Blood

blood testing, blood clotting

**Laboratory exercise and seminar in medical physiology**

Home preparation, study materials and learning objectives

**Learning objectives – what you should learn?**

• List hematological parameters and interpret their values

• Explain the consequences of their changes on other physiological parameters

• Define the individual steps participating in hemostasis and the balance between the pro- and anticoagulant state of the organism

**Study materials**

* Lecture
* Textbook L. Constanzo – Physiology, 6th or 7th edition
  + Pages 222-223
* Textbook Guyton and Hall – Medical Physiology, 13th edition
  + Chapter 36 and 37

<https://www.youtube.com/watch?v=cVE7rtO83Dk>

<https://www.youtube.com/watch?v=ZMf3DyN5zJw>

**Home preparation**

**Fill the table:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **Definition** | **Values** | **Limit in men** | **Limit in Women** |
| **RBC** |  |  |  |  |
| **Hgb** |  |  |  |  |
| **Htc** |  |  |  |  |
| **MCV** |  |  |  |  |
| **MCH** |  |  |  |  |
| **MCHC** |  |  |  |  |
| **RDW** |  |  |  |  |
| **RET** |  |  |  |  |
| **WBC** |  |  |  |  |
| **PLT** |  |  |  |  |
| **APTT** |  |  |  |  |
| **Quick** |  |  |  |  |
| **INR** |  |  |  |  |

**Bonus material**

https://www.youtube.com/watch?v=9\_1Wz\_DZE-8

**Materials for the practical exercise**

**I. Determination of haematocrit**

The hematocrit expresses the volume fraction of erythrocytes out of the total blood volume. In men, the value of HTK is 0.39-0.51, in women 0.33-0.47. The determination of the hematocrit value is important for many clinical aspects, including the calculation of baseline red blood cell values.

In determining the HTK, we determine the volume percentage of erythrocytes out of the total volume of centrifuged blood. After centrifugation, the individual blood components are separated by weight. The lowest column is the erythrocyte column, above it is a thin layer of leukocytes and platelets, and the highest is the plasma column.

**Answer the questions:**

Which factors can affect the hematocrit value? Name several physiological and several pathological causes.

Is a higher hematocrit value clearly a positive phenomenon? Justify.

**II. Blood clotting**

**1. Anticoagulation**

Inhibition of coagulation by antithrombin III:

Antithrombin forms complexes with factors IIa,IXa, Xa, XIa and XIIa and thereby inhibits its enzymatic activity. Its effect is substantially (by orders of magnitude) enhanced by its cofactor heparin.

**Anticoagulant therapy**

Therapeutic administration of heparin is used to prolong coagulation times, for example, in patients with deep vein thrombosis.

Administration of coumarins (Warfarin) also prolongs the coagulation time. It prevents the synthesis of factors II, VII, IX and X in the liver by inhibiting the vitamin K required for their final carboxylation. Unfortunately, coumarins do not only prevent the carboxylation of coagulation factors, but also of anticoagulant factors: protein C and protein S. This is the reason for the risk of inducing a transient increase in coagulation during the initial administration of coumarins.

**Preparation of non-clotting blood in vitro**

Blood clotting often needs to be prevented for laboratory examination of the blood sample (e.g. determination of the number of blood elements, haemoglobin concentration, sedimentation rate, etc.).

**2. Fibrinolysis**

Fibrinolysis, endothelial recanalisation and endothelial regeneration are important components of haemostasis. This is evidenced by the fact that one of the first players in the activation of coagulation, kallikrein, activates fibrinolysis right at the beginning of the cascade.

The proteolytic enzyme plasmin is responsible for the cleavage of fibrin. Its precursor plasminogen is a component of plasma. The plasminogen activator is released from the damaged tissue. The activity of platelets (coagulum retraction) and the content of their granules (endothelial growth factors, PDGF - platelet derived growth factor ) as well as the reaction of surrounding tissues are also important for repair.

**Answer the questions:**

For which examinations clotting blood is used and for which non-clotting blood is used, please explain.

What substances would you use to prepare non-clotting blood? Explain their mechanism of action.

What is the difference between anticoagulant and fibrinolytic therapy?

In what situations would you use it?

What are the risks of disproportionate anticoagulation and fibrinolytic therapy?

When is topical and when is total use of these agents appropriate?

**III. Coagulation tests**

**Determination of thromboplastin (also used prothrombin) time (Quick test)**

Determination of the prothrombin time tests the external coagulation system represented by factor VII, calcium ions and activated tissue thromboplastin. In vivo, the activator of factor VII is tissue factor in the subendothelium and phospholipids from damaged tissue; in vitro, it is administered in excess as complete thromboplastin, a proteinlipid complex derived from human brain tissue. For the test, we need the subject's decalcified plasma, which is obtained by saturating calcium ions with sodium citrate added to venous blood in a 1:9 ratio (0.5 ml sodium citrate and 4.5 ml blood) and then centrifuging for 10 min at 2000 rpm. The prothrombin time is determined by monitoring the time taken for the first fibrin fibre to form after mixing decalcified plasma, complete thromboplastin and calcium ions in calcium chloride solution at 37 °C. The resulting time depends on the concentration of the different coagulation factors of the external and common system (VII, V, X, II and I ).

The range of physiological values of prothrombin time is 12-15 s. To standardize the test result, the International Normalized Ratio (INR) is used with a physiological range of 0.9-1.2. It is the ratio of the prothrombin time value of the test sample to the prothrombin time of standard (physiological) plasma.According to the INR values, anticoagulation therapy is corrected and monitored.

**Activated partial thromboplastin time (APTT)**

The activated partial thromboplastin time test monitors the efficiency of the internal coagulation system. The kit prepared for this test contains a kaolin-kephalin complex, in which kaolin represents the negative surface, and kephalin contains tissue factor and replaces platelet phospholipid, necessary for activation of factor X. The plasma prepared for this test must therefore not only be decalcified but also free of platelets (centrifugation at least 10 min at 2 000 rpm).

The partial thromboplastin time depends mainly on the initial events of haemocoagulation in the internal system, namely factors XII, XI, IX and VIII.Only secondarily does it capture the efficiency of the factors of the common system, i.e., X, V, II, and I.

The normal time of the test is 28-40 s. The time is again compared with that of standard plasma and their ratio should have a physiological value of 0.83-1.3 (APTTR).

**Answer the questions:**

What will be the result of the Quick test and APTT in hemophilia A?

What will be the result of APTT in the absence of f. VII?

Can hemorrhagic manifestations occur when the intestinal flora is disturbed?

Does impaired fat resorption in the small intestine affect hemocoagulation?

What is blood serum?

Prepared using material from the Department of Physiology, 1st Faculty of Medicine, Charles University.