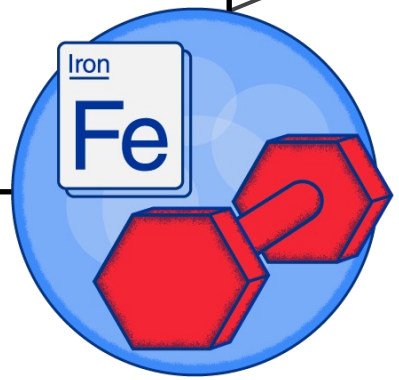
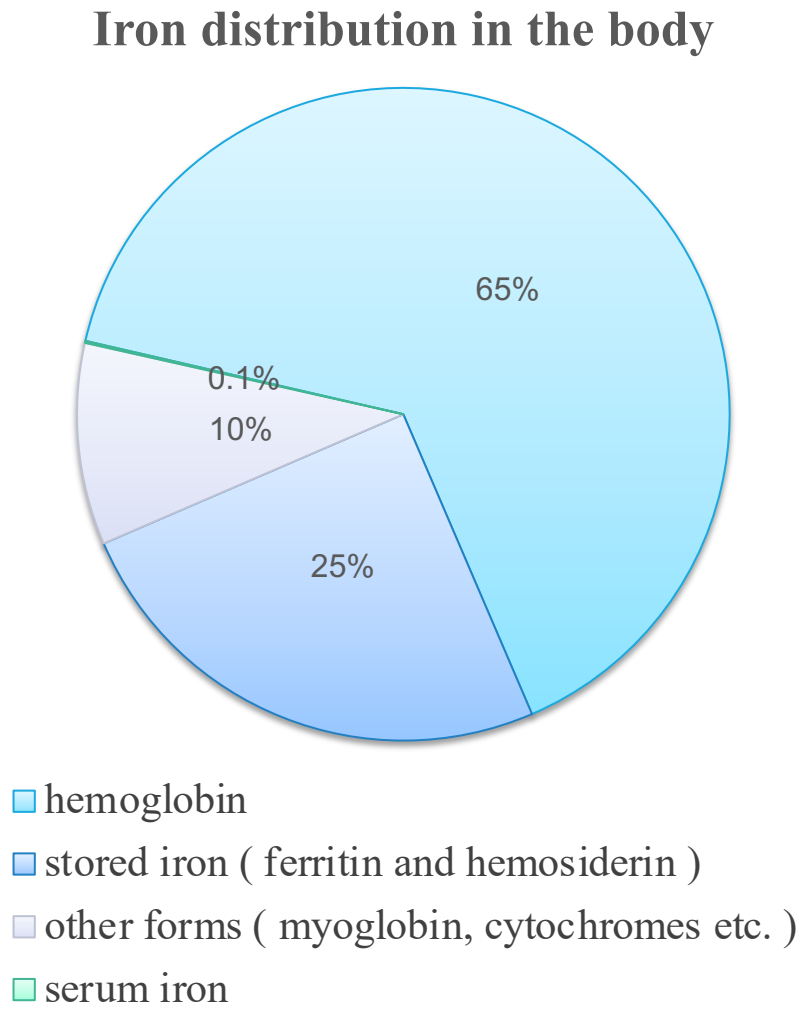
Blood Biochemistry BCH 471[Practical]

Lab (6) Quantitative Determination of Serum Iron, (UIBC), and (TIBC)



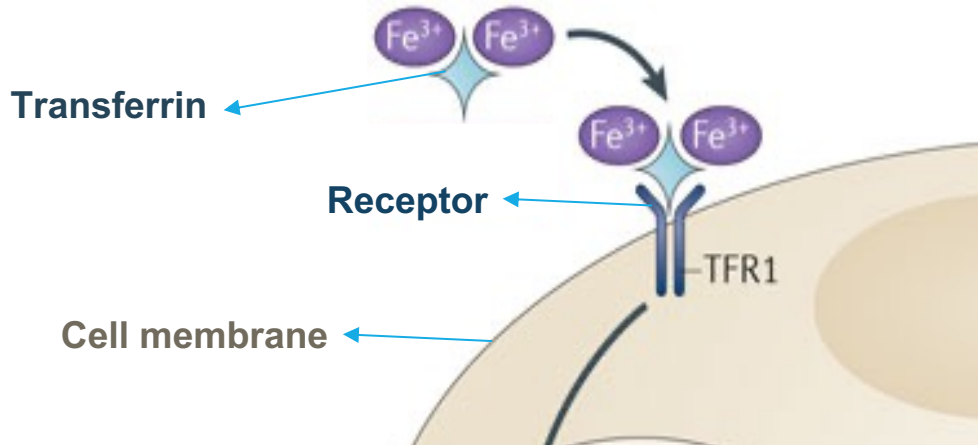
Iron in The Body

- **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.**
- Iron occurs in the body in three forms: functional, storage, and transport form.
- **Serum iron** is defined as Fe (III) bound to transferrin and to a much lesser extent to some other serum proteins.



