

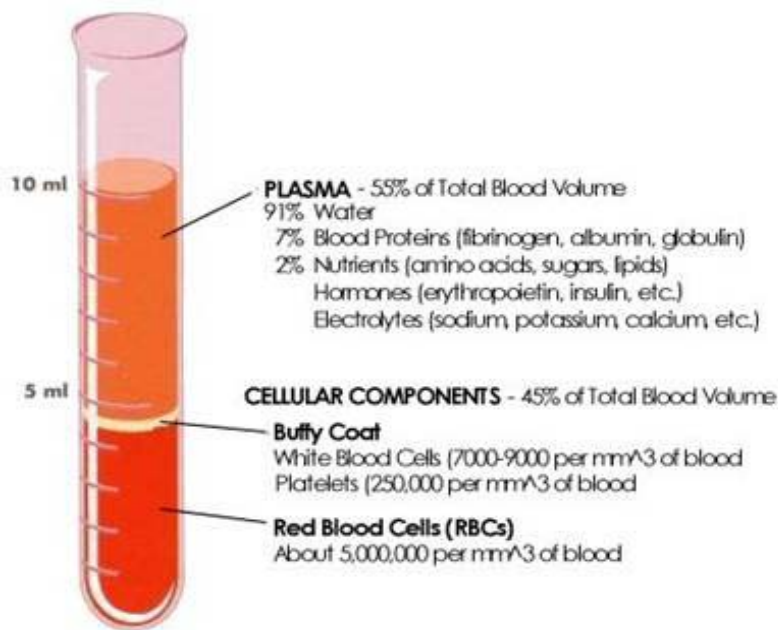
**Practical lab 13**  
**Hematocrit. Hemoglobin – identification, spectroscopy,**  
**crystallographic method (Teichman crystals)**

**Hematocrit**

Definition: The **hematocrit (Ht or HCT)** or **packed cell volume (PCV)** is the proportion of blood volume that is occupied by red blood cells.

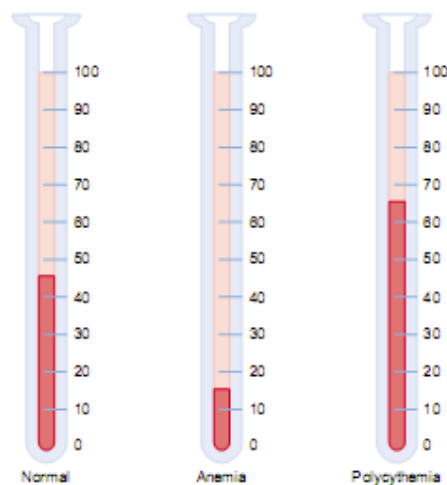
Method and principle: blood cells are heavier than plasma, so they can be separated according to mass by centrifugation. Blood is sampled on anticoagulant and placed in a hematocrit tube of 100 mm in height. The blood is then centrifuged at 3000 rpm for 5-10 min, the result being (as you can see in the figure below) a red column, represented by packed red blood cells, the height of which represents the hematocrit and a transparent column represented by plasma. The buffy coat in-between represents white blood cells and platelets.

Reading: read the height of the red column on the tube; because the height of the tube is 100 mm, the value read on the tube represents a percentage value.



Normal values:

- Women – 38% (41% ± 6%)
- Men – 42% (46.5% ± 6)
- New-born -50%



A normal hematocrit (HCT) value defines the state of normocytomia, a smaller value – oligocytomia, a greater value – polycytomia..

