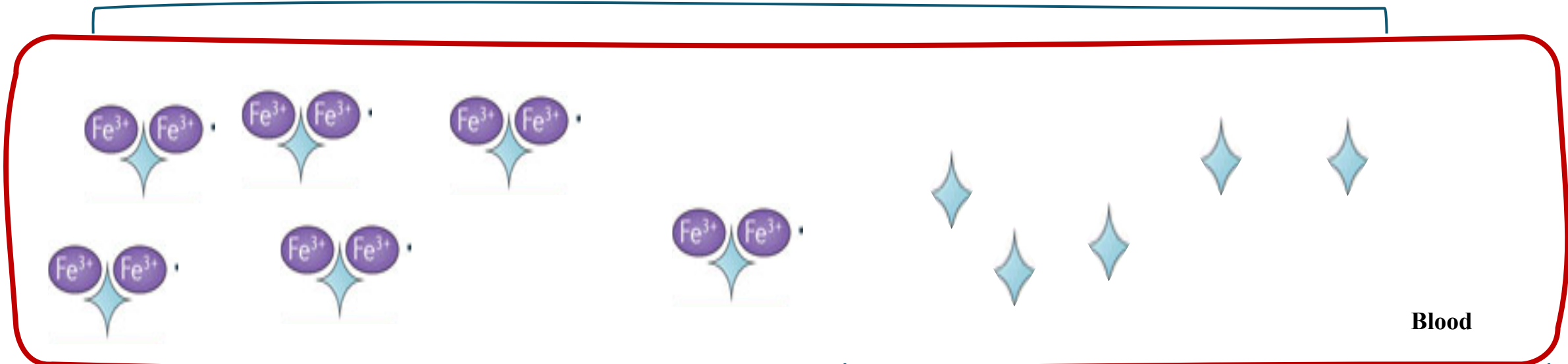
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- It is a medical laboratory test that measures the blood's capacity to **bind iron with transferrin**.
- It measures the total amount of iron that can be bound by proteins in the blood, which indirectly measures transferrin.
- It is calculated by **adding** serum iron and unsaturated iron binding capacity (**UIBC**)
- **UIBC** is the portion of transferrin that has not yet been saturated with iron.
- It is most frequently used along with a serum iron test to evaluate people suspected of having either iron deficiency anemia or iron overload (hemochromatosis)



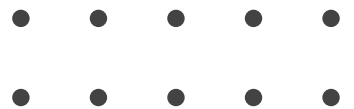
# Serum Iron, TIBC and UIBC

**Total iron-binding capacity**



**Serum Iron**

**Unsaturated serum iron binding capacity**





# Practical Part

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# Principle

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- **Serum iron:** The iron is dissociated from its Fe-III-transferrin complex by addition of acidic buffer containing hydroxylamine which **reduces the Fe(III) to Fe(II)**.
- Then the **chromogenic agent (PDTS)** form a highly **colored Fe(II) complex** that is measured spectrophotometrically at **565nm**.
- The intensity of color is **directly proportional** to the concentration of iron.
- **UIBC:** Determined by adding **Fe(II)** to serum so that it binds to unsaturated iron binding site on **transferrin**.
- The **excess Fe(II)** react with **PDTS** to form color complex which is measured spectrophotometrically at **565nm**.
- **The difference between the amount of Fe(II) added and the amount of Fe(II) measured represent the UIBC.**
- **TIBC:** is determined by **adding serum iron to UIBC value**.

# Principle

- **Serum Iron:**

- **Fe-III-transferrin complex**

Acidic Buffer  
→

**Fe(II)**

PDTS  
→

**Colored Fe(II) complex**

Measure absorbance at 565nm.

- **UIBC:**

- **Unsaturated iron binding site on transferrin**

Adding excess Fe(II) to serum  
→

**Fe-III-transferrin complex**

+

**The excess Fe(II)**

PDTS  
↓

**Colored Fe(II) complex**

Measure absorbance at 565nm.

**UIBC = Amount of excess Fe(II) added - amount of Fe(II) measured**

- **TIBC: serum iron + UIBC**

# Method

Serum Iron ( $A^1$ , $A^0$ )			
	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 ( $A''$ , $A'$ )			
	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
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Mix and incubate at 37°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 ( $A''$ ).

# Calculations

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**Serum iron Conc. In test ( $\mu\text{g}/\text{dl}$ )=**

$[(A^1 - A^0) \text{ test} / (A^1 - A^0) \text{ Std}] \times \text{Std. iron Conc.}$

**Serum UIBC In test ( $\mu\text{g}/\text{dl}$ )=**

$\text{Std. iron Conc.} - \{ [(A'' - A') \text{ test} / (A'' - A') \text{ Std}] \times \text{Std. iron Conc.} \}$

**Serum TIBC In test ( $\mu\text{g}/\text{dl}$ )= Serum iron + Serum UIBC**

**Transferrin saturation (%)=**  $[\text{Serum iron Concentration} / \text{TIBC}] \times 100$

**Note: the Std. iron Conc. = 500  $\mu\text{g}/\text{dl}$**

## Normal Ranges

- Serum iron (50 -160  $\mu\text{g}/\text{dl}$ )
- TIBC (250 - 450  $\mu\text{g}/\text{dl}$ )
- UIBC (120 – 470  $\mu\text{g}/\text{dl}$ )
- Transferrin saturation (20% – 55%)

# Results

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## Defect in Serum iron

- **Serum iron is low** in iron deficiency anemia whether due to:
  - insufficient intake, malabsorption, blood loss or inability to retrieve storage iron.
- **Serum iron concentration is high** when:
  - Bone marrow cannot utilize iron, hemolysis, increased absorption or defects in storage capabilities.
  - High values are also found in severe hepatitis due to release from liver cells.

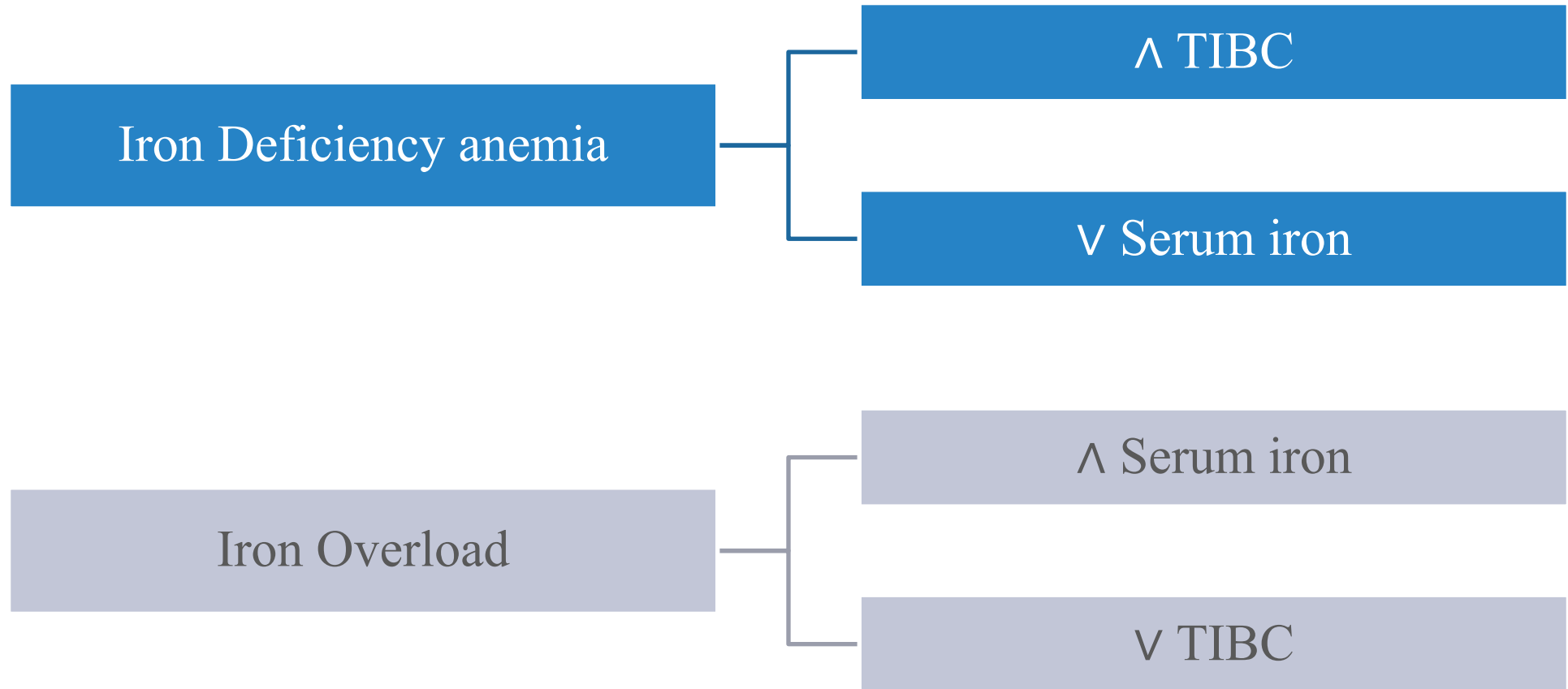
## Defect in Total iron binding capacity (TIBC)

Increase in iron deficiency anemia.

**Decrease** in hemochromatosis, malignant or rheumatic fever.

# Results

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Stored Iron ↑ transferrin ↓ TIBC ↓  
Stored Iron ↓ transferrin ↑ TIBC ↑