Iron Transportation

- **Iron** is carried in 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 (a condition called, Atransferrinemia)
- **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 ↑

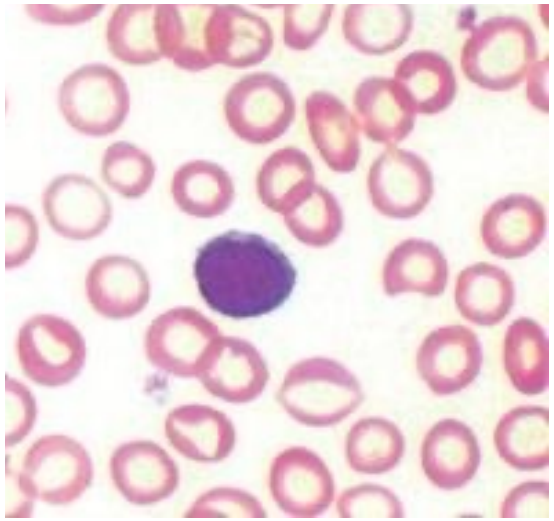


Fig. 2. microcytic hypochromic anemia

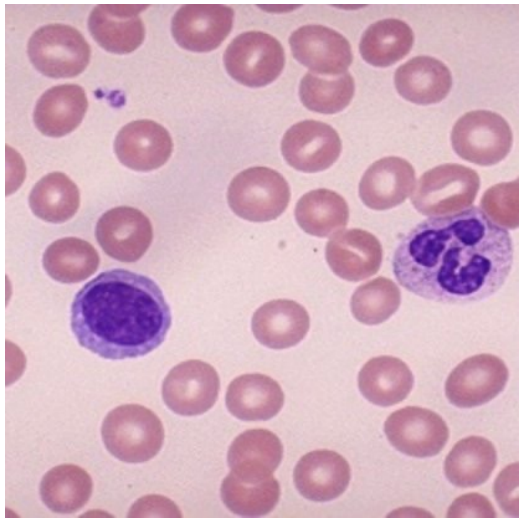


Fig. 3. normal blood smear

Iron Level in Blood

- 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)**

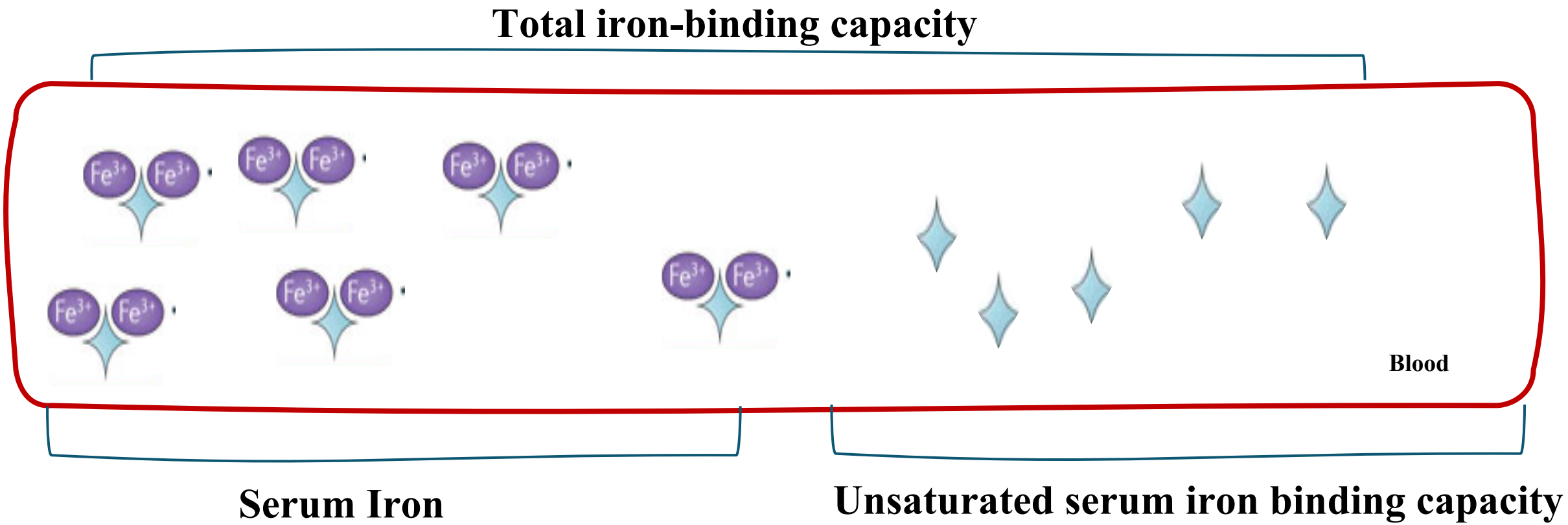
- TIBC or UIBC may be ordered along with serum iron when it appears that a person has **iron deficiency** or **overload.**
- These tests may be ordered when there are signs of **anemia**, especially when a CBC is performed and shows red blood cells that are **microcytic** and **hypochromic** and the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

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

Total Iron-binding Capacity (TIBC)

- 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



Practical Part 💉

Objectives

1) To determine the level of serum iron and UIBC using a kit.