Variations of hematocrit:

1. With red cell count: - decrease in anemia, increase in poliglobulia
2. With place of sampling : venous blood has higher hematocrit than arterial blood, but lower than splenic blood.
3. With hydration status – hemodilution vs hemoconcentration
4. With erythropoiesis/ erythrolysis ratio

Amongst the erythrocyte parameters : hematocrit, hemoglobin, red cell count, we can establish the diagnosis of anemia. The type of anemia is established by taking into account the erythrocyte indices (MCV, MCH, MCHC)

MCV =mean cell volume of one RBC= HCT/red cell count = 80 -100fL

MCH= mean cell hemoglobin in one RBC= Hb concentration/ red cell count= 27- 32 pg

MCHC= mean hemoglobin concentration in all RBCs =hemoglobin conc/HCT= 320-360 g/L

With regard to these indices we can classify anemias into the following categories:

- I. normocromic, normocitic anemia:
  - acute hemoraging
  - aplastic/hypoplastic anemia
  - leucemya
  - renal/ hepatic disease
- II. hipocromic, microcytic anemia:
  - Iron deficient anemia
  - Cronic loss of blood
  - Hemolytic anemia
  - Cronic inflamation anemia
- III. normocromic, megalocytic anemia:
  - Anemie through B12deficit
  - Anemie through folic acid deficit

## HEMOGLOBIN

Hemoglobin (Hb) – major function in transporting blood gases.

Hemoglobin: a cromoprotein

Molecular weight = 68000da

has 4 subunits, each having 17000da :

- HEM (prosthetic group):
  - Fe<sup>2+</sup>
  - tetrapirolic nucleus (same as in chlorophyll and B12 Vitamin)
- GLOBIN (polypeptide)

$\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  = types of polypeptide chains.

### Hb types

	HbA	HbF	HbA <sub>2</sub>
Structure	$\alpha_2\beta_2$	$\alpha_2\gamma_2$	$\alpha_2\delta_2$
%	96-98	0,5-0,8	1,5-3,2

The conversion from HbF to HbA occurs 3-6 month after birth.

65% of Hb is synthesized in erythroblasts

35% of Hb is synthesized in reticulocytes.

## Hem and Hb synthesis

- I. Krebs cycle -> succinyl coenzyme A (SucCoA)
- II. SucCoA + glicin ->  $\delta$  amino-levulinic acid (ALA)
- III. 2 ALA -> Porphobilinogen (pirolic nucleus)
- IV. 4 Pirobs -> Protoporphirine IX (PP IX)
- V. PP IX +  $Fe^{++}$  -> HEM ( $Fe^{++}$  is inserted in the center of PP IX)
- VI. 4 Hem + polipeptide (globin) -> hemoglobin chain ( $\alpha$  or  $\beta$ )
- VII. 2  $\alpha$  chains + 2  $\beta$  chains -> Hb A

## Globin synthesis

- dimeric structure
- 2 polypeptide pairs

There are:

- 2  $\alpha$  chains having 141 aa
- 2  $\beta$  chains having 146 aa.

Changing the sequence of aa leads to - type  $\gamma$  (146 aa, 37 different from type  $\beta$ )  
- type  $\delta$  (146 aa, 10 different)  
- tip  $\epsilon$

The protein has an  $\alpha$  helix spatial structure. The synthesis takes place in ribosomes, where the polypeptide  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  chains are formed.

The 2 dimmers ( $\alpha_2$  &  $\beta_2$ ) form together with hem HbA. There are also  $A_1$ ,  $A_2$ ,  $A_3$  types.

HbA<sub>3</sub> can be found in elder, having modified  $\beta$  chains.

HbF (fetal) has a high binding capacity for oxygen.

HbU (embryo type) – Gowers – has  $\alpha_2$ ,  $\epsilon_2$  chains.

## Iron metabolism

Total iron amount in the body =  $4 \pm 0,5g$ .

It is divided in the following compartments:

- hemoglobin – 67%
- iron stored as ferritin and hemosiderin – 27%
- myoglobin – 3,5%
- oxidative enzymes – 0,2%
- plasma

## Iron absorption

Minimum requirements:

- newborn: 10 mg/24h
- children: 5 mg/24h
- young women: 20mg/24h
- pregnant women: 30mg/24h
- women after menopause: 10mg/24h
- men: 10mg/24h

## Identification of hemoglobin

### Principle:

comparing clorhemine obtained from the tested blood with the Sahli hemoglobinometer clorhemine standard solution.

### Necessary material:

- Sahli hemoglobinometer consisting in:
  - 2 lateral tubes with the standard solution – brown color.
  - 1 central tube used for the test – it has a gradation from 10 to 140 Sahli units (100<sup>th</sup> degree equals 16g Hb%)

- annexed: a capillary pipette for blood maneuvering (with a 20 µl sign) + 1 distilled water pipette + 1 glass stirring stick.
- HCl N/10 solution
- Distilled water

Protocol:

- Put in the test tube HCl N/10 until 10<sup>th</sup> division.
- Add with the capillary pipette 20 µl blood.
- Brownish Chlorhemine results.
- Dilute the resulted solution with distilled water till it gets the same color with the standard probe.
- Read the digits where the level has raised – meaning the HB amount in Sahli units.
- In order to transform it in grams, calculate as follows:

$$\begin{array}{r} 100 \dots\dots\dots 16 \text{ g} \\ \text{read value} \dots\dots\dots X \text{ g} \end{array}$$

$$X = 16 \times \text{read value} / 100$$

Normal values:

- men – 15-17g%
- women – 13,5-15,5g%
- newborn – 17-20g.

**Hb Spectroscopy**

Principle:

Spectroscopy is based on the difference between the absorption spectra of hemoglobin and its related compounds.

Necessary material:

- spectroscope with direct vision
- tubes
- hemoglobin & derived solutions
- crystals of sodium hydrosulfite
- saturated potassium ferriocyanide.

Protocol:

- **oxyhemoglobin spectra (OHb)**
  - diluted blood solution (2-3 drops in 20 ml distilled water)
  - in contact with the air the blood turns bright red (Hb gets saturated with oxygen.)
  - put the tube in front of the spectroscope
  - the oxyhemoglobin absorption spectra has 2 bands:
    - a wide yellow one (577 nm)
    - a narrow green one (536 – 556 nm)
- **Reduced hemoglobin spectra**
  - put in the HbO<sub>2</sub> – 10 ml solution + a couple of crystals of sodium hydrosulfite.
  - the color turns violet red, due to the reduced Hb.
  - one can see a single green yellow band = Stokes band (530-548 nm)
- **Carboxihemoglobin spectra (HbCO)**
  - Carboxihemoglobin is formed by the coordinative binding of CO to hem's iron. It is a reversible reaction. The affinity of Hb for CO is 210 times higher than for O<sub>2</sub>. If we breathe CO – intoxication– 40% carboxihemoglobin = lethal value.
  - there are 2 bands:
    - A yellow one (564-579 nm)

- A green one (530-548 nm)
- both of them have a violet trend.
- **Met hemoglobin spectra**
- put 5-6 blood drops in 20 ml water.
- add 8-10 drops of saturated potassium ferriocyanide solution. The solution gets dark brown due to the formed met hemoglobin.
- it has 4 absorption bands:
  - A narrow red one (620-630 nm) – specific for met hemoglobin.
  - 2 pale yellow green bands
  - a wide blue one (486-578 nm)
- met hemoglobin is improper for oxygen transport – a certain degree of hypoxia is reached.
  - Normally, erythrocytes contain 0,1-0,4 met hemoglobin;.

### **The crystallographic method (Teichman crystals)**

#### Principle:

Hemoglobin treated with “arising” HCl (resulted from the reaction of NaCl + CH<sub>3</sub>COOH) → chlorhemine

Chlorhemine can be identified as brownish diamond shaped crystals = Teichman crystals

#### Necessary material:

- a microscope slide, a glass slide cover, gas burner, microscope, blood, glacial acetic acid to which NaCl 1% has been added.

#### Protocol:

- Pour 1 blood drop on the slide.
- dry it by heating, but not over 40-45 degrees C – to avoid protein denaturation. Test it on the back of your hand.
- after add 1 drop of glacial acetic acid and NaCl 1% and cover it with the glass slide cover.
- heat it until it boils. Due to chlorhemine the probe gets a brown color.
- cool it and put it under the microscope. It is suggested to cool it slower in order to get bigger crystals.

#### Discussion:

- the method is used in forensic medicine in order to identify blood stains.
- the crystals show the presence of blood without giving any information about the species.