2) Understand the obtained values, and make a diagnosis.

Principle

- **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 (hydroxylamine))→ **Fe(II)** —(PDTS (chromogenic agent))→ **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)**

Fe-III-transferrin complex + The excess Fe(II) —(PDTS)→ **Colored Fe(II) complex**
Measure absorbance at 565nm.

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

- **TIBC: serum iron + UIBC**

Method

Serum Iron (A^0, A^1)			
	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 colour reagent	0.05 ml to all tubes

Mix and incubate at 37ºC 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

Serum iron Conc. In test (µg/dl)=

[(A^1- A^0) test/ (A^1-A^0) Std] x Std. iron Conc.

Serum UIBC In test (µg/dl)=

Std. iron Conc. – { [(A``- A`) test/ (A``-A`) Std] x Std. iron Conc.}

Serum TIBC In test (µg/dl)= Serum iron + Serum UIBC

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

Note: the Std. iron Conc. = 500 µg/dl

Normal Ranges

- Serum iron (50 -160 µg/dl)
- TIBC (250 - 450 µg/dl)
- UIBC (120 – 470 µg/dl)
- Transferrin saturation (20% – 55%)

Results

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.

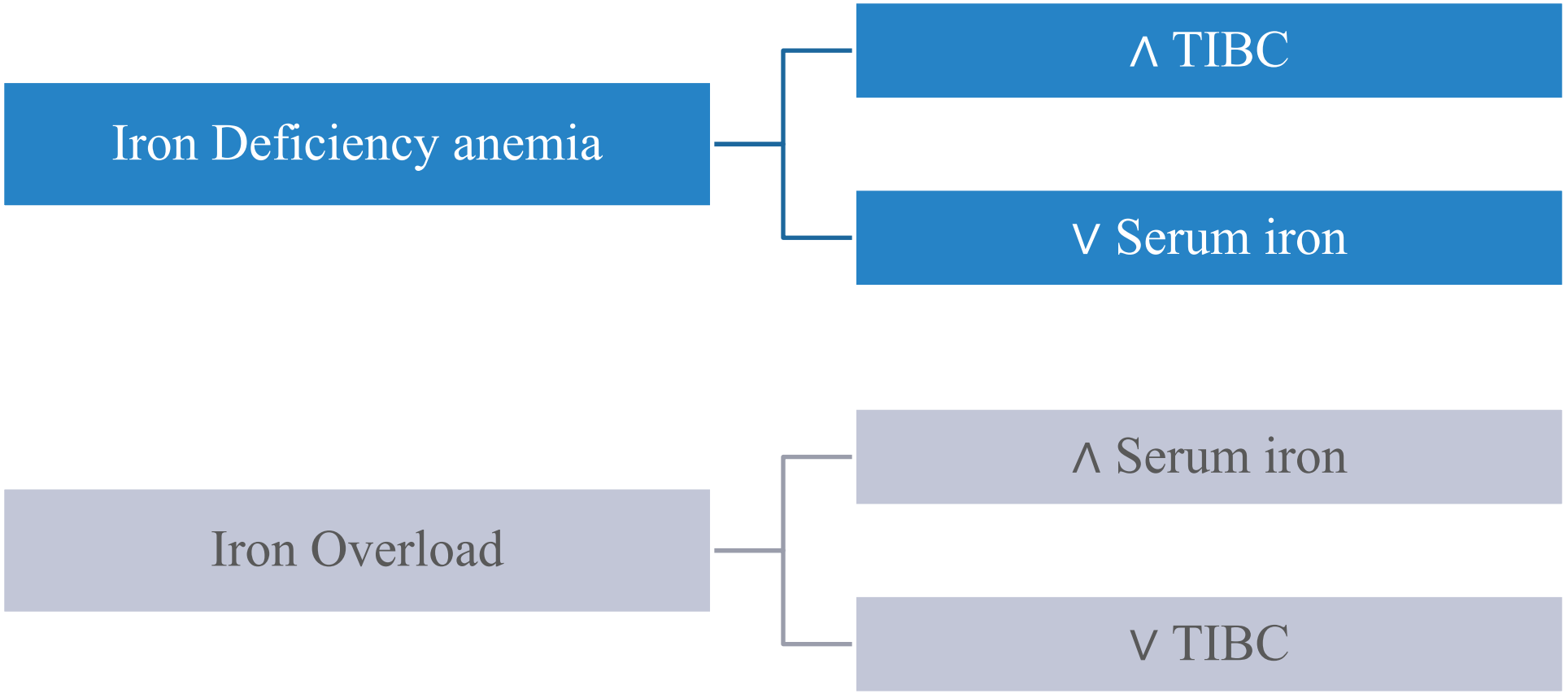
- **Serum iron concentration is high** when:
  - Bone marrow cannot utilize iron, hemolysis, increased absorption or defects in storage capabilities.
  - High values are also found in severe hepatitis due to release from liver cells.

Defect in Total iron binding capacity (TIBC)

Increase in iron deficiency anemia.

Decrease in hemochromatosis, malignant or rheumatic fever.

Results



Stored Iron ↑ transferrin ↓ TIBC ↓

